

Recognising and defining a new crown clade within Stromboidea Rafinesque, 1815 (Mollusca, Gastropoda)

Stephen J. Maxwell¹, Aart M. Dekkers²,
Tasmin L. Rymer^{1,3}, Bradley C. Congdon^{1,3}

1 College of Science and Engineering, James Cook University, Cairns Qld 4870, Australia **2** Oasestraat 79, 1448 NR Purmerend, The Netherlands **3** Centre for Tropical Environmental and Sustainability Sciences, James Cook University, Cairns Qld 4870, Australia

Corresponding author: Stephen J. Maxwell (stephen.maxwell@my.jcu.edu.au)

Academic editor: *Thierry Backeljau* | Received 8 March 2019 | Accepted 12 June 2019 | Published 29 July 2019

<http://zoobank.org/DABE488F-B6AA-4E8A-90B2-020A856852AF>

Citation: Maxwell SJ, Dekkers AM, Rymer TL, Congdon BC (2019) Recognising and defining a new crown clade within Stromboidea Rafinesque, 1815 (Mollusca, Gastropoda). ZooKeys 867: 1–7. <https://doi.org/10.3897/zookeys.867.34381>

Abstract

This paper defines a new crown clade Neostromboidea to separate the Strombidae, Rostellariidae, and Seraphsidae from their sister families Struthiolariidae and Aporrhaidae. There is significant value to understanding evolutionary processes within Stromboidea to recognise the universal similarity in the position of the eye on the end of peduncles and a diminished cephalic tentacle that arises from the middle to the end on that peduncle. This is in contrast to other members of the Stromboidea where the eye is located at the base of the cephalic tentacle. These physiological differences represent two set of organisms with divergent and independent evolutionary life histories and therefore these differences need to be identifiable within the nomenclature to bring meaning to the way we name things.

Keywords

Aporrhaidae, Rostellariidae, Seraphsidae, Strombidae, Struthiolariidae

Introduction

Current Stromboidea Rafinesque, 1815 systematics has suffered from the effects of taxonomic inflation that has destroyed the evolutionary contextualisation that was once found within the historical nomenclature (Abbott 1960). This paper brings back

that evolutionary contextualisation with the recognition of a new clade. There is a long history of morphologically based division with the Stromboidea. Early studies classified the Mollusca Linné, 1758 in terms of gross anatomy, with the radula being the dominating feature in some classifications (Troschel 1856–1863; Mörch 1866; Cooke 1895; Thiele 1931), while other classifications were based on the structure and positioning of the mantle cavity and the buccal mass, or movement of the sole of the foot (MacDonald 1857; Cooke 1885, 1927). The historically recognised recent members of the Strombidae Rafinesque, 1815 (s. l.) included the now separated Rostellariidae Gabb, 1868 and Seraphsidae Jung, 1974, both of which share a universal similarity in the positioning of the eye on the end of the peduncle, and a diminished cephalic tentacle that arises from the middle to the end on the peduncle. This contrasts with other members of the Stromboidea, the outgroups Struthiolariidae Gabb, 1868 and Aporrhaidae Gray, 1850, where the eye is located at the base of the cephalic tentacle, which is not reduced (Figure 1).

This study argues for the division of the crown clade Stromboidea based on shared morphological synapomorphies between families within this clade, which indicate a level of divergent and independent evolutionary life histories. This separation is needed

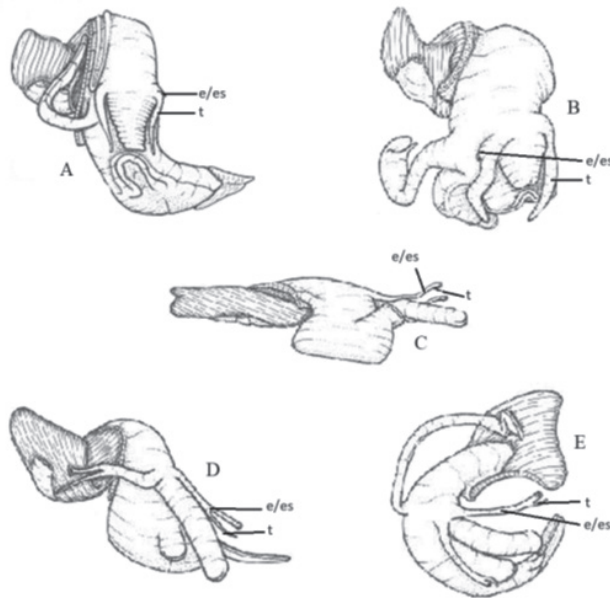


Figure 1. The anatomy of representatives of the five families with Stromboidea Rafinesque, 1815 indicating the eye (e) eye stalk (es) and the tentacle (t) **A** Struthiolariidae Gabb, 1868 – *Tylospira scutulata* (Gmelin, 1791) (Simone 2005, fig. 254) **B** Aporrhaidae Gray, 1850 – *Aporrhais occidentalis* (Beck, 1836) (Simone 2005, fig. 297) **C** Seraphsidae Jung, 1974 – *Terebellum terebellum* (Linné, 1758) (Simone 2005, fig. 231) **D** Rostellariidae Gabb, 1868 – *Tibia insulaechorab* (Röding, 1798) (Simone 2005, fig. 249) **E** Strombidae Rafinesque, 1815 – *Strombus gallus* Linné, 1758 (Simone 2005, fig. 164).

to assist in resolving the higher order systematics of the Stromboidea to enable a more focused approach to understanding relationships and ancestral morphological states and patterns. There is a requirement for a name-bearing reference point that brings together the historically recognised members of the Strombidae that have now been divided into three separate families, and to distinguish those families from the other Stromboidea taxa, to achieve basal resolution of the crown clade through a clear definition and diagnosis enabling the separation from sister taxa, thus enabling an evolutionary meaning to be brought to the nomenclature of the clade.

The aim of this paper is to divide the superfamily Stromboidea by morphological evidence into two distinctive evolutionary crown clades. Crown clades are clades that are defined based on living taxa. The crown clade focussed upon here contains the families Seraphsidae, Strombidae, and Rostellariidae.

Systematic part

Mollusca Linné, 1758

Caenogastropoda Cuvier, 1797

Sorbeoconcha Ponder & Lindberg, 1987

Stromboidea Rafinesque, 1815

Neostromboidea, new clade

Type. The genus *Strombus* Linné, 1758.

Definition: The clade is nested within Stromboidea, with the characteristics outlined in the diagnosis, and contains taxa more closely related to *Strombus pugilis* Linné, 1758 (Strombidae) *Terebellum terebellum* (Linné, 1758) (Seraphsidae) and *Tibia fusus* (Linné, 1758) (Rostellariidae) than Struthiolariidae Gabb, 1868 and Aporrhaidae Gray, 1850.

Diagnosis: The animal possesses eyes on the end of the peduncles. The cephalic tentacle is also located on the peduncle towards the distal end. The radula has a central rachidian tooth with three lateral teeth either side. The foot is laterally compressed, with a defined propodium and a metapodium. The shell form changes upon maturation with the development of an outer lip structure.

Remarks: Neostromboidea is well supported in previous revisions and studies on the phylogeny of Stromboidea (Figure 2; Latiolais et al. 2006; Simone 2005). Simone (2005) marked this clade as “node 9” and noted that it was monophyletic within the Stromboidea. Latiolais et al. (2006) used Aporrhaidae as the outgroup in their analysis, which demonstrated a significant genetic distance between the taxa Strombidae and Aporrhaidae. Neostromboidea brings a higher level of resolution to the nomenclature by restoring the cladistic understanding and evolutionary meaning that had been lost as a consequence of taxonomic inflation (Abbott 1960; Simone 2005; MolluscaBase 2019).

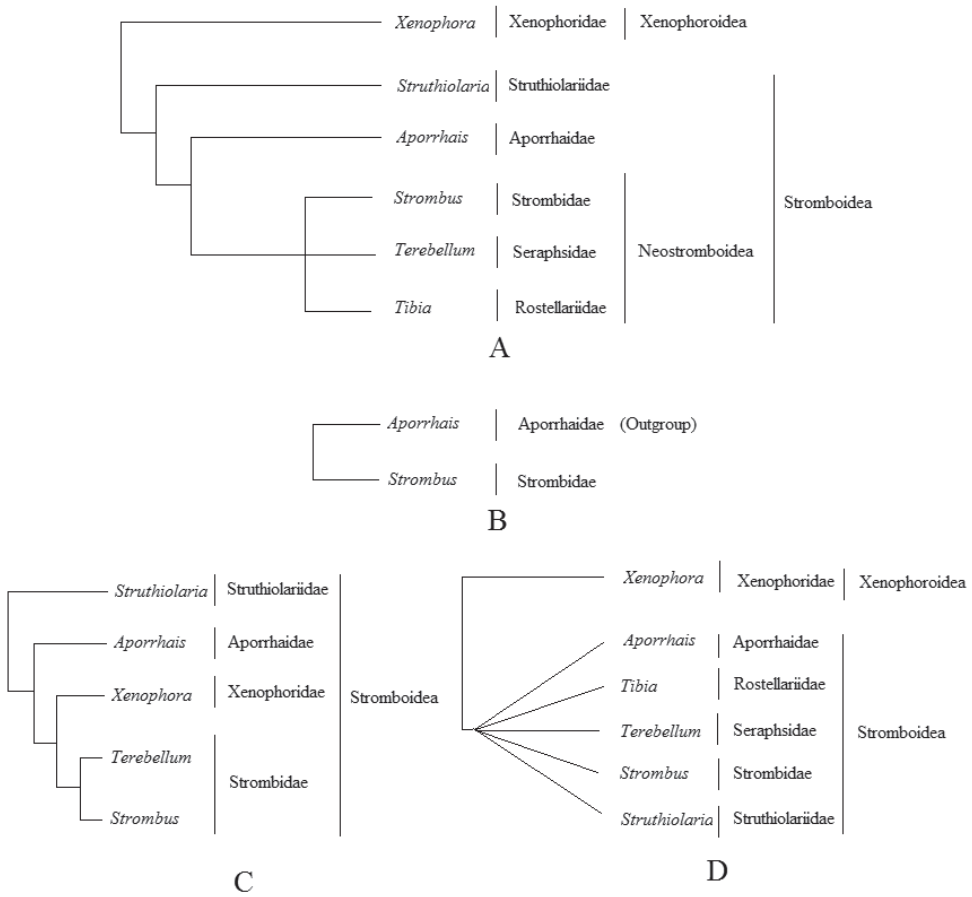


Figure 2. The new phylogeny of Stromboidea **A** and alternatives found from previous revisions **B** molecular analysis after Latiolais et al. (2006: 440, fig. 2) **C** anatomical analysis after Simone 2005: 261, fig. 388 **D** cladogram based on the nomenclature after MolluscaBase (2019).

Discussion

The Neostromboidea falls within the clade Stromboidea which belongs to the highly variable invertebrate Gastropoda (Mollusca). The ancestral resolution of Neostromboidea is extremely unstable, with many conflicting views on the exact ancestors that provide a division between Neostromboidea and the two immediate outgroups Struthiolariidae and Aporrhaidae (Gabb 1869; Roy 1994; Kiel and Bandel 2002; Bandel 2007). It is postulated that *Phyllocheilus* Gabb, 1868 and *Pterodonta* d'Orbigny, 1843 form the shared common ancestor of the new clade and the Aporrhaidae and Struthiolariidae based on gross morphology and the similarities with known stromboi- dal ancestors of Rostellariidae.

The Cretaceous clade Pugnellidae Kiel & Bandel, 1999, which is considered an ancestor of Strombidae (Wenz 1938; Sohl 1960), is somewhat fluid in its content. However, recent revisions have clarified the taxonomic position of Pugnellidae, which is now considered to be a descendant of the Aporrhaidae (Kiel and Bandel 1999). This position is based on the structure of the protoconch, the low height of the teleoconch, the lack of ornamentation typical of Strombidae, the presence of a posterior rostrum with a groove, and, importantly, the extension of the callus from the inner lip, which covers a greater portion of the teleoconch (Popenoe 1983; Kiel and Bandel 1999).

Morphologically, recent members of the Struthiolariidae and Aporrhaidae differ from Neostromboidea in having a broad rather flattened foot, as well as eyes on the base of the tentacles rather than on peduncles as with the Neostromboidea (Gardner 1875). These recent members also differ for the most part in their feeding processes, whereby the animal lies buried and extends its proboscis to 'grasp' at potential food items, or they are filter feeders (Purchon 1977; Savazzi 1988, 1991). However, the buried grasping feeding habit is not a significant distinguishing characteristic separating Struthiolariidae and Aporrhaidae from the Seraphsidae (Jung and Abbott 1967). Given the general instability of the aporrhaid group, it is not within the scope of this study to argue inclusiveness or provide a definition for that complex.

Conclusions

The Neostromboidea incorporates those taxa that developed a basal sinus on the shell outer lip in conjunction with eyes placed on peduncles. The co-evolution of this shell structure and morphological trait allowed the eyestalk to protrude whilst the animal remained aperture face down on the substrate, protecting the soft parts from exposure. Furthermore, the movement of the cephalic tentacle towards the distal end of the eyestalk, thus protruding out from the basal sinus, enables the animal to achieve sensory awareness without any of the soft parts being exposed. There is much greater resolution within the Stromboidea with the recognition and naming of this clade, enabling researchers to focus on the evolution of either of the two divergent evolutionary trajectories of that clade's members.

References

- Abbott RT (1960) The genus *Strombus* in the Indo-Pacific. *Indo-Pacific Mollusca* 1: 33–146.
- Bandel K (2007) About the larval shell of some Stromboidea, connected to a review of the classification and phylogeny of the Strombimorpha (Caenogastropoda). *Freiberger Forschungshefte C* 524: 97–206.
- Cooke AH (1895) Molluscs. In: Harmer SF, Shipley AE (Eds) *The Cambridge Natural History*. Volume III, Macmillan and Co, London, 1–459.

- Cuvier G (1797) Tableau Élémentaire de l'Histoire Naturelle des Animaux. Baudouin, Paris, 710 pp. <https://doi.org/10.5962/bhl.title.11203>
- d'Orbigny MA (1852) Prodrome de paléontologie stratigraphique universelle des animaux mollusques et rayonnés faisant suite au cours élémentaire de paléontologie et de géologie stratigraphiques, Volume 3. Victor Masson, Paris, 189 pp. <https://doi.org/10.5962/bhl.title.45605>
- Gabb WM (1868) An attempt at a revision of the two families Strombidae and Aporrhaidae. American Journal of Conchology 4: 137–149.
- Gabb WM (1869). Notes on the genera *Alaria*, *Diarthema*, *Dicroloma*, being a supplement to “An attempt at a revision of the Strombidae and Aporrhaidae”. American Journal of Conchology 5: 19–23.
- Gardner JS (1875) On the Gault Aporrhaidae. The Geological Magazine 2: 49–56.
- Jung P (1974) A revision of the family Seraphsidae (Gastropoda: Strombacea). Palaeontographica Americana 7: 4–72.
- Jung P, Abbott RT (1967) The Genus *Terebellum* (Gastropoda: Strombacea). Indo-Pacific Mollusca 1: 445–454.
- Kiel S, Bandel K (1999) The Pugnellidae, a new stromboidean family (Gastropoda) from the Upper Cretaceous. Paläontologische Zeitschrift 73: 47–58.
- Kiel S, Bandel K (2002) About some aporrhaid and strombid gastropods from the Late Cretaceous. Paläontologische Zeitschrift 76: 83–97.
- Latiolais JM, Taylor MS, Roy K, Helleberg ME (2006) A molecular phylogenetic analysis of strombid gastropod morphological diversity. Molecular Phylogenetics and Evolution 41: 436–444.
- Linné C (1758) Systema Naturae per Regna Tria Naturae Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis, vol 1 (10th edn). Laurentii Salvii, Holmiae, 823 pp. <https://doi.org/10.5962/bhl.title.542>
- MacDonald JD (1857) Observations on the natural affinities and classification of Gastropoda. Proceedings of the Royal Society of London 8: 384–393.
- MolluscaBase (2019) MolluscaBase. Stromboidea Rafinesque, 1815. World Register of Marine Species. <http://www.marinespecies.org> [02/06/2019]
- Mörch OAL (1866) On the modern classification of Mollusks (translated G.W. Tryon). American Journal of Conchology 2: 83–86.
- Ponder WF, Lindberg DR (1997) Towards a phylogeny of gastropod mollusks: An analysis using morphological characters. Zoological Journal of the Linnean Society 119: 83–265.
- Popenoe WP (1983). Cretaceous Aporrhaidae from California: Aporrhainae and Arrhoginae. Journal of Paleontology 57: 742–765.
- Purchon RD (1977) The Biology of the Mollusca (2nd edn). In: Kerkut GA (Ed.) International Series of Monographs in Pure and Applied Science: Division: Zoology. Pergamon Press, Oxford, 560 pp.
- Rafinesque CS (1815) Analyse de la Nature, ou Tableau de l'Univers et des Corps Organisés. Aux dépens de l'auteur, Palerme, 244 pp. <https://doi.org/10.5962/bhl.title.106607>

- Roy K (1994) Effects of the Mesozoic Marine Revolution on the taxonomic, morphologic and biogeographic evolution of a group: aporrhaid gastropods during the Mesozoic. *Paleobiology* 20: 274–296.
- Savazzi, E. (1991) Constructional morphology of strombid gastropods. *Lethaia* 24: 311–331.
- Savazzi E (1988) Burrowing behaviour in recent Indo-Pacific strombid gastropods. *Neues Jahrbuch für Geologie und Paläontologie Monatshefte* 1988: 415–430.
- Simone LR (2005) Comparative morphological study of representatives of the three families of Stromboidea and the Xenophoroidea (Mollusca, Caenogastropoda), with an assessment of their phylogeny. *Arquivos de Zoologia* 37: 141–267. <https://doi.org/10.11606/issn.2176-7793.v37i2p141-267>
- Sohl NF (1960) Archaeogastropoda, Mesogastropoda and stratigraphy of the Ripley, Owl Creek, and Prairie Bluff formations. United States Geological Survey Professional Paper 331A: 1–151.
- Thiele J (1931) *Handbuch der Systematischen Weichtierkunde*. Neudruck der Ausgabe, Stuttgart, 1154 pp.
- Troschel FH (1856-1863) *Das Gebiss der Schnecken zur Begründung einer natürlichen Classification*. Nicolaische Verlagsbuchhandlung, Berlin, 409 pp. <https://doi.org/10.5962/bhl.title.11262>
- Wenz W (1938) Gastropoda Teil 1: Allgemeiner Teil und Prosobranchia. In Schindewolf H (Ed) *Handbuch der Paläozoologie* 6: 721–1639.

A new species and new record of the cryptobiotic ant genus *Ponera* Latreille, 1804 (Hymenoptera, Formicidae) from Hong Kong

Mac P. Pierce¹, Chi-Man Leong¹, Benoit Guénard¹

¹ *The University of Hong Kong, School of Biological Sciences, Kadoorie Biological Sciences Building, Pok Fu Lam Road, Hong Kong SAR, China*

Corresponding author: Mac P. Pierce (macppierce@gmail.com)

Academic editor: M. Borowiec | Received 13 May 2019 | Accepted 9 July 2019 | Published 29 July 2019

<http://zoobank.org/34B58A66-6B98-47D4-9D9A-A8B119818EA5>

Citation: Pierce MP, Leong C-M, Guénard B (2019) A new species and new record of the cryptobiotic ant genus *Ponera* Latreille, 1804 (Hymenoptera, Formicidae) from Hong Kong. ZooKeys 867: 9–21. <https://doi.org/10.3897/zookeys.867.36139>

Abstract

Despite its small size, Hong Kong hosts a surprising level of ant diversity. Through faunal studies on arthropods conducted in Hong Kong over recent years, a new record and species of the genus *Ponera* have been discovered, which are introduced here. *Ponera guangxiensis* Zhou, 2001 is reported for the first time from Hong Kong, and *Ponera tudigong* **sp. nov.** is here described as a new species, easily distinguishable from other *Ponera* species and unique within the genus for its four mandibular teeth.

Keywords

Asia, biodiversity, description, Ponerinae, taxonomy

Introduction

The cryptic ant genus *Ponera* Latreille, 1804, contains 59 valid species at present (Bolton 2019; Leong et al. 2019). Known mostly from the Old World, the bulk of its diversity occurs in the tropical to subtropical Oriental realm (Janicki et al. 2016; Guénard et al. 2017). The tropical diversity of *Ponera* in the Oriental realm and further south into the Oceanian and Australian realms contrasts with its absence as native species in the Afrotropical and Madagascan realms, and its limited distribution within the Neotropical realm, where it is limited to two species in the northern part of Mesoamerica (Branstetter and Longino 2019). Relatively little is known of the biology and ecology of the species of *Ponera*, owing

to its cryptobiotic lifestyle and paucity of collection. In general, *Ponera* has small colonies (ca. hundreds of individuals), is most commonly found underneath rocks and rotting logs, and is likely a generalist predator of small arthropods (Schmidt and Shattuck 2014).

At present only one species of *Ponera*, *P. sinensis* Wheeler, 1928, is known from Hong Kong and the surrounding Guangdong province. Through faunal studies conducted in Hong Kong using pitfall traps and sifted leaf-litter, two newly recorded species have been found: *P. guangxiensis* Zhou, 2001, and *P. tudigong* sp. nov., newly described in this paper. We include here accounts of the two species, the description of *P. tudigong* sp. nov., and an update to the key to East Asian species of *Ponera* presented by Leong et al. (2019).

Materials and methods

Material examined

Twenty-nine individuals collected from Hong Kong were examined (Figure 1). All non-type material has been deposited in the Insect Biodiversity and Biogeography Laboratory (IBBL) at the University of Hong Kong. The type specimens of *P. tudigong* have been deposited at the California Academy of Sciences (CAS). Images and specimen data were uploaded to AntWeb.org (2018) and are available in the supplementary specimen file.

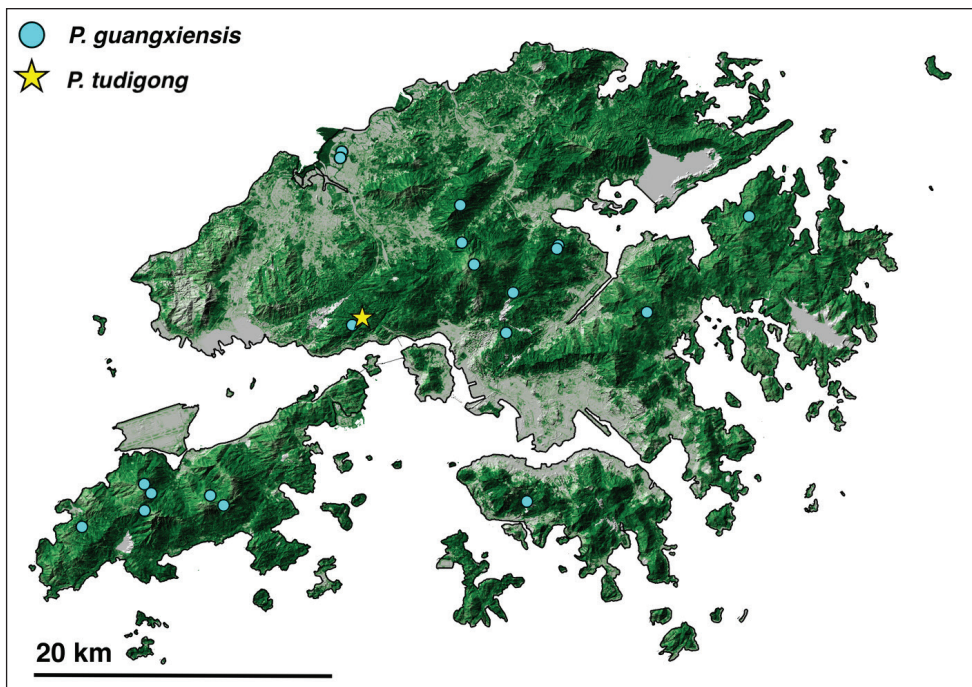


Figure 1. Topographic map of Hong Kong, with tree cover shown in green (darker color indicates greater tree cover, lighter color indicates less). Collection records for Hong Kong *Ponera* species are: *P. guangxiensis* (light-blue circle), *P. tudigong* sp. nov. (yellow star).

Morphology

Morphological measurements were done using a Leica S8AP0 stereomicroscope at 80 × magnification. Measurements used are detailed below and follow those used in Leong et al. (2019), with the exception of the addition of mandible length (MaL) and change of total length (TL) which are described below. Weber's length (WL) has been changed to mesosomal length (ML). Measurements are reported in millimeters to two decimal places. Repeated measurements on a single individual resulted in an accuracy of 0.01 mm. Individuals of *Ponera guangxiensis* from different regions of Hong Kong were measured to account for population variability. Terminology follows Taylor (1967), Harris (1979), Bolton (1994), and Keller (2011).

Imaging

Specimen images were taken using a Leica DFC450 digital camera mounted on a Leica M205C dissecting microscope, and processed using Leica Application Suite V4 software.

| | |
|-------------|--|
| ATL | Abdominal tergum III length. Maximum length of 3 rd abdominal tergum (= 1 st gastral tergum), measured from the center of the anterior margin to the center of the posterior margin, in dorsal view. |
| ATW | Abdominal tergum III width. Maximum width of the 3 rd abdominal tergum (= 1 st gastral tergum), measured as a straight line from one lateral margin to the other, in dorsal view. |
| HL | Head length. Maximum length of the head, measured as a straight line between the center of the posterior cephalic margin and the center of the anterior clypeal margin, in full-face view. |
| HW | Head width. Maximum width of the head, measured as a straight line from one lateral margin to the other, in full-face view. |
| MaL | Mandible length. Maximum length of the mandible, measured as a straight line from the mandibular insertion to the tip of the apical tooth, with the mandible in dorsal view. |
| ML | Mesosomal length. Maximum length of the mesosoma, measured as a straight line from the anterior-most point of the pronotum to the posterior basal angle of the metapleuron, in profile view. |
| PeH | Petiole height. Maximum height of the petiole, measured as a straight line from the ventral margin of the subpetiolar process to the dorsal margin of the petiole, in profile view. |
| PeNL | Petiole node length. Length of the node of the petiole, measured as a straight line from the anterior margin of the petiole immediately above the dorsal base of the anterior petiolar tubercle to the posterior margin of the petiole, in profile view. |
| PeW | Petiole width. Maximum width of the petiole, measured as a straight line from one lateral margin of the petiole to the other, in dorsal view. |

| | |
|-------------|--|
| PrW | Pronotal width. Maximum width of the pronotum, measured as a straight line from one lateral margin of the pronotum to the other, in dorsal view. |
| SL | Scape length. Maximum length of the scape, measured as a straight line from the base of the scape (excluding the basal neck and condyle). |
| TL | Total length. Maximum horizontal length of the specimen from the anterior-most extent of the mandibles to the posterior tip of the gaster, measured in profile view. |
| ATI | Abdominal tergum III (= 1 st gastral tergum) index. $ATL/ATW \times 100$. |
| CI | Cephalic index. $HW/HL \times 100$. |
| DPeI | Dorsal petiole index. $PeW/PeNL \times 100$. |
| LPeI | Lateral petiole index. $PeNL/PeH \times 100$. |
| PeI | Petiole index. $PeW/PrW \times 100$. |
| SI | Scape index. $SL/HW \times 100$. |

Species accounts

Ponera tudigong sp. nov.

<http://zoobank.org/37401E74-D228-4D0B-AD52-995EDF69C40A>

Figs 2–6

Type material. Holotype, worker: Hong Kong Special Administrative Region, Tai Lam Country Park, 22.38079N, 114.05378E, ± 45 m, 249 m above sea level (a.s.l.), 8 November 2017 (collection code Pi-MPP-43-07, pitfall) [CAS, unique specimen identifier CASENT0872070].

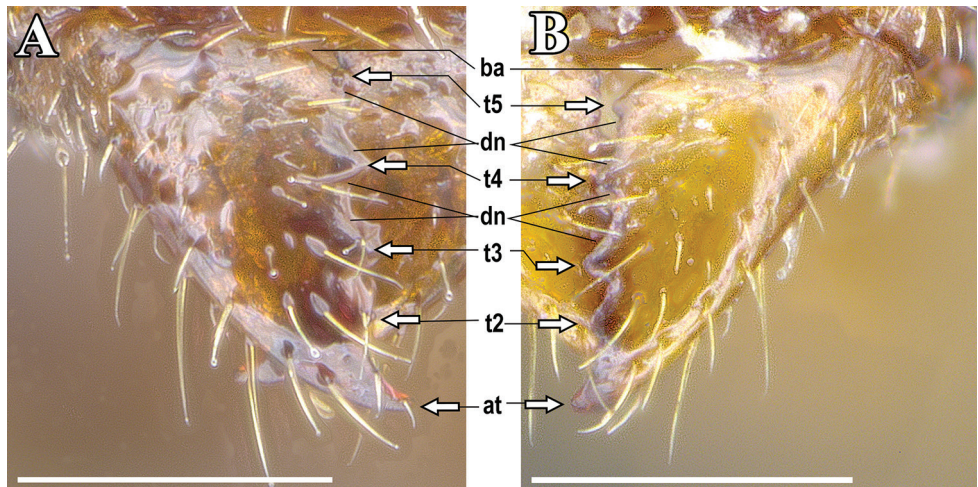


Figure 2. Mandible of *Ponera tudigong* sp. nov. **A** Queen (ANTWEB1009670) **B** worker (ANTWEB1009796). Abbreviations: ba: basal angle of mandible; t: tooth number; at: apical tooth of mandible; dn: denticle; sensu Bolton (1994).



Figure 3. *Ponera tudigong* sp. nov., worker (ANTWEB1009796). Head in full-face view.

Non-type material. Queen: same data as holotype worker (collection code Pi-MPP-43-05, pitfall) [CAS, CASENT0872071].

Geographic range. Hong Kong S.A.R., China.

Diagnosis (worker). *Ponera tudigong* can be distinguished from the other species in the genus by the following characters: masticatory margin of the mandible with a small denticle and four enlarged teeth (Figure 2), an apomorphic character for this new species (see Leong et al. 2019); petiole relatively thick in lateral view (LPeI 60) with the anterodorsal margin of the node of the petiole projecting slightly forward; metanotal groove forming clearly incised suture in dorsal view (Fig. 4A); eyes absent.

Measurements and indices (mm). Worker ($n = 1$): ATL 0.43, ATW 0.46, HL 0.59, HW 0.51, MaL 0.29, ML 0.82, PeH 0.39, PeNL 0.23, PeW 0.32, PrW 0.38, SL 0.43, TL 2.3. Indices: ATI 92, CI 85, DPeI 138, LPeI 60, PeI 85, SI 84.

Queen ($n = 1$): ATL 0.54, ATW 0.54, HL 0.65, HW 0.56, MaL 0.41, ML 1.03, PeH 0.43, PeNL 0.25, PeW 0.35, PrW 0.47, SL 0.48, TL 2.5. Indices: ATI 101, CI 85, DPeI 140, LPeI 59, PeI 75, SI 85.

Description of worker. *Head.* In full face view, head subrectangular (Fig. 3), longer than broad (CI: 85) with shallowly concave occipital margin, and slightly convex lateral margins. Anterior clypeal margin nearly straight, with a low and blunt medial tooth. Eyes absent. Mandibles subtriangular, with a small denticle ($t5$) and four triangular enlarged teeth ($t1$ to $t4$) on the masticatory margin (Fig. 2A). Antennal



Figure 4. *Ponera tudigong* sp. nov., worker (ANTWEB1009796). **A** Dorsal view **B** lateral view.

scapes, when laid back, nearly reaching occipital corners of head; average ratio of the length of antennomeres

$$\frac{7\text{th}}{6\text{th}} : \frac{8\text{th}}{6\text{th}} : \frac{9\text{th}}{6\text{th}} : \frac{10\text{th}}{6\text{th}} = 1.08 : 1.42 : 1.92 : 2.32$$

Funicular segments of antennae incrassate, increasing in length and breadth distally.

Mesosoma. In profile view, mesosomal dorsum slightly convex. Promesonotal suture and metanotal groove both present and clearly incised. Mesopleuron distinct, separated from mesonotum by a clearly incised promesonotal articulation. In dorsal view, lateral margins of pronotum and mesonotum rounded, lateral margins of propodeum slightly concave. Propodeal corner in lateral view forming a blunt angle.

Metasoma. In profile view, petiole sub-rectangular and remarkably thick compared to other species in the genus (LPeI = 59.5), with anterior and posterior margins straight and parallel; anterodorsal corner nearly right-angled, posterodorsal corner blunt; anterodorsal corner higher than posterodorsal corner. Subpetiolar process sub-rectangular in shape, with a large and circular anterior fenestra, extending across the entire ventral margin of the petiole. Posterior portion of subpetiolar process with a well-developed pair of blunted teeth. In dorsal view, petiolar node sub-oval, clearly wider than long (DPeI = 138.1), with a slightly convex anterior margin and slightly concave posterior



Figure 5. *Ponera tudigong* sp. nov., queen (ANTWEB1009670). Head in full-face view.

margin. In dorsal view, abdominal tergum III (= 1st gastral tergum) wider than long (ATI = 91.8), and in profile view with a bluntly rounded anterodorsal corner.

Sculpture. Entirety of head punctate, covered by closely and evenly spaced fine puncturing. Mandibles smooth. Sculpturing of mesosoma and metasoma relatively light, ranging from punctulate to imbricate. Mesosomal and metasomal dorsum nearly smooth.

Pilosity. Head and antennae covered by fine golden-colored pubescence. Mandibles with sparse, short, erect to sub-erect filiform hairs. Pilosity of mesosoma consisting of a fine pubescence, similar to that of the head. Fine and sparse pubescence covering each metasomal segment, becoming sparser after the fourth abdominal segment (= 2nd gastral segment). Short, golden, sub-erect to erect filiform hairs present on all metasomal segments, becoming longer and denser posteriorly from the fifth abdominal segment (= 3rd gastral segment).

Color. Coloration of entire body of the individual, excluding the legs, ranging from light to dark reddish-brown. Legs light brown.

Description of queen. The description of the queen of *P. tudigong* follows that of the worker caste, with the following differences:

Body size larger than that of the worker caste, with large, sub-circular compound eyes, 0.15 mm in diameter, present on the anterior part of the head, roughly one-third the distance to the posterior margin. Three distinct ocelli present on the posterior part of the head. Mandibles similar to worker caste (Fig. 2).



Figure 6. *Ponera tudigong* sp. nov., queen (ANTWEB1009670). **A** Lateral view **B** dorsal view.

Sub-petiolar process of the queen with a subquadrate anterior fenestra, a low and blunt downward-projecting anterior angle, and a downward-projecting square medial tooth. Abdominal tergum III (= 1st gastral tergum) as long as wide (ATI: 101).

Sculpturing, coloration, and pilosity of the queen is similar to that of the worker caste, with sculpturing slightly more pronounced.

Etymology. This new species is named in honor of Tudigong (土地公), the lord of the soil and the ground, a widely venerated Chinese deity.

Comments. *Ponera tudigong* is known from a single location in Tai Lam country park, Hong Kong SAR. The worker and queen were collected in separate pitfall traps 5 meters apart during the same sampling period. They were initially recognized as being distinct from the other local *Ponera* species, *P. sinensis* and *P. guangxiensis*, based on the

mandible and shape of its petiole and the absence of eyes, and was later determined to be a new species. It is the only known *Ponera* species with four mandibular teeth, all other species having either three (most of *Ponera* species), five (*P. pentodontos* Xu, 2001), or seven (*P. taylori* Bharti & Wachkoo, 2012).

Nothing is known about the biology or life history of *P. tudigong*. Like most species of *Ponera*, it is likely cryptobiotic and subterranean, as the absence of eyes might suggest. The fact that our specimens were captured in surface pitfall traps may suggest that the species does come to the surface at least occasionally, maybe at night, and possibly to forage or for reproduction purposes, given that a wingless queen was captured in addition to the worker. Of course, soil disturbance during trap emplacement may also be responsible.

Ponera tudigong is known from only a single collection event, despite a significant amount of leaf-litter and pitfall-trap sampling done throughout Hong Kong in recent years. This suggests that the species is indeed quite rare. The habitat it was collected from is a restored secondary forest, and it was collected within one meter of a well-used hiking trail.

Ponera guangxiensis Zhou, 2001

Fig. 7

Ponera guangxiensis Zhou, 2001A: 37, 227, figs 33, 34 (w.) CHINA. Indomalaya.

Geographic range. China: Guangxi province, Hong Kong S.A.R (new record); Vietnam.

Measurements and indices (mm). Worker (n = 6): ATL 0.38–0.44, ATW 0.48–0.51, HL 0.54–0.58, HW 0.49–0.53, MaL 0.31–0.36, ML 0.74–0.81, PeH 0.38–0.41, PeNL 0.19–0.22, PeW 0.31–0.32, PrW 0.38–0.40, SL 0.38–0.42, TL 2.19–2.38. Indices: ATI 79–91, CI 85–94, DPel 143–167, LPel 46–54, Pel 78–84, SI 75–86.

Queen (n = 1): ATL 0.50, ATW 0.57, HL 0.59, HW 0.53, MaL 0.34, ML 0.91, PeH 0.44, PeNL 0.21, PeW 0.38, PrW 0.46, SL 0.44, TL 2.55. Indices: ATI 88, CI 89, DPel 182, LPel 46, Pel 82, SI 85.

Diagnosis. See Leong et al. (2018).

Material examined. Hong Kong Special Administrative Region (HKSAR), Kam Shan Country Park, New Territories, 22.37089N, 114.14839E, 18 October 2017 (KS S1-R, Winkler sifter) [IBBL, ANTWEB1016325]. HKSAR, Tai Lam Country Park, New Territories, 22.37598N, 114.04713E, 3 November 2017 (TL S2-C (B), Winkler sifter) [IBBL, ANTWEB1016405]. HKSAR, Ngong Ping, Lantau Island, 22.25403N, 113.9105E, 9 November 2017 (NP S1-C, Winkler sifter) [IBBL, ANTWEB1016486]. HKSAR, Ngong Ping, Lantau Island, 22.26537N, 113.91491E, 9 November 2017 (NP S2-C, Winkler sifter) [IBBL, ANTWEB1016506]. HKSAR, Ngong Ping, Lantau Island, 22.27144N, 113.9103E, 9 November 2017 (NP S3-C, Winkler sifter) [IBBL, ANTWEB1016519] (1 worker, 1 dealate queen). HKSAR, Pak Sha O, New Territories, 22.44743N, 114.3082E, 17 November 2017 (PSO S1-

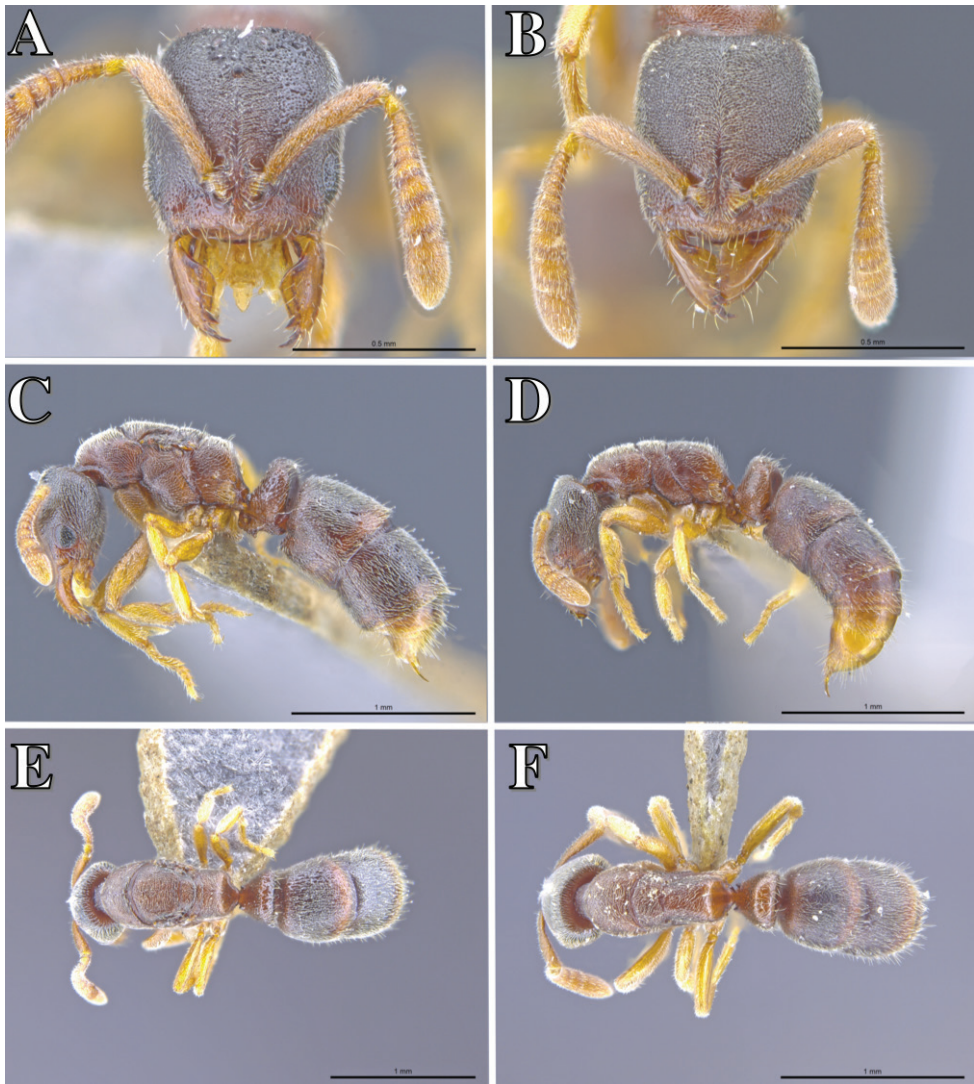


Figure 7. *Ponera guangxiensis* Zhou, 2001, worker (ANTWEB1016982) and queen (ANTWEB1016609). **A** Queen head, full-face view **B** worker head, full-face view **C** queen, lateral view **D** worker, lateral view **E** queen, dorsal view **F** worker, dorsal view.

R, Winkler sifter) [IBBL, ANTWEB1016555] (1 worker, 1 dealate queen). HK-SAR, Ling Wui Shan, Lantau Island, 22.24333N, 113.86932E, 4 December 2017 (LWS S1-C, Winkler sifter) [IBBL, ANTWEB1016609]. HKSAR, Ling Wui Shan, Lantau Island, 22.24333N, 113.86932E, 4 December 2017 (LWS S1-R, Winkler sifter) [IBBL]. HKSAR, Ngong Ping, Lantau Island, 22.25403N, 113.9105E, 9 November 2017 (NP S1-R, Winkler sifter) [IBBL]. HKSAR, Ngong Ping, Lantau Island, 22.25403N, 113.9105E, 9 November 2017 (NP S3-R, Winkler sifter) [IBBL] (1 worker, 1 dealate queen). HKSAR, Tai Lam Country Park, New Territories, 22.37598N, 114.04713E, 3 November 2017 (TL S2-R, Winkler sifter) [IBBL].

HKSAR, Ngong Ping, Lantau Island, 22.25396499N, 113.910478E, 428 m a.s.l., 9 November 2017 (IAS-0162, hand collected) [IBBL, ANTWEB1009802; ANTWEB1009629; ANTWEB1009803] (3 workers). HKSAR, Tai Po Kau, New Territories, 22.42833N, 114.18273E, 171 m a.s.l., 2 June 2017 (IAS-0215, hand collected) [IBBL, ANTWEB1016982; ANTWEB1009685; ANTWEB1016939] (3 workers). HKSAR, Tai Po Kau, New Territories, 22.426138N, 114.181783E, 14 July 2015 (RHL-HK-TPK-T1WM, Winkler sifter) [IBBL, RHL00642]. HKSAR, Sunset Peak, Lantau Island, 22.263923N, 113.953762E, 3 June 2015 (RHL-HK-LSP-T3WM, Winkler sifter) [IBBL, RHL00758]. HKSAR, Tai To Yan, New Territories, 22.454795N, 114.118215E, 7 August 2015 (RHL-HK-TYF-T1WJ, Winkler sifter) [IBBL, RHL01441]. HKSAR, Tai To Yan, New Territories, 22.454795N, 114.118215E, 7 August 2015 (RHL-HK-TYF-T1WM, Winkler sifter) [IBBL, RHL01467]. HKSAR, Aberdeen Reservoir, Hong Kong Island, 22.26N, 114.162E, 26 June 2015 (RHL-HK-ABR-T4WM, Winkler sifter) [IBBL, RHL02059]. HKSAR, Kadoorie Farm and Botanical Garden, New Territories, 22.4302N, 114.1192E, 14 September 2015 (RHL-HK-KFBG-T1WM, Winkler sifter) [IBBL, RHL02550]. HKSAR, Mau Ping Wood, New Territories, 22.3844N, 114.241E, 20 October 2015 (RHL-HK-MTLW-T1W J, Winkler sifter) [IBBL, RHL02599]. HKSAR, Mai Po Nature Reserve, New Territories, 22.49N, 114.04E, 10 m a.s.l., 13 September 1993 [KFBG, ANTWEB1015483; ANTWEB1015484] (2 workers). HKSAR, Sunset Peak South, Lantau Island, 22.2573N, 113.9622E, 670 m a.s.l., 8 October 1996 (Winkler sifter) [KFBG, ANTWEB1016030]. HKSAR, Mai Po Nature Reserve, New Territories, 22.486N, 114.039E, 10 m a.s.l., 13 September 1993 (Pitfall and bait). HKSAR, Shing Mun Reservoir, New Territories, 22.39718N, 114.15273E, 230 m a.s.l., 6 July 2011 (PSW16625-05, Winkler sifter). HKSAR, Tai Mo Shan, New Territories, 22.41595N, 114.12722E, 775 m a.s.l., 5 July 2011 (PSW16616-03, Winkler sifter).

Comments. *Ponera guangxiensis* is relatively common in collections within Hong Kong. It is most commonly collected in native secondary forests, either in leaf litter samples or by searching under stones or rotting logs. It appears to be widespread in Hong Kong, though no observations on its biology or ecology have been made.

Ponera sinensis Wheeler, 1928

Ponera sinensis Wheeler, 1928c: 6 (w.) CHINA (Hong Kong). Indomalaya.

Geographic range. Guangxi province, Yunnan province, Taiwan, Hong Kong S.A.R.

Diagnosis. See Wheeler (1928), Wilson (1957), Taylor (1967), and Leong et al. (2019).

Comments. To the best of our knowledge, the only confirmed specimen of *P. sinensis* from Hong Kong is the holotype, collected by Professor F. Silvestri from an unknown location, and described by Wheeler (1928). Despite numerous faunal studies in Hong Kong over recent years, no further specimens of *P. sinensis* have been identified. On the contrary, the vast majority of *Ponera* specimens collected from Hong Kong

represent the newly recorded *P. guangxiensis*. Whether this represents an underlying ecological change or simply an artefact of sampling remains unclear, and future sampling in Hong Kong may yield new specimens of *P. sinensis*.

Update to the identification key of East Asian *Ponera* species

The following is an update to the identification key to East Asian species of *Ponera* originally provided by Leong et al. (2019), which contains 31 key couplets for 32 species. *Ponera tudigong* sp. nov., described here, is placed in a new couplet inserted after the 4th couplet in Leong et al. (2019), which has been slightly modified to reflect this. The new couplet has been numbered as 4', to discern it from couplet 4 in the original key, and to leave the remainder of the original key unaffected.

- 1 In lateral view, petiolar node very thick with convex posterior margin; with upper portion of posterior margin bulging.....2
- In lateral view, petiolar node not as developed and presenting straight to convex posterior margin; with upper portion of posterior margin not bulging.....3
- 2 Petiolar node in dorsal view with slightly convex anterior margin and slightly concave posterior margin. Smaller species, HW: 0.54–0.60 mm. Body color dark.....*P. rishen* Terayama, 2009
- Petiolar node in dorsal view with strongly convex anterior margin and strongly concave posterior margin. Larger species, HW: 0.65–0.75 mm. Body color reddish brown.....*P. takaminei* Terayama, 1996
- 3 Eye large, each consisting of 20 or more facets. Larger species, HW: ca. 0.68 mm.....*P. kohmoku* Terayama, 1996
- Eye small, each consisting of 10 or fewer facets. Size variable4
- 4 Masticatory margin of mandible with 5 subequal large teeth. Smaller species, HW: 0.53–0.55 mm.....*P. pentodontos* Xu, 2001
- Masticatory margin of mandible with 3 or 4 enlarged teeth. Size variable.....4'
- 4' Masticatory margin of mandible with 4 enlarged teeth followed by a small denticle.....*P. tudigong* sp. nov.
- Masticatory margin of mandible with 3 enlarged teeth followed by a series of small to indistinct denticles; the number of denticles variable5

Acknowledgements

We thank Dr. Zheng-Hui Xu, Dr. Shan-Yi Zhou, and Dr. Mamoru Terayama for providing images and specimens of Asian *Ponera* species for comparison. We thank Kit Lam Tang Wilfred for assistance in imaging and preparing the specimens. We thank members of the IBBL for general support and discussion. We also thank Jack Longino, Gabriela Camacho, and Peter Hawkes for helpful comments that improved the manuscript. This study was funded by the Environment and Conservation Fund of the Hong Kong SAR, ECF Project 32/2015.

References

- Bolton B (1994) Identification guide to the ant genera of the world. Harvard University Press.
<http://www.hup.harvard.edu/catalog.php?isbn=9780674442801>
- Bolton B (2019) An online catalog of the ants of the world. <http://antcat.org> [accessed 28 January 2019]
- Branstetter MG, Longino JT (2019) Ultra-conserved element phylogenomics of new world *Ponera* (Hymenoptera: Formicidae) illuminates the origin and phylogeographic history of the endemic exotic ant *Ponera exotica*. *Insect Systematics and Diversity* 3(2): 1; 1–13.
<https://doi.org/10.1093/isd/ixz001>
- Guénard B, Weiser M, Gomez K, Narula N, Economo EP (2017) The Global Ant Biodiversity Informatics (GABI) database: a synthesis of ant species geographic distributions. *Myrmecological News* 24: 83–89. https://doi.org/10.25849/myrmecol.news_024:083
- Harris RA (1979) Glossary of surface sculpturing. *Occasional Papers in Entomology, State of California, Department of Food and Agriculture* 28: 1–31.
- Janicki J, Narula N, Ziegler M, Guénard B, Economo EP (2016) Visualizing and interacting with large-volume biodiversity data using client-server web-mapping applications: the design and implementation of antmaps.org. *Ecological Informatics* 32: 185–193. <https://doi.org/10.1016/j.ecoinf.2016.02.006>
- Keller RA (2011) A phylogenetic analysis of ant morphology (Hymenoptera: Formicidae) with special reference to the poneromorph subfamilies. *Bulletin of the American museum of natural history* 355: 1–90. <https://doi.org/10.1206/355.1>
- Leong CM, Guénard B, Shiao SF, Lin CC (2019) Taxonomic revision of the genus *Ponera* Latreille, 1804 (Hymenoptera: Formicidae) of Taiwan and Japan, with a key to East Asian species. *Zootaxa* 4594(1): 1–86. <https://doi.org/10.11646/zootaxa.4594.1.1>
- Leong CM, Lin CC, Shiao SF, Nguyen DD, Eguchi K (2018) Taxonomic notes on *Ponera guangxiensis* Zhou, 2001 (Hymenoptera: Formicidae: Ponerinae), with a new distribution record from Vietnam and the first description of queen, male, and larva. *Revue Suisse de Zoologie* 152: 201–215. <https://zenodo.org/record/1414195#.XFUtEM8zbOQ>
- 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>
- Taylor RW (1967) A monographic revision of the ant genus *Ponera* Latreille (Hymenoptera, Formicidae). *Pacific Insects Monograph* 13: 1–112.
- Wheeler WM (1928) Ants collected by Professor F. Silvestri in China. *Bollettino del Laboratorio di Zoologia Generale e Agraria della Reale Scuola Superiore d'Agricoltura. Portici* 22: 3–38.
- Wilson EO (1957) The *tenuis* and *selenophora* groups of the ant genus *Ponera* (Hymenoptera: Formicidae). *Bulletin of the Museum of Comparative Zoology* 116(6): 355–386.
- Xu Z (2001) A systematic study on the ant genus *Ponera* Latreille (Hymenoptera: Formicidae) of China. *Entomotaxonomia* 23(1): 51–60. <https://europepmc.org/abstract/cba/347241>
- Zhou SY (2001) Ants of Guangxi. Guangxi Normal University Press, Guilin, China, 255 pp. [In Chinese]

New and little known Latindiinae (Blattodea, Corydiidae) from China, with discussion of the Asian genera and species

Lu Qiu¹, Zong-Qing Wang¹, Yan-Li Che¹

¹ Institute of Entomology, College of Plant Protection, Southwest University, Chongqing, China

Corresponding author: Yan-Li Che (lilyche@swu.edu.cn)

Academic editor: Eliana Cancellato | Received 8 May 2019 | Accepted 13 July 2019 | Published 29 July 2019

<http://zoobank.org/58656EC4-7BE6-4135-8C9A-F66A95B97EF9>

Citation: Qiu L, Wang Z-Q, Che Y-L (2019) New and little known Latindiinae (Blattodea, Corydiidae) from China, with discussion of the Asian genera and species. ZooKeys 867: 23–44. <https://doi.org/10.3897/zookeys.867.35991>

Abstract

A new Latindiinae, *Brachylatindia xui* **gen. et sp. nov.**, is described and illustrated from Tibet, China. The new genus *Beybienkonus* **gen. nov.** is established to include *Beybienkonus acuticercus* (Bey-Bienko, 1957), **comb. nov.** The Asian Latindiinae is discussed with a total of six genera included. A checklist of Asian species and a key to the Asian genera of Latindiinae are provided.

Keywords

Brachylatindia, *Beybienkonus*, Homopteroidea, *Ipolatta*, *Ctenoneura*

Introduction

In the Orthopteran catalogue (Princis 1963), twelve genera (*Latindia* Stål, 1860, *Paralatindia* Saussure, 1868, *Ipisoma* Bolívar, 1893, *Ctenoneura* Hanitsch, 1925, *Compsoodes* Hebard, 1917, *Homopteroidea* Shelford, 1906, *Melestora* Stål, 1860, *Bucolion* Rehn, 1932, *Biolleya* Saussure, 1897, *Buboblatta* Hebard, 1920, *Ipolatta* Karny, 1914, *Stenoblatta* Walker, 1868) were included in the family Latindiidae (now Latindiinae), two of which (*Biolleya* and *Stenoblatta*) were later transferred to the Blaberidae (Roth 2003). For the remaining genera, Roth (2003) did not specifically list any of them in Latindiinae. Only two of the remaining ten genera were regarded as Latindiinae, viz. *Latindia* and *Buboblatta*, while the remaining eight were treated as subfamily unsettled

genera (Beccaloni 2014). None of the subsequent papers treated these remaining genera as Latindiinae, although all acknowledged that some of these genera may be truly related to Latindiinae (Gutiérrez 2012; Djernæs et al. 2015; Qiu, Che and Wang 2016; Wang et al. 2017). Except for problems at the generic level, the status of Latindiinae was also inconclusive. In their recent phylogenetic papers on Blattodea, both Djernæs et al. (2015) and Wang et al. (2017) indicated that Latindiinae may be upgraded to the family Latindiidae.

Three Asian genera were historically included in Latindiinae (Princis 1963), but were later excluded, i.e., *Homopteroidea*, *Ctenoneura*, and *Ipolatta*. Members of *Ctenoneura* are unique among cockroaches for their absence of the genital hook, and differ from Latindiinae by the asymmetrical subgenital plate, single stylus, more complex venation, and the apterous female (Qiu et al. 2017). However, *Homopteroidea* and *Ipolatta* were excluded from Latindiinae without providing any reason; recent papers now indicate that the Asian Latindiinae are more diverse than previously thought: Qiu et al. (2016) reported the genus *Sinolatindia* from China and Lucañas (2018) described genus *Gapudipentax* from the Philippines.

Since Latindiinae species are small and unnoticeable, specimens are difficult to obtain. Recently, we obtained some living individuals and specimens from Yunnan and Tibet, China. All materials were collected from the rotten wood. We thus take this opportunity to carefully study them and report upon this little known subfamily from China. The status of *Homopteroidea* and *Ipolatta* are reconsidered, and a checklist of the Latindiinae species from Asia as well as a key to the Asian genera are provided.

Materials and methods

The specimens from China are all deposited in the Institute of Entomology, Southwest University, Chongqing, China (SWU). We also examined specimens of the *Homopteroidea* collection in Oxford University Museum of Natural History, Oxford, UK (OUM). Those specimens include the lectotype and two paralectotypes of *Homopteroidea shelfordi* (ORTH0206 1/4, 3/4–4/4), the paratype of *Ctenoneura aberrans* (= *Homopteroidea aberrans*) (ORTH342 2/2), the holotype of *Homopteroidea maculate* (ORTH363), the lectotype of *Homopteroidea minor* (ORTH389 1/2), and slides studied by Roth (1995a), viz. slide 272 (tegmina and a wing), slide 273 (male genitalia and subgenital plate) of *Homopteroidea nigra*, and slide 271 (a tegmen), and slide 278 (male genitalia) of *Homopteroidea brachyptera*.

The definition of Latindiinae here follows that of Qiu et al. (2016) and Wang et al. (2017). Morphological terminology used in this paper mainly follows Roth (2003), genitalia terms follow Klass (1997), and venation terms follow Li et al. (2018).

The genital segments of the examined specimens were dipped in 10% NaOH and observed in glycerine jelly using a Motic K400 stereomicroscope and a Leica M205A stereomicroscope. Venation drawings were made with the aid of Adobe Photoshop CS6, a Leica M205A stereomicroscope and a Motic K400 stereomicroscope. Photo-

graphs of the habitus and characters were made using a Leica M205A stereomicroscope. All photographs were modified in Adobe Photoshop CS6.

For pairing and comparison, we selected seven samples representing different morphologies and genders from different locations to sequence their COI genes. The COI sequences are deposited in the National Center for Biotechnology Information GenBank (accession numbers MN116495, MN116496, MN116497, MN116498, MN116499, MN116500, MN116501). The extraction procedure was according to the Hipure Tissue DAN Mini Kit. Total DNA was stored at -20 °C. Primers for the amplifications are COI-F3 (5'-CAACYAATCATAAAGANATTGGAAC-3') and COI-R3 (5'-TAAACTTCTGGRTGACCAAARAATCA-3'). The amplification conditions were as follows: initial denaturation at 98 °C for 2 min, followed by 35 cycles of 10s at 98 °C, 10s for 51 °C, and 15s for 72 °C, with final extension of 2 min at 72 °C. Laboratory reagents were provided by TsingKe Co, Ltd., China. All voucher specimens are deposited at SWU. The genetic divergence value was quantified based on the Kimura 2-parameter (K2P) distance model (Kimura 1980), using MEGA 7 (Tamura et al. 2013) with 1000 bootstrap replicates.

Taxonomy

Brachylatindia gen. nov.

<http://zoobank.org/8A2734A8-F5DD-4133-955C-8749BE50AA14>

Type species. Here designated: *Brachylatindia xui* sp. nov.

Diagnosis. Small, brachypterous, smooth but with sparse micro spines. Head oval, ocelli absent; pronotum roundly triangular, meso- and meta- notum somewhat reduced; front femur type C₂, tarsal claws simple, arolia present; male with a gland at the centre of 4th tergum; subgenital plate of female valved.

This new genus resembles *Gapudipentax* Lucañas, 2018, but it can be readily distinguished from the latter by the following characters: 1) head sub-oval, while head triangular in *Gapudipentax*; 2) pronotum triangular with rounded edges, smooth, with indistinct micro setae, while pronotum pentagonal with rounded edges, distinct pubescent in *Gapudipentax*; 3) male with subtriangular tegmen, while male with subquadrate tegmen in *Gapudipentax*; 4) front femur with two long apical spine at hind margin, while front femur without any long apical spines in *Gapudipentax*; 5) tarsal claw not serrated, while tarsal claw serrated in *Gapudipentax*; and 6) male with tergal gland, while male without tergal gland in *Gapudipentax*.

Generic description. Body small, smooth, sexual dimorphism indistinct, both brachypterous. Male: head longer than width, oval, vertex not exposed. Ocelli absent. Pronotum triangular with rounded edges, with indistinct and micro setae. Meso- and meta- notum reduced, narrowed, median of both slightly extended. Tegmina reduced, reaching only up to the middle of the 2nd tergum; wing reduced, very small (flightless). Front femur type C₂, apex without spine; mid- and hind femora each with a spine

at apex and a spine at apical portion of hind margin. Tarsomere 1 longer than the rest of tarsomeres combined. Pulvilli absent. Tarsal claws simple, symmetrical. Arolia present. Abdomen with 4th tergum specialised, with a gland medially. Supra-anal plate trapezoidal, with large hyaline area, apex concave; paraprocts hooked at apical portions; cerci each with a small spine at apex. Subgenital plate symmetrical; styli simple, similar. Genitalia complex, with long and robust genital hook (L3), R2 elongate.

Female: similar to male. Tegmina reduced. Metanotum normal, wings absent. Supra-anal plate subtriangular, apex emarginated. Subgenital plate valved, medial slit entire, through the apex to the base.

Geographical distribution. China (Tibet).

Etymology. *Brachys* (Greek for short) + *latindia* refers to a Latindiinae cockroach with brachypterous tegmina.

***Brachylatindia xui* sp. nov.**

<http://zoobank.org/EAF2ADE4-6D04-4179-8DA1-2BB66F34F300>

Figs 1–3, 11A

Type material. **Holotype**, male (SWU): **CHINA: Tibet (= Xizang):** Upper Zayü (= Shangchayu) Town [上察隅镇], Zayü (= Chayu) County [察隅县], Nyingchi (= Linzhi) City [林芝市], alt. 1900 m, 9.VII.2016, Hao Xu et Jian-Yue Qiu leg. **Paratype:** 1 nymph (SWU), same data as holotype.

Diagnosis. As for the genus (vide supra).

Description. Male (holotype). General: measurements (mm): body length (vertex to abdomen tip): 6.2, pronotum length × width: 2.2 × 3.0, tegmen length: 2.1. Size small, brownish yellow, tegmina and wings reduced (Fig. 1A, B). **Head:** oval, with very sparse setae, brownish yellow. Vertex convex, sheltered under pronotum. Eyes small, wide apart; interocular space much greater than the distance between antennal sockets. Ocelli absent. Frons smooth, two very shallow spots situated between the lower parts of the antennal sockets. Antennal sockets small, each with a row of setae at upper margin. Antennae dark brown, long, 7.4 mm, longer than the body length. Clypeus small, nearly trapezoidal, ante-clypeus and post-clypeus not indicated. Labrum small, sub-triangular, apex blunt. Maxillary palpi moderate (Fig. 2B). **Pronotum:** brownish yellow, lateral parts sub-transparent. Smooth, surface without pubescence, but very sparse micro setae (cannot be observed by naked eyes, but visible under microscope). Shape subtriangular, widest near the hind angles, apex rounded, hind angles rounded (Fig. 2A). **Mesonotum and metanotum.** Both somewhat reduced; mesonotum semi-oval, apical margin thickened; apical margin of metanotum protruded, almost reaching to half of the 1st tergum, the protruded part quadrated and thickened (Fig. 2E). **Tegmina and wings:** both reduced, flightless. Tegmen smooth, almost reaching the edge of the 2nd tergum, lobate, apex rounded; venation reduced, main veins simple. Wing small, triangular, venation indistinct (Figs 1A, 2F). **Legs:** smooth, setose, whitish yellow, tibiae and tarsi brownish yellow. Front

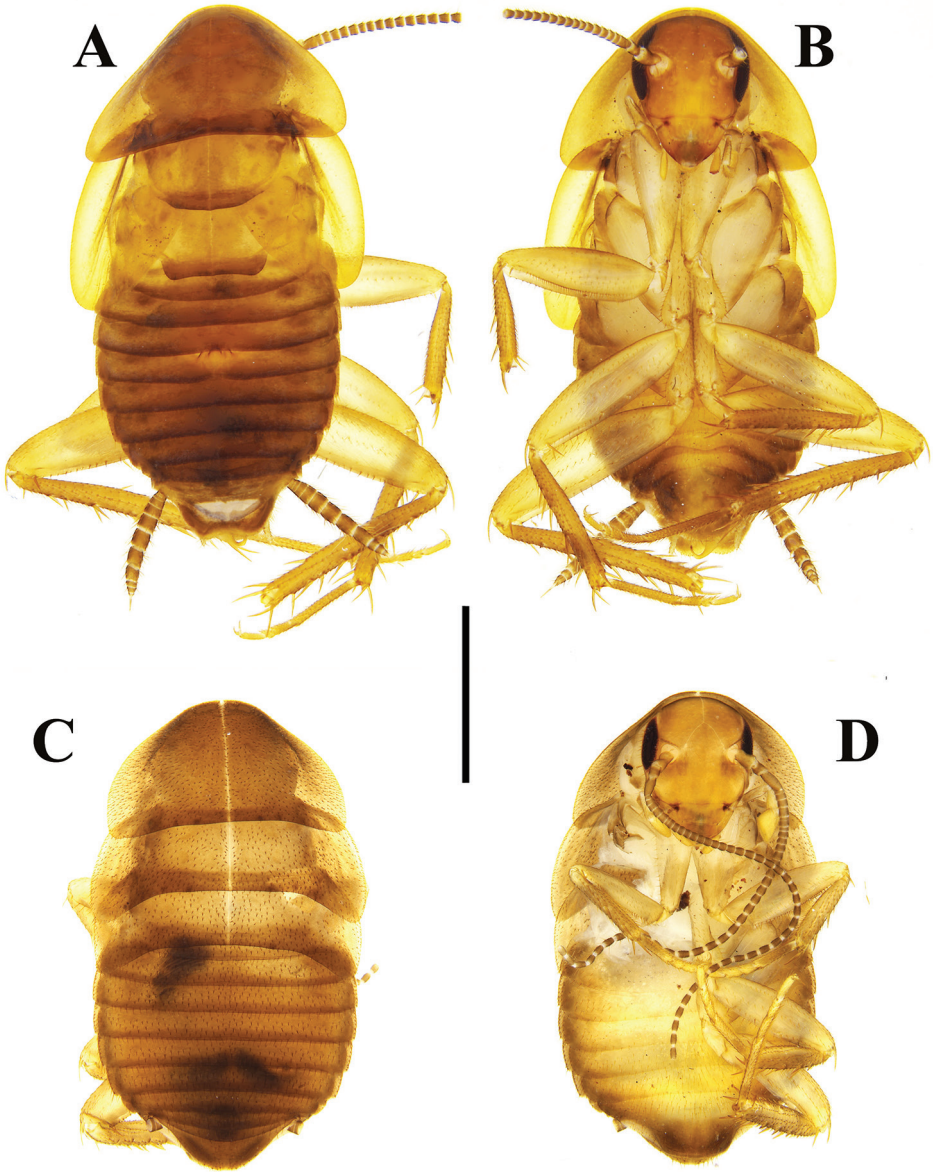


Figure 1. A–D *Brachylatindia xui* gen. et sp. nov. **A** male holotype, dorsal view **B** same, ventral view **C** nymph paratype, dorsal view **D** same, ventral view. Scale bar: 2 mm.

femur with a row of small spines at hind margin, ending with a long spine and a short spine near apex (type C_2) (Fig. 2C). In middle and hind femur, each femur with a row of sparse spines at hind margin, ending with one long spine; one long spine appearing at the apex of anterior margin. Tibiae normally with some long spines and short setae. Tarsi covered with many spines; the length of tarsus 1 longer than the total length of

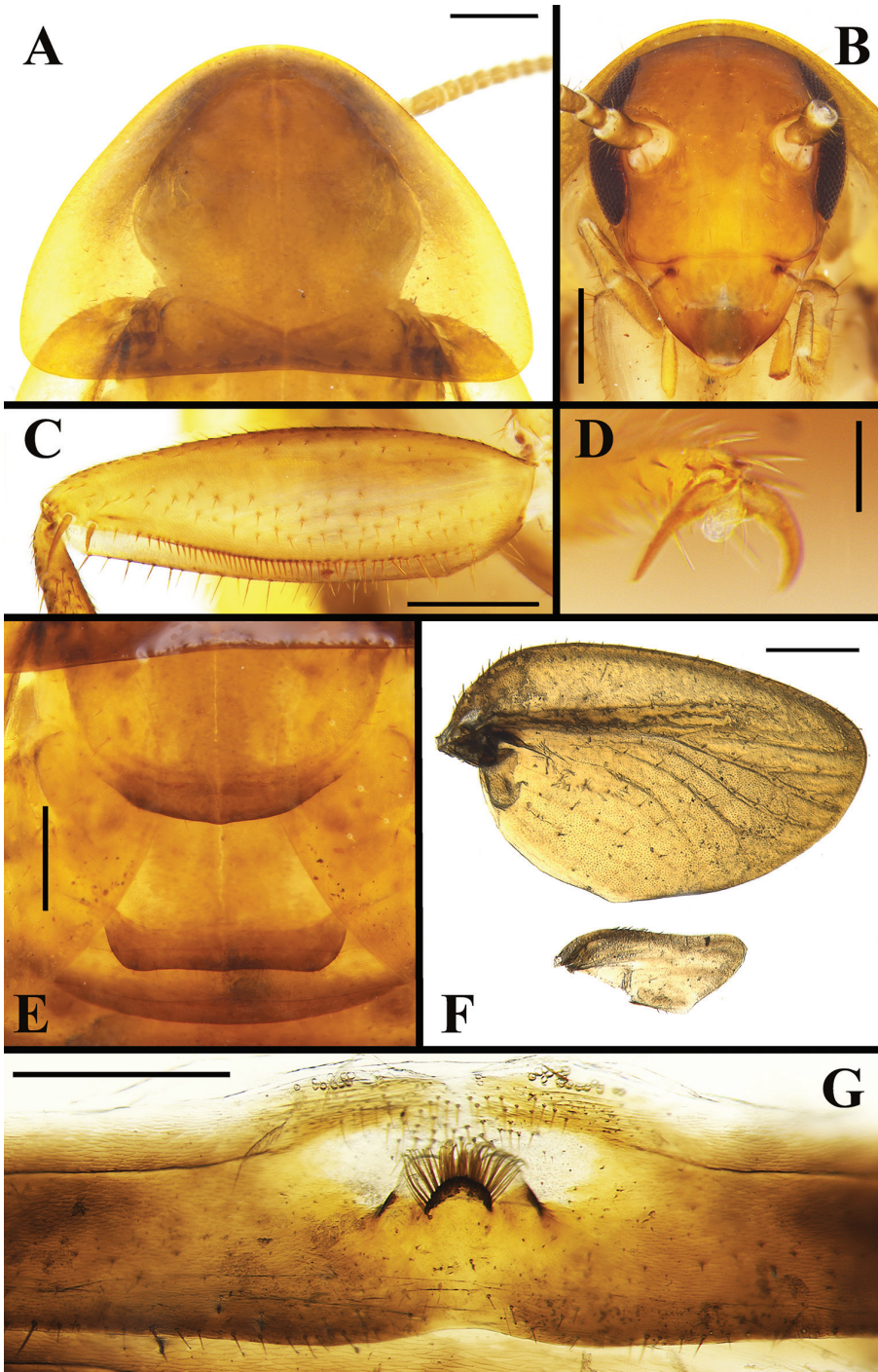


Figure 2. *Brachylatindia xui* gen. et sp. nov., male holotype **A** pronotum, dorsal view **B** head, ventral view **C** front femur **D** tarsal claw **E** meso- and meta-notum **F** right tegmen and wing **G** tergal modification in the 7th tergum of abdomen. Scale bars: 0.5 mm (**A–C, E–G**); 0.1 mm (**D**).

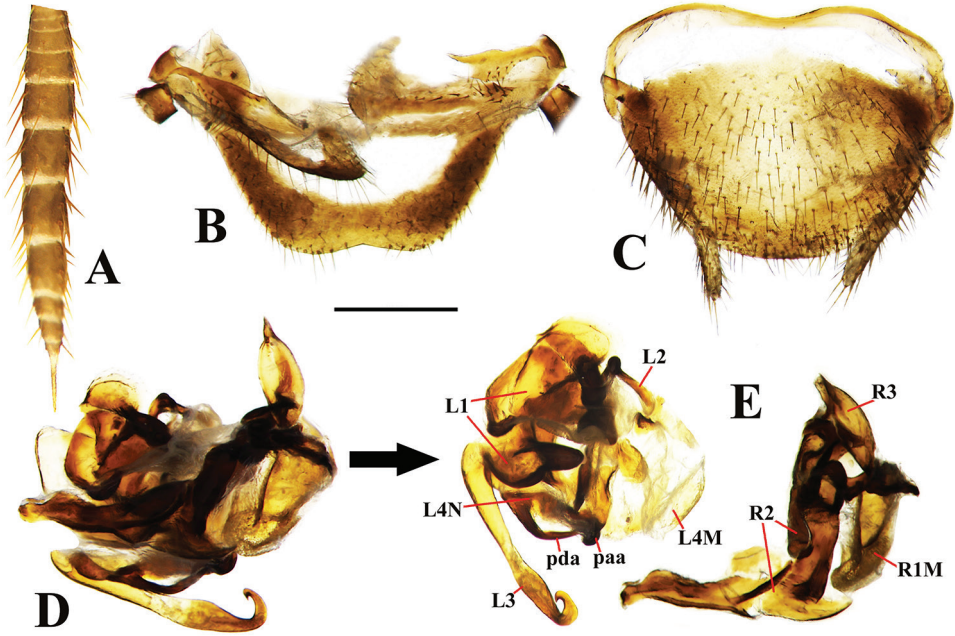


Figure 3. *Brachylatindia xui* gen. et sp. nov., male holotype **A** cercus **B** supra-anal plate, ventral view **C** subgenital plate, ventral view **D** genitalia, original position **E** genitalia (dissected). Scale bar: 0.5 mm.

tarsi 2 to 5; tarsal claws normal, symmetrical, small; arolia minute (Fig. 2D). **Abdomen:** smooth, brownish, 4th tergum specialised, with a gland medially, hind margin of 4th tergum thinned and slightly concave in the middle (Fig. 2G). Supra-anal plate with a large transparent area medially, apex widely concave, margin setose; paraprocts with long setae; cerci long, lateral portions setose, apex with a spine (Figs 3A–B). Subgenital plate setose; styli cylindrical (Fig. 3C). **Genitalia:** complex. **Left phallomere:** L1 large, consists of two irregular sclerites, the ventral one with a stick-like process on the left, apex round; L2 small, elongate and curved, median with a lamina; L3 very robust, apical portion enlarged, then thinner and curved toward apex, apex sharp; L4N with pda and paa well developed, long and sharp; L4M thin, transparent. **Right phallomere:** R1M and R3 small; R2 with two distinct elongate sclerites, the ventral one short, stick-like, apex enlarged and rounded, the dorsal one extremely long, lying across the whole phallomere (Fig. 3D, E).

Female. Unknown.

Nymph. Similar to the adult, but body densely pubescent (Fig. 1C, D).

Ootheca. Unknown.

Natural history. Individuals were collected from the rotten wood (H. Xu et J.-Y. Qiu, pers. comm.) (Fig. 11A).

Distribution. China (Tibet) (Fig. 13).

Etymology. This new species is named after Dr. Hao Xu, one of the collectors of this new species, for his efforts in collecting this cockroach.

***Brachylatindia* sp.**

Figs 4, 5, 11B

Material examined. 1 female (SWU), **CHINA: Tibet:** Jialongba [加龙坝], Suotong Village [索通村], Guxiang Township [古乡], Bomê (= Bomi) County [波密县], Nyingchi City [林芝市], alt. 2300 m, 23.VII.2016, Jian-Yue Qiu et Hao Xu leg.

Diagnosis. Body small, brownish yellow (Fig. 4A, B). Body length (vertex to abdomen tip): 7.4 mm, pronotum length (midline) × width (the widest points): 2.3 × 3.0 mm, tegmen length: 2.1 mm. Head oval (Fig. 5B), pronotum smooth, triangular (Fig. 5A). Mesonotum reduced, trapezoidal; metanotum wide, not reduced (Fig. 5E). Tegmina short, triangular, venation simple (Fig. 5F); wings absent. Front femur type C₂ (Fig. 5C), arolia present but small (Fig. 5D). Abdomen without tergal modification. Supra-anal plate with a yellowish area medially, apex narrowed, concave, margin setose (Fig. 5G); subgenital plate valved (Fig. 5H).

Natural history. This species was collected from the rotten wood from the forest of Guxiang, Bomi (H. Xu et J.-Y. Qiu, pers. comm.) (Fig. 11B).

Geographical distribution. China (Tibet) (Fig. 13).

Remarks. This species is similar to the male of *Brachylatindia xui* sp. nov., but its front femur has a right-angle protrusion near the base (Fig. 5C), mesonotum is trapezoidal, metanotum is not reduced (Fig. 5E), tegmina are larger, and the wings are absent. These differences may be sexually dimorphic, so to further verify they are different species, we sequenced the COI genes of *B. xui* sp. nov. and this female specimen (GenBank access numbers MN116501 and MN116499, respectively, for the male and nymph specimens of *B. xui* sp. nov., MN116496 for this female specimen). The results show that the divergence between the two species is 15.7% (0% between the holotype and paratype of *B. xui* sp. nov.). This result indicates that this specimen is not the female of *Brachylatindia xui* sp. nov.

***Beybienkonus* gen. nov.**

<http://zoobank.org/2108E9F0-AFE5-41CB-95BE-C829B30DCFE4>

Type species. *Beybienkonus acuticercus* (Bey-Bienko, 1957) comb. nov.

Diagnosis. This new genus is unique by having two robust spines on each hind femur and one curved robust spine at the apex of each hind tibia in male. The hind legs of male are robust. Apex of each cercus with a long spine in both sexes. Both brachypterous and macropterous types are present in this genus.

Generic description. Body large for Latindiinae, smooth; both brachypterous and macropterous types are present.

Brachypterous male. Head longer than width, oval, vertex slightly exposed. Ocelli represented as two white spots. Pronotum semi-oval, smooth. Meso- and meta-notum slightly reduced, median not extended. Tegmina reduced, reaching up to half of abdomen; wing reduced, small and elongate (flightless). Front femur type C₁; mid- and

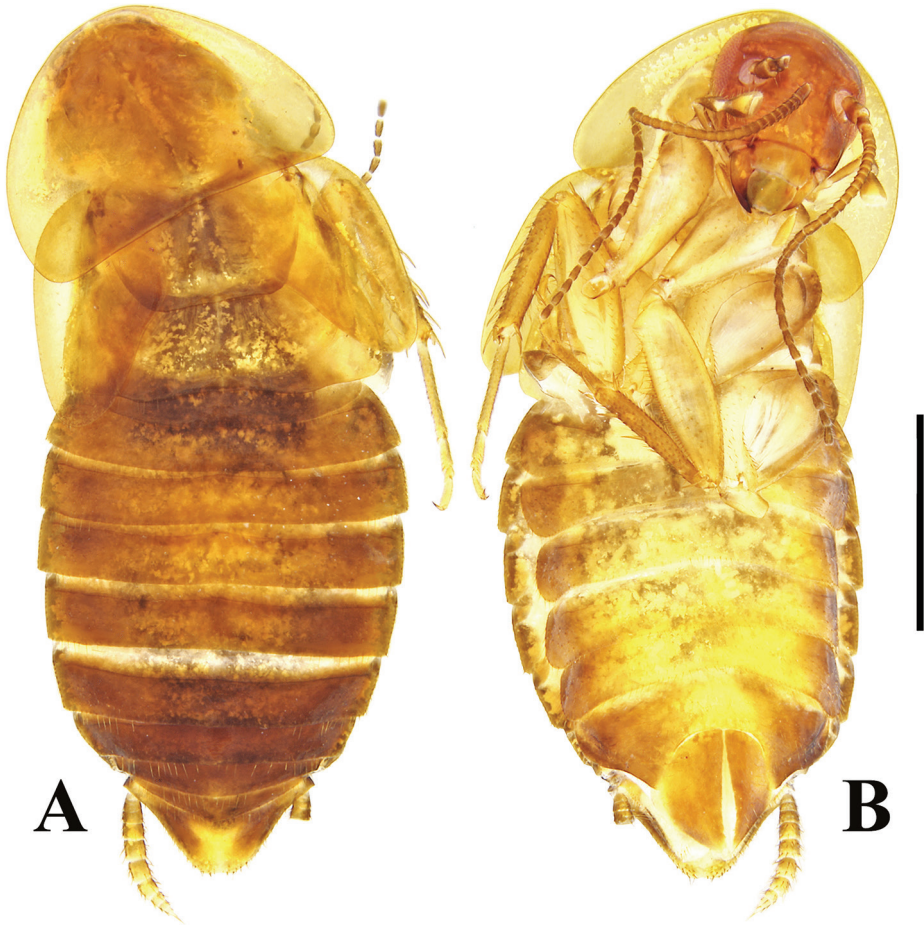


Figure 4. *Brachylatindia* sp., female from Tibet **A** dorsal view **B** ventral view. Scale bar: 2 mm.

hind femora each with a spine at apex and two spines at apical portion of hind margin, the two spines of hind femur well developed, robust. Hind tibia with a robust long spine and a thin long spine at apex. Tarsomere 1 longer than the rest of tarsomeres combined. Pulvilli absent. Tarsal claws simple, symmetrical; arolia absent. Abdomen without tergal modification. Supra-anal plate narrowly triangular, with large hyaline area medially, apex with two rounded lobes; paraprocts simple; cerci smooth dorsad, setose ventrad, each with a long spine at apex. Subgenital plate symmetrical; styli simple, similar. Genitalia with small genital hook (L3), right phallomere large.

Macropterous male. Unknown.

Brachypterous female. Similar to brachypterous male. Tegmina more reduced, not exceeding the half of abdomen, wing much more reduced, very small (flightless). Hind legs not as robust as male, spines normal, not enlarged. Supra-anal plate sub-triangular, apex emarginated, margin setose, ventral surface setose. Subgenital plate valved, medial slit entire through the apex to the base.

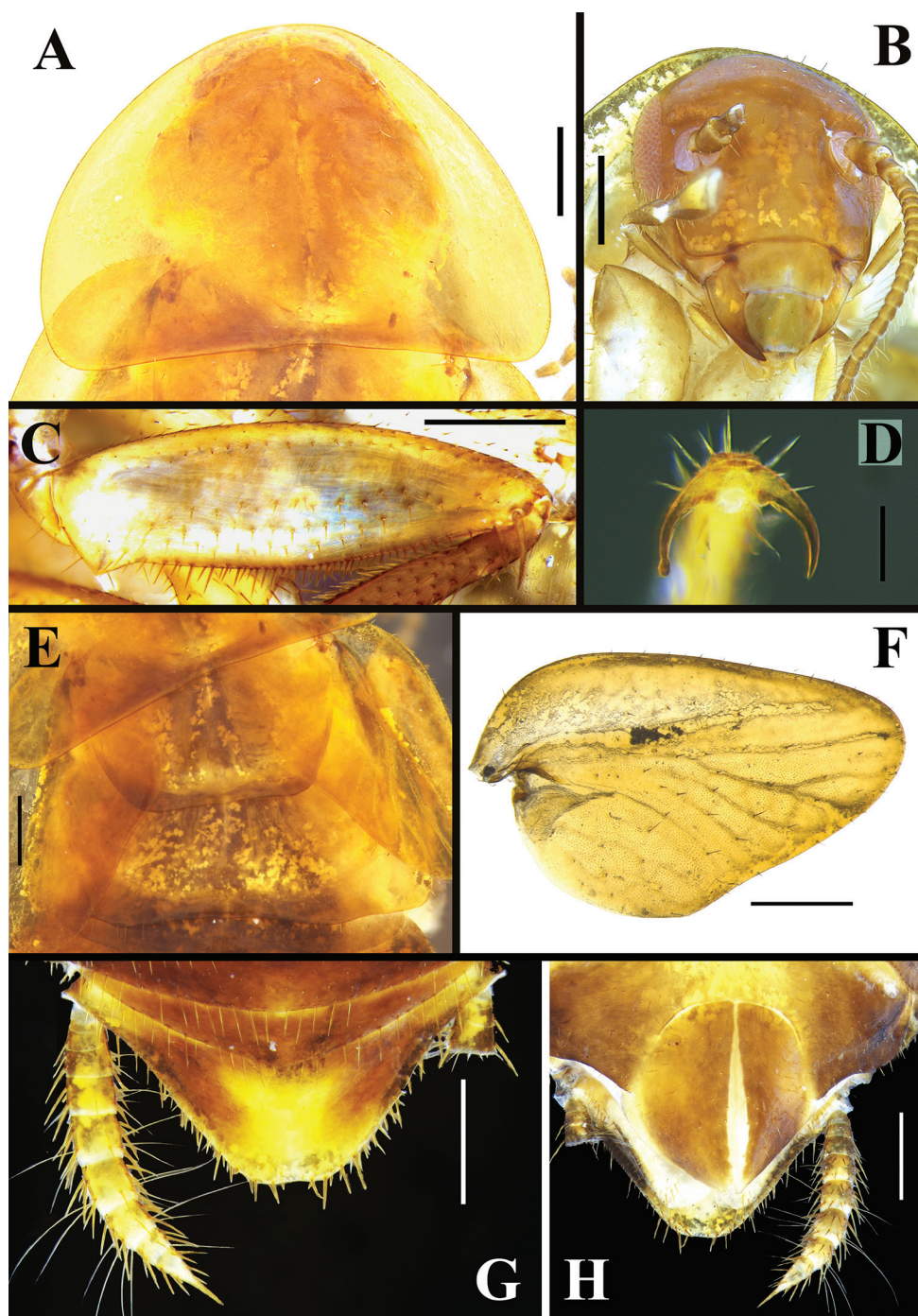


Figure 5. *Brachylatindia* sp., female from Tibet **A** pronotum, dorsal view **B** head, ventral view **C** front femur **D** tarsal claw **E** meso- and meta-notum **F** right tegmen **G** supra-anal plate, dorsal view **H** subgenital plate, ventral view. Scale bars: 0.5 mm(**A–C**, **E–H**); 0.1 mm (**D**).

Macropterous female. Body relatively narrowed. Pronotum oval, small. Tegmina and wings fully developed exceeding the end of abdomen (capable of flight). The remaining characters similar to the brachypterous female.

Geographical distribution. China (Yunnan and Tibet).

Etymology. Named after Bey-Bienko G.Y., the Russian entomologist, who first reported the type species *Beybienkonus acuticercus* (Bey-Bienko, 1957) comb. nov. from Yunnan, China.

***Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov.**

Figs 6–10, 11C–F

Ctenoneura acuticercus Bey-Bienko, 1957: 896 (original description); Princis 1963: 101 (catalogue); Roth 1993: 87; Feng et al. 1997: 34 (catalogue); Qiu et al. 2017: 297.

Material examined. CHINA: Yunnan: 5 males (brachypterous), 3 females (macropterous), 10 females (brachypterous), and more than 30 nymphs (under rearing): around Mangyun Township [芒允乡], Yingjiang County [盈江县], Dehong Prefecture [德宏景颇族自治州], 24°34'N, 97°45'E, alt. ca. 800–1300 m, 27.II–11.III.2018, Gui-Chang Liu (local people) leg.; **Tibet:** 3 females (brachypterous): Gelin Village [格林村], Bengbeng Township [背崩乡], Medog County [墨脱县], Nyingchi City [林芝市], alt. 1600 m, 15.VII.2016, Hao Xu et Jian-Yue Qiu leg. (all in SWU).

Description. Brachypterous male. General: measurements (mm): body length (vertex to abdomen tip): 10.1–10.6, pronotum length (midline) × width (the widest points): 3.1–3.2 × 4.6–4.8, tegmen length: 4.9–5.1, tegmen width: 2.7–2.8. Size small, body smooth, brownish yellow (Fig. 6 A, B). **Head:** longer than width. Vertex slightly exposed under pronotum, convex, darker than the remaining part of head. Eyes small, wide apart; interocular space much greater than the distance between ocelli and antennal sockets. Ocelli represented as two white spots, situated above antennal sockets. Frons smooth, two brown spots situated between the lower parts of the antennal sockets. Antennal sockets small. Antennae dark brown, shorter than the body length (8.6–10.3 mm). Face smooth, large. Clypeus small, nearly trapezoidal, the edge between ante-clypeus and post-clypeus indistinct. Labrum small, sub-triangular. Maxillary palpi long (Fig. 8A). **Pronotum:** light brownish yellow, lateral parts sub-transparent. Smooth, surface without pubescence, but very sparsely with micro setae (can't be observed by naked eyes, even easily overlooked under microscope). Shape semi-oval, widest at 1/4 from the base, hind angles round (Fig. 8B). **Tegmina and wings:** both reduced, flightless. Tegmen smooth, brown, reaches to the 4th or 5th tergum, nearly rectangular, apical portion slightly protruding, overall outline slightly rounded; venation simple. Wing small, elongate, curved, reaching the apex of 2nd or 3rd tergum (Figs 6A, 9C, D). **Legs:** smooth, brownish yellow, sparsely covered with short setae, hind legs robust. Front femur with a row of small spines at hind margin, ending with a long spine



Figure 6. A–D *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov., brachypterous individuals from Yunnan **A** male, dorsal view **B** same, ventral view **C** female, dorsal view **D** same, ventral view. Scale bar: 2 mm.

at apex (type C_1) (Fig. 8F), while in middle and hind femur without row of spines at hind margin; in middle and hind legs, each femur with a long spine at anterior apex and two long spines at hind apex; in hind femur, the two spines at hind apex extremely



Figure 7. A–B *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov., macropterous female from Yunnan **A** dorsal view **B** ventral view. Scale bar: 2 mm.

robust and long. Tibia with a spinous protrusion at apex (small in front and middle tibiae, extremely long in hind tibia); surface of tibia normally with some long spines, the spines at tibial apex longer than the spines at tibial surface; in hind tibia, two of the apical spines extremely long (one is thin and straight, the other is robust and curved) (Fig. 8D). Tarsi covered with many spines; the length of tarsus 1 sub-equal to the total length of tarsus 2 to 5; tarsal claws normal, symmetrical, moderate in size; arolia absent (Fig. 8G). **Abdomen:** smooth, brownish, terga without modification, lateral margins with small spinous pubescence. Supra-anal plate (Fig. 10A) pubescent, narrowly triangular, apex with two rounded lobes; paraprocts simple; cerci long, smooth and without pubescence dorsally, with pubescent ventrally, apex with a very long and sharp spine (Fig. 8H, I). Subgenital plate simple, sparsely pubescent, base with rough setae laterally; styli slender (Fig. 10B). **Genitalia: Left phallomere:** L1 consists of two irregular sclerites, the dorsal one slice-like, the ventral one with three unequal-sized protrusions; L2 thick, straight; L3 small, curved, S-shaped; L4N simple, straight; L4M small, slice-like. **Right phallomere:** large. R3 and R1M elongate; R2 with two sclerites, the ventral one stout, irregularly rounded, the dorsal one extremely long, irregular, lays across the whole phallomere (Figs 10C–D).

Brachypterous female. Measurements (mm): body length: 8.9–10.5, pronotum length \times width: 3.0–3.2 \times 4.5–4.7, tegmen length: 4.4–4.7, tegmen width: 2.5–2.7. Generally similar to the brachypterous male, but eyes slightly smaller than that of the male, antennae shorter than the body length (Fig. 6C, D). Tegmen shorter, apex

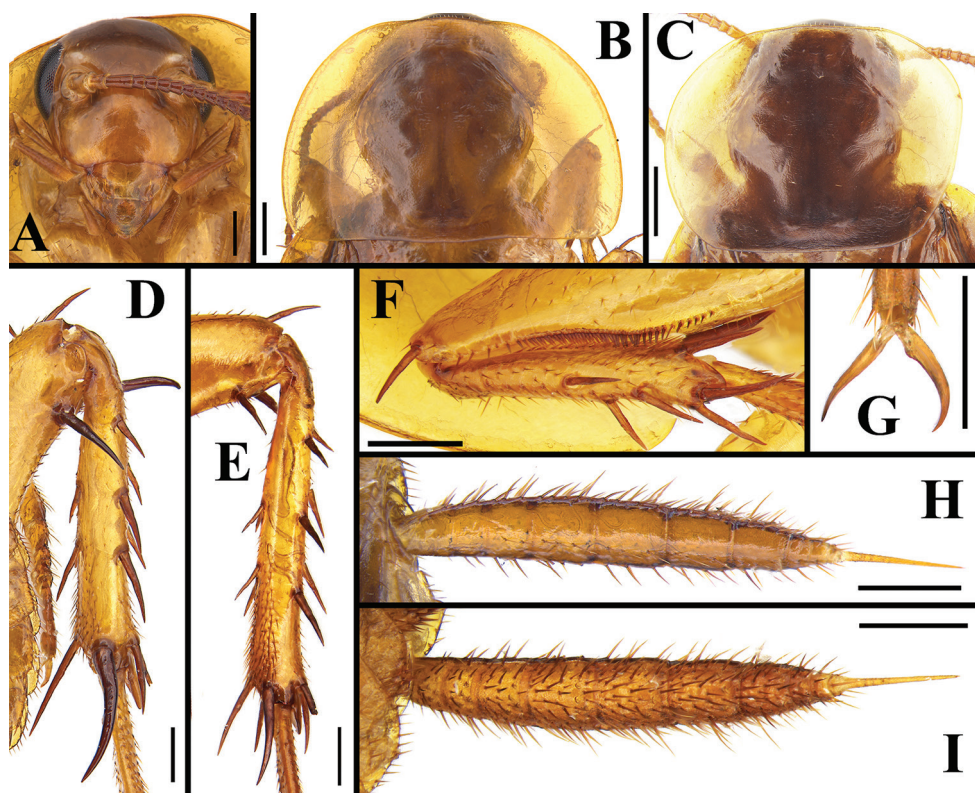


Figure 8. *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov., individuals from Yunnan **A** head, brachypterous male, ventral view **B** pronotum, brachypterous male, dorsal view **C** pronotum, macropterous female, dorsal view **D** part of the hind leg, male **E** part of the hind leg, female **F** front femur, male **G** tarsal claw, male **H** cercus, male, dorsal view **I** cercus, male, ventral view. Scale bars: 0.5 mm.

truncated, only reaching half of the 3rd or 4th tergum; wing much more reduced, only reaching the 1st tergum; venation simple (Fig. 9E, F). Spines on legs normal, not elongated or enlarged (Fig. 8E). Supra-anal plate trapezoid-shaped, apex rounded, median slightly concaved, margin and ventral surface setose (Fig. 10E). Subgenital plate valved (Fig. 10F).

Macropterous male. Unknown.

Macropterous female. Measurements (mm): body length: 9.2–9.4, total length: 10.9–12.5, pronotum length × width: 2.3–2.6 × 3.2–3.6, tegmen length: 8.9–10.1, tegmen width: 2.9–3.1 (Figs 7A–B). Head and legs the same as the brachypterous females. Pronotum sub-oval, wider than long, hind margin slightly truncated (Fig. 8C). Tegmina and wings fully developed. Tegmen with a thick and simple ScP, ScP area articulated with many small veins; R with four branches; M with two long branches that are parallel with the main vein; CuA bifurcated at basal half, near the middle, an isolated vein present, with many articulated cross veins connecting it with the main vein of CuA. Wing with a single ScP and RA; RP simple, with many thin veins; M

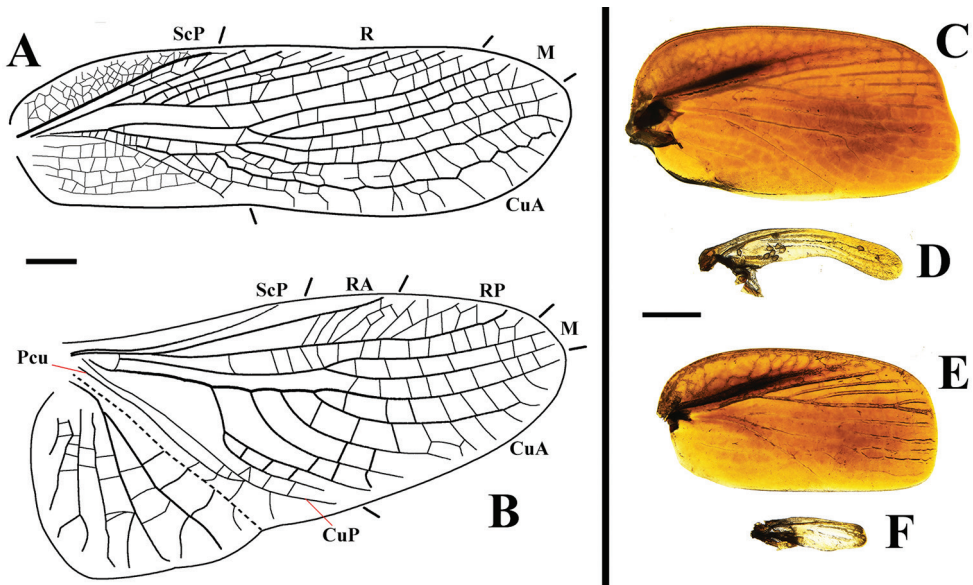


Figure 9. Tegmina and wings of *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov., individuals from Yunnan **A** tegmen, macropterous female **B** wing, macropterous female **C** tegmen, brachypterous male **D** wing, brachypterous male **E** tegmen, brachypterous female **F** wing, brachypterous female. Scale bars: 0.5 mm.

bifurcated medially; CuA with four curved and parallel branches; CuP and Pcu simple (Fig. 9A, B). Abdomen slightly narrower than the brachypterous female.

Nymph. Large nymphs light brownish yellow, sub-transparent, densely pubescent (Fig. 12A).

Ootheca. Flat, rounded, with only two eggs, dense serrations present at the keel (Fig. 12D).

Natural history. Individuals were captured from rotten wood, or under the barks of the rotten wood (Fig. 11C, D). Under the lab condition, individuals can feed on bread crumbs and apple pieces; one can prevent the others from grabbing its food by kicking (by the strong hind legs), or fast running away with food (food were carried by front legs). Females were noticed producing oothecae in April (Fig. 12C), the nymphs were very fast hatched around 10–15 days.

Distribution. China (Yunnan and Tibet) (Fig. 13).

Remarks. Bey-Bienko (1957) described *Ctenoneura acuticercus* based on two females from Yunnan, China. From the original description, *C. acuticercus* is characterised by the smooth pronotum, absence of intercalary vein and arolia, triangular supra-anal plate with emarginate, valvular subgenital plate, and cerci with a large spine apically. Bey-Bienko (1957) himself had indicated *C. acuticercus* is related to *Ctenoneura aberrans* Hanitsch, 1928. However, *C. aberrans* had been moved to genus *Homopteroidea* since this species is quite different from *Ctenoneura* (Roth 1995a). Later, Qiu et al. (2017) doubted *C. acuticercus* Bey-Bienko, 1957 to be a *Ctenoneura* species according to the absent intercalary vein and arolia, and the female *Ctenoneura* was found to be

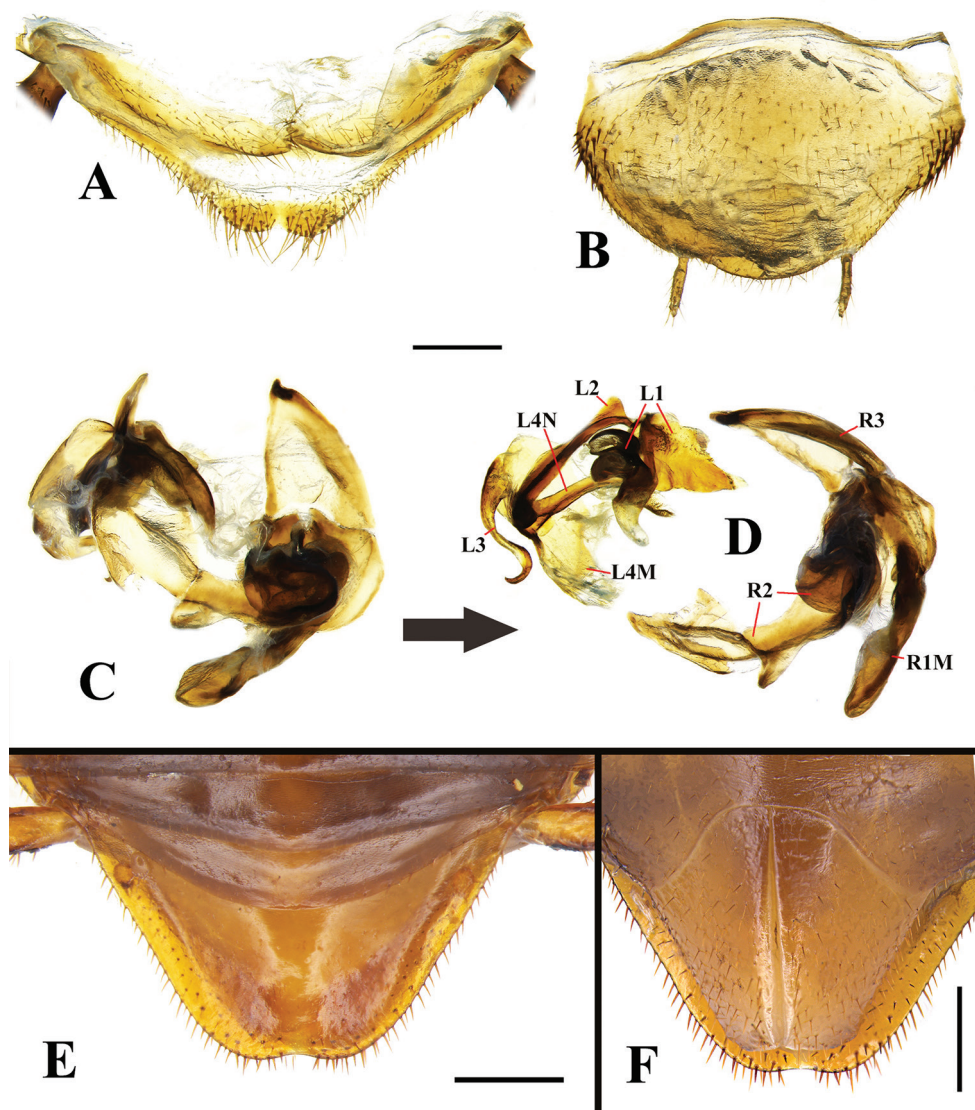


Figure 10. *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov., individuals from Yunnan **A** supra-anal plate, male, ventral view **B** subgenital plate, male, ventral view **C** genitalia, male, original position **D** genitalia, male (dissected) **E** supra-anal plate, female, dorsal view **F** subgenital plate, female, ventral view. Scale bars: 0.5 mm.

apterous. However, due to no specimens of *Ctenoneura acuticercus* being available, the problem remained unsolved.

Recently we obtained abundant living individuals of *Ctenoneura acuticercus* from Yingjiang, Yunnan. These roaches were captured from the same locality in the rotten woods. We noticed that this species displays polymorphism. Most individuals are

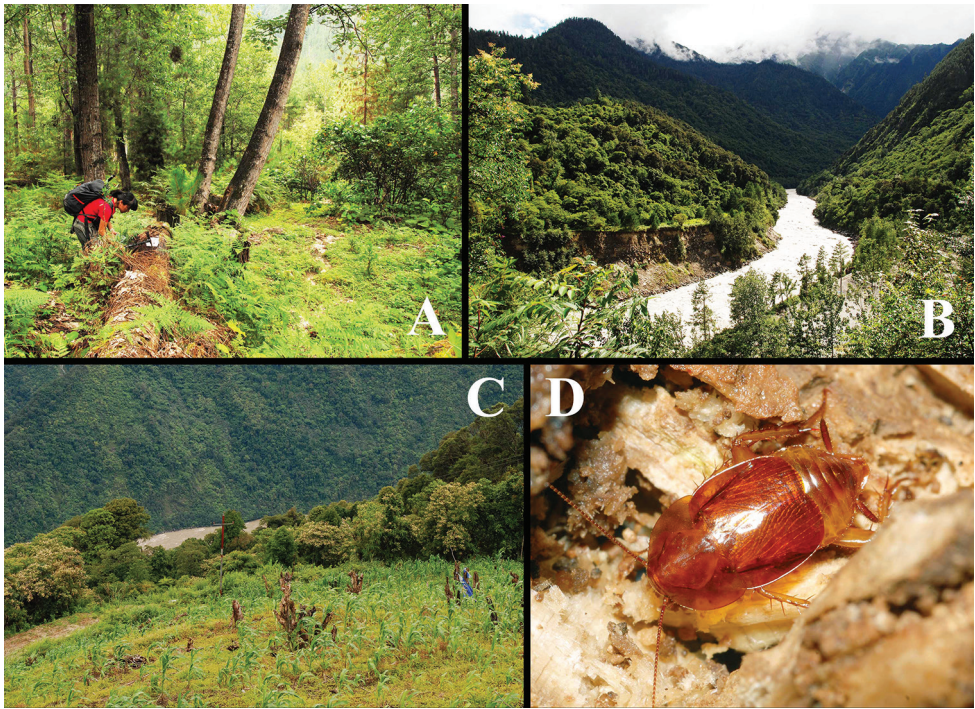


Figure 11. Habitats of Latindiinae from China **A** habitat of *Brachylatindia xui* gen. et sp. nov., Shangchayu, Chayu, Tibet **B** habitat of *Brachylatindia* sp., Guxiang, Bomi, Tibet **C–D** habitat of *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov., Motuo, Tibet **D** a living *B. acuticercus* (Bey-Bienko, 1957), comb. nov., found in rotten wood. All photographs by Hao Xu.

brachypterous, while individuals were very rarely macropterous in the material we examined (Fig. 12B). We compared the brachypterous and macropterous individuals both by morphological features and the COI sequences (one brachypterous male, one brachypterous female and one macropterous female were sequenced, GenBank access numbers MN116497, MN116498 and MN116500, respectively). Both results showed that the brachypterous and the macropterous individuals are conspecific: 1) morphologically, the brachypterous individuals and the macropterous individuals show no differences but in the shape of pronotum and the length of tegmina and wings; and 2) the divergence of COI sequences between the brachypterous male and the macropterous female is 0%, and the divergence between the brachypterous female and the macropterous female is 0.2%. Thus, we confirmed the brachypterous and the macropterous individuals are the same species. Meanwhile, we also sequenced one of the Tibetan specimens by COI (GenBank access number MN116495), and found the divergence between the Tibetan specimen and the Yunnan specimen is only 4.3%–4.4%. Thus, we can confirm that the Tibet individuals are conspecific with the Yunnan individuals.

After a carefully study of *Ctenoneura acuticercus*, we readily confirmed that this species should be excluded from the genus *Ctenoneura* by the winged female, the complex

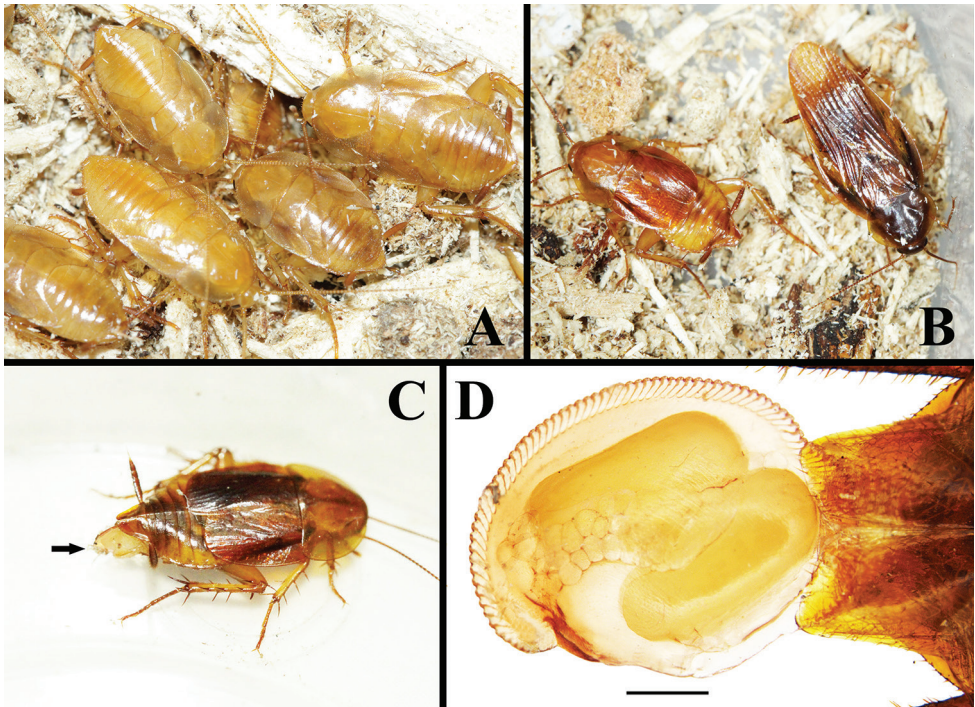


Figure 12. *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov. from Yingjiang, Yunnan, under lab conditions **A** nymphs **B** macropterous and brachypterous females. **C** brachypterous female with ootheca (black arrow indicated) **D** ootheca. Scale bar: 0.5 mm. All photographs by Lu Qiu.

male genitalia with genital hook, and the simplified venation without intercalary vein; and it does not belong to any of the other genera in Corydiidae. We herein establish genus *Beybienkonus* gen. nov. to accommodate *C. acuticercus*. Thus, *Beybienkonus acuticercus* (Bey-Bienko, 1957), comb. nov. is proposed.

Discussion

In Asia, Latindiinae genera were poorly recorded. Princis (1963) listed only three genera (*Homopteroidea*, *Ipolatta*, *Ctenoneura*) in Latindiidae (now Latindiinae). Only *Ctenoneura* was recently studied and proved to be different from Latindiinae (Qiu et al. 2017), while the other two should be kept as members of Latindiinae.

Homopteroidea Shelford currently contains eight species, all of which are restricted to Southeast Asia (Fig. 13). We examined the *Homopteroidea* collection of OUM and consulted former papers (Hanitsch 1929; Roth 1995a; Roth 1995b; Qiu et al. 2016). *Homopteroidea* is proved to belong to Latindiinae by the small and wide apart eyes, simplified venation, the dense fringe-like spinules on the hind margin of front femur,

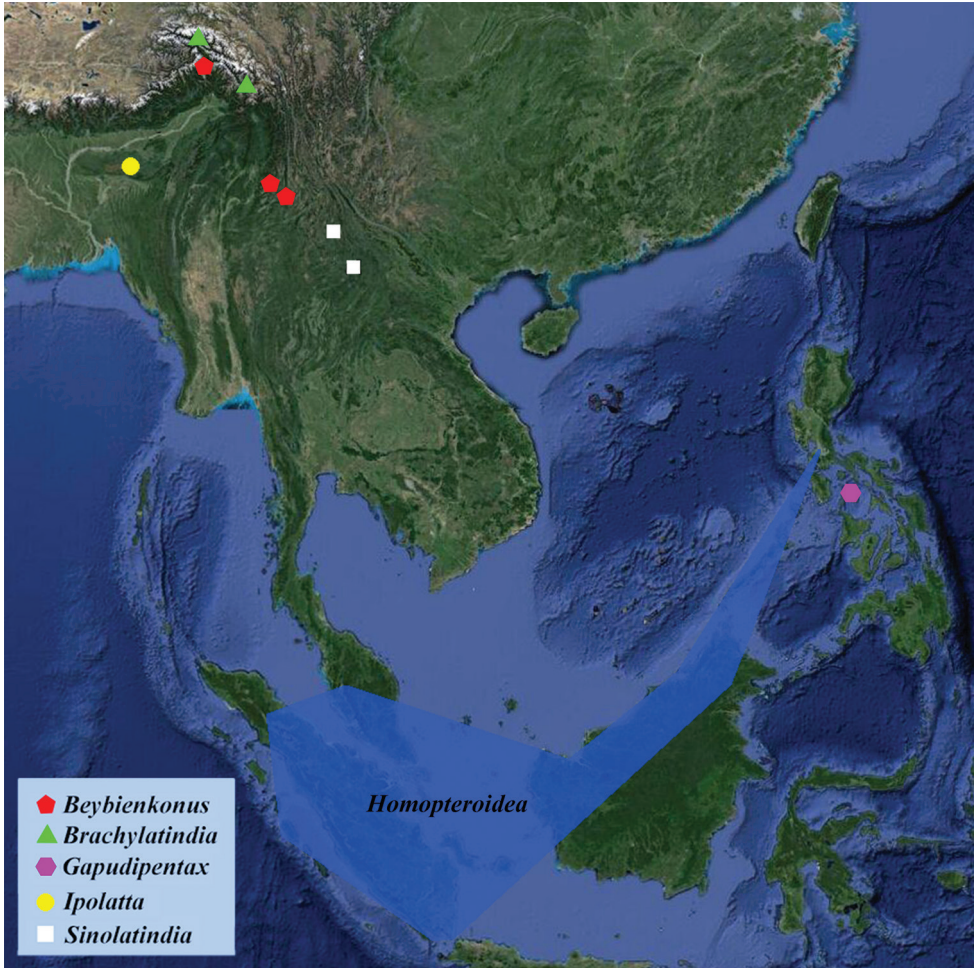


Figure 13. Distribution map of Latindiinae from Asia.

the large white macula at medial supra-anal plate, simplified subgenital plate of male, the complex male genitalia, and the longitudinal incision at subgenital plate of female (valved). This genus is unique among the Latindiinae for the “presutural vein” in tegmina and distinct transparent “presutural zone” in right tegmen (Hanitsch 1929; Roth 1995a; Roth 1995b). However, one “aberrant” species, *Homopteroidea aberrans* (Hanitsch, 1928), has no separate presutural vein or hyaline presutural zone, which therefore requires further study to confirm its status.

Ipolatta Karny only contains one species, *via. Ipolatta paradoxa* Karny, 1914, the type specimen is reported from Assam (Karny 1914) (Fig. 13). This genus is characterised by the strongly transverse head (with truncated vertex), discoid and large pronotum (hind margin truncated), horny and veinless tegmina (which exceed the

abdomen), and shortened wings. Its supra-anal plate is transverse, and subgenital plate is described as “profunde fissa (= deeply split)”. The head shape of *Ipolatta* resembles that of *Latindia*, *Sinolatindia* and *Gapudipentax*; the character in the subgenital plate indicates that the holotype is a female and is identical to the characters of female Latindiinae. Thus we consider *Ipolatta* as a Latindiinae genus. Nevertheless, this genus is only known from the original description, and its real identity needs further confirmation.

Qiu et al. (2017) also mentioned that *Ctenoneura gigantea* Roth, 1993 was “aberrant” in *Ctenoneura*. This species was described based on one none-abdomen individual from Perak, Malaysia (Roth 1993). The wing venation exhibited in Roth (1993) is in general the Latindiinae type, so we would determine this species to be a Latindiinae. Lacking specimens to study, it may be a new genus, but for now its status remains unsolved.

Checklist of Latindiinae from Asia

***Homopteroidea* Shelford, 1906**

| | |
|--|---------------------------------|
| <i>Homopteroidea biramiata</i> Roth, 1995 | Indonesia; Malaysia |
| <i>Homopteroidea brachyptera</i> Roth, 1995 | Indonesia |
| <i>Homopteroidea maculata</i> Hanitsch, 1929 | Indonesia; Malaysia; Philippine |
| <i>Homopteroidea minor</i> Hanitsch, 1933 | Malaysia; Indonesia |
| <i>Homopteroidea nigra</i> Shelford, 1906 | Malaysia; Indonesia |
| <i>Homopteroidea nodipennis</i> (Karny, 1926) | Malaysia; Indonesia |
| <i>Homopteroidea shelfordi</i> Hanitsch, 1925 | Malaysia; Indonesia |
| <i>Homopteroidea aberrans</i> (Hanitsch, 1928) | Indonesia; Malaysia |

***Ipolatta* Karny, 1914**

| | |
|--------------------------------------|---------------|
| <i>Ipolatta paradoxa</i> Karny, 1914 | India (Assam) |
|--------------------------------------|---------------|

***Sinolatindia* Qiu, Che et Wang, 2016**

| | |
|--|----------------|
| <i>Sinolatindia petila</i> Qiu, Che & Wang, 2016 | China (Yunnan) |
|--|----------------|

***Gapudipentax* Lucañas, 2018**

| | |
|---|-----------------------|
| <i>Gapudipentax guiting</i> Lucañas, 2018 | Philippines (Sibuyan) |
|---|-----------------------|

***Brachylatindia* Qiu, Wang & Che, gen. nov.**

| | |
|---|---------------|
| <i>Brachylatindia xui</i> Qiu, Wang & Che, sp. nov. | China (Tibet) |
|---|---------------|

***Beybienkonus* Qiu, Wang & Che, gen. nov.**

| | |
|---|-----------------------|
| <i>Beybienkonus acuticercus</i> (Bey-Bienko, 1957), comb. nov. | China (Yunnan, Tibet) |
|---|-----------------------|

Key to the known genera of Latindiinae from Asia

- 1 Right tegmen usually with a hyaline presutural zone *Homopteroidea*
- Right tegmen without hyaline presutural zone **2**
- 2 Tegmina veinless, or with indistinct venation **3**
- Tegmina with distinct venation **4**
- 3 Tegmina horny, veinless *Ipolatta*
- Tegmina somewhat hyaline, venation absent in male, indistinct in female
..... *Gapudipentax*
- 4 Arolia absent, male without tergal modification **5**
- Arolia present, male with tergal modification *Brachylatindia*
- 5 Body large, smooth, tarsal claws simple, apex of cerci with a distinct long
spine *Beybienkonus*
- Body small, pubescent, tarsal claws serrated, apex of cerci without a long
spine *Sinolatindia*

Acknowledgements

We sincerely thank Dr. Hao Xu (Hunan Agricultural University, Changsha), Dr. Jian-Yue Qiu (SWU) and Mr. Gui-Chang Liu (Yingjiang, Yunnan) for their field works. Latindiinae species are very difficult to find; without their help, this work would not have been finished. We are also grateful to Dr. John Richard Schrock (Department of Biological Sciences, Emporia State University) for revising the manuscript before submission. We thank Amoret Spooner and Katherine Child (all OUM) for providing photographs of the *Homopteroidea* collection from OUM, and Dr. Cristian Caballes Lucañas (University of the Philippines Los Baños) for providing information about *Gapudipentax*. Sonia Lopes (Federal University of Rio de Janeiro) is acknowledged for her valuable comments. This research was funded by the National Natural Sciences Foundation of China (Nos. 31772506, 31872271).

References

- Beccaloni GW (2014) Cockroach Species File Online. Version 5.0/5.0. World Wide Web electronic publication. <http://Cockroach.SpeciesFile.org> [accessed 27 March 2018]
- Bey-Bienko GY (1957) Blattoidea of Szechuan and Yunnan. Communication I. Entomologicheskoe Obozrenie 36: 895–915.
- Djernæs M, Klass KD, Eggleton P (2015) Identifying possible sister groups of Cryptocercidae + Isoptera: A combined molecular and morphological phylogeny of Dictyoptera. Molecular Phylogenetics and Evolution 84: 284–303. <https://doi.org/10.1016/j.ympev.2014.08.019>

- Gutiérrez E (2012) New species of the genus of cockroaches *Compsodes* (Dictyoptera, Blattaria, Corydiidae) from the Dominican Republic. *Solenodon* 10: 52–62.
- Hanitsch R (1929) Fauna Sumatrensis, Blattidae (Beitrag No. 63). *Tijdschrift voor Entomologie* 72: 263–302.
- Feng PZ, Guo YY, Woo FC (1997) Cockroaches of China, Species and Control. China Science & Technology Press, 206 pp.
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. <https://doi.org/10.1007/BF01731581>
- Klass KD (1997) The external male genitalia and the phylogeny of Blattaria and Mantodea. *Bonner Zoologische Monographien* 42: 1–341.
- Li XR, Zheng YH, Wang CC, Wang ZQ (2018) Old method not old-fashioned: parallelism between wing venation and wing-pad tracheation of cockroaches and a revision of terminology. *Zoomorphology* 2018: 1–15. <https://doi.org/10.1007/s00435-018-0419-6>
- Lucañas CC (2018) A new Latindiinae cockroach, *Gapudipentax guiting* gen. et sp. n. (Blattodea: Corydiidae: Latindiinae) from the Philippines. *Halteres* 9: 151–156.
- Princis K (1963) Blattariae: Suborde Polyphagoidea: Fam.: Homoeogamiidae, Euthyrrhaphidae, Latindiidae, Anacompsidae, Atticolidae, Attaphilidae; Subordo Blaberoidea: Fam. Blaberidae. In: Beier M (Ed.) *Orthopterorum Catalogus. Pars 4. Uitgeverij Dr. W. Junk, 's-Gravenhage*, 77–172.
- Qiu L, Che Y, Wang Z (2016) *Sinolatindia petila* gen. n. and sp. n. from China (Blattodea, Corydiidae, Latindiinae). *ZooKeys* 596: 27–38. <https://doi.org/10.3897/zookeys.596.8332>
- Qiu L, Che YL, Wang ZQ (2017) Contribution to the cockroach genus *Ctenoneura* Hanitsch, 1925 (Blattodea: Corydioidea: Corydiidae) with descriptions of seven new species from China. *Zootaxa* 4237(2): 265–299. <https://doi.org/10.11646/zootaxa.4237.2.3>
- Roth LM (1993) Revision of the cockroach genus *Ctenoneura* Hanitsch (Blattaria, Polyphagidae). *Tijdschrift voor Entomologie* 136: 83–109.
- Roth LM (1995a) Revision of the cockroach genus *Homopteroidea* Shelford (Blattaria, Polyphagidae). *Tijdschrift voor Entomologie* 138: 103–116.
- Roth LM (1995b) Description of a new species of *Ctenoneura* Hanitsch from Sabah (Blattaria, Polyphagidae). *Tijdschrift voor Entomologie* 138: 117–119.
- Roth LM (2003) Systematics and phylogeny of cockroaches (Dictyoptera: Blattaria). *Oriental Insects* 37: 1–186. <https://doi.org/10.1080/00305316.2003.10417344>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology & Evolution*. 30: 2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Wang ZQ, Shi Y, Qiu ZW, Che YL, Lo N (2017) Reconstructing the phylogeny of Blattodea: robust support for interfamilial relationships and major clades. *Scientific Reports* 7: 3903. <https://doi.org/10.1038/s41598-017-04243-1>

The genus *Dixa* (Diptera, Dixidae) in Croatian lotic habitats, with a checklist of species and relationships with the fauna of neighbouring countries

Marija Ivković¹, Lara Ivanković²

1 Division of Zoology, Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia **2** Zeleni trg 2, 10000 Zagreb, Croatia

Corresponding author: Marija Ivković (marija.ivkovic@biol.pmf.hr)

Academic editor: Art Borkent | Received 29 May 2019 | Accepted 18 July 2019 | Published 29 July 2019

<http://zoobank.org/0207C27C-66FB-4D6B-B5E7-907241991898>

Citation: Ivković M, Ivanković L (2019) The genus *Dixa* (Diptera, Dixidae) in Croatian lotic habitats, with a checklist of species and relationships with the fauna of neighbouring countries. ZooKeys 867: 45–54. <https://doi.org/10.3897/zookeys.867.36613>

Abstract

Invertebrate surveys in Croatia conducted between 2005 and 2018 included 39 sampling sites yielding bycatch samples of Dixidae (Diptera). All records of this family from the territory of Croatia are summarized, including previously unpublished data. Collections contained six species of *Dixa* Meigen – *D. dilatata* Strobl, *D. maculata* Meigen, *D. nebulosa* Meigen, *D. nubilipennis* Curtis, *D. puberula* Loew, and *D. submaculata* Edwards, with *Dixa dilatata* reported from Croatia for the first time. Information relating to the ecoregions in which species were found and specific species traits are provided. Compared to neighbouring countries, the Croatian species assemblage is most similar to the fauna of Italy and least similar to that of Serbia and Montenegro.

Keywords

Meniscus midges, aquatic insects, faunistics, ecology

Introduction

The Dixidae, or meniscus midges, are one of the smallest families of Diptera in Europe, with only two genera, *Dixa* Meigen and *Dixella* Dyar and Shannon, and 32 species recorded (Oosterbroek 2007; Pape and Beuk 2012). Approximately 190 species are

recognized worldwide (Wagner et al. 2008; Pape et al. 2011; Moulton 2016, 2017). They are nematocerous flies belonging to the superfamily Culicoidea, which also includes the Culicidae, which they most closely resemble (Wiegmann et al. 2011; Bor-kent 2012). Adults are small, frail, and do not feed. They remain near their biotopes (streams, ponds), and rest in the vegetation. Males of some species form swarms. Eggs are deposited in masses at the water's edge, and the life cycle includes four larval instars and the pupa. The larvae are filter feeders that rest on the water surface where they take on a characteristic, reversed U-shaped posture. Pupation takes place on emergent substrates. Larvae are feeding on microorganisms and decaying plant or animal material trapped in the water column or on the surface film (Wagner et al. 2008, Wagner 2011). Larvae of *Dixa* prefer running water, while those of *Dixella* occur in both stagnant or slow-moving water (Oosterbroek 2007). Some species are restricted to bog or mesotrophic lakes and are appropriate bioindicators. Species diversity is highest in springs and in headwater streams (Wagner et al. 2008). Dixid larvae are sometimes a significant component of invertebrate drift in streams (Elliott and Tullett 1977; Sertić Perić et al. 2014). They are extremely sensitive indicators of the presence of surfactant or oil-borne pollutants in streams (Thomas 1979). Larval mortality increases with decreasing surface tension of water (Fowler et al. 1997). Disney (1999) published an exceptionally fine compilation of West Palaearctic Dixidae that can be used worldwide as a basic information resource.

So far there have only been two studies dealing with Dixidae in Croatia, and the only records are those in Ivković et al. (2017) from the Krka River and Ivanković et al. (2019) from Plitvice Lakes National Park resulting from a study of the emergence patterns and ecological preferences of Dixidae.

Materials and methods

Study site. Croatia is a relatively small country with a surface area of 56,594 km² situated at the crossroads of Central and Mediterranean Europe and the Balkan Region. It is divided into two ecoregions, the Dinaric western Balkan (ER5) and the Pannonian lowland (ER11) (Illies 1978), and forms part of two drainage basins, the Black Sea Basin and the Adriatic Sea Basin.

Specimen records. This paper is based on unpublished data from our own research and published data gleaned from the literature. Each record was georeferenced using ArcGIS software. The literature used for identifications included Shtakel'berg (1989) and Disney (1999). We followed the current classification of Pape and Beuk (2012). Locality records are listed for each species. A list of locality names including latitude, longitude, altitude, and number code for each locality is given in Table 1, and a map with all sites plotted is provided as Figure 1. Specimens were collected from lotic freshwater habitats throughout Croatia. Adult specimens were collected using emergence traps (details in Ivković et al. 2013), sweep nets, yellow pan traps and aspirators, whereas larvae were collected by Surber sampler (25 × 25 cm) and kick-net sampler (25 × 25 cm). Larval samples were collected as a result of several macroinvertebrate surveys conducted between 2005 and

Table 1. Sampling sites in Croatia. Ecoregions are taken from Illies (1978); Dinaric western Balkan (5) and Pannonian lowland (11).

| Site Name | Site ID | Latitude | Longitude | Elevation (m) | Ecoregion |
|---|---------|------------|------------|---------------|-----------|
| Boščak Stream | 1 | 46°25'45"N | 16°35'48"E | 145 | 11 |
| Bistrec Stream, Rakovnica | 2 | 46°21'50"N | 16°39'43"E | 145 | 11 |
| Bistrec Stream | 3 | 46°20'17"N | 16°48'34"E | 145 | 11 |
| Kotoribski kanal | 4 | 46°20'53"N | 16°48'41"E | 130 | 11 |
| Plitvica, Upper Reach | 5 | 46°18'20"N | 16°43'21"E | 205 | 11 |
| Spring Šumi, Zagorje | 6 | 46°11'19"N | 16°09'27"E | 390 | 11 |
| River Reka, upper reach | 7 | 46°10'33"N | 16°03'38"E | 170 | 11 |
| Kraljevec, Medvednica Mountain | 8 | 45°52'03"N | 15°56'45"E | 420 | 11 |
| Djedovica by Rupnica, Papuk Mountain | 9 | 45°36'17"N | 17°31'54"E | 365 | 11 |
| Brzaja, before N. Zvečeva, Papuk Mountain | 10 | 45°33'17"N | 17°30'53"E | 500 | 11 |
| Stream Kovačica, Papuk Mountain | 11 | 45°31'12"N | 17°40'51"E | 360 | 11 |
| River Duboka rijeka, Papuk Mountain | 12 | 45°30'26"N | 17°32'53"E | 355 | 11 |
| Headwater of Dubočanka, Papuk Mountain | 13 | 45°30'17"N | 17°44'03"E | 670 | 11 |
| Dubočanka, Papuk Mountain | 14 | 45°29'11"N | 17°40'42"E | 585 | 11 |
| River Riječina, upper reach | 15 | 45°24'30"N | 14°25'30"E | 380 | 5 |
| Velika Belica | 16 | 45°28'27"N | 14°48'12"E | 430 | 5 |
| Headwater of Dobra River | 17 | 45°25'28"N | 14°57'04"E | 539 | 5 |
| River Bukovačka Dobra | 18 | 45°25'23"N | 14°57'15"E | 515 | 5 |
| Mrežnički Brig, Mrežnica River | 19 | 45°25'34"N | 15°29'51"E | 130 | 5 |
| River Dretulja, Plaški | 20 | 45°05'03"N | 15°22'09"E | 370 | 5 |
| River Korana in Korana village, NP Plitvice | 21 | 44°55'33"N | 15°37'09"E | 390 | 5 |
| Stream Sartuk, NP Plitvice | 22 | 44°55'57"N | 15°33'10"E | 765 | 5 |
| Stream Plitvica, NP Plitvice | 23 | 44°54'08"N | 15°36'27"E | 555 | 5 |
| Tufa barrier Novakovića Brod, NP Plitvice | 24 | 44°54'08"N | 15°36'38"E | 505 | 5 |
| Tufa barrier Kozjak-Milanovac, NP Plitvice | 25 | 44°53'39"N | 15°36'32"E | 545 | 5 |
| Lake Kozjak, NP Plitvice | 26 | 44°52'40"N | 15°37'07"E | 535 | 5 |
| Tufa barrier Labudovac, NP Plitvice | 27 | 44°52'17"N | 15°35'59"E | 630 | 5 |
| Middle reach of Crna rijeka, NP Plitvice | 28 | 44°50'22"N | 15°35'59"E | 665 | 5 |
| Upper reach of Crna rijeka, NP Plitvice | 29 | 44°50'10"N | 15°36'30"E | 670 | 5 |
| Upper reach of Bijela rijeka, NP Plitvice | 30 | 44°50'04"N | 15°33'33"E | 715 | 5 |
| Spring of Bijela rijeka, NP Plitvice | 31 | 44°49'58"N | 15°33'25"E | 720 | 5 |
| Kosovčica Spring | 32 | 43°56'27"N | 16°15'09"E | 255 | 5 |
| River Kosovčica | 33 | 44°01'39"N | 16°12'45"E | 220 | 5 |
| River Orašnica | 34 | 44°03'40"N | 16°13'59"E | 225 | 5 |
| River Krka above the mouth of River Kosovčica | 35 | 44°02'24"N | 16°13'27"E | 215 | 5 |
| River Krka below the mouth of River Kosovčica | 36 | 44°01'40"N | 16°12'19"E | 210 | 5 |
| Roški Slap, Krka River, NP Krka | 37 | 43°54'13"N | 15°58'29"E | 55 | 5 |
| Skradinski buk, Krka River, NP Krka | 38 | 43°48'14"N | 15°57'52"E | 25 | 5 |
| Čikotina Lada, River Cetina | 39 | 43°31'59"N | 16°44'40"E | 250 | 5 |

2018. Specimens were preserved in 80% or 96% ethanol (EtOH). For identification of adults, male and female terminalia were dissected, if needed. In some cases, terminalia (and preceding abdominal segments) were cleared in 10% KOH, neutralized with acetic acid, and rinsed in water to improve visualization and facilitate identification. For larvae, all available structural characters were used for identification. Taxonomic diversity was considered at the level of species according to Pape and Beuk (2012). European ecoregions were defined according to the *Limnofauna Europaea* (Illies 1978).

Data analysis. A list of species was compiled from all specimen data (Table 2). Comparison of species richness and assemblage composition with surrounding countries (Italy, Hungary, Serbia and Montenegro) was conducted by compiling species lists for those countries taken from the *Fauna Europaea* (Pape and Beuk 2012). Slovenia

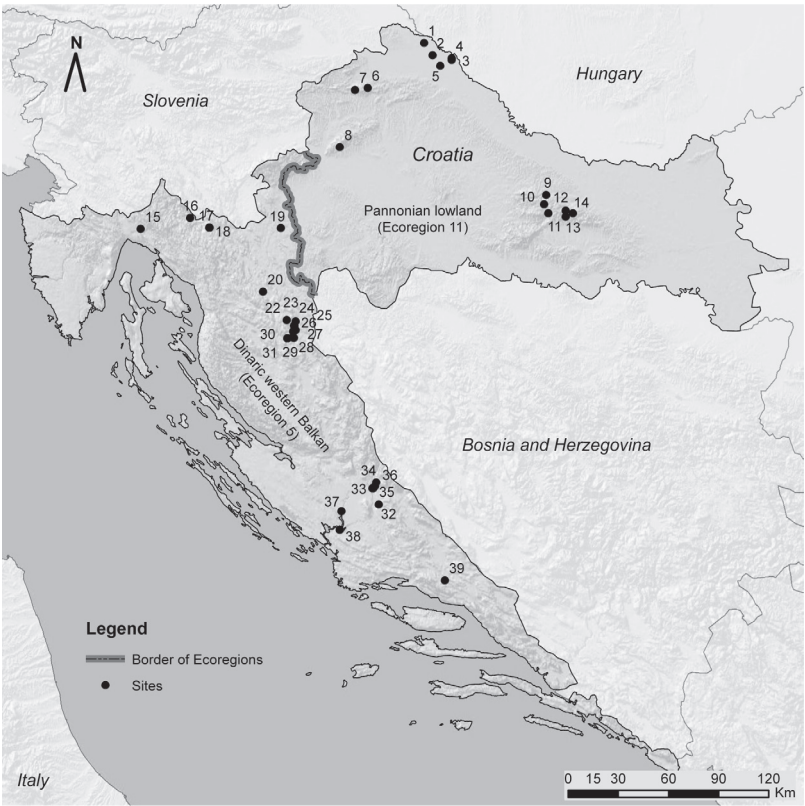


Figure 1. Sampling sites of *Dixa* recorded from Croatia (see Table 1 for codes).

and Bosnia and Herzegovina were not included in the comparison matrix, as there are no *Dixa* species records (Pape and Beuk 2012). A species-by-country matrix was constructed and a Sørensen Index of Similarity of each pairwise comparison calculated using Primer v6 software (Clarke and Gorley 2006).

Table 2. Croatian *Dixa*. Species niche traits. Key: habitat type 1 = spring or eucrenal zone, 2 = stream, 3 = river, 4 = tufa barrier (barrage lake outlet); voltinism U = univoltine, B = bivoltine, M = multivoltine; occurrence Sp = Spring, Su = Summer, A = Autumn, AYR = all year round; distribution in Europe wd = widely distributed. NA - not applicable. European Ecoregions are taken from Illies (1978); Dinaric western Balkan (5), and Pannonian lowland (11).

| Species | Species niche traits | | | | |
|---------------------------------------|----------------------|-----------|------------|--------------|-----------|
| | Habitat type | Voltinism | Occurrence | Distribution | Ecoregion |
| <i>Dixa dilatata</i> Strobl, 1900 | 1, 4 | NA | NA | wd | 5 |
| <i>Dixa maculata</i> Meigen, 1818 | 1, 2, 4 | U | Sp, Su, A | wd | 5, 11 |
| <i>Dixa nebulosa</i> Meigen, 1830 | 1, 2, 3 | U, B | Sp, Su, A | wd | 5, 11 |
| <i>Dixa nubilipennis</i> Curtis, 1832 | 1, 4 | U | Su | wd | 5 |
| <i>Dixa puberula</i> Loew, 1849 | 1, 2, 3, 4 | U, B, M | AYR | wd | 5, 11 |
| <i>Dixa submaculata</i> Edwards, 1920 | 1, 2, 4 | U, B, M | AYR | wd | 5, 11 |

Results and discussion

List of *Dixa* species of Croatia

The following format is used for the distribution data: literature references (name of the site and in parentheses the citation of the reference and site ID); new records (life stage in which the identifications were made, i.e., adult ♂, ♀ and larvae, name of the site and in parentheses the site ID, date of collection and the collector). All the sites and their numbers are listed in Table 1.

Genus *Dixa* Meigen, 1818

Dixa dilatata Strobl, 1900

New records. • 1 larva; Stream Sartuk, NP Plitvice (22); 10 Sep. 2009. • 1♂; Roški Slap, Krka River, NP Krka (37); 29 Mar. 2011; M. Ivković leg.

Remarks. This species is newly recorded from Croatia.

Dixa maculata Meigen, 1818

Literature references. • tufa barrier Novakovića Brod, NP Plitvice (Ivanković et al. 2019) (24) • tufa barrier Kozjak-Milanovac, NP Plitvice (Ivanković et al. 2019) (25) • tufa barrier Labudovac, NP Plitvice (Ivanković et al. 2019) (27) • upper reach of Crna rijeka, NP Plitvice (Ivanković et al. 2019) (29) • upper reach of Bijela rijeka, NP Plitvice (Ivanković et al. 2019) (30).

New record. • 1 larva; Dubočanka, Papuk Mountain (14); 9 Sep. 2009.

Dixa nebulosa Meigen, 1830

Literature references. • River Korana in Korana village, NP Plitvice (Ivanković et al. 2019) (21) • Stream Plitvica, NP Plitvice (Ivanković et al. 2019) (23) • tufa barrier Novakovića Brod, NP Plitvice (Ivanković et al. 2019) (24) • tufa barrier Kozjak-Milanovac, NP Plitvice (Ivanković et al. 2019) (25) • tufa barrier Labudovac, NP Plitvice (Ivanković et al. 2019) (27) • upper reach of Bijela rijeka, NP Plitvice (Ivanković et al. 2019) (30) • Roški Slap, Krka River, NP Krka (Ivković et al. 2017) (37) • Skradinski buk, Krka River, NP Krka (Ivković et al. 2017) (38).

New records. • 1 larva; Boščak Stream (1); 15 Apr. 2010 • 1 larva; Bistrec Stream (3); 13 Jul. 2010 • 33 larvae; same site; 16 Sep. 2010 • 1 larva; same site; 18 May 2016 • 48 larvae; Kotoribski kanal (4); 18 May 2016 • 2 larvae; Plitvica, Upper Reach (5); 25 May 2009 • 10 larvae; same site; 13 Jul. 2010 • 13 larvae; Djedovica by Rupnica,

Papuk Mountain (9); 9 Sep. 2009 • 1 larva; River Duboka rijeka, Papuk Mountain (12); 9 Sep. 2009 • 1♀; same site, 18.ix.2012, M. Ivković leg. • 1♀; Dubočanka, Papuk Mountain (14); 18 Sep. 2012; M. Ivković leg. • 1 larva; River Riječina, upper reach (15); 23 Sep. 2009 • 1 larva; Velika Belica (16); 24 Nov. 2009 • 1 larva; Headwater of Dobra River (17); 24 Sep. 2009 • 8♂ and 3♀; Mrežnički Brig, Mrežnica River (19); 10 Sep. 2011; M. Ivković leg. • 6 larvae; River Dretulja, Plaški (20); 9 Oct. 2009 • 3 larvae; Lake Kozjak, NP Plitvice (26); 18 Jul. 2018 • 15 larvae; same site; 16 Sep. 2016 • 4 larvae; River Kosovčica (33); 12 Jun. 2012 • 2 larvae; River Krka above the mouth of River Kosovčica (35); 19 Sep. 2012 • 2 larvae; River Krka below the mouth of River Kosovčica (36); 20 Sep. 2012 • 1♂; Roški Slap, Krka River, NP Krka (37); 29 Mar. 2011; M. Ivković leg. • 2♂; same site; 28 Apr. 2011; M. Ivković leg. • 1♂ and 1♀; Čikotina Lađa, River Cetina (39); 2005; M. Ivković leg.

Dixa nubilipennis Curtis, 1832

Literature references. • River Korana in Korana village, NP Plitvice (Ivanković et al. 2019) (21) • Roški Slap, Krka River, NP Krka (Ivanković et al. 2017) (37).

New record. • 1♀; Kosovčica Spring (32); 18 Nov. 2010; M. Ivković leg.

Dixa puberula Loew, 1849

Literature references. • River Korana in Korana village, NP Plitvice (Ivanković et al. 2019) (21) • Stream Plitvica, NP Plitvice (Ivanković et al. 2019) (23) • tufa barrier Novakovića Brod, NP Plitvice (Ivanković et al. 2019) (24) • tufa barrier Kozjak-Milanovac, NP Plitvice (Ivanković et al. 2019) (25) • tufa barrier Labudovac, NP Plitvice (Ivanković et al. 2019) (27) • middle reach of Crna rijeka, NP Plitvice (Ivanković et al. 2019) (28) • upper reach of Crna rijeka, NP Plitvice (Ivanković et al. 2019) (29) • upper reach of Bijela rijeka, NP Plitvice (Ivanković et al. 2019) (30) • spring of Bijela rijeka stream, NP Plitvice (Ivanković et al. 2019) (31) • Roški Slap, Krka River, NP Krka (Ivanković et al. 2017) (37).

New records. • 2♂ and 1♀; Spring Šumi, Zagorje (6); 15 Jul. 2014; M. Ivković leg. • 1♂; same site; 9 Oct. 2014; M. Ivković leg. • 1 larva; Kraljevec, Medvednica Mountain (8); 21 Jun. 2006 • 1♀; Brzaja, before N. Zvečeva, Papuk Mountain (10); 14 Jun. 2012; M. Ivković leg. • 1♀; Stream Kovačica, Papuk Mountain (11); 14 Jun. 2012; M. Ivković leg. • 3♂ and 5♀; Dubočanka, Papuk Mountain (14); 13 Jun. 2012; M. Ivković leg. • 1 larva; River Bukovačka Dobra (18); 18 Jun. 2006 • 1♂ and 1♀; Stream Plitvica, NP Plitvice (23); 28 Jun. 2007; M. Ivković leg. • 1♂; tufa barrier Novakovića Brod, NP Plitvice (24); 29 May 2007; M. Ivković leg. • 2♂ and 1♀, same site, 28 Jun. 2007; M. Ivković leg. • 1♀; tufa barrier Kozjak-Milanovac, NP Plitvice (25); 28 Jul. 2007; M. Ivković leg. • 2♂; tufa barrier Labudovac, NP Plitvice (27); 29 May 2007; M. Ivković leg. • 1♀; Roški Slap, Krka River, NP Krka (37); 17 Sep. 2010; M. Ivković

leg. • 1♂; same site; 16 Dec. 2010; M. Ivković leg. • 3♀; same site; 29 Mar. 2011; M. Ivković leg. • 84♂ and 43♀; same site; 28 Apr. 2011; M. Ivković leg. • 3♂ and 6♀; same site; 29 May 2011; M. Ivković leg. • 1♂; same site; 13 Oct. 2011; M. Ivković leg.

***Dixa submaculata* Edwards, 1920**

Literature references. • tufa barrier Novakovića Brod, NP Plitvice (Ivanković et al. 2019) (24) • tufa barrier Kozjak-Milanovac, NP Plitvice (Ivanković et al. 2019) (25) • middle reach of Crna rijeka, NP Plitvice (Ivanković et al. 2019) (28) • upper reach of Crna rijeka, NP Plitvice (Ivanković et al. 2019) (29) • upper reach of Bijela rijeka, NP Plitvice (Ivanković et al. 2019) (30) • spring of Bijela rijeka, NP Plitvice (Ivanković et al. 2019) (31).

New records. • 2 larvae; River Reka, upper reach (7); 8 Nov. 2009 • 2 larvae; Kraljevec, Medvednica Mountain (8); 21 Jun. 2006 • 1 larva; River Orašnica (34); 16 Jul. 2009 • 1 larva; River Krka above the mouth of River Kosovčica (35); 19 Sep. 2012.

Species richness and assemblage composition

In total six species of *Dixa* (Table 2) are recorded from Croatia, collected from 39 sites (Fig. 1, Table 1). *Dixa nebulosa* is found at the greatest number of sites (25) while *Dixa dilatata* was the rarest, found only at two sites. All six species occur in the Dinaric western Balkan (Ecoregion 5), while four species occur in the Pannonian lowland (Ecoregion 11). All recorded *Dixa* species are widely distributed in Europe (Table 2). Six of the 12 recognized *Dixa* species in Europe (Pape and Beuk 2012) are now reported from Croatia. Some of the species, e.g., *Dixa puberula* and *D. nebulosa*, may eventually prove to be members of a species complex, rather than a single species (J.K. Moulton and R. Wagner, pers. comm.). Available seasonal phenological data (Table 2) revealed two species (*Dixa puberula* and *Dixa submaculata* Edwards) are multivoltine and present all year round. *Dixa maculata* and *D. nebulosa* occurred from spring through autumn. *Dixa nubilipennis* was only collected during summer.

Italy and Hungary have seven and five, respectively, recorded species of *Dixa*, while Serbia and Montenegro each have only a single recorded species, *Dixa nebulosa* (Fig. 2). The Sørensen Index of Similarity showed that the *Dixa* fauna of Croatia is most similar to that of Italy followed by Hungary, whereas it is least similar to that of Serbia and Montenegro (Table 3). These results were to be expected due to the low number of species recorded for Serbia and Montenegro.

Comparing our list of Croatian species with published records in the *Fauna Europaea* (Pape and Beuk 2012) revealed that none of the six species treated here were previously recorded from Croatia until Ivanković et al. (2019) reported *D. maculata*, *D. nebulosa*, *D. nubilipennis*, *D. puberula*, and *D. submaculata*. Herein, we report *Dixa dilatata* as new to the dixid fauna of Croatia.

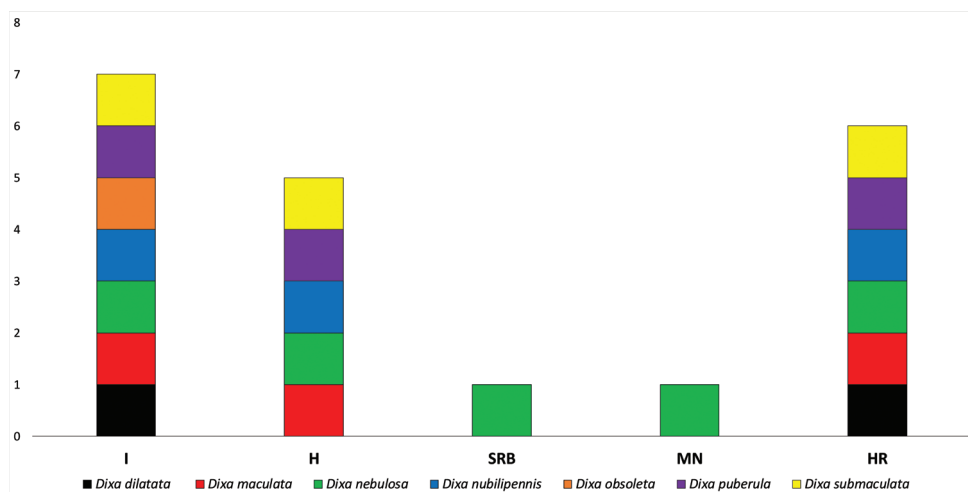


Figure 2. Comparison of the Croatian *Dixa* assemblage with the fauna of neighbouring countries.

Table 3. Sørensen Index of Similarity between *Dixa* assemblages for surrounding countries in relation to Croatia. Key: I = Italy (301 338 km²), H = Hungary (93 030 km²), SRB = Serbia (88 361 km²), MN = Montenegro (13 812 km²) HR = Croatia (56 594 km²).

| | I | H | SRB | MN | HR |
|-----|-------|-------|-------|-------|----|
| I | 0 | | | | |
| H | 83.33 | 0 | | | |
| SRB | 25 | 33.33 | 0 | | |
| MN | 25 | 33.33 | 100 | 0 | |
| HR | 92.38 | 90.91 | 28.57 | 28.57 | 0 |

Concluding remarks

All the recorded species have a wide European distribution and none is restricted to Croatia or to the Balkan Region. There may be a few more species of *Dixa* yet to be recorded, and, because of the high endemism of the Dinaric area (Ivković and Plant 2015) and especially of the aquatic Diptera (Ivković et al. 2012; Pont and Ivković 2013; Kvitte et al. 2013; Kvitte and Ivković 2018), it is possible that undescribed species of *Dixa* may yet be found. In the future, collecting should be focused not only on lotic habitats but also on lentic habitats so that *Dixella* species can also be studied.

Acknowledgements

We would like to thank Dr Igor Stanković for his help with the ArcGIS software and production of the map. We would like to thank Dr Adrian C. Pont for correcting the English language. We would like to thank all the people working in the Animal Ecology Laboratory at the Department of Biology for collecting many macrozoobenthos

samples from which Dixidae larvae were isolated. We are especially grateful to reviewer J.K. Moulton and subject editor Art Borkent for improving the manuscript with their helpful comments and corrections.

References

- Borkent A (2012) The pupae of Culicomorpha – morphology and a new phylogenetic tree. *Zootaxa* 3396: 1–98. <https://doi.org/10.11646/zootaxa.3398.1>
- Disney RHL (1999) British Dixidae (Meniscus midges) and Thaumaleidae (Trickle midges): Keys with ecological notes. Freshwater Biological Association, Scientific Publication No. 56, Ambleside, 129 pp.
- Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, 192 pp. <http://www.primer-e.com/index.html>
- Elliott JM, Tullett PA (1977) The downstream drifting of larvae of *Dixa* (Diptera: Dixidae) in two stony streams. *Freshwater Biology* 7: 403–407. <https://doi.org/10.1111/j.1365-2427.1977.tb01688.x>
- Fowler JA, Withers ID, Dewhurst F (1997) Meniscus midges (Diptera: Dixidae) as indicators of surfactant pollutants. *Entomologiste* 116: 24–27.
- Illies J (1978) Limnofauna Europaea. A checklist of the Animals inhabiting European Inland Waters, with Account of their Distribution and Ecology. G. Fischer, Stuttgart and Swets & Zeitlinger, Amsterdam, 532 pp.
- Ivanković L, Ivković M, Stanković I (2019) Perennial phenology patterns and ecological traits of Dixidae (Insecta, Diptera) in lotic habitats of a barrage lake system. *Limnologica* 76: 11–18. <https://doi.org/10.1016/j.limno.2019.03.001>
- Ivković M, Miliša M, Previšić A, Popijač A, Mihaljević Z (2013) Environmental control of emergence patterns: case study of changes in hourly and daily emergence of aquatic insects at constant and variable water temperatures. *International Review of Hydrobiology* 98: 104–115. <https://doi.org/10.1002/iroh.201301483>
- Ivković M, Plant A (2015) Aquatic insects in the Dinarides: identifying hotspots of endemism and species richness shaped by geological and hydrological history using Empididae (Diptera). *Insect Conservation and Diversity* 8: 302–312. <https://doi.org/10.1111/ica.12113>
- Ivković M, Plant A, Horvat B (2012) A new species of *Wiedemannia* (Diptera: Empididae: Clinocerinae) from Balkan Peninsula. *Zootaxa* 3478: 581–585.
- Ivković M, Pont AC, Kvifte GM, Mihaljević Z (2017) Emergencijske značajke vodenih dvokrilaca (Insecta, Diptera) sedrenih barijera NP “Krka”. In: Marguš D (Ed.) Zbornik radova sa znanstveno-stručnog skupa vizija i izazovi upravljanja zaštićenim područjima prirode u Republici Hrvatskoj: Aktivna zaštita i održivo upravljanje u Nacionalnom parku “Krka”. Kerschoffset, Zagreb, 196–207.
- Kvifte GM, Ivković M (2018) New species and records of the *Pericoma trifasciata* group from Croatia (Diptera: Psychodidae). *Zootaxa* 4486: 076–082. <https://doi.org/10.11646/zootaxa.4486.1.5>
- Kvifte GM, Ivković M, Klarić A (2013) New records of moth flies (Diptera: Psychodidae) from Croatia, with the description of *Berdeniella keroveci* sp.nov. *Zootaxa* 3737: 57–67. <https://doi.org/10.11646/zootaxa.3737.1.4>

- Moulton JK (2016) The *Dixa inextricata* Dyar & Shannon (Diptera: Dixidae) species group, with two new cryptic species from the eastern Nearctic Region. *Zootaxa* 4121: 458–472. <https://doi.org/10.11646/zootaxa.4121.4.6>
- Moulton JK (2017) The true identity of *Dixa modesta* Johannsen (Diptera: Dixidae) resolved: synonymy of *Dixa similis* Johannsen, designation of the *Dixa ubiquita* species group, and description of three new eastern Nearctic species. *Zootaxa* 4216: 247–260. <https://doi.org/10.11646/zootaxa.4216.3.3>
- Oosterbroek P (2007) The European Families of the Diptera: Identification, diagnosis, biology. KNNV Publishing, Utrecht, 204 pp.
- Pape T, Beuk P (2012) Fauna Europaea. <https://www.faunaeur.org> [accessed 20 May 2019]
- Pape T, Blagoderov V, Mostovski MB (2011) Order Diptera Linnaeus, 1758. In: Zhang Z-Q (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. *Zootaxa* 3148: 222–229. <https://doi.org/10.11646/zootaxa.3148.1.42>
- Pont AC, Ivković M (2013) The hunter-flies of Croatia (Diptera: Muscidae, genus *Limnophora* Robineau-Desvoidy). *Journal of Natural History* 47: 1069–1082. <https://doi.org/10.1080/00222933.2012.750775>
- Sertić Perić M, Dražina T, Špoljar M, Radanović I, Primc B, Habdija I. (2014) Meiofauna constitute a considerable portion of invertebrate drift among moss-rich patches within a karst hydrosystem. *Biologia* 69: 363–380. <https://doi.org/10.2478/s11756-013-0323-y>
- Shtakel'berg AA (1989) Family Dixidae. In: Brill EJ (Ed.) Keys to the Insects of the European Part of the USSR, Volume V, Diptera and Siphonaptera. Amerind publishing Co. Pvt. Ltd., New Delhi, 209–215.
- Thomas A (1979) Diptères torrenticoles peu connus. VI. Les Dixidae du sud-ouest de la France (Nematocera) (*Dixa puberula* Loew, 1849. Écologie, microhabitat et intérêt pratique pour le dépistage des pollutions per les stations touristiques de montagne). *Bulletin de la Société d'Histoire Naturelle de Toulouse* 115: 242–268.
- Wagner R (1997) Diptera Dixidae, Meniscus flies. In: Nilsson A (Ed.) Aquatic Insects of North Europe, A taxonomic Handbook, Vol. 2. Apollo Books, Stenstrup, 145–148.
- Wagner R (2011) Dixidae (Meniscus Midges). In: Wagner R, Marxsen J, Zwick P, Coc EJ (Eds) Central European Stream Ecosystems. Wiley-VCH Verlag & Co. KGaA, Weinheim, 380–381. <https://doi.org/10.1002/9783527634651>
- Wagner R, Barták M, Borkent A, Courtney G, Goddeeris B, Haenni J-P, Knutson L, Pont AC, Rotheray GE, Rozkošný R, Sinclair B, Woodley N, Zatwarnicki T, Zwick P. (2008) Global diversity of dipteran families (Insecta Diptera) in freshwater (excluding Simuliidae, Culicidae, Chironomidae, Tipulidae and Tabanidae). *Hydrobiologia* 595:489–519. <https://doi.org/10.1007/s10750-007-9127-9>
- Wiegmann BM, Trautwein MD, Winkler IS, Barr NB, Kim J-W, Lambkin C, Bertone MA, Cassel BK, Bayless KM, Heimberg AM, Wheeler BM, Peterson KJ, Pape T, Sinclair BJ, Skevington JH, Blagoderov V, Caravas J, Kutty SN, Schmidt-Ottm U, Kampmeier GE, Thompson FC, Grimaldi DA, Beckenbach AT, Courtney GW, Friedrich M, Meier R, Yeates DK (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences of the United States of America* 108: 5690–5695. <https://doi.org/10.1073/pnas.1012675108>

DNA barcoding of *Deltocephalus* Burmeister leafhoppers (Cicadellidae, Deltocephalinae, Deltocephalini) in China

Hong Zhang¹, Yalin Zhang², Yani Duan¹

1 School of Plant Protection, Anhui Agricultural University, Hefei, Anhui Province 230036, China **2** Key Laboratory of Plant Protection Resources and Pest Management of the Ministry of Education, Entomological Museum, Northwest A&F University, Yangling, Shaanxi Province 712100, China

Corresponding author: Yani Duan (duanyani@hotmail.com)

Academic editor: Mick Webb | Received 9 April 2019 | Accepted 10 July 2019 | Published 29 July 2019

<http://zoobank.org/4F29DD6F-38BA-446A-85F2-E57C8BC17529>

Citation: Zhang H, Zhang Y, Duan Y (2019) DNA barcoding of *Deltocephalus* Burmeister leafhoppers (Cicadellidae, Deltocephalinae, Deltocephalini) in China. ZooKeys 867: 55–71. <https://doi.org/10.3897/zookeys.867.35058>

Abstract

We investigated the feasibility of using the DNA barcode region in identifying *Deltocephalus* from China. Sequences of the barcode region of the mitochondrial COI gene were obtained for 98 specimens (*Deltocephalus vulgaris* – 88, *Deltocephalus pulicaris* – 5, *Deltocephalus uncinatus* – 5). The average genetic distances among morphological and geographical groups of *D. vulgaris* ranged from 0.9% to 6.3% and among the three species of *Deltocephalus* ranged from 16.4% to 21.9% without overlap, which effectively reveals the existence of a “DNA barcoding gap”. It is important to assess the status of these morphological variants and explore the genetic variation among Chinese populations of *D. vulgaris* because the status of this species has led to taxonomic confusion because specimens representing two distinct morphological variants based on the form of the aedeagus are often encountered at a single locality. Forty-five haplotypes (*D. vulgaris* – 36, *D. pulicaris* – 5, *D. uncinatus* – 4) were defined to perform the phylogenetic analyses; they revealed no distinct lineages corresponding either to the two morphotypes of *D. vulgaris* or to geographical populations. Thus, there is no evidence that these variants represent genetically distinct species.

Keywords

COI, genetic distance, morphological variant

Introduction

China contains threatened biodiversity hotspots, including one spanning the Palearctic and Oriental regions and containing a high level of species diversity (Lin et al. 2010). In these regions, accurate identification of extant species is of great significance, although the taxonomic expertise is limited. Traditionally, identification of most species has been based on morphology. However, the availability of inexpensive DNA sequencing technology now provides additional tools not only for routine species identification but also for testing the validity of morphology-based species concepts. DNA barcoding is a simple, effective tool, that can identify and delimit species, including some complex taxa, rapidly and accurately using a standard short DNA sequence of the mitochondrial cytochrome c oxidase I (COI) (Hebert et al. 2003, 2004b; Ward et al. 2005; Hajibabaei et al. 2006). This method has been widely recognized and accepted in molecular phylogenetic studies (Hebert et al. 2003). The COI-based identification system has achieved remarkable success discriminating species across numerous animal groups, including birds (Hebert et al. 2004b), fishes (Hubert et al. 2008), and the insect orders Lepidoptera (Hebert et al. 2004a; Hajibabaei et al. 2006; Yang et al. 2012; Ashfaq et al. 2013), Ephemeroptera (Ball et al. 2005), and Hymenoptera (Smith et al. 2008). But this technology has also failed to identify species accurately under certain circumstances. For example, in a study of 449 species of Diptera and using 1333 COI sequences, Meier et al. (2006) obtained an identification success rate below 70% due to extensive overlap in inter- and intraspecific genetic distances. Within the dipteran family Caliphoridae, Whitworth et al. (2007) found that only 60% of species tested could be identified reliably.

Deltocephalini feed on grasses and sedges and are diverse and abundant in grassland ecosystems. This group contains 73 genera and 613 species around the world. *Deltocephalus*, type genus of this tribe contains 62 species distributed in the Old World and New World. Some species of this genus can transmit pathogenic diseases to economically important plants and are important economic pests; therefore, tools are needed for their rapid and accurate identification. Four species are described from China, two of them transmit pathogenic diseases. Identification of leafhopper species in most genera now requires dissection and examination of the male genitalia. However, some taxonomically problematic species apparently exhibit substantial intraspecific variation in male genital structures, and this causes confusion among taxonomists. One such practical example is *D. vulgaris*, which has well-documented morphological differences in the shape of the aedeagus (Figs 2, 3). Dash and Viraktamath (1998) first reported morphological variation in this species when they reviewed the genus *Deltocephalus* from India. Webb and Viraktamath (2009) also reported two forms of the aedeagus despite many shared morphological features in the species. Zhang and Duan (2011) redescribed *D. vulgaris* with detailed drawings and photos, illustrating these obvious morphological differences.

Based on DNA barcoding of leafhoppers, 63 barcodes from 45 species in Japan (15 subfamilies and 37 genera without Deltocephalini) were analysed (Kamitani 2011). DNA barcodes from 546 adult specimens of leafhoppers, planthoppers and treehoppers (Hemiptera, Auchenorrhyncha) were obtained from Barrow Island and analysed (Gopurenko et al. 2013). Species determination of members in the genus *Aphrodes* (Hemiptera, Cicadellidae) based on vibrational signals, mitochondrial DNA and morphology were performed (Bluemel et al. 2014). A total of 1482 specimens based on DNA barcodes of Nearctic Auchenorrhyncha (Insecta, Hemiptera) were studied by Foottit et al. (2014). The boundaries of seven closely related species of the evacanthine leafhopper genus *Bundera* (Cicadellidae, Evacanthinae) based on DNA barcoding, morphology and hyperspectral reflectance profiling was investigated by Wang et al. (2016), and a revision of the genus *Orosius* (Cicadellidae, Deltocephalinae, Opsiini) based on morphological and DNA barcoding was undertaken by Fletcher et al. (2017). Although, DNA barcoding research has been applied to these groups of leafhoppers, until now, a few molecular data are available for *Deltocephalus*. Therefore, a better understanding of *Deltocephalus*, and particularly the variation of *D. vulgaris* based on molecular data, is urgently needed.

In this study, we studied 98 COI sequences from three species of *Deltocephalus*. DNA barcoding data were used to investigate genetic variation of Chinese populations of *D. vulgaris* and to determine whether the morphological variants previously identified in this species represent distinct lineages. Our specific aims were to test the feasibility of using DNA barcoding data for identification of species of *Deltocephalus*, to determine the levels of the genetic variation within *D. vulgaris*, and to preliminarily discuss its possible correlation with morphological variation and biogeographic patterns.

Material and methods

Taxon sampling

A total of 98 specimens of *Deltocephalus* (*D. vulgaris* – 88, *D. pulicaris* – 5, *D. uncinatus* – 5) were collected with an insect sweep net in the daytime and by a light trap at night. Specimens were all collected directly into 95% or 100% ethanol and stored in -80 °C prior to study. The sample included *D. vulgaris*, *D. uncinatus* and *D. pulicaris* to facilitate comparison of inter- to intraspecific genetic variation in this group. *Deltocephalus vulgaris* specimens were divided into 11 groups based on their morphological differences and different geographical distributions in China (Table 1, Figs 1–3). Voucher specimens were deposited in the Key Laboratory of Plant Protection Resources and Pest Management of Ministry of Education, Entomological Museum, Northwest A&F University, Yangling, Shaanxi Province, China (NWAUFU) and the School of Plant Protection, Anhui Agricultural University, Hefei, Anhui Province, China (AAU).

Table 1. List of samples studied and their relevant information.

| Species | Group code | Sample size | Individual code | Haplotype | Locality | GenBank accession |
|--------------------|------------|-------------|-----------------|-----------|-------------------------------------|-------------------|
| <i>D. vulgaris</i> | YNA | 8 | YNA1 | Hap1 | Banhong Town, Yunnan Province | MK764780 |
| | | | YNA2 | Hap2 | Banhong Town, Yunnan Province | MK764781 |
| | | | YNA3 | Hap3 | Banhong Town, Yunnan Province | MK764782 |
| | | | YNA4 | Hap2 | Banhong Town, Yunnan Province | MK764783 |
| | | | YNA5 | Hap4 | Banhong Town, Yunnan Province | MK764784 |
| | | | YNA6 | Hap2 | Banhong Town, Yunnan Province | MK767485 |
| | | | YNA7 | Hap1 | Banhong Town, Yunnan Province | MK764786 |
| | | | YNA8 | Hap2 | Banhong Town, Yunnan Province | MK764787 |
| | YNB | 13 | YNB1 | Hap5 | Banhong Town, Yunnan Province | MK764788 |
| | | | YNB2 | Hap1 | Banhong Town, Yunnan Province | MK764789 |
| | | | YNB3 | Hap5 | Banhong Town, Yunnan Province | MK764790 |
| | | | YNB4 | Hap5 | Banhong Town, Yunnan Province | MK764791 |
| | | | YNB5 | Hap5 | Banhong Town, Yunnan Province | MK764792 |
| | | | YNB6 | Hap5 | Banhong Town, Yunnan Province | MK764793 |
| | | | YNB7 | Hap5 | Banhong Town, Yunnan Province | MK764794 |
| | | | YNB8 | Hap6 | Banhong Town, Yunnan Province | MK764795 |
| | | | YNB9 | Hap7 | Banhong Town, Yunnan Province | MK764796 |
| | | | YNB10 | Hap8 | Banhong Town, Yunnan Province | MK764797 |
| | | | YNB11 | Hap5 | Banhong Town, Yunnan Province | MK764798 |
| | | | YNB12 | Hap5 | Banhong Town, Yunnan Province | MK764799 |
| | | | YNB13 | Hap5 | Banhong Town, Yunnan Province | MK764800 |
| | ZJA | 7 | ZJA1 | Hap9 | Lin'an County, Zhejiang Province | MK764801 |
| | | | ZJA2 | Hap10 | Lin'an County, Zhejiang Province | MK764802 |
| | | | ZJA3 | Hap11 | Lin'an County, Zhejiang Province | MK764803 |
| | | | ZJA4 | Hap12 | Lin'an County, Zhejiang Province | MK764804 |
| | | | ZJA5 | Hap13 | Lin'an County, Zhejiang Province | MK764805 |
| | | | ZJA6 | Hap12 | Lin'an County, Zhejiang Province | MK764806 |
| | | | ZJA7 | Hap12 | Lin'an County, Zhejiang Province | MK764807 |
| | ZJB | 8 | ZJB1 | Hap14 | Kowloon Mountain, Zhejiang Province | MK764808 |
| | | | ZJB2 | Hap10 | Kowloon Mountain, Zhejiang Province | MK764809 |
| | | | ZJB3 | Hap15 | Kowloon Mountain, Zhejiang Province | MK764810 |
| | | | ZJB4 | Hap12 | Kowloon Mountain, Zhejiang Province | MK764811 |
| | | | ZJB5 | Hap16 | Kowloon Mountain, Zhejiang Province | MK764812 |
| | | | ZJB6 | Hap17 | Kowloon Mountain, Zhejiang Province | MK764813 |
| | | | ZJB7 | Hap18 | Kowloon Mountain, Zhejiang Province | MK764814 |
| | | | ZJB8 | Hap19 | Kowloon Mountain, Zhejiang Province | MK764815 |
| | FJA | 7 | FJA1 | Hap20 | Shajian Town, Fujian Province | MK764816 |
| | | | FJA2 | Hap20 | Shajian Town, Fujian Province | MK764817 |
| | | | FJA3 | Hap5 | Shajian Town, Fujian Province | MK764818 |
| | | | FJA4 | Hap21 | Shajian Town, Fujian Province | MK764819 |
| | | | FJA5 | Hap5 | Shajian Town, Fujian Province | MK764820 |
| | | | FJA6 | Hap20 | Shajian Town, Fujian Province | MK764821 |
| | | | FJA7 | Hap5 | Shajian Town, Fujian Province | MK764822 |
| | FJB | 7 | FJB1 | Hap22 | Shajian Town, Fujian Province | MK764823 |
| | | | FJB2 | Hap20 | Shajian Town, Fujian Province | MK764824 |
| | | | FJB3 | Hap20 | Shajian Town, Fujian Province | MK764825 |
| | | | FJB4 | Hap20 | Shajian Town, Fujian Province | MK764826 |
| | | | FJB5 | Hap20 | Shajian Town, Fujian Province | MK764827 |
| | | | FJB6 | Hap8 | Shajian Town, Fujian Province | MK764828 |
| | | | FJB7 | Hap23 | Shajian Town, Fujian Province | MK764829 |

| Species | Group code | Sample size | Individual code | Haplotype | Locality | GenBank accession |
|---------------------|------------|-------------|-----------------|-----------|------------------------------------|-------------------|
| <i>D. vulgaris</i> | HNA | 9 | HNA1 | Hap24 | Jianfeng Mountain, Hainan Province | MK764830 |
| | | | HNA2 | Hap8 | Jianfeng Mountain, Hainan Province | MK764831 |
| | | | HNA3 | Hap8 | Jianfeng Mountain, Hainan Province | MK764832 |
| | | | HNA4 | Hap8 | Jianfeng Mountain, Hainan Province | MK764833 |
| | | | HNA5 | Hap8 | Jianfeng Mountain, Hainan Province | MK764834 |
| | | | HNA6 | Hap25 | Jianfeng Mountain, Hainan Province | MK764835 |
| | | | HNA7 | Hap8 | Jianfeng Mountain, Hainan Province | MK764836 |
| | | | HNA8 | Hap26 | Jianfeng Mountain, Hainan Province | MK764837 |
| | | | HNA9 | Hap27 | Jianfeng Mountain, Hainan Province | MK764838 |
| | HNB | 8 | HNB1 | Hap20 | Jianfeng Mountain, Hainan Province | MK764839 |
| | | | HNB2 | Hap28 | Jianfeng Mountain, Hainan Province | MK764840 |
| | | | HNB3 | Hap29 | Jianfeng Mountain, Hainan Province | MK764841 |
| | | | HNB4 | Hap30 | Jianfeng Mountain, Hainan Province | MK764842 |
| | | | HNB5 | Hap8 | Jianfeng Mountain, Hainan Province | MK764843 |
| | | | HNB6 | Hap31 | Jianfeng Mountain, Hainan Province | MK764844 |
| | | | HNB7 | Hap8 | Jianfeng Mountain, Hainan Province | MK764845 |
| | | | HNB8 | Hap8 | Jianfeng Mountain, Hainan Province | MK764846 |
| | GDB | 9 | GDB1 | Hap32 | Patio Hill, Guangdong Province | MK764847 |
| | | | GDB2 | Hap8 | Patio Hill, Guangdong Province | MK764848 |
| | | | GDB3 | Hap8 | Patio Hill, Guangdong Province | MK764849 |
| | | | GDB4 | Hap8 | Patio Hill, Guangdong Province | MK764850 |
| | | | GDB5 | Hap8 | Patio Hill, Guangdong Province | MK764851 |
| | | | GDB6 | Hap20 | Patio Hill, Guangdong Province | MK764852 |
| | | | GDB7 | Hap8 | Patio Hill, Guangdong Province | MK764853 |
| | | | GDB8 | Hap8 | Patio Hill, Guangdong Province | MK764854 |
| | | | GDB9 | Hap8 | Patio Hill, Guangdong Province | MK764855 |
| | GXA | 4 | GXA1 | Hap33 | Lingyun County, Guangxi Province | MK764856 |
| | | | GXA2 | Hap1 | Lingyun County, Guangxi Province | MK764857 |
| | | | GXA3 | Hap34 | Lingyun County, Guangxi Province | MK764858 |
| | | | GXA4 | Hap20 | Lingyun County, Guangxi Province | MK764859 |
| | GXB | 8 | GXB1 | Hap35 | Shangsi County, Guangxi Province | MK764860 |
| | | | GXB2 | Hap20 | Shangsi County, Guangxi Province | MK764861 |
| | | | GXB3 | Hap32 | Shangsi County, Guangxi Province | MK764862 |
| | | | GXB4 | Hap1 | Shangsi County, Guangxi Province | MK764863 |
| | | | GXB5 | Hap5 | Shangsi County, Guangxi Province | MK764864 |
| | | | GXB6 | Hap5 | Shangsi County, Guangxi Province | MK648065 |
| | | | GXB7 | Hap20 | Shangsi County, Guangxi Province | MK764866 |
| | | | GXB8 | Hap36 | Shangsi County, Guangxi Province | MK764867 |
| <i>D. pulicaris</i> | XJ | 5 | XJ1 | Hap37 | Altay City, Xinjiang Province | MK764868 |
| | | | XJ2 | Hap38 | Altay City, Xinjiang Province | MK764869 |
| | | | XJ3 | Hap39 | Altay City, Xinjiang Province | MK764870 |
| | | | XJ4 | Hap40 | Altay City, Xinjiang Province | MK764871 |
| | | | XJ5 | Hap41 | Altay City, Xinjiang Province | MK764872 |
| <i>D. uncinatus</i> | YN | 5 | YN1 | Hap42 | Menglong Town, Yunnan Province | MK764873 |
| | | | YN2 | Hap43 | Menglong Town, Yunnan Province | MK764874 |
| | | | YN3 | Hap43 | Menglong Town, Yunnan Province | MK764875 |
| | | | YN4 | Hap44 | Menglong Town, Yunnan Province | MK764876 |
| | | | YN5 | Hap45 | Menglong Town, Yunnan Province | MK764877 |

Note: individual code with province initials and A or B and number; A and B are representative of two different morphological variants of *D. vulgaris* respectively.

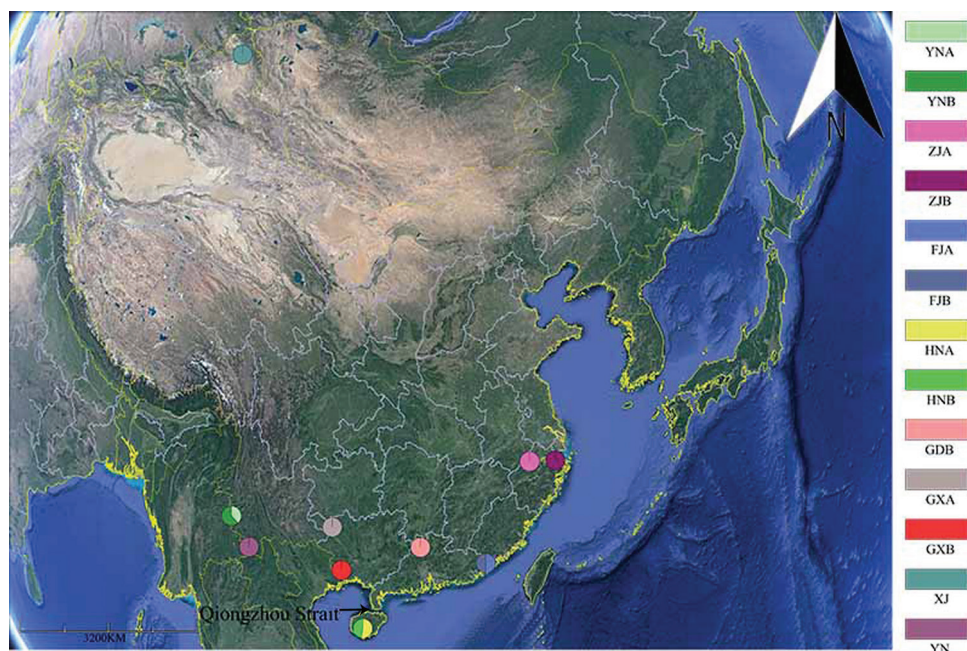


Figure 1. Distribution of *Deltocephalus* in China, codes same as in Table 1.

Morphology

Morphological observations were made using an Olympus SZX10 stereoscopic microscope (Olympus Corporation, Tokyo, Japan). All photographs and drawings were modified with Adobe Photoshop CS.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the whole abdomen of each leafhopper using the EasyPure Genomic DNA Kit (EE101; Transgen, Beijing, China) following the manufacturer's instructions with the following modifications: abdomen incubated at 55 °C for about 20 hours, and with a nondestructive DNA extraction procedure to allow subsequent morphological observation. Genomic DNA extracts were stored in a freezer at -20 °C.

The barcode region (630bp) of the COI gene was amplified using primer combination (Folmer et al. 1994), LCO1490 (5'-GGT CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') by the standard polymerase chain reaction (PCR) method. Total reaction volume was 25 µl, containing 12.5 µl of 2×Taq MasterMix, 8.5 µl of double distilled water (ddH₂O), 2 µl of forward and reverse primer (1 µl, respectively), and 2 µl of DNA template solution. The following thermal cycling protocol was used: an initial denaturation step at 94 °C

for 3 min, followed by 5 cycles of denaturation at 94 °C for 1 min, annealing at 45 °C for 1.5 min and extension at 72 °C for 1.5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 53.5 °C for 1 min and extension at 72 °C for 1 min, with a final extension of at 72 °C for 5 min, and ending with incubation at 12 °C.

The PCR products were examined using 1% agarose gel electrophoresis with ethidium bromide stain to check for successful amplification. The successful PCR products were sent to Beijing Tsingke Biotechnology Co., Ltd (China) for sequencing of both strands using the original PCR primers. All sequences collected in this study have been submitted to GenBank and accession numbers are shown in Table 1.

Molecular data analysis

The forward and reverse chromatograms were proofread and then assembled and edited using DNASTAR software (DNASTAR, Madison, Wisconsin, USA). Multiple sequence alignments were performed by CLUSTAL X 2.0.21 (Thompson et al. 1997; Jeanmougin et al. 1998). Primer sequences were manually deleted with BIOEDIT 7.0.9.0 (Hall 1999). To ensure that the correct target gene fragment was obtained, all sequences were checked in NCBI by Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). To ensure nonexistence of stop codons and pseudogenes, the nucleotide sequences were translated to amino acids by MEGA 7 (Kumar et al. 2016). Sequence composition analyses were performed in MEGA 7. Pairwise genetic distances were calculated using the Kimura 2-parameter (K2P) model in MEGA 7 (Kimura 1980). Haplotypes were defined by DNASP 5.0 (Librado and Rozas 2009). The detailed statistics for haplotypes are shown in Table 1. The substitution saturation tests of 45 haplotype sequences segments were conducted in DAMBE 5.3.74 (Xia 2013) by comparing the index of substitution saturation (Iss) with critical values (Iss.c). To construct phylogenetic trees, neighbor joining (NJ), minimum evolution (ME), Bayesian inference (BI) and maximum likelihood (ML) analyses were performed. NJ and ME analyses (Saitou and Nei 1987) were performed in MEGA 7 under K2P substitution model. Branch support was measured using 1000 replicates in each analysis (Felsenstein 1985). Results were summarized as 50% majority consensus trees in MEGA 7. BI analysis was performed in MRBAYES 3.1.2 (Ronquist and Huelsenbeck 2003). The best-fit nucleotide evolution substitution model was selected by JMODELTEST 2.1.7 (Darriba et al. 2012). The Bayesian information criterion (BIC) was used to compare substitution models. The HKY+G model of nucleotide evolution was used. Two replicate runs with four independent Markov chain Monte Carlo (MCMC) chains (one cold chain and three hot chains) to conduct for 2 million generations, with trees sampled every 1000 generations with default parameter values. The average standard deviation of split frequency was lower than 0.01, indicating that the sampling of posterior distribution was adequate. The average standard deviation of split frequencies and Potential Scale Reduction Factor (PSRF) were used for examining convergence.

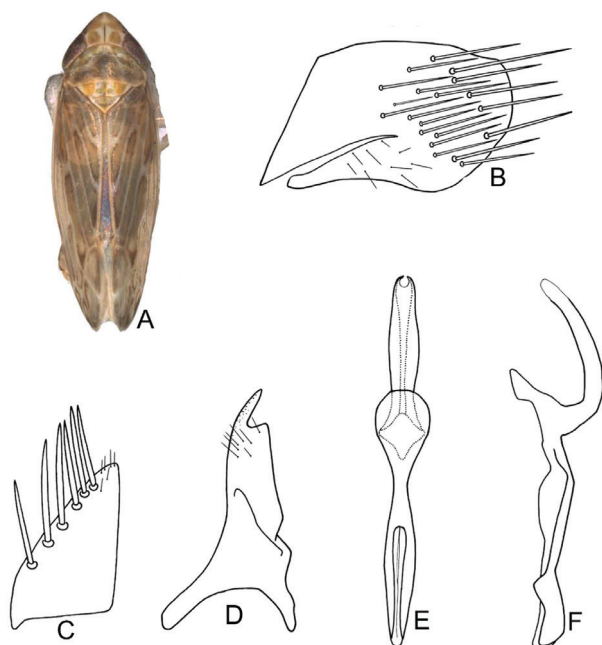


Figure 2. Morphological variant marked with A for *D. vulgaris* **A** habitus in dorsal view **B** subgenital plate **C** subgenital plate **D** style **E** aedeagus and connective, dorsal view **F** aedeagus and connective, lateral view (after Zhang and Duan 2011).

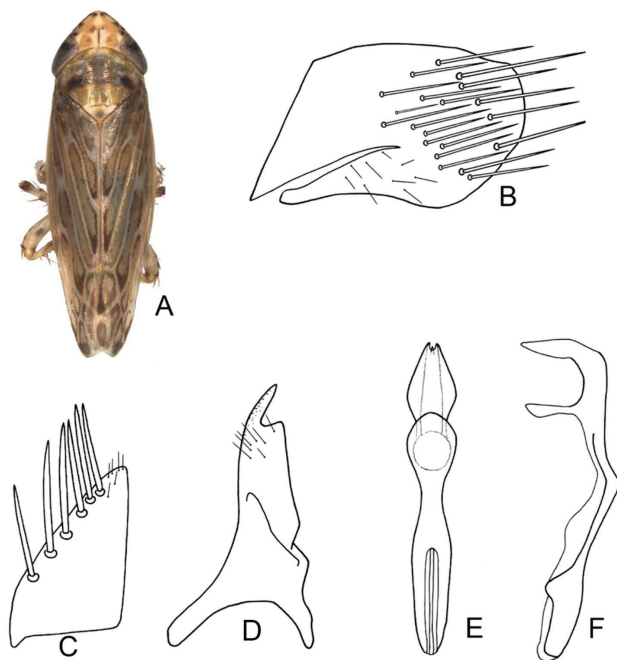


Figure 3. Morphological variant marked with B for *D. vulgaris* **A** habitus in dorsal view **B** subgenital plate **C** subgenital plate **D** style **E** aedeagus and connective, dorsal view **F** aedeagus and connective, lateral view (after Zhang and Duan 2011).

The stationarity was determined in TRACER 1.5 (Rambaut and Drummond 2009) by plotting the log-likelihood values versus generation number and the effective sample sizes >200 for all parameters. After stationarity had been reached, the first 25% trees were discarded as burn-in and a 50% majority-rule consensus tree with the posterior probability considered as node support values was constructed by summarizing the remaining trees. ML analysis was performed in RAXMLGUI 1.3.1, a graphical front-end for RAXML (Silvestro and Michalak 2012). All ML analyses with thorough bootstrap were run 10 times starting from random seeds under the GTRGAMMA model. The bootstrap support value (BS) was evaluated by analysis with 1000 replicates. All tree topologies were displayed in FIGTREE 1.4 (Rambaut 2009).

Results

Morphological variation of *D. vulgaris*

Our specimens from China included representatives of both previously reported morphotypes of the aedeagus of *D. vulgaris*. They also exhibited a range of more subtle variation in the curvature of the aedeagal shaft in lateral view. Under the current morphology-based concept, this species can nevertheless be identified by the colour pattern and the presence of a shallow apical notch on the aedeagus in posterior view.

Sequence composition

The COI sequences are 630bp in length after alignment and trimming. Details of nucleotide composition are listed in Table 2. As is typical for insect mtDNA, the gene is AT-rich (Liu et al. 2012).

Table 2. The average nucleotide composition of the COI sequences of *Deltocephalus*.

| Group/Species | T (%) | C (%) | A (%) | G (%) | A+T (%) |
|---------------------|-------|-------|-------|-------|---------|
| YNA | 32.8 | 18.8 | 34.0 | 14.1 | 56.8 |
| YNB | 33.1 | 18.3 | 33.5 | 15.1 | 66.6 |
| ZJA | 32.8 | 19.0 | 34.2 | 14.4 | 67.0 |
| ZJB | 32.9 | 18.9 | 34.1 | 14.1 | 67.0 |
| FJA | 33.0 | 18.3 | 33.8 | 14.9 | 66.8 |
| FJB | 33.0 | 18.4 | 34.1 | 14.5 | 67.1 |
| HNA | 33.1 | 18.4 | 33.9 | 14.6 | 67.0 |
| HNB | 33.0 | 18.3 | 34.0 | 14.7 | 67.0 |
| GDB | 33.0 | 18.3 | 34.0 | 14.7 | 67.0 |
| GXA | 33.0 | 18.4 | 33.9 | 14.7 | 66.9 |
| GXB | 33.0 | 18.3 | 33.9 | 14.8 | 66.9 |
| A total of A | 33.0 | 18.6 | 34.0 | 14.5 | 67.0 |
| A total of B | 33.0 | 18.4 | 33.9 | 14.7 | 66.9 |
| A total of A and B | 33.0 | 18.5 | 33.9 | 14.6 | 66.9 |
| <i>D. pulicaris</i> | 33.7 | 20.9 | 30.6 | 14.8 | 64.3 |
| <i>D. uncinatus</i> | 35.2 | 18.0 | 32.0 | 14.9 | 57.2 |

Substitution saturation test

The results of haplotype sequences for the substitution saturation test indicate the value of Iss is smaller than Iss.c; namely, little substitutional saturation was detected, which is strongly informative for constructing phylogenetic trees.

Analysis of the genetic distance and phylogenetic trees

The average genetic distances among morphological and geographical groups of *D. vulgaris* ranged from 0.9% to 6.3% and among species of *Deltocephalus* ranged from 16.4% to 21.9% without overlap (Table 3). This effectively reveals the existence of “DNA barcoding gap” and indicates the variation among morphological and geographical groups of *D. vulgaris* have not reached species level. Forty-five haplotypes (*D. vulgaris* – 36, *D. pulicaris* – 5, *D. uncinatus* – 4) were defined to perform phylogenetic analyses. The phylogenetic analyses based on NJ, ME, BI and ML methods nearly yielded identical trees except for the slight change of the position of a few individuals of *D. vulgaris* and bootstrap values (Figs 4, 5). *Deltocephalus vulgaris* haplotypes grouped into several distinct clades. However, these groups included individuals of both morphotypes and formed a distinct monophyletic clade with strong support value (BS(NJ) = 100, BS(ME) = 100, PP = 1, BS(ML) = 97) with no obvious biogeographic structure. Furthermore, different morphotypes of *D. vulgaris* share the same haplotype (Table 1). Thus, the COI sequence data suggest that previous authors were correct in treating the two morphotypes of *D. vulgaris* as belonging to the same species.

Table 3. Kimura 2-parameter genetic distances between groups/species of *Deltocephalus*.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| YNA | | | | | | | | | | | | |
| YNB | 0.047 | | | | | | | | | | | |
| ZJA | 0.041 | 0.063 | | | | | | | | | | |
| ZJB | 0.041 | 0.057 | 0.017 | | | | | | | | | |
| FJA | 0.045 | 0.011 | 0.063 | 0.056 | | | | | | | | |
| FJB | 0.043 | 0.029 | 0.047 | 0.043 | 0.023 | | | | | | | |
| HNA | 0.042 | 0.031 | 0.049 | 0.046 | 0.026 | 0.031 | | | | | | |
| HNB | 0.044 | 0.014 | 0.062 | 0.056 | 0.007 | 0.022 | 0.023 | | | | | |
| GDB | 0.043 | 0.019 | 0.057 | 0.052 | 0.012 | 0.023 | 0.024 | 0.009 | | | | |
| GXA | 0.045 | 0.023 | 0.050 | 0.046 | 0.021 | 0.030 | 0.032 | 0.021 | 0.024 | | | |
| GXB | 0.045 | 0.022 | 0.055 | 0.052 | 0.019 | 0.031 | 0.032 | 0.020 | 0.023 | 0.028 | | |
| <i>D. pulicaris</i> | 0.207 | 0.219 | 0.206 | 0.204 | 0.212 | 0.206 | 0.210 | 0.210 | 0.209 | 0.212 | 0.213 | |
| <i>D. uncinatus</i> | 0.171 | 0.171 | 0.169 | 0.168 | 0.166 | 0.164 | 0.165 | 0.165 | 0.164 | 0.168 | 0.168 | 0.219 |

Note: the values indicate average intergroup and interspecific distances.

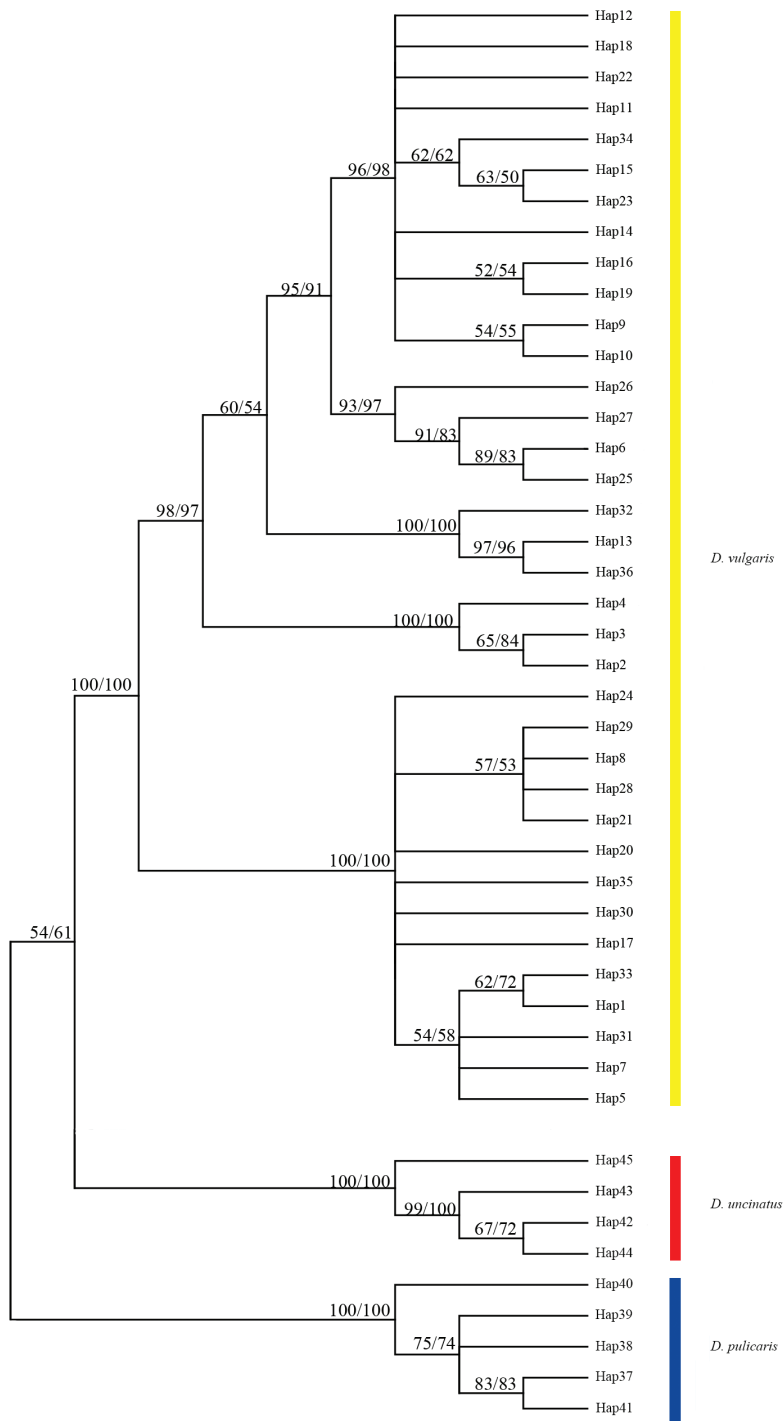


Figure 4. NJ/ME tree of 45 COI haplotypes. The node support: NJ/ME bootstrap values. Bootstrap values of less than 50 are not displayed.

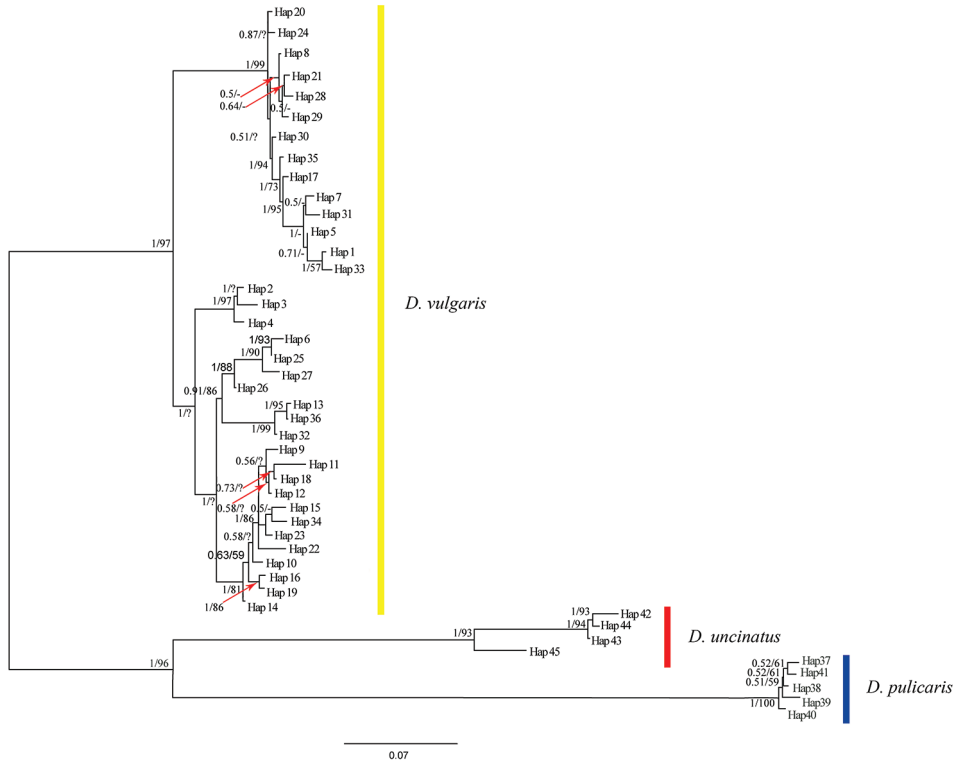


Figure 5. BI/ML tree of 45 COI haplotypes. The node support: BI posterior probabilities/ML bootstrap values. Posterior probabilities and bootstrap values under 0.5 and 50 are shown “-”. “?” means the positions of the different individual of *D. vulgaris* in ML tree is slightly different from those in BI tree.

Discussion

DNA barcoding as a standardised method to provide rapid and accurate species demarcation and has been widely applied in identifying and delimiting taxa since it was first reported by Hebert et al. (2003). Two standard criteria have generally been accepted in delimiting species using COI-based DNA barcodes. Based on the existence of a DNA barcoding gap, the feasibility of COI-based DNA barcoding depends on the fact that genetic distances among species are usually much higher than distances within species, without overlap. Different numbers of single species always form an independent clade in a phylogenetic tree (Wiens and Penkrot 2002; Hebert et al. 2003). Our analysis of COI sequences of *Deltocephalus* suggests a low level of genetic variation among morphotypes and geographical populations of *D. vulgaris*, and even different morphotypes of *D. vulgaris* share the same haplotype (e.g., YNA1 and YNB2; FJA1 and HNB1). The intergroup average genetic distances (0.9%–6.3%) of *D. vulgaris* among morphotypes and geographical populations is distinctly lower than that among species of *Deltocephalus* (16.4%–21.9%), without overlap. The phylogenetic tree (Figs 4, 5) recovered three independent lineages representing each of the three species with moderate to high support val-

ues. The genetic distances among a few morphotypes and geographical populations of *D. vulgaris* exceeded the 3% standard threshold (e.g., ZJA and HNB; YNA and HNA). The more detailed genetic distances between groups/species of *Deltocephalus* are summarized in Table 3. However, all individuals of *D. vulgaris* grouped into a single clade with strong support comprising several subordinate clades but with no obvious correspondence to morphological or geographic groups. Furthermore, different morphotypes from the same and different geographical distributions of *D. vulgaris* share the same haplotype (Table 1). We consider that the intraspecific genetic distance of a 3% standard threshold can be an inconsistent standard in different groups and maternal inheritance of mitochondrial genes can be affected in the process of evolution by the same mode of inheritance as Wolbachia infection, which also may result in a higher divergence in host mtDNA (Frezal and Leblois 2008; Muñoz et al. 2011). The low level of variation among morphotypes and geographical populations of *D. vulgaris* supports the notion that they represent a single species.

Differences in morphological characteristics, especially in male genitalia, have been the most reliable standard for discriminating among complex groups for many years. However, some cases of intraspecific variation in genital structures have been reported and these have led to uncertainty in the status of species and morphotypes. Mutanen et al. (2007) reported the male genital features that are most accepted and widely used standards to delimit species have been doubted in comparative study on male genital variation in *Pammene luedersiana* (Lepidoptera, Tortricidae). Yang et al. (2014) found 31 morphological variants in six species of *Mogannia* (Cicadidae, Cicadinae), but analysis of molecular data revealed low levels of intraspecific variation, although these morphological features have routinely been used to delimit species in this group. On the other hand, Wang et al. (2016) delimited seven different species of *Bundera* (Cicadellidae, Evacanthinae) based on molecular data, but only very minor morphological differences were found among six of these species. We are gradually becoming aware that similar morphological variation may have a different significance in different groups of leafhoppers and morphology-based species concepts may require confirmation using other kinds of data. DNA barcoding can efficiently complement morphology-based taxonomy and improve accuracy and rapidity in species identification.

Deltocephalus vulgaris, including 88 individuals in this study, and mainly representing two different forms of the aedeagus, were confirmed to be a single species grouped into a single clade with strongly support value in its phylogenetic trees (Figs 4, 5). Individuals collected both at the same place and time and different times and places have the same two forms of the aedeagus (e.g., FJA2 and FJB2; YNB10 and HNA2), which indicates forms are not related to temperature, humidity, precipitation, day length, altitude or latitude.

Our study shows a low intraspecific genetic distance between Guangdong and Hainan populations of *D. vulgaris* in southern China, suggesting that the Qiongzhou Strait (Fig. 1), a well-known biogeographic barrier has not significantly restricted gene flow for this species and they even share the same haplotype (Table 1). One logical assumption to explain this discovery is that Hainan and Guangdong arose earlier than the Qiongzhou Strait historically. Therefore, *D. vulgaris* freely exchanged genes when Guangdong and Hainan had been connected.

In the present study, lack of apparent correlation between morphology and COI haplotype is consistent with the hypothesis that the observed morphological variation is intraspecific. Nevertheless, we acknowledge the possibility that two different leaf-hopper species may share the same, or similar, COI haplotype. Thus, study of other genes may, in the future, reveal higher levels of divergence between the two forms and support recognition of some morphological variants as separate species.

Acknowledgements

We express our sincere thanks to M. D. Webb, the Natural History Museum, London, UK and Dr C.H. Dietrich, Department of Entomology, University of Illinois, USA for reading the manuscript and making some suggestions. We also thank Dr J. R. Schrock, Emporia State University, USA for revising the manuscript. This study is supported by the National Natural Science Foundation of China (31000968), Anhui Provincial Natural Science Foundation (1608085MC55) and Anhui Provincial Colleges and Universities Natural Science Foundation (KJ2015A006).

References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215(3): 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Ashfaq M, Akhtar S, Khan AM, Adamowicz SJ, Hebert PDN (2013) DNA barcode analysis of butterfly species from Pakistan points towards regional endemism. *Molecular Ecology Resources* 13(5): 832–843. <https://doi.org/10.1111/1755-0998.12131>
- Ball SL, Hebert PDN, Burian SK, Webb JM (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society* 24(3): 508–524. <https://doi.org/10.1899/04-142.1>
- Bluemel JK, Derlink M, Pavlovčič P, Russo I-RM, King RA, Corbett E, Sherrard-Smith E, Blejec A, Wilson MR, Stewart AJA, Symondson WOC, Virant-Doberlet M (2014) Integrating vibrational signals, mitochondrial DNA and morphology for species determination in the genus *Aphrodes* (Hemiptera, Cicadellidae). *Systematic Entomology* 39: 304–324. <https://doi.org/10.1111/syen.12056>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9(8): 772. <https://doi.org/10.1038/nmeth.2109>
- Dash PC, Viraktamath CA (1998) A review of the Indian and Nepalese grass feeding leaf-hopper genus *Deltocephalus* (Homoptera, Cicadellidae) with description of new species. *Hexapoda* 10(1–2): 1–59.
- Felsenstein J (1985) Confidence limits on phylogenies an approach using the bootstrap. *Evolution* 39(4): 783–791. <https://doi.org/10.2307/2408678>

- Fletcher M, Löcker H, Mitchell A, Gopurenko D (2017) A revision of the genus *Orosius* Distant (Hemiptera, Cicadellidae) based on male genitalia and DNA barcoding. *Austral Entomology* 56: 198–217. <https://doi.org/10.1111/aen.12224>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294–299.
- Footitt RG, Maw E, Hebert PDN (2014) DNA barcodes for Nearctic Auchenorrhyncha (Insecta, Hemiptera). *PLoS ONE* 9: e101385. <https://doi.org/10.1371/journal.pone.0101385>
- Frezal L, Leblois R (2008) Four years of DNA barcoding: current advances and prospects. *Infection Genetics and Evolution* 8(5): 727–736. <https://doi.org/10.1016/j.meegid.2008.05.005>
- Gopurenko D, Fletcher M, Löcker H, Mitchell A (2013) Morphological and DNA barcode species identifications of leafhoppers, planthoppers and treehoppers (Hemiptera, Auchenorrhyncha) at Barrow Island. *Records of the Western Australian Museum Supplement* 83(1): 253–285. <https://doi.org/10.18195/issn.0313-122x.83.2013.253-285>
- Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* 103(4): 968–971. <https://doi.org/10.1073/pnas.0510466103>
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B* 270(1512): 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004a) Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptus fulgurator*. *Proceedings of the National Academy of Sciences of the United States of America* 101(41): 14812–14817. <https://doi.org/10.1073/pnas.0406166101>
- Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004b) Identification of birds through DNA barcodes. *PLoS Biology* 2(10): 1657–1663. <https://doi.org/10.1371/journal.pbio.0020312>
- Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, Burrridge M, Watkinson D, Dumont P, Curry A, Bentzen P, Zhang JB, April J, Bernatchez L (2008) Identifying Canadian freshwater fishes through DNA barcodes. *PLoS ONE* 3: e2490. <https://doi.org/10.1371/journal.pone.0002490>
- Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with Clustal X. *Trend in Biochemical Sciences* 23: 403–405. [https://doi.org/10.1016/S0968-0004\(98\)01285-7](https://doi.org/10.1016/S0968-0004(98)01285-7)
- Kamitani S (2011) DNA barcodes of Japanese leafhoppers. *Esakia* 50: 81–88.
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16(2): 111–120. <https://doi.org/10.1007/BF01731581>

- 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>
- Librado PJR, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25(11): 1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Lin LH, Ji X, Diong CH, Du Y, Lin CX (2010) Phylogeography and population structure of the Reeves's Butterfly Lizard (*Leiolepis reevesii*) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 56(2): 601–607. <https://doi.org/10.1016/j.ympev.2010.04.032>
- Liu S, Li MF, Ye J, Wang Y, Wang CL (2012) The molecular phylogenetic analysis of *Panaeus* based on 16S rRNA and COI sequences. *Journal of Biology* 29(5): 37–42.
- Meier R, Shiyang K, Vaidya G, Peter KLN (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* 55(5): 715–728. <https://doi.org/10.1080/10635150600969864>
- Muñoz AG, Baxter SW, Linares M, Jiggins CD (2011) Deep mitochondrial divergence within a *Heliconius* butterfly species is not explained by cryptic speciation or endosymbiotic bacteria. *BMC Evolutionary Biology* 11(1): 358. <https://doi.org/10.1186/1471-2148-11-358>
- Mutanen M, Rytönen S, Lindén J, Sinkkonen J (2007) Male genital variation in a moth *Pammene luedersiana* (Lepidoptera, Tortricidae). *European Journal of Entomology* 104(2): 259–265. <https://doi.org/10.14411/eje.2007.040>
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12): 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4(4): 406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- Smith MA, Rodriguez JJ, Whitfield JB, Deans AR, Janzen DH, Hallwachs W, Hebert PDN (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology and collections. *Proceedings of the National Academy of Sciences of the United States of America* 105(34): 12359–12364. <https://doi.org/10.1073/pnas.0805319105>
- Rambaut A (2009) FigTree Version 1.3.1. <http://tree.bio.ed.ac.uk/software/figtree/>
- Rambaut A, Drummond AJ (2009) Tracer Version 1.5.0. <http://beast.bio.ed.ac.uk/software/tracer/> [Accessed on: 2015-3-10]
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25(24): 4876–4882. <https://doi.org/10.1093/nar/25.24.4876>

- Wang Y, Nansen C, Zhang YL (2016) Integrative insect taxonomy based on morphology, mitochondrial DNA and hyperspectral reflectance profiling. *Zoological Journal of the Linnean Society* 177: 378–394. <https://doi.org/10.1111/zoj.12367>
- Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B* 360(1462): 1847–1857. <https://doi.org/10.1098/rstb.2005.1716>
- Webb MD, Viraktamath CA (2009) Annotated check-list, generic key and new species of Old World *Deltocephalini* leafhoppers with nomenclatorial changes in the *Deltocephalus* group and other *Deltocephalinae* (Hemiptera, Auchenorrhyncha, Cicadellidae). *Zootaxa* 2163: 1–64.
- Whitworth TL, Dawson RD, Magalon H, Baudry E (2007) DNA barcoding cannot reliably identify species of the blowfly genus *Protophormia* (Diptera, Calliphoridae). *Proceedings of the Royal Society B* 274(1619): 1731–1739. <https://doi.org/10.1098/rspb.2007.0062>
- Wiens JJ, Penkrot TA (2002) Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* 51(1): 69–91. <https://doi.org/10.1080/106351502753475880>
- Xia X (2013) DAMBE5: a comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution* 30(7): 1720–1728. <https://doi.org/10.1093/molbev/mst064>
- Yang MS, Chen X, Huo WX, Wei C (2014) Morphological variation versus genetic divergence: a taxonomic implication for *Mogannia* species (Cicadidae, Cicadinae). *Systematics and Biodiversity* 12(4): 456–472. <https://doi.org/10.1080/14772000.2014.946980>
- Yang ZF, Landry JF, Handfield L, Zhang YL, Solis MA, Handfield D, Scholtens B, Mutanen M, Nuss M, Hebert PDN (2012) DNA barcoding and morphology reveal three cryptic species of *Anania* (Lepidoptera, Crambidae, Pyraustinae) in North America, all distinct from their European counterpart. *Systematic Entomology* 37: 686–705. <https://doi.org/10.1111/j.1365-3113.2012.00637.x>
- Zhang YL, Duan YN (2011) Review of the *Deltocephalus* group of leafhoppers (Hemiptera, Cicadellidae, Deltocephalinae) in China. *Zootaxa* 2870: 1–47. <https://doi.org/10.11646/zootaxa.2870.1.1>

Description of a new species of cynopoeciline killifish (Cyprinodontiformes, Aplocheilidae), possibly extinct, from the Atlantic Forest of south-eastern Brazil

Wilson J.E.M. Costa¹

¹ *Laboratory of Systematics and Evolution of Teleost Fishes, Institute of Biology, Federal University of Rio de Janeiro, Caixa Postal 68049, CEP 21941-971, Rio de Janeiro, Brazil*

Corresponding author: Wilson J.E.M. Costa (wcosta@acd.ufrj.br)

Academic editor: M.E. Bichuette | Received 21 February 2019 | Accepted 12 July 2019 | Published 29 July 2019

<http://zoobank.org/D786D97E-BA45-4EFC-8F83-22D14904A90A>

Citation: Costa WJEM (2019) Description of a new species of cynopoeciline killifish (Cyprinodontiformes, Aplocheilidae), possibly extinct, from the Atlantic Forest of south-eastern Brazil. ZooKeys 867: 73–85. <https://doi.org/10.3897/zookeys.867.34034>

Abstract

Specimens found between 1985 and 1988 in the Magé River Basin, south-eastern Brazil were misidentified as *L. splendens*. The recent rediscovery of other specimens in the Estrela River Basin near the type locality of *L. splendens* has clarified the species' concept, making it possible to recognise the Magé River Basin specimens as a new species. The new species is herein described as *Leptopanchax sanguineus* **sp. nov.** and is distinguished from all other cynopoecilines by a unique colour pattern in males, including red bars with sinuous margins. It was collected in a well-preserved, temporary shallow swampy area within dense moist forest, but since 1990 the species has not been found again. *Leptopanchax sanguineus* **sp. nov.** is one of three species of cynopoeciline killifishes living in lowland moist forests of the coastal plains of Rio de Janeiro State, where the greatest diversity of endemic cynopoecilines is concentrated. Each of these species has been recorded a single time in the last 30 years, a surprisingly low record attributable to intense deforestation during the last several decades resulting in small fragmented lowland moist forests of today. This study indicates that seasonal killifishes adapted to uniquely live in this kind of habitat should be regarded with special concern in studies evaluating conservation priorities.

Keywords

Biodiversity, conservation, moist tropical forest, systematics, taxonomy

Introduction

The Atlantic Forest of south-eastern Brazil encompasses one of the most species-rich biota in the world, with a high diversity of plants and animals (Myers et al. 2000). Although the greatest part of the original Atlantic Forest was extirpated in the last three centuries and consequently several endemic species became endangered or even extinct, new species are still being recognised in recent years (Tabarelli et al. 2005; Costa 2016a). Aplocheilid killifishes are represented in the Atlantic Forest by 14 genera and over 45 valid species (Costa 2009, 2014, 2016b; Costa and Amorim 2013, 2014; Costa et al. 2014), of which six genera and 18 species belong to the tribe Cynopoecilini (Costa 2008, 2016b; Ferrer et al. 2014). Like several other South American and African aplocheiloids, cynopoecilines are seasonal killifishes, which uniquely have their entire life cycle restricted to temporary pools and swamps formed during rainy seasons (Myers 1942; Costa 2002a, 2009).

The greatest species diversity of cynopoecilines is concentrated in the coastal plains of Rio de Janeiro State, south-eastern Brazil (i.e., eight valid endemic species in three genera, of which two genera are endemic), with most taxa consisting of miniature species not surpassing 25 mm standard length (SL) and exhibiting high diversification of morphological traits (Costa 2008, 2016a). Different kinds of vegetation formations sheltering distinct seasonal killifish habitats are present in this region, including temporary pools in seasonally dry forests and coastal restingas, and seasonal swamps in dense moist forests (Costa 1995, 2009, 2016a). This region also contains the greatest occurrence of cynopoeciline species threatened with extinction in South America, some of them critically endangered or presumably extinct (Costa 2002b, 2009, 2012), with most taxa poorly represented in ichthyological collections and not collected in recent years (Costa 2016a).

Among areas of endemism for seasonal killifishes in the Rio de Janeiro coastal plains is the area encompassing river basins draining the southern flank of the coastal mountain range, Serra do Mar, and flowing into the Baía de Guanabara (i.e. Guanabara Bay area in Costa 2009, 2012; hereafter GBA). Three species of seasonal killifishes have been reported to occur in the GBA: *Leptolebias marmoratus* (Ladiges, 1934), *Leptopanchax opalescens* (Myers, 1942) and *Leptopanchax splendens* (Myers, 1942). This area was formerly occupied by a dense moist forest, but presently, after over 60 years of intense deforestation, the forest is restricted to small enclaves within urban areas. All three species were primarily recorded from the Estrela River Basin, in the western portion of the GBA (Myers 1942). Whereas *L. opalescens* was found in open vegetation habitats close to the forest border, *L. marmoratus* and *L. splendens* were found only within the dense moist forest (Myers 1942; Costa et al. 1988; Costa 2009). All three species were considered extinct after extirpation of known habitats in the 1950s, but *L. marmoratus* and *L. opalescens* were rediscovered some decades after, in neighbouring basins of western GBA (Costa 2002c, 2013). Costa and Lacerda (1988) redescribed *L. splendens* on the basis of old collections deposited in museums. They also provided colour photographs of a male tentatively identified as *L. splendens* and its habitat, consisting of a well-preserved forest area in the Magé River Basin, eastern portion of the GBA. However, the recent rediscovery of a population of *L. splendens* near its type locality in the Rio Estrela ba-

sin, over 60 years after its last record, has shown that this species is probably endemic to the Estrela River Basin in the western part of the GBA (Costa et al. 2019) and is not conspecific with specimens from the Magé River Basin in the eastern part of the GBA. Specimens of both populations differ greatly in several morphological characters, including fin ray, scale and vertebra counts, extent and relative position of fins, presence of filamentous rays on unpaired fins, presence of contact organs on male pectoral fin, presence of dermosphenotic bone, and male colour pattern. The new species from the Magé River Basin, which was misidentified as *L. splendens* in the last three decades (Costa and Lacerda 1988; Costa 2002b, 2009), is herein described.

Material and methods

The description of the new species was based on specimens collected over 30 years ago and then preserved for study, deposited in the ichthyological collections of the Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MNRJ) and Museu de Zoologia, Universidade de São Paulo, São Paulo (MZUSP). Colouration characters were analysed and described based on photographs taken from a male collected in 1985 (published in Costa and Lacerda 1988: fig.1) and notes taken from direct observation in aquaria of live specimens collected in 1985 and 1987; colouration characters were also checked in photographs of live specimens born in aquaria, published in Costa (1995: fig. 113) and Seegers (2000: fig. S31853-4). Since no new photographs are available, a coloured pencil drawing based on available material and rigorously following fish proportions and colours was made to illustrate the new species. Measurements and counts follow Costa (1988). Measurements are presented as percentages of SL, except for those related to head morphology, which are expressed as percentages of head length. Fin ray counts include all elements. Osteological data were taken from cleared and stained specimens prepared following Taylor and Van Dyke's (1985) protocol; the abbreviation C&S indicates specimens prepared for osteological observation and preserved in glycerine. Terminology for frontal squamation followed Hoedeman (1958), and for the cephalic neuromast series followed Costa (2001). Comparative material is listed in Costa (2016a) and Costa et al. (2019). The map illustrating species distribution was generated using QGIS Geographic Information System, Open Source Geospatial Foundation Project.

Results

Leptopanchax sanguineus sp. nov.

<http://zoobank.org/C106BC45-F740-432D-9288-881A721BD7EF>

Figs 1, 2A, Table 1

Holotype. MNRJ 51331, male, 20.9 mm SL; Brazil: Rio de Janeiro State: Magé Municipality: temporary swamp within dense moist forest in a private reserve (Reserva



Figure 1. *Leptopanchax sanguineus* sp. nov., MNRJ 51331, holotype, male, 20.9 mm SL. Scale bar: 5 mm.

Table 1. Morphometric data of *Leptopanchax sanguineus* sp. nov.

| | Holotype | Paratypes | |
|-----------------------------|----------|-----------|------|
| | male | male | male |
| Standard length (mm) | 20.9 | 20.8 | 20.6 |
| % of standard length | | | |
| Body depth | 25.9 | 27.4 | 26.2 |
| Caudal peduncle depth | 14.9 | 16.1 | 15.6 |
| Pre-dorsal length | 61.0 | 61.8 | 59.7 |
| Pre-pelvic length | 52.4 | 53.1 | 50.6 |
| Length of dorsal-fin base | 25.7 | 25.1 | 26.4 |
| Length of anal-fin base | 31.4 | 29.0 | 31.4 |
| Caudal-fin length | 35.3 | –* | 34.7 |
| Pectoral-fin length | 22.3 | 22.5 | 21.2 |
| Pelvic-fin length | 9.1 | 9.4 | 8.9 |
| Head length | 29.6 | 29.8 | 31.1 |
| % of head length | | | |
| Head depth | 76.7 | 77.4 | 77.4 |
| Head width | 71.0 | 75.2 | 69.5 |
| Snout length | 10.2 | 11.1 | 10.9 |
| Lower jaw length | 20.9 | 21.6 | 17.3 |
| Eye diameter | 36.1 | 36.5 | 33.8 |

Particular do Patrimônio Natural Campo Escoteiro Geraldo Hugo Nunes), Magé River Basin, near the village of Citrolândia, 22°34'57"S, 43°02'08"W, altitude about 30 m above sea level (a.s.l.); M. T. C. Lacerda and K. Tanizaki, August 1987.

Paratypes. MNRJ 11413, 2 males, 20.6–20.8 mm SL; MZUSP 38443, 1 male, about 20 mm SL, 1 female, about 15 mm SL (C&S); collected with holotype.

Diagnosis. *Leptopanchax sanguineus* differs from other cynopoeilines, except *L. splendens*, by the presence of red bars on the whole flank in males (vs. absence); uniquely in *L. sanguineus*, the bars are broad, wider than the interspace width (vs. narrow, half interspace width or less) and have sinuous margins (vs. straight). *Leptopanchax sanguineus* is further distinguished from *L. splendens* by having 15 dorsal-fin rays (vs. 12–14), 6 pelvic-fin rays (vs. 5), 27 scales on the longitudinal series and 9 on the transverse series (vs. 24–25 and 7, respectively), 29 vertebrae (vs. 26–27), pelvic fin tip posteriorly reaching the anal fin in males (vs. reaching urogenital papilla), pelvic-fin bases

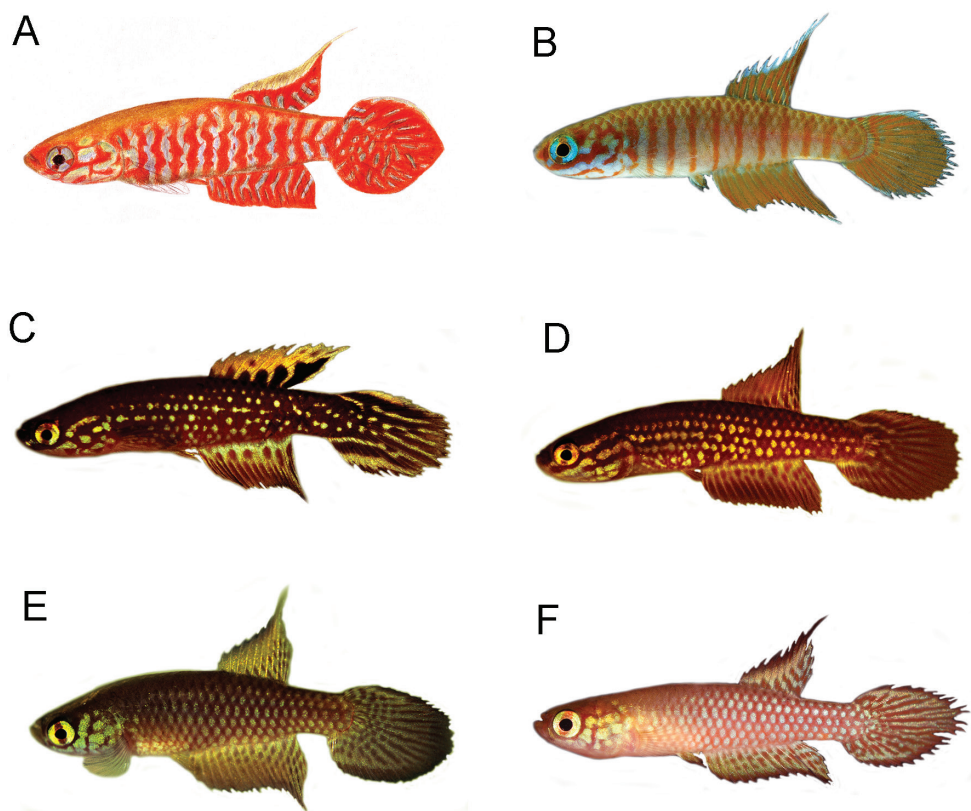


Figure 2. Male fin morphology and life colour patterns in *Leptopanchax*. **A** coloured pencil drawing illustrating *L. sanguineus* sp. nov. in life, about 20 mm SL **B** *L. splendens*, UFRJ 6902, 22.7 mm SL **C** *L. aureoguttatus*, UFRJ 6331, 22.3 mm SL **D** *L. itanhaensis*, UFRJ 6453, 20.7 mm SL **E** *L. citrinipinnis*, UFRJ 8899, 20.6 mm SL **F** *L. opalescens*, UFRJ 8986, 20.2 mm SL.

medially separated, in close proximity (vs. medially united), absence of filamentous rays on the caudal fin (vs. short filamentous rays on the posterior margin of the caudal fin in males), presence of a golden stripe on the distal margin of the dorsal fin in males (vs. white stripe), absence of contact organs on the male pectoral fin (vs. presence) and absence of the dermosphenotic bone (vs. presence). *Leptopanchax sanguineus* also differs from *L. splendens* and all other cynopoecilines by the presence of a small red spot on the posterior portion of the iris (vs. spot absent).

Description. Morphometric data appear in Table 1. Body slender, sub-cylindrical. Greatest body depth at vertical just anterior to pelvic-fin base. Dorsal and ventral profiles of head and trunk slightly convex, approximately straight on caudal peduncle. Head narrow, subtriangular in lateral view. Jaws short, teeth numerous, conical, irregularly arranged; outer teeth hypertrophied, inner teeth small and numerous. Vomerine teeth absent. Urogenital papilla cylindrical and short in males, slightly projecting body-wall outside, and pocket-shaped in females.

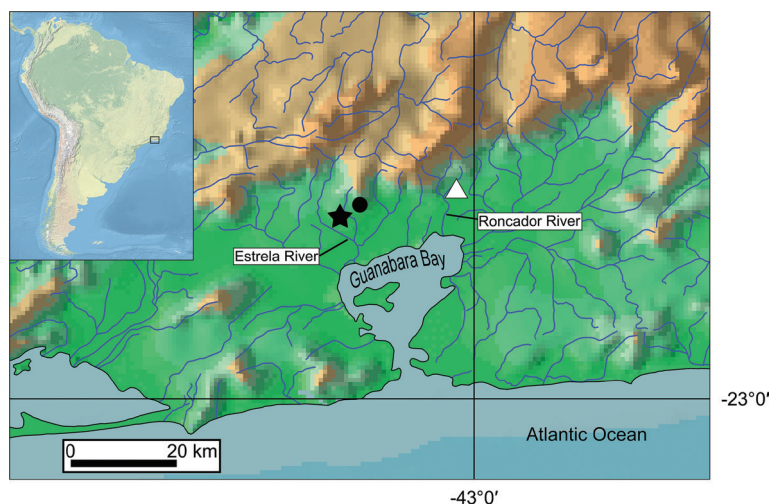


Figure 3. Geographical distribution of *L. sanguineus* sp. nov. (white triangle) and *L. splendens* (black symbols: star, type locality; dot, 2018 collection site).

Dorsal fin subtriangular, pointed and terminating in short filamentous ray in males, its tip posteriorly reaching vertical through caudal-fin base; dorsal fin slightly pointed to rounded in females. Anal fin sub-rectangular, pointed and longer posteriorly in males, rounded in females. Caudal fin elliptical to sub-lanceolate in males, slightly longer than deep, often posteriorly terminating in minute tip; caudal fin elliptical in females. Pectoral fin elliptical, posterior margin reaching between base of pelvic-fin base and anus. Pelvic fin small, tip reaching anal-fin origin; pelvic-fin bases separated, medially in close proximity. Dorsal-fin origin at vertical between base of 4th and 5th anal-fin rays. Dorsal-fin rays 15; anal-fin rays 18; caudal-fin rays 28; pectoral-fin rays 15; pelvic-fin rays 6. No contact organs on fins. Four neuromasts on caudal-fin base. Total vertebrae 29.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 20 % of caudal-fin base; no scales on dorsal, anal and pectoral-fin bases. Frontal squamation E-patterned; E-scales not overlapping medially; supraorbital scales absent. Longitudinal series of scales 27; transverse series of scales 9; scale rows around caudal peduncle 12. Three, or four, minute contact organ per scale of ventral portion of flank in males. Cephalic neuromasts: supraorbital 1 + 10; parietal 1; anterior rostral 1, posterior rostral 1; infraorbital 1 + 15; preorbital 3; otic 1, post-otic 2; supratemporal 1; median opercular 1, ventral opercular 1; pre-opercular 11, mandibular 7; lateral mandibular 4, paramandibular 1.

Colouration in life. Males. Flank light metallic blue with 12 or 13 red bars, wider than interspace, margins sinuous producing overall zigzag shape. Dorsum pale yellowish brown, venter pale blue with red bars. Head light blue to greenish blue on opercle, with red reticulation; red stripe between orbit and middle opercle. Jaws red. Ventral surface of head pale blue, scale margin red. Iris bright blue, with dark reddish-brown bar through orbit centre, and small red spot on its posterior margin. Unpaired fins red



Figure 4. Habitat of *L. sanguineus* sp. nov. in 1988.

with metallic blue to greenish-blue vertical vermiculate marks; broad golden stripe on distal margin of dorsal fin. Pelvic fin red with bright blue margin. Pectoral fin hyaline.

Females. Flank pale brownish grey. Dorsum pale brown, venter white. Head side grey, with pale golden iridescence on opercle. Iris yellow, with dark brownish grey bar through orbit centre, and small red spot on its posterior margin. Fins hyaline.

Colouration in alcohol. In both sexes, specimens with head and flank pale brown; fins hyaline in females, hyaline with pale brown pigmentation in males.

Distribution and habitat. *Leptopanchax sanguineus* is known from specimens collected between 1985 and 1987, from a single locality (Fig. 3). The collection site was situated in a dense moist forest, consisting of a well-preserved fragment of about 200,000 m², of the original forest that formerly occupied the plains surrounding the coastal mountain range. This forest is a campsite used by a group of Boy Scouts (Campo Escoteiro Geraldo Hugo Nunes). It is drained by small streams belonging to the

Magé River Basin (also known as Roncador River Basin). *Leptopanchax sanguineus* was found in temporary swamp channels situated in small depressions but not directly in contact with surrounding streams. These channels were shallow, about 20 cm deep, with clear, slightly yellow water, and no aquatic vegetation (Fig. 4) (see also Costa 1995: figs 128, 129). The water was acid, pH usually 4.8 to 6.0 after rains (Tanizaki et al. 1991), and the bottom composed of dense litter.

In 1988, some killifish breeders tried to breed *L. sanguineus* in aquaria, but offspring contained only male specimens (J. C. Ghisolfi pers. comm. 1990). Between 1989 and 2000, annual attempts were made to collect the species again. Using GPS, the exact point of the original collection was recorded and new sites were sampled, but no specimen was found. In 2001, monthly collections were made but again no specimen of *L. sanguineus* was found. The shallow temporary swamp channels disappeared, probably as a result of the lowering of the water table caused by the diversion of waters from the streams to supply an ornamental fish farm in the vicinity of the forest (Costa 2009). Sporadic attempts to find this species in other localities of the Magé River Basin, including its upper and lower courses were also unsuccessful. These attempts were directed for all areas with suitable environmental conditions for seasonal killifishes inhabiting moist forests (i.e., flooded forested plains). Since 2009, the Campo Escoteiro Geraldo Hugo Nunes became officially protected by the Brazilian Government when it was recognised as a private natural heritage reserve. However, *L. sanguineus* has not been found since 1987 and it is possibly extinct in the area.

Etymology. The name *sanguineus*, from the Latin, meaning blood-coloured, is an allusion to the predominantly red colouration in males, unique among Neotropical killifishes.

Discussion

Morphological data of *L. sanguineus*, then identified as *L. splendens*, were used in a phylogenetic analysis of the Cynopoecilini (Costa 2016a), justifying its inclusion in the genus *Leptopanchax* (Costa 2016b). *Leptopanchax sanguineus* shares with other congeners a golden distal stripe on the dorsal fin in males and vermiculate marks on the caudal fin in males (Fig. 2A). These apomorphic character states are present in all species placed by Costa (2016b) in *Leptopanchax*, including *L. citrinipinnis*, the type species of the genus (Fig. 2C–F), although the golden stripe on the dorsal fin is not always well delimited and vermiculate marks on the caudal fin may acquire different shapes. A third synapomorphy described by Costa (2016b) to diagnose *Leptopanchax* is the presence of an elliptical caudal in males, longer than deep (Fig. 2C–F), a condition that is not clearly attributed to *L. sanguineus*, in which the caudal fin is shorter and its posterior margin is slightly pointed (Fig. 2A). Among species of *Leptopanchax*, *L. sanguineus* was considered by Costa (2016b) to be closer to *L. aureoguttatus* (Cruz, 1974) and *L. itanhaensis* (Costa, 2008), which also have a horizontal red stripe between the orbit and the preopercle in males (Fig. 2A, C, D). *Leptopanchax sanguineus* differs from all other cynopoecilines by having red bars on the male flank that are wider than the interspace

and have sinuous margins, and the presence of a small red spot on the posterior portion of the iris (Fig. 2A).

The recent rediscovery of *L. splendens* poses some incongruence in its positioning. This species also has a red stripe between the orbit and the preopercle and shares with *L. sanguineus* the presence of red bars on the whole flank and blue iris in males (Fig. 2B), but it does not have the character states diagnostic for *Leptopanchax*. In *L. splendens*, there is no broad golden distal stripe on the male dorsal, but a narrow bluish white stripe, and there are no vermiculate marks on the male caudal fin, which is pale orange, with a few pale red bars on the basal region (Fig. 2B). On the other hand, the series of narrow straight red bars on the male flank in *L. splendens* (Fig. 2B) may be non-homologous to the irregular broad red bars in *L. sanguineus* (Fig. 2A), but similar to the narrow straight bars present on the anterior portion of the male flank in *Mucurilebias leitaoi* (Cruz & Peixoto, 1991), which also exhibits a narrow white stripe on the dorsal fin (Cruz and Peixoto 1991; Costa 2014). Therefore, based on available data, the phylogenetic position of *L. splendens* remains unclear, and possibly it is not closely related to *L. sanguineus*, although both species are endemic to neighbouring river basins (Fig. 3). The uncertain phylogenetic position of *L. splendens* is also reinforced by the presence of a dermosphenotic bone and male contact organs on the male pectoral fin, which are plesiomorphic conditions for cynopoecilines. These two plesiomorphic character states occur in species of the genera *Mucurilebias* Costa, 2014 and *Notholebias* Costa, 2008, but not in species of the clade comprising *Leptopanchax*, *Campellolebias* Vaz-Ferreira & Sierra, 1974, *Cynopoecilus* Regan, 1912, and *Leptolebias* Myers, 1952, in which the dermosphenotic and pectoral-fin contact organs are always absent (Costa 2016a).

Leptopanchax sanguineus, known from a single locality, is a typical moist-forest species, an ecological adaptation considered to have arisen three times independently among cynopoecilines (Costa 2016a). Presently, only six cynopoeciline species are adapted to live in this kind of habitat: *Cynopoecilus notabilis* Ferrer, Wingert & Malabarba, 2014, *Leptolebias marmoratus*, *Leptop. aureoguttatus*, *Leptop. itanhaensis*, *Leptop. sanguineus*, and *Leptop. splendens*. Records of cynopoeciline forest-dwellers are extremely rare when compared with records of cynopoeciline species found in open vegetation habitats. For example, in southern Brazil, species of *Cynopoecilus*, subgenus *Cynopoecilus*, that are always found in open vegetation habitats, are frequently sampled in field studies (Costa 2002c; Costa et al. 2016). Contrastingly, the only species of the subgenus *Poecilopanchax* Costa, 2016, *C. notabilis*, is known from a single locality (Ferrer et al. 2014). Similarly in south-eastern Brazil, where a rich species diversity of cynopoecilines is concentrated (Costa 2009), species of the genus *Notholebias*, which are found in open vegetation habitats, are known from several localities (Costa 1988) and are often recorded in the literature (e.g. Costa and Amorim 2013), whereas each of the three species endemic to the BGA adapted to life within dense forests, *Leptol. marmoratus*, *Leptop. sanguineus* and *Leptop. splendens*, were recorded a single time in the last 30 years of continuous field studies directed to killifish habitats in the region (Costa and Lacerda 1988; Costa 2002c; present study). Actually, this discrepancy in field records is associated with the intense deforestation process in the Atlantic Forest (Costa 2008). Several reserves have protected moist and semi-deciduous forests along

the coastal mountain range (Serra do Mar), but lowland moist forests, where killifishes are found, were greatly extirpated during the last two centuries and are presently represented by rare small fragments. Therefore, killifish species uniquely inhabiting lowland moist forests should be regarded with special concern in evaluation studies for conservation priorities.

Key to identification of *Leptopanchax* species

- 1 Flank in males with continuous longitudinal rows of iridescent light blue to yellowish green spots on each scale (Fig. 2E–F); no horizontal red stripe on head side (Fig. 2E–F); dorsal-fin origin in vertical between base of seventh and ninth anal-fin rays.....**2**
- Flank in males with interrupted zones of iridescent marks (Fig. 2A–D); horizontal red stripe between orbit and preopercle in males (Fig. 2A–D); dorsal-fin origin in vertical between base of second and sixth anal-fin rays.....**3**
- 2 Well-delimited dark-red stripe on distal margins of dorsal and anal fins in males (Fig. 2F).....***L. opalescens***
- Diffuse dark-reddish brown pigmentation on distal margins of dorsal and anal fins in males (Fig. 2E).....***L. citrinipinnis***
- 3 Iris bright greenish yellow in males (Fig. 2C, D); no bars on flank (Fig. 2C, D).....**4**
- Iris bright blue in males (Fig. 2A, B); red bars on flank in males (Fig. 2A, B).....**5**
- 4 Two dark-red stripes along entire dorsal and ventral submarginal parts of caudal fin in males (Fig. 2C); male caudal-fin stripes branching posteriorly (Fig. 2C); dark red to dark brown short transverse bars on basal portion of dorsal fin in males (Fig. 2C).....***L. aureoguttatus***
- Two dark-red stripes on anterior portion of dorsal and ventral submarginal parts of caudal fin in males (Fig. 2D); male caudal-fin stripes not branching posteriorly (Fig. 2D); small brownish orange spots on basal portion of dorsal fin in males (Fig. 2D).....***L. itanhaensis***
- 5 Flank in males with narrow red bars, narrower than interspace width, with straight margins (Fig. 2B); distal margin of dorsal fin with narrow bluish white stripe in males (Fig. 2B); 12–14 dorsal-fin rays; 5 pelvic-fin rays; 24–25 scales on longitudinal series; 7 scales on transverse series; pelvic fin tip posteriorly reaching urogenital papilla; pelvic-fin bases medially united; with filamentous rays on distal margin of dorsal fin and posterior margin of caudal fin in males (Fig. 2B).....***L. splendens***
- Flank in males with broad red bars, wider than interspace width, with sinuous margins (Fig. 2A); distal margin of dorsal fin with broad golden stripe in males (Fig. 2A); 15 dorsal-fin rays; 6 pelvic-fin rays; 27 scales on longitudinal series; 9 scales on transverse series; pelvic fin tip posteriorly reaching anal fin in males; pelvic-fin bases medially separated; absence of filamentous rays on fins (Fig. 2A).....***L. sanguineus* sp. nov.**

Acknowledgements

Thanks are due to M. Britto and C. Moreira for hospitality and assistance during visits to their institution, to J. L. Mattos for photographing *L. splendens*, P. F. Amorim for assistance in the laboratory, and to M. Moutinho for supporting several field studies in the Campo Escoteiro Geraldo Hugo Nunes. A former version of the manuscript befitted from the criticisms provided by P. Buckup and J. Maldonado. This study was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Ministério de Ciência e Tecnologia, grant number 307349/2015-2) and FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro, grant number E-26/202.954/2017).

References

- Costa WJEM (1988) Sistemática e distribuição do complexo de espécies *Cynolebias minimus* (Cyprinodontiformes, Rivulidae), com a descrição de duas espécies novas. *Revista Brasileira de Zoologia* 5: 557–570. <https://doi.org/10.1590/S0101-81751988000400004>
- Costa WJEM (1995) Pearl killifishes, the Cynolebiatinae: systematics and biogeography of the neotropical annual fish subfamily (Cyprinodontiformes: Rivulidae). TFH, Neptune City, 128 pp. <https://doi.org/10.2307/1446910>
- Costa WJEM (2001) The neotropical annual fish genus *Cynolebias* (Cyprinodontiformes: Rivulidae): phylogenetic relationships, taxonomic revision and biogeography. *Ichthyological Exploration of Freshwaters* 12: 333–383.
- Costa WJEM (2002a) The seasonal fish genus *Nematolebias* (Cyprinodontiformes: Rivulidae: Cynolebiatinae): taxonomic revision with description of a new species. *Ichthyological Exploration of Freshwaters* 13: 41–52.
- Costa WJEM (2002b) Peixes anuais brasileiros: diversidade e conservação. Editora da UFPR, Curitiba, 238 pp.
- Costa WJEM (2002c) *Leptolebias marmoratus* (Cyprinodontiformes: Rivulidae: Cynolebiatinae): rediscovery and redescription of a rare, miniaturized forest dwelling seasonal fish from southeastern Brazil. *Ichthyological Exploration of Freshwaters* 13: 379–384.
- Costa WJEM (2002c) The annual fish genus *Cynopoecilus* (Cyprinodontiformes: Rivulidae): taxonomic revision, with descriptions of four new species. *Ichthyological Exploration of Freshwaters* 13: 11–24.
- Costa WJEM (2008) Monophyly and taxonomy of the Neotropical seasonal killifish genus *Leptolebias* (Teleostei: Aplocheiloidi: Rivulidae), with the description of a new genus. *Zoological Journal of the Linnean Society* 153: 147–160. <https://doi.org/10.1111/j.1096-3642.2008.00380.x>
- Costa WJEM (2009) Peixes aplocheilóideos da Mata Atlântica brasileira: história, diversidade e conservação/ Aplocheiloid fishes of the Brazilian Atlantic Forest: history, diversity and conservation. Museu Nacional UFRJ, Rio de Janeiro, 172 pp.
- Costa WJEM (2012) Delimiting priorities while biodiversity is lost: Rio's seasonal killifishes on the edge of survival. *Biodiversity and Conservation* 21: 2443–2452. <https://doi.org/10.1007/s10531-012-0301-7>

- Costa WJEM (2013) *Leptolebias opalescens*, a supposedly extinct seasonal killifish from the Atlantic Forest of south-eastern Brazil, rediscovered 31 years after its last record (Cyprinodontiformes: Rivulidae). *Ichthyological Exploration of Freshwaters* 23: 357–358.
- Costa WJEM (2014) A new genus of miniature cynolebiasine from the Atlantic Forest and alternative biogeographical explanations for seasonal killifish distribution patterns in South America (Cyprinodontiformes: Rivulidae). *Vertebrate Zoology* 64: 23–33.
- Costa WJEM (2016a) Inferring evolution of habitat usage and body size in endangered, seasonal cynopoeiline killifishes from the South American Atlantic Forest through an integrative approach (Cyprinodontiformes: Rivulidae). *PLoS ONE* 11(7): e0159315. <https://doi.org/10.1371/journal.pone.0159315>
- Costa WJEM (2016b) Comparative morphology and classification of South American cynopoeiline killifishes (Cyprinodontiformes: Aplocheilidae), with notes on family-group names used for aplocheiloids. *Vertebrate Zoology* 66: 125–140. http://www.senckenberg.de/files/content/forschung/publikationen/vertebratezoology/vz66-2/03_verttebrate_zoology_66-2_costa_125-140.pdf
- Costa WJEM, Amorim PF (2013) Delimitation of cryptic species of *Notholebias*, a genus of seasonal miniature killifishes threatened with extinction from the Atlantic Forest of south-eastern Brazil (Cyprinodontiformes: Rivulidae). *Ichthyological Exploration of Freshwaters* 24: 63–72.
- Costa WJEM, Amorim PF (2014) Integrative taxonomy and conservation of seasonal killifishes, *Xenurolebias* (Teleostei: Rivulidae), and the Brazilian Atlantic Forest. *Systematics and Biodiversity* 12: 350–365. <https://doi.org/10.1080/14772000.2014.918062>
- Costa WJEM, Amorim PF, Aranha GN (2014) Species limits and DNA barcodes in *Nematolebias*, a genus of seasonal killifishes threatened with extinction from the Atlantic Forest of south-eastern Brazil, with description of a new species (Teleostei: Rivulidae). *Ichthyological Exploration of Freshwaters* 24: 225–236.
- Costa WJEM, Amorim PF, Mattos JLO (2016) A new species of inseminating seasonal killifish of the *Cynopoeilus melanotaenia* complex from southern Brazil (Cyprinodontiformes: Rivulidae). *Biodiversity Data Journal* 4: e6888. <https://doi.org/10.3897/BDJ.4.e6888>
- Costa WJEM, Lacerda MTC (1988) Identité et redescription de *Cynolebias sandrii* et de *Cynolebias fluminensis* (Cyprinodontiformes, Rivulidae). *Revue Française de Aquariologie et Herpetologie* 14: 127–132.
- Costa WJEM, Lacerda MTC, Tanizaki K (1988) Description d'une nouvelle espèce de *Cynolebias* des plaines côtières du Brésil sud-oriental (Cyprinodontiformes, Rivulidae). *Revue Française d'Aquariologie et Herpetologie* 15: 21–24.
- Costa WJEM, Mattos JLO, Amorim PF (2019) Rediscovery of *Leptopanchax splendens* (Cyprinodontiformes: Aplocheilidae), a seasonal killifish from the Atlantic Forest of south-eastern Brazil that was recently considered extinct. *Journal of Fish Biology*. <https://doi.org/10.1111/jfb.13898>
- Cruz CAG, Peixoto OL (1991) Descrição de uma nova espécie de peixe anual do Estado da Bahia (Cyprinodontiformes, Rivulidae). *Revista Brasileira de Zoologia* 7: 637–641. <https://doi.org/10.1590/S0101-81751990000400017>

- Ferrer J, Wingert JM, Malabarba LR (2014) Description of a new species and phylogenetic analysis of the subtribe Cynopoecilina, including continuous characters without discretization (Cyprinodontiformes: Rivulidae). *Zoological Journal of the Linnean Society* 172: 846–866. <https://doi.org/10.1111/zoj.12190>
- Hoedeman JJ (1958) The frontal scalation pattern in some groups of toothcarps (Pisces, Cyprinodontiformes). *Bulletin of Aquatic Biology* 1: 23–28.
- Myers GS (1942) Studies on South American fresh-water fishes I. *Stanford Ichthyological Bulletin* 2: 89–114.
- Myers N, Mittermeir RA, Mittermeir CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. <https://doi.org/10.1038/35002501>
- Seegers L (2000) Killifishes of the world: New World killis. ACS GmbH, Mörfelden-Walldorf, 224 pp.
- Tabarelli M, Pinto LP, Silva JMC, Hirota M, Bedê L (2005) Challenges and opportunities for biodiversity conservation in the Brazilian Atlantic Forest. *Conservation Biology* 19: 695–700. <https://doi.org/10.1111/j.1523-1739.2005.00694.x>
- Tanizaki K, Torres JPM, Barros AAM (1991) Limnology of a Temporary Swamp in a Tropical Forest. In: *Anais do I Simpósio Internacional de Estudos Ambientais em Florestas Tropicais Úmidas*, Manaus, 1990. Sociedade Brasileira para a Valorização do Meio Ambiente, Rio de Janeiro, 13–18.
- Taylor WR, Van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybio* 9: 107–109. <http://sfi.mnhn.fr/cybio/numeros/1985/92/01-Taylor%5b92%5d107-119.pdf>

An online interactive identification key to common pest species of Aspidiotini (Hemiptera, Coccoomorpha, Diaspididae), version 1.0

Scott A. Schneider^{1,2,3}, Michael A. Fizdale⁴, Benjamin B. Normark^{2,3}

1 *Systematic Entomology Laboratory, USDA, Agricultural Research Service, Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, Maryland, USA* **2** *Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, Massachusetts, USA* **3** *Department of Biology, University of Massachusetts, Amherst, Massachusetts, USA* **4** *School of Natural Sciences, Hampshire College, Amherst, Massachusetts, USA*

Corresponding author: Scott A. Schneider (scott.schneider3@usda.gov)

Academic editor: R. Blackman | Received 27 March 2019 | Accepted 19 July 2019 | Published 30 July 2019

<http://zoobank.org/D826AEF6-55CD-45CB-AFF7-A761448FA99F>

Citation: Schneider SA, Fizdale MA, Normark BB (2019) An online interactive identification key to common pest species of Aspidiotini (Hemiptera, Coccoomorpha, Diaspididae), version 1.0. ZooKeys 867: 87–96. <https://doi.org/10.3897/zookeys.867.34937>

Abstract

Aspidiotini is a species-rich tribe of armored scale insects that includes several polyphagous and specialist pests that are commonly encountered at ports-of-entry to the United States and many other countries. This article describes a newly available online interactive tool that can be used to identify 155 species of Aspidiotini that are recognized as minor to major pests or that are potentially emergent pests. This article lists the species and features included with a description of the development and structure of the key. The interactive key is free to access at https://idtools.org/id/scales/aspidiotini/about_index.php.

Keywords

Agriculture, armored scale insects, ITS, Lucid, plant quarantine

Introduction

Armored scales (Hemiptera: Diaspididae) are the largest family of scale insects, accounting for approximately one-third of species diversity in the infraorder Coccoomorpha. They are among the most invasive insects in the United States (Miller et al. 2005)

and are responsible for considerable agricultural damage, estimated to cost roughly \$1–2 billion USD in damage and management expenses each year (Miller and Davidson 2005). They are frequently encountered at ports-of-entry to the U.S. and other countries but are difficult to identify because their morphology is highly derived, and specimens require labor-intensive, skilled preparation as slide mounts. Few systematists are trained in their preparation and identification (Hardy 2013).

The tribe Aspidiotini is one of the larger subdivisions within this family, comprising approximately one-quarter of all described armored scales, ca. 720 species of 2,600 in total (~ 28%) (García Morales et al. 2016). The tribe includes many agricultural pests. Miller and Davidson (1990) compiled a list of global armored scale pests, including 199 species, of which 81 belong to Aspidiotini (41%). They subsequently published a list of pests in the United States (Miller and Davidson 2005), which included 43 species from Aspidiotini of 110 in total (39%). Similarly, Beardsley Jr and Gonzalez (1975) listed what they considered the principal armored scale pests of the world, 14 of 43 are from Aspidiotini (35%). Compared to other armored scale tribes, a disproportionate number of species from this group are pestiferous. From Miller and Davidson's (1990) list, pests belonging to Diaspidini account for 23% (46) of those listed, Lepidosaphidini account for 18% (35), and Parlatoriini for 7% (13). Tribal classifications follow the current, phylogenetically informed framework proposed by Normark et al. (2019).

The classification of aspidiotine genera does not conform to current estimates of phylogenetic relationships and presents a complex revisionary challenge. Recent molecular phylogenetic estimates have revealed paraphyly of several genera (Schneider et al. 2018), including pest-rich genera, where taxonomic changes could hinder identification efforts. Interactive identification keys are useful tools in this regard because they operate independently of hierarchical classification and are thus robust to changes in combination. Online keys offer the advantage of adaptability to reflect nomenclatural changes and reduce time invested in producing and publishing updated dichotomous keys, which are usually organized around genera and are limited in regional scope. This article describes a newly available online interactive key to 155 commonly encountered species from Aspidiotini. Many of the species included have broad geographical distributions and the tool is intended to be applicable toward an international audience. However, the list is based largely upon quarantine interceptions from the United States; thus, the species representation has some inherent biases. The key is designed for the identification of the adult female stage, which has been cleared of body contents and slide-mounted (see Miller and Davidson 2005; Wilkey 1990 for slide mounting protocols).

The suite of traits that define tribe Aspidiotini includes early paternal genome elimination, one-barred macroduct filaments, one pair of setae on the antennae of adult females, and a lack of pores near the anterior spiracles (see Fig. 1) (Andersen et al. 2010; Schneider et al. 2018). Users should note that the key does not include all species of Aspidiotini and could thus result in false positive identifications if the specimen under consideration is not included. For this reason, it is important to compare the specimen against voucher collections, descriptions, and illustrations. If any question remains regarding the identification of a specimen, a specialist should be consulted. The first version of the key comprises 22% of species in tribe Aspidiotini.

Project description

The conception of this key is the product of a workshop held at the University of Massachusetts Amherst (UMass) in 2014, organized and hosted by BBN and SAS. Expert identifiers from the United States Department of Agriculture (D.R. Miller), California Department of Food and Agriculture (G.W. Watson, J.W. Dooley), Florida Division of Plant Industry (I.C. Stocks), and Auburn University (N.B. Hardy) were consulted in compiling a list of species and characters for inclusion in the key.

Many of the species (entities) included are commonly considered to be pests (Beardsley Jr and Gonzalez 1975; Miller and Davidson 1990; 2005; Williams and Watson 1988), while several others are recognized as potential emerging pests based on quarantine interception records and input from expert identifiers. The key provides a link for each species to their respective records in ScaleNet (<http://scalenet.info/>), where the user can find additional information on nomenclatural history, recorded hosts and natural enemies, geographic distribution, references, and additional notes (García Morales et al. 2016). The user is encouraged to regularly check ScaleNet records for up-to-date classification information.

The list of characters (features) in this key was composed during the UMass workshop. Some features were adapted from a draft key to the armored scale genera of Australia written by N.B. Hardy. The current version includes 82 features and 195 character states (Table 1). Character states were coded using a combination of published written species descriptions, published illustrations, and slide-mounted voucher material from the entomology collection at UMass and the National Museum of Natural History's (NMNH) collection in Beltsville, MD. The task of character coding was divided between SAS and MHF; each was responsible for coding a set of characters for all species to establish consistency in the coding scheme. Illustrations and images of character states are included in the key to aid the user in interpreting alternative options. Illustrations of the general morphology of aspidiotines, labeled with descriptive terminology, are provided (Figs 1, 2) in lieu of a glossary.

Table 1. Features used to separate species in version 1.0 of the interactive key. Lobes L1–L4 correspond with the pygidial lobes from the median pair through the fourth pair. Interlobular spaces are referred to as S0 (between median lobes), S1 (between median and second lobes), S2 (between second and third lobes), and S3 (between third and fourth lobes).

| Location | Features |
|---------------------|--|
| General features | Pores near anterior spiracles (presence); body shape (overall); prosoma (constrictions, sclerotized protuberance, processes); lobes L2–L4 (presence); plates / gland-spines (presence); paraphyses (presence, longest length, relative lengths); perivulvar pores (presence); anus (shape, size, relative distance to apex, relative position of vulva); pygidium shape (angle, configuration of apex); dorso-medial pygidial sclerotization (pattern, distinct unsclerotized strip arising from S2, dorsal sclerotized bars near anterior margin of pygidium) |
| Abdominal segment 8 | Lobe L1 (fusion, distance between lobes, orientation, shape at apex, # median notches, # lateral notches); basal sclerites (presence, length, shape); plates S0 (presence); paraphyses S0 (presence, shape); macroduct between L1 (presence, length); dorsal seta of L1 (relative length) |
| Abdominal segment 7 | Lobe L2 (presence, shape at apex, # median notches, # lateral notches); plates S1 (number, shape, fringing, relative length); paraphyses S1 (presence, shape, relative length, relative width); dorsal ducts S1 (pore furrow presence and width, size of orifices, sclerotization of rim) |
| Abdominal segment 6 | Lobe L3 (presence, shape at apex, # median notches, # lateral notches); plates S2 (number, shape, fringing, relative length); paraphyses S2 (presence, shape, relative length, relative width); dorsal ducts S2 (pore furrow presence and width, size of orifices, sclerotization of rim) |
| Abdominal segment 5 | Lobe L4 (presence); plates / gland-spines S3 (number, shape); macroducts S3 (pore furrow presence, marginal ducts presence, size and orientation of marginal ducts, submarginal ducts presence, submedial ducts presence); paraphyses S3 (presence) |

Aspidiotine General Morphology

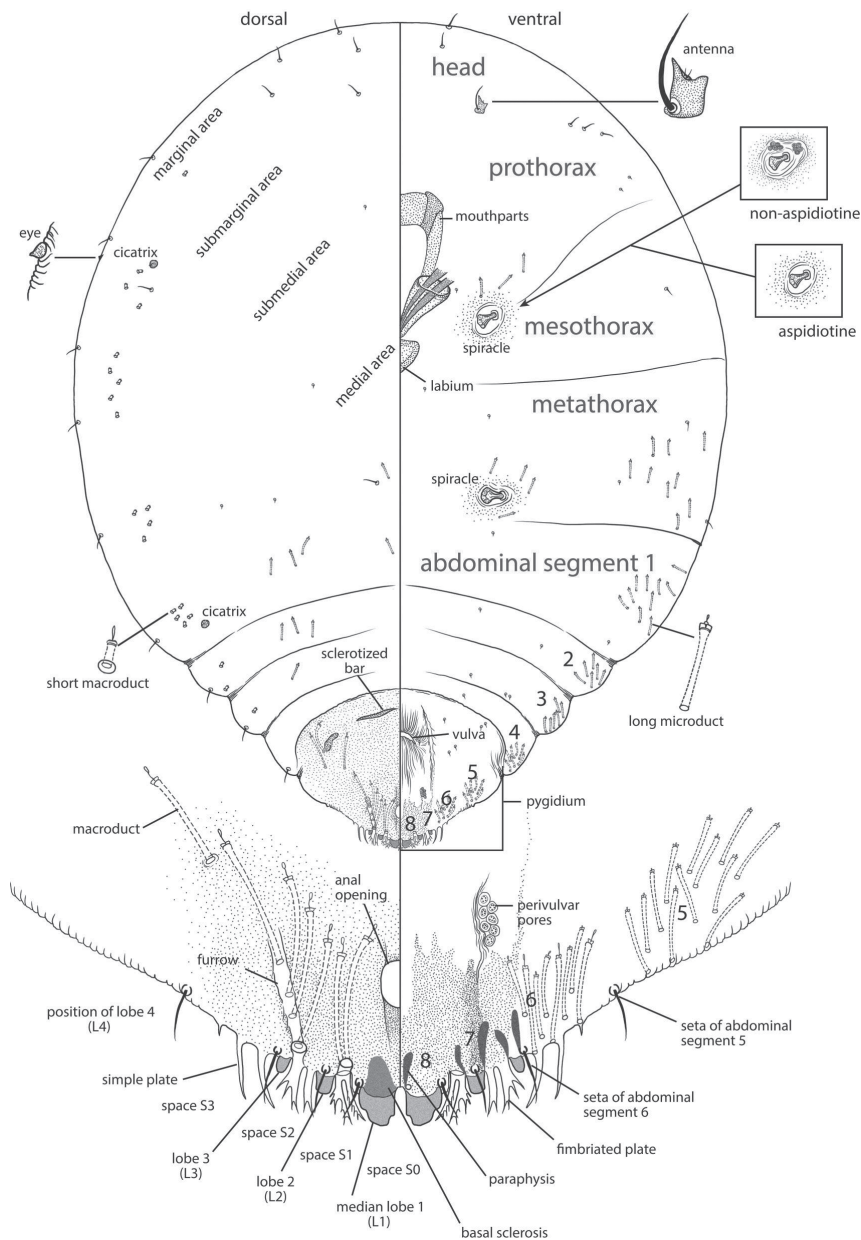


Figure 1. Aspidiotine general morphology. This diagram exemplifies a composite aspidiotine species, illustrating major anatomical features, body segmentation, and traits that a user would encounter in the key. The illustration orients users to the appearance of slide-mounted specimens and terminology used to describe their features. The illustration is based on a similar image presented by Miller and Davidson (2005), their Figure 3. Illustration by Taina Litwak.

Aspidiotine Pygidial Morphology

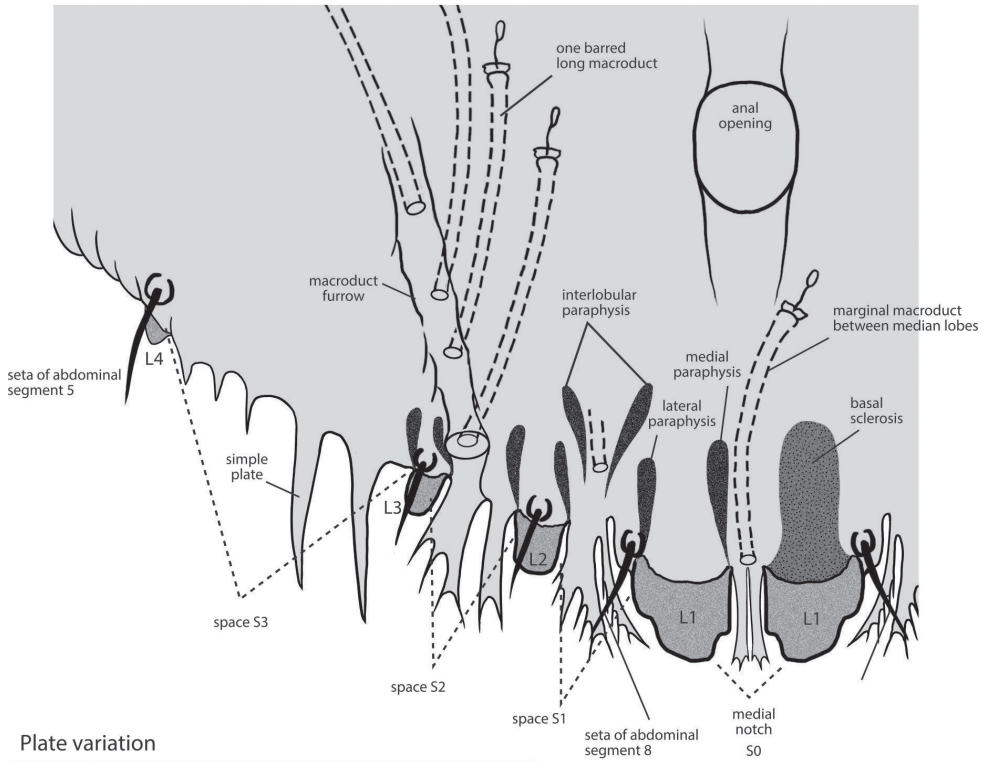
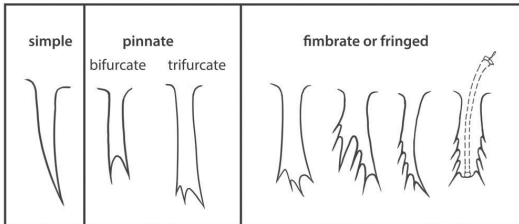


Plate variation



Lobe variation

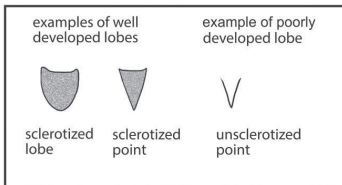


Figure 2. Aspidiotine pygidial morphology. This diagram provides an enlarged view of the general pygidial morphology of aspidiotines. This serves as another guide to the appearance of anatomical features and their terminology. Illustration by Taina Litwak.

List of terminal taxa included in the current version

- Acutaspis*: *A. agavis* (Townsend & Cockerell), *A. albopicta* (Cockerell), *A. aliena* (Newstead), *A. morrisonorum* Kosztarab, *A. paulista* Hempel, *A. perseae* (Comstock), *A. reniformis* (Cockerell), *A. scutiformis* (Cockerell), *A. umbonifera* (Newstead).
- Aonidiella*: *A. aurantii* (Maskell), *A. citrina* (Coquillett), *A. comperei* McKenzie, *A. gracilis* (Balachowsky), *A. inornata* McKenzie, *A. orientalis* (Newstead), *A. replicata* (Lindinger), *A. taxus* Leonardi, *A. tsugae* Takagi.
- Aspidaspis*: *A. arctostaphyli* (Cockerell & Robbins), *A. densiflorae* (Bremner), *A. florenciae* (Coleman).
- Aspidiella*: *A. hartii* (Cockerell), *A. sacchari* (Cockerell), *A. zingiberi* (Takahashi).
- Aspidiotus*: *A. atomarius* (Hall), *A. cryptomeriae* Kuwana, *A. destructor* Signoret, *A. elaeidis* Marchal, *A. excisus* Green, *A. fularum* Balachowsky, *A. hedericola* Leonardi, *A. kellyi* Brain, *A. nerii* (Bouche), *A. pothos* Takagi, *A. rigidus* Reyne.
- Chentraspis unilobis* (Maskell).
- Chortinaspis*: *C. subchortina* (Laing), *C. subterranea* (Lindinger).
- Chrysomphalus*: *C. ansei* (Green), *C. aonidum* (Linnaeus), *C. bifasciculatus* Ferris, *C. dictyospermi* (Morgan), *C. diversicolor* (Green), *C. fodiens* (Maskell), *C. nepenthivorus* Smith-Pardo, Evans & Dooley, *C. pinnulifer* (Maskell), *C. propisimus* Banks.
- Clavaspidiotus apicalis* Takagi.
- Clavaspis*: *C. coursetiae* (Marlatt), *C. covilleae* (Ferris), *C. disclusa* Ferris, *C. herculeana* (Cockerell & Hadden), *C. subsimilis* (Cockerell), *C. texana* Ferris, *C. ulmi* (Johnson).
- Comstockaspis perniciosus* (Comstock).
- Davidsonaspis aguacatae* (Evans, Watson & Miller).
- Diapspidiotus*: *D. aesculi* (Johnson), *D. africanus* (Marlatt), *D. alni* (Marchal), *D. ancylus* (Putnam) [bark and leaf forms], *D. armenicus* (Borchsenius), *D. caucasicus* (Borchsenius), *D. coniferarum* (Cockerell), *D. degeneratus* (Leonardi), *D. elaeagni* (Borchsenius), *D. forbesi* (Johnson), *D. fraxini* (McKenzie), *D. gigas* (Ferris), *D. hunteri* (Newell), *D. juglansregiae* (Comstock), *D. leguminosum* (Archangelskaya), *D. lenticularis* (Lindinger), *D. liquidambaris* (Kotinsky), *D. maleti* (Vayssi re), *D. marani* (Zahradnik), *D. mcombi* McKenzie, *D. osborni* (Cockerell), *D. ostreaeformis* (Curtis), *D. prunorum* (Laing), *D. pyri* (Lichtenstein), *D. shastae* (Coleman), *D. slavonicus* (Green), *D. sulci* (Balachowsky), *D. transcaspensis* (Marlatt), *D. turanicus* (Borchsenius), *D. uvae* (Comstock), *D. wuenni* (Lindinger), *D. zonatus* (Frauenfeld).
- Dynaspidiotus*: *D. abieticola* (Koroneos), *D. abietis* (Schrank), *D. apachea* (Ferris), *D. britannicus* (Newstead), *D. californicus* (Comstock), *D. ephedrarum* (Lindinger), *D. rhodesiensis* (Hall), *D. tsugae* (Marlatt).
- Hemiberlesia*: *H. andradae* Okusu & Normark, *H. candidula* (Cockerell), *H. cyanophylli* (Signoret), *H. diffinis* (Newstead), *H. flabellata* Ferris, *H. ignobilis* Ferris, *H. lataniae* (Signoret), *H. mendax* McKenzie, *H. musae* Takagi & Yamamoto, *H. neodiffinis* Miller & Davidson, *H. ocellata* Takagi & Yamamoto, *H. oxycoccus* (Woglum), *H. palmae* (Cockerell), *H. pitysophila* Takagi, *H. popularum* (Marlatt), *H. rapax* (Comstock).

Lindingaspis: *L. ferrisi* McKenzie, *L. floridana* Ferris, *L. musae* (Laing), *L. picea* (Male-notti), *L. rossi* (Maskell), *L. williamsi* Balachowsky.

Melanaspis: *M. bromiliae* (Leonardi), *M. delicata* Ferris, *M. enceliae* (Ferris), *M. glom-erata* (Green), *M. inopinata* (Leonardi), *M. leivasi* (Costa Lima), *M. nigropunc-tata* (Cockerell), *M. obscura* (Comstock), *M. smilacis* (Comstock), *M. tenebricosa* (Comstock).

Morganella: *M. conspicua* (Brain), *M. longispina* (Morgan).

Mycetaspis: *M. apicata* (Newstead), *M. defectopalus* Ferris, *M. personata* (Comstock).

Neoselenaspis *silvaticus* (Lindinger).

Oceanaspidiotus spinosus (Comstock).

Octaspidiotus: *O. australiensis* (Kuwana), *O. multipori* (Takahashi), *O. stauntoniae* (Takahashi), *O. subrubescens* (Maskell), *O. tamarindi* (Green).

Pseudischinaspis bowreyi (Cockerell).

Rhizaspidiotus: *R. canariensis* (Lindinger), *R. dearnessi* (Cockerell), *R. donacis* (Leonardi).

Saharaspis ceardi (Balachowsky).

Selenaspis: *S. albus* McKenzie, *S. articulatus* (Morgan), *S. ferox* Lindinger, *S. kamerunicus* Lindinger, *S. spinosus* Laing.

Targionia: *T. arthrophyti* (Archangelskaya), *T. parayuccarum* Munting, *T. vitis* (Signoret).

Varicaspis fiorineides (Newstead).

Technical specifications

Web location: https://idtools.org/id/scales/aspidiotini/about_index.php

Platform: a website

Web Server: CentOS

Programming language: PHP 5 and MySQL

Application version: 1.0

Data base: MySQL

Data: 1.0

Language: English

License for use of the key: Attribution-Non-commercial

Use of the primary data: available upon request.

The key was built in Lucid Builder 3.5 (<http://lucidcentral.org>, Queensland, Australia). It is hosted online on the Identification Technology Program (ITP) webserver (<http://idtools.org/>) and deployed using a JavaScript Player (Schneider et al. 2019). ITP is part of the USDA Animal and Plant Health Inspection Service Plant Protection and Quarantine division (APHIS PPQ). The key is linked to additional scale insect identification resources available on this platform at <http://idtools.org/id/scales/index.php> and described in Miller et al. (2014).

The first feature encountered in the key, “pores near anterior spiracles,” will help confirm if a species is included within tribe Aspidiotini. This tool is only suitable for

identifying specimens that lack pores near the anterior spiracles. An alternative key for the identification of armored scale genera, developed by J. Dooley and R. Dones (2008), is available at http://keys.lucidcentral.org/keys/v3/Dones_Lourdes/homepage.htm.

Pygidial segmentation is described and depicted such that each lobe is associated with the interlobular space immediately posterior to it. The pygidial lobes are actually located near the midpoint of each abdominal segment, and each segment encompasses portions of the adjacent anterior and posterior interlobular spaces. This key employs an alternative representation purely for organizational purposes; each abdominal segment is associated with a single lobe and a single interlobular space to avoid confusion. The illustrations of pygidial segmentation (Fig. 3) refer to the region considered for each feature and serve as a guide for the user; note that they are an imperfect depiction of abdominal segmentation as it is usually recognized.

Conclusions

The interactive key to species of Aspidiotini is useful for identifying major and minor pests as well as several potential pests from this tribe. This key facilitates species-level identification for a challenging group where specialized training and access to uncommon reference materials is usually required. The intent is to provide a user-friendly, accessible tool that simplifies the task of identification by minimizing the need to consult multiple dichotomous keys, which are often limited in zoogeographical or taxonomic scope. The digital format easily allows for updates to the classification, list of features, and species representation in future versions. Species are linked to a continuously updated, comprehensive database of scale insect taxonomic and biological information,

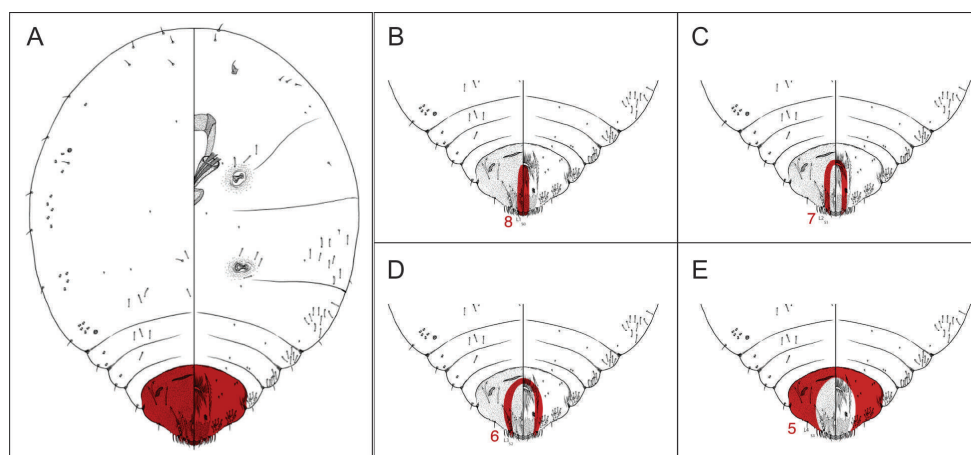


Figure 3. Abdominal segmentation. This diagram shows pygidial segmentation as it is defined for the purposes of this key. The panels highlight (A) the pygidium (B) abdominal segment 8 (C) abdominal segment 7 (D) abdominal segment 6 and (E) abdominal segment 5. Illustrations by Taina Litwak.

ScaleNet. The targeted audience includes an international group of scientists, identifiers at ports-of-entry and government agencies from the local to national level. Both the key and this article are freely available to access.

Acknowledgements

We thank the following contributors and attendees from the 2014 workshop at UMass Amherst for their time and expertise, without which this resource would not exist: Douglass R. Miller, Gillian W. Watson, John W. Dooley, Ian C. Stocks, Nate B. Hardy, Hedda Monaghan, and Akiko Okusu. The workshop and key development were supported by an NSF grant awarded to BBN (DEB-1258001). SAS was supported in part by a NIFA Hatch Fund (2013-1000785), also awarded to BBN. We thank Amanda Redford (USDA APHIS PPQ) for assistance with hosting the key on the ITP platform, <http://idtools.org>. Taina Litwak (USDA ARS SEL) produced the line art presented in this article and in the interactive key. The authors have declared that no competing interests exist. The authors have no support to report. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture; USDA is an equal opportunity provider and employer.

References

- Andersen JC, Wu J, Gruwell ME, Gwiazdowski R, Santana SE, Feliciano NM, Morse GE, Nor-mark BB (2010) A phylogenetic analysis of armored scale insects (Hemiptera: Diaspididae), based upon nuclear, mitochondrial, and endosymbiont gene sequences. *Molecular Phylogenetics and Evolution* 57: 992–1003. <https://doi.org/10.1016/j.ympev.2010.05.002>
- Beardsley Jr JW, Gonzalez RH (1975) The biology and ecology of armored scales. *Annual Review of Entomology* 20: 47–73 <https://doi.org/10.1146/annurev.en.20.010175.000403>
- Dooley J, Dones R (2008) Armored scales of the world: Compendium and key to the genera of the Diaspididae (Sternorrhyncha, Hemiptera). http://keys.lucidcentral.org/keys/v3/Dones_Lourdes/homepage.htm [accessed March 27, 2019]
- García Morales M, Denno BD, Miller DR, Miller GL, Ben-Dov Y, Hardy NB (2016) ScaleNet: A Literature-based model of scale insect biology and systematics. *Database* 2016: bav118. <https://doi.org/10.1093/database/bav118>
- Hardy NB (2013) The status and future of scale insect (Coccoidea) systematics. *Systematic Entomology* 38: 453–458. <https://doi.org/10.1111/syen.12022>
- Miller DR, Davidson JA (1990) A list of the armored scale insect pests. In: Rosen D (Ed.) *Armoured scale insects: their biology, natural enemies and control*. Elsevier, Amsterdam, 299–306.
- Miller DR, Davidson JA (2005) *Armored scale insect pests of trees and shrubs* (Hemiptera: Diaspididae). Cornell University Press, Ithaca, NY, 442 pp.

- Miller DR, Miller GL, Hodges GS, Davidson JA (2005) Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on US agriculture. *Proceedings of the Entomological Society of Washington* 107: 123–158
- Rung A, Miller D, Parikh G (2014) Scale Insects, edition 2, a tool for the identification of potential pest scales at U.S.A. ports-of-entry (Hemiptera, Sternorrhyncha, Coccoidea). *ZooKeys* 431: 61–78. <https://doi.org/10.3897/zookeys.431.7474>
- Normark BB, Okusu A, Morse GE, Peterson DA, Itioka T, Schneider SA (2019) Phylogeny and classification of armored scale insects (Hemiptera: Coccomorpha: Diaspididae). *Zootaxa* 4616: 1–98. <https://doi.org/10.11646/zootaxa.4616.1.1>
- Schneider SA, Fizdale M, Normark BB (2019) Key to commonly intercepted Aspidiotini (Hemiptera: Diaspididae). https://idtools.org/id/scales/aspidiotini/about_index.php [accessed March 27.2019]
- Schneider SA, Okusu A, Normark BB (2018) Molecular phylogenetics of Aspidiotini armored scale insects (Hemiptera: Diaspididae) reveals rampant paraphyly, curious species radiations, and multiple origins of association with *Melissotarsus* ants (Hymenoptera: Formicidae). *Molecular Phylogenetics and Evolution* 129: 291–303. <https://doi.org/10.1016/j.ympev.2018.09.003>
- Wilkey R (1990) Collection, preservation and microslide mounting. In: Rosen D (Ed.) *Armored Scale Insects – Their Biology, Natural Enemies and Control*. Elsevier, Amsterdam, 345–349.
- Williams DJ, Watson GW (1988) *The Scale Insects of the Tropical South Pacific Region. Part 1 The Armoured Scale Insects (Diaspididae)*. CAB International, Wallingford, 290 pp.

Notes on braconid wasps (Hymenoptera, Braconidae) parasitising on *Agrilus mali* Matsumura (Coleoptera, Buprestidae) in China

Liang Ming Cao^{1*}, Yan Long Zhang^{1*}, Cornelis van Achterberg²,
Zhi Yong Wang³, Xiao Yi Wang¹, Wen Xia Zhao¹, Zhong Qi Yang¹

1 The Key Laboratory of Forest Protection, State Forestry and Grassland Administration of China, Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China

2 Institute of Insect Sciences, Zhejiang University, Hangzhou 310058, China **3** Leshan Normal University, Leshan, 614000, China

Corresponding author: Zhong-Qi Yang (yangzhqi@126.com)

Academic editor: Jose Fernandez-Triana | Received 14 May 2019 | Accepted 3 July 2019 | Published 30 July 2019

<http://zoobank.org/E02E4D43-F6C4-47A5-AAD1-CDF02FF37C51>

Citation: Cao LM, Zhang YL, van Achterberg C, Wang ZY, Wang XY, Zhao WX, Yang ZQ (2019) Notes on braconid wasps (Hymenoptera, Braconidae) parasitising on *Agrilus mali* Matsumura (Coleoptera, Buprestidae) in China. ZooKeys 867: 97–121. <https://doi.org/10.3897/zookeys.867.36170>

Abstract

Braconid parasitoids reared from *Malus sieversii* and *Malus domestica* trees in NW China infested by *Agrilus mali* Matsumura (Coleoptera, Buprestidae) are illustrated and discussed. Six species were found parasitising *Agrilus mali* in NW China, namely, *Atanycolus ivanowi* (Kokujev) (Braconinae), *Doryctes undulatus* (Ratzeburg), *Pareucorystes varinervis* Tobias, *Polystenus rugosus* Foerster, *Spathius sinicus* Chao, and *Spathius brevicaudis* Ratzeburg (Doryctinae). All listed species are newly recorded parasitoids of *Agrilus mali*. *Pareucorystes varinervis* and *Spathius brevicaudis* are new records for the Chinese fauna, but *Spathius brevicaudis* has been recorded from Taiwan before. Both sexes of *Spathius brevicaudis* are redescribed here to allow inclusion in the recent revision of the Chinese *Spathius* species. An identification key to the six braconid parasitoids of *Agrilus mali* in NW China is provided.

Keywords

Agrilus mali, Braconidae, China, new record, parasitoid wasps, *Malus sieversii*

* These authors contributed equally to this work.

Introduction

The apple buprestid, *Agrilus mali* (Coleoptera: Buprestidae), is considered to be a dangerous pest in China of apple trees. Recently, a large area of wild *Malus sieversii* has been killed by *Valsa mali* Miyabe et Yamada and *Cytospora mandshurica* Miura, after infection by *A. mali* (Figure 1A, B, C). Its larvae feed under the bark on the phloem, which weakens the nutrient transportation and results in depressed and dark-coloured dead bark over the affected areas (Figure 1D). Heavy infestations result in dead branches and, eventually, in the death of the whole tree (Figure 1D). Besides apple trees, also other fruit trees (e.g., crab-apple, pear, peach, and cherry) are attacked.

Adult, egg and larval stages of *Agrilus mali* are shown in Figure 2; *A. mali* is widely distributed in Asian Russia, Japan, the Korean peninsula, and north China, and is a common species in apple orchards. In 1995 it was reported attacking the endangered wild apple (*Malus sieversii*) in Xinyuan County, Ili Kazakh Autonomous Prefecture, Xinjiang Province (Wang et al. 1995). This tertiary relict is the sole ancestor of most cultivars of the domesticated apple, *Malus domestica*. With its high genetic diversity, the forest is seen as the most significant national gene pool of apple, even to the world.

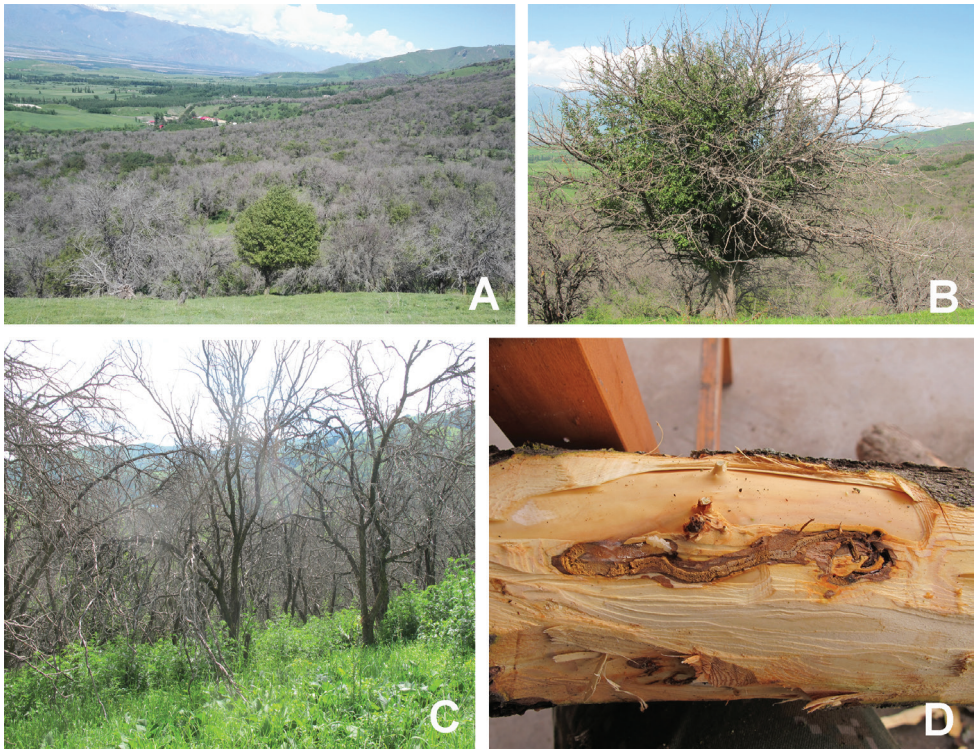


Figure 1. **A** Trees of *Malus sieversii* damaged by *A. mali* **B** A single tree of *Malus sieversii* damaged by *A. mali* **C** Dead branches because of *A. mali* **D** Trunk of *Malus sieversii* damaged by *A. mali*.

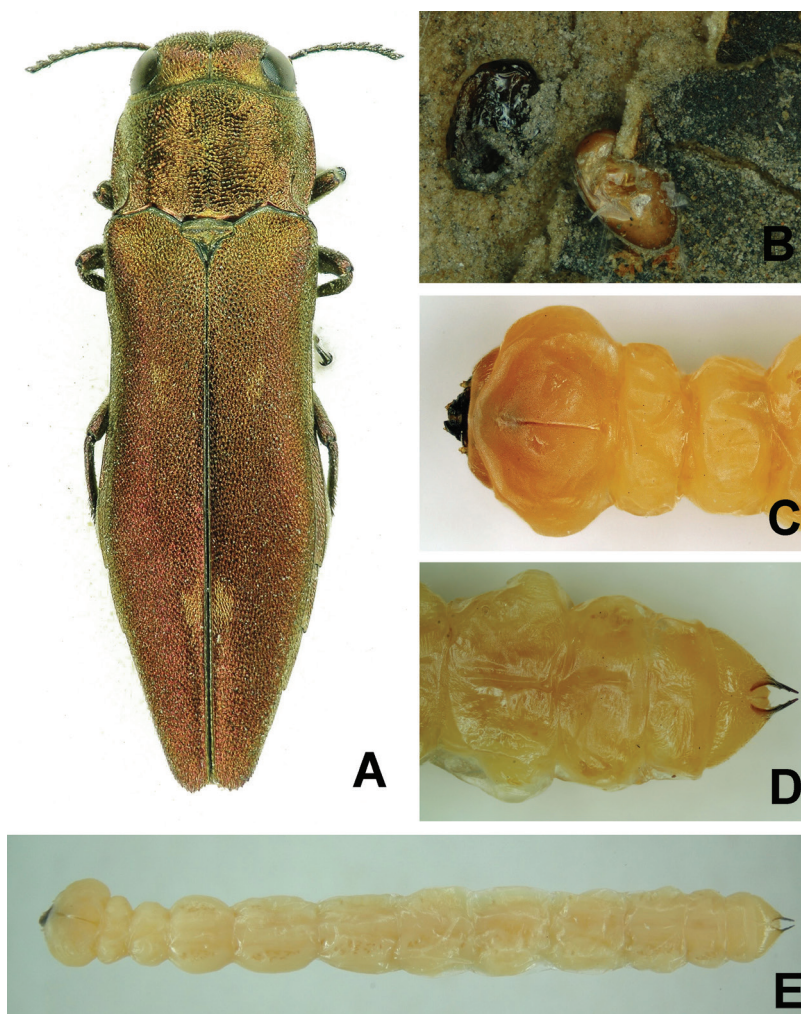


Figure 2. **A** Adult of *A. mali* **B** Eggs of *A. mali* **C** Head and thorax of larva, dorsal view **D** Abdominal tip of larva, ventral view **E** Larva of *A. mali*, ventral view.

Recently, *A. mali* became the major pest of *M. sieversii* and 48.6% of the Xinjiang wild apple forest was damaged, over 4866.67 m², and in some areas like Xinyuan County and Gongliu City, most trees died.

Using organic insecticides has allowed the control of *A. mali* in orchards, but in the wild this is ineffective as the trees are scattered over a vast area. The damage of *A. mali* is erratic, wide-spread, and frequent spraying of chemicals will be another threat to the vulnerable local ecosystem. Therefore, biological control is considered as the best countermeasure and a survey about the natural enemies of *A. mali* has been carried out in recent years.

Materials and methods

This study is based on specimens retained in the Entomological Museum of Chinese Academy of Forestry (Beijing, China) and the Naturalis Biodiversity Center (Leiden, the Netherlands). Natural enemy surveys of *A. mali* were conducted in Xinjiang, Shaanxi and Qinghai Provinces from 2011 to 2018. Trunk bark of stressed trees was peeled off to search for *A. mali* larvae and associated parasitoids. The larvae and possible parasitoid cocoons were placed singly in vials (12 mm in diameter and 75 mm in length), each containing a piece of filter paper dipped in distilled water for moisture. The vials were plugged tightly with sterilised cotton and maintained at 22–25 °C in the rearing room. Parasitoid cocoons were successively reared to adults. Specimens were examined with a Nikon SZH 1500 stereomicroscope. Photographs were taken with an Olympus CX31 microscope with the UV–C Optical Totally Focuses System developed by Beijing United Vision Technology Co., Ltd. Terminology and measurements follow van Achterberg (1993).

Taxonomy

Braconinae

Atanycolus ivanowi (Kokujev, 1898)

Figures 3A, B, 4, 5

Vipio (*Atanycolus*) *ivanowi* Kokujev, 1898: 364.

Bracon (*Vipio*) *sculpturatus* Thomson, 1892: 1800; Wang et al., 2009. Syn. by Belokobylskij et al., 2003: 369.

Atanycolus signatus Szépligeti, 1901, 33: 176. Syn. by Papp, 1960.

Material examined. 4♀, 20♂, China, Xinjiang Province, Gongliu City, Mohuer County, 25.VI.2006, 1325 m altitude, 43°15'27"N, 82°47'56"E, Yang ZhongQi leg. The collected cocoons were adhered to mature dead larvae of *A. mali*. 86♀, 78♂, same locality and biological data, but collected 25.VI.–24.VII.2015 by Wang ZhiYong.

Hosts. Larva of *Agrilus mali* (**new record**) (Buprestidae). *Arhopalus syriacus* Reitter, *Leptura rubra* Linnaeus, *Monochamus galloprovincialis* Olivier *Osphrantheria inaurata* Holzschuh, *Tetropium fuscum* Fabricius, *T. gabrieli* Weise (Cerambycidae); *Anthaxia auralenta* Fabricius, *Chrysobothris solieri* Gory et Laporte, *Lampra mirifica* Mulsant, *Melanophila decastigma* Fabricius, *M. picta* Pallas, *Sphenoptera tappesi* Marseul (Buprestidae).

Distribution. China (Xinjiang); Armenia; Austria; Azerbaijan; Croatia; Czech Republic; Finland; France; Germany; Greece; Hungary; Italy; Japan; Kazakhstan; Russia; Slovakia; Switzerland; Tajikistan; Turkey; Turkmenistan; Ukraine; Uzbekistan.

Remarks. *Atanycolus ivanowi* is clearly characterised by the sculpture of the first to fourth metasomal tergites (Fig. 4E). Wang et al. (2009) reported *A. ivanowi*

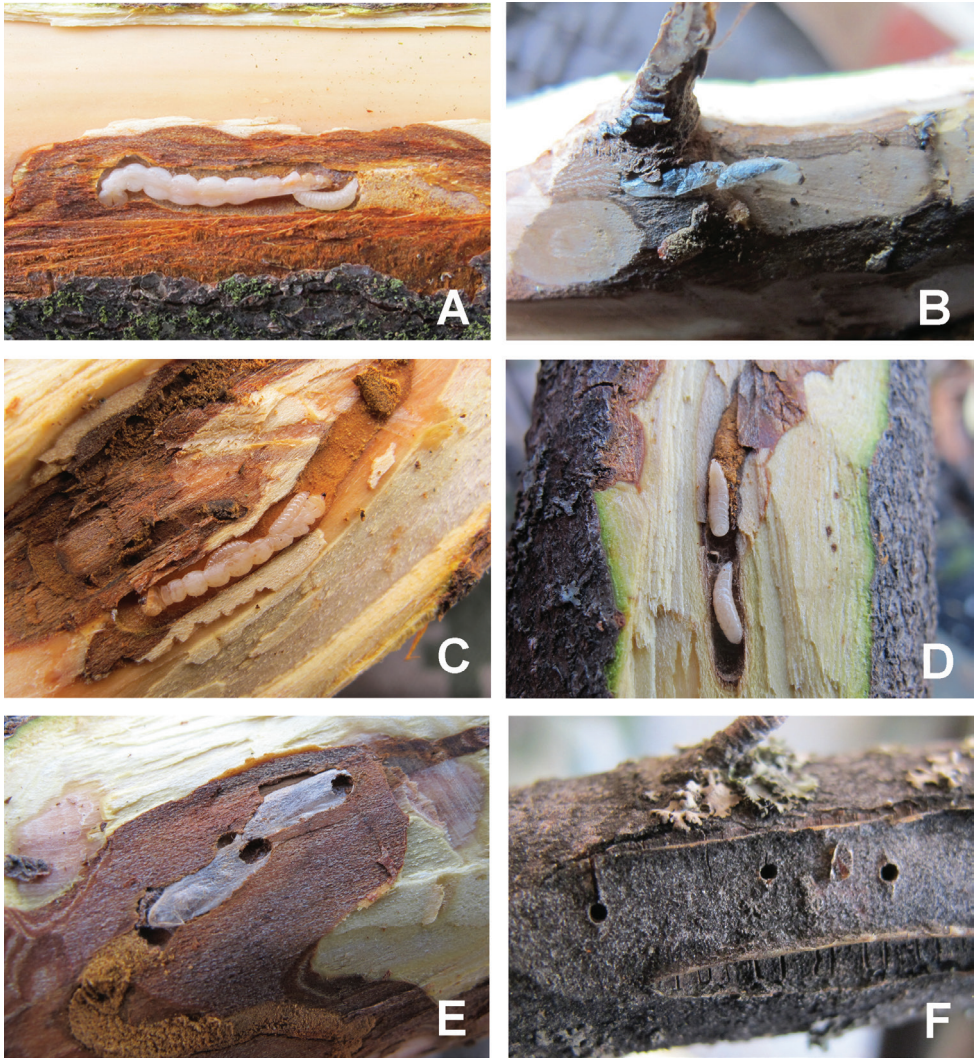


Figure 3. **A** Larva of *Atanycolus ivanowi* on larva of *A. mali* **B** Cocoons of *Atanycolus ivanowi* **C** Third instar larvae of *Doryctes undulatus* **D** Fourth instar larvae of *Doryctes undulatus* **E** Empty cocoons of *Doryctes undulatus* **F** Emergence hole of *Doryctes undulatus*.

(as *A. sculpturatus*) for the first time from Xinjiang and also provided a key to Chinese fauna of *Atanycolus* Foerster.

Genus *Atanycolus* are ectoparasitoids of wood borers, and are usually solitary (Figure 3A), very rarely there are two individuals on one host. *A. ivanowi* is widely distributed in the Central and South Palearctic region. This parasitoid is mostly found on the mature larva of *A. mali*. When full grown, the larva will spin a cocoon in the gallery of *A. mali*, ca. 10 days later the adult will emerge by biting a small round hole in the bark.

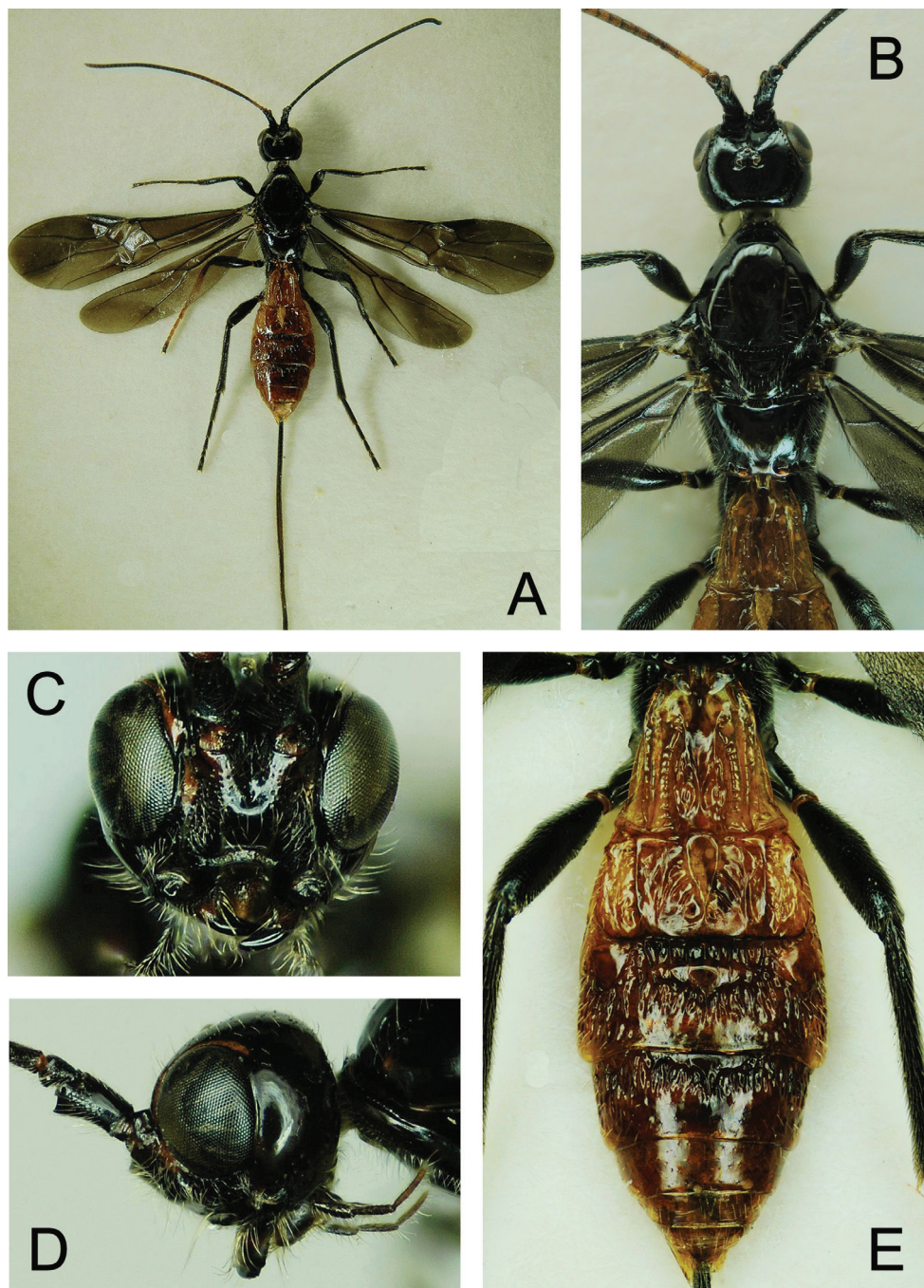


Figure 4. *Atanycolus ivanowi* ♀ **A** Habitus, dorsal view **B** head and mesosoma, dorsal view **C** head, frontal view **D** head, lateral view **E** metasoma, dorsal view.

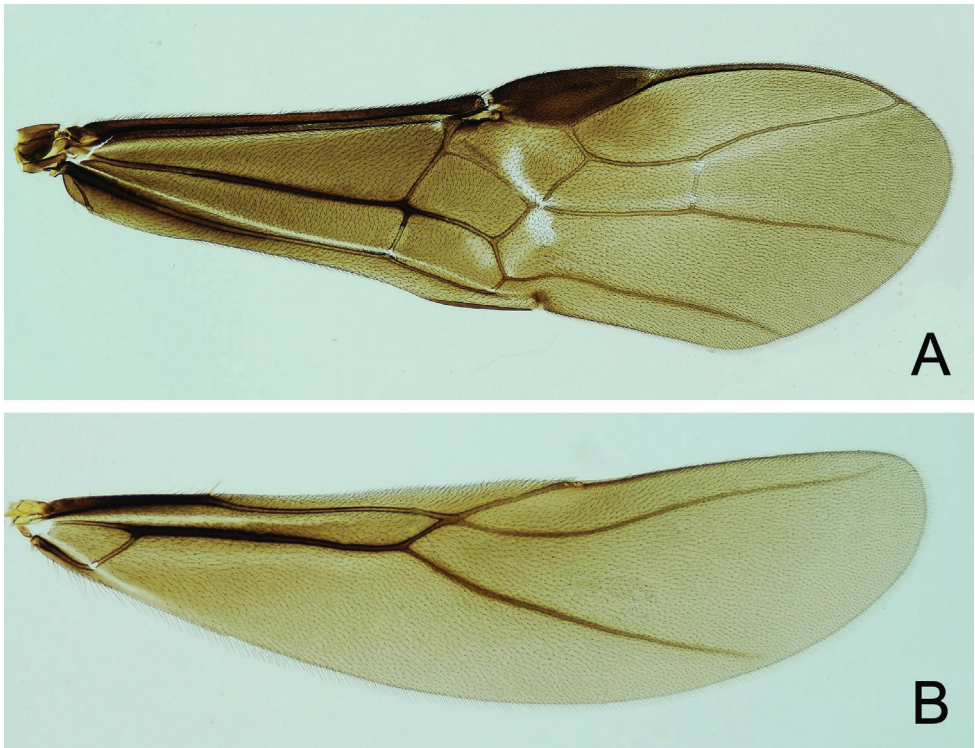


Figure 5. *Atanycolus ivanowi* ♀ **A** Forewing **B** Hind wing.

We found two generations per year of *A. ivanowi* in our experimental fields. The first generation lasts ca. 40 days from late March to May, and many adults can be seen during May to July. The larvae of the overwintering generation can be seen before August and later only cocoons can be found. Obviously, this parasitoid overwinters in the cocoon stage.

In total, we collected 86 females and 78 males of *A. ivanowi* in 2015 from one site (Xinjiang Province, Gongliu City, Mohuer County), which shows that the approximate ratio of female and male is 11/10. The natural parasitisation rate is approximately 26.7% and according to our investigations, *A. ivanowi* has the maximum population on *A. mali*. Obviously, *A. ivanowi* should be protected in order to increase the biodiversity of the forests, in which *Malus sieversii* is the main component.

Doryctinae

Doryctes undulatus (Ratzeburg, 1852)

Figures 3C, D, E, F, 6, 7, 8

Bracon undulatus Ratzeburg, 1852: 35.

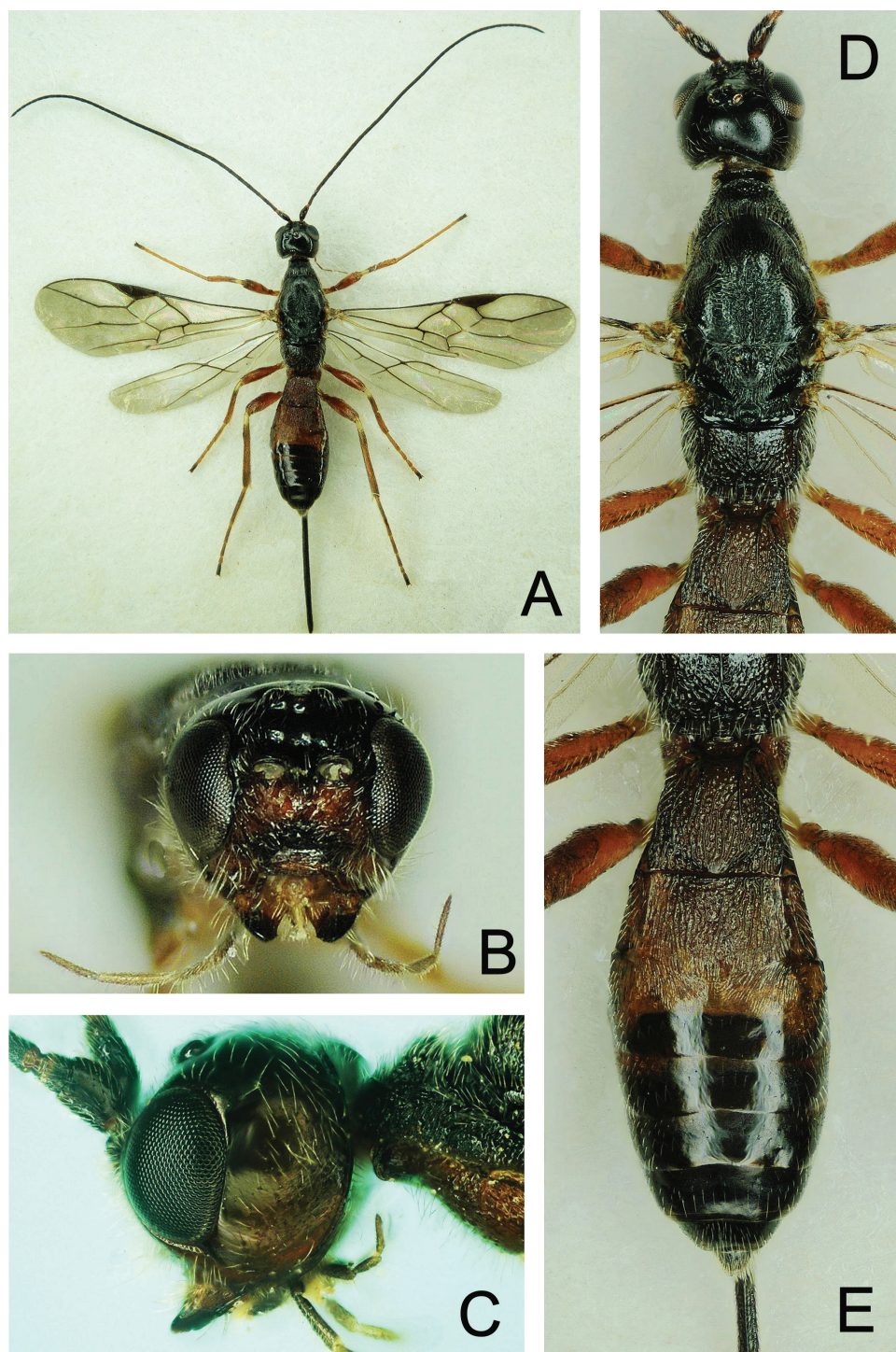


Figure 6. *Doryctes undulatus* ♀ **A** Habitus, dorsal view **B** Head, frontal view **C** Head, lateral view **D** Head and mesosoma, dorsal view **E** Metasoma, dorsal view.

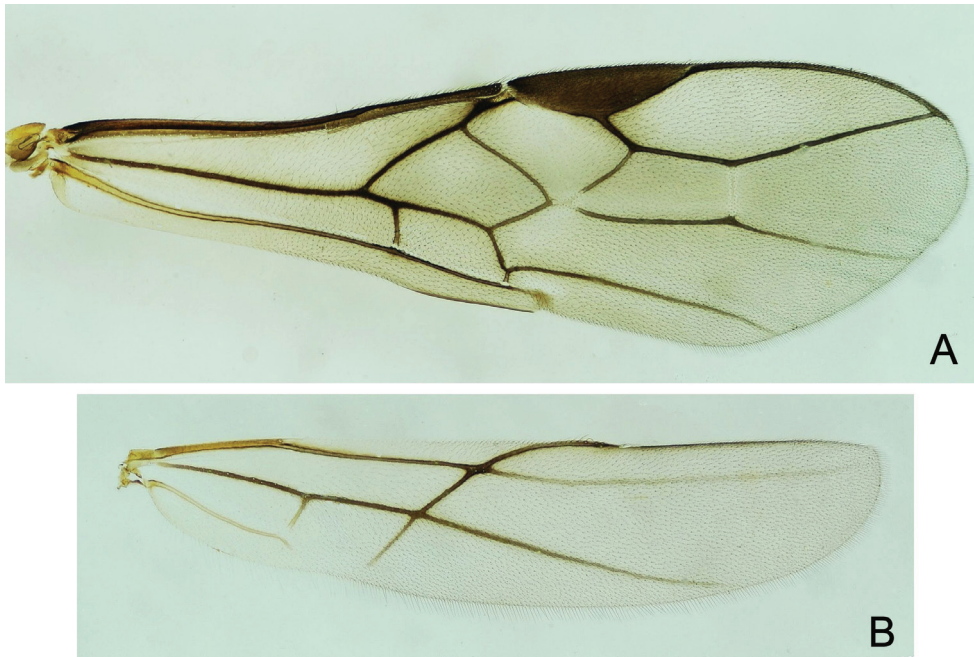


Figure 7. *Doryctes undulatus* ♀ **A** Forewing **B** Hind wing.

Doryctes undulatus: Reinhard, 1865: 256; Marshall, 1888: 237; Shenefelt et Marsh, 1976: 1294; Belokobylskij, 1998: 63; Belokobylskij et Maeto, 2009: 128; Belokobylskij et al., 2012: 47.

Doryctes brachyurus: Marshall, 1888: 238; Shenefelt et Marsh, 1976: 1279; Papp, 1984: 175.

Material examined. 2♀, China, Xinjiang Province, Gongliu City, Mohuer County, 8.VIII.2008, 1325 m altitude, 43°13'25"N, 82°45'16"E, Yang ZhongQi leg. 1♀, China, Xinjiang Province, Xinyuan County, Halabula, 16.VI.2011, 46°12'16"N, 82°59'20"E, Yang ZhongQi leg., 25.VI.2011, hatched out from a mature larvae of *A. mali*. 3♀, China, Xinjiang Province, Xinyuan County, Halabula, 16.VI.2011, 46°12'16"N, 82°59'20"E, Yang ZhongQi leg., 10.VII.2011, hatched out from a mature larvae of *A. mali*. 1♂, China, Xinjiang Province, Xinyuan County, Halabula, 16.VI.2011, 46°12'16"N, 82°59'20"E, Yang ZhongQi leg., 25.VII.2011, hatched out from a mature larvae of *A. mali*. 1♂, China, Xinjiang Province, Gongliu City, Mohuer County, 15.VI.2011, 1325 m altitude, 43°13'25"N, 82°45'16"E, Tang YanLong, Wang Zhi-Yong & Yang ZhongQi leg., 8.VII.2011, hatched out from a mature larvae of *A. mali*.

Hosts. Larva of *Agrilus mali* (new record) (Buprestidae). *Axinopalpis gracilis* Krynicki, *Grammoptera ruficornis* Fabricius, *Molorchus kiesewetteri* Mulsant et Rey, *M. umbellatarum* Schreber, *Pogonocherus decoratus* Germar, *P. fasciculatus* DeGeer, *P. hispidulus* Piller, *P. hispidus* (Linnaeus), *Tetrops praeustus* (Linnaeus) (Cerambycidae);

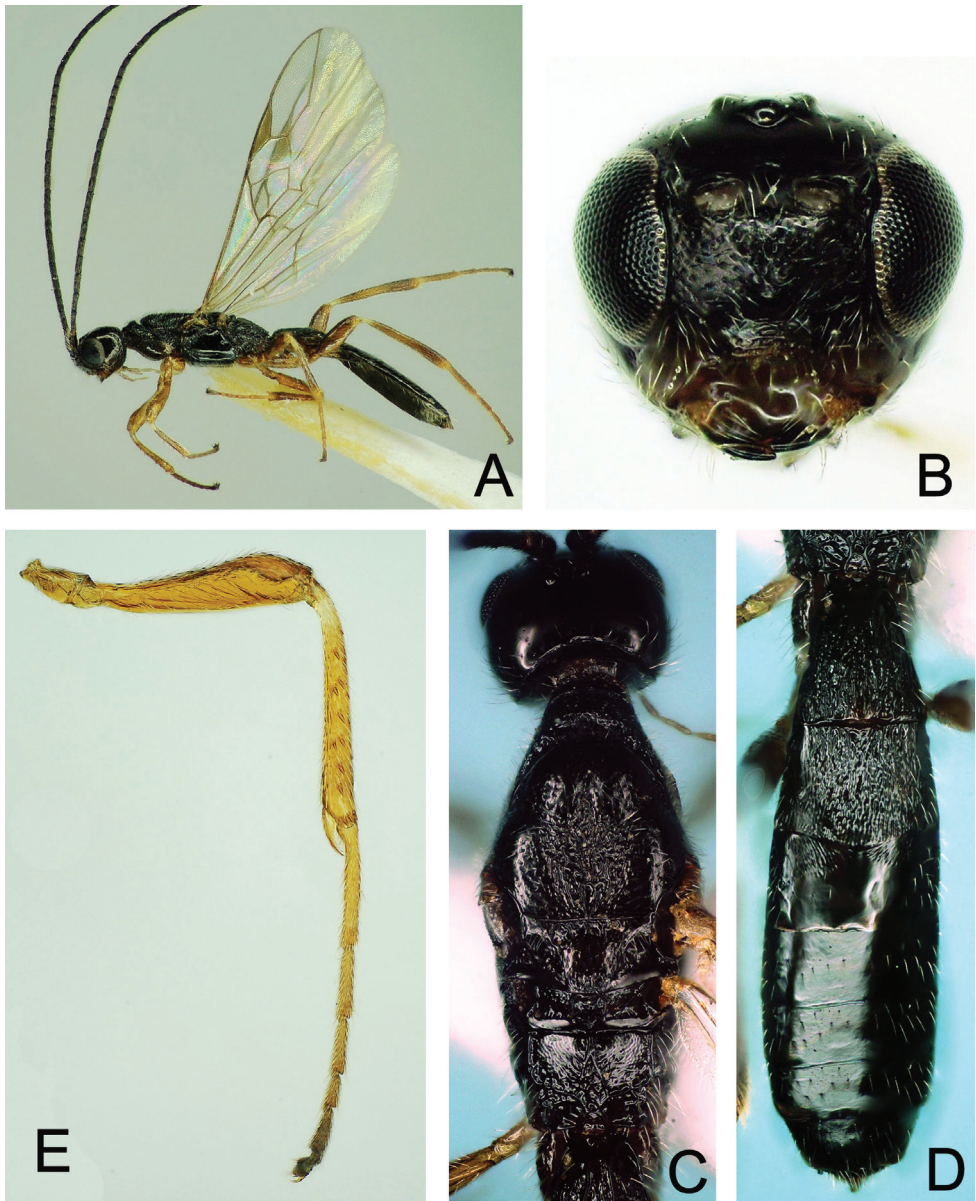


Figure 8. *Doryctes undulatus* ♂ **A** Habitus, lateral view **B** Head, frontal view **C** Head and mesosoma, dorsal view **D** Metasoma, dorsal view **E** Left fore leg.

Agrilus convexicollis Redtenbacher, *A. cuprescens* Menetries, *A. mendax* Mannerheim *A. viridis* (Linnaeus) *Anthaxia tuerki* Scopoli (Buprestidae); *Magdalis armigera* Geoffroy, *M. ruficornis* (Linnaeus), *Pityogenes bidentatus* (Herbst) (Curculionidae).

Distribution. China (Xinjiang, Heilongjiang, Jilin, Gansu); Bulgaria; France; Germany; Hungary; Italy; Japan; Kazakhstan; Korea; Lithuania; Moldova; Mongolia; Poland; Russia; Slovakia; Sweden; Switzerland; United Kingdom.

Remarks. According to the detailed redescription of Japanese specimens (Belokobylskij et Maeto, 2009) and the key to this genus for China (Belokobylskij et al. 2012), our specimens from Xinjiang are quite the same, although there are still some tiny differences present, e.g., hind femur much stronger, 2.8 times as long as the maximum width in lateral view (vs. hind femur 2.9–3.2 times longer than wide in Japan); female body colour is stable reddish brown and male body is totally black (vs. reddish brown to almost black in Japan); extend of sculpture of third tergite varies from 1/4 to 1/2 basally, but semi-circular striation is always present, which is the main character to separate it from *D. striatellus* (Belokobylskij et Maeto 2009). After extensive comparison, we consider the differences to be intra-specific variation of *D. undulatus*. *Agrilus mali* is newly reported as host of *D. undulatus*. On average, a host larva is parasitised by two larvae of *D. undulatus* as ectoparasitoid (Figure 3C, D, E, F).

***Pareucorystes varinervis* Tobias, 1961 (new record for China)**

Figures 9, 10

Pareucorystes varinervis Tobias, 1961: 529.

Pareucorystes depressus Fischer, 1966: 323.

Material examined. 10♀, 1♂, China, Qinghai Province, Hualong County, 17.VI.2008, 36°05'42"N, 102°15'43"E, Yang ZhongQi leg.

Host. Larva of *Agrilus mali* (**new record**) (Buprestidae). *Agrilus angustulus* Illiger, *A. auricollis* Kiesenwetter, *A. convexicollis* Redtenbacher, *A. laticornis* Illiger, *A. sulcicollis* Lacordaire, *A. viridis* (Linnaeus) (Buprestidae); *Tetrops praeustus* (Linnaeus) (Cerambycidae).

Distribution. China (Qinghai); Azerbaijan; Bulgaria; Canary Islands; Czechoslovakia; France; Hungary; Italy; Kazakhstan; Russia; Slovakia; Ukraine.

Remarks. This species is mainly specialised on *Agrilus* species; if the population density could be enlarged, it would be a potential biological control agent. The species is also a new to the Chinese fauna, and a new parasitoid of *A. mali*.

***Polystenus rugosus* Foerster, 1863**

Figures 11, 12

Polystenus rugosus Foerster, 1863: 237; Shenefelt et Marsh, 1976: 1361; Papp, 1984: 182; Belokobylskij et Tobias, 1986: 34; Belokobylskij, 1998: 74; Belokobylskij et Maeto, 2009: 409; Tang et al., 2014: 6.

Corystes aciculatus Reinhard, 1865: 259.

Eucorystes aciculatus Marshall, 1888: 204.

Eucorystoides aciculatus Ashmead, 1900: 368; Shenefelt et Marsh, 1976: 1354; Papp, 1984: 182.

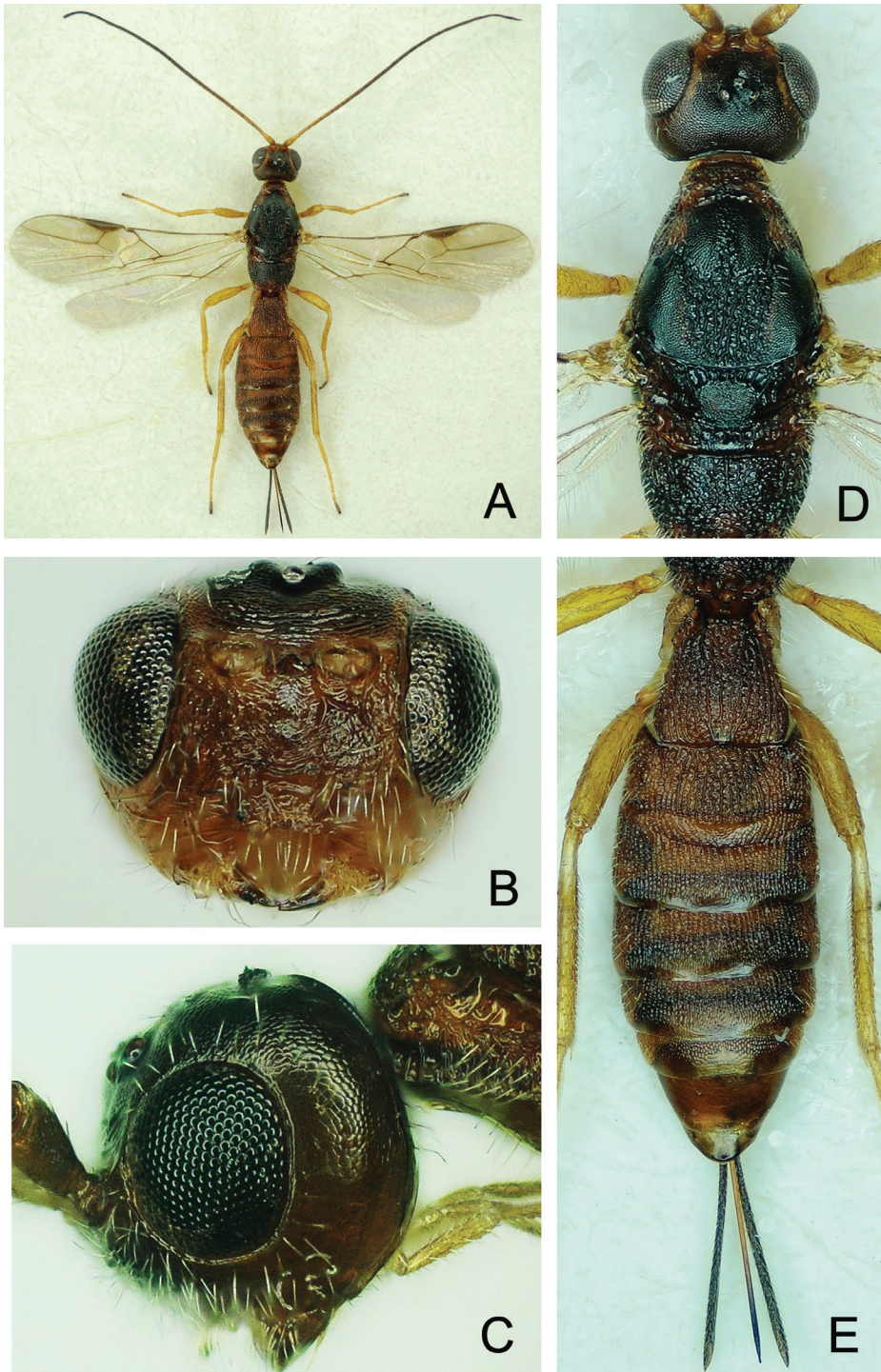


Figure 9. *Pareucorystes varinervis* ♀ **A** Habitus, dorsal view **B** Head, anterior view **C** Head, lateral view **D** Head and mesosoma, dorsal view **E** Metasoma, dorsal view.



Figure 10. *Pareucorystes varinervis* ♂ **A** Forewing **B** Hind wing.

Material examined. 1♀, China, Shaanxi Province, Yijun County, 29.VII.2006, 35°23'56"N, 109°06'41"E, Yang ZhongQi leg, 28.VII.2006, hatched out from a mature larva of *A. mali*. 1♂, China, Xinjiang Province, Gongliu City, Mohuer County, 26.VI.2006, 1325 m altitude, 43°13'25"N, 82°45'16"E, Yang ZhongQi leg, 13.VII.2006, hatched out from a mature larvae of *A. mali*.

Hosts. Larva of *Agrilus mali* (**new record**) (Buprestidae). *Agrilus angustulus* Illiger, *A. auricollis* Kiesenwetter, *A. sulcicollis* Lacordaire, *A. viridis* (Linnaeus), *Anthaxia manca* Linnaeus, *Coraebus bifasciatus* Olivier (Buprestidae); *Sinoxylon sexdentatum* Olivier (Bostrichidae).

Distribution. China (Xinjiang, Shaanxi, Henan, Zhejiang, Taiwan?), Austria; Czech Republic; Germany; Hungary; Italy; Japan; Kazakhstan; Korea; Liechtenstein; Poland; Russia; Slovakia; Switzerland; Tajikistan; Ukraine.

Remarks. This species is newly reported for Xinjiang and Shaanxi and *A. mali* is a new host record.

Spathius sinicus Chao, 1957

Figures 13, 14

Spathius sinicus Chao, 1957: 3; 1977: 209; Chen & Shi, 2004: 162; Tang et al., 2015: 106; Yu et al., 2016.

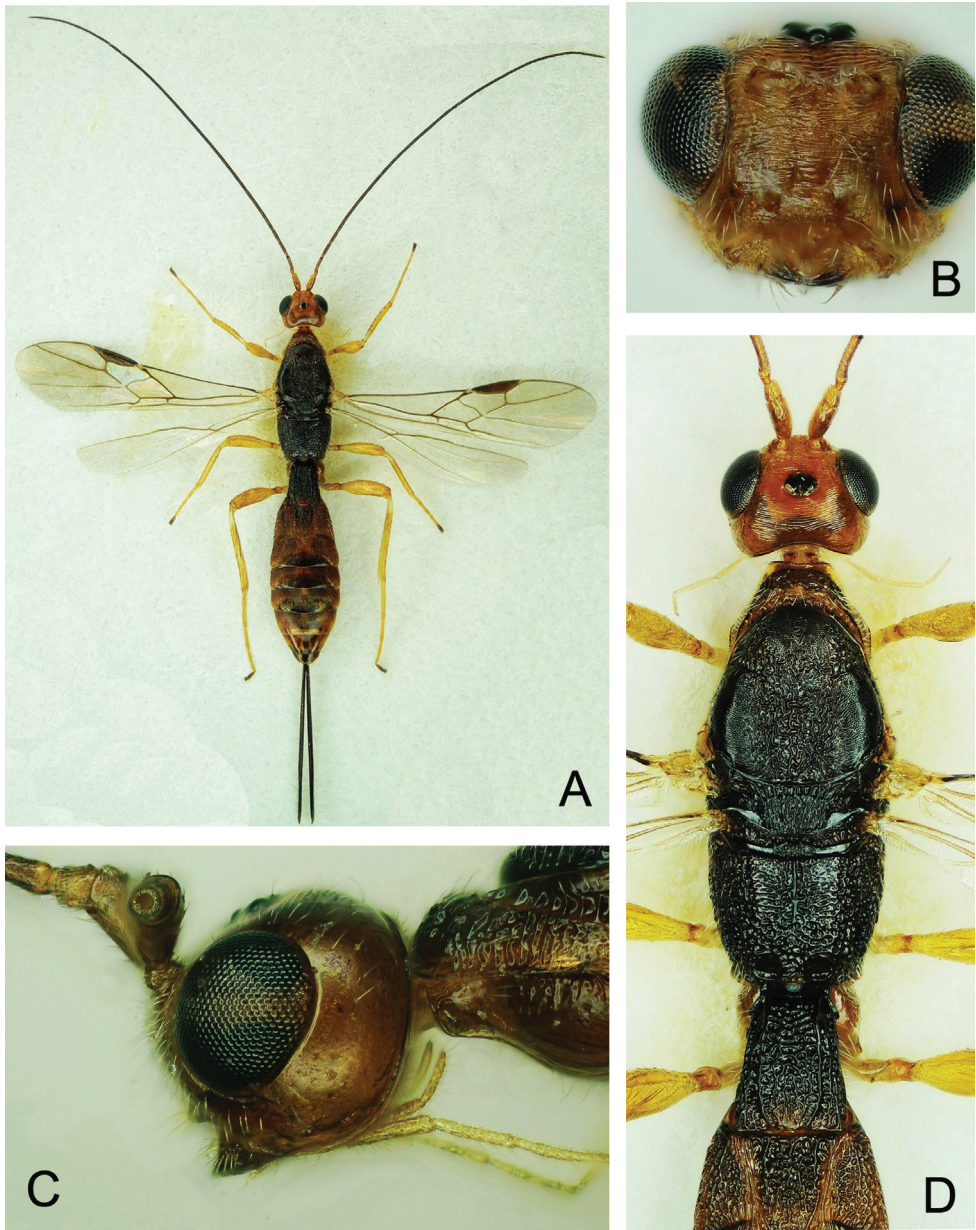


Figure 11. *Polystenus rugosus* ♀ **A** Habitus, dorsal view **B** Head, anterior view **C** Head, lateral view **D** Head and mesosoma, dorsal view.

Material examined. 6♀, China, Xinjiang Province, Gongliu City, Mohuer County, 15.VI.2011, 1325 m altitude, 43°13'25"N, 82°45'16"E, Zhang YanLong, Wang Zhi-Yong & Yang ZhongQi leg. 8.VII.2011, hatched out from mature larvae of *A. mali*.

Host. Larva of *A. mali* (new record) (Buprestidae).

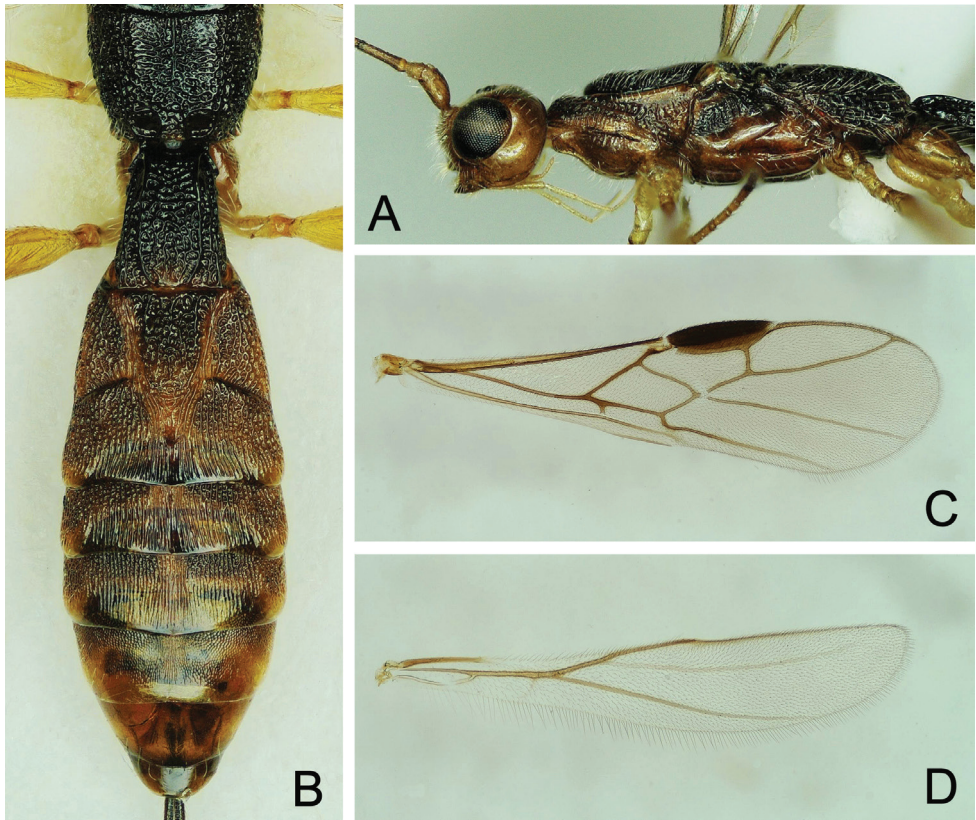


Figure 12. *Polystenus rugosus* ♀ **A** Head and mesosoma, lateral view **B** Metasoma, dorsal view **C** Forewing **D** Hind wing.

Distribution. China (Xinjiang, Fujian, Heilongjiang, Hunan, Shanghai, Jilin, Tianjin, Zhejiang); Japan.

Remarks. This species is widely distributed in China and *A. mali* is the first reported of a host. It is peculiar that during several years of investigation, only 6 individuals have been found on *A. mali* at one tree, which indicates that it is an occasional parasitism.

***Spathius brevicaudis* Ratzeburg, 1844 (new record in China mainland)**

Figures 15–18

Spathius brevicaudis Ratzeburg, 1844: 49; Nixon, 1943: 202; Belokobylskij, 1996: 188.

Material examined. 1 ♀, 1 ♂, China, Xinjiang Province, Gongliu City, Mohuer County, 26.VI.2006, 1325 m altitude, 43°13'25"N, 82°45'16"E, Zhang YanLong, Wang Zhi-Yong & Yang ZhongQi leg., 12.VII.2006, hatched out from mature larvae of *A. mali*.

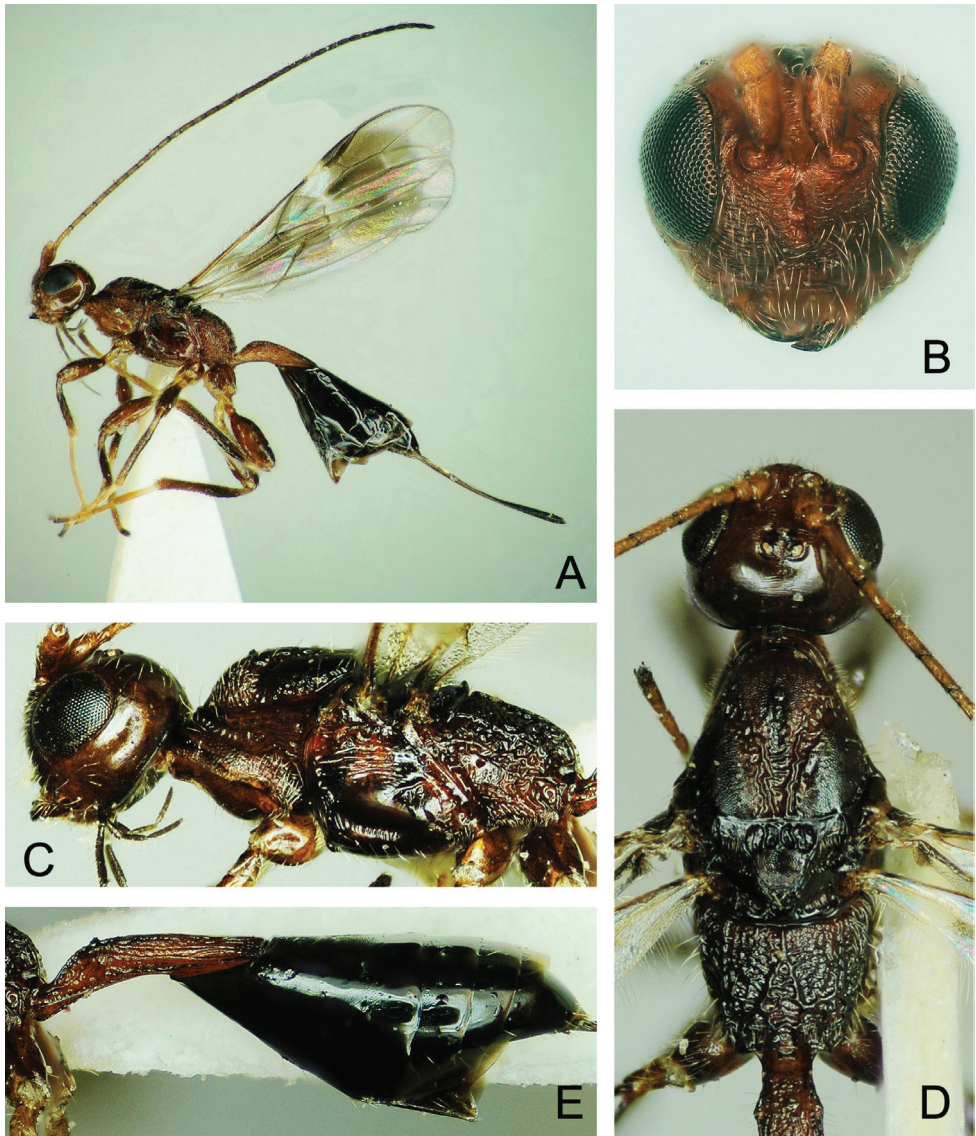


Figure 13. *Spathius sinicus* ♀ **A** Habitus, lateral view **B** Head, anterior view **C** Head and mesosoma, lateral view **D** Head and mesosoma, dorsal view **E** Metasoma, lateral view.

Redescription. Body length, ♀/♂ = 2.9/2.8 mm; forewing length, ♀/♂ = 2.34/2.25 mm.

Colour (Female). Head dark brown, basal half of antenna yellow, its apical half brown; mesoscutal lobes, scutellum dark brown, pronotum brown; metasoma dark brown except first and second metasomal segments, basal portion of third metasomal segment yellow; fore wing partly weakly darkened; legs yellow (Fig. 15A, B).



Figure 14. *Spathius sinicus* ♀ **A** Metasoma, dorsal view **B** Forewing **C** Hind wing.

Head. Median length 0.7 times of its width in dorsal view; vertex broad, surface rough, with low (fine) sculptures and rare white setae (Fig. 15D); length between posterior margin of lateral ocellus and occipital carina half of head length in dorsal view; occipital carina median portion concave, reversed V-shaped (Fig. 15D); length of eye: length of temple in dorsal view = 4.3: 4; eyes small, slightly protruding bilaterally; OOL: OD: POL = 3: 1: 1.8; ocellar area distinctly differentiated, slightly swollen; width of head 1.1 times of height in front view, distance between eyes $1.3 \times$ height of eye (Fig. 15C); face distinctly and irregularly striate, covered with white setae; malar space $0.6 \times$ height of eye; height of clypeus $0.4 \times$ its width, exterior margin of clypeus straight; basally mandible broad, apical portion black, blunt and robust; hypoclypeal depression deeply concave; antenna 28 segmented, scape twice length of first flagellar segment, and twice its maximum width; first flagellar segment $5.3 \times$ its maximum width, as long as second flagellar segment; last antennal segment acute apically.

Mesosoma. Length of mesosoma $1.9 \times$ its width and $1.6 \times$ its height in lateral view (Fig. 16A); pronotal depression with short carinae; mesoscutum distinctly elevated above pronotum (Figs 15D, 16A). Mesoscutum nearly equilateral triangular, median length $0.9 \times$ its maximum width; median and lateral lobes of mesoscutum with scaly sculpture; notauli and centre of mesoscutum deep and light-coloured with strong carinae (Fig. 15D); mesopleuron distinctly striate in upper $1/3$ near pronotum and tegula, posterior $2/3$ with scaly sculpture, epicnemial carina bent, episternal area taproot-shaped (Fig. 16A). Precoxal sulcus broad, length $1.67 \times$ its width, with four longitudinal carinae inside. Scutellum flat and triangular, apical $1/3$ of scutellum with scaly sculpture; scutellar sulcus $1/3$ of scutellum length, with seven longitudinal carinae and separated small concave depressions; metanotum broad, dorsally

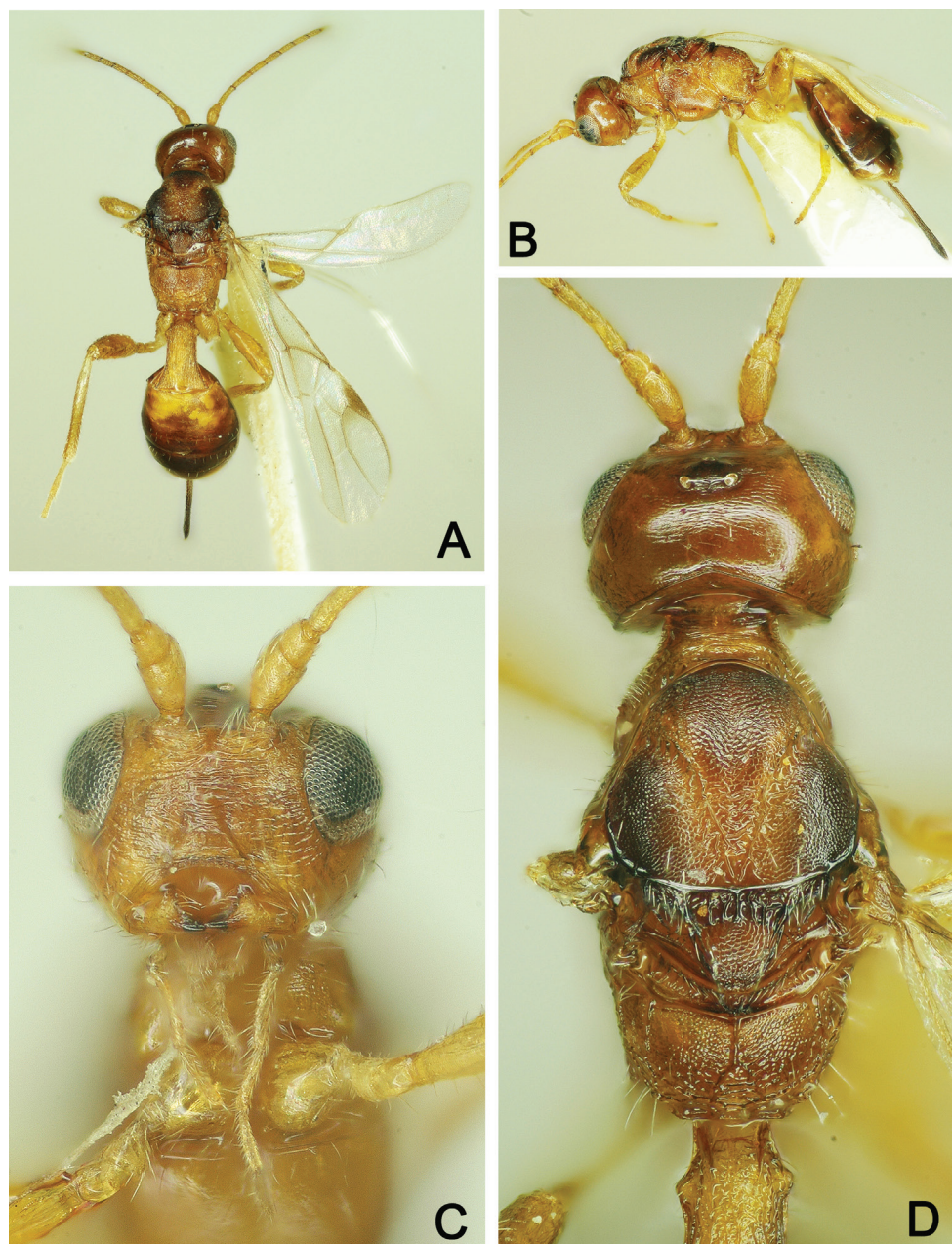


Figure 15. *Spathius brevicaudis* ♀ **A** Habitus, dorsal view **B** Habitus, lateral view **C** Head, anterior view **D** Head and mesosoma, dorsal view.

concave, laterally with several longitudinal carinae, posterior margin slightly curved; propodeum weakly oblique posteriorly (lateral view), with scaly sculpture, medio-longitudinal carina bifurcates at basal third, posterior half of propodeum with irregular carinae.

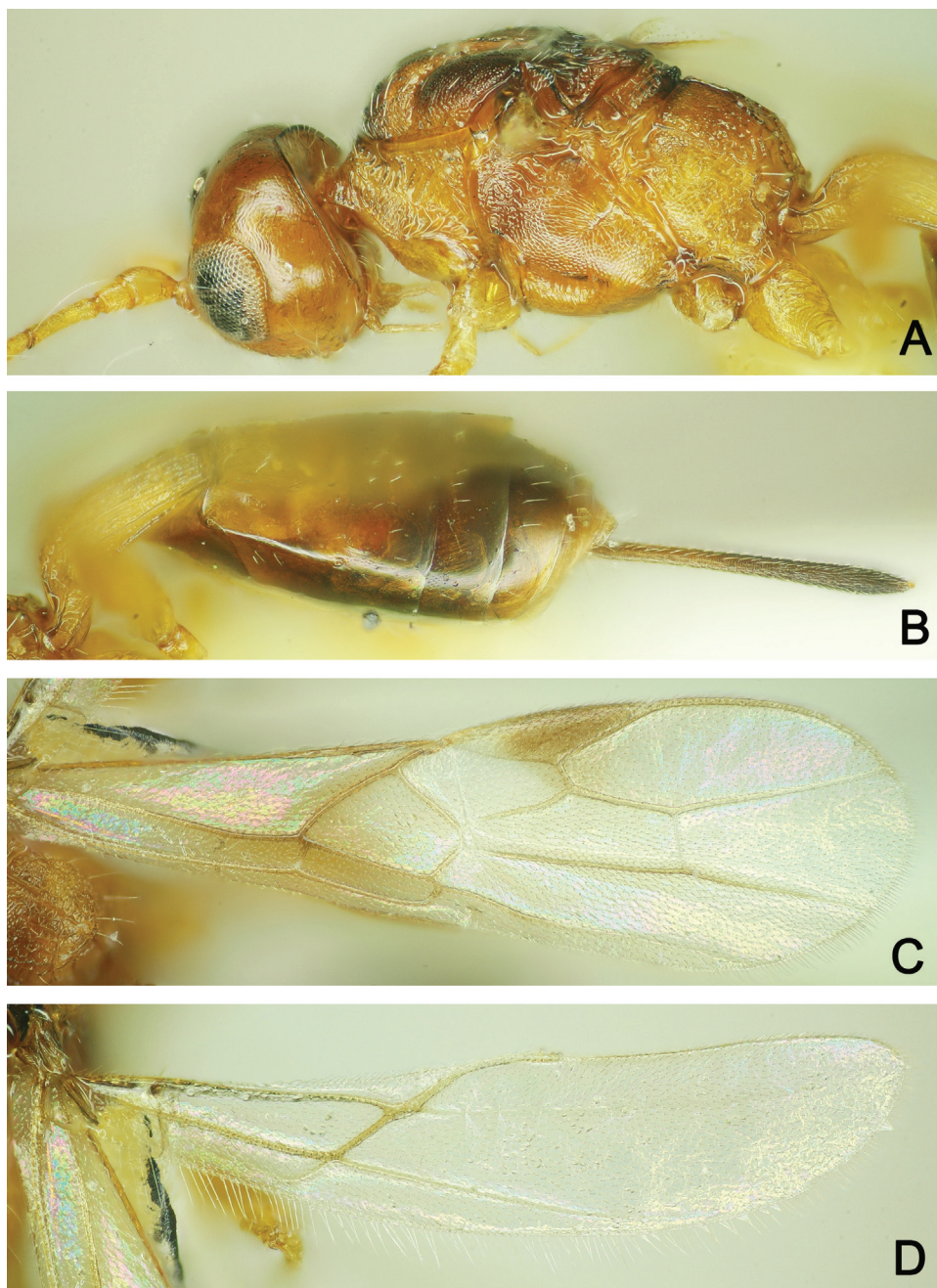


Figure 16. *Spathius brevicaudis* ♀ **A** Head and mesosoma, lateral view **B** Metasoma, lateral view **C** Fore wing **D** Hind wing.

Legs. Fore femur 0.8 times as long as fore tibia and 3.75 times as long as its width, fore tibia 8.0 times of its width, outside with a row of spines and apex with comb of spines, ratio of fore tarsal segments I–V = 1.4:0.7:0.5:0.3:0.6; mid femur

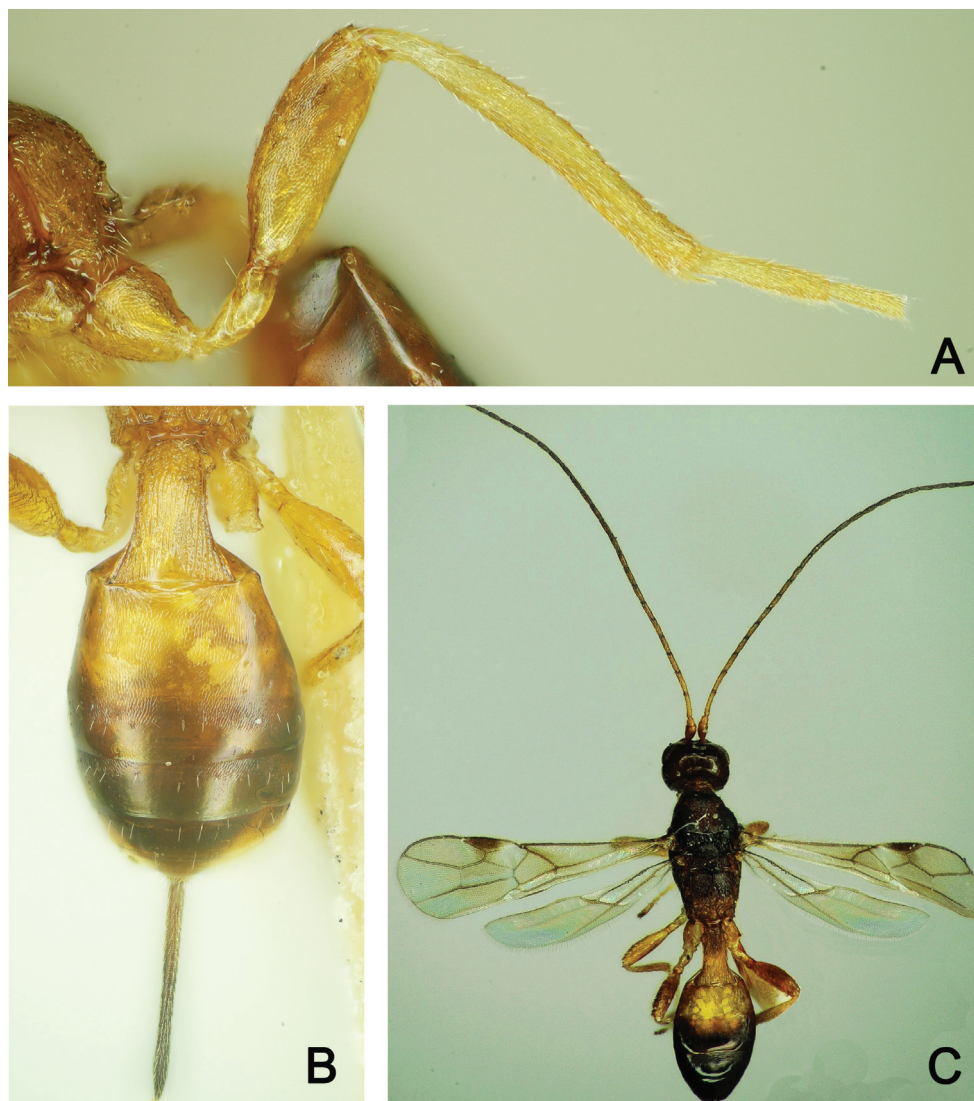


Figure 17. *Spathius brevicaudis* **A** ♀, Left hind leg, lateral view **B** ♀, metasoma, dorsal view **C** ♂, habitus, dorsal view.

0.8 times of mid tibia, ratio of mid tarsal segments I–V = 7:5:4:5:7; hind femur 2.7 times of its width, 0.8 times as long as hind tibia, ratio of hind tarsal segments I–V = 1.5:0.8:0.5:0.4:0.8 (Fig. 17A).

Wings. Fore wing with faintly brown along veins in basal half, apical half of fore wing largely subhyaline, its length 3.2 times of width; pterostigma 4.0 times as long as its maximum width; vein 1-R1 1.3 times of pterostigma, vein r originates at middle of pterostigma; vein SR1 8.5 times as long as vein r and straight; vein r nearly 1/4 of

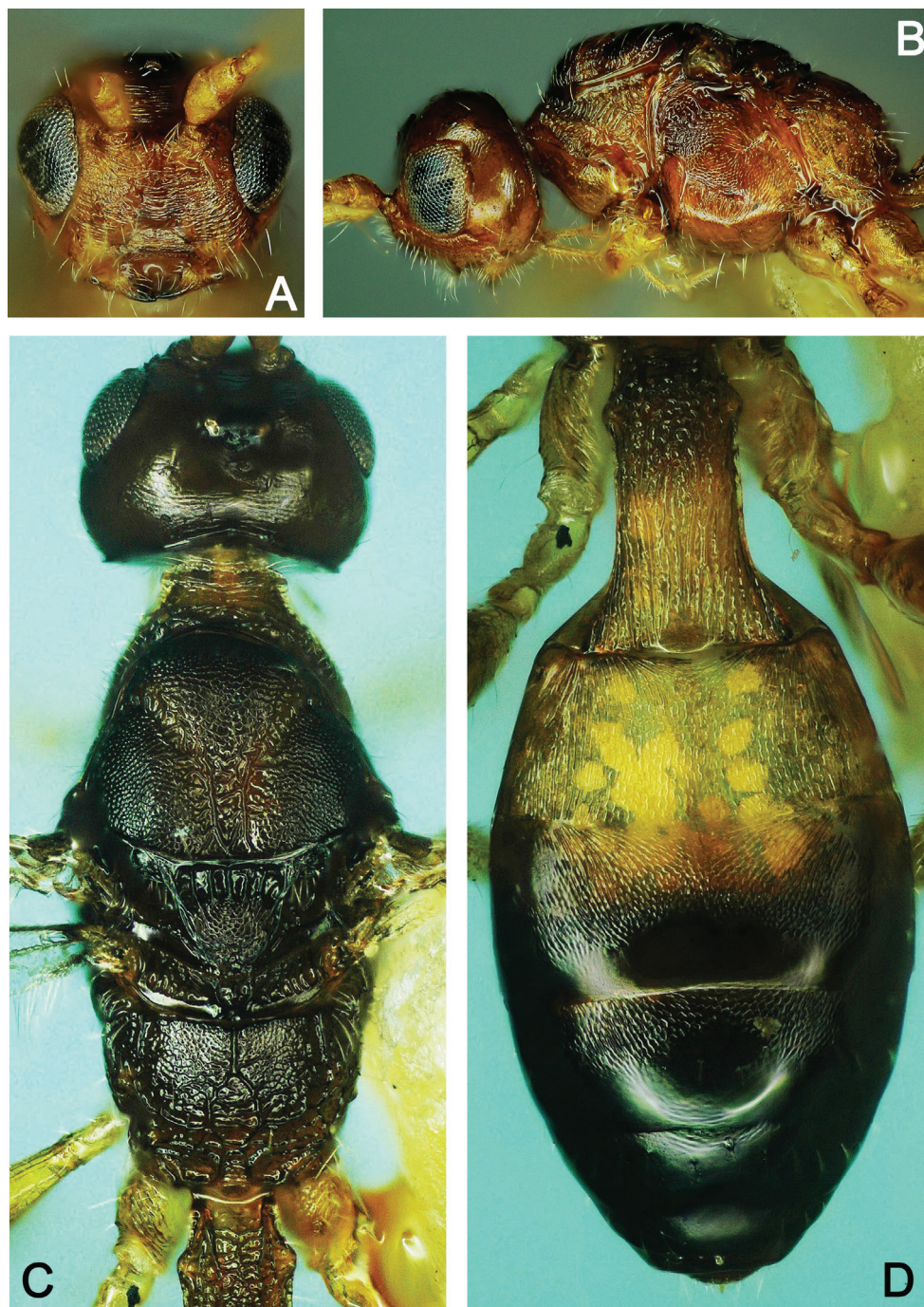


Figure 18. *Spathius brevicaudis* ♂ **A** Head, frontal view **B** Head and mesosoma, lateral view **C** Head and mesosoma, dorsal view **D** Metasoma, dorsal view.

vein 2-SR, vein cu-a perpendicular to vein CU1, vein m-cu enters second submarginal cell; meeting point of veins 2-SR, 2-M and 2-SR+M finely sclerotised, veins reduced; vein 1-SR+M straight, vein 1-SR 1/3 length of vein 1-M; vein r-m weakly sclerotised, nearly invisible; veins 3-M and CU1a extending to wing margin (Fig. 16C). Length of hind wing $5.0 \times$ its width (Fig. 16D).

Metasoma. First tergite length $1.55 \times$ its maximum apical width in dorsal view, apical 2/3 with regular longitudinal striae, basal 1/3 rugulose; in lateral view first tergite very robust, spiracular tubercles located at basal 1/4, laterally with erect white long setae, apical 1/3 of laterotergites visible; second tergite largely rugulose and with several large round yellow spots; basal 2/3 of third tergite striate-rugulose, apical 1/3 smooth; fourth tergite basally 1/4 with longitudinal rugulosity; fifth and sixth tergites smooth. Length of setose part of the ovipositor sheath $0.7 \times$ length of metasoma, $0.38 \times$ length of fore wing, and $0.3 \times$ length of body (Figs 16B, 17B).

Male. Body length 2.8 mm, otherwise similar to female (Figs 17C, 18), but pigmentation and metasomal sculpture more developed than in female.

Distribution. China (Xinjiang, Taiwan); Austria; Azerbaijan; Bulgaria; Czech Republic; Denmark; France; Georgia; Germany; Hungary; Italy; Japan; Kazakhstan; Korea; Moldova; Mongolia; Poland; Romania; Russia; Slovakia; Sweden; Switzerland.

Host. Larva of *Agrilus mali* (new record), *Agrilus viridis* (Linnaeus), *Anthaxia manca* Linnaeus, *A. quadripunctata* (Linnaeus), *Bostrichus bidens* Fabricius (Buprestidae); *Acanthocinus griseus* (Fabricius), *Arhopalus coreanus* Sharp, *Exocentrus lusitanus* (Linnaeus) (Cerambycidae); *Blastophagus minor* (Hartig), *B. piniperda* (Linnaeus), *Carphoborus minimus* (Fabricius), *Ceutorhynchus quadridens* (Panzer), *Dryocoetes autographus* (Ratzeburg), *Hylesinus fraxini* Panzer, *Ips acuminatus* (Gyllenhal), *I. typographus* (Linnaeus), *Lixus bidens* Fabricius, *Magdalis frontalis* Gyllenhal, *M. violacea* (Linnaeus), *Niphades variegatus* Roelofs, *Onthotomicus angulatus* Eichhoff, *Phloeotribus rhododactylus* (Marsham), *Pissodes notatus* Fabricius, *P. obscurus* Roelofs, *Pityogenes bidentatus* (Herbst), *P. chalcographus* (Linnaeus), *Pityophthorus micrographus* (Linnaeus), *Polygraphus subopacus* Thomson, *Rynchaenus fagi* (Linnaeus), *R. pilosus* Fabricius, *R. quercus* (Linnaeus) *R. salicis* (Linnaeus), *R. testaceus* Muller, *Scolytus intricatus* (Ratzeburg), *S. koenigi* Schewyrew, *S. laevis* Chapuis, *S. mali* (Bechstein), *S. multistriatus* (Marsham), *S. rugulosus* (Muller), *Shirahoshizo insidiosus* Roelofs, *Sh. pini* Morimoto, *Sh. rufescens* Roelofs (Curculionidae); *Xiphydria longicollis* (Geoffroy) (Xiphydriidae).

Remarks. Among all the parasitoids of *A. mali* we found in the past years, only two specimens of *S. brevicaudis* were recorded, which indicates that it is an occasional parasitoid of this host. This species is here recorded as new for continental China, after Belokobylskij (1996) reported it from Taiwan. Its identification is based on Ratzeburg's original description, Nixon's redescription, and reared material from Europe seen by the second author. *S. brevicaudis* may be confused with *S. rubidus* (Rossi), but *S. brevicaudis* has vein M+CU1 of fore wing straight or nearly so (weakly to moderately sinuate in *S. rubidis*), wing membrane with a faintly infusate patch or band below pterostigma (with a distinct dark patch or band below pterostigma, rarely reduced

in small specimens) and basal pale spot of pterostigma weakly differentiated (basal pale spot of pterostigma distinctly differentiated in dark specimens). Although, the differences are minor, all related to the fore wing and sometimes gradual, we prefer to recognise *S. brevicaudis* as separate species till molecular data will become available. The main reason for this is that reared series show these minor differences to be stable enough for separation both species in north-western Europe.

Key to braconid parasitoids of *Agrilus mali* in northwest China

- 1 Occipital and prepectal carinae absent (Fig. 4D) ***Atanycolus ivanowi* (Kokujev)**
- Occipital and prepectal carinae present (Figs 6C, 8C, 9C, 11C, 13C, 15D) **2**
- 2 Forewing with two submarginal cells, because vein r-m of fore wing is completely absent (except sometimes in *Pareucorystes*) (Figs 9A, 10A, 12C) **3**
- Forewing with three submarginal cells, because vein r-m is weakly developed (Figs 7A, 14B, 16C) **4**
- 3 Metasomal tergites 2+3 with V-shaped pale area and without posteriorly curved transverse groove (Fig. 12B) ***Polystenus rugosus* Foerster**
- Metasomal tergites 2+3 without V-shaped pale area and with posteriorly curved transverse groove (Fig. 9E) ***Pareucorystes varinervis* Tobias**
- 4 Vein m-cu of fore wing entering first submarginal cell (antefurcal: Fig. 7A); first metasomal tergite sessile (Fig. 6E) ***Doryctes undulatus* (Ratzeburg)**
- Vein m-cu of fore wing entering second submarginal cell (postfurcal: Figs 14B, 16C); first tergite petiolate (Figs 14A, 17B) **5**
- 5 Forewing partly strongly infuscated (Fig. 14B); first metasomal tergite elongate and slender, approx. twice as long as its apical width (Fig. 14A) ***Spathius sinicus* Chao**
- Forewing weakly infuscated, subhyaline (Fig. 16C); first tergite comparatively short and broad, approx. 1.5 × as long as its apical width (Fig. 17B) ***Spathius brevicaudis* Ratzeburg**

Acknowledgements

We sincerely thank Dr. Jose Fernandez-Triana (Canadian National Collection of Insects, Ottawa, Canada) and Dr. Sergey Belokobylskij (Zoological Institute, Russian Academy of Sciences, St Petersburg, Russia) for their critical reading of and helpful comments on the manuscript. This research is financially supported by the Special Fund for Forest Scientific Research in the Public Welfare (Grant No. 201404403) and the National Key R&D Program of China (2016YFC0501503-1-1).

References

- van Achterberg C (1993) Illustrated key to the subfamilies of the Braconidae (Hymenoptera: Ichneumonoidea). Zoologische Verhandelingen, Leiden 283: 1–189. <https://www.repositorio.naturalis.nl/document/150389>
- Ashmead WH (1900) Classification of the ichneumon flies, or the superfamily Ichneumonoidea. Proceedings of the United States National Museum 23: 1–120. <https://doi.org/10.5479/si.00963801.23-1206.1>
- Belokobylskij SA (1996) A contribution to the knowledge of the Doryctinae of Taiwan (Hymenoptera: Braconidae). Zoosystematica Rossica 5: 153–191. <https://www.cabi.org/isc/abstract/19971105200>
- Belokobylskij SA (1998) Subfamily Doryctinae. In: Lehr PA (Ed.) Key to Insects of the Russian Far East. Neuropteroidea, Mecoptera, Hymenoptera. Part 4. Dal'nauka, Vladivostok, 50–109.
- Belokobylskij SA, Taeger A, van Achterberg C, Haeselbarth E, Riedel M (2003) Checklist of the Braconidae (Hymenoptera) of Germany. Beiträge zur Entomologie 53: 341–435. <https://doi.org/10.21248/contrib.entomol.53.2.341-435>
- Belokobylskij SA, Tang P, He JH, Chen XX (2012) The genus *Doryctes* Haliday, 1836 (Hymenoptera: Braconidae, Doryctinae) in China. Zootaxa 3226: 46–60. <https://doi.org/10.11646/zootaxa.3226.1.2>
- Belokobylskij SA, Tobias VI (1986) Subfam. Doryctinae. In: Medvedev G.S. (ed.) Key to Insects of the USSR European part. Hymenoptera. Leningrad, Nauka 3(4): 21–72.
- Belokobylskij SA, Maeto K (2009) Doryctinae (Hymenoptera, Braconidae) of Japan (Fauna mundi. Vol. 1). Warszawska Drukarnia Naukowa, Warszawa, 806 pp. [197 figs, 16 photographs]
- Chao HF (1957) On south-eastern Chinese braconid-flies of the subfamily Spathinae (Braconidae). Transactions of the Fujian Agricultural College 4: 1–18.
- Chao HF (1977) A study on Chinese braconid wasps of the tribe Spathiini (Hymenoptera: Braconidae, Doryctinae). Acta Entomologica Sinica 20(2): 205–216.
- Chen JH, Shi QX (2004) Systematic studies on Doryctinae of China (Hymenoptera: Braconidae). Fujian Science and Technology Publishing House, Fujian, 274 pp.
- Dalla Torre CG (1898) Catalogus Hymenopterorum, 4, Braconidae. G. Engelmann, Leipzig, 323 pp.
- Fischer M (1966) Über gezuchtete Braconiden aus Europa (Hymenoptera). Zeitschrift für Angewandte Entomologie 58: 323–339. <https://doi.org/10.1111/j.1439-0418.1966.tb04349.x>
- Foerster A (1863) Synopsis der Familien und Gattungen der Braconiden. Verhandlungen des Naturhistorischen Vereins der Preussischen Rheinlande und Westfalens 19: 225–288.
- Kokujev NR (1898) Symbolae ad cognitionem Braconidarum Imperii Rossici et Asiae Centralis. Trudy Russkago Entomologicheskago Obshchestva [Horae Societatis Entomologicae Rossicae] 32: 345–411.
- Marshall TA (1888) Les Braconides. In: André E (Ed.) Species des Hyménoptères d'Europe et d'Algérie. 4: 7–609. <https://doi.org/10.5962/bhl.title.10281>

- Nixon GEJ (1943) A revision of the Spathiinae of the old world (Hymenoptera, Braconidae). Transaction of the Royal Entomological Society of London 93(2): 173–495. <https://doi.org/10.1111/j.1365-2311.1943.tb00434.x>
- Papp J (1960) A revision of the tribe Braconini Ashmead from the Carpathian Basin (Hymenoptera, Braconidae). Annales Historico-Naturales Musei Nationalis Hungarici 52: 367–380. <https://eurekamag.com/research/037/703/037703223.php>
- Papp J (1984) Contributions to the braconid fauna of Hungary, V. Doryctinae. (Hymenoptera: Braconidae). Folia Entomologica Hungarica 45: 173–185.
- Ratzeburg JTC (1852) Die Ichneumoniden der Forstinsecten in forstlicher und entomologischer Beziehung. Dritter Band, Berlin, 272 pp. <https://doi.org/10.5962/bhl.title.11094>
- Reinhard H (1865) Beiträge zur Kenntnis einiger Braconiden-Gattungen. Drittes Stück. Berliner Entomologische Zeitschrift 9: 241–267. <https://doi.org/10.1002/mmnd.18650090309>
- Shenefelt RD, Marsh PM (1976) Braconidae 9 Doryctinae. Hymenopterorum Catalogus (nova editio) Pars 13: 1263–1424.
- Szépligeti G (1901) A palaearktibus Braconidák meghatarozó táblázatai. [Bestimmungstabelle der palaearktischen Braconiden.] Termeszeti Tud. Kozl., Allattani Kozlem 33: 174–184, 261–288.
- Tang P, Belokobylskij S, Chen XX (2014) The genus *Polystenus* (Hymenoptera: Braconidae: Doryctinae) in China, with descriptions of two new species. Journal of Insect Science 14(66): 1–11. <https://doi.org/10.1093/jis/14.1.66>
- Tang P, Belokobylskij S, Chen XX (2015) *Spathius* Nees, 1818 (Hymenoptera: Braconidae, Doryctinae) from China with a key to species. Zootaxa 3960(1): 1–132. <https://doi.org/10.11646/zootaxa.3960.1.1>
- Tobias VI (1961) A new genus of the tribe Doryctini (Hymenoptera: Braconidae) and its taxonomic importance. Zoologicheskii Zhurnal 40(4): 529–535. [in Russian with English summary]
- Thomson CG (1892) XLIV, Bidrag till Braconidernas Kannedom. Opuscula Entomologica 16: 1659–1751.
- Wang CX, Zhao JT, Sui JZ (1995) *Agrilus mali* Matsumura happened in Xinjiang. (in Chinese). Xinjiang Agricultural Sciences 5: 225–226. http://xueshu.baidu.com/usercenter/paper/show?paperid=371481e3f2a0639a6d504f7ba5b1276d&site=xueshu_se
- Wang YP, Shi M, Chen XX, He JH (2009) The genus *Atanycolus* Foerster (Hymenoptera, Braconidae, Braconinae) in China, with description of one new species. ZooKeys 27: 31–41. <https://doi.org/10.3897/zookeys.27.251>
- Yu DS, van Achterberg C, Horstmann K (2016) Taxapad 2015: World Ichneumonoidea, Taxonomy, biology, morphology and distribution. Vancouver. <http://www.taxapad.com> [accessed 9 October 2018]

Three new species of the genus *Neophyllomyza* Melander (Diptera, Milichiidae) from China, with a revised key to the Chinese species

Yu-Qiang Xi¹, Ding Yang², Xin-Ming Yin¹

1 Department of Entomology, Henan Agricultural University, No. 95 Wenhua Road, Jinshui District, Zhengzhou 450003, Henan Province, China **2** Department of Entomology, China Agricultural University, No. 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China

Corresponding author: Xin-Ming Yin (xinmingyin@126.com)

Academic editor: Owen Lonsdale | Received 16 May 2019 | Accepted 19 July 2019 | Published 30 July 2019

<http://zoobank.org/910730A9-C37F-4DCC-98D4-3E92F07AB9FE>

Citation: Xi Y-Q, Yang D, Yin X-M (2019) Three new species of the genus *Neophyllomyza* Melander (Diptera, Milichiidae) from China, with a revised key to the Chinese species. ZooKeys 867: 123–137. <https://doi.org/10.3897/zookeys.867.36247>

Abstract

Three new species of the genus *Neophyllomyza*, *N. clavipalpis* **sp. nov.**, *N. motuoensis* **sp. nov.**, and *N. obtusa* **sp. nov.**, are described from China. A revised key to the six Chinese species of *Neophyllomyza* is also presented.

Keywords

Diptera, Milichiidae, *Neophyllomyza*, morphology, taxonomy

Introduction

Neophyllomyza Melander, 1913 is a small genus in the subfamily Phyllomyzinae with 13 known species, which are distributed worldwide. There are three Palearctic species (Villeneuve 1920; Hendel 1924; Iwasa 2019), three Oriental species (Xi and Yang 2014), two Afrotropical species (Lamb 1914; Séguéy 1938), two Australian species (Hendel 1907; Curran 1936), two Nearctic species (Melander 1913; Brochu and Wheeler 2009), and one Neotropical species (Williston 1896). Adults of *Neophyllomyza* are small acalyptrate flies. *Neophyllomyza* species are kleptoparasites, with adults mostly sucking at the prey of spiders or insects (Robinson and Robinson 1977; Sivinski and Stowe 1980).

Three species have been recorded from China (Xi and Yang 2014), *N. luteipalpis*, *N. lii*, and *N. tibetensis*. In this study, all known Chinese species are reviewed, and three new species are described.

Materials and methods

Genitalia preparations were made by removing and macerating the apical portion of the abdomen in glacial acetic acid, then rinsing them in distilled water before storing them in glycerine filled microvials. After examination, genitalia were transferred to fresh glycerine and stored in a microvial on the pin below the specimen or moved to an ethanol tube together with the wet specimens. Specimens examined were deposited in the Entomological Museum of Henan Agricultural University (HAU), Zhengzhou; the Entomological Museum of China Agricultural University (CAU), Beijing. The general terminology follows McAlpine (1981) and Brake (2000). The following abbreviations are used:

| | | | |
|------------|---------------------------|---------------|------------------------|
| asc | apical scutellar seta(e), | prsc | prescutellar seta(e), |
| pa | postalar seta(e), | ia | intraalar seta(e), |
| bsc | basal scutellar seta(e), | sa | supraalar seta(e), |
| pos | postsutural seta(e), | kepsts | katepisternal seta(e), |
| dc | dorsocentral seta(e), | S | sternite, |
| prs | presutural seta(e), | npl | notopleural seta(e), |
| h | humeral seta(e), | T | tergite. |

Taxonomy

Neophyllomyza Melander, 1913: 243.

Type species. *Neophyllomyza quadricornis* Melander, 1913

Diagnosis. Body small, 1.0–1.6 mm, brownish to dark brown. Postocellar setae cruciate or converging; paired cruciate setae present along the middle of the front; fronto-orbital setae extending to anterior margin of frons, the upper ones diverging, the lower converging; face excavated, cheeks narrow; palpus enlarged, compressed, apical setulae usually present; lunule small, bare.

Neophyllomyza clavipalpis sp. nov.

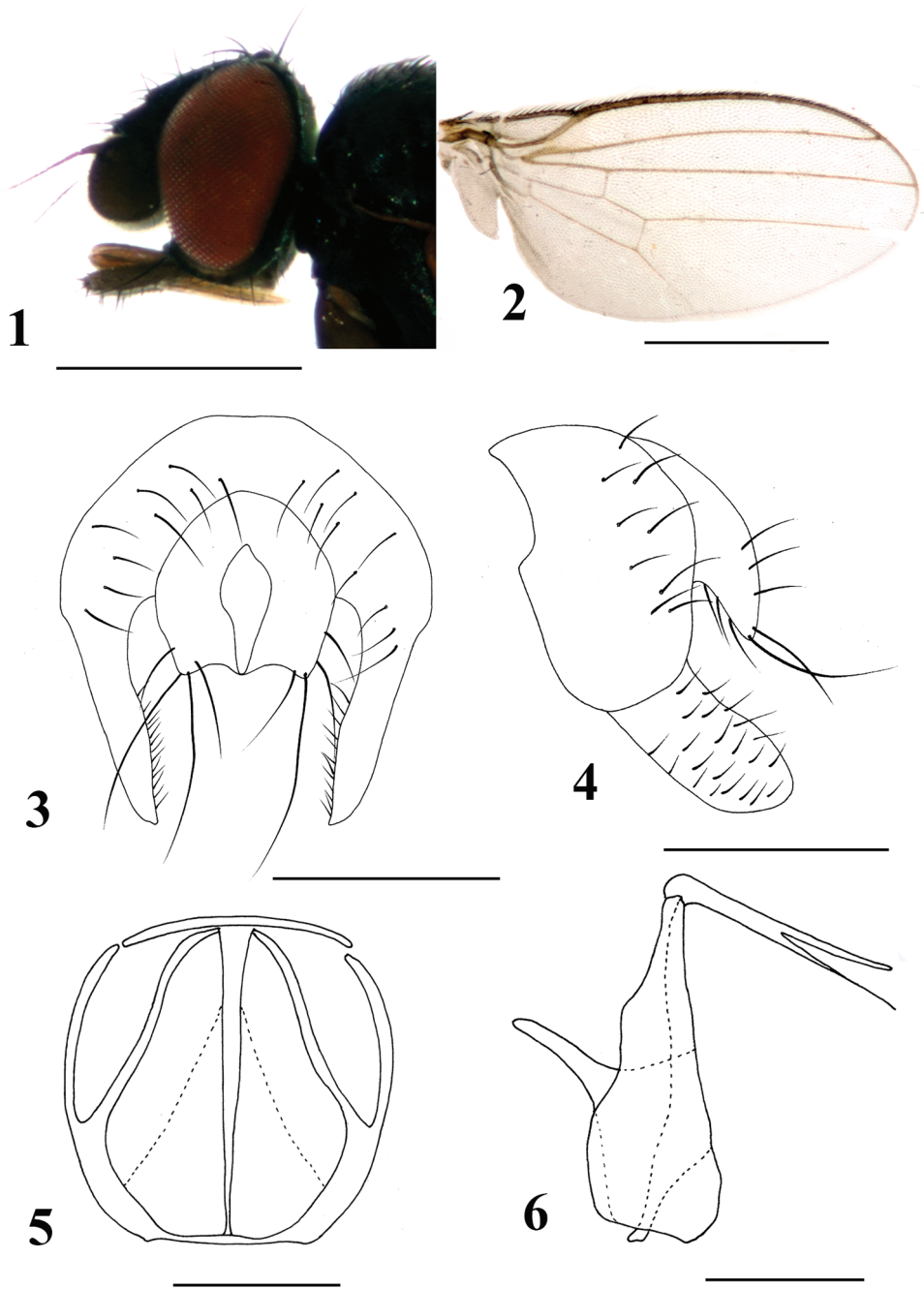
<http://zoobank.org/78E8652E-9140-4927-9FA4-1A2D9E6BFB10>

Figures 1–6

Diagnosis. Gena relatively narrowed, approximately one-twelfth eye height; palpus brown, apically with black sparse setae (Fig. 1). Epandrium irregularly horseshoe-shaped with strong setae; surstylus elongate and margin with dense setulae. Hypandrium narrowed; phallapodeme rod-like. (Fig. 5).

Description. *Male.* Body length 1.4–1.5 mm; wing length 1.3–1.4 mm.

Head (Fig. 1) darkish black with greyish microtomentum; orbital plate satiny blackish brown without microtomentum, ocellar triangle darkish black without mi-



Figures 1–6. *Neophyllomyza clavipalpis* sp. nov. (male). **1** Head, lateral view **2** wing **3** epandrium, cerci, and surstyli, posterior view **4** epandrium, cerci, and surstyli, lateral view **5** genitalia, posterior view (hypandrium; phallapodemic; subepandrial) **6** genitalia in lateral view (hypandrium; phallapodemic; subepandrial). Scale bars: 0.5 mm (**1, 2**); 0.1 mm (**3–6**).

crotomentum; lunule small, blackish with black margin. Posterior eye margin ventrally diverging from head margin; eye 1.4 times as high as long, gena approximately one-twelfth of eye height. Setae and setulae on head black; ocellar triangle with two ocellar setae and three short setae; frons with two orbital and two frontal setae, orbital setae laterocline and frontal setae mediocline, three interfrontal setae; postocellar setae converging. Vibrissal angle relatively blunt; vibrissa strong, located above the level of lower margin of eye. Antenna darkish black, with microtomentum; pedicel with short black setae at middle and margin, setulae at margin longer than others, longest one approximately four times longer than others; first flagellomere with pubescence, approximately quadrate, apical margin smooth; arista two times as long as first flagellomere, black, pubescent very short. Proboscis geniculate, brownish-yellow, margin without setulae. Palpus rod-like, with blunt apex, narrow, approximately 0.2 mm, four times longer than wide; brown with short dense brownish pubescence, margin with black sparse setae.

Thorax black with grey microtomentum, except scutum shiny blackish-brown with sparse black microtomentum; scutellum darkish brown with grey microtomentum. Setae and setulae on thorax black; one h, two dc, one prsc, two npl, one prs, two pa, one kepsts (setulae at forward position); scutellum 1.5 times wider than long, with pair of asc and bsc, asc 2.5 times longer than bsc. Legs slender, darkish brown. Setae and setulae on legs black. Mid tibia with one black preapical dorsal seta. Wing hyaline (Fig. 2), unspotted; veins brown; Sc strong; M_1 between r-m and dm-cu a little longer than dm-cu. Calypter yellowish, with dense brownish microtrichiae, margin with brownish setulae. Knob of halter brownish, stalk brown.

Abdomen darkish brown with grey microtomentum. Setae and setulae on abdomen black; TII-TV with setae, marginal setae slightly longer than others; sternites with sparse black setulae. SII generally luniform, SIII and SIV broad with middle narrower, SIV wider than SIII; SV very shallowly falciform, apical margin extends to basal margin. Male genitalia (Figs 3–6): epandrium irregularly falciform with strong setae; surstylus elongate and margin with dense setulae; hypandrium narrowed, ribbon pattern; phallapodeme pear-shaped; subepandrial sclerite well developed; cercus wide with long setae.

Female. Unknown.

Type material. Holotype: ♂, **China:** Jilin, Antu, Changbai Mountain (42°27'14.20"N, 128°08'15.98"E; 1100 m), 26.VIII.2015, Yu-Qiang Xi (HAU). Paratype: 1 ♂, same data as holotype (HAU).

Distribution. China (Jilin).

Etymology. The specific name refers to the shape of the palpus.

Remarks. This new species is somewhat similar to *N. acyglossa* (Villeneuve), but can be differentiated from the latter by the following features: M_1 between r-m and dm-cu 1.8 times as long as dm-cu; knob of halter brownish, stalk brown; epandrium irregularly luniform. In *N. acyglossa*, M_1 between r-m and dm-cu is 1.2 times as long as dm-cu; knob of halter dark brown, stalk blackish brown; epandrium very finely crescentic (Villeneuve 1920).

***Neophyllomyza motuoensis* sp. nov.**

<http://zoobank.org/19C2D14C-E401-4577-90CD-A5E107C771AA>

Figures 7–12

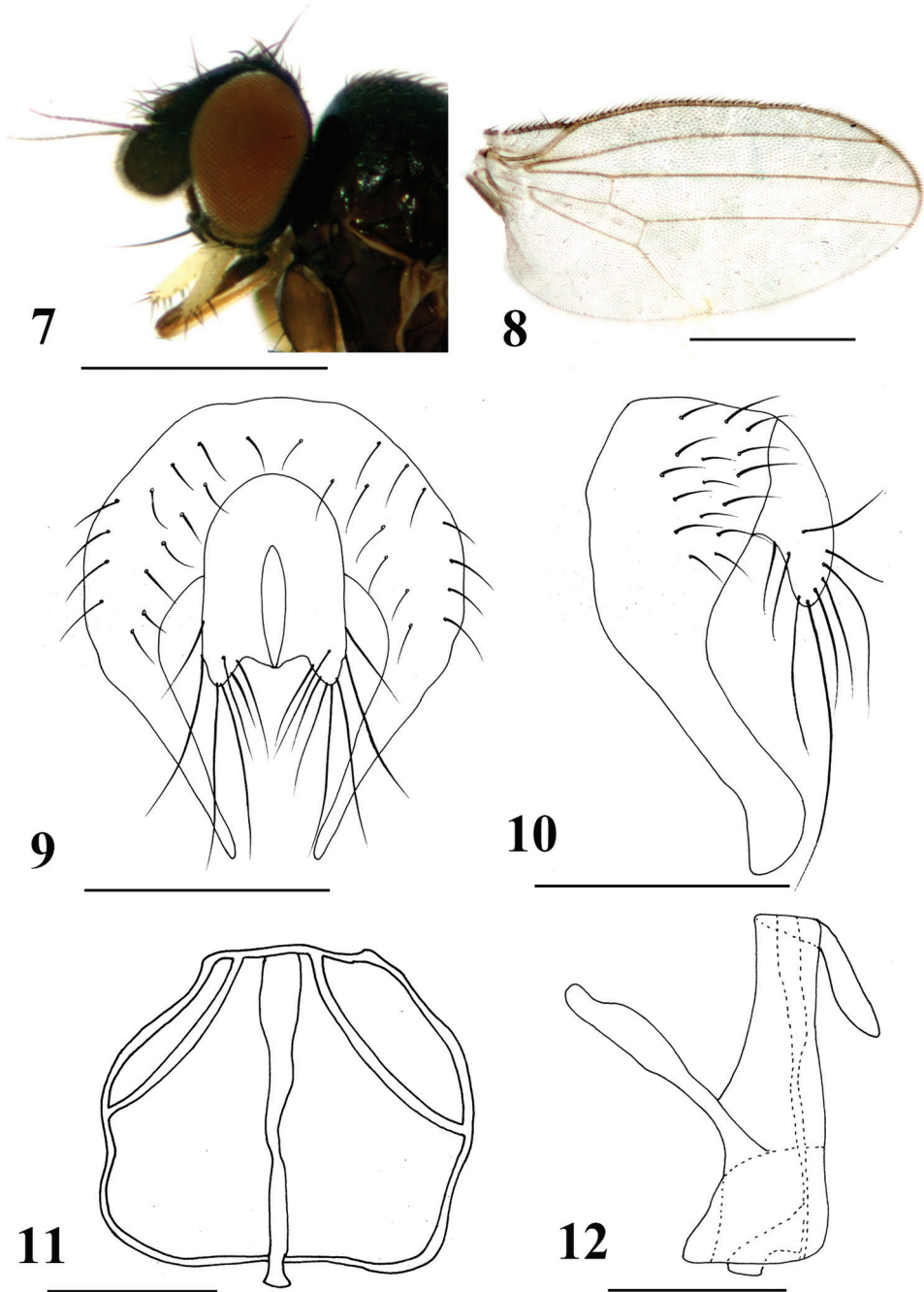
Diagnosis. Gena relatively narrowed, approximately one-ninth of eye height; palpus yellowish with short dense brownish pubescence (Fig. 7). Surstylus elongate, apically slightly blunted; hypandrium narrowed, irregularly quadrate; subepandrial sclerite well-developed. Cercus enlarged with long setae (Figs 9–12).

Description. *Male.* Body length 1.2–1.5 mm; wing length 1.3–1.6 mm.

Head (Fig. 7) blackish-brown with greyish microtomentum; orbital plate subshiny blackish-brown without microtomentum; ocellar triangle darkish brown without microtomentum; lunule small, darkish yellow with black margin. Posterior eye margin ventrally diverging from head margin; eye 1.5 times as high as long, gena approximately one-ninth of eye height. Setae and setulae on head black; ocellar triangle with two ocellar setae and three short setae; frons with two orbital and two frontal setae, orbital setae laterocline and frontal setae mediocline, three interfrontal setae; postocellar setae converging. Vibrissal angle relatively blunt; vibrissa strong, located at the level of lower margin of eye. Antenna darkish brown with microtomentum; pedicel with short black setae at middle and margin, setulae at margin longer than others, longest one approximately 2.5 times longer than others; first flagellomere with pubescence, approximately quadrate; arista 2.5 times as long as first flagellomere, darkish brown, distinctly short pubescence. Proboscis geniculate, darkish brown, margin without setulae. Palpus oblong with blunt apex in lateral view, approximately 0.2 mm, 4 times longer than wide; yellowish with short dense brownish pubescence, apically with short and long sparse black setae.

Thorax darkish brown with grey microtomentum, except scutum shiny blackish-brown with sparse black microtomentum; scutellum darkish brown with grey microtomentum. Setae and setulae on thorax black; one h, two dc, one prsc, two npl, one prs, one sa, one pa, one kepts (a row of setulae at forward position); scutellum 1.5 times wider than long, with pair of asc and bsc, asc 2.5 times longer than bsc. Legs slender, darkish brown except tarsi yellow. Setae and setulae on legs black. Mid tibia with one black preapical dorsal seta. Wing hyaline (Fig. 8); veins brown; Sc strong; M_1 between r-m and dm-cu longer than dm-cu. Calypter yellowish, with dense brownish microtrichae, margin with brownish setulae. Knob of halter yellowish white, stalk brownish.

Abdomen darkish brown with grey microtomentum. Setae and setulae on abdomen black; TII–T V with setae, rowed marginal setae slightly longer than others; sternites with sparse setulae. SII generally luniform, apically slightly blunt; SIII oblong; SIV irregularly quadrate; SV falciform, apical margin extends to basal margin extremely at mid. Male genitalia (Figs 9–12): epandrium irregularly semi-circular, with strong setae; surstylus elongate, apically slightly blunted, margin without dense setulae; hypandrium narrowed, irregularly quadrate; subepandrial sclerite well-developed; phallapodeme linearly pear-shaped. Cercus enlarged with long setae.



Figures 7–12. *Neophyllomyza motuoensis* sp. nov. (male). **7** Head, lateral view **8** wing **9** epandrium, cerci, and surstyli, posterior view **10** epandrium, cerci, and surstyli, lateral view **11** genitalia, posterior view (hypandrium; phallapodemic; subepandrial) **12** genitalia, lateral view (hypandrium; phallapodemic; subepandrial). Scale bars: 0.5 mm (**7,8**); 0.1 mm (**9–12**).

Female. Body length 1.3–1.6 mm; wing length 1.4–1.6 mm. Similar to male. Female terminalia: TVIII distinctly narrowed, blackish with dense short microtomentum. Supra-anal plate irregularly quadrate; subanal plate luniform, apically wide and blunt, with sparse setulae. Cercus long, apically nearly circular; black, with sparse setulae.

Type material. Holotype: ♂, **China:** Tibet, Motuo, 80 K (29°39'20.75"N, 95°29'17.59"E, 1000 m), 23.VII.2012, Xuan-Kun Li (CAU). Paratypes: 1 ♂, 1 ♀, same data as holotype (CAU); 1 ♂, 2 ♀♀, China, Tibet, Motuo, Beibeng (29°14'30.75"N, 95°10'18.59"E, 700 m), 30.VII.2012, Wen-Liang Li (CAU).

Distribution. China (Tibet).

Etymology. The specific name refers to the type locality of Motuo County.

Remarks. This new species is somewhat similar to *N. lii* Xi et Yang, 2014 but can be differentiated from the latter by the following features: postocellar setae converging; three interfrontal setae; scutellum 1.5 times wider than long, with pair of asc and bsc, asc 2.5 times longer than bsc. In *N. lii*, postocellar setae cruciate; four interfrontal setae; scutellum two times wider than long, with pair of asc and bsc, asc three times longer than bsc (Xi and Yang 2014).

***Neophyllomyza obtusa* sp. nov.**

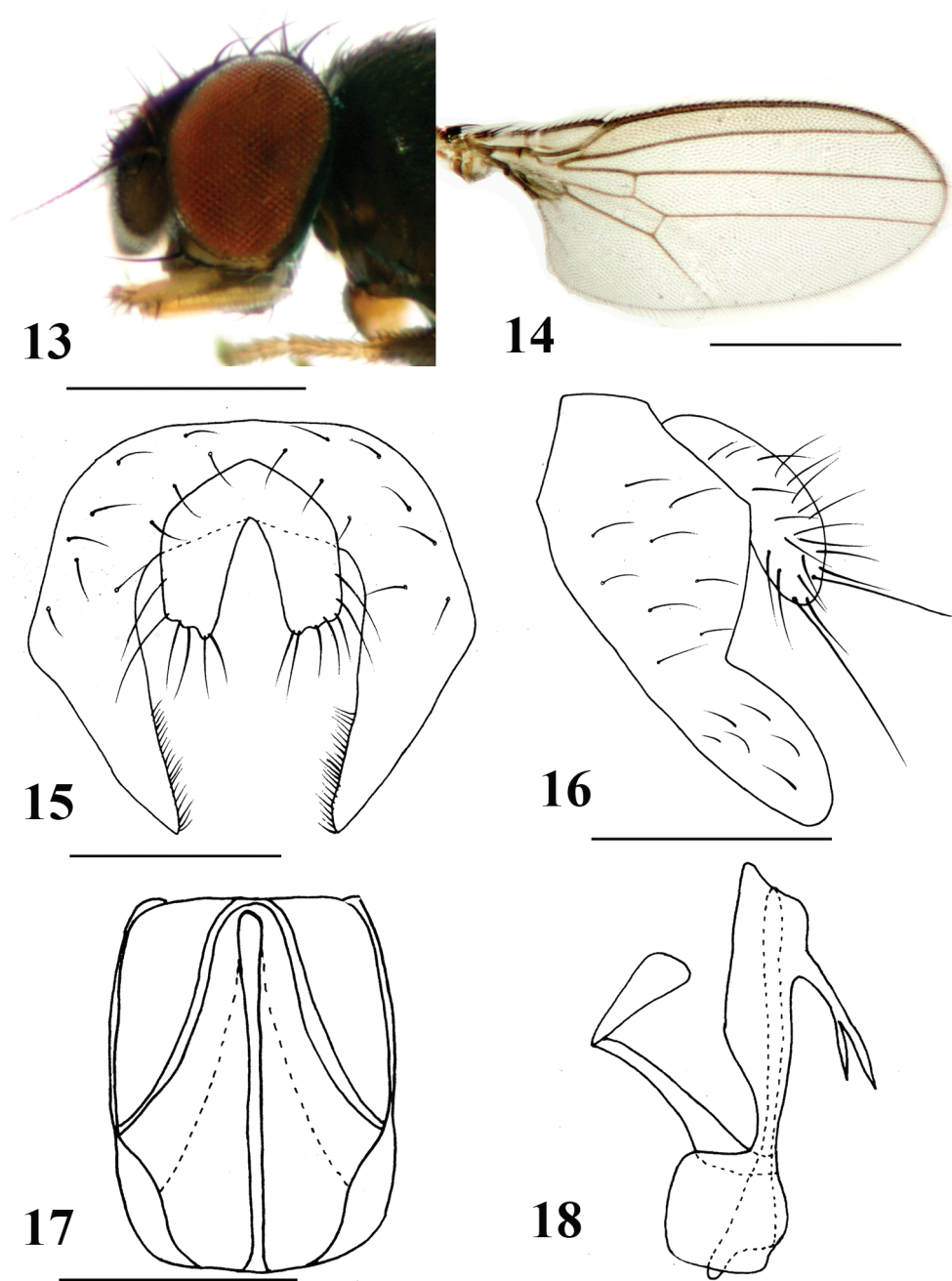
<http://zoobank.org/1ABDE481-E006-43CD-BADB-BBA0722E53C6>

Figures 13–18

Diagnosis. Gena narrowed, approximately one-fifteenth eye height; palpus darkish yellow and terminal with short and long sparse black setae (Fig. 13). Surstylus wide, apically blunt; hypandrium narrowed, finely luniform; subepandrial sclerite well-developed, irregularly quadrate. Cercus enlarged with long setae (Figs 15–18).

Description. *Male.* Body length 1.1–1.2 mm; wing length 1.1–1.2 mm.

Head (Fig. 13) black with greyish microtomentum; orbital plate subshiny black without microtomentum; ocellar triangle black without microtomentum; lunule small, yellow with brown margin. Posterior eye margin ventrally diverging from head margin; eye 1.4 times as high as long, gena approximately one-fifteenth eye height. Setae and setulae on head black; ocellar triangle with two ocellar setae and three short setae; frons with two orbital and two frontal setae, orbital setae laterocline and frontal setae mediocline, three interfrontal setae; postocellar setae cruciate. Vibrissal angle blunt; vibrissa strong, located at level of lower margin of eye. Antenna brown with microtomentum; pedicel with short black setulae at middle and margin, setulae at margin longer than others, longest one approximately 3.5 times longer than others; first flagellomere with pubescence, irregularly circular; arista four times as long as first flagellomere, brown, distinctly pubescent. Proboscis long, geniculate, darkish yellow, margin with short sparse black setulae. Palpus oblong with blunt apex in lateral view, less than 0.2 mm, four times longer than wide; darkish yellow, with short dense brownish pubescence, apically with short and long sparse black setae.



Figures 13–18. *Neophyllomyza obtusa* sp. nov. (male). **13** Head, lateral view **14** wing **15** epandrium, cerci, and surstyli, posterior view **16** epandrium, cerci, and surstyli, lateral view **17** genitalia, posterior view (hypandrium; phallapodemic; subepandrial) **18** genitalia, lateral view (hypandrium; phallapodemic; subepandrial). Scale bars: 0.5 mm (**13**, **14**); 0.1 mm (**15–18**).

Thorax darkish brown with grey microtomentum, except mesonotum shiny darkish brown with sparse black microtomentum; scutellum blackish-brown with grey microtomentum. Setae and setulae on thorax black; one h, two dc, one prsc, two npl, one prs, one pos, one ia, one sa, one pa, one kepsts (setulae at forward position); scutellum 1.6 times wider than long, with pair of asc and bsc, asc three times longer than bsc. Legs slender, darkish brown, except tarsi darkish yellow. Setae and setulae on legs black. Mid tibia with 1 black preapical dorsal seta. Wing hyaline (Fig. 14); veins brown; Sc strong; M_1 between r-m and dm-cu longer than dm-cu. Calypter yellowish with dense brownish microtrichae, margin with brownish setulae. Knob of halter brown, stalk brownish.

Abdomen darkish brown with grey microtomentum. Setae and setulae on abdomen black; TII-T V with setae at posterior 3/4, marginal setae longer than others; sternites with sparse setulae. SII generally luniform, apical margin slightly blunt; SIII oblong; SIV very broadly broad with middle narrower, apical margin slightly wider than basal margin; SV broadly broad with middle narrower, the apical margin smooth and depressed falciform. Male genitalia (Figs 15–18): epandrium irregularly luniform, with strong setae; surstylus wide, apically blunt; hypandrium narrowed, very shallowly falciform; subepandrial sclerite well-developed, irregularly quadrate; phallapodemic sclerite linearly irregularly quadrato-rhombiform. Cercus enlarged with long setae.

Female. Unknown.

Type material. Holotype: ♂, **China:** Guangxi, Fangchenggang, Shangsi (21°54'27.25"N, 107°54'18.81"E, 1400 m), 17.V.2013, Xing-Yue Liu (CAU). Paratypes: 2 ♂♂, same data as holotype (CAU).

Distribution. China (Guangxi).

Etymology. The specific name refers to the shape of the vibrissal angle.

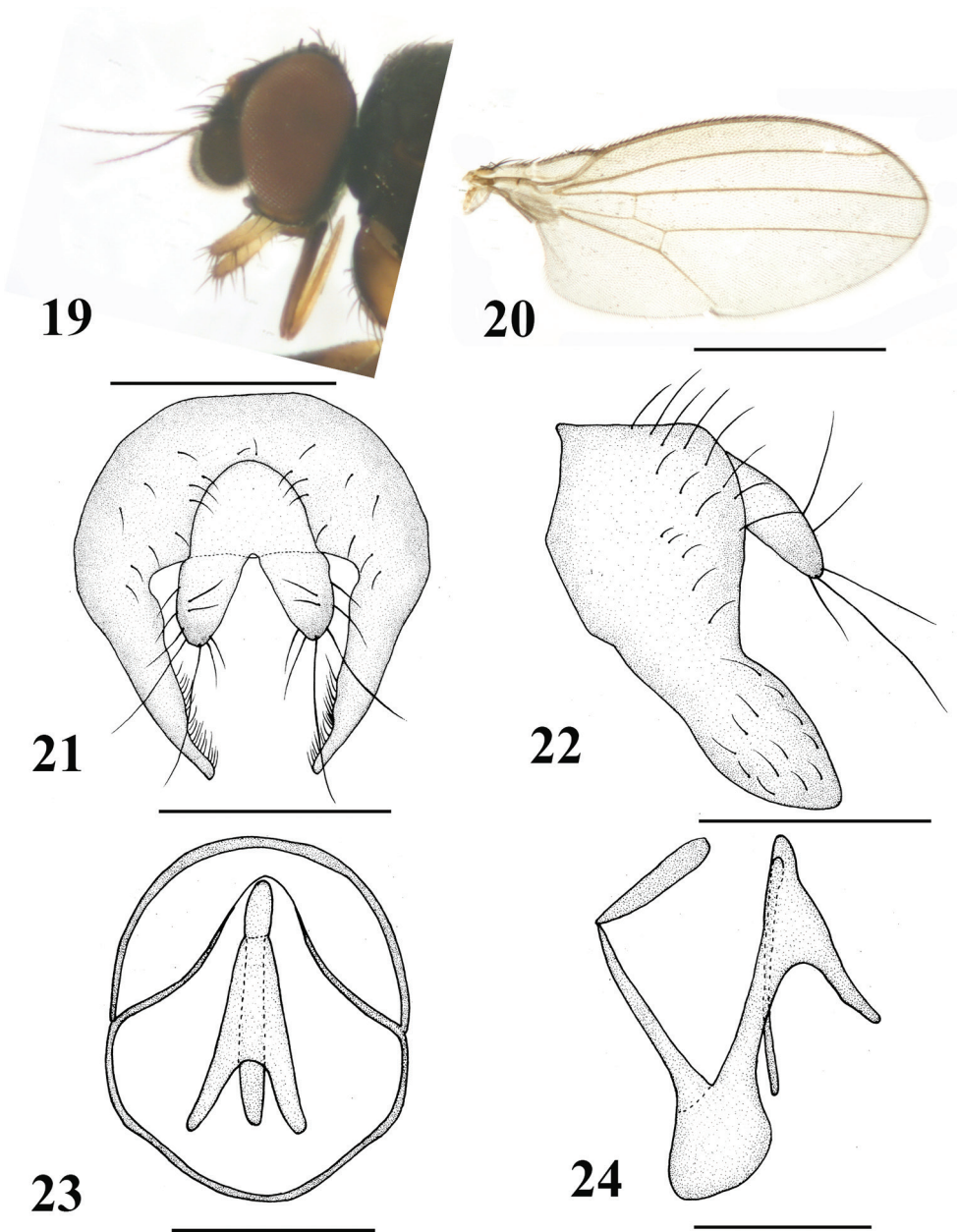
Remarks. This new species is somewhat similar to *N. leanderi* (Hendel, 1924), but can be differentiated from the latter by the following features: knob of halter brown, stalk brownish; surstylus wide, apically blunt; cercus wide. In *N. leanderi*, knob of halter yellowish, stalk yellow; surstylus relatively narrow and long; cercus long and thin (Hendel 1924).

Neophyllomyza luteipalpis Xi & Yang, 2014

Figures 19–24

Neophyllomyza luteipalpis Xi et Yang, 2014: 1641. Type-locality: China (Yunnan).

Diagnosis. Body, 1.4–1.6 mm; wing, 1.4–1.6 mm. Gena very narrowed, approximately one-nineteenth eye height. Palpus darkish yellow, apically brownish and with strong setae. Epandrium irregularly hemispherical with strong setae; surstylus elongate and margin with dense setulae; cercus bifurcated and with long setae.



Figures 19–24. *Neophyllomyza luteipalpis* Xi & Yang, 2014 (male). **19** Head, lateral view **20** wing **21** epandrium, cerci, and surstyli, posterior view **22** epandrium, cerci, and surstyli, lateral view **23** genitalia, posterior view (hypandrium; phallapodemic; subepandrial) **24** genitalia, lateral view (hypandrium; phallapodemic; subepandrial). Scale bars: 0.5 mm (**19, 20**); 0.1 mm (**21–24**).

Material. 1 ♂, **China:** Yunnan, Baoshan, Baihualing (25°17'32.85"N, 98°48'23.08"E; 1575 m), 12.VII.2012, Wen-Liang Li (CAU); 1 ♂, **China:** Yunnan, Yingjiang, Xima (24°36'56.63"N, 97°39'23.09"E; 1390 m), 5.V.2012, Wen-Liang Li (CAU); 1 ♂, **China:** Yunnan, Tengchong, Guangming (25°29'15.92"N, 98°32'28.86"E; 1835 m), 7.V.2012, Wen-Liang Li (CAU); 3 ♀♀, **China:** Yunnan, Baoshan, Baihualing (25°17'32.85"N, 98°48'23.08"E; 1575 m), 16.V.2012, Wen-Liang Li (CAU).

Distribution. China (Yunnan).

Neophyllomyza lii Xi & Yang, 2014

Figures 25–30

Neophyllomyza lii Xi et Yang, 2014: 1643. Type-locality: China (Yunnan).

Diagnosis. Body, 1.4–1.6 mm; wing, 1.2–1.4 mm. Gena narrowed, approximately one-twelfth eye height; palpus brownish; apically with both short and long sparse black setae; epandrium irregularly hemispherical, with strong setae; surstylus elongate with apex slightly acute, margin with dense setulae; cercus bifurcated and elongate, with long setae.

Material. 1 ♂, **China:** Yunnan, Yingjing, Tongbiguan (24°36'56.63"N 97°39'23.09"E, 1340 m), 1.V.2012, Wen-Liang Li (CAU); 1 ♂, **China:** Yunnan, Baoshan, Dahooping (25°19'18.84"N, 100°08'32.04"E, 1925 m), 11.V.2012, Wen-Liang Li (CAU).

Distribution. China (Yunnan).

Neophyllomyza tibetensis Xi & Yang, 2014

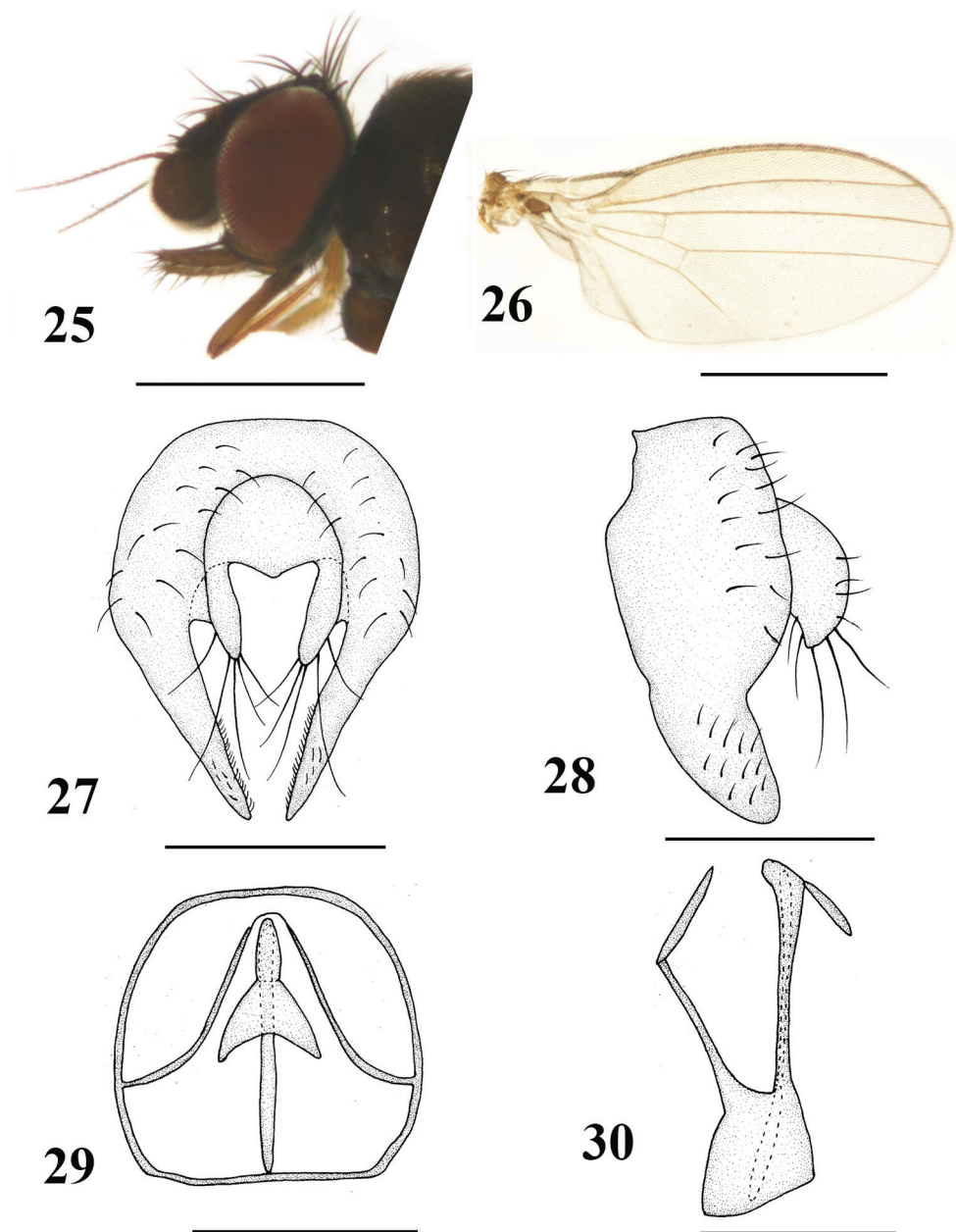
Figures 31–36

Neophyllomyza tibetensis Xi et Yang, 2014: 1646. Type-locality: China (Tibet).

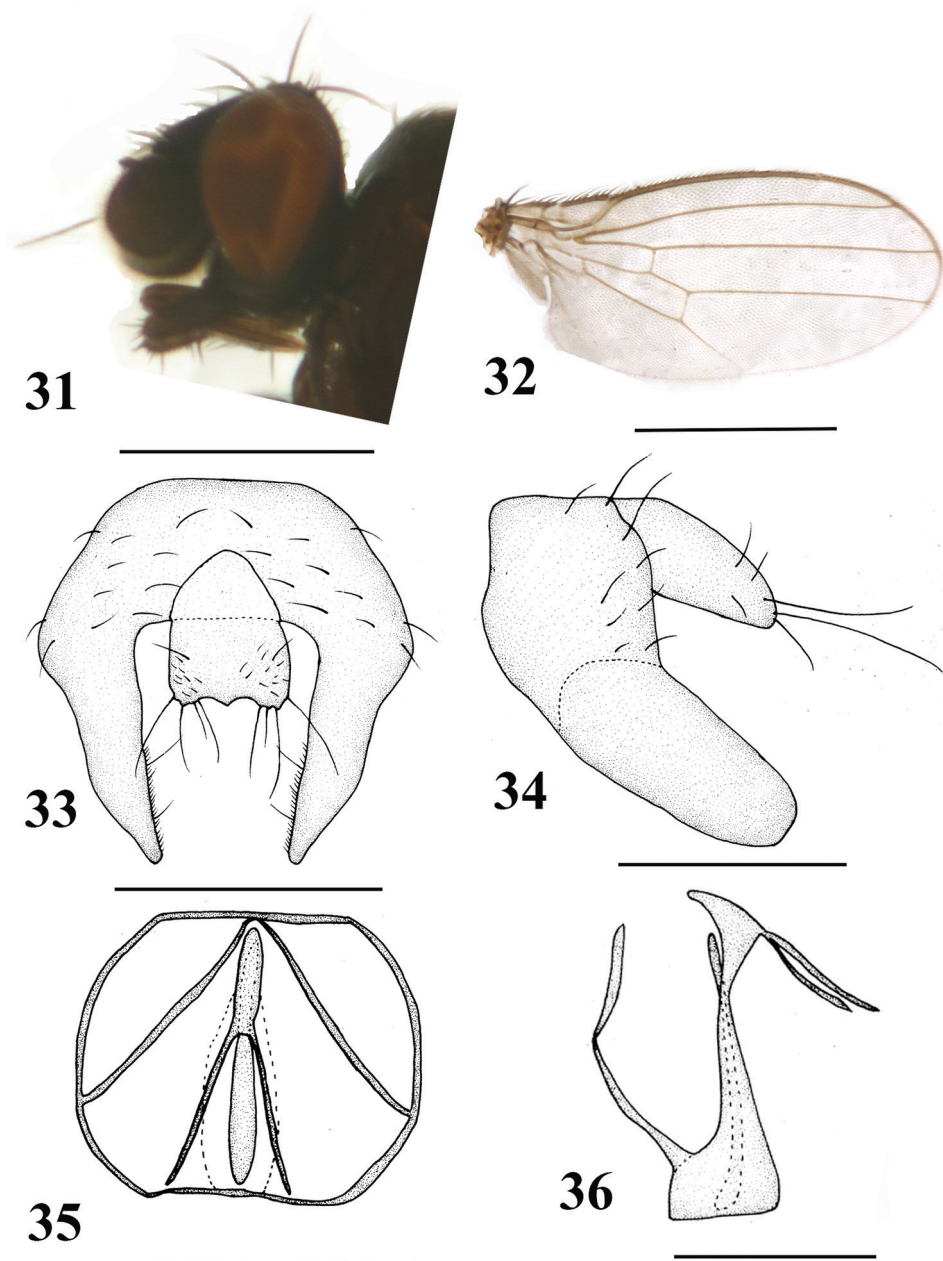
Diagnosis. Body, 1.2–1.4 mm; wing, 1.2–1.4 mm. Gena relatively narrowed, approximately one-eighth eye height. Palpus darkish brown and apically with sparse black setae. Epandrium irregularly falciform, with strong setae; surstylus elongate and slightly blunted apically, margin with dense setulae; cercus broad with long setae.

Material. 1 ♂, **China:** Tibet, Linzhi, Bayi Town (29°38'32.58"N 94°21'32.55"E, 825 m), 5. V. 2012, Wen-Liang Li (CAU); 1 ♀, **China:** Tibet, Linzhi, Bayi Town (29°38'32.58"N, 94°21'32.55"E, 810 m), 30. VI. 2012, Xuan-Kun Li (CAU).

Distribution. China (Tibet).



Figures 25–30. *Neophyllomyza lii* Xi & Yang, 2014 (male). **25** Head, lateral view **26** wing **27** epanthrium, cerci, and surstyli, posterior view **28** epanthrium, cerci, and surstyli, lateral view **29** genitalia, posterior view (hypandrium; phallopodemic; subepandrial) **30** genitalia, lateral view (hypandrium; phallopodemic; subepandrial). Scale bars: 0.5 mm (**25**, **26**); 0.1 mm (**27–30**).



Figures 31–36. *Neophyllomyza tibetensis* Xi & Yang, 2014 (male). **31** Head, lateral view **32** wing **33** epandrium, cerci, and surstyli, posterior view **34** epandrium, cerci, and surstyli, lateral view **35** genitalia, posterior view (hypandrium; phallapodemic; subepandrial) **36** genitalia, lateral view (hypandrium; phallapodemic; subepandrial). Scale bars: 0.5 mm (**31**, **32**); 0.1 mm (**33–36**).

Key to Chinese species (males) of *Neophyllomyza*

| | | |
|---|--|--------------------------------|
| 1 | Postocellar setae cruciate | 2 |
| – | Postocellar setae converging | 4 |
| 2 | Four interfrontal setae; eye 1.6 times as high as long | |
| | <i>N. luteipalpis</i> Xi & Yang | |
| – | Three interfrontal setae; eye 1.4 times as high as long | 3 |
| 3 | M_1 between r-m and dm-cu 1.8 times as long as dm-cu; gena approximately one-twelfth of eye height | <i>N. lii</i> Xi & Yang |
| – | M_1 between r-m and dm-cu 1.5 times as long as dm-cu; gena approximately one-fifteenth of eye height | <i>N. obtusa</i> sp. nov. |
| 4 | Arista 2 times as long as first flagellomere; vibrissa strong, located above the level of lower eye margin | <i>N. clavipalpis</i> sp. nov. |
| – | Arista 2.5 times as long as first flagellomere; vibrissa strong, located at the level of lower eye margin | 5 |
| 5 | Gena approximately one-ninth of eye height; M_1 between r-m and dm-cu 1.8 times as long as dm-cu | <i>N. motuoensis</i> sp. nov. |
| – | Gena approximately one-seventh of eye height; M_1 between r-m and dm-cu 1.3 times as long as dm-cu | <i>N. tibetensis</i> Xi & Yang |

Faunistic remarks

Chinese species of *Neophyllomyza* are mainly distributed in Tibet and Yunnan Province, and *N. obtusa* sp. nov. and *N. clavipalpis* sp. nov. occur in Guanxi and Jilin Provinces; all of these represent new provincial records for *Neophyllomyza* in China. There are five species distributed in the Palearctic Region (Villeneuve 1920; Hendel 1924; Xi and Yang 2014; Iwasa 2019), and three species (*N. clavipalpis* sp. nov., *N. motuoensis* sp. nov., and *N. tibetensis* Xi et Yang) from China in the Palearctic Region. Three Chinese species are distributed in the Oriental Region, *N. obtusa* sp. nov., *N. luteipalpis* Xi et Yang, and *N. lii* Xi et Yang (Xi and Yang 2014). With further research, more species of *Neophyllomyza* will be found, because the Chinese fauna of Milichiidae is extraordinarily rich.

Acknowledgements

Authors express their sincere thanks to Dr. Wenliang Li (Luoyang), Dr. Xuankun Li (Beijing), and Prof. Xingyue Liu (Beijing), for their kind help and for collecting the specimens. This work was supported by the National Natural Science Foundation of China (grant number 31672333), Natural Science Youth Innovation Fund of Henan Agricultural University (grant number KJCX2019A11) and the Agricultural Technology System of Henan (grant number S2014-11-G03).

References

- Brake I (2000) Phylogenetic systematics of the Milichiidae (Diptera, Schizophora). *Entomologia Scandinavica* 57 (Supplement): 1–120.
- Brochu K, Wheeler TA (2009) Systematics and ecology of the Nearctic species of *Neophyllomyza* (Diptera: Milichiidae). *Canadian Entomologist* 141: 103–111. <https://doi.org/10.4039/n09-001>
- Curran CH (1936) The Templeton Crocker Expedition to the western Polynesian and Melanesian islands, 1933. *Proceedings of the California Academy of Sciences* 22: 1–66.
- Hendel F (1907) Neue und interessante Dipteren aus dem kaiserlichen Museum in Wien. *Wiener entomologische Zeitung* 26: 223–245. <https://doi.org/10.5962/bhl.part.8886>
- Hendel F (1924) Neue europäische *Phyllomyza*-Arten (Dipt. Milichiidae). *Deutsche entomologische Zeitschrift* 1924: 405–408. <https://doi.org/10.1002/mmnd.48019240505>
- Iwasa M (2019) Contribution to the knowledge of the Japanese Milichiidae (Diptera), with descriptions of two new species. *Zootaxa* 4551(5): 571–582. <https://doi.org/10.11646/zootaxa.4551.5.5>
- Lamb CG (1914) The Percy Sladen Trust expedition to the Indian Ocean in 1905, under the leadership of Mr. Stanley J, Gardiner MA. Vol. 5. NO. XV. *Transactions of the Linnean Society of London* 16: 307–372. <https://doi.org/10.1111/j.1096-3642.1913.tb00152.x>
- McAlpine JF (1981) Morphology and terminology-adults. In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM (Eds) *Manual of Nearctic Diptera*. Vol. 1, Agriculture Canada Monograph 27: 9–63.
- Melander AL (1913) A synopsis of the dipterous groups Agromyzinae, Milichiinae, Ochthiphilinae, and Geomyzinae. *Journal of the New York Entomological Society* 21: 243–244.
- Robinson MH, Robinson B (1977) Associations between flies and spiders: bibliocommensalism and dipsoparasitism? *Psyche* 84: 150–157. <https://doi.org/10.1155/1977/26019>
- Séguy E (1938) Diptera I. Nematocera et Brachycera. In: Jeannel R (Eds) *Mission Scientifique de l'Omo*. Vol. 4 (Zoologie). *Mémoires du Muséum nationale d'Histoire naturelle (A)* 8: 319–380.
- Sivinski J, Stowe M (1980) A kleptoparasitic cecidomyiid and other flies associated with spiders. *Psyche* 87: 337–348. <https://doi.org/10.1155/1980/27685>
- Villeneuve J (1920) *Vichyia acyglossa*, espèce et genre nouveaux de la famille des Milichiinae (Dipt. Muscidae). *Bulletin Société entomologique d'Égypte* 1920: 69–70.
- Williston SW (1896) On the Diptera of St. Vincent (West Indies). *Transactions of the Entomological Society of London* 1896: 253–446. <https://doi.org/10.1111/j.1365-2311.1896.tb00965.x>
- Xi YQ, Yang D (2014) *Neophyllomyza* (Diptera: Milichiidae) recorded from China with descriptions of three new species. *Florida Entomologist* 97: 1640–1647. <https://doi.org/10.1653/024.097.0438>

***Thraumata*, a new genus from South America with description of a new species from Peru (Lepidoptera, Noctuidae)**

Paul Z. Goldstein¹, Alberto Zilli²

1 Systematic Entomology Laboratory, USDA, National Museum of Natural History, E-502, P.O. Box 37012, MRC 168, Washington, DC 20013-7012, USA **2** Life Sciences, Insects Division, The Natural History Museum, Cromwell Road, London SW7 5BD, UK

Corresponding author: Paul Z. Goldstein (drpzgoldstein@gmail.com)

Academic editor: D. Lafontaine | Received 30 July 2018 | Accepted 14 June 2019 | Published 31 July 2019

<http://zoobank.org/9765595E-1A20-4418-B5C6-AC4B6F587CE5>

Citation: Goldstein PZ, Zilli A (2019) *Thraumata*, a new genus from South America with description of a new species from Peru (Lepidoptera, Noctuidae). ZooKeys 867: 139–160. <https://doi.org/10.3897/zookeys.867.28728>

Abstract

Thraumata **gen. nov.** is described to accommodate three South American species, two previously placed in *Phuphena* Walker, 1858, namely *Thraumata petrovna* (Schaus, 1904), **comb. nov.** and *Thraumata subvenata* (Schaus, 1914), **comb. nov.**; and one, *Thraumata peruviansia* **sp. nov.**, newly described from Peru. Although the larval biology is unknown, these species share several features that suggest their placement in Eriopinae and, as a consequence, a potential association with ferns (Pteridophyta) as larval host plants.

Keywords

Phuphena, Noctuidae, owlet moths, Peru, systematics

Introduction

Phuphena Walker, 1858 appears to represent a polyphyletic assemblage that includes a morphologically homogeneous, putatively monophyletic core group of 10 described species: *P. cilix* (Druce, 1898), *P. constricta* Dognin, 1912, *P. diagona* Hampson, 1908, *P. multilinea* Schaus, 1911, *P. obliqua* (Smith, 1900), *P. parallela* (Hampson, 1904), *P. proselyta* Schaus, 1921, *P. transversa* (Schaus, 1894), *P. tura* (Druce, 1889), *P. zelotypa* Schaus, 1911 and the type species *P. fusipennis* Walker, 1858. Outside this assemblage

are *Phuphena costata* Schaus, 1914, which will be treated elsewhere, and two of the species treated here, *P. petrovna* (Schaus, 1904) and *P. subvenata* Schaus, 1914. Of the 15 available names for species in the genus *Phuphena*, William Schaus described eight in six publications between 1894 and 1921. Three of these species he originally placed in other genera, in some cases denoting the generic name with a question mark, and among these was *P. petrovna* (Schaus, 1904), which Schaus (1904) described in *Leptina* Guenée 1852 nec Meigen 1830 (= *Baileya* Grote, 1895) (Nolidae). In the course of revising *Phuphena* we identified *P. petrovna*, *P. subvenata*, and an undescribed species similar to *P. petrovna* as missing features diagnostic of typical *Phuphena* species while sharing a number of conspicuous features not observed elsewhere in the genus. Our primary purpose here is to describe the new species and a new genus *Thraumata* gen. nov. to accommodate all three species, and as a possible addition to the Eriopinae, now under study. *Phuphena* is currently recognized within the Phosphilini and, although this placement will be treated more thoroughly in a separate revision of *Phuphena*, we address a number of features here that seem to contradict it. We further discuss several characters that bear both on the placement of both *Phuphena* and *Thraumata*, and include a diagnosis of each genus.

Materials and methods

Genitalic preparations follow Clarke (1941) in part and Lafontaine (2004), but staining with chlorazol black and slide-mounting in Euparal. Vesicae were everted with a microsyringe prior to fixation. Dissections followed either an overnight room-temperature soak or a brief 15-minute heated soak in supersaturated sodium hydroxide solution and were examined under dissecting microscopes prior to slide-mounting. Wing preparations followed the procedure modified from Jaeger (2017): following an overnight soak in a small stender dish with enough 50% EtOH to cover the wings and 10 drops of 6% NaCl, ethanol and bleach were added as needed, depending on the ease with which scales could be cleared. Wings were then stained overnight in Eosin Y. Images were taken using Microptics and Visionary Digital imaging systems and images were manipulated with Adobe Photoshop and Illustrator (Adobe Systems, Mountain View, CA). Images of *T. petrovna* vesicae were taken in glycerin, held in a sectioned plexiglass cylinder affixed to a slide. Measurements were made with the aid of an ocular micrometer. Forewing (FW) length was measured from the center of the axillary area up to the apex of the forewing. Terminology generally follows Forbes (1954) and Lafontaine (1998, 2004).

Prior to dissection, the abdomens of several specimens underwent a 24-hour soak in a 10% proteinase K solution in the Laboratory of Analytical Biology at USNM. DNA extractions and partial DNA barcodes were then generated at the Biodiversity Institute of Ontario.

Exemplars used for comparative purposes included specimens of generotypes within Phosphilini and Eriopinae, including *Phuphena*, *Phosphila* Hübner, 1818, *Acherdoia* Walker, 1865, and *Callopistria* Hübner, [1821].

Repository abbreviations

The following abbreviations refer to collections from which specimens forming the basis of this study originated:

AMNH American Museum of Natural History, New York, USA
NHMUK [formerly, BMNH] The Natural History Museum, London, UK
USNM National Museum of Natural History [formerly, United States National Museum], Washington, District of Columbia, USA

Systematics

Phuphena Walker, 1858

Type species. *Phuphena fusipennis* Walker, 1858 by monotypy.

Diagnosis. Species of *Phuphena* diverge with respect to wing pattern, but most share a bounded medial area. Orbicular and reniform stigmata sometimes absent or indistinct; outer forewing margin smooth in the majority of species but crenulate in *cilix*. Hindwing upperside coloration uniform. Male genitalia of *Phuphena* distinct, the valvae uniquely reduced, with no evidence of clasping architecture, narrow for most of their length, and in most species swollen apically to form a knob-like cucullus, giving them a club-shaped appearance, with a corona comprising an unorganized arrangement of setae. Vinculum V-shaped, slightly truncate in some species, and extending below the base of the valves for a distance equal to or greater than the width at its broadest point. The phallus is asymmetrically sclerotized, often with a patch of scale-like cornuti, and the vesica without well-developed cornuti or diverticula, but with a corresponding ventro-distal strip of scale-like cornuti. Basal abdominal brushes present but not visibly subdivided as in *Callopietria* Hübner, [1821] (type species: *Noctua juvenina* Stoll, 1782); a smaller pair on A8, and eversible saccular hair pencils completely absent. Female genitalia simple, the corpus bursae lemon-shaped, with no appendix or signa, the ductus seminalis attached apically, to the distal (anterior) end of the corpus. This distinguishes *Phuphena* from all *Thraumata* spp. and from *Callopietria juvenina*, in which the ductus arises from the proximal (posterior) end of the corpus, but this character varies among other *Callopietria* species.

Thraumata gen. nov.

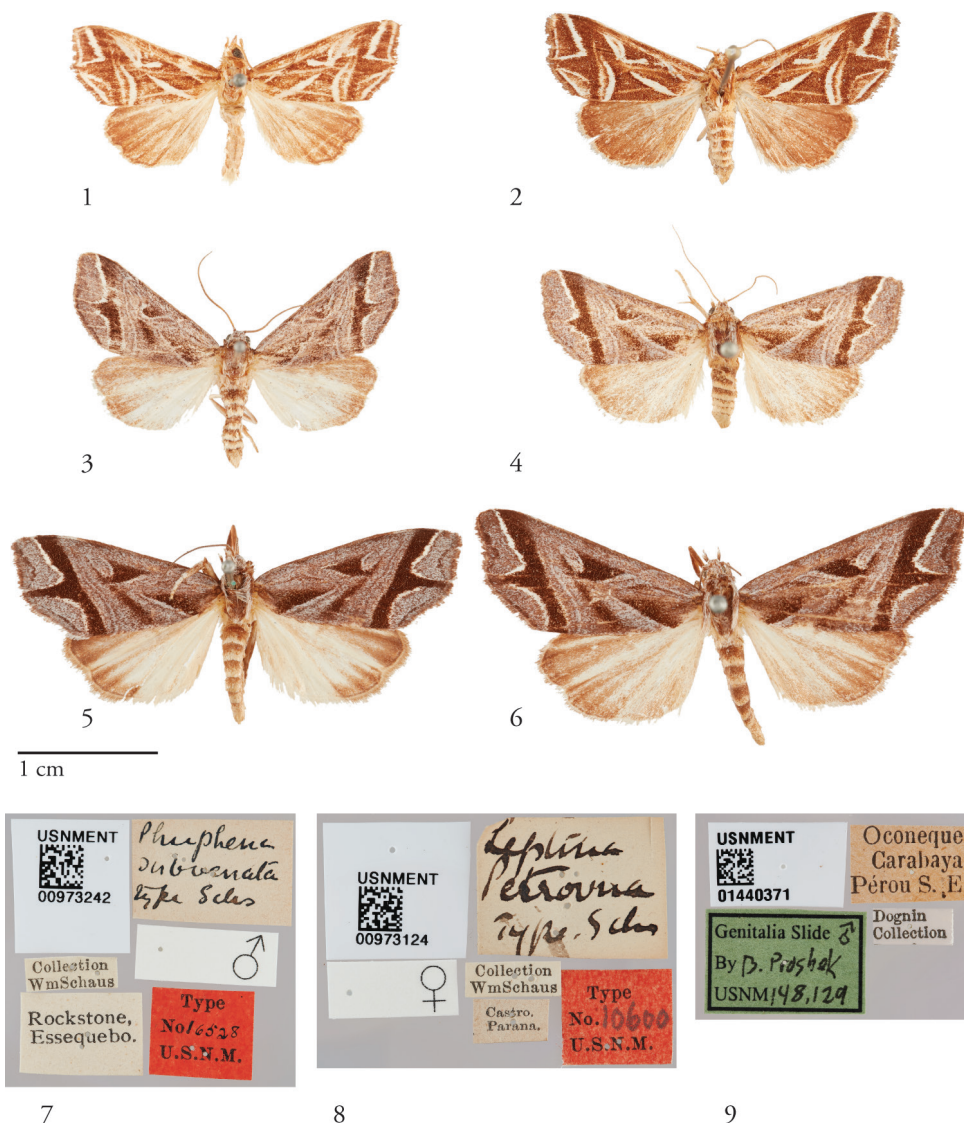
<http://zoobank.org/EFC5C009-2693-43AB-BDF5-3507B02E5FB3>

Type species. *Leptina? petrovna* Schaus, 1904, by present designation.

Etymology. *Thraumata* is the nominative neuter plural form of the Greek *θραύμα*, meaning shard, fragment, or potsherd, in reference to the fragmented appearance of the forewing pattern.

Diagnosis. Species of *Thraumata* are readily distinguished from *Phuphena* and *Calloplistria* by the fragmented appearance of forewing pattern elements, the differential coloration of the hindwing upperside, paler towards the base with more darkly shaded margins, and the configuration of male and female genitalia, as follows: Valves without clasper complex (as in *Phuphena*, *Calloplistria*, and *Phosphila*), but tapered and not clublike, unlike *Phuphena*, and with no differentiated cucullus or corona. Vinculum squared, less V-shaped than in *Phuphena*, extending below the valves for a distance less than the width at its broadest point. Vesica with multiple basal lobes and without cornuti. Ventrally recurved basal abdominal brushes present in *subvenata*; saccular coremata present and, except in *subvenata*, less developed than in *Calloplistria*. The female genitalia are distinct, the corpus bursae without signa, elongate and bowed, not lemon-shaped as in *Phuphena*, the ductus bursae attaching postero-dorsally to the corpus, and the ductus seminalis arises from the posterior end of the corpus as in *Calloplistria juvenina*, not at the anterior apex as in all known *Phuphena* and several *Calloplistria* species.

Description. **Head.** Frons fully scaled; eye large, hairless; antenna filiform, shortly setose-ciliate in both sexes; labial palpus slightly upturned and densely scaled, third segment reduced compared to the first and second segments; proboscis well developed. **Thorax.** Vestiture variously brown or grayish-brown scaling intermixed with white. **Wings.** Forewing elongate, not broadly rounded, the apex acute and the outer margin slightly angled at the middle; distal field crossed by conspicuous dark fascia outwardly produced at middle with triangular projection. Hindwing elongate too, slightly produced in correspondence to vein M3. Sexual dimorphism most obvious in hindwing, female with slightly darker shading towards wing base than male, but this character is neither discrete nor reliable. Underside of both wings brown or grayish-brown, the most prominent marking that of the toothed forewing submarginal line; Hindwing with 1–3 elongate pale streaks. A clearly expressed M2 visible on hindwing, arising from the discoidal cell closer to M3 than in *Calloplistria*, possibly representing an autapomorphic condition for *Thraumata*. **Legs.** Outwardly pale, silvery white in *petrovna* and *peruviansia* sp. nov.; otherwise brown or with a mixture of brown and whitish scales. Foretibial epiphysis rugose; a single pair of striped mid-tibial spurs, two pair on hind-tibia; 3 rows of tibial spines on all legs. **Abdomen.** Vestiture usually paler than on thorax and concolorous with hindwing surface; abdominal segments typically pale distally, more darkly banded at the anterior end of each segment, with darker scaling more uniformly diffuse below; typical triline brush organs absent except in *T. subvenata* comb. nov., which bears a pair of tufts on male sternum A2, without pockets or levers; other species exhibit raised lateral flanges on sternum A2; posterior margin of 8th sternite incised; rods extending from base of sternum A8 to pleurae. **Male genitalia.** Tegumen raised at base of uncus; vinculum short relative to tegumen; paratergal sclerite visible but fused with tegumen. Valve tapered, not extending beyond tegumen, finely setose throughout and most heavily towards base. Uncus setose, swollen apically, terminating in a small point. Juxta tightly joined with valve and transtilla. Phallus asymmetrically sclerotized, narrow in its basal half; vesica with multiple bubble-like



Figures 1–9. Dorsal habitus of *Thraumata* species. **1** *T. subvenata* Holotype ♂, Guyana, USNMMENT00973242 **2** *T. subvenata*, USNMMENT01440366♀, Suriname **3** *T. petrovna* ♂ USNMMENT01440358, Brazil **4** *T. petrovna* Holotype ♀ USNMMENT00973124, Costa Rica **5** *T. peruviansia* Holotype ♂ USNMMENT01440371, Peru **6** *T. peruviansia* ♀ USNMMENT01440374, Peru **7–9** Holotype specimen labels of *Thraumata* species **7** *Phuphena subvenata* Schaus **8** *Leptina petrovna* Schaus **9** *Thraumata peruviansia* sp. nov.

subbasal diverticula and a weakly to heavily sclerotized para-basal plate or lip from the distal end of the phallus; fremen of spermatophore with a well-developed nipple. **Female genitalia.** Ductus bursae short, membranous, without colliculum; appendix bursae present at posterior end of corpus bursae, but not well differentiated; ductus

seminalis arising from appendix bursae; corpus bursae oblong, without signa. Posterior apophyses rodlike, distally modified faintly, if at all. The bowed, ventrally facing configuration of the intersegmental membrane between A8 and A9 deformed so as to orient the corpus bursa at an angle with respect to the ventrally facing papillae anales.

Immature stages. Unknown.

Distribution. Recorded from northern South America, southeastern Brazil, southern Peru, Bolivia, and Argentina along the eastern Andean slopes (Fig. 16).

Key to species of *Thraumata* based on habitus

- 1 Ground color of FW predominantly dark brown, all markings conspicuously highlighted in silvery white..... *T. subvenata*
- FW dominated by gray, with dark brown fascia in distal field bearing outwardly produced tooth, white lining confined to outer edge of this fascia (submarginal line)..... **2**
- 2 FW length less than 13 mm (males) and 14 mm (females), toothed projection not reaching termen..... *T. petrovna*
- FW length greater than 14 mm (males) and 15 mm (females), toothed projection extended to termen *T. peruviansia*

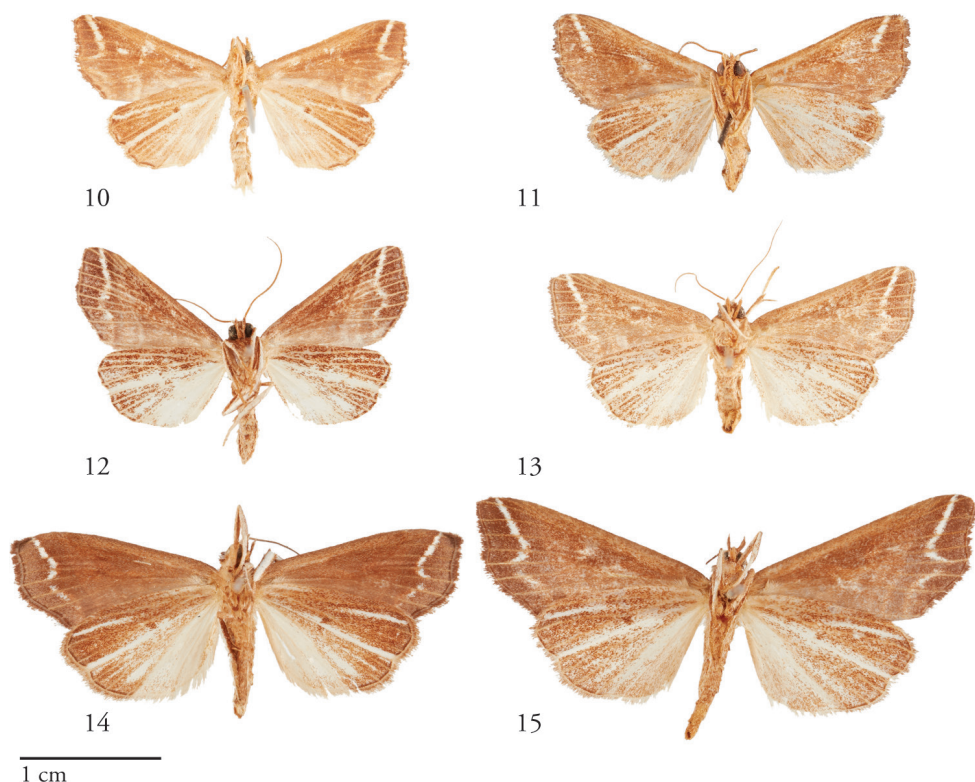
Thraumata petrovna (Schaus, 1904), **comb. nov.**

Figs 3, 4, 12, 13 (habitus); Fig. 17 (wing venation); Figs 18–20 (male genitalia); Fig. 25 (male abdomen); Fig. 29 (female genitalia)

Leptina? petrovna Schaus, 1904: 152. Type locality: Brazil [Rio de Janeiro], Petropolis. Figure: Hampson (1908: 597, pl. 121, fig. 23).

Material examined. Type material. BRAZIL: Holotype ♀ (USNM). Collection Wm Schaus, Castro, Parana, Type No. 10600, *Leptina petrovna* Type. Schs, USNMENT00973124.

Other material examined. BRAZIL (34♂, 39♀): **Paraná** (7♂, 7♀): *Males*: Banhado, Quatro Barras, Paraná, BRASIL – 800 m, 22.V.1971, Becker & Laroca, USNMENT01440489; Banhado, Quatro Barras, Paraná, BRASIL – 800 m 22.V.1971, Becker & Laroca; *Phuphena petrovna* (Schs, 1904), USNMENT01440447; Banhado, Quatro Barras, Paraná, BRASIL – 800 m, 27.II.1971, Becker & Laroca; *Phuphena petrovna* (Schs, 1904), USNMENT01440439; Castro. Parana.; *petrovna* Schs; Collection Wm Schaus, USNMENT01440466; Castro [ex coll. E.D. Jones] [NHMUK]; Castro, May 97 (E.D. Jones) [ex coll. Rothschild] [NHMUK]; Castro [ex coll. Joicey] [NHMUK]. *Females*: 2♀ Castro [ex coll. E.D. Jones] [NHMUK]; Castro, Febr. 97 (E.D. Jones) [ex coll. Rothschild] [NHMUK]; Castro, May 97 (E.D. Jones) [ex coll. Rothschild] [NHMUK]; Castro (E. D. Jones) [ex coll. Joicey] [NHMUK]; Castro.



Figures 10–15. Ventral habitus of *Thraumata* species. **10** *T. subvenata* Holotype ♂, Peru, NHMUK010606200 **11** *T. subvenata*, USNMENT01440366 ♀, Suriname **12** *T. petrovna* ♂ USNMENT01440358, Brazil **13** *T. petrovna* Holotype ♀ USNMENT00973124, Costa Rica **14** *T. peruvien-sia* Holotype ♂ USNMENT01440371, Peru **15** *T. peruvien-sia* ♀ USNMENT01440374, Peru.

Parana.; *Leptina* [?] *petrovna* Schs Schs 99, USNMENT01440329; Becker Coll. No. 4573, Morumbi, Morrestes Paranà BRASIL 18.VII.1970 Becker & Laroca; *Phuphena petrovna* (Schs. 1904), USNMENT01440394. **Minas Gerais** (5♂, 3♀) **Males**: Campo bello Rio Zikan, USNM Dissection 148134, USNMENT01440385, BOLD Process ID LNAUW3136-17; Campo bello Rio Zikan; Noct 324 [illeg.] 15.4-31, USNM ♂ 41,204 SAB, USNMENT01440393; Campo bello Rio Zikan, Noct ♀ [sic] 324 [illeg.] 12.4-31, USNMENT01440370; [Ouro] Preto [ex coll. Joicey] [NHMUK]; Uberaba [ex coll. Le Moulte] [NHMUK]. **Females**: BRASIL: MG 1400 m Serra do Cipó 17–19. iv.1991, V.O. Becker coll., Becker Coll. No. 77884, USNMENT01440362; BRASIL: MG 1400 m Serra do Cipó 17–19.iv.1991, V.O. Becker coll., Becker Coll. No. 77884, USNMENT01440368; BRASIL: MG 1400 m Serra do Cipó 17–19.iv.1991, V.O. Becker coll., Becker Coll. No. 77884, USNMENT01440352. **Bahia** (1♀): BRASIL: BA Camacã, 400–700 m 21–30.ix.1991, V.O. Becker coll., Becker Coll. No. 83540, USNMENT01440364. **Santa Catarina** (7♂, 12♀): **Males**: Rio Vermelho, 830 m, June 1936 (A. Maller) [ex coll. Rothschild] [NHMUK]; Hansa Humboldt, 60 m, July 1936 (A. Maller) [ex coll. Rothschild] [NHMUK]; Brazil, Santa Catarina: Rio Ver-

melho VII-44 Anton Maller, USNMENT01463998 [AMNH]; Brazil, Sta. Catarina: Nova Teutonia, July 1–5 1951, Plauman, USNMENT01463709 [AMNH]; Brazil, Sta. Catarina: Nova Teutonia Plauman, USNMENT01463996 [AMNH]; The Sperry Collection, 23-7-48, Neuvo Teutonia, Brazil Plaumann coll., USNMENT01463920 [AMNH]; Rio Vermelho St. Cath., Brazil November 1947, dissection 148363, USNMENT01422625 [AMNH]. *Females*: Nova Bremen, 250 m, July 37 (F. Hoffmann) [ex coll. Rothschild] [NHMUK]; *idem*, Aug. 37, F. Hoffmann [ex coll. Rothschild] [NHMUK]; Neu Bremen, Rio Laeiss, Nov. 1935 (F.H. Hoffmann) [ex coll. Rothschild] [NHMUK]; *idem*, April 1936 (F.H. Hoffmann) [ex coll. Rothschild] [NHMUK]; Hansa Humboldt, 60 m, July 1936 (A. Maller) [ex coll. Rothschild] [NHMUK]; Becker Coll. No. 52409, BRASIL: SC, Scara, 300 m, 27.i.1983, V.O. Becker coll., USNM Dissection 148127, USNMENT01440363; Brasilien Nova Teutonia Fritz Plaumann, 27°80.0N Br. 52–53°Westl. L., 13, USNMENT01440353; Brazil, Sta. Catarina: Nova Teutonia July 12–17, 1948, F. Plaumann, USNMENT01463849 [AMNH]; The Sperry Collection, July 4, 1951, Neuvo Teutonia, Brazil Plaumann, coll., USNMENT01463774 [AMNH]; Collection of Grace H. and John L. Sperry Nova Teutonia Brazil, 24.7.48 Plaumann, USNMENT01463737 [AMNH]; Collection of Grace H. and John L. Sperry Brasilien Nova Teutonia, 27.7.1948, 87°11 [illeg.] 52°23, L. Fritz Plaumann, 300 [illeg.], 500 [illeg.], USNMENT01463784 [AMNH]; St Catherines Brazil, F. Johnson Collector, USNMENT01440404. **Espírito Santo** (1♀): USNMENT01440369 ♀. **Rio de Janeiro** (10♂, 7♀): *Males*: Rio (Derg.) [2♂ ex coll. Rothschild; 1♂ ex coll. Joicey] [NHMUK]; 2♂ Rio Janeiro [ex coll. Walsingham] [NHMUK]; Rio de Janeiro, Organ Mts, near Tijuca / *Leptina petrovna* Schaus [ex coll. S. R. Wagner] [NHMUK]; 2♂ Petropolis [ex coll. Rothschild] [NHMUK]; Petropolis (M. Doer) [ex coll. Walsingham] [NHMUK]; Teresopolis, 13–22.iii.1957 [ex coll. Kettlewell] [NHMUK]. *Females*: Rio (Derg.) [1♀ ex coll. Rothschild; 1♀ ex coll. Joicey] [NHMUK]; Petropolis, 5-7-76 [ex coll. Joicey] [NHMUK]; Teresopolis, c. 2750 ft., 14–18.xii.57 (E. P. Wiltshire) [ex coll. Wiltshire] [NHMUK]; Pico d'Itatiaia, 28.iii–1. iv.1958 [ex coll. Kettlewell] [NHMUK]; Petropolis, Brazil.; *petrovna* Schs; Collection Wm Schaus, USNMENT01440482; Petropolis, Brazil.; *petrovna* Schs; Collection Wm Schaus; USNM Dissection 148128, USNMENT01440346. **Distrito Federal** (1♂): Becker Coll. No. 40221; Planaltina, DF. BRASIL-1000 m 15.V.1982, V.O. Becker coll. 15°35'S, 47°42'W; *Phuphena petrovna* (Schs, 1904), USNMENT01440358. **Goiás**: (1♀) Becker Coll. No. 20293; Formosa, Goiás BRASIL-800 m, 19.III.1977 V.O. Becker col., *Phuphena petrovna* (Schs. 1904), USNMENT01440386. **São Paulo** (2♂, 3♀): *Males*: São Paulo [ex coll. E.D. Jones] [NHMUK]; São Paulo [ex coll. Gerrard] [NHMUK]. *Females*: São Paulo (D. Jones); São Paulo [ex coll. Rothschild] [NHMUK]; São Paulo [ex coll. Gerrard] [NHMUK]. **ARGENTINA** (1♂, 3♀): *Male*: Argentina, Prov. Jujuy Dept. Ledesma 5–7.5 km W of Rt. 34 near entrance Parque Nacional Callilegua 1600 m, 14.II.1991; mesic forest along river, K. Johnson et al, USNMENT01463799 [AMNH]. *Females*: Argentina, Prov. Jujuy Dept. Capital; Baptist Mission[,] Camp at Rio Lozano, 1600 m, 28.II.1991; riparian woodland with steep forested slopes, K. Johnson et al., USNMENT01463997 [AMNH]; Argentina,

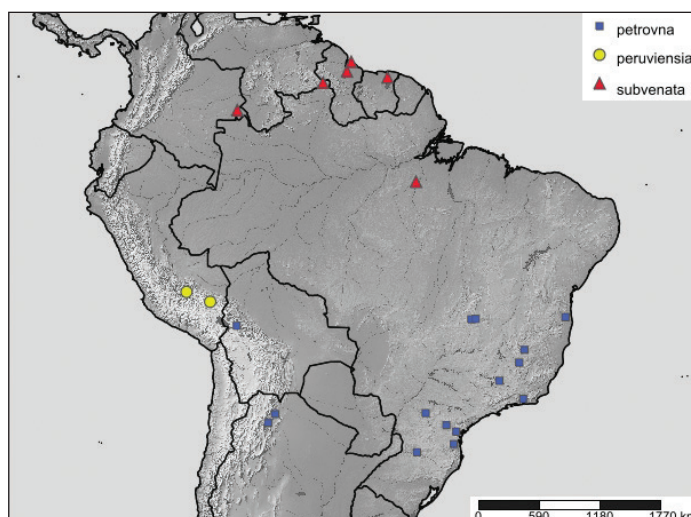


Figure 16. Known distribution of *Thraumata* species.

Prov. Salta Dept. La Caldera; La Caldera–Jujuy Prov. border, Rt. 9, km 20, km post 1642: “La Cargadera”, 1450 m, 12.II.1991, mesic forest, K. Johnson et al., USNMENT01463797 [AMNH]; Argentina, Prov. Salta Dept. La Caldera; La Caldera–Jujuy Prov. border, Rt. 9, km 20, km post 1642: “La Cargadera”, 1450 m, 12.II.1991, mesic forest, K. Johnson et al., USNMENT01463840 [AMNH]. **BOLIVIA** (1♂, 1♀): Rio Songo, 750 m [via coll. Fassl ex coll. Oberthür] [NHMUK]; Rio Songo, 750 m [via coll. Fassl ex coll. Oberthür] [NHMUK]. **NO LOCALITY DATA** (1♂, 1♀): [no data] [ex coll. E.D. Jones] [NHMUK].

Diagnosis. This and the following species are readily differentiated from *T. subvenata* by the gray-brown ground coloration and less prominent white markings throughout the forewing. *Thraumata petrovna* is, on average, the smallest of the three species, and although its wing pattern closely tracks that of the larger *peruviansia*, the postmedial tooth of the forewing is shorter, not reaching the wing margin; the vesica is distinctly more sclerotized, bearing a pronounced lateral band and a conspicuous, rugose plate.

Redescription. Head. Vertex and frontal scaling predominantly white but with a mixture of light gray-brown scales throughout the vertex, frons and palpi; frontal scales elongate. **Thorax.** Vestiture a mix of white and grayish-brown scales. **Wings.** Forewing lengths: males 10.8–12.9 mm ($N=13$, $\bar{x}=12.2$ mm, $M=12.5$ mm); females (including holotype 12.1 mm) 11.0–13.7 mm ($N=22$, $\bar{x}=12.4$ mm, $M=12.5$ mm). Forewing ground color predominantly gray, with darker chocolate-brown buff in lower basal field, both below and between stigmata, and in toothed fascia of distal field; hindwing pale, brownish-gray towards margin; forewing and hindwing underside with diffuse chocolate-brown scaling, the shading on the hindwing concentrated between veins, fading towards hind margins on both forewing and hindwing; hindwing underside with a single conspicuous median pale streak. **Legs.** Outwardly pure white; otherwise

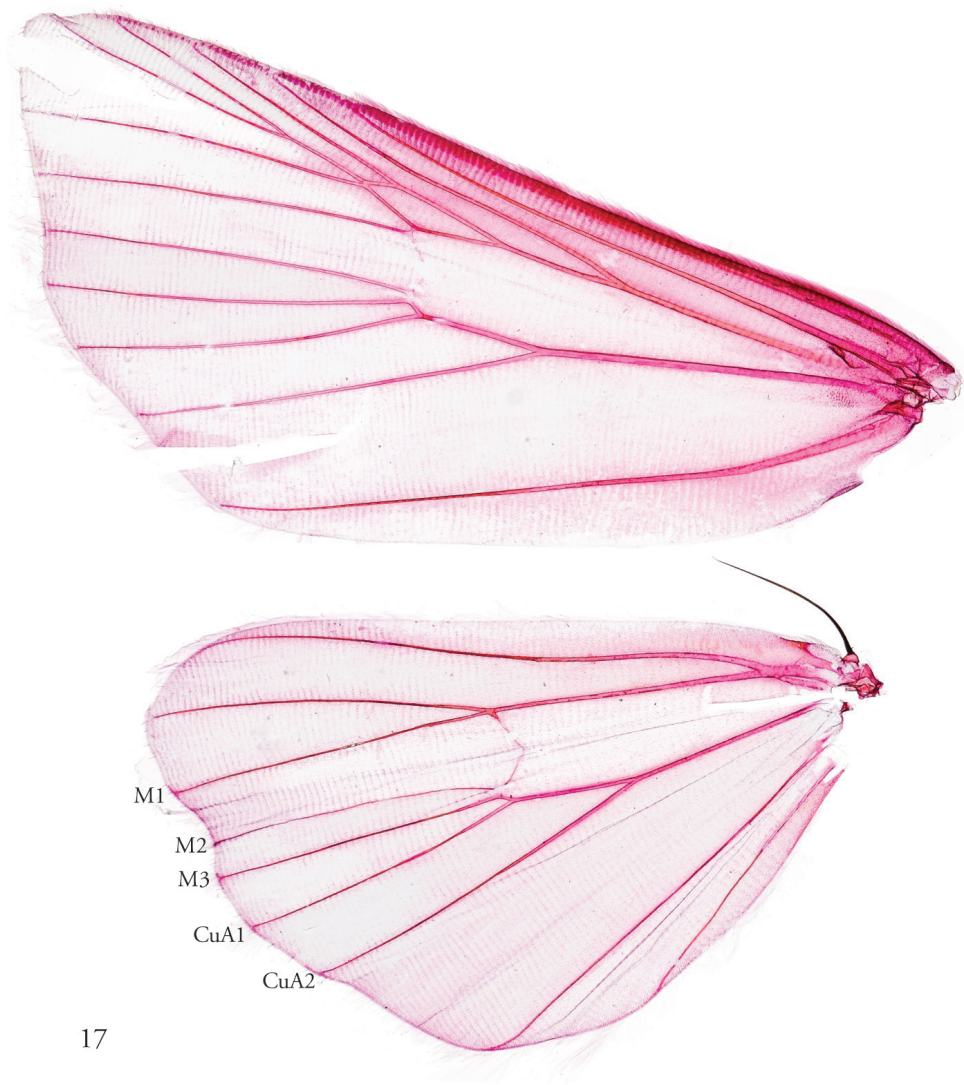


Figure 17. Wing venation of *T. petrovna*, male, USNMENT01440439, wing preparation 148365.

light brown. **Abdomen.** Abdominal segments with cream-colored scales distally, darker brown-banded at the anterior end of each segment; brown scaling more uniformly diffuse below. Abdominal brushes lacking. **Male genitalia.** Tegumen rooflike, a distinct vertex and a pitch $>45^\circ$. Saccus bluntly rounded. Phallus heavily sclerotized, swollen and rugose along its outer edge; vesica with two rudimentary subbasal diverticula, and para-basal plate arising from distal end of the phallus. **Female genitalia.** Posterior apophyses roughly $1.3\times$ as long as anterior apophyses; papillae anales densely setose.

Immature stages. Unknown.

Etymology. Not explained, but evidently in reference to the type locality Petropolis ("City of Peter").

Distribution. Southeastern Brazil and along the eastern slopes of the Bolivian and Argentinian Andes.

Remarks. A partial barcode sequence (658bp – 286n = 372bp) was generated from a Brazilian male specimen USNMENT01440385 corresponding to USNM dissection 148134 (Fig. 18), BOLD sample ID USNM_PG_H02. Schaus' (1904) original placement of *petrovna* in what is now a synonym of the nolid genus *Baileya* may simply reflect the quadrifine appearance of its hindwing.

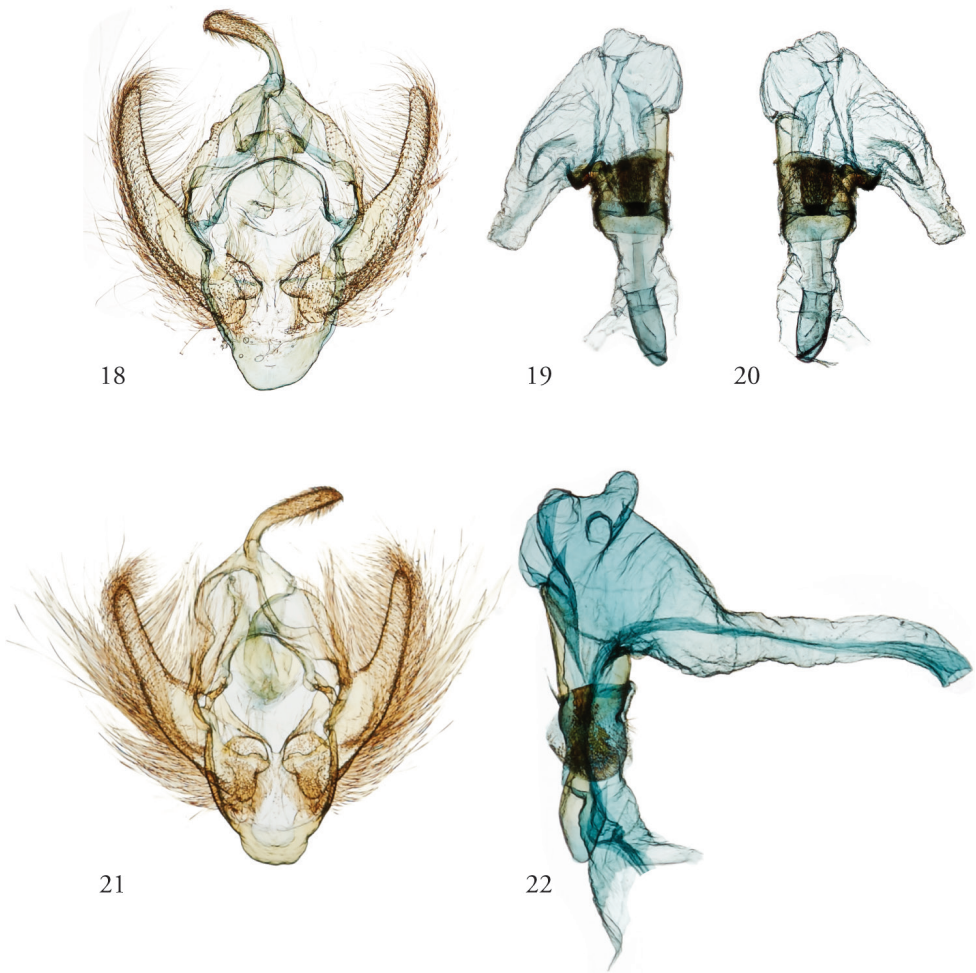
***Thraumata peruviansia* sp. nov.**

<http://zoobank.org/FBC0A9B5-7D7F-44F9-AFD9-5F3BC2B352F9>

Figs 5, 6, 14, 15 (habitus); Figs 21, 22 (male genitalia); Fig. 26 (male abdomen); Fig. 28 (female genitalia)

Material examined. Type material. PERU (11♂, 6♀): **Holotype** ♂ (USNM), Oconeque Carahaya Perou S. E.; Dognin Collection; USNMENT01440384. **Paratypes** (10♂, 6♀; USNM, NHMUK, AMNH): *Males*: same data as holotype, Dissection 148129, USNMENT01440371, BOLD Process ID LNAUW3137-17; same data as holotype, USNMENT01440341; Quinton, Carabaya, Peru, 5000 ft, i 1905. G. Ockenden [Joicey Bequest Brit. Mus. 1934-120] [NHMUK]; 2♂ Oconeque, Carabaya, Peru. 5000 ft, ii 1905, G. Ockenden; 1♂, Joicey Bequest Brit. Mus. 1934-120; 1♂, Rothschild Bequest B.M. 1939-1 [NHMUK]; 2♂ Oconeque, Carabaya, 7000 ft., dry s., July 1904 (G. Ockenden), Rothschild Bequest B.M. 1939-1 [NHMUK]; 3♂ Oconeque S. E. Peru, Feb'05 7000 ft, G. Ockenden [2♂, 67. 20. Ex Coll. Ed. Brabant 1920 / Rothschild Bequest B.M. 1939-1; 1♂ – 1909-89. [NHMUK]. *Females*: same data as holotype, Dissection 148130, USNMENT01440374; PERU: Cusco Machu Picchu 2885 m 5.II.1959 J.F.G. Clarke, USNMENT01440443; Macchu Pichu [sic] Peru II-11 1966 B. Heineman [AMNH]; Oconeque, Carabaya, Peru. 5000 ft, ii 1905, G. Ockenden; Joicey Bequest Brit. Mus. 1934-120 [NHMUK]; Oconeque, Carabaya, 7000 ft., dry s., July 1904 (G. Ockenden), Rothschild Bequest B.M. 1939-1 [NHMUK]; Oconeque S. E. Peru Feb'05, 7000 ft G. Ockenden, 67. 20. Ex Coll. Ed. Brabant 1920/Rothschild Bequest B.M. 1939-1 [NHMUK]

Diagnosis. *Thraumata peruviansia* is most readily distinguished from the other two species by the combination of its larger size and distinctive vesica. In this and the previous species, the dominant ground color of forewing is gray, darker in the present species than in *T. petrovna*. This feature, the less pronounced white lining of pattern elements, particularly in basal and medial fields of forewing, and the comparatively reduced abdominal scent tufts in the male most readily distinguish this species pair from the more uniformly brown ground coloration of *T. subvenata*. In the new species the triangular tooth projecting from the dark brown distal fascia of the forewing is longer



Figures 18–22. *Thraumata* male genitalia. **18–20** *T. petrovna* dissection 148363, USNMENT01422625, Brazil **18** Apparatus **19, 20** Phallus **21, 22** *T. peruviansia*, dissection 148129, USNMENT01440371 **21** Apparatus **22** Phallus.

than in *T. petrovna*, and there are at least two distinct white streaks on the hindwing underside instead of one.

Description. **Head.** Vestiture as in *T. petrovna*. **Thorax.** Vestiture a mix of grayish-brown scales intermixed with white, especially within tegulae. **Wings.** Forewing lengths: males (including holotype 15.3 mm) 14.2–15.3 mm ($N = 3$, $\bar{x} = 15.0$ mm, $M = 12.5$ mm); females, 15.5–16.8 mm ($N = 3$, $\bar{x} = 16.1$ mm, $M = 16.1$ mm). Forewing coloration dominated by gray scaling suffused with white, lines for the most part obscured, although in some individuals antemedial and postmedial lines still discernible as faint, thin pale gray lines. Four areas of more uniform, chocolate-brown scaling: an elongate, obtuse subtriangular smudge in lower basal field from wing base to antemedial line; an oblique band in place of median shading between from reniform

stigma and inner margin, dark filling between orbicular and reniform stigmata, and in fascia bearing outwardly projected median tooth of distal field, this fascia lined externally with silvery white. Forewing underside nearly uniform chocolate brown excepting paler white inner marginal area; the shading on the hindwing underside interrupted by two-three conspicuous pale streaks, fading towards wing base. **Legs.** White outward, otherwise as brown as in previous species. **Abdomen.** Abdominal segments predominantly gray, ringed with pale cream-colored scales distally; abdominal brushes lacking. **Male genitalia.** Sacculus squared. Sclerotized lateral band on phallus less pronounced than in *petrovna*, vesica with three rudimentary subbasal diverticula and reduced para-basal plate from distal end of the phallus. **Female genitalia.** Ductus bursae quite short relative to distended corpus bursae. Anterior and posterior apophyses roughly co-equal. Papillae anales densely setose. As in other *Thraumata*, the ductus seminalis arises from a bulge at the posterior end of the corpus bursae.

Immature stages. Unknown.

Etymology. The name *peruviensia* is a neuter plural adjective, in reference to the country of origin of the new species, in coordination with the generic name *Thraumata*.

Distribution. Southeastern Peru.

Remarks. This species is very similar in appearance to *T. petrovna* but consistently larger and occurring at higher altitudes. An incomplete barcode sequence (658bp – 88n = 570bp) was generated from a 1905 male specimen, USNMENT01440371 corresponding to USNM dissection 148129 (Figs 21, 22), BOLD sample ID USNM_PG_H03.

***Thraumata subvenata* (Schaus, 1914), comb. nov. (provisional position)**

Figs 1, 2, 10, 11 (habitus); Figs 23, 24 (male genitalia); 27 (male abdomen); Fig. 30 (female genitalia); Figs 31–34 (morphological details).

Phuphena subvenata Schaus, 1914: 486. Type locality: [Guyana] British Guiana: Essequibo River, Rockstone.

Material examined. Type material. GUYANA: Holotype ♂ (USNM). Collection Wm Schaus, Rockstone, Essequibo, Type 16528 USNM, *Phuphena subvenata* Type Schs, USNMENT00973242. **Paratype. GUYANA:** 1♂ Rockstone, Essequibo; *Phuphena subvenata* Schs, W. Schaus, 1912-38. [NHMUK];

Other material examined (3♂, 7♀). **GUYANA:** 1♀ Demerara; Rothschild Bequest, B.M. 1939-1 [NHMUK]; 1♀ Demerara; 75; Adams Bequest. BM 1912-399., NHMUK010914717, slide NHMUK010314585 [NHMUK]; 1♂ Brit. Guiana, Kaialam [illeg.], 26.V.1929, [illeg.] T.D.A. Cockerell, Pres. by Imp. Bur. Ent. Brit. Mus 1930-188., NHMUK010914410, slide NHMUK010314636 [NHMUK]; 1♂ Georgetown Br. Guiana; July; A Busck coll, Not in BM 1925, USNM Dissection 148139, USNMENT01440389, BOLD Process ID LNAUW3138-17. **SURINAME** (1♀): Geldersland [sic], Surinam River.; Collection Wm Schaus, USNMENT01440366. **VENEZUELA** (1♀): Venezuela, Bolivar: Guaiquinima tepui, camp



Figures 23, 24. *Thraumata subvenata* **23** Apparatus, dissection 148139, USNMENT01440389
24 Phallus, NHMUK010201223, slide NHMUK010314637, Colombia.



Figures 25, 26. *Thraumata* male abdomens. **25** *T. peruviansia* dissection 148134, USNMENT01440385
26 *T. peruviansia* dissection 148129, USNMENT01440371, Brazil.

1, 5°55'N, 63°30'W, 1150 m, Feb. 24–28, 1990, D. Grimaldi [AMNH]. **COLOMBIA** (1♂, 1♀): Ober Rio Negro, Ost. Colomb., 800 m., Coll. Fassl; Ex. Oberthür Coll. Brit. Mus. 1927-3.; (♂) NHMUK010201223; slide NHMUK010314637 [NHMUK]. **BRAZIL** (2♀): Pará (A. M. Moss); Rothschild Bequest, BM, 1939-1, NMHUK010916076 [NHMUK].



Figure 27. *Thraumata subvenata* NHMUK010201223, slide NHMUK010314637, Colombia.

Diagnosis. The predominantly golden-brown coloration of the forewing upperside and the prominent white edging of its markings readily distinguished this species from *T. petrovna* and *T. peruviansia*. Valvae are more broadly articulated with the vinculum than in the other species, the costal edge $<1/2$ the length of the outer edge. Unlike the two predominantly gray-brown species, *T. subvenata* also bears basal abdominal brushes and what appear to be well-developed eversible coremata on the sacculus.

Redescription. Head. Proboscis edged with papillae and a micro-serrated ridge. Antennae ciliate; eyes hairless; palpi extending above eyes. Frons, vertex and palpi with



Figures 28, 29. *Thraumatina* female genitalia. **28** *T. peruviansia* dissection 148130, USNMMENT01440374 USNM, Brazil **29** *T. petrovna* dissection 148127, USNMMENT01440363, Peru.

a mix of brown and white scales; terminal segment of the palpus whitish. **Thorax.** Vestiture predominantly light brown, a whitish V formed by two lines of whitish scales within the tegulae. **Wings.** Forewing length, males ($N = 2$), 11.5 (holotype)–12.0 mm; females ($N = 3$), 12.1 mm, 12.4 mm, 13.0 mm. Forewing coloration dominated by golden-brown scaling; the differential white shadowing, of various pattern elements, particularly the postmedial and subterminal lines, renders a fractured appearance consisting of streaks and sickle-shaped forms: two basal white streaks, 2 crescent-shaped medial markings bisected by brown inner line and two, more expansive, subterminal forms, each enclosing a brown center. These latter four simply bracket the broad, dentate subterminal band homologous to that in the previous two species. **Legs.** Predominantly brown, paler outwardly but not silvery white. Male fore-femur with a thick, cream-colored pencil of long scales (Figs 31, 32), absent in females and therefore likely a scent tuft. **Abdomen.** Posterior edges of abdominal segments ringed with cream-colored scales. Males with several secondary sexual structures, including distal scale tufts borne by the lateral rods of 8th sternite and a pair of mixed gray and brown abdominal brushes on A2 sternite, visible in situ on the holotype as arching inward and overlapping above the midline (Fig. 33). The holotype was clearly damaged and



30

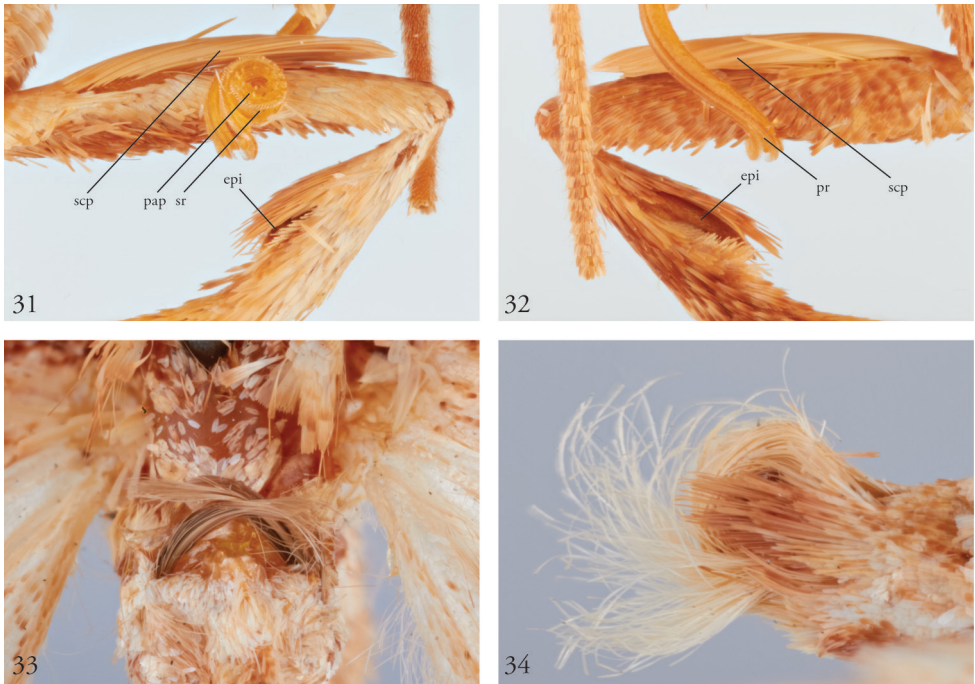
Figure 30. *Thraumata subvenata*, female genitalia. NHMUK010914717, Guyana.

repaired poorly, with abdomen re-attached upside down, such that ventral brushes appear dorsal. Tufts of elongate hairs present on 8th sternite (Fig. 34). **Male genitalia.** Relative to the rest of the moth, valvae the largest of the three species; costal and ventral edges swollen to form an inner crest; a bundle of stiff hairs inserted on the infero-lateral corners of valvae; valvae fused to vinculum along more than half their length. Vesica with three rudimentary subbasal diverticula, para-basal plate at the distal end of the phallus weakly sclerotized, appearing faintly crenulate. **Female genitalia.** Anterior and posterior apophyses comparable in length. Antrum well developed, cuplike. Relative to its congeners, *T. subvenata* has a longer ductus bursae, with the ductus seminalis arising from a bulge along the ductus bursae, posterior to the corpus.

Immature stages. Unknown.

Distribution. Known only from northern South America, specifically Colombia, Venezuela, Guyana, Suriname and Brazil (Pará).

Remarks. Both the valvae and the legs of *subvenata* males are equipped with more conspicuous scent tufts than either of the two sister species. An incomplete barcode sequence (658bp – 88n = 570bp) was generated from a 1925 male specimen from Guyana USNMENT01440389 corresponding to USNM dissection 148139 (Figs 21, 22), BOLD sample ID USNM_PG_H04.



Figures 31–34. *Thraumata subvenata*, holotype (male), foreleg, proboscis, and abdominal structures. **31** Right foreleg, outside lateral view, indicating femoral scale pencil (sp), epiphysis (ep), and proboscis (pr) with papillae (pap) and serrated lateral ridge (sr) **32** Right foreleg, inside lateral view, indicating femoral scale pencil (sp) and epiphysis (ep) **33** Ventral brushes on A2 **34** Abdominal terminus, showing distal scale tufts.

Discussion

The boundaries of the Eriopinae have yet to be established as several of the characters used to circumscribe the subfamily are either widespread or have been characterized vaguely, and are likely to be revisited during the course of ongoing studies. For present purposes, we suggest the possibility that placement of *Thraumata* within the Eriopinae on the basis of characters shared by *Callopietria* (= *Eriopus* Treitschke, 1825). These include the tapered, ear-shaped valves without a linear corona, the presence in *T. subvenata* of small saccular coremata on the inner face of the valves, and narrow sclerotization along the basal half of the phallus, which dissipates in the distal third (cf. Holloway 1989; Yen and Wu 2009). The “ballooning” of the tegumen noted in several *Callopietria* can also be seen, particularly in *T. subvenata* (Fig. 23), which is the only *Thraumata* with basal abdominal hair pencils (Fig. 27) known in most *Callopietria* species but absent from Phosphilini. The configuration of the male genital capsule of *Thraumata* differs sharply from that of *Phuphena*, in which a uniformly slender stalk-like valve devoid of basal coremata ends in a clubbed cucullus with a diffuse corona of stout setae. *Thraumata* and *Phuphena* share with *Callopietria* conspicuous tufting of the dorsal side of valves,

but differ in the position of the ductus seminalis with respect to the bursa copulatrix (posterior in *Thraumata* and anterior on the fundus bursae in *Phuphena*; this character varies in *Calloplistria* as noted above), and in the oblique orientation of the orbicular and reniform stigmata towards one another in *Thraumata*. *Thraumata* may also be differentiated from *Calloplistria* by the short incrassate uncus and, at least in the case of the species pair *petrovna* and *peruviensia*, by elongate wings and the origin of hindwing M2 in close proximity to M3. In addition to the developed saccular and abdominal coremata, several features of *T. subvenata* are sufficiently reminiscent of *Calloplistria* to consider either its placement in *Thraumata* tentative, or the possibility that the whole of *Thraumata* are phylogenetically embedded subgenerically within *Calloplistria*. These include the configuration of the juxta, with its deep mesial invagination and broad lateral plates; less elongate wings than in *T. petrovna* or *T. peruviensia*; and the position of M2 further from M3 than in the other two species of *Thraumata*. The position of the ductus seminalis of *T. subvenata* on the posterior side of the corpus bursae is similar to that of other *Thraumata*, but differs in that it arises from a bulge emerging from the ductus bursae itself, not from the corpus. Both these configurations differ from that of *Phuphena*, in which the ductus seminalis arises from the distal end of the corpus. In *Calloplistria juvenina*, the ductus seminalis is described as arising from sclerotized wrinkles on the posterior end of the bursa (Fibiger and Hacker 2007: 51), but in our observations the area in the immediate vicinity of the base of the ductus seminalis is membranous, and variants of all these character states may be observed elsewhere in the genus (e.g., *C. phaeogona* Hampson, 1908; cf. Yen and Wu 2009:28, Fig. 14B).

The pleural tufts on A8 of *T. subvenata* (Fig. 27) are absent from its congeners, although in these the rods remain expressed. Such variation among close relatives is not unusual or inconsistent with the lability of secondary sexual characters elsewhere in the Noctuidae (e.g., Apameini, Phlogophorini) or elsewhere in the Lepidoptera (e.g., Pyraloidea, cf. Goldstein et al. 2013). The hair pencils on A2 of *T. subvenata* (Figs 27, 33) are consistent with Fibiger and Lafontaine's (2005: 41) statement to the effect that such brushes in Eriopinae are "thought not to be homologous with [those] of other noctuids," since they lack typical pockets and apodemes. They are not, however, divided into sub-pencils or otherwise consistent with Poole's (1995: 18) description of eriopine abdominal brushes. The more diffuse tufts at the base of the valves on *T. subvenata* (Fig. 23) are comparable to the eversible saccular coremata mentioned by Fibiger and Lafontaine as well as Holloway (1989) as features of Eriopinae. Other such features adduced by Yen and Wu (2009) and visible in *Thraumata* include the "ladder-shaped" (or H-shaped) antero-medially incised male 8th abdominal sternite; scale tufts associated with the paired pleural rods at the base of sternum A8 (only in *T. subvenata*, Fig. 27); the absence of the clasper complex from the valve (Figs 18, 21, 23); short vinculum (especially in *T. subvenata*, Fig. 23); narrow, asymmetrically sclerotized phallus (Figs 19, 20); and, in the female, a membranous, oblong corpus bursae (Figs 28–30). None of these can be considered uniquely derived within Noctuidae, however.

Fibiger and Lafontaine (2005: 41) placed *Phuphena* in Phosphilini based on the simple valve and spiny vesica, but this assignment may be problematic. Although the vesica of *Phosphila turbulenta* Hübner, 1818 (the type species of *Phosphila*) is similar in shape to those of *Phuphena*, it is spineless. Where vesical spines do occur *Phosphila* (e.g., in *P. dogmatica* Dyar, 1916), they are fine and enclose the basal portion of the vesica. Spines on the vesicae of *Phuphena*, on the other hand, are confined to a ventral band of scale-like cornuti. The valves of typical *Phuphena* are stalk-like, swollen or slightly swollen apically, whereas those of *Phosphila* are more expansive and gradually tapered. *Phuphena* species do share a number of taxonomically widespread features (or losses thereof) with Phosphilini, including naked, lashless eyes, and elongate, membranous corpus bursae without signa. Similarities in the male genitalia include the absence of a free pleural sclerite, the valves simplified in such a way that they are without clasping architecture, and the corona (if so termed) comprises an apical, unarranged concentration of setae (cf. “hairlike setae”; Poole 1995: 21). The dimensions of *Thraumata* valvae are more similar to those of *Callopietria* than *Phosphila*, i.e. tapered and not narrowed throughout or clublike. The phalli of *Thraumata* share the pattern of sclerotization seen in both *Phuphena* and *Callopietria*. Although we hasten to add that both *Phuphena* and *Thraumata* are significantly smaller-bodied than most Phosphilini, they do not bear any immediately obvious similarities with *Acherdoa ferraria*, which possesses highly reduced male genitalic features. As Poole (1995: 21) described, adult Phosphilini also lack basal abdominal hair pencils of any kind, and although this character is notoriously homoplastic throughout the Noctuidae, and appears polymorphically in both *Thraumata* and *Callopietria*, its absence is consistent throughout Phosphilini, and its presence consistent among all known *Phuphena*.

DNA barcode data, including partial sequences of the 658bp COI barcode region obtained for all three species of *Thraumata*, provisionally support the monophyly of both *Phuphena* and *Thraumata* and unite them with the eriopine genera *Callopietria* and *Argyrosticka*. But these results must be viewed skeptically, given their incompleteness (570bp for both *T. peruviansia* and *T. subvenata*, and 372bp for *T. petrovna*; see Material examined above for BOLD process ID codes of available sequences). Although the larvae of *Phuphena* have thus far been associated exclusively with ferns and differ in many other respects from the gaudy, semi-gregarious larvae of Phosphilini, which have been recorded only from Smilacaceae, we have as yet only been able to examine larvae indirectly, through images. Without specimens, none of the larval characters discussed by Crumb (1956), Beck (1960, 1999–2000), Poole (1995), Fibiger and Lafontaine (2005), or Yen and Wu (2009) that might support or refute their placement in Eriopinae can be examined critically, and to our knowledge no reared larvae of *Thraumata* have been described or imaged. These circumstances reinforce the need for a thorough revision of the current concept of the Eriopinae as the analyses of groups long placed in the Amphipyrrinae proceed (Keegan et al. 2019). While we find it likely that the taxonomic diversity of New World Eriopinae is broader than previously supposed, a more precise circumscription of the group will ultimately depend on a combination of additional larval information and molecular phylogenetic data.

Acknowledgements

Ben Proshek (USDA) prepared many of the dissections and figures. David Grimaldi, Courtney Richenbacher, and Suzanne Rab Green (all AMNH) kindly provided loans of specimens. Nick Silversen and Scott Miller (USNM) coordinated the barcoding effort with Meredith Miller and Sean Prosser (Biodiversity Institute of Ontario). The authors are especially grateful for the comments of Don Lafontaine and two anonymous reviewers that improved the content of this manuscript. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; USDA is an equal opportunity provider and employer.

References

- Beck H (1960) Die Larvalsystematik der Eulen (Noctuidae). Abhandlungen zur Systematik der Insekten 4. Akademie Verlag, Berlin, 406 pp.
- Beck H (1999–2000) Die Larven der europäischen Noctuidae. 4 vols. Herbiopoliana, Buchreihe zur Lepidopterologie 5. Verlag U. Eitschberger, Marktleuthen, 859, 447, 336, 512 pp.
- Clarke JFG (1941) The preparation of the slides of the genitalia of Lepidoptera. Bulletin of the Brooklyn Entomological Society 36: 149–161.
- Crumb SE (1956) The larvae of the Phalaenidae. United States Department of Agriculture, Technical Bulletin 1135: 1–356. [pl. 1–11]
- Fibiger M, Hacker HH (2007) Noctuidae Europaeae 9. Amphipyridae – Xyleninae. Entomological Press, Sorø, 410 pp.
- Fibiger M, Lafontaine JD (2005) A review of the higher classification of the Noctuoidea (Lepidoptera) with special reference to the Holarctic fauna. *Esperiana* 11: 7–92.
- Forbes WTM (1954) The Lepidoptera of New York and Neighboring States. III. Noctuidae. Memoir 329, Cornell University Agricultural Experiment Station, Ithaca, New York.
- Goldstein PZ, Metz MA, Solis MA (2013) Phylogenetic systematics of *Schacontia* Dyar with descriptions of eight new species (Lepidoptera: Crambidae). *Zookeys* 291: 27–81. <https://doi.org/10.3897/zookeys.291.3744>
- Hampson GF (1908) Catalogue of the Lepidoptera Phalaenae in the British Museum. Volume 7. Taylor and Francis, London, 709 pp. [108–122 pls]
- Holloway JD (1989) The Moths of Borneo Part 12: Family Noctuidae, trifine subfamilies: Noctuinae, Heliethinae, Hadeninae, Acronictinae, Amphipyridae, Agariinae, 57–226. [+ 404 figs in 38 plates + 8 colour plates]
- Jaeger CM (2017) Phylogeny of Tortricidae (Lepidoptera): A morphological approach with enhanced whole mount techniques. Masters Thesis, Mississippi State University, 117 pp.
- Keegan K, Lafontaine JD, Wahlberg N, Wagner DL (2019) Towards resolving and redefining Amphipyridae (Lepidoptera: Noctuoidea, Noctuidae): a massively polyphyletic taxon. *Systematic Entomology* 44: 451–464. <https://doi.org/10.1111/syen.12336>

- Lafontaine JD (1998) The Moths of America North of Mexico (Fascicle 27.3.) – Noctuoidea, Noctuidae (part): Noctuinae, Noctuini. Wedge Entomological Research Foundation, Washington, DC, 348 pp.
- Lafontaine JD (2004) The Moths of America North of Mexico (Fascicle 27.1.) – Noctuoidea, Noctuidae (part): Noctuinae, Agrotini. Wedge Entomological Research Foundation, Washington, DC, 385 pp.
- Poole RW (1995) Noctuoidea: Noctuidae (part), Cuculliinae, Stiriinae, Psaphidinae (Part). In: Dominick RB, et al. (Eds) The Moths of America North of Mexico Fasc. 26.1. The Wedge entomological Research Foundation, Washington, DC, 1–249. [pls A–V, 1–5]
- Schaus W (1904) New species of American Heterocera. Transactions of the American Entomological Society 30: 135–178.
- Schaus W (1914) New species of noctuid moths from tropical America. Proceedings of the United States National Museum 46: 485–549. <https://doi.org/10.5479/si.00963801.2039.485>
- Yen S-H, Wu S (2009) Biota Taiwanica. Hexapoda: Lepidoptera, Noctuoidea, Noctuidae, Eriopinae. National Sun Yat-Sen University & National Science Council, 60 pp. [20 pls.]