

Subdivision of the neotropical Prisopodinae Brunner von Wattenwyl, 1893 based on features of tarsal attachment pads (Insecta, Phasmatodea)

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Abstract

The euplantulae of species from all five genera of the Prisopodinae Brunner von Wattenwyl, 1893 were examined using scanning electron microscopy with the aim to reveal the significance of attachment pads regarding their phylogenetic relationships. The split into the conventional two sister groups is supported by the two-lobed structure of the euplantulae with a smooth surface in the Prisopodini and a nubby surface microstructure in the Paraprisopodini. The two lineages are well distinguishable by this feature, as well as by the shape of the euplantulae themselves. The functional importance of the attachment pad surface features is discussed.

Keywords

Phasmatodea, tarsus, euplantulae, Neotropis, scanning electron microscopy

Introduction

The Prisopodinae Brunner von Wattenwyl, 1893, which occur exclusively in Central and South America, were erected by Karny (1923) and include various species that were later transferred to other subfamilies. Günther (1953) placed the whole group

as a subordinate taxon of the Pseudophasmatinae Rehn, 1904. This view is supported as well by Bradley and Galil (1977) as by more recent phylogenetic analyses (Bradler 2009, Goldberg et al. 2015). Zompro (2004) recovered the Prisopodinae and arranged it to the two remaining tribes Prisopodini Brunner von Wattenwyl 1893, which includes *Damasippus* Stål, 1875, *Dinelytron* Gray, 1835 and *Prisopus* Peletier de Saint Fargeau & Serville, 1828, and Paraprisopodini Zompro, 2004 with *Melophasma* Redtenbacher, 1906 and *Paraprisopus* Redtenbacher, 1906. Bradler (2009) found support for monophyletic Pseudophasmatinae, including the genus *Prisopus* based on distinctive characters of the mouthparts. Goldberg et al. (2015) recovered the species *Melophasma antillarum* (Caudell, 1914) as a member of the Pseudophasmatinae based on molecular data. These findings provide preliminary evidence that the two groups Paraprisopodini and Prisopodini are related to the Pseudophasmatinae. We assume that the former Prisopodinae are a subordinate group within the Pseudophasmatinae, but suppose that the subordinate lineages Prisopodini and Paraprisopodini are sister groups considering their characteristic egg morphology (Zompro 2004).

Various attachment devices have evolved on the tarsi and pretarsi of hexapods (Beutel and Gorb 2001, 2006). As attachment systems underlie adaptations to the substrate and the ecology, their appearance and specific structure reflect aspects of the evolution of the species. The phylogenetic relevance of attachment pads has been previously demonstrated for the Hexapoda in general (Beutel and Gorb 2001, 2006) and with emphasis on the Phasmatodea and Mantophasmatodea (Beutel and Gorb 2006, 2008), as well as for such subgroups as the Dermaptera (Haas and Gorb 2004) and the Plecoptera (Nelson 2009). As hypothesised by Gottardo et al. (2015) the micro-morphological surface of the euplantulae might bear phylogenetic relevant features as well. In the present study, the euplantulae of taxa from all five genera included in the Prisopodinae were examined. The general shape of the euplantulae and their surface microstructure were compared with the aim of uncovering relationships between the species included in the corresponding tribes. These characters are discussed with the aim to achieve a more accurate characterisation of the two lineages and to evaluate the monophyly and phylogenetic position of this group.

Methods

One species per genus has been examined from dried specimens using scanning electron microscopy (SEM). Living animals were anaesthetised with CO₂ and then decapitated. The right metatarsi were dissected at the level of the tibia and fixated in 2.5% glutaraldehyde in PBS buffer on ice on a shaker for 24 h. To soften and reactivate the attachment pads from the tarsi of dried insects, the legs were cut off, rehydrated in a relaxing chamber for 24 h, and then stored in a 10% solution of lactic acid (Gladun and Gumovsky 2006). The tarsi remained in the solution for 24–48 h and then fixated in 2.5% glutaraldehyde in PBS buffer on ice on a shaker for 24 h. Fixated samples were dehydrated in an ascending alcohol series and critical-point dried. The dried samples

were mounted on aluminium stubs and sputter-coated with a 15 nm thick layer of gold-palladium. Specimens were observed in the scanning electron microscope (SEM) Hitachi S4800 (Hitachi High-Technologies Corp., Tokyo, Japan) at 7 kV of acceleration voltage. Further species were examined in a stereo microscope to ensure the consistency of the SEM findings. A comprehensive list of the examined specimens is provided in the appendix.

Results

Paraprisopodini

Zompro (2004) characterised the species of the Paraprisopodini by their elongated abdomen and the shortening of tegmina and alae in comparison to the Prisopodini as synapomorphies. The two groups, *Melophasma* and *Paraprisopus*, are distributed in Northern South America (Brock et al. 2016).

Melophasma antillarum (Caudell 1914)

The tarsi of *M. antillarum* consist of very broad tarsomeres bearing large, roundish euplantulae. The arolium is smaller than the euplantulae. The euplantulae form two separated lobes diverging in lateral direction of the tarsus (Fig. 1A). The flexible adhesive cuticle of the euplantulae is limited to the distal part of the tarsomere forming a clearly cut attachment pad (Fig. 1B). The euplantula surface at high magnification of the SEM reveals small conical outgrowths of the epicuticle (Fig. 1C).

Paraprisopus merismus (Westwood, 1859)

Similar to *M. antillarum*, the euplantulae of *P. merismus* cover a proportionally significant area of the ventral side of tarsomeres, but form hemispherical attachment pads. The arolium is likewise reduced in size (Fig. 1D). All euplantulae form two lobes (Fig. 1E). Although the surface microstructure of the pads consists of similar nubs as in *M. antillarum*, the density of the nubs is lower (Fig. 1F).

Prisopodini

The representatives of the Prisopodini are also distributed in Central and South America (Brock et al. 2016). This lineage is distinguished from the Paraprisopodini by their long tegmina, which project beyond the second abdominal segment. Additionally, they possess eggs with the operculum inserted dorsally at a positive angle, whereas the eggs of the Paraprisopodini have the operculum inserted at the anterior end (Zompro 2004).

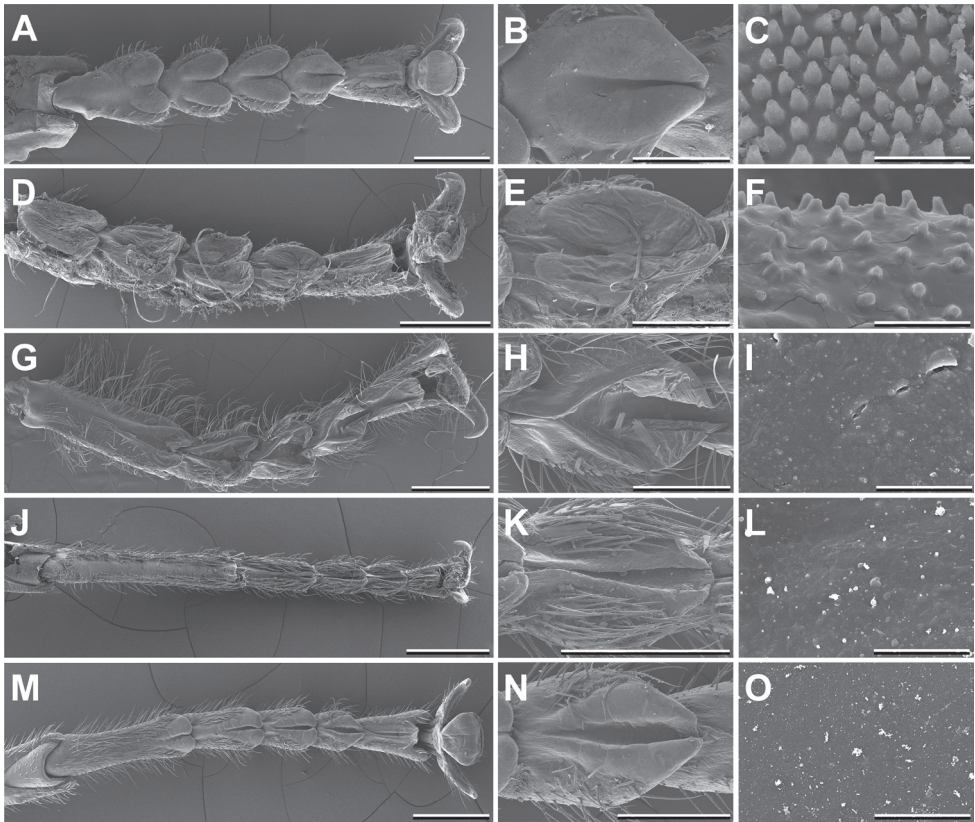


Figure 1. Scanning electron micrographs of the tarsal morphology of different Prisopodinae species. From left to right: Overview; Fourth euplantula; Adhesive microstructure. Scale bars: 1 mm; 300 μ m; 5 μ m. *Melophasma antillarum*, female (**A–C**). *Paraprisopus merismus*, female (**D–F**). *Prisopus horstokkii*, female (**G–I**). *Dinelytron grylloides*, female (**J–L**). *Damasippus* sp., female (**M–O**).

Prisopus horstokkii (Haan, 1842)

In general the tarsus of *Pr. horstokkii* has a similar appearance to other Prisopodini, but in detail the tarsal morphology of *Prisopus* reveals unique characters in comparison to the other Prisopodinae. The tarsus is not symmetrical as in the other species, but broadened apically. Additionally, the tarsal setae on the dorsal side of the tarsomeres are much longer in comparison to the other examined genera. The euplantulae of this species are similar to the other Prisopodini, with euplantulae consisting of two bars (Fig. 1G). Each euplantula follows the entire tarsomere length and forms an elliptical pad with a groove (Fig. 1H). In *Pr. horstokkii*, the surface of the euplantulae is smooth without any microstructural elevations (Fig. 1I).

***Dineilytron grylloides* Gray, 1835**

In comparison to the tarsi of the Paraprisopodini, the tarsus and the euplantulae of *D. grylloides* are more slender. Except the first tarsomere, the euplantulae consist of two thin bars traversing the tarsomere and dividing it centrally. The euplantula on the long basitarsus is limited to a small bilobed pad (Fig. 1J). In contrast to the Paraprisopodini and to various other phasmids (Beutel and Gorb 2008), the membranous attachment pad intersects the entire tarsomere (Fig. 1K). The surface microstructure of the euplantulae of *D. grylloides* is completely smooth in contrast to that of the Paraprisopodini (Fig. 1L).

***Damasippus* sp.**

The examined specimens are captive-bred from individuals which have been found in Monteverde, Costa Rica and which do not belong to any described species. Similar to *D. grylloides*, the tarsi of this *Damasippus* sp. individual are slender with a long basitarsus. The euplantulae consist of two bars as well (Fig. 1M). The two bars reach out to each other at the proximal and distal tips, forming an elliptical attachment pad, surrounding a groove in the middle (Fig. 1N). The surface of the euplantulae is smooth, as in the other genera of the tribe (Fig. 1O).

Discussion

In Zompro (2004), the erection of the Paraprisopodini and the characterisation of the lineages within the Prisopodinae have been done without further discussion of the distinguishing features. The Paraprisopodini are considered by Zompro (2004) the more 'basal' tribe of both, because of the shortened tegmina in comparison to the Prisopodini. The tegmina of closely related outgroup taxa (the remaining Pseudophasmatinae) are shorter than of representatives of this group. So the consideration of this shortening as a synapomorphy as brought up by Zompro (2004) is less consistent than considering the elongated tegmina and specialised appearance of the euplantulae in the Prisopodini as synapomorphies.

Due to the shortening of the basitarsus observed in the Paraprisopodini the entire tarsal chain looks shorter than in the Prisopodini. With such geometry, the adhesive force is generated nearer to the body of the insect, which might provide some advantage for controlling attachment and detachment. The Paraprisopodini are camouflaged well in resting position with their legs pulled towards their body. Longer legs on the contrary may be useful for taking longer strides and therefore run faster (Chapman 1998).

All species of the Prisopodinae bear a euplantula on the tarsomere V, which is not the case in all species of the Phasmatodea (Vallotto et al. 2016). Considering the need to attach strongly to the substrate, an additional attachment pad is helpful to increase the attachment force (Gottardo et al. 2015). As the euplantulae are known to generate strong friction (Bußhardt et al. 2012; Labonte and Federle 2013), the use of an additional fifth euplantula might result in a higher total friction force.

The two lineages can be distinguished by the morphological features of the tarsi. The Paraprisopodini bear round, bilobed attachment pads with a nubby adhesive ultrastructure, which correspond to the shape found in many other species of the Phasmatodea (cf. Beutel and Gorb 2008; Vallotto et al. 2016). These two character states are supposed to be a ground plan for the Euphasmatodea (Gottardo et al. 2015) and therefore are suggested to be plesiomorphic regarding the group examined herein. However the arolium being smaller than the euplantulae is a character state not present in the ground plan of the Euphasmatodea, judging on the species covered by the literature (e.g. Beutel and Gorb 2008; Gottardo et al. 2015; Vallotto et al. 2016), and might represent an apomorphy on the level of the Paraprisopodini. Bradley and Galil (1977) suggested *Melophasma* being a synonym of *Paraprisopus* without reasoning the synonymisation. This has been ignored by the following publications dealing with *Melophasma*, but is another suggestion of monophyletic Paraprisopodini.

The Prisopodini's euplantulae on the contrary consist of two thin bars, which are interpreted as an apomorphy of this lineage and support the monophyly of it. Additionally the lack of nubs on the euplantulae is not part of the ground plan in the Euphasmatodea (Gottardo et al. 2015) and is a further potential apomorphy of the Prisopodini. As the euplantulae of the Paraprisopodini match the suggested ground plan of the Euphasmatodea, namely nubby euplantular ultrastructures and roundish bilobed euplantulae, the monophyly of the Prisopodinae sensu Zompro (2004) still has to be validated. Lacking a broader taxon sampling covering closely related outgroups, a synapomorphy of the Prisopodinae sensu Zompro (2004) cannot be found in the attachment devices. A possible synapomorphy of this group is found in the egg morphology, namely a specialised longitudinal area on the ventral surface and the operculum 'inserted at an angle' (Zompro 2004). Additionally the tegmina of both lineages are elongated in comparison to the remaining Pseudophasmatinae. Although the tegmina are shorter in the Paraprisopodini in comparison to the Prisopodini, they are of median length regarding the even shorter tegmina of the Pseudophasmatinae.

The examined species of *Damasippus* is found in Costa Rica in dampy and windy habitats. The flying adults are in need of effective attachment organs in order to adhere securely on different substrates when landing, since a fall to the ground would cause troubles to the large animals living up in the tree canopies. The flight of the examined species is not sufficiently effective to return to the foliage without high efforts, but their specialisation to the food plants necessitates a distribution close to them. Considering the slightly concave shape of the euplantulae in this lineage, they may function as a suc-

tion cup, generating strong attachment force on rather smooth substrates. It is plausible to assume that, if the bars meet together, they form an ellipse and seal the surrounded volume. The generation of the suction effect can be presumably performed by haemolymph pressure control within the euplantulae (cf. Shvanvich 1949; Denning et al. 2014).

Additionally, both lineages differ significantly in their surface microstructure. So far the nubby surface of the Paraprisopodini is exclusively found in other species with reduced wings. The apterous species *Neohirasea maerens* (Brunner von Wattenwyl, 1907), *Aretaon asperrimus* (Redtenbacher, 1906) (Beutel and Gorb 2008), *Carausius morosus* (Sinéty, 1901) (Bußhardt et al. 2012), and *Conlephasma enigma* Gottardo & Heller, 2012 (Gottardo and Heller 2012) bear nubby surface structures. The species *Orthomeria kangi* Vallotto, Bresseel, Heitzmann & Gottardo, 2016 (Vallotto et al. 2016), *Medauroidea extradentata* (Brunner von Wattenwyl, 1907) (Bußhardt et al. 2012), *Hermarchus leytenensis* Zompro, 1997 (Gottardo and Vallotto 2012), and *Eurycantha calcarata* Lucas, 1869 (Gottardo et al. 2015) include both winged and un-winged taxa which all possess smooth attachment structures. Furthermore, pointed acanthae on the euplantulae in *Timema nevadense* Strohecker, 1966 (Gorb and Beutel 2008) and small elevated hexagons in *Dallaiphasma eximius* Gottardo, 2011 (Gottardo 2011) have been previously reported. It is hypothesised that the evolution of different attachment microstructures might reflect phylogenetic patterns (Gottardo et al. 2015). For a proper outgroup comparison of the attachment devices and a more comprehensive comparison of the attachment microstructures a comprehensive study with broader taxon sampling is necessary.

From the functional point of view, smooth phasmid attachment pads demonstrate strong adhesive and frictional performance on smooth substrates, whilst the nubby pad surface seems to be the adaption to a broader range of substrate textures (Bußhardt et al. 2012).

The species *M. antillarum* bears euplantulae, which are known to mainly generate friction, but possesses a reduced arolium, which generates adhesion (Labonte and Federle 2013). The increased role of friction in this species reveals the likeliness of this species to use the tarsi in sliding direction instead of relying on the attachment force directed orthogonally from the ground. In the resting position, representatives of the Paraprisopodini pull their short legs towards their body and rely primarily on friction during attachment. This is reflected in their substrate preferences: they usually occupy thinner branches with small curvature radii. In contrast, individuals from the Prisopodini, which rather prefer flat substrates, rather stretch their legs away from the body, raising the friction contribution due to lowering the angle between the tarsus and the substrate, needed for a proper attachment to the substrate. Together with a comprehensive study of the adhesive structures of the Phasmatodea to evaluate the phylogenetic distribution of these features, we suggest some experimental studies measuring the adhesive properties of different attachment ultrastructures. These may also help to scrutinise the assumptions on the functional morphology of the taxa examined herein.

Conclusions

Within the Prisopodinae two types of attachment pads are found coherently for the two previously suggested lineages (Paraprisopodini and Prisopodini). It is shown here that characters of attachment pads are useful for distinguishing these lineages. The Paraprisopodini bear big and roundish bilobed euplantulae, as most other known Euphasmatodea, whilst the Prisopodini bear two-bared euplantulae with a groove intersecting the entire tarsomere as an apomorphy. Additionally, the two lineages can be distinguished by the micromorphology of the pad surface. Whilst the Paraprisopodini bear nubby euplantulae with specific densities of nubs, the Prisopodini's euplantulae are smooth without any micromorphological features. Both macroscopical and microscopical characters contribute to the differentiation of the two lineages, which formerly were distinguished by the tegmina only. The use of the pad surface microstructure for the phylogeny of these groups is suggested in this study for the first time. To validate the monophyly of the former Prisopodinae and their location within the Pseudophasmatinae a more comprehensive study of the attachment ultrastructures of the Phasmatoidea in combination with upcoming transcriptome analyses are suggested.

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Appendix

Abbreviations

Coll. TB	Private collection of Thies Büscher, Kiel, Germany
NHMUK	Natural History Museum London, UK
NMW	Natural History Museum Vienna, Austria
OUMNH	University Museum of Natural History Oxford, UK
ZFMK	Zoologisches Forschungsinstitut und Museum „Alexander Koenig“, Bonn, Germany
HT	Holotype
ST	Syntype
PT	Paratype
SEM	Scanning electron microscope

List of examined specimens

Damasippus sp.; coll. TB: 2♂♂, 2♀♀; one female examined via SEM

Damasippus sp.; NHMUK: 1♂, 3♀♀

Damasippus batesianus (Westwood, 1859); OUMNH, HT: 1♂

Damasippus discoidalis Redtenbacher, 1906; ZFMK: 1♂

Damasippus fuscipes Redtenbacher, 1906; NHW, ST: 2♂♂, 1♀

Damasippus fuscipes Redtenbacher, 1906; NHMUK, ST: 1♂

Damasippus striatus Redtenbacher, 1906; OUMNH: 1♀

Damasippus zymbraeus (Westwood, 1859); OUMNH, ST: 2♂♂, 1♀

Damasippus zymbraeus (Westwood, 1859); OUMNH: 1 nymph

Dineilytron agrion Westwood, 1859; NHMUK, HT: 1♂

Dineilytron agrion Westwood, 1859; OUMNH: 1♂

- Dinelytron grylloides* Gray, 1835; coll. TB: 1♀; examined via SEM
- Melophasma antillarum* coll. TB: 6♂♂, 6♀♀; one female examined via SEM
- Melophasma vermiculare* Redtenbacher, 1906; NHW, ST: 2♀♀
- Paraprisopus* sp.; NHMUK: 3♂♂
- Paraprisopus merismus* (Westwood, 1859); coll. TB: 1♀; examined via SEM
- Paraprisopus merismus* (Westwood, 1859); NHMUK, HT: 1♂
- Paraprisopus foliculatus* Redtenbacher, 1906; NHW, ST: 1♀
- Prisopus ariadne* Hebard, 1923; NHMUK: 1♀
- Prisopus berosus* Westwood, 1859; NHMUK, ST: 2♂♂
- Prisopus berosus* Westwood, 1859; OUMNH: 1♂
- Prisopus cepus* Westwood, 1859; OUMNH, HT: 1♂
- Prisopus cepus* Westwood, 1859; OUMNH: 2♀♀, 1 nymph
- Prisopus cornutus* Gray, 1835; OUMNH: 1♂
- Prisopus cornutus* Gray, 1835; NHMUK: 1♂
- Prisopus horridus* (Gray, 1835); OUMNH: 1♀
- Prisopus horstokkii* (Haan, 1842); coll. TB: 3♂♂, 1♀; one female examined via SEM
- Prisopus horstokkii* (Haan, 1842); NHMUK: 3♂♂, 2♀♀
- Prisopus phacellus* Westwood, 1859; NHMUK, HT: 1♂
- Prisopus phacellus* Westwood, 1859; NHMUK: 2♂♂, 1♀
- Prisopus phacellus* Westwood, 1859; OUMNH: 2♀♀
- Prisopus sacratus* (Olivier, 1792); OUMNH: 3♂♂, 2♀♀, 2 nymphs
- Prisopus sacratus* (Olivier, 1792); NHMUK: 5♂♂, 4♀♀
- Prisopus sacratus* (Olivier, 1792); ZFMK: 1♂
- Prisopus spiniceps* Burmeister, 1838; OUMNH: 1♀

Taxonomy of *Macromotettixoides* with the description of a new species (Tetrigidae, Metrodorinae)

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Abstract

Descriptions of the flying organs and generic characteristics of the genus *Macromotettixoides* Zheng, Wei & Jiang are currently imprecise. *Macromotettixoides* is reviewed and compared with allied genera. A re-description is undertaken and a determination key is provided to *Macromotettixoides*. *Macromotettixoides parvula* Zha & Wen, **sp. n.** from the Guizhou Karst Region, China, is described and illustrated with photographs. Observations on the ecology and habits of the new species are recorded. Four current species of *Hyboella* Hancock are transferred to *Macromotettixoides*. Variations of the flying organs and tegminal sinus in the Tetrigidae are discussed, which will help to describe them accurately.

Keywords

China, determination key, ecology, habit, *Hyboella*, Karst Region, Orthoptera, revision, Tetridoidea

Introduction

The originally monotypic genus *Macromotettixoides* (Orthoptera: Tetrigidae: Metrodorinae) was erected by Zheng et al. in 2005 with *M. jiuwanshanensis* Zheng, Wei & Jiang, 2005 as its type species. Deng et al. (2014) gave a systematic study of this

genus, which dealt with eight known species. Simultaneously Zheng (2013a) introduced another new species, *M. wuyishana* Zheng, 2013. Recently Deng (2016) in his dissertation transferred *Apterotettix lativertex* Zheng, Ou & Zhang, 2012 to this genus and assigned a new name, *M. longling* (Zheng, Ou & Zhang, 2012) for it, because the epithet ‘*lativertex*’ had already been used in this genus, also described another new species, *M. jinggangshanensis* Deng, 2016. As a result, *Macromotettixoides* currently includes 11 species, which are all distributed in China.

Based on absent tegmen (accordingly tegminal sinus is absent or inconspicuous), Zheng et al. (2005) erected *Macromotettixoides* to distinguish from *Macromotettix* Günther, 1939; and for the reason of shortened hind process and with broad and arcuate apex, Zheng et al. (2012) erected *Pseudomacromotettix* Zheng, Li & Lin, 2012 to separate from *Macromotettixoides*. To these three allied genera there are some problems we are facing: 1) descriptions to both flying organs and tegminal sinus are ambiguous; 2) descriptions of generic characteristics of *Macromotettixoides* are vague (Deng et al. 2014, Deng 2016); and 3) features of partial species of *Hyboella* Hancock, 1915 are also in accord with *Macromotettixoides*, but the researches above seldom dealt with *Hyboella*.

In this study *Macromotettixoides* is examined and compared it with allied genera. At the same time *Macromotettixoides parvula* Zha & Wen, sp. n., from Guizhou Karst Region, China, is described and illustrated with photographs. Some aspects of ecology and observations of habits of the new species are also recorded.

Materials and methods

Specimens were photographed using a stereo microscope (Olympus Corporation, SZX16, Tokyo, Japan), ecological pictures were photographed using a Nikon Coolpix P520 camera. Morphological terminology and measurement landmarks follow Zheng (2005) and Tumbrinck (2014). Measurements are given in millimeters (mm). Type specimens are deposited in the Specimen Room of the School of Life Sciences, Huaibei Normal University, Huaibei, Anhui Province, China.

Taxonomy

Macromotettixoides Zheng, Wei & Jiang, 2005

Macromotettixoides Zheng, Wei & Jiang 2005: 366; Zheng 2005: 176; Deng et al. 2007: 160, 2014: 548; Deng 2011: 543, 2016: 155.

Type species. *Macromotettixoides jiuwanshanensis* Zheng, Wei & Jiang, 2005 by original designation

Redescription. Size small and stout. Vertex nearly at the same or slightly below the level of anterior margin of pronotum, and decidedly wider than width of one

eye; longitudinal furrow (instead of scutellum in most genera of Cladonotinae) relatively shallow, equal to or wider than diameter of scapus (similar to Cladonotinae); antennae filiform, inserted between or below lower margin of eyes. Pronotum roof-like or nearly at the same level; median carina conspicuous, sometime weakly, but not strongly lamellate; hind process short, not surpassing apex of hind femur, apex acute or acutely rounded; posterior angle of lateral lobe turning outwards (differ from Tetigininae (directed downwards and contiguous to the body)), apex truncated or roundly truncated (differ from Scelimeninae (directed sideward as an acute triangle process or a long acute spine)); ventral sinus present, tegminal sinus absent or very inconspicuous; external lateral carina surpassing middle of lower margin of pronotum. Flying organs abbreviated: tegmina invisible; hind wings invisible in most species, visible but never reaching middle of hind process in few species. Female ovipositor narrow and long.

Distribution. China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hubei, Hunan, Jiangxi, Sichuan, Yunnan, Taiwan).

Key to species of *Macromotettixoides* (16 species)

- 1 Hind wings very small and hidden beneath pronotum, invisible or barely visible..... 2
- Hind wings slightly elongate and nearly reaching middle of hind femur, visible 15
- 2 Pronotum flattened, nearly at the same level..... 3
- Pronotum, in lateral view, distinctly roof-like 5
- 3 Anterior margin of pronotum broadly arcuate forward; humeral angles also broadly arcuate (Hainan) *M. hainanensis* (Liang, 2002), **comb. n.**
- Anterior margin of pronotum truncated; humeral angles obtuse angled 4
- 4 Vertex 1.4 times as wide as one eye; prozonal carinae contracted backward; hind process reaching middle of hind femur (Taiwan) *M. taiwanensis* (Liang, 2000), **comb. n.**
- Vertex 2.0 times as wide as one eye; prozonal carinae parallel; hind process reaching knee of hind femur (Guizhou) *M. parvula* **sp. n.**
- 5 Anterior margin of pronotum obtusely angled forward 6
- Anterior margin of pronotum truncated 10
- 6 Upper margin of pronotum wholly arcuate in lateral view; hind process reaching apex of hind femur 7
- Upper margin of pronotum, in lateral view, arcuate only before humeral angles while straight or undulated behind humeral angles; hind process not reaching apex of hind femur 9
- 7 Vertex 3.0 times as wide as one eye, anterior margin obtusely angled (Fujian) *M. wuyishana* Zheng, 2013a
- Vertex 2.1-2.3 times as wide as one eye, anterior margin arcuate 8
- 8 Vertex together with frontal costa right angled; humeral angles obtusely angled, interhumeral carina absent; middle of posterior margin of female sub-

- genital plate with a triangular protrusion (Guangxi)
 ***M. jiuwanshanensis* Zheng et al., 2005**
- Vertex together with frontal costa rounded; humeral angles absent, paired interhumeral carinae presented; posterior margin of female subgenital plate three-tooth-like (Jiangxi) ***M. jinggangshanensis* Deng, 2016**
- 9 Longitudinal furrow between antennal grooves 1.6 times as wide as diameter of scapus; pronotal disc with many net-like wrinkles; humeral angles indistinct; lower margins of fore and mid femora a little undulate (Guangxi)
 ***M. lativertex* Deng et al., 2014**
- Longitudinal furrow between antennal grooves as wide as diameter of scapus; pronotal disc smooth; humeral angles arcuate; lower margins of fore and mid femora straight (Jiangxi) ***M. brachynota* Zheng & Shi, 2009**
- 10 Lower margins of fore and mid femora undulated **11**
- Lower margins of fore and mid femora straight **12**
- 11 Antenna inserted below lower margin of eyes; prozonal carinae parallel; apex of hind process narrow (Sichuan) ***M. undulatifemura* Deng et al., 2012**
- Antenna inserted between lower margin of eyes; prozonal carinae contracted backward; apex of hind process relatively wide, concave in the middle (Yunnan) ***M. curvimarginus* (Zheng & Xu, 2010), comb. n.**
- 12 Vertex 2.0–2.14 times as wide as one eye; interhumeral carina absent **13**
- Vertex 1.3–1.6 times as wide as one eye; paired interhumeral carinae presented **14**
- 13 In lateral view upper margin of pronotum before shoulders strongly arcuate; humeral angles obtusely rounded (Hubei) ***M. wufengensis* Zheng et al., 2009**
- In lateral view upper margin of pronotum straight; humeral broadly arcuate (Hunan) ***M. badagongshanensis* (Zheng, 2013b), comb. n.**
- 14 Vertex 1.3 times as wide as one eye; antenna inserted below lower margin of eyes; in lateral view upper margin of pronotum strongly arcuate before humeral angles while straight behind humeral angles (Fujian)
 ***M. zhengi* Deng, 2011**
- Vertex 1.6 times as wide as one eye; antenna inserted between lower margin of eyes; in lateral view upper margin of pronotum nearly straight (Yunnan)
 ***M. longling* (Zheng et al., 2012)**
- 15 Vertex 2.0 times as wide as one eye; hind process reaching middle of hind femur; lower margin of mid femur undulate (Yunnan)
 ***M. cliva* Zheng et al., 2006**
- Vertex 1.5 times as wide as one eye; hind process reaching two-thirds of hind femur; lower margin of mid femur straight (Guizhou)
 ***M. aelytra* (Zheng et al., 2002), nymph***

*Note: according to descriptions (antegenicular denticle and genicular denticle have not been separated) and drawings of Zheng et al. (2002) and Zheng (2005), the type specimen (only one female) of *M. aelytra* (Zheng, Li & Shi, 2002) (synonym: *Hyboella*

aelytra Zheng, Li & Shi, 2002 (Zheng et al. 2006)) should be a nymph. Validity of *M. aelytra* requires more material to confirm its characters, and herein we temporarily place the species in the key.

***Macromotettixoides parvula* Zha & Wen, sp. n.**

<http://zoobank.org/F2EFC917-2D8A-4A9A-89E8-DD3A3EA6861D>

Figs 1–2

Diagnosis. With extremely small size, *Macromotettixoides parvula* sp. n. can easily be separated from other species of the genus. Other differences are listed in the key to species of *Macromotettixoides*.

Description. Female. Body size extremely small.

Head. Face and vertex rough, covered with large and small granules. Vertex nearly at the same level but uneven, 2.0 times as wide as one eye, a little contracted forward, protruding forward and slightly surpassing anterior margin of eyes; anterior margin broadly arcuate and depressed, anterior part of lateral carina distinctly folded upward and reaching top of eyes; medial carina distinct and erected in anterior half, but absent in posterior half; paired fossulae deep, behind fossulae vertex slightly elevated on both sides (Fig. 2b). In lateral view face slightly oblique, fastigium (vertex together with frontal costa) rounded and protruding forward; fascial carinae smooth, between lateral ocelli concave, between antennal grooves widely and obtusely triangular forward (Fig. 2d); in frontal view fascial carinae diverged in the middle of inner margin of eyes, longitudinal furrow wide and shallow and nearly forming into a scutellum, between antennal grooves 1.5 times as wide as diameter of scapus (Fig. 2a). Antenna filiform and short, 17-segmented, inserted decidedly below lower margin of eyes, segment 11 longest, 5.0 times as long as wide (Fig. 2a, d). Eyes globose and protruding, over level of anterior margin of pronotum, lateral ocelli placed at lower one third of inner margin of eyes (Fig. 2a).

Thorax. Pronotum disc very coarse, covered with big and small granules and many thick and net-like wrinkles (Fig. 1). Anterior margin truncated and wide, paired extralateral carinae indistinct (Fig. 2b); pronotum in the center between posterior sulcus and humeral angles slightly elevated, otherwise nearly at the same level. Median carina entire and thick, between sulci distinctly elevated with swollen base, behind humeral angles with a protrusion, the posterior protrusions lower and indistinct; in lateral view upper margin undulate, the first sinusoidal wave occur between sulci, longest and highest, in the middle with a deep concavity, followed by the second wave behind humeral angles, the posterior waves low and nearly at the same height. Prozonal carinae conspicuous, thick, erected and parallel (Fig. 2b); humeral angles obtuse angled and low, pronotum disc behind humeral angles covered with many reticular wrinkles; hind process reaching knees (three males and two females) or barely surpass apex of hind femur (one female), apex sharp-rounded; external lateral carina straight, distinctly surpassing middle of lower margin and reaching two-thirds of hind femur, folded upward

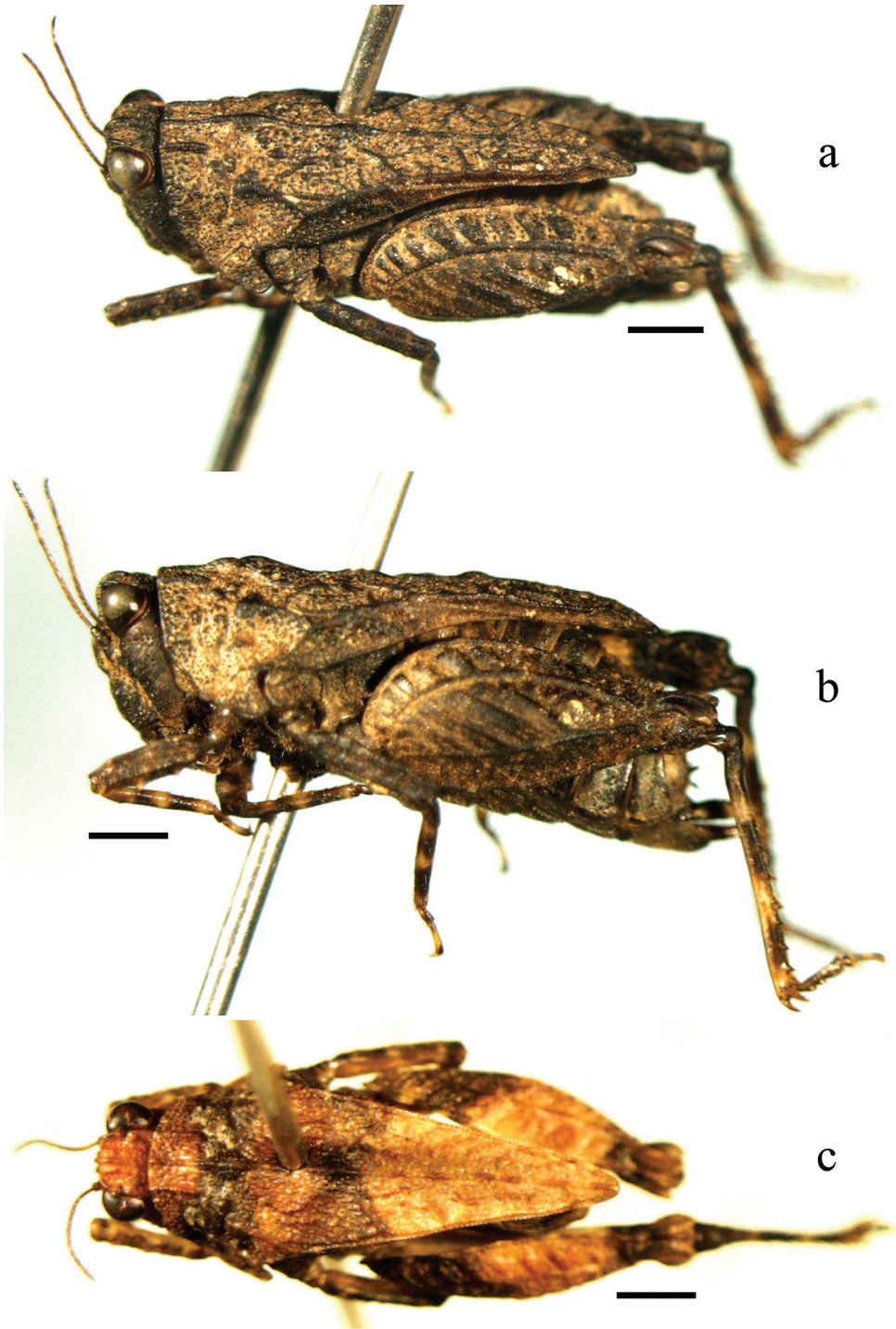


Figure 1. *Macromotettixoides parvula* sp. n. **a** oblique-lateral view of female body **b** lateral view of female body **c** dorsal view of male body. Scale bars 1.0 mm.

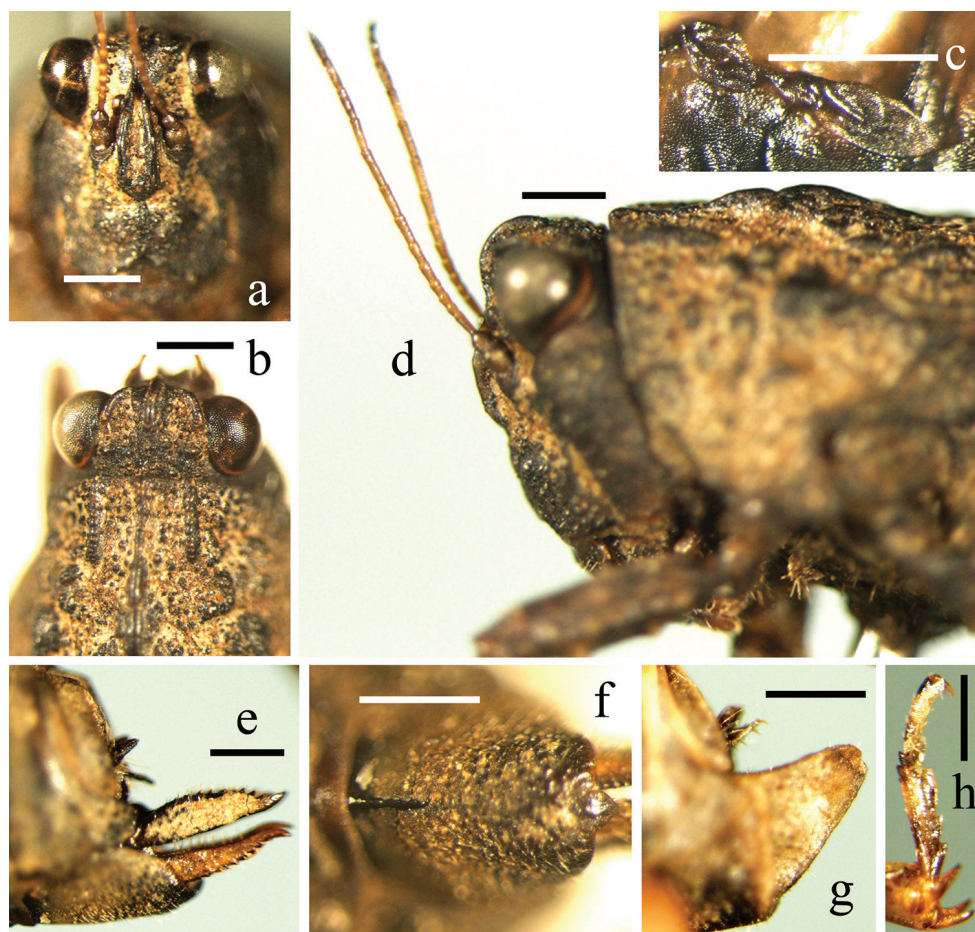


Figure 2. *Macromotettixoides parvula* sp. n. **a** frontal view of female head **b** dorsal view of female head **c** left-lateral view of female tegmen and hind wing after uncovering the pronotum **d** lateral view of female head and anterior pronotum **e** lateral view of female ovipositor and subgenital plate **f** ventral view of female subgenital plate **g** lateral view of male subgenital plate **h** left-lateral view of female hind tarsus. Scale bars 0.5 mm.

indistinctly behind humeral angles; lateral carina of pronotum before apex curved inward; posterior angle of lateral lobe of pronotum extending turning outwards, margin smooth, apex truncated and anterior margin of apex rounded; posterior margin of each lateral lobe with one concavity. Tegmina and wings extremely degenerated, long and oval, apices acute, wing distinctly larger than tegmen, both hidden beneath pronotum and invisible (Fig. 2c). Margins of all femora serrate except base of upper margin of hind femur, upper margins of fore and mid femora nearly straight while lower margins with two teeth (basal and middle) each; hind femur stout, 2.3 times as long as wide, upper margin before antegenicular denticle with a small tooth, other teeth on upper and lower margins indistinct; antegenicular denticle slightly isolated,



Figure 3. Habitat environment of *Macromotettixoides parvula* sp. n. **a** a gully in a broad-leaved forest **b** border of a stream. Pictures were photographed by Lingsheng Zha in China, Guizhou, Leishan, Leigongshan Mountain, 2 Aug 2016.

low, apex or nearly right angled or a little sharp, genicular denticle finger-like, extending backward and apex obtuse; margins of fore and mid tibiae straight; two inner margins of hind tibia serrate, terminal part slightly wider than basal part, outer/inner side with 6-7/4-6 spines; first segment of hind tarsus 1.35 times as long as second plus third, first and second pulvilli small and apices sharp, third pulvillus large and apex obtuse (Fig. 2h).

Abdomen. Ovipositor: upper valva about 4.0 times as long as wide, upper margin arcuate, sub-base widest, in the middle slightly distorted inward, then slightly turn outward and at last inward again; outer margins of upper and lower valvae with saw-like teeth, but base of upper valva smooth (Fig. 2e). Subgenital plate: length nearly equal to width, median carina distinct in anterior part, posterior margin nearly truncated and in the middle triangularly protruding which is slightly folded inward (Fig. 2f).

Coloration. Body dark or dark brown (Fig. 1). Antennae brown, color of terminal 3-5 segments dark, color of the two segments of before and after the longest segment a little light (Fig. 2d). Sometimes both the posterior part of pronotum and the posterior part of outer side of hind femur brown. All tibiae with three yellowish brown rings each, but basal and middle rings of hind tibia large. More or less, infrascapular area, teeth on lower margins of fore and mid femora, upper and lower margin of hind femur, and outer sides of all femora maculated with yellowish brown.

Male. Slightly smaller than female (Fig. 1). Vertex also 2.0 times as wide as one eye; antenna 16 segmented, segment 10 longest. Subgenital plate short cone-shaped, apex nearly truncated, upper apex bifurcate and forming into two obtuse and very short teeth (Fig. 2g). Other characters same as female.

Measurements. Length of body ♂5.8–6.2 mm, ♀7.5–8.3 mm; length of pronotum ♂5.8–6.0 mm, ♀6.3–7.0 mm; length of hind femur ♂4.1–4.3 mm, ♀4.2–4.5 mm; length of antenna ♂, ♀2.6–2.8 mm.

Type material. Holotype female, China, Guizhou, Leishan, Leigongshan Mountain, N26°22'18.25", E108°11'28.06", 1430 m alt, 2 Aug. 2016, collected by

Lingsheng ZHA. Paratypes: three males and two females, Leigongshan Mountain, 1300–1600 m alt, 1–3 Aug. 2016, collected by Lingsheng ZHA.

Ecology and habits. Specimens of *Macromotettixoides parvula* sp. n. were collected and observed among low and sparse shrubs with fall-leaf layers in gullies, slopes and a dry stream bed in humid rainforests of Karst Region (Fig. 3). They are very small and not easy to find; they move quickly and they like to jump into shrubs when being disturbed. They mainly feed on humus. We infer their adults may prefer to stay in sandy soil, because body surfaces of most specimens are covered tightly by sandy soil (Zha et al. 2016a, fig. 1a, b).

Etymology. The new species epithet '*parvula*' means body size is extremely small.

Distribution. China (Guizhou). Only found in Leigongshan Mountain (Leishan County).

Discussion

Flying organs and tegminal sinus in Tetrigidae

According to Zha et al. (2016b), hind wings of Tetrigidae can be divided into four types: 'normal' (developed, nearly reach apex of hind process or more), 'abbreviated' (never reach middle of hind process, but distinctly longer than tegmen), 'vestigial' (shorter than tegmen) and 'apterous' (absent, degenerated completely). In *Macromotettixoides* and *Pseudomacromotettix*, we believe their tegmina are presented and their hind wings belong to the 'abbreviated' (Fig. 2c) or 'vestigial' type, so using 'absent' to describe their small flying organs is exactly not suitable if not uncovering pronota. In this report, we use 'invisible' to replace 'absent'/'wanting' for describing tegmen and wing. To some species of 'abbreviated' or 'vestigial' type, a little visible parts of their fly organs may vary distinctly even become invisible among the same species, which should not be considered as a valuable taxonomic character (Zha et al. 2016b). We also believe tegminal sinus varies according to tegmen strictly during evolution. In other words, normal tegmen means that the tegminal sinus is conspicuous; on the contrary, invisible or a little visible tegmen has determined that the tegminal sinus is absent or shallow.

Relationships between *Macromotettixoides* and its allied genera

In order to clarify relationship between *Macromotettixoides* and its allied genera, we summarize their main differences, based on their known species, as in Table 1. Undoubtedly, *Macromotettixoides* is most similar to *Pseudomacromotettix* and *Macromotettix* (see Introduction and Table 1).

According to Hancock (1915), Günther (1939), Zheng (2005) and Deng (2016), the typical characteristic of *Hyboella* is a pronotum distinctly humpbacked and elevated before the shoulders while depressed and flattened behind shoulders. This characteristic can separate *Hyboella* from *Pseudomacromotettix*, *Macromotettix*, *Cotysoides* Zheng &

Table 1. Main differences between *Macromotettixoides* and its allied genera.

Characters Genera	Vertex and anterior margin of pronotum		Antenna inserted above, between or below lower margin of eye			Tegminal sinus		External lateral carina reaching lower margin of pronotum	
	Nearly at the same level	Vertex distinctly higher	Lower 1/3 of inner margin	Between or slightly below	Far away below	Absent or inconspicuous	Presented	Middle or more	Before middle
<i>Pseudomacromotettix</i>	✓			✓		✓		✓	
<i>Macromotettixoides</i>	✓			✓		✓		✓	
<i>Macromotettix</i>	✓			✓			✓	✓	
<i>Hyboella</i>	✓		✓	✓		✓?	✓	✓	✓
<i>Corysoides</i>	✓		✓				✓		✓
<i>Bolivariettix</i>	✓			✓			✓		✓
<i>Mazarredia</i>		✓		✓			✓		✓
<i>Xistrella</i>		✓			✓		✓		✓

Jiang, 2000, and *Bolivaritettix* Günther, 1939 where their pronota are wholly roof-like or nearly at the same level. Notably, partial species of *Macromotettixoides* also have this similar character (see the key)! The type species of *Hyboella*, *H. tentata* Hancock, 1915, not only possesses this typical characteristic, but also has a conspicuous tegminal sinus and normal flying organs. Therefore, only depending upon the conspicuous tegminal sinus and normal flying organs can one separate *Hyboella* from *Macromotettixoides* (Table 1). In light of this, we suggest that species currently placed in *Hyboella* whose tegminal sinus is absent (accordingly, the tegmen is invisible), and also whose hind wing is 'abbreviated' or 'vestigial', should be transferred to *Macromotettixoides*. Just as in species of *Macromotettixoides*, we also believe no 'apterous' species occur in *Hyboella*.

Herein we transfer the related Chinese species of *Hyboella* whose tegminal sinuses are all absent (their flying organs are all invisible); also their pronota do not meet the typical characteristic of *Hyboella* (wholly roof-like or flattened), into *Macromotettixoides* as follows:

Macromotettixoides badagongshanensis (Zheng, 2013b), comb. n. = *Hyboella badagongshanensis* Zheng, 2013b;

M. curvimarginus (Zheng & Xu, 2010), comb. n. = *H. curvimarginus* Zheng & Xu, 2010;

M. hainanensis (Liang, 2002), comb. n. = *H. hainanensis* Liang, 2002;

M. taiwanensis (Liang, 2000), comb. n. = *H. taiwanensis* Liang, 2000.

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Taxonomic study on specimens of the genus *Micrencaustes* deposited in the Bernice P. Bishop Museum (Coleoptera, Erotylidae)

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Abstract

Specimens of the genus *Micrencaustes* deposited in Bernice P. Bishop Museum were studied. Two new species *Micrencaustes* (*Mimencaustes*) *rotundimaculata* sp. n. and *Micrencaustes* (*Mimencaustes*) *serrati-maculata* sp. n. are described and illustrated. A key to worldwide species of the subgenus *Mimencaustes* is provided.

Keywords

Philippines, Australia, key, new species, taxonomy

Introduction

The genus *Micrencaustes* was established by Crotch (1876) for *Encaustes lunulata* (MacLeay, 1825). This genus includes two subgenera and 42 species, distributed mainly in Asia and Australia (Heller 1918; Chûjô and Chûjô 1989). It is characterized by small antennal clubs, coarse eyes, a very broad terminal segment of the maxillary palpus, and possession of procoxal lines. The subgenus *Mimencaustes* Heller has mesocoxal lines, whereas subgenus *Micrencaustes* does not. Recent taxonomic work on the genus includes Osawa and Chûjô (1990), Li and Ren (2006) and Meng et al. (2014).

In the past, M. Chûjô (1968a, b) examined specimens of Erotylidae in the Bernice P. Bishop Museum (BPBM), Honolulu, Hawaii, U.S.A. He mainly studied specimens from Thailand, Laos, Vietnam, and southern China. He mentioned only one species of the genus *Micrencaustes*, *M. liturata* MacLeay. In 2016, the first author researched the specimens of *Micrencaustes* in BPBM, and this paper presents the result of this study. The material examined included 108 specimens representing 15 species. Among them, seven and eight species belonged to the subgenera *Micrencaustes* and *Mimencaustes*, respectively. Two new species of the subgenus *Mimencaustes* were described and illustrated. One new species, *Micrencaustes* (*Mimencaustes*) *rotundimaculata* sp. n., was collected from Philippines. The other new species, *Micrencaustes* (*Mimencaustes*) *serraticulata* sp. n., was collected from Australia.

Materials and methods

Morphological examinations were carried out with a Nikon SMZ1500 stereomicroscope. To examine the genitalia, the abdominal segments were detached from the body after softening in hot water. All measurements are given in millimeters. Holotypes and a paratype are deposited in BPBM. Morphological terminology follows Wegrzynowicz (1997) and Skelley and Leschen (2007). The following abbreviations are used in the text: **pl**, pronotum length; **pw**, pronotum width.

Taxonomy

Key to the worldwide species of the subgenus *Mimencaustes* Heller

- | | | |
|---|---|-------------------------------------|
| 1 | Body entire dark, without marks..... | 2 |
| – | Body with marks..... | 3 |
| 2 | Body strongly shining..... | <i>M. (M.) papuana</i> Heller |
| – | Body weakly shining..... | <i>M. (M.) debaani</i> (Castelnau) |
| 3 | The marks on pronotum and elytron..... | 4 |
| – | The marks only on pronotum or elytron..... | 6 |
| 4 | Pronotum without black spots in the mark ... | <i>M. (M.) serraticulata</i> sp. n. |
| – | Pronotum with one or two black spots in the mark..... | 5 |
| 5 | Head with an irregular red mark between eyes, antennomere III almost equal to antennomere IV..... | <i>M. (M.) torquata</i> Gorham |
| – | Head without mark between eyes, antennomere III approx 1.5 times as long as antennomere IV..... | <i>M. (M.) taiwana</i> Araki |
| 6 | Pronotum with marks..... | 7 |
| – | Elytron with marks..... | 8 |
| 7 | Head without orange mark, prosternal femoral lines surpassing the front edge of coxae..... | <i>M. (M.) acridentata</i> Li & Ren |

- Head with orange mark, prosternal femoral lines reaching the front edge of coxae..... *M. (M.) rensbiae* Meng, Ren & Li
- 8 Basal mark of elytron with two black spots near anterior border9
- Basal mark of elytron without black spots near anterior border10
- 9 Pronotum with impunctate longitudinal median areas; every tibia with outer edge of apex acutely toothed..... *M. (M.) michioi* Osawa
- Pronotum without impunctate longitudinal median areas; mesotibia with outer edge of apex acutely toothed ... *M. (M.) biomaculata* Meng, Ren & Li
- 10 Basal mark of elytron emarginated on posterior border
..... *M. (M.) wunderlichi* Heller
- Basal mark of elytron not emarginated on posterior border.....11
- 11 Elytron with posterior mark longitudinally oval..... *M. (M.) dajaca* Heller
- Elytron with posterior mark rounded..... *M. (M.) rotundimaculata* sp. n.

***Micrencaustes (Mimencaustes) rotundimaculata* sp. n.**

<http://zoobank.org/08D1B989-6F0B-4388-ACB5-B223BBA52082>

Type material. Holotype. male, PHILIPPINES: Camarines Sur, Mt. Iriga, 13.4158°N, 123.4211°E, alt. 500–600m, 22 April 1962, H.M. Torrevillas leg. Paratype. 1 female, PHILIPPINES: Queznn, Queznn Park Tayahas [Note: The spelling on the label is wrong. Quezon, Quezon Park Tayabas is correct.], 14.6509°N, 121.0443°E, alt. 305m. 5 June 1932, F.C. Hadden leg.

Diagnosis. Body elongated, widest at base of elytra, general color dark. Each elytron with two orange marks; anterior mark almost reaching lateral and basal margins, quadrate; the posterior one placed before the apex, rounded. Clypeus with the anterior border shaped like a concave “V”. Antennomere III approx. 1.4 times as long as IV; relative lengths of antennomeres II–XI: 11: 25: 18: 18: 18: 17: 16: 19: 12: 16. The terminal segment of maxillary palpus triangular, with sides rounded, nearly 2.9 times as wide as long. The elytron with strong striae, intervals finely and sparsely punctured. Mesoventrite with a median transverse rectangular depression.

Description. *Body* (Fig. 1) elongate, moderately convex, length: 15.0–16.0mm, width: 5.0–5.2mm; widest at base of elytra, general color dark, moderately shining. Each elytron with two orange marks; anterior mark almost reaching lateral and basal margins, quadrate; the posterior one placed before the apex, rounded, not touching either margin.

Head (Fig. 2) strongly and sparsely punctured, densely punctured behind the eyes, with ocular lines. Clypeus strongly and rather densely punctured, with anterior border “V” concave, with a fovea on each side of the base. Eyes large, moderately prominent and coarsely faceted. Antennae (Fig. 3) extending to posterior border of pronotum; antennomere III nearly 1.4 times as long as IV; antennomere VIII slightly shorter than VII; antennomere IX triangular; antennomere X crescent-shaped; antennomere XI almost fan-shaped; relative lengths of antennomeres II–XI: 11: 25: 18: 18: 18: 17: 16: 19: 12: 16. The terminal

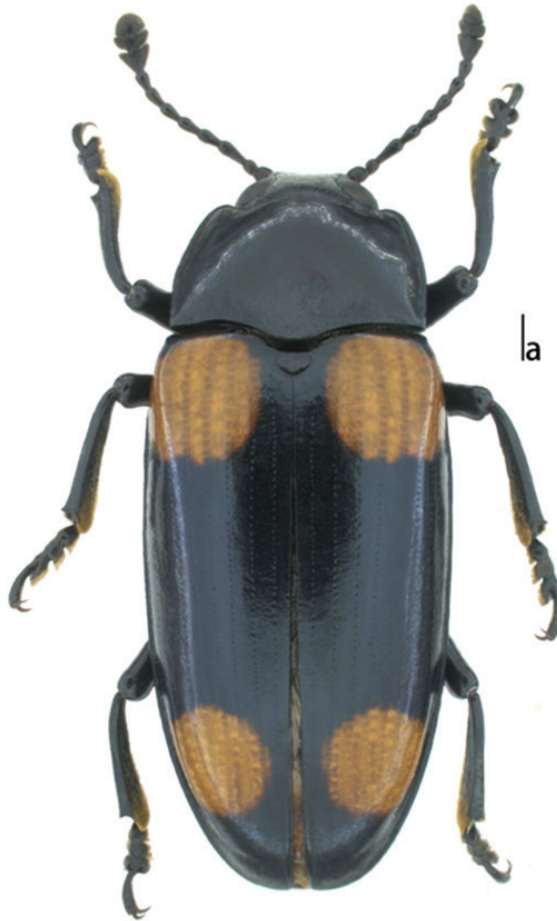


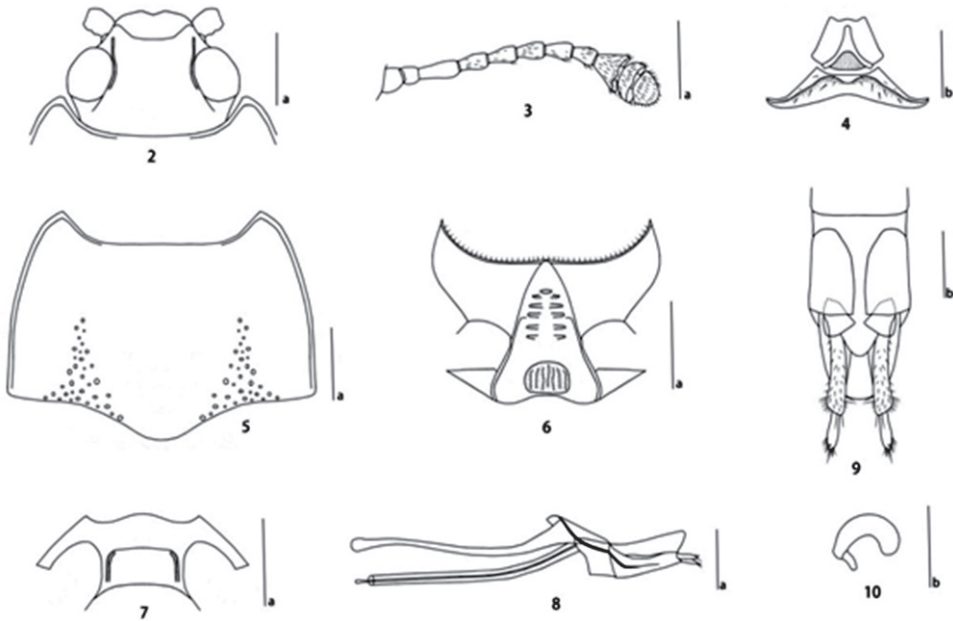
Figure 1. Habitus of *Micrencaustes* (*Mimencaustes*) *rotundimaculata* sp. n. Scale bar 1.0 mm.

segment of maxillary palpus triangular, with sides rounded, nearly 2.9 times as wide as long. Mentum (Fig. 4) triangular, with long golden setae, both sides concave; submentum (Fig. 4) depressed on each side of front area, without puncture, with long golden setae.

Pronotum (Fig. 5) widest at basal third ($pl/pw = 0.76\text{--}0.77$); sides almost parallel on posterior third, and slightly narrowing toward apex. Pronotum finely and densely punctured; with a group of coarse punctures on each side of base. Anterior angles projected; posterior angles nearly rectangular. Pronotal anterior margin concave and basal margin weakly sinuate.

Prosternum (Fig. 6) with shallow and oblique rugulae on lateral areas.

Prosternal process triangular, produced into a blunt point at apex, emarginate at posterior border, finely punctured at front, with a rounded depression in the middle of base, covered longitudinal wrinkles. Prosternal femoral lines almost straight, converging anteriorly and slightly exceeding the front edge of coxae.



Figures 2–10. *Micrencaustes (Mimencastes) rotundimaculata* sp. n. **2** head **3** antenna **4** mentum and submentum **5** pronotum **6** prosternum **7** mesoventrite **8** aedeagus in lateral views **9** female genitalia in ventral view **10** female spermatheca. Scale bars **a** 1.0 mm, **b** 0.5 mm.

Scutellum pentagonal, with fine and sparse punctures.

Elytra widest near base, then gradually narrowing to apex. Each elytron with nine striae, the outside stria short; strial punctures stronger at base, gradually weakened apically and disappearing before extremity; intervals finely punctured and wrinkled.

Mesoventrite (Fig. 7) broad, with a median transverse rectangular depression, coxal lines short, sternum with fine and sparse punctures.

Metaventrte finely and sparsely punctured, with a longitudinal depression in the middle of basal seven eighths.

Abdomen densely punctured, covered with short golden hairs.

Male genitalia (Fig. 8) with median lobe weakly curved, truncated at apex in lateral view; median strut 1.73 times as long as median lobe.

Female genitalia (Fig. 9) with narrow styli at apex of coxite, covered with setae at apex. Female spermatheca kidney-shaped (Fig. 10).

Distribution. Philippines (Camarines Sur and Quezon).

Remarks. *Micrencaustes (Mimencastes) rotundimaculata* is most similar to *Micrencaustes (Mimencastes) dajaca* Heller, 1918, due to the similar form and pattern of the elytron. The new species can be distinguished from it by: the elytron with an anterior quadrate mark, and a rounded posterior mark; clypeus with the anterior border shaped like a concave “V”; mesoventrite with a median transverse rectangular depression; and the elytron with strong striae, intervals finely and sparsely punctured. In contrast, *Mi-*

crencaustes (*Mimencaustes*) *dajaca* has two elongate rounded marks on each elytron; clypeus with anterior border feebly emarginated; mesoventrite with a transverse arched depression in the middle; elytron with weak striae, intervals finely and densely punctured.

Etymology. The species is named for having the posterior band of the elytron rounded.

***Micrencaustes* (*Mimencaustes*) *serratimaculata* sp. n.**

<http://zoobank.org/FECAA575-EF10-4F16-BD8D-99EAB4019A37>

Type material. Holotype. Female, Australia: Queensland, Hambledon [Note: Now named Edmonton], 17.0165°S, 145.7487°E, November 1921, Pemberton leg.

Diagnosis. Body oblong oval, convex, general color dark, shiny. Pronotum with one transverse, zigzag dark red mark. Each elytron with two dark red bands. Clypeus anterior border feebly emarginated. Antennae short, antennomere III 1.7 times as long as IV; relative lengths of antennomeres II–XI: 22: 58: 34: 36: 34: 34: 30: 42: 27: 30. The terminal segment of maxillary palpus triangular, with rounded sides, nearly 2.9 times as wide as long. Pronotum widest at middle, with a group of coarse punctures on each side of base. Scutellum almost triangle, not transverse. Mesoventrite broad, with coxal lines. Abdomen finely and closely punctured. The last segment of abdomen covered with very large punctures along the outside edge. Mesotibia with outer edge of apex acutely toothed.

Description. *Body* (Fig. 11) oblong oval, convex, length: 20.0 mm, width: 7.0 mm; general color dark, shining. Pronotum with one transverse, zigzag dark red mark occupying the sides and most of the center, with three waves at anterior border, with posterior border four waves. Each elytron with two dark red bands; anterior band at the base surrounding the humeral angle, leaving a black part at humerus; posterior band near the apex, neither touching the side nor suture, with posterior border curved.

Head (Fig. 12) finely and sparsely punctured on vertex, strongly and sparsely punctured behind the vertex, with ocular lines. Clypeus strongly and sparsely punctured, with anterior border feebly emarginated, with a fovea on each side of base. Eyes large, moderately prominent laterally, coarsely faceted. Antennae (Fig. 13) short, approaching posterior border of pronotum; antennomere III 1.7 times as long as IV; antennomere VIII slightly shorter than VII; antennomere IX triangular; antennomere X crescent-shaped; antennomere XI semicircle; relative lengths of antennomeres II–XI: 22: 58: 34: 36: 34: 34: 30: 42: 27: 30. The maxillary palpus terminal segment triangular, sides rounded, nearly 2.9 times as wide as long. Mentum (Fig. 14) with plate triangular, both sides concave; submentum (Fig. 14) depressed on each side of middle area, with strong puncture and a few long golden setae.

Pronotum (Fig. 15) widest at middle (pl/pw = 0.71); sides slightly curved, strongly margined, with some coarse punctures on the surface; anterior margin straight in the middle, margined behind eyes; basal margin weakly sinuate. Pronotum finely and sparsely punctured; with a group of coarse punctures on each side of base. Anterior angles projected; posterior angles obtuse.



Figure 11. Habitus of *Micrencaustes* (*Mimencaustes*) *serratimaculata* sp. n. Scale bar 1.0 mm.

Prosternum (Fig. 16) sparsely punctured laterally, with some shallow and oblique rugulae; a depression in the middle area; surface with short golden setae.

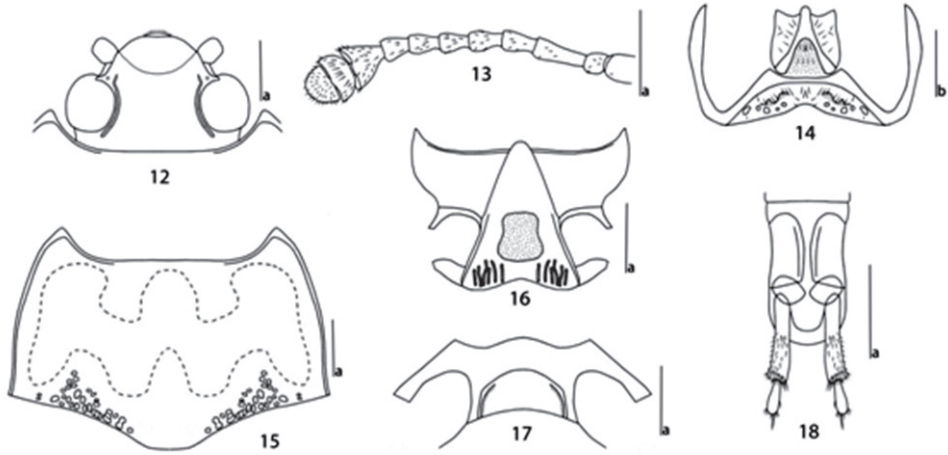
Prosternal process triangular, produced into a blunt point at apex, emarginated on posterior border, sparsely punctured on point, with longitudinal rugulae at front. Prosternal femoral lines converging anteriorly and slightly exceeding to the front edge of coxae.

Scutellum almost triangle, posterior angle blunt, and surface with fine and sparse punctures.

Elytra widest near base, then gradually narrowing to apex. Each elytron with seven striae; intervals finely punctured and wrinkled.

Mesoventrite (Fig. 17) broad, almost no punctures, with a transverse arched depression medially, with coxal lines; sternum with fine and sparse punctures.

Metaventricle finely and sparsely punctured, with a longitudinal depression on posterior seven eighth.



Figures 12–18. *Micrencaustes* (*Mimencaustes*) *serratimaculata* sp. n. **12** head **13** antenna **14** mentum and submentum **15** pronotum **16** prosternum **17** mesoventrite **18** female genitalia in ventral view. Scale bars **a** 1.0 mm, **b** 0.5 mm.

Abdomen finely and closely punctured, covered with short golden hairs, with smooth areas laterally on the surface of abdominal segments. The last segment of abdomen covered very large punctures along the outside edge.

Mesotibia with outer edge of apex acutely toothed.

Female genitalia (Fig. 18) with narrow styli at apex of coxite, and styli rounded apically, covered with setae at apex. Female spermatheca was not found.

Distribution. Known only from the type locality (Australia: Queensland, Edmonton).

Remarks. *Micrencaustes* (*Mimencaustes*) *serratimaculata* is most similar to *Micrencaustes* (*Micrencaustes*) *gigas* MacLeay 1887, due to the similar form and color pattern of body. The new species can be distinguished from it by the mesosternum with coxal lines; very shiny body surface; scutellum not transverse; and the abdomen finely and closely punctured. *Micrencaustes* (*Micrencaustes*) *gigas* is without mesocoxal lines, only moderately shiny, scutellum is transverse, and the ventral surface is sparsely punctured.

Etymology. The species is named for the zigzag mark on pronotum.

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Description of a new species of *Euderus* Haliday from the southeastern United States (Hymenoptera, Chalcidoidea, Eulophidae): the crypt-keeper wasp

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Abstract

A new species of the genus *Euderus* Haliday, *Euderus* set **sp. n.**, is described and illustrated from the southeastern United States, where it parasitizes the crypt gall wasp, *Bassetia pallida* Ashmead, 1896, on live oaks in the genus *Quercus* (subsection *Virentes*). This is the 1st species of the genus reported from the southeastern United States to parasitize cynipid gall wasps and the 3rd species of the genus reported to attack cynipids in North America. Modified sections of the identification keys to subgenera and species of *Euderus* (Yoshimoto, 1971) are included to integrate the new species.

Keywords

Bassetia pallida, Chalcidoidea, Cynipidae, *Euderus*, Eulophidae, *Quercus geminata*, *Quercus virginiana*, new species

Introduction

The genus *Euderus* Haliday, 1844 is a group of parasitic wasps in the family Eulophidae (Hymenoptera) with approximately 77 described species (Yoshimoto 1971, Noyes 2016). The genus has a cosmopolitan distribution, where it is found in North and South America, Europe, Asia, Australia, and many isolated island archipelagos, including the Hawaiian islands, Micronesia, the Canary Islands, and the Seychelles archipelago (Ahmad 1976, Askew et al. 2001, Báez and Askew 1999, Gates et al. 2002, Gibson et al. 2006, Goolsby et al. 2001, Gunasena and Harris 1998, Herting 1973, Thompson 1955, Yoshimoto 1971).

Yoshimoto (1971) produced the last revision of the genus *Euderus* for North America, north of Mexico. The revisions built off of Nearctic catalogues by Peck (1951, 1963) and Burks (1967), with new additions based on material deposited in the Canadian National Collection, material loaned from the Natural History Museum, London, Philadelphia Academy of Sciences, and the Museum of Comparative Zoology, Harvard University. Based on this revision, there are 22 species reported from North America north of Mexico, with 12 residing in the coastal southeastern U.S. (Florida, Alabama, Mississippi, Louisiana, and Texas; Yoshimoto 1971, Noyes 2016). According to Yoshimoto (1971), the biological records of *Euderus* from the U.S. indicate that most species are host specific on pupae of leaf tying, leaf mining, twig and fruit boring Lepidoptera (Tortricidae or Gelechiidae) and stem boring and other herbivorous Coleoptera (Buprestidae, Cerambycidae, and Curculionidae). Rarely, *Euderus* has been reported to attack gall-making Hymenoptera (Cynipidae) or exhibit hyperparasitism on other Hymenopteran parasitoids (Ichneumonidae). Detailed host records from field observation across the genus are summarized in Table 1 of Yoshimoto (1971).

Here, we report the first species of this genus from the southeastern United States to attack cynipid gall wasps, where it is associated with the crypt gall wasp *Bassetia pallida* Ashmead, 1896 (Hymenoptera; Cynipidae) on live oaks (*Quercus*; subsection *Virentes*), including *Quercus virginiana* and *Q. geminata*. We modify the key published by Yoshimoto (1971) to include the new species and add a correction to the key to subgenera.

Materials and methods

Field collections and lab husbandry

For the type locality, branches of the sand live oak, *Quercus geminata*, infested with the asexual generation of the crypt gall wasp, *Bassetia pallida*, were collected July 15, 2014, August 1, 2015, and October 1, 2015, in Inlet Beach, Florida (Lat/Long: 30.273663, -86.001911). Additional populations were collected across the U.S. Gulf coast on *Q. geminata* and *Q. virginiana* in 2014, 2015, and 2016 (see Table 1). Branches were placed in clear plastic cups, covered with a coffee filter and rubber band, and maintained outside in natural temperature and humidity conditions in a constantly shaded walkway at Rice University in Houston, Texas (Lat/Long: 29.717030, -95.401279). Emergence of all in-

Table 1. Confirmed localities for *E. set* associated with *B. pallida* galls on live oaks. (LA = lab emergence from a *B. pallida* gall; D = found during dissection of *B. pallida* gall).

Location	Lat/Long	Host plant	Collection method	N
Inlet Beach, FL	30.273663, -86.001911	<i>Q. geminata</i>	LA, D	158
Lake Lizzie, FL	28.227718, -81.179641	<i>Q. geminata</i>	D	12
Ochlocknee Bay, FL	29.922913, -84.411060	<i>Q. geminata</i>	D	7
Jekyll Island, GA	31.073975, -81.424541	<i>Q. virginiana</i>	LA	1
Gautier, MS	30.382323, -88.611080	<i>Q. virginiana</i>	D	3
Delcambre, LA	29.968115, -91.981863	<i>Q. virginiana</i>	D	2
Morgan City, LA	29.693581, -91.159113	<i>Q. virginiana</i>	D	1
Humble, TX	29.998392, -95.184455	<i>Q. virginiana</i>	LA	19
Rice Univ., TX	29.716882, -95.401928	<i>Q. virginiana</i>	LA, D	27

dividuals was monitored regularly for a year or dissected out of *B. pallida* crypt galls in the lab. All individuals were preserved in 96% EtOH and frozen in a -80°C ultrafreezer.

Morphological descriptions and type material locations

Descriptions of the species have been made under a Leica M125 Stereoscope, with lighting achieved through a Leica LED5000 SLI - Spotlight illumination with 2 HiPower LEDs and a Leica TL5000 Transmitted Light Base with Rottermann Contrast TM, brightfield and two sided darkfield. For images, 75-150 stacked photographs were produced by a Canon 7D Mark II (Canon USA, Melville, NY), with a Mitutoyo M Plan Apo 10x objective mounted onto the Canon EF Telephoto 70 – 200mm zoom lens, which was mounted on a Stackshot Automated Focus Stacking Macro Rail (Cognysis Inc., Traverse City, MI). The Canon MT–24EX Macro Twin Lite Flash with custom made diffusers was used to minimize hot spots. Images were processed using Zerene Stacker (Zerene Systems LLC., Richland, VA) and plates were finished with Adobe Illustrator CC. Pictures of slide-mounted wings were taken using a Leica ICC50W camera.

Morphological nomenclature follows Gibson et al. (1997), Yoder et al. (2010), and Hymenoptera Anatomy Consortium (2016). The identification key is modified from the key to subgenera and species of the genus *Euderus* in Yoshimoto (1971).

Type material is deposited in the American Museum of Natural History (AMNH) (Curator: Dr. James Carpenter; Collection Assistant: Christine LeBeau). AMNH specimen identification codes: Holotype - AMNH_IZC 00238642; 8 paratypes - AMNH_IZC 00238643 – 00238650.

Complementing morphological taxonomy with molecular barcodes

When samples were of sufficient quality for genetic work, we complemented morphological taxonomy with molecular barcodes (e.g., Smith et al. 2008, 2012, Forbes and

Funk 2013, Forbes et al. 2016). Genomic DNA from two individuals from the Inlet Beach, FL population were extracted using DNeasy Blood and Tissue kits (Qiagen Inc., Valencia, CA). We used a pair of degenerate primers to amplify a segment of the mitochondrial cytochrome oxidase (mtCOI) gene using standard PCR protocols (Smith et al. 2008). Primers used were COI pF2: 5' - ACC WGT AAT RAT AGG DGG DTT TGG DAA - 3' and COI 2437d: 5' - GCT ART CAT CTA AAW AYT TTA ATW CCW G - 3', developed by Simon et al. (1994) and modified by Kaartinen et al. (2010). We treated amplified fragments with Exonuclease I (New England Biolabs, Ipswich, MA) and Shrimp Alkaline Phosphatase (Fermentas Life Sciences, Glen Burnie, MD) and sequenced in both forward and reverse directions on an ABI 3730 DNA analyzer using BigDye 3.1 sequencing chemistry (ThermoFisher Inc., Waltham, MA). We edited raw sequences and assembled forward and reverse reads using Geneious v.6.1.8 (Kearse et al. 2012). The final sequences were 703bp and 745bp in length, a function of amplification and sequencing success. We ran each sequence through the “identification request” module on the Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007) to identify the highest percentage matches from previously identified taxa. All sequences were deposited in GenBank (accession numbers provided below).

Results

Details on the on the type material and type locality are provided, with a thorough description with images, a differential diagnosis of the new species, and a modification to the identification key published by Yoshimoto (1971) that distinguishes this new species from closely related species. We also provide a correction to the key to subgenera published in Yoshimoto (1971). In addition, we provide a brief description of etymology and information on the distribution, natural history, biology, and results of the mtDNA DNA barcoding analysis. In Supplemental File 1, we provide further details of the morphological, geographic, and ecological distinguishing features of this new *Euderus* species, *Euderus set*, which differentiate it from (1) ecologically similar *Euderus* species attacking cynipid gall wasps, (2) geographically overlapping *Euderus* species in the coastal southeastern United States, (3) geographically proximate *Euderus* species from the Caribbean, and (4) morphologically similar species within the *Euderus* genus. We also provide the DNA sequence data to complement the morphological taxonomy in Suppl. material 1.

Taxonomy

Euderus Haliday, 1844

Euderus Haliday, 1844. Trans. Ent. Soc. Lond. 3: 298.

Type-species. *Entedon amphis* Walker.

***Euderus set* Egan, Weinersmith, & Forbes, sp. n.**

<http://zoobank.org/44A7E50B-5E80-4A60-9170-DD1B9A7221B7>

Figs 1–2 (3 panels per figure)

Material examined. Holotype, ♀, Inlet Beach, FL, collected July 15, 2015 by Scott P. Egan, AMNH ID#: AMNH_IZC 00238642

Allotype, same data as holotype

Paratypes, 2 ♂, same locality as holotype

Specimens from each collection locality (Table 1).

Description. FEMALE. Length 1.6 – 2.3 mm. Holotype 2.3 mm

Color. Head, scrobal depression, pedicel, flagellum, mesoscutum, scutellum, coxae and metasoma metallic, olive green to turquoise to iridescent blue (colors depends on lighting and age of specimen); antennal scape white to yellow; femora and tibiae concolorous with mesoscutum but color lightens apically; tarsi white, except terminal segment dark brown (Fig. 1A, B).

Head. Head in fresh specimens as wide as mesosoma; in dorsal view 2.9 times as broad as long; eyes prominent and bare; vertex, frons, and clypeus reticulate; vertex and upper frons distributed with white bristles; scrobal depression extends from slightly below anterior ocellus to level of lower eye margin, smooth above torulus and striolate below; toruli located in lower third of scrobal depression; clypeus short, subquadrate, only slightly longer than wide; malus sulcus inconspicuous and 0.44 times eye length; mandibles with three teeth. Antennal scape 3.5 times as long as broad and 0.6 times eye length; Flagellum with nine segments, with anellus two-segmented, funicle four-segmented, and clava three-segmented. Funicular segments each with 2 rows of thick, mostly non-overlapping bristles (Fig. 2B). Relative length of scape, pedicel, anelli, funicle 1, 2, 3, and 4, and clava 1, 2, and 3 as 38, 12, 2, 24, 22, 22, 20, 16, 12, 8, respectively; two anelli with the same length but relative breadth of first anellus to second anellus as 6, 8.

Mesosoma. Pronotum, mesoscutum and scutellum reticulate; pronotum short in dorsal view, with six brown bristles at margin with mesoscutum; mesoscutum sparsely setose and setae inconspicuous; scutellum with many short setae and two pairs of strong setae in posterior third. Mesoscutum 0.95 as long as broad, mid-lobe convex, notauli complete and deep; axillae slightly advanced, their anterior tip extending to the approximate midpoint of the mesoscutum. Scutellum moderately convex and length 0.80 times length of mesoscutum. Propodeum 0.24 times length of scutellum and with strong median carina (Fig. 1B); callus with 6–7 bristles (Fig. 2A).

Wings. Forewing broad, extending past apex of gaster, marginal ciliae short; basal cell bare; submarginal vein with 6 dorsal bristles; postmarginal vein 1.3x length of stigmal vein; 3 admarginal hairs on left wing, four admarginal hairs on right wing; stigmal vein short and with large stigmus with 6 hairs on surface; 5 strongly-defined hair lines reaching dorsal and apical margin of forewing, with 3–4 additional less strongly defined hair lines that may or may not reach wing margin; 1 hair at median of radial cell (Fig. 2A). Hindwing 0.80 times length of forewing; hindwing moderately and evenly setose; hind marginal ciliae long.

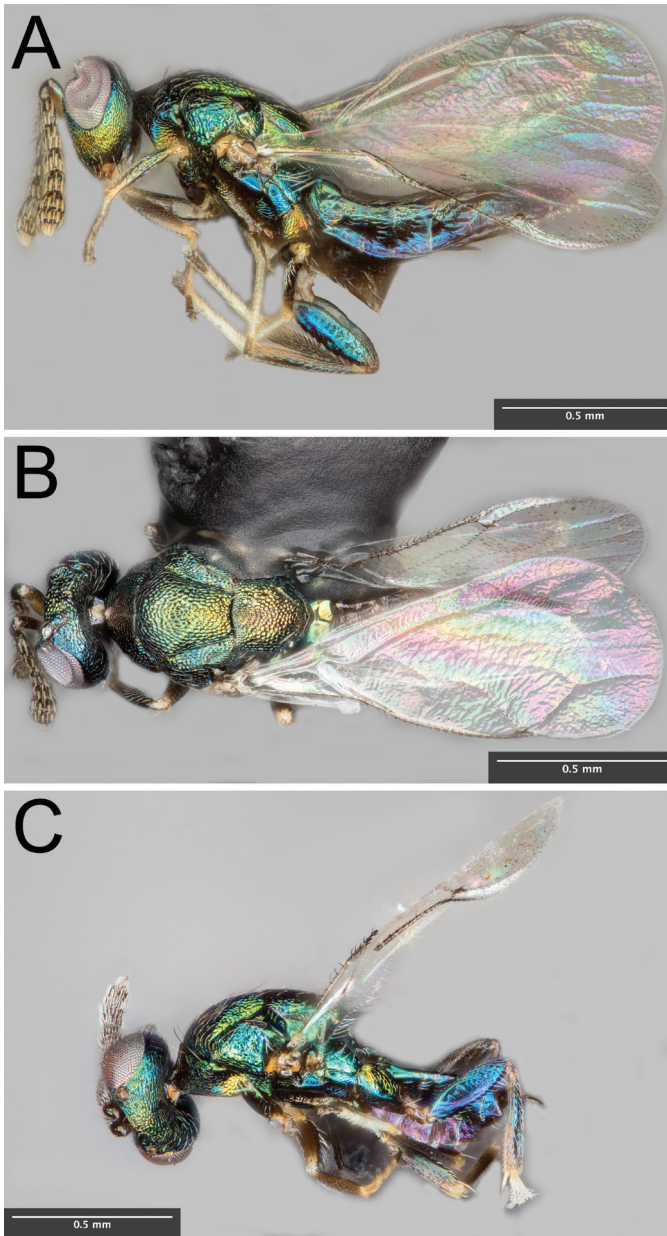


Figure 1. **A** Lateral habitus of female *Euderus seti* **B** Dorsal habitus of female *Euderus seti* **C** Lateral habitus of male *Euderus seti*.

Metasoma. Metasoma with petiole hidden in dorsal view; gaster elongate, 2.6 times as long as broad, and as long or longer than head and mesosoma combined; scattered white setae at posterior margins of each tergite becoming more dense towards

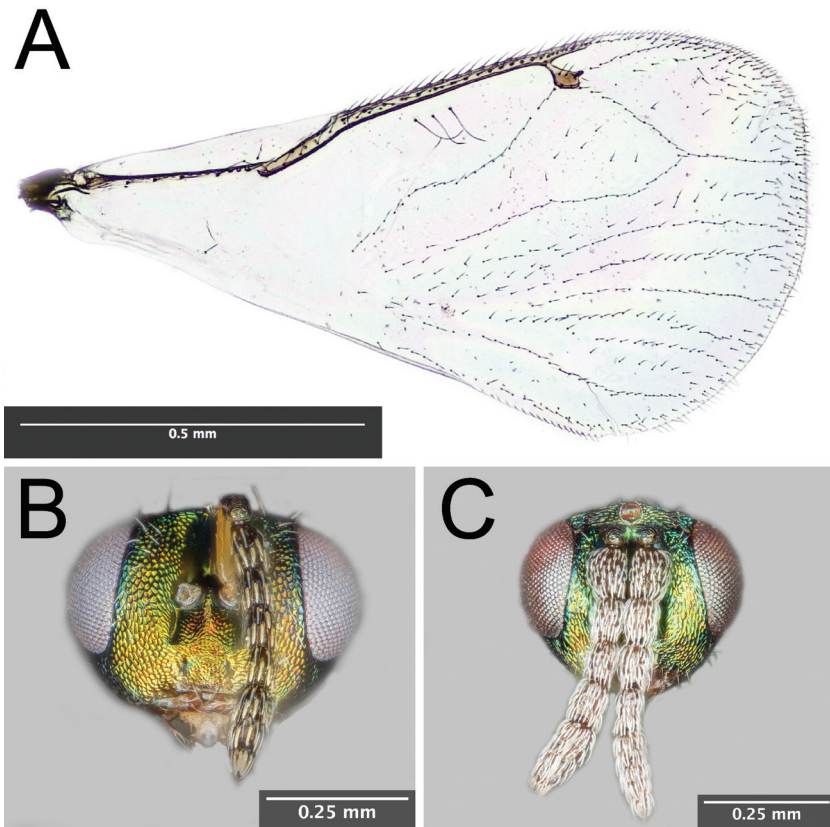


Figure 2. **A** Right forewing of male *Euderus set* **B** Anterior view of female *Euderus set* head **C** Anterior view of male *Euderus set* head.

the apex of the gaster; each cercus with two long bristles. Ovipositor extends slightly beyond apex of gaster (Fig. 1A).

MALE. Length 1.2 – 1.6 mm. Antennae inserted in middle of face (Fig. 2C). Funicular segments each with 3 loosely ordered rows of fine bristles. Gaster ovate; shorter than head and thorax combined. Other characters similar to female.

Etymology. Named after the ancient Egyptian god *Set*, whose mythological stories mirror the natural biology of *E. set*. *Set* was the god of evil and chaos (Pinch 2004) and was reported to have control over evil animals like hyenas and serpents, just as *E. set* manipulates the behavior of its host, which is a parasite of trees (see *Biology* section below). The god *Set* is also reported to have trapped his brother Osiris in a crypt to kill him, later retrieving the body and chopping it up into small piece, which also fits with *E. set*, the crypt-keeper wasp, which kills its host in a crypt, and devours the host from the inside out, leaving major sections of exoskeleton (i.e., body parts) chopped up and distributed in the crypt (Weinersmith et al., in revision).

Diagnosis. Two changes are required for the new species, *E. set*, to be included in the North American key to species of the genus *Euderus* by Yoshimoto (1971). First, a change is required to the key to subgenera of *Euderus*, where characters referring to the male flagellum should be removed. Yoshimoto (1971) used whorls of long hairs on the male flagellum as a diagnostic trait to discriminate between subgenera *Neoeuderus* and *Euderus*, but at that time only a single male specimen of subgenus *Neoeuderus* was available for study, and this individual was from the species *E. viridilineatus* for which no females had been found. As the male *E. set*. do not have antennae with pronounced whorls of long hairs, this is not an appropriate diagnostic trait for the subgenus.

We propose the following revision to the Yoshimoto (1971) key to subgenera:

- 4 Apical margin of fore wing with 5 hair lines; female antenna inserted at level of lower margin of eye; male antenna inserted about middle of face....***Neoeuderus***
- Apical margin of fore wing with 3–4 hair lines (Fig. 2A); antenna of male and female inserted slightly above or at level of lower margin of eye (Fig. 2B, C)
.....***Euderus***

Secondly, Yoshimoto's (1971) key to the subgenus *Neoeuderus* should be revised as follows:

- 3 Neck region of stigmal vein short, stigma large (1.0) with 6–10 scattered hairs on surface, postmarginal vein between 1.2 and 1.5 times length of stigma vein; radial cell with one or more scattered hairs**4**
- Neck of stigmal vein elongate, stigma small (0.5), surface with 4 hairs; postmarginal vein 2 times length of stigma vein; radial cell bare
.....***multilineatus* (Girault)**
- 4 Surface of stigma with 9–10 scattered hairs; submarginal vein with 7–8 dorsal bristles; 6 admarginal hairs; callus with 12 scattered hairs; apical region of radial cell with >1 scattered hairs***crawfordi* Peck**
- Surface of stigma with 4–6 scattered hairs; submarginal vein with 6 dorsal bristles; 3–4 admarginal hairs; radial cell with 1 hair at approximately its median; (Fig. 2A); callus with 6–7 scattered hairs (Fig. 1A, C);***set***

Molecular barcodes to complement morphological taxonomy. The two female *Euderus set* mtDNA-COI sequences were 98% identical to each another and each was most similar to other previously identified *Euderus* in the BOLD database. Sequence 1 was 88.4% identical to *Euderus sp.* D0703 on BOLD and sequence 2 was 89.8% identical to another *Euderus sp.* on BOLD (Ratnasingham and Hebert 2007). See Supplemental File 1 for the two specific mtDNA sequences.

Distribution. Type locality for *E. set* is Inlet Beach, Florida, U.S. (Lat/Long: 30.273663, -86.001911), where it emerged from a stem crypt gall on *Quercus geminata* induced by the crypt gall wasp, *Bassetia pallida*. We have also collected *B. pallida* galls from live oaks across the Gulf coast of the southeastern United States, where additional *E. set* have been found, including additional sites in Georgia, Florida, Mississippi, Louisiana, and Texas (see Table 1). Generally, we expect *E. set* to be restricted to

the range of live oaks (*Quercus*; subsection *Virentes*; Cavender-Bares et al. 2015) upon which *Bassettia pallida* induce galls (Melika and Abrahamson 2007).

Biology. The genus *Euderus* Haliday is a small group of chalcidoid wasps belonging to the family Eulophidae (Burks 2003) where the majority are reported to be primary parasitoids of arthropods at all stages of development (Burks 1979, Burks 2003, Noyes 2016). *E. set* parasitizes the crypt gall wasp *Bassettia pallida* Ashmead, 1896, which forms galls on American live oaks in the genus *Quercus* and the subsection *Virentes* across the southeastern United States (Ashmead 1896, Melika and Abrahamson 2007, Egan et al. 2013). There are six to eight different cynipid gall wasps that are highly specialized and form galls on this same live oak complex (Egan et al. 2013). We have reared out many of the parasitoids from this large community, including clearly documenting the community from another gall former, *Belonocnema treatae* (Forbes et al. 2016), but this is the first time we have observed a member of the genus *Euderus* in this system after two decades of work (Lund et al. 1996, Egan and Ott 2007, Egan et al. 2012, Egan et al. 2013, Egan, unpubl. data).

Published records almost certainly underestimate the diversity of subgenus *Neoeuderus* in North America, and many other species in the subgenus may also be specialist parasitoids of oak galling cynipids. Yoshimoto (1971) noted that the biological records of the Nearctic *Euderus* indicate that most species are host specific, while also cautioning that host records for *Euderus* are patchy and the result of field observation, which only represent the most common species where there are substantial rearing records. While Yoshimoto noted just four members of subgenus *Neoeuderus*, the current work adds a fifth, and we have recently reared another from the honey comb leaf gall wasp, *Callirhytis fava*, on pin oak in Iowa. While this undescribed *Euderus* parasitoid of *C. fava* has not yet been extensively studied, it is most similar in appearance to *E. set*, *E. crawfordi*, and *E. multilineatus*. If this truly is a different species, then three of the six species in subgenus *Neoeuderus* are known parasitoids of the oak-associated Cynipidae.

Emergence of *E. set* in the lab from field-collected *B. pallida* galls was concentrated from February to March coincident with new leaf growth of the host plants and adult maturation and emergence of the asexual generation *Bassettia pallida* (Melika and Abrahamson 2007, Egan, unpubl. data). We also observed a smaller pulse in September and October, which could have been a natural occurrence, or induced by harvesting galled tissue and bringing it into a controlled environment. Regardless of harvest time (August or October), a similar emergence window was observed in February and March.

Euderus set is strongly associated with a behavioral phenotype in its host, the crypt gall wasp, *Bassettia pallida*, where infected gall wasps cut an emergence hole through the gall tissue as an adult, but then die and remain partially in the crypt to plug the emergence hole with its head (Weinersmith et al., in revision). When *E. set* emerges, it cuts an emergence hole directly through the head capsule plugging the hole (Weinersmith et al., in revision). The host's behavioral phenotype may benefit *E. set* by making it easier for the adult stage to emerge from the crypt (as it now only has to emerge through the parasitoid's head capsule, rather than through the tree stem itself; Weiner-

smith et al., in review). This putative behavioral manipulation of the host by its parasitoid *E. set* is the first time this has been described by the species-rich and economically important Chalcidoidea and is also the inspiration behind both the scientific name, *E. set*, and the common name, the crypt-keeper wasp.

In addition to *E. set*, we have also reared eleven additional natural enemy species from *Bassetia pallida* galls on live oaks (*Quercus*; subsection *Virentes*), including two inquilines (genera *Synergus* and *Ceroptres*) and nine parasitoids including three species from the genus *Sycophila*, two species from genus *Ormyrus*, one each from the genera *Eurytoma*, *Acaenacis*, and *Brasema*, as well as a parasitoid from the platygastriid subfamily Platygastriinae that we have not yet been able to key to genus. The natural enemy community requires further description.

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Supplementary material I

Morphological, geographic, and ecological distinguishing features of *Euderus set*

Authors: Scott P. Egan, Kelly L. Weinersmith, Sean Liu, Ryan D. Ridenbaugh, Y. Miles Zhang, Andrew A. Forbes

Data type: PDF File

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Three new species of the killifish genus *Melanorivulus* from the central Brazilian Cerrado savanna (Cyprinodontiformes, Aplocheilidae)

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Abstract

Three new species are described from the Neotropical region comprising the Cerrado savannas of the central Brazilian plateaus, which is among the most important biodiversity centres in the world. These species are considered closely related to *M. dapazi* from the same region, with which they share the presence of a rudimentary interarcual cartilage and a dark reddish brown distal margin on the male anal fin. The group comprising *M. dapazi* and the three new species is here named as the *M. dapazi* species group. *Melanorivulus ignescens* **sp. n.**, from the upper Rio Araguaia basin, is distinguished from all other species of the *M. dapazi* group by the anal-fin colour pattern in males; *M. flavipinnis* **sp. n.** and *M. regularis* **sp. n.** from the Rio Paraguai basin are distinguished from all other congeners of the *M. dapazi* group by the colour pattern of the caudal fin and number of scales in the longitudinal series, respectively. All the new species are further unambiguously diagnosed by unique combinations of morphological characters, including meristic and morphometric data, and colour patterns. This study reinforces the importance of using live colour patterns to diagnose species and species groups of the genus *Melanorivulus*, but also indicates that osteological characters may be informative for species diagnosis. This study confirms the high diversity of species of *Melanorivulus* in the central Brazilian Cerrado plateaus already reported in previous studies, indicating that endemic species are often restricted to short segments of a single river drainage.

Keywords

Biodiversity hotspot, morphology, osteology, systematics, taxonomy

Introduction

The region comprising the Cerrado savannas of central Brazil has been considered among the most important biodiversity hotspots in the world (Myers et al. 2000), although many organisms endemic to this region were insufficiently sampled and poorly known until recent years (Costa et al. 2016). A typical component of the Cerrado fauna is the killifish genus *Melanorivulus* Costa, 2006, with species inhabiting the Veredas, a Cerrado ecosystem consisting of small streams running in shallow valleys, often exhibiting the buriti-palm *Mauritia flexuosa* along their banks (e.g., Costa 2007a; Oliveira et al. 2012). Probably as a consequence of small size, usually not surpassing 45 mm of total length, species of *Melanorivulus* occurring in this ecosystem were not represented in collections until recent years, with the great majority of the approximately 40 species occurring in the central Brazilian Cerrado being described only after 2005 (e.g., Costa 2012; Costa et al. 2016).

The greatest diversity among species of *Melanorivulus* endemic to the Cerrado is concentrated in the central-western Brazilian plateaus, which range in altitudes from 400 to 1,100 m above sea level (asl), in the Caiapó mountain range (Costa 2012). This area is drained by the upper tributaries of the Rio Araguaia, flowing north and belonging to the Amazonas–Tocantins river system, and the upper Paraguai and Paraná river basins, flowing southwest and south, respectively, and belonging to the Paraná–Paraguay–Uruguay river system. A total of 12 species have been recorded for this area, of which four are endemic to the Araguaia basin, one to the Paraguai basin, and seven to the Paraná basin (Costa 1989, 2005, 2006a, 2007a–b, 2008, 2012). During a recent expedition to this area, three new species were collected, one from the upper Araguaia basin and two from the Paraguai basin. All the three new species are considered to be closely related to *M. dapazi*, endemic to the Paraguai basin, by all sharing a rudimentary interarcual cartilage and a dark reddish brown stripe on the distal margin of the anal fin in males (vs. interarcual cartilage well-developed and never a similar stripe on the anal-fin distal margin; see Discussion below). This assemblage is hereafter called the *M. dapazi* species group and the three new species are herein described.

Material and methods

Specimens were captured with small dip nets (40 × 30 cm) and were euthanized soon after collection. Representative live specimens were kept alive for nearly 24 hours, photographed, and then euthanized. Euthanasia was conducted in a buffered solution of tricaine methanesulfonate (MS-222) at a concentration of 250 mg/l, for a period of about 10 minutes, i.e., until opercular movements ceased. Specimens were fixed in formalin for a period of 10 days, and then transferred to 70% ethanol. Collections were made with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) and methods for euthanasia were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit

number: 01200.001568/2013-87). Material is deposited in Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ) and Coleção Ictiológica do Centro de Ciências Agrárias e Ambientais, Universidade Federal do Maranhão, Chapadinha (CICCAA).

Descriptions of colouration of living fish were based on photographs of both sides of individuals. Photographs were taken in small aquaria around 24 hours after collection. Additional direct observations were made with fish in small transparent plastic bottles just after collection. Measurements and counts follow Costa (1988). Measurements are presented as percentages of standard length (SL), except for those related to head morphology, which are expressed as percentages of head length. Fin-ray counts include all elements. Four specimens, two males and two females, were cleared and stained for osteological analysis using the methods presented in Taylor and Van Dyke (1985); the abbreviation C&S in lists of material indicates those specimens that were prepared for osteological examination. Terminology for osteological structures followed Costa (2006b), for frontal squamation Hoedeman (1958), and for cephalic neuromast series Costa (2001). Osteological characters used in species descriptions are those that show variability within *Melanorivulus* (e.g., Costa 2016). Herein, geographical localities involved terms popularly adopted in the local region to compose names of geographical accidents (e.g., rio, ribeirão) allowing more accurate identifications of localities in the field and avoiding common mistakes when tentatively translating them to English; following this reasoning, Rio Paraguai is used instead of Paraguay River. New species descriptions are listed according to their type localities, from north to south.

Results

Melanorivulus ignescens sp. n.

<http://zoobank.org/01008AFD-8842-4DED-8E5F-4D7DF54463F9>

Figs 1–2, Table 1

Holotype. UFRJ 6875, male, 27.7 mm SL; Brazil: Mato Grosso state: Guiratinga municipality: stream tributary to Rio Bandeira, Rio das Garças drainage, Rio Araguaia drainage, 16°21'54"S, 53°47'58"W, altitude approximately 520 m asl, road MT-270, approximately 3 km southwest of the village of Guiratinga; W. J. E. M. Costa et al., 11 August 2016.

Paratypes. UFRJ 6876, 13 males, 15.8–25.5 mm SL, 18 females, 17.7–23.4 mm SL; UFRJ 6877, 2 males, 24.0–25.1 mm SL, 2 females, 22.4–23.4 mm SL (C&S); CICCAA00277, 1 male, 20.6 mm SL, 1 female, 18.6 mm SL; collected with holotype.

Diagnosis. *Melanorivulus ignescens* is distinguished from all other species of the *M. dapazi* group by having the anal fin, in adult males, bright reddish orange (vs. yellow in *M. dapazi*, *M. flavipinnis*, and *M. regularis*). Also distinguished from all other congeners of the *M. dapazi* group by the following combination of character states: 5–6 pelvic-fin rays (vs. 7 in *M. dapazi* and *M. regularis*); 29–31 scales in longitudinal series (vs. 35–37 in *M. regularis*); female caudal spot inconspicuous in live fish (vs. conspicuous in *M.*



Figure 1. *Melanorivulus ignescens* sp. n., holotype, UFRJ 6875, male, 27.7 mm SL. Photograph by W.J.E.M. Costa.

dapazi and *M. regularis*); caudal fin, in males, without red bars and distinctive orange margin (vs. with red bars in *M. regularis* and *M. flavipinnis*, with broad bright orange band along the whole margin in *M. dapazi*); in females, ventral surface of the head with dark grey spots, often forming short stripe on the chin (vs. without dark grey spots in *M. dapazi*); caudal-fin short, its length 26.8–33.1% SL (vs. long, its length 34.1–38.7% SL in *M. flavipinnis*). Also distinguished from all other species of the *M. dapazi* group by having a constriction on the metapterygoid (vs. constriction absent).

Description. Morphometric data appear in Table 1. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth at vertical just in front of pelvic-fin base. Dorsal and ventral profiles of trunk almost straight to slightly convex in lateral view; dorsal and ventral profiles of caudal peduncle nearly straight. Head moderately wide, sub-triangular in lateral view, dorsal profile nearly straight, ventral profile convex. Jaws short, snout weakly pointed in lateral view.

Dorsal and anal fins short, extremity slightly pointed in males, rounded in females. Caudal fin oval, slightly longer than deep. Pectoral fin rounded, posterior margin reaching vertical at 80–90% of length between pectoral-fin and pelvic-fin bases. Pelvic fin small, tip reaching between urogenital papilla and base of 1st anal-fin ray in males, reaching between anus and urogenital papilla in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin on vertical through base of 8th anal-fin ray. Dorsal-fin rays 9–11; anal-fin rays 13–15; caudal-fin rays 30–31; pectoral-fin rays 13; pelvic-fin rays 5–6. No contact organs on fins.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 25% of caudal-fin base; no scales on dorsal and anal-fin bases. Frontal squamation F-patterned, rarely E-scale anteriorly overlapping F-scale; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Longitudinal series of scales 29–31;



Figure 2. *Melanorivulus ignescens* sp. n., paratype, UFRJ 6876, female, 23.4 mm SL. Photograph by W.J.E.M. Costa.

Table 1. Morphometric data of *Melanorivulus ignescens*.

	holotype	paratypes	
	male	males (n = 9)	females (n = 6)
Standard length (mm)	27.7	20.1–25.5	21.4–24.7
Percent of standard length			
Body depth	22.1	21.3–22.3	20.5–22.8
Caudal peduncle depth	13.7	12.9–14.2	12.6–13.4
Pre-dorsal length	77.1	74.0–78.5	74.7–77.0
Pre-pelvic length	55.9	55.7–57.9	55.8–57.8
Length of dorsal-fin base	13.5	11.2–12.7	10.9–12.8
Length of anal-fin base	23.8	19.8–21.6	19.4–21.0
Caudal-fin length	33.1	29.9–32.9	26.8–32.0
Pectoral-fin length	21.4	19.1–21.5	18.0–20.6
Pelvic-fin length	10.8	9.4–11.1	8.2–9.7
Head length	27.6	27.2–30.9	27.2–30.0
Percent of head length			
Head depth	67.5	62.0–70.4	63.3–70.9
Head width	70.9	66.2–73.3	69.6–76.8
Snout length	13.2	10.9–13.5	12.1–14.1
Lower jaw length	21.6	15.9–19.5	15.3–21.4
Eye diameter	32.3	32.4–35.9	32.5–34.7

transverse series of scales 9; scale rows around caudal peduncle 16. No contact organs on scales. Cephalic neuromasts: supraorbital 3 + 3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1 + 11 + 1, preorbital 2, otic 1, post-otic 1–2, supratemporal 1, median opercular 1, ventral opercular 1, pre-opercular 2 + 4, mandibular 2–3 + 1, lateral mandibular 1, paramandibular 1.

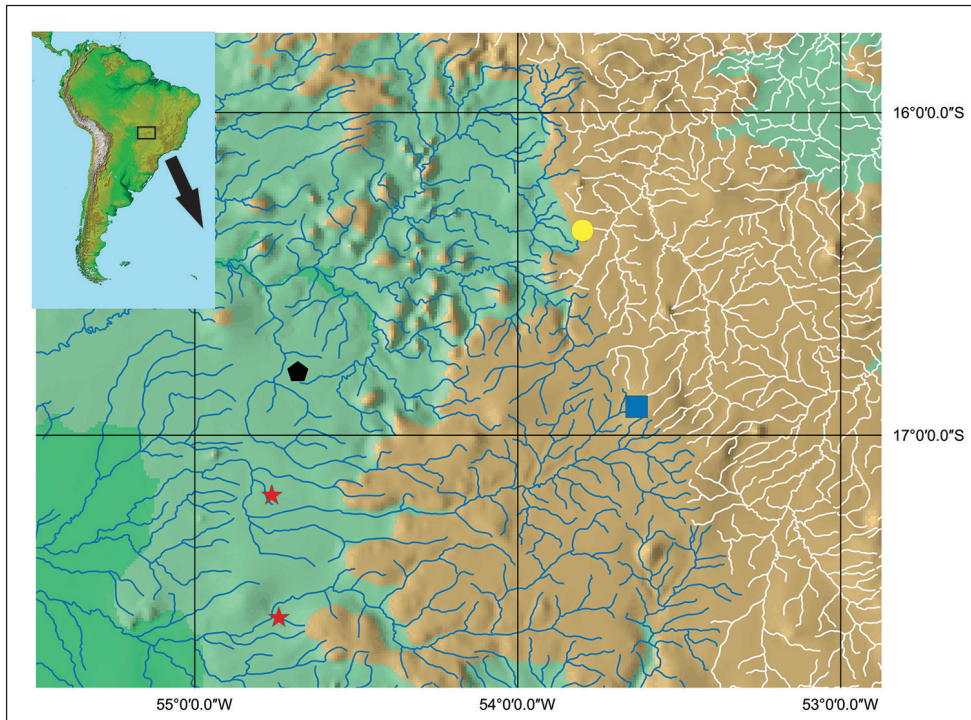


Figure 3. Geographical distribution of killifishes of the *Melanorivulus dapazi* species group. Yellow circle: *M. ignescens*; black pentagon: *M. flavipinnis*; blue square: *M. regularis*; red star: *M. dapazi*. Blue river drainage: Paraguay; white river drainage: Araguaia.

Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight. Ventral process angulo-articular short, pointed. Ventral process of palatine short, slightly contacting quadrate. Mesopterygoid slender, posterior tip not reaching metapterygoid. Metapterygoid sub-rectangular, with constriction on middle portion. Dorsal portion of preopercle short and pointed, channel rudimentary. Basihyal sub-triangular, greatest width 50% of length; basihyal cartilage nearly 15% of total basihyal length. Six branchiostegal rays. Second pharyngobranchial teeth absent. Interarcual cartilage rudimentary. Fourth ceratobranchial teeth present, continuously arranged. Gill-rakers on first branchial arch 1 + 8. Vomerine teeth 2–4. Dermosphenotic present. Ventral process of posttemporal absent. Second proximal radial of dorsal fin between neural spines of 19th and 21st vertebrae, first proximal radial of anal fin between pleural ribs of 13th and 15th vertebrae. Total vertebrae 30–31.

Colouration. Males. Flank metallic green-blue to metallic light green, sometimes purple-blue above anal fin; oblique narrow orangish red bars irregularly arranged, often forming chevron-like marks anteriorly directed; horizontal rows of reddish orange dots on anteroventral part of flank, between bases of pectoral and pelvic fins; pale dark grey blotches on postorbital region mainly visible when fish is exposed to strong light. Dorsum light brown with black dots, venter white. Dorsal portion of head side light

brown, ventral portion white; pale golden iridescence on opercular region. Jaws dark grey. Iris pale yellow, sometimes with dark brown bar on anterior and posterior portions. Dorsal fin light yellow with two or three oblique dark red bars on posterior portion of fin. Anal fin reddish orange in adult exemplars to yellowish orange in juveniles, basal portion bluish white, distal region becoming gradually dark red-brown, distal margin with high concentration of melanophores. Caudal fin light yellow, often with faint orange spots on middle portion; sometimes pale bluish posterior margin. Pectoral fin hyaline. Pelvic fin orange.

Females. Side of trunk and head similar to males, but with paler colours. Ventral surface of head white, with dark grey spots often forming short stripe on chin. Dorsal fin pale yellow, with transverse series of grey spots; broad dark grey to black band on distal margin. Anal fin green-yellow, basal portion light blue with small red spots. Caudal fin pale yellow, with three or four dark grey bars, often interrupted; small black spot, smaller than pupil, on dorso-basal portion of fin overlapping anterior-most bar, more conspicuous in preserved specimens; broad dark grey to black band on whole fin margin.

Distribution. Known only from the type locality area, a small stream tributary to the Rio Bandeira, Rio das Garças drainage, upper Rio Araguaia basin, central Brazil, altitude approximately 520 m asl (Fig. 3).

Etymology. From the Latin, *ignescens* (becoming inflamed), an allusion to the orange anal fin in males.

Melanorivulus flavipinnis sp. n.

<http://zoobank.org/6A6F3FA1-5867-4293-BCC6-D1782B35566C>

Figs 4–5, Table 2

Holotype. UFRJ 6881, male, 28.5 mm SL; Brazil: Mato Grosso state: Rondonópolis municipality: stream tributary of Rio Anhumas, Rio São Lourenço drainage, Rio Paraguai basin, 16°48'16"S, 54°40'52"W, altitude approximately 420 m asl, road BR-070; W. J. E. M. Costa et al., 13 August 2016.

Paratypes. UFRJ 6882, 2 males, 25.0–26.6 mm SL, 5 females, 22.3–39.4 mm SL; UFRJ 6883, 2 males, 22.5–25.6 mm SL, 2 females, 24.2–26.4 mm SL (C&S); CICC AA00279, 1 male, 25.7 mm SL, 1 female, 25.3 mm SL; collected with holotype.

Diagnosis. *Melanorivulus flavipinnis* differs from all other species of the *M. dapazi* group by the presence, in males, of seven or eight narrow red bars on the caudal fin, irregularly shaped and sometimes interconnected (vs. five or six dark red-brown regularly shaped and never interconnected bars in *M. regularis*; four or fewer short rudimentary bars, sometimes absent, in *M. dapazi*; bars always absent in *M. ignescens*) and by the caudal fin, in females, being yellow on the middle portion and reddish orange on marginal region (vs. yellow to pale pink on the whole fin in the remaining species). Also distinguished from all other congeners of the *M. dapazi* group by the following combination of character states: 5–6 pelvic-fin rays (vs. 7 in *M. dapazi* and *M. regularis*); 30–32 scales in longitudinal series (vs. 35–37 in *M. regularis*); female caudal spot



Figure 4. *Melanorivulus flavipinnis* sp. n., holotype, UFRJ 6881, male, 28.5 mm SL. Photograph by W.J.E.M. Costa.

inconspicuous in live fish (vs. conspicuous in *M. dapazi* and *M. regularis*); caudal fin, in males, without distinctive orange margin (vs. with broad bright orange band along the whole margin in *M. dapazi*); anal fin, in males, yellow (vs. reddish orange in *M. ignescens*); in females, ventral surface of head with dark grey spots, often forming short stripe on chin (vs. without dark grey spots in *M. dapazi*); caudal-fin long, its length 34.1–38.7% SL (vs. short, length 26.8–33.1% SL in *M. ignescens*). Also distinguished from all other species of the *M. dapazi* by the fourth ceratobranchial teeth arranged in two separate sections along the bone surface (vs. continuously arranged).

Description. Morphometric data appear in Table 2. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth at vertical just in front of pelvic-fin base. Dorsal and ventral profiles of trunk almost straight to slightly convex in lateral view; dorsal and ventral profiles of caudal peduncle nearly straight. Head moderately wide, sub-triangular in lateral view, dorsal profile nearly straight, ventral profile convex. Jaws short, snout weakly pointed in lateral view. Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight.

Dorsal and anal fins short, tip slightly pointed in males, rounded in females. Caudal fin oval, longer than deep. Pectoral fin rounded, posterior margin reaching vertical at approximately 80–90% of length between pectoral-fin and pelvic-fin bases. Pelvic fin small, tip reaching between base of first and third anal-fin rays in males, reaching urogenital papilla in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin on vertical through base of 8th anal-fin ray. Dorsal-fin rays 9–10; anal-fin rays 14–15; caudal-fin rays 30–31; pectoral-fin rays 13; pelvic-fin rays 5–6. No contact organs on fins.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 25% of caudal-fin base; no scales



Figure 5. *Melanorivulus flavipinnis* sp. n., paratype, UFRJ 6882, female, 27.8 mm SL. Photograph by W.J.E.M. Costa.

Table 2. Morphometric data of *Melanorivulus flavipinnis*.

	holotype	paratypes	
	male	males (n = 5)	females (n = 7)
Standard length (mm)	28.5	22.5–26.6	22.9–28.4
Percent of standard length			
Body depth	21.3	21.7–22.7	20.9–23.1
Caudal peduncle depth	13.3	13.2–14.3	12.8–14.1
Pre-dorsal length	72.3	73.0–76.2	73.8–76.4
Pre-pelvic length	53.7	52.6–55.1	53.0–56.8
Length of dorsal-fin base	14.6	11.2–13.7	10.4–13.4
Length of anal-fin base	24.7	21.4–25.2	20.6–23.3
Caudal-fin length	36.1	34.1–38.2	34.4–38.7
Pectoral-fin length	20.7	19.9–23.3	20.3–21.7
Pelvic-fin length	12.4	10.6–13.3	8.5–11.7
Head length	26.3	26.1–28.1	26.5–28.1
Percent of head length			
Head depth	67.0	65.5–71.3	66.0–73.5
Head width	69.4	67.4–72.7	70.9–76.2
Snout length	13.4	12.9–15.3	13.1–15.6
Lower jaw length	17.5	18.4–20.0	17.8–19.5
Eye diameter	33.4	32.3–35.2	28.8–35.8

on dorsal and anal-fin bases. Frontal squamation F-patterned, rarely E-scale anteriorly overlapping F-scale; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Longitudinal series of scales 30–32; transverse series of scales 8; scale rows around caudal peduncle 16. No contact organs on scales. Cephalic neuromasts: supraorbital 3 + 3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1 + 10–11 + 1, preorbital 2, otic 1, post-otic 1, supratemporal 1,

median opercular 1, ventral opercular 1, pre-opercular 2 + 4, mandibular 2–3 + 1, lateral mandibular 1, paramandibular 1.

Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight. Ventral process angulo-articular short, pointed. Ventral process of palatine short, slightly contacting quadrate. Mesopterygoid slender, posterior tip not reaching metapterygoid. Metapterygoid sub-rectangular, with constriction on middle portion. Dorsal portion of preopercle short and pointed, channel rudimentary. Basihyal sub-triangular, greatest width about 50% of length; basihyal cartilage about 15–25% of total basihyal length. Six branchiostegal rays. Second pharyngobranchial teeth absent. Interarcual cartilage rudimentary. Fourth ceratobranchial teeth present, continuously arranged. Gill-rakers on first branchial arch 1 + 8. Vomerine teeth 2. Dermosphenotic present. Ventral process of posttemporal absent. Second proximal radial of dorsal fin between neural spines of 19th and 21st vertebrae, first proximal radial of anal fin between pleural ribs of 13th and 15th vertebrae. Total vertebrae 30–31.

Colouration. Males. Flank metallic green-blue to metallic light blue, sometimes purple-blue above anal fin; oblique narrow orangish red bars irregularly arranged, often forming chevron-like marks anteriorly directed; short light red stripe on humeral region; horizontal rows of reddish orange dots on antero-ventral part of flank, between bases of pectoral and pelvic fins; pale dark grey blotches on postorbital region mainly visible when fish is exposed to strong light. Dorsum light yellowish-brown with black dots, venter white. Dorsal portion of head side light brown, ventral portion white; pale golden iridescence on opercular region. Jaws dark grey. Iris pale yellow, sometimes with dark brown bar on anterior and posterior portions. Dorsal fin light yellow with seven or eight narrow oblique red bars, often forming reticulate pattern on distal portion of fin. Anal fin pale blue on its proximal half, with faint oblique red bars, light yellow in its distal half, distal region becoming gradually dark reddish brown on marginal border, distal margin with high concentration of melanophores. Caudal fin bright yellow, more intensely pigmented on dorsal and ventral portions, with seven or eight narrow red bars, irregularly shaped and sometimes interconnected. Pectoral fin yellowish hyaline. Pelvic fin light blue with orangish brown anterior margin.

Females. Side of trunk and head similar to males, but with paler colours. Ventral surface of head white, with dark grey spots often forming short stripe on chin. Dorsal fin pale yellow, with oblique grey bars; broad dark grey to black band on distal margin. Anal fin green-yellow, basal portion light blue with small red spots. Caudal fin pale yellow on middle portion, reddish orange on marginal region, with five to seven dark grey bars, often interconnected; small black spot, smaller than pupil, on dorso-basal portion of fin overlapping anterior-most bar, conspicuous only in preserved specimens; broad dark grey to black band on whole fin margin.

Distribution. Known only from the type locality, a small stream tributary to the Rio Anhumas, Rio São Lourenço drainage, Rio Paraguai basin, central Brazil, altitude approximately 420 m asl (Fig. 3).

Etymology. From the Latin, *flavipinnis* (yellow fins), referring to the bright yellow colouration of the caudal fin in males.

***Melanorivulus regularis* sp. n.**

<http://zoobank.org/6DE3B93B-9257-4557-B4C1-20B4D2BD05FE>

Fig 6, Table 3

Holotype. UFRJ 6878, male, 26.9 mm SL; Brazil: Mato Grosso state: Alto Graças municipality: Ribeirão da Sobra, upper Rio Itiquira drainage, Rio Paraguai basin, 16°54'41"S, 53°37'55"W, altitude approximately 750 m asl, road BR-364; W. J. E. M. Costa et al., 5 August 2016.

Paratypes. UFRJ 6879, 4 males, 24.4–33.3 mm SL, 9 females, 22.3–33.8 mm SL; UFRJ 6880, 2 males, 25.3–31.2 mm SL, 2 females, 23.7–28.4 mm SL (C&S); CICC AA00278, 1 male, 24.7 mm SL, 1 female, 25.9 mm SL; collected with holotype.

Diagnosis. *Melanorivulus regularis* is distinguished from all other species of the *M. dapazi* group by the presence, in males, of five or six dark reddish brown, regularly shaped and never interconnected bars on the caudal fin (vs. seven or eight narrow red bars, irregularly shaped and sometimes interconnected in *M. flavipinnis*; four or fewer short rudimentary bars, sometimes absent, in *M. dapazi*; bars always absent in *M. ignescens*). Also distinguished from all other congeners of the *M. dapazi* group by the following combination of character states: 7 pelvic-fin rays (vs. 5–6 in *M. flavipinnis* and *M. ignescens*); 35–37 scales in longitudinal series (vs. 29–32 in *M. flavipinnis* and *M. ignescens*); caudal fin, in females, pale (vs. yellow on the middle portion and reddish orange on marginal region in *M. flavipinnis*); female caudal spot conspicuous in live exemplars fish (vs. inconspicuous in *M. flavipinnis* and *M. ignescens*); caudal fin, in males, without distinctive orange margin (vs. with broad bright orange band along the whole margin in *M. dapazi*); anal fin, in males, yellow (vs. reddish orange in *M. ignescens*); in females, ventral surface of head with dark grey spots, often forming short stripe on chin (vs. without dark grey spots in *M. dapazi*). Also distinguished from all other congeners of the *M. dapazi* group by having 32 vertebrae (vs. 29–31).

Description. Morphometric data appear in Table 3. Body slender, sub-cylindrical anteriorly, slightly deeper than wide, compressed posteriorly. Greatest body depth at vertical just in front of pelvic-fin base. Dorsal and ventral profiles of trunk almost straight to slightly convex in lateral view; dorsal and ventral profiles of caudal peduncle nearly straight. Head moderately wide, sub-triangular in lateral view, dorsal profile nearly straight, ventral profile convex. Jaws short, snout weakly pointed in lateral view. Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight.

Dorsal and anal fins short, tip slightly pointed in males, rounded in females. Caudal fin oval, slightly longer than deep. Pectoral fin rounded, posterior margin reaching vertical at around 80% of length between pectoral-fin and pelvic-fin bases. Pelvic fin small, tip reaching between urogenital papilla and base of 1st anal-fin ray in males, reaching anus in females; pelvic-fin bases medially in close proximity. Dorsal-fin origin on vertical through base of 8th anal-fin ray. Dorsal-fin rays 10–11; anal-fin rays 14–15; caudal-fin rays 31–33; pectoral-fin rays 13–14; pelvic-fin rays 7. No contact organs on fins.



Figure 6. *Melanorivulus regularis* sp. n., holotype, UFRJ 6878, male, 26.9 mm SL. Photograph by W.J.E.M. Costa.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation extending over anterior 25% of caudal-fin base; no scales on dorsal and anal-fin bases. Frontal squamation F-patterned, rarely E-scale anteriorly overlapping F-scale; E-scales not overlapping medially; scales arranged in regular circular pattern around A-scale without exposed margins. Longitudinal series of scales 35–37; transverse series of scales 9; scale rows around caudal peduncle 16. No contact organs on scales. Cephalic neuromasts: supraorbital 3 + 3, parietal 1, anterior rostral 1, posterior rostral 1, infraorbital 1 + 10–11 + 1, preorbital 1–2, otic 1, post-otic 1, supratemporal 1, median opercular 1, ventral opercular 1, pre-opercular 2 + 4, mandibular 2–3 + 1, lateral mandibular 1, paramandibular 1.

Jaw teeth numerous, conical, irregularly arranged, outer teeth larger and slightly curved, inner teeth straight. Ventral process angulo-articular short, pointed. Ventral process of palatine short, slightly contacting quadrate. Mesopterygoid slender, posterior tip not reaching metapterygoid. Metapterygoid sub-rectangular, with constriction on middle portion. Dorsal portion of preopercle short and pointed, channel rudimentary. Basihyal sub-triangular, greatest width near 55% of length; basihyal cartilage the 20% of total basihyal length. Six branchiostegal rays. Second pharyngobranchial teeth absent. Interarcual cartilage rudimentary. Fourth ceratobranchial teeth present, continuously arranged. Gill-rakers on first branchial arch 1 + 7–8. Vomerine teeth 2–5. Dermosphenotic present. Ventral process of posttemporal absent. Second proximal radial of dorsal fin between neural spines of 19th and 21st vertebrae, first proximal radial of anal fin between pleural ribs of 14th and 15th vertebrae. Total vertebrae 32.

Colouration. Males. Flank light metallic blue; oblique narrow orange-red bars irregularly arranged, often forming chevron-like marks anteriorly directed; horizontal rows of reddish orange dots on antero-ventral part of flank, between bases of pectoral and pelvic fins; dark brown pigmentation concentrated on postorbital, overlapped by black dots on superficial layer of skin. Dorsum light yellowish-grey, venter white. Dor-

Table 3. Morphometric data of *Melanorivulus regularis*.

	holotype	paratypes	
	male	males (n = 7)	females (n = 7)
Standard length (mm)	26.9	24.4–33.3	23.7–33.8
Percent of standard length			
Body depth	23.2	21.9–22.6	21.6–23.0
Caudal peduncle depth	13.4	12.7–13.9	12.8–13.6
Pre-dorsal length	73.9	72.7–76.5	73.8–76.9
Pre-pelvic length	55.4	53.8–58.0	54.3–57.5
Length of dorsal-fin base	13.6	11.5–14.5	12.2–13.8
Length of anal-fin base	22.1	20.4–24.1	18.8–21.1
Caudal-fin length	34.1	32.7–33.7	31.7–34.9
Pectoral-fin length	21.4	20.0–22.2	19.2–21.5
Pelvic-fin length	10.7	9.6–12.4	8.4–10.2
Head length	27.2	25.3–27.5	24.9–27.2
Percent of head length			
Head depth	69.6	67.9–73.1	69.6–80.4
Head width	73.5	71.1–77.2	75.5–82.9
Snout length	15.2	13.6–17.1	13.4–16.2
Lower jaw length	20.1	16.6–20.7	20.1–23.7
Eye diameter	29.3	30.1–33.5	30.8–34.2

sal portion of head side light brown, ventral portion white; pale golden iridescence on opercular region. Jaws dark grey. Iris pale yellow to pale brown. Dorsal fin pale yellow with four or five narrow red bars on posterior portion of fin. Anal fin orangish-yellow, basal portion white, posterior portion pale blue with two or three faint red oblique bars; distal region becoming gradually dark red-brown, distal margin with high concentration of melanophores. Caudal fin pale blue to pale yellow, with five or six dark red-brown regularly shaped bars, ventral portion light yellow without bars, ventral margin orangish-brown. Pectoral fin yellowish-hyaline. Pelvic fin pale blue with brown anterior margin.

Females. Side of trunk and head similar to males, but with paler colours. Ventral surface of head white, with dark grey spots often forming short stripe on chin. Dorsal fin pale yellow, with three or four bars on posterior region; broad dark grey to black band on distal margin. Anal fin pale yellow, basal portion light blue. Caudal fin pale yellow, with four or five dark grey bars; small black spot, slightly smaller than pupil, on dorso-basal portion of fin; broad dark grey to black band on whole fin margin.

Distribution. Known only from the type locality, Ribeirão da Sobra, an upper tributary of the Rio Itiquira, Rio Paraguai basin, central Brazil, in altitude about 750 m asl (Fig. 3).

Etymology. From the Latin, *regularis* (regular), a reference to the caudal fin bars in males, regularly shaped and arranged on fin.

Key to the species of the *Melanorivulus dapazi* group

- 1 In females, ventral surface of head with dark grey spots, often forming short stripe on chin; in males, caudal fin never with broad bright orange band along entire margin **2**
- In females, ventral surface of head without dark grey spots; in males, caudal fin with broad bright orange band along margin ***M. dapazi***
- 2 7 pelvic-fin rays; 35–37 scales in longitudinal series; female caudal spot conspicuous in live fish ***M. regularis***
- 5–6 pelvic-fin rays; 29–32 scales in longitudinal series; female caudal spot inconspicuous in live fish **3**
- 4 Caudal fin, in males, without bars; caudal fin, in females, pale yellow; anal fin, in males, bright red-orange; caudal-fin length 26.8–33.1% SL ***M. ignescens***
- Caudal fin, in males, with 7–8 red bars; caudal fin, in females, pale yellow on middle portion and orange on marginal portion; anal fin, in males, light yellow; caudal-fin length 34.1–38.7% SL ***M. flavipinnis***

Discussion

Morphological characters indicate that all three new species here described are more closely related to *M. dapazi* than to other congeners, with these four species comprising the *M. dapazi* group. In all species of this group, the interarcual cartilage is rudimentary, nearly equal in size to the adjacent cartilage at the tip of the first epibranchial (Fig. 7). In other species of *Melanorivulus*, the cartilage is well-developed, larger than first epibranchial cartilage, and around one fourth the length of the first epibranchial (e.g., Costa 2016: fig. 4). In addition, species of the *M. dapazi* group share the presence of a dark red-brown distal margin on the male anal fin (Figs 1, 4, 6), a condition not found in other congeners. A molecular phylogeny of *Melanorivulus* (Costa et al. 2016) supports *M. dapazi* as a sister group to a clade including species of the *M. decoratus* group, but the three species here described were not included in that analysis. The *M. decoratus* species group is diagnosed by the presence of five branchiostegal rays (vs. six) and a narrow basihyal, its width around 35% of the longitudinal length (vs. 45–60%). The *M. decoratus* species group is comprised of three miniature species not surpassing 20 mm SL: *M. atlanticus* Costa, Bragança & Ottoni, 2015, from the coastal plains of northeastern Brazil, *M. decoratus* Costa, 1989, from the middle Rio São Francisco Basin, and *M. jalapensis* Costa, 2010 from the middle Rio Tocantins drainage (Costa 1989, 2010; Costa et al. 2015).

Relationships among species of the *M. dapazi* group remain unclear. *Melanorivulus flavipinnis*, endemic to the Paraguai basin, is possibly more closely related to *M. ignescens*, endemic to the Araguaia basin, than to *M. dapazi* and *M. regularis* that like *M. flavipinnis* are endemic to the Paraguai basin. Among species of the *M. dapazi* group, only in *M. flavipinnis* and *M. ignescens* there are five or six rays in the pelvic

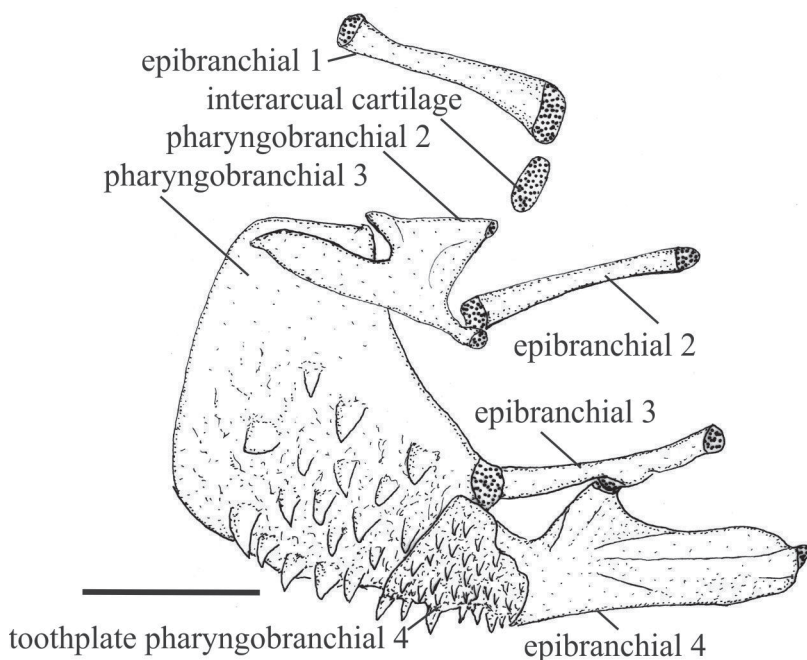


Figure 7. Dorsal branchial arches, left side, ventral view, of *Melanorivulus flavipinnis*, paratype, UFRJ 6883, 25.6 mm SL. Larger stippling indicates cartilage. Scale bar: 0.5 mm.



Figure 8. *Melanorivulus dapazi*, UFRJ 11203, female, 22.2 mm SL. Photograph by W.J.E.M. Costa.

fin. In addition, in both species the spot on the basal portion of the female caudal fin is inconspicuous in live fish (Figs 2 and 5) and poorly visible in preserved specimens. In *M. dapazi* and *M. regularis*, there are seven pelvic-fin rays and the female caudal spot is conspicuous and delimited in live (Fig. 8) and preserved specimens, conditions considered plesiomorphic for *Melanorivulus* (Costa, 2016). The unique pigmentation pattern on the ventral surface of the head in females that is shared by *M. flavipinnis*,

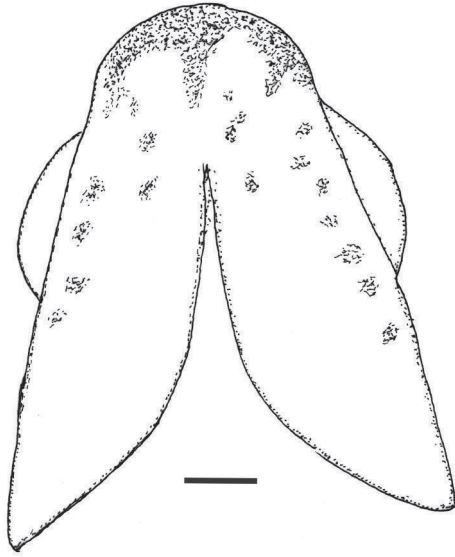


Figure 9. Diagrammatic representation of the colour pattern on the ventral surface of the head in females of *Melanorivulus regularis*, UFRJ 6879, 28.5 mm SL. Scale bar: 1 mm.

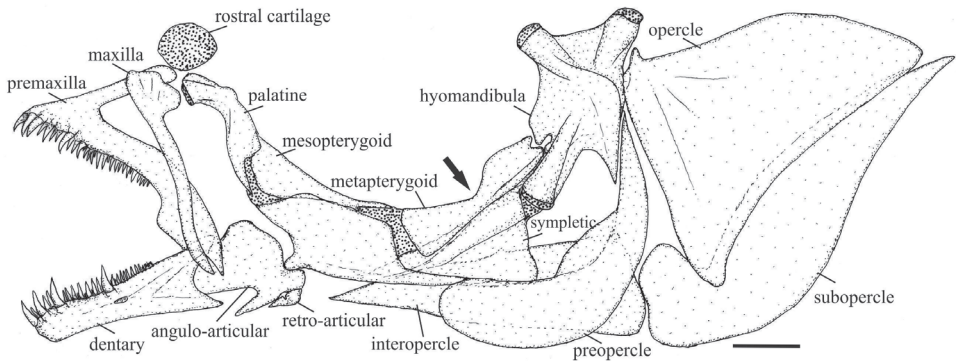


Figure 10. Jaws, jaw suspensorium and opercular apparatus, left side, lateral view, of *Melanorivulus ignescens*, paratype, UFRJ 6877, 25.1 mm SL. Larger stippling indicates cartilage. Scale bar: 0.5 mm.

M. ignescens, and *M. regularis* (Fig. 9), may be indicative of close relationships among these three species.

Costa (2016) discussed the importance of using live colour pattern characters to diagnose species and species groups of *Melanorivulus*, showing high congruence with molecular data. In that study, particular attention was given to patterns involving the caudal fin, which contained a high concentration of phylogenetically informative characters, useful to delimit most species of the *M. zygonectes* group. Concordantly, the present study shows that colour patterns documented from live fish is an accurate tool to recognise species of the *M. dapazi* group (see key for species identification above).

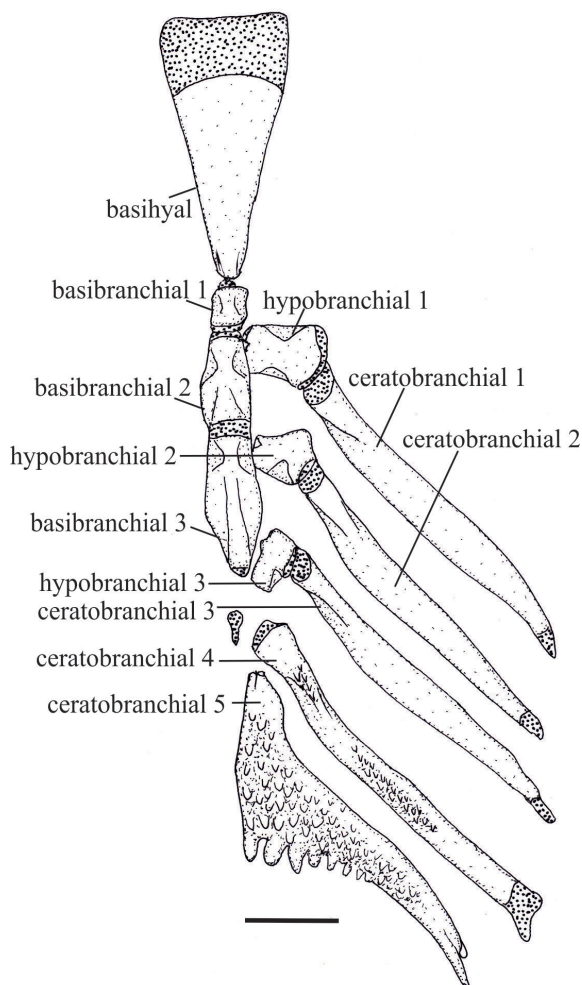


Figure 11. Basihyal and ventral branchial arches, right and median portion, dorsal view, of *Melanorivulus flavipinnis*, paratype, UFRJ 6883, 25.6 mm SL. Larger stippling indicates cartilage. Scale bar: 0.5 mm.

Osteological characters have been used to infer relationships among species groups of *Melanorivulus* and for diagnostic purposes (e.g., Costa 2016; this study). The present study shows that osteological characters may be also useful to diagnose single species. The unique shape of the metapterygoid recorded for *M. ignescens*, with a constriction in its middle portion (Fig. 10), and the unique arrangement of teeth on the fourth ceratobranchial in *M. flavipinnis*, exhibiting a median gap (Fig. 11), are not present in other congeners. In addition, *M. regularis* differs from other species of the *M. dapazi* group by having 32 vertebrae (vs. 29–31 in other species). Thus, although checking osteological characters in larger specimen samples is often not practicable, it is recommendable that osteology be included in taxonomical studies of *Melanorivulus* to complement species diagnoses.

Recent killifish inventories in the area of the central Brazilian plateaus drained by the upper tributaries of the Araguaia, Paraná and Paraguai river basins have revealed an unexpected high diversity of species of the genus *Melanorivulus* (e.g., Costa 2012). The present study confirms this high diversity, indicating once again that species inhabiting the region have small geographical ranges, often restricted to short segments of a single river drainage. For example, among species endemic to the Paraguai basin, *M. regularis* was found in a single locality of the Rio Itiquira drainage, at approximately 750 m asl, whereas the present field survey indicated that at altitudes around 450 m asl of the same drainage, the only species found was *M. dapazi*, which also occurs in similar altitudes of the neighbouring areas included in the Rio Correntes drainage (Costa 2005). On the other hand, *M. flavipinnis* here described from the Rio São Lourenço drainage at approximately 420 m asl, is substituted by *M. cyanopterus* (Costa, 2005), at altitudes of approximately 250 m asl (Costa 2005). The last species is a member of the *M. punctatus* group, distantly related to the *M. dapazi* group and geographically widespread in the lower Paraguai river basin (Costa et al. 2016). Recent studies with other vertebrates occurring in the Cerrado indicate that high species diversity in the region is correlated with the topographical reorganization during the Miocene, which generated geographical isolation of ancestral populations in plateaus and peripheral depressions (Prado et al. 2012; Guarnizo et al. 2016). This paleogeographical scenario may explain the present distribution of distinct species of *Melanorivulus* along different altitudinal zones of river drainages.

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Revising the distribution of a threatened goby, *Apocryptodon punctatus* (Perciformes, Oxudercidae), in Japan with the discovery of an isolated population

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Abstract

Five specimens of a threatened goby, *Apocryptodon punctatus* (21.2–40.1 mm in standard length), were collected at a mudflat site of Kushima City, Miyazaki Prefecture, Kyushu, southern Japan over two seasons, autumn (September 2015) and spring (April 2016). A review of distributional records of *A. punctatus* revealed that this population represents the southernmost record of the species in Japanese waters, and is isolated ca. 200 km south-southwest from the nearest point of the main range of the species along the Pacific coast of Japan. Publicising this population will help conserve it and its vulnerable habitat.

Keywords

Endangered species, estuarine fish, Gobioidae, Red List, tidal flat, voucher

Introduction

Apocryptodon punctatus Tomiyama, 1934, an estuarine gobiid fish of the subfamily Oxudercinae (Murdy 1989), now the family Oxudercidae (Nelson et al. 2016), is known from central to western Japan (Inui 2015), the western coast of South Korea (Kim et al. 1986, Youn 2002) and Taiwan (Chen and Fang 1999). This species inhabits silty mudflat environments utilizing the burrows of alpheid shrimps (Dôtu 1961; Koyama et al. 2017). It is an ecological indicator for natural tidal flat environments (Suzuki 2003) and recent ecological studies have shown habitat preferences and symbiotic partner specificity for the species (Koyama et al. 2016, 2017). Although the species is considered threatened because of the vulnerability of its habitat (Iwata 1997, Suzuki 2003), recent increases in field sampling efforts have resulted in the discovery of several new locality records (Inui 2015). Consequently, the species' conservation status in the Red Data Book for wildlife of Japan has dropped from "Endangered (EN)" in 2007 to "Vulnerable (VU)" in 2015. However, this does not reflect an improvement in the species' habitat, and habitat degradation remains a concern (Inui 2015). Sato and Aizawa (1992) summarized literature and specimen records of the species at that time, including a distribution map of the species (with 13 points on the map); the distribution showed a belt-like shape from central to western Japan with sparse distribution in central Japan. Of these records, the southernmost distributional point (Nichinan City, southern part of Miyazaki Prefecture) was clearly separated from the main distribution, ca 200 km to the south. However, this record was based on a juvenile specimen (Masahiro Aizawa personal communication) and there has been no further record from Miyazaki Prefecture until very recently (Inui 2015). Given the lack of known populations in the Miyazaki Prefecture, the origin of the Nichinan City specimen was uncertain. In recent faunal diversity surveys of estuaries along the coasts of Miyazaki Prefecture, the authors collected several specimens of *A. punctatus* from Kushima City, located 20 km southwest of Nichinan City. Distributional records of threatened species and their publicity are important contributors to policy decisions regarding the conservation of vulnerable species and their habitats (Inui and Koyama 2014). Furthermore, unusual occurrences of a species may indicate a biodiversity hotspot or other unusual conditions (Hiscock 2014). This note describes the specimens of *A. punctatus* from Kushima City, and reports on the habitat in order to elucidate the population status at this site. Additionally, in order to understand the range of *A. punctatus* in Japan, recent records of the species were reviewed, including unpublished specimen data that supported distributional information for each prefecture in Inui (2015).

Materials and methods

Specimens of *A. punctatus* were collected using hand nets from a mudflat estuary of Kushima City, the southernmost part of Miyazaki Prefecture, Kyushu, southern Japan (detailed information of the locality omitted for conservation purposes), in the Japa-

nese autumn (= end of summer, September 27, 2015) and spring (April 8, 2016). The specimens were immediately killed by placing them in a mixture of environmental water and ice in a plastic bag. Thereafter, the fishes were fixed in 10% formalin and subsequently preserved in 70% ethanol. Color photographs when fresh (Fig. 1) were taken after approximately 30 minutes in fixation. All specimens and photos were deposited in the ichthyological collection and image database of the Kanagawa Prefectural Museum of Natural History (KPM-NI for specimens, KPM-NR for photos). The following five specimens were examined (photo numbers in parentheses): KPM-NI 40542 (KPM-NR 166524), 26.6 mm SL (standard length), 27 Sep. 2015, collected by A. Murase and Y. Miyazaki; KPM-NI 40543 (KPM-NR 166525), 21.2 mm SL, same data as KPM-NI 40542; KPM-NI 40558 (KPM-NR 166539), 28.9 mm SL, 8 April 2016, collected by A. Murase and R. Miki; KPM-NI 40559 (KPM-NR 166540), 35.6 mm SL, same data as KPM-NI 40558; KPM-NI 40560 (KPM-NR 166541), 40.1 mm SL, same data as KPM-NI 40558.

Counts and P-V relation (i.e., the relationship between pterygiophores of the dorsal fins and vertebrae) followed Akihito (1984), with vertebrae data taken from radiographs. Caudal-fin rays included those on hypural plates. Measurements were made with needle-point calipers to the nearest 0.1 mm, according to Hubbs and Lagler (2004) except for the following: body depth, vertical distance from anal-fin origin to second dorsal-fin base; prepelvic length, distance from snout tip to anterior margin of base of pelvic-fin spine; preanal length, distance from snout tip to anal-fin origin; caudal peduncle depth, depth at vertical trough point of attachment of last anal-fin membrane to caudal peduncle; pectoral-fin length, length of longest ray; pelvic-fin length, distance from base of pelvic-fin spine to posteriormost extremity of fin.

In order to elucidate the condition of the environment, salinity and water temperature in tidal pools on the mudflat where the specimens of *A. punctatus* were captured were measured at the time of fish sampling using YK-31SA (Sato Shouji Inc., Kawasaki) and TT-508 (Tanita, Tokyo). In addition, the percentages of silt and clay were calculated following Koyama et al. (2016a) using a sediment core of 2.5 cm depth and 5 cm diameter. These water environment and sediment samplings were repeated five times and 20 times on each collection date (27 September 2015 and 8 April 2016) respectively.

Inui (2015) updated the distribution of *A. punctatus* in Japanese waters and listed the names of prefectures where the species has been recorded. While several new prefecture records were included, Inui (2015) provided no specimen data supporting the records. In order to accurately document the known distribution of *A. punctatus*, a list of localities of the species was summarized on the basis of published literature and the second author's unpublished specimen data that had been used for the distributional update in Inui (2015). Those specimens were deposited in the Tokushima Prefectural Museum (TKPM-P). Of the prefectures listed as localities of *A. punctatus* by Inui (2015), Mie and Kagoshima Prefectures have no museum specimen records (the collected specimens are either uncatalogued or missing); locality details in those prefectures follow Ryutei Inui's unpublished data.



Figure 1. Lateral view of a fresh specimen of *Apocryptodon punctatus*, KPM-NI 40559, 35.6 mm SL, collected from a mudflat estuary of Kushima City, Miyazaki Prefecture, Kyushu, Southern Japan. Top, photo number KPM-NR 166540B; bottom, photo number KPM-NR 166540A.

Results

Description

Counts and measurements of *Apocryptodon punctatus* collected from Kushima City are shown in Table 1. P-V relation 3/I II II I 0/9 (based on only the three larger specimens due to the obscurity of pterygiophores in the smaller specimens). A supraorbital pore present just behind eye.

Body elongate and more compressed posteriorly. Eyes small and prominent dorsally. Mouth large, horizontal and its posterior edge positioned behind a vertical line from posterior edge of eye. First and second dorsal fins close and connected by small membrane.

Head and body dark yellow dorsally, lower part of head and trunk white, tail greyish white ventrally. Bold black bar horizontally across centre of preopercular and opercula (bar posteriorly oblique reaching to posterodorsal edge of operculum in larger specimens). Dark bar on occipital region across dorsal edge (bar shape differs between individuals, being a simple bar, fine arch, or eyeglass-like spot). Two dark bars present across dorsal edge of nape, laterally appearing as dark spots. Dark bar (may be wedge like) across anterior part of 1st dorsal-fin base; three dark bars across region from end of 1st dorsal-fin base to centre of 2nd dorsal-fin base. Blotch present across posterior base of 2nd dorsal fin. These bars or blotch on dorsal edge appearing as a simple spot, line or saddle-like spot in lateral view. Dark blotch or spot present on upper anteriormost part of region beneath pectoral-fin (absent in KPM-NI 40542). Five small dark blotches (two anteriormost vertically oblong in shape and latter three shorter or circular) present on body axis from

Table 1. Counts and proportional measurements of *Apocryptodon punctatus* ($n = 5$) from Kushima City, Miyazaki Prefecture, Kyushu, southern Japan.

Standard length (mm)	21.2–40.1
Counts	
Dorsal-fin rays	VI–I, 22
Anal-fin rays	22 or 23
Pectoral-fin rays	22 or 23
Pelvic-fin rays	I, 5
Caudal-fin rays (upper + lower)	7 + 6
Vertebrae (precaudal + caudal)	10 + 26
In % of standard length	
Total length	125.6–128.7
Head length	29.2–31.2
Snout length	7.9–9.3
Upper-jaw length	15.2–16.0
Interorbital width	0.2–0.5
Orbit diameter	5.2–6.4
Body depth	12.6–14.0
Predorsal length	38.5–40.1
Prepelvic-fin length	29.2–30.1
Preanal-fin length	58.5–60.8
Caudal-peduncle length	4.8–5.7
Caudal-peduncle depth	7.6–8.2
Length of 1 st dorsal-fin base	15.6–17.0
Length of 2 nd dorsal-fin base	40.8–41.4
Length of anal-fin base	36.5–38.8
Pectoral-fin length	16.0–17.5
Pelvic-fin length	17.5–19.3
Length of 1 st spine of 1st dorsal fin	9.7–10.4
Length of 2 nd spine of 1st dorsal fin	10.7–11.3
Length of 1 st spine of 2 nd dorsal fin	7.7–9.0
Length of 1 st soft-ray of 2 nd dorsal fin	9.5–11.1
Length of 1 st anal-fin soft-ray	5.2–5.9
Length of 2 nd anal-fin soft-ray	6.9–7.5

trunk to caudal-fin base, connected to each other with dark horizontal lines. Dorsal-fin rays dark yellow. First dorsal-fin entirely transparent but 2nd dorsal-fin slightly darker posterodorsally with numerous horizontal dark yellow spots on lower part of fin. Anal-fin rays nearly transparent, whitish. Lower edge of anal fin with white margin, becoming broader anteriorly in larger specimens; area above white region blackish, darker posterodorsally (darker area separated into two separate wide blotches in KPM-NI 40559; Fig. 1). Caudal-fin rays nearly transparent; upper and central area of fin dark yellow, lower part blackish. Pectoral fin nearly transparent, dark yellow; lower part of fin blackish with white lower margin. Pelvic fin entirely whitish with nearly transparent membrane.

Habitat

The Kushima City specimens of *A. punctatus* were captured on a small mudflat zone (ca 200 m²) that at low tide reveals a small stream flowing into the mouth of a wider river. The mean salinity level of the location was 2.7 ± 0.1 (\pm SD, ranging 2.6–2.9) and 2.3 ± 0.1 (2.1–2.5) in autumn and spring respectively. It is considered a polyhaline environment (sensu McLusky and Elliott 2004) over the two seasons, while the nearby stream had zero salinity at the sampling time. Mean water temperature was $29.6 \pm 1.6^\circ\text{C}$ (27.8–31.5°C) and $21.8 \pm 0.5^\circ\text{C}$ (21.4–22.5°C) in autumn and spring respectively. The mean ratio of silt and clay in the location was $31.7 \pm 7.5\%$ (17.7–45.8%) and $35.7 \pm 6.3\%$ (19.4–45.1%) in autumn and spring respectively. The mean ratio of sand ($66.0 \pm 1.5\%$ and $59.5 \pm 5.2\%$ in autumn and spring respectively) was greater than that of silt and clay, and that of gravel ($2.3 \pm 1.5\%$ and $4.8 \pm 1.5\%$ in autumn and spring respectively) was clearly less than that of silt and clay showing that the composition of sediments in the location was mainly sand, with some silt and clay and almost no gravel.

Distribution

The distribution records of *A. punctatus* are summarized in Table 2 and each locality is mapped in Fig. 2 (45 localities).

Discussion

The five specimens collected from Kushima City corresponded well with the earlier descriptions of *A. punctatus* in Tomiyama (1934), Murdy (1989), Sato and Aizawa (1992) and Akihito et al. (2013) in having the following diagnostic characters: dorsal-fin element VI-I, 22; mouth large, its posterior edge clearly positioned behind posterior edge of eye; five small dark blotches (two anteriormost vertical, oblong shape) present on body axis from trunk to caudal-fin base, connected by dark horizontal lines. The blotches on the lateral body of the Kushima City specimens differ somewhat with the descriptions in Tomiyama (1934) and Murdy (1989) in having circular blotches on the posterior part of the body. These earlier authors observed larger specimens (40–80 mm in total length, TL, in the former, 49–67 mm SL in the latter) for their description whilst the specimens in the present study ranged from 21.2–40.1 mm SL. The observed coloration differences may be ontogenetic or geographic variation, as mentioned in Matsui et al. (2011).

The first comprehensive review of distributional records of *A. punctatus* since Sato and Aizawa (1992) resulted in many more localities than the 13 of Sato and Aizawa (1992), and included the northernmost (Maizuru Bay, Kyoto) and easternmost (Watarai-gun, Mie) records. The primary distribution of *A. punctatus* is across mainland Honshu and the northwestern part of Kyushu, appearing as a diagonal band through

Table 2. Distribution records of *Apocryptodon punctatus* from Japanese waters based on literature and voucher sources.

Locality			Source	
Region	Prefecture	City, town or island	Literature	Voucher
Pacific coast (including Seto Inland Sea)	Mie	Watarai-gun	Inui (2015)	US
	Wakayama	Kainan City	Senou and Kitamura (1982)	MS
		Arida City	Kishino and Nomoto (2000)	MS
		Tanabe City	Suzuki and Wada (1999)	MS
	Tokushima	Tokushima City	Sato and Aizawa (1992)	MS
		Anan City	Present study	adMS
	Kochi	Kochi City	Miyake et al. (2006)	MS
		Suzaki City	Okamura (2002)	P
		Shimanto City	Okamura (2002)	Non
	Miyazaki	Nichinan City	Sato and Aizawa (1992)	MS
		Kushima City	Present study	adMS
Seto Inland Sea	Hyogo	Tatsuno City	Present study	adMS
		Ako City	Suzuki and Masuda (1993)	MS
		Okayama City	Present study	adMS
	Okayama	Kurashiki City	Present study	adMS
		Kasaoka City	Dôtu (1961)	US
		Fukuyama City (east)	Yoshigou and Nakamura (2002)	MS
	Hiroshima	Fukuyama City (west)	Yoshigou (2001)	MS
		Innoshima City	Yoshigou (2001)	MS
	Yamaguchi	Yamaguchi City	Present study	adMS
		Ube City	Present study	adMS
		Sanyo-onoda City	Present study	adMS
		Shimonoseki City	Present study	adMS
	Kagawa	Marugame City	Present study	adMS
	Ehime	Saijyo City	Present study	adMS
		Ainan-cho Town	Present study	adMS
	Fukuoka	Kitakyushu City	Present study	adMS
		Yukuhashi City	Present study	adMS
	Oita	Nakatsu City	Present study	adMS
Sea of Japan and East China Sea	Kyoto	Maizuru City	Matsui et al. (2011)	MS
	Yamaguchi	Nagato City	Mori (1995)	US
		Fukutsu City	Inui et al. (2012)	US
	Fukuoka	Itoshima City	Inui et al. (2012)	US
		Yanagawa City	Tomiyama (1934)	Holotype
		Higashimatsuura-gun	Fujii and Asayama (2013)	US
	Saga	Imari City	Fujii and Asayama (2013)	P, US
		Kishima-gun	Sato and Aizawa (1992)	MS
		Kashima City	Dôtu (1961)	Non
		Tsushima Island	Yoshigou and Nakamura (2003)	MS
	Nagasaki	Matsuura City	Sato and Aizawa (1992)	MS
		Goto Islands	Yoshigou and Nakamura (2003)	MS
		Uto City	Sato and Aizawa (1992)	MS
	Kumamoto	Yatsushiro City	Koyama et al. (2016a)	US
		Izumi City	Inui (2015)	US
	Kagoshima	Akune City	Inui (2015)	US

Voucher: adMS, additional museum specimens in the present study; MS, museum specimens; P, photo; US, uncataloged specimens.

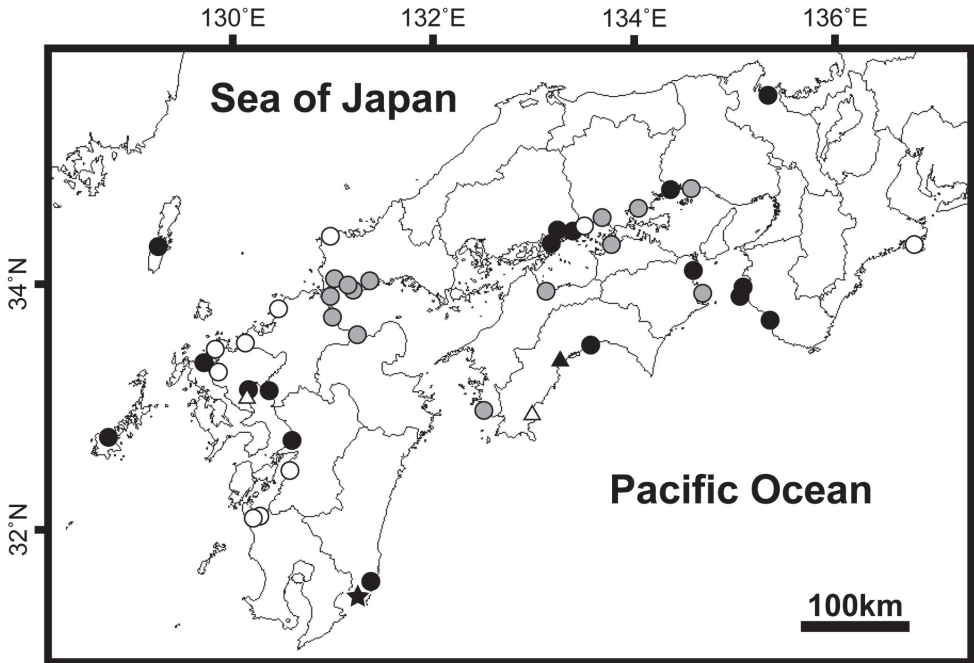


Figure 2. Distribution map of *Apocryptodon punctatus* in Japanese waters. Solid star indicates the present record from Kushima City, Miyazaki Prefecture, Kyushu; solid circles, records with voucher specimens deposited in a museum; gray circle, records with additional museum specimens in the present study; open circles, records with uncatalogued specimens; solid triangle, photo record; open triangles, literature records without a voucher.

western Japan (Fig. 2). This distributional review also revealed that the specimens from Kushima City, Miyazaki Prefecture, represent the southernmost records within Japanese waters, being ca 200 km south-southwest of Ainan-cho, Ehime Prefecture, the closest point on the Pacific coast (Fig. 2, Table 2). The authors' recent survey of fish fauna in thirty estuaries along 100 km of the coast of Miyazaki Prefecture found *A. punctatus* in the estuary of Kushima City only (Atsunobu Murase unpublished data). The record from Nichinan City (Sato and Aizawa 1992) was based on a single juvenile specimen (LIAIP1985-325, collected on 4 Oct. 1985). In addition, there have been no further records of the species and, in recent years, the preferred habitat of *A. punctatus* in the city has been largely lost (Masahiro Aizawa, personal communication). On the other hand, several specimens have been captured over two seasons (autumn and spring) and other individuals observed (Atsunobu Murase unpublished data) on the mudflat estuary of Kushima City, located ca. 20 km south of Nichinan City. This mudflat has a recorded salinity of 2.1–2.9 at low tide, maintaining a higher salinity level than an adjacent stream (zero salinity), and is composed of a maximum of more than 45% silt and clay (on average more than 35% and 31% in spring and autumn respectively). The occurrence rate of *A. punctatus* reaches its maximum at a silt and clay

level of around 60%, in the estuary of Kuma-gawa River, western Kyushu (Koyama et al. 2016, Akihiko Koyama, pers. comm.). The silt and clay levels recorded in this study are approaching the level recorded in that previous study. Furthermore, several unidentified alpheid shrimps that may be symbiotic partners for *A. punctatus* (Dôtu 1961, Koyama et al. 2017) have been observed in this mudflat (Atsunobu Murase unpublished data). *Apocryptodon punctatus* matures at 60 mm TL in Ariake Bay, western Kyushu (Dôtu 1961), but the maximum size recorded in the present study was 50.5 mm TL (KPM-NI 40560). Temperate fish species often have a smaller size at maturity in lower latitudes (i.e., warmer conditions) than higher latitudes (Kuriwa et al. 2014, Trip et al. 2014, Stocks et al. 2015). Kushima City is located more than 100 km south-southeast of Ariake Bay and has warmer conditions in the coastal zone given its proximity to the warm Kuroshio Current (mean surface water temperature in February ca. 19°C in the former vs ca 14°C in the latter: Japan Oceanographic Data Center 2016); this latitudinal size variation could therefore be evident in *A. punctatus*. In addition, the strong and warm Kuroshio Current, which hinders dispersion of fishes from north to south (Matsuura and Senou 2012, Kuriwa et al. 2014), flows northward off the coast of southern Miyazaki, and no population of *A. punctatus* has been found near Kushima City until now. Therefore, it is reasonable to conclude that the population of *A. punctatus* from an estuary of Kushima City reproduces locally, and is isolated from other populations in Japanese waters.

Miyazaki Prefecture has lost large areas of estuarine tidal flats since the 1980's mainly due to the restriction of Hitotsuba lagoon and the development of Miyazaki Port near Miyazaki City (Miura et al. 2005, Miura 2008). It is possible that the preferable estuarine habitat for *Apocryptodon punctatus* has been lost in the prefecture except for the estuary of Kushima City. Fishes are a good indicator of estuarine ecosystem health, and are useful to assess and monitor anthropogenic impacts (Whitfield and Elliott 2002, Harrison and Whitfield 2004). Species such as *A. punctatus*, which has a relatively sparse distribution and specificity for habitat and a symbiotic partner, can be used as indicator species for environmental monitoring in estuaries by coupling their occurrence with biodiversity and functional parameters (e.g. biomass, water quality, etc). That aside, it is important that the threatened status of this and other species is recognized and that preferable habitat is maintained. This is all the more important when a population is isolated, such as the present case. Urgent action is required to conserve this population, with the first step being to record Kushima City (Miyazaki Prefecture) in the Red Data Book as the southernmost limit for *A. punctatus* in Japanese waters.

Other materials. Catalogue number and collection data of additional museum specimens for distributional records of *A. punctatus* in Fig. 2 and Table 2 are as follows (all the specimens collected by Ryutei Inui and his colleagues, specimen size expressed in SL)—Tokushima Prefecture: TKPM-P 23222 (1, 47.4 mm, Anan City, 16 Apr. 2011); Hyogo Prefecture: TKPM-P 24521 (1, 27.7 mm, Tatsuno City, 17 Nov. 2011); Okayama Prefecture: TKPM-P 24549 (1, 49.5 mm, Okayama City, 29 Apr. 2011), TKPM-P 24550 (3, 37.7–43.4 mm, Okayama City, 16 Nov. 2011), TKPM-P 24551

(1, 52.2 mm, Kurashiki City, 2 June 2011); Yamaguchi Prefecture: TKPM-P 24637 (2, 48.7–50.3 mm, Shimonoseki City, 27 July 2011), TKPM-P 24638 (2, 25.7–40.7 mm, Sanyo-onoda City, 8 Aug. 2010), TKPM-P 24639 (1, 43.3 mm, Sanyo-onoda City, 27 July 2011), TKPM-P 24640 (1, 43.5 mm, Ube City, 27 July 2011), TKPM-P 24641 (1, 44.5 mm, Yamaguchi City, 28 July 2011), TKPM-P 24642 (1, 40.7 mm, Yamaguchi City, 26 Apr. 2009); Kagawa Prefecture: TKPM-P 23514 (1, 21.3 mm, Marugame City, 6 Oct. 2011); Ehime Prefecture: TKPM-P 23567 (1, 25.3 mm, Ainan-cho Town, 12 Oct. 2011), TKPM-P 23568 (1, 35.7 mm, Saijyo City, 15 July 2011), TKPM-P 23569 (1, 16.5 mm, Saijyo City, 20 Sep. 2008); Fukuoka Prefecture: TKPM-P 25279 (3, 17.9–55.0 mm, Kitakyushu City, 11 Aug. 2011), TKPM-P 25280 (4, 17.4–18.3 mm, Kitakyushu City, 11 Aug. 2011), TKPM-P 25281 (1, 16.7 mm, Yukuhashi City, 11 Aug. 2011), TKPM-P 25282 (1, 16.9 mm, Yukuhashi City, 11 Aug. 2011), TKPM-P 25283 (42.9 mm, Yukuhashi City, 8 June 2006), TKPM-P 25284 (1, 48.6 mm, Kitakyushu City, 24 Mar. 2008); Oita Prefecture: TKPM-P 25068 (1, 58.5 mm, Nakatsu City, 12 Aug. 2011).

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A new species of *Pristimantis* (Amphibia, Anura, Craugastoridae) from a montane forest of the Pui Pui Protected Forest in central Peru (Región Junín)

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Abstract

A new species of frog of the genus *Pristimantis* is described from a montane forest between 1700 and 1800 m a.s.l. of the Pui Pui Protected Forest (Región Junín) in central Peru. *Pristimantis ashaninka* **sp. n.** is described based on five adult females (snout–vent length 23.1–26.7 mm) and ten juveniles (snout–vent length 10.6–13.4). It differs from its congeners by having the skin on dorsum shagreen with many conical tubercles giving it a spinose appearance, lacking a tympanum, having groin, anterior and posterior surfaces of thighs uniformly grayish brown, and a pale bronze iris with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris. Among the Peruvian *Pristimantis* that lack a tympanum, *P. ashaninka* **sp. n.** is morphologically most similar to *P. lirellus*, *P. martiae*, and *P. rhabdocnemus*. However, 16S DNA barcoding revealed clear distinctions between all four species of *Pristimantis*.

Keywords

Andes, montane forests, anuran diversity, *Pristimantis ashaninka* new species, Peru

Introduction

The Pui Pui Protected Forest (Bosque de Protección Pui Pui, hereafter PPPF) is located in the Región Junín (Provincias Chanchamayo, Jauja, Concepción, and Satipo), was created 1985, and protects 60,000 hectares (30% montane forest, 70% puna habitats) between 1700 and 4500 m a.s.l. (SERNANP 2010). We surveyed the herpetofauna of the Pui Pui Protected Forest (Fig. 1) in montane forests and high Andean grasslands (puna) between 2012 and 2014 with the aim to record the amphibian and reptile species richness and to evaluate their conservation status. The collected specimens included several new species of craugastorid frogs. New species of craugastorid frogs are frequently discovered and scientifically described from Peru (e.g., Chávez and Catenazzi 2016, Padial et al. 2016, Shepack et al. 2016). Integrative taxonomy, the use of different character sets (e.g., morphological and molecular), has been very helpful to identify and describe new species (Padial et al. 2010) in this complicated group of frogs which has a high species diversity (487 species of which 127 occur in Peru, AmphibiaWeb 2016), cryptic diversity and often phenotypic polymorphism. Herein, we describe a new species of *Pristimantis* (*Pristimantis* sp. Pui Pui in Lehr et al. in press) from a montane forest between 1700 and 1800 m a.s.l in the PPPF.

Methods

Morphological characters. The format for the description follows Lynch and Duellman (1997), except that the term dentigerous processes of vomers is used instead of vomerine odontophores (Duellman et al. 2006), and diagnostic characters are those of Duellman and Lehr (2009). Taxonomic classification follows Hedges et al. (2008) and Heinicke et al. (2009), except that we followed Pyron and Wiens (2011) for family placement, and Padial et al. (2014) for names of *Pristimantis* species groups. Specimens were fixed in 96% ethanol and stored in 70% ethanol. Liver tissues of three specimens (NMP6V 75063–65) were taken for genetic analyses (see Lehr et al. in press, GenBank accession numbers KY006110–12). Sex and maturity of specimens were identified through dissections of gonads. Specimens with a SVL ≤ 13.4 mm were considered juveniles when gonads were too small to distinguish between sexes. We measured the following variables to the nearest 0.1 mm with digital calipers under a stereomicroscope: snout–vent length (SVL, straight length distance from tip of snout to vent), tibia length (TL, distance from the knee to the distal end of the tibia), foot length (FL, distance from proximal margin of inner metatarsal tubercle to tip of Toe IV), head length (HL, from angle of jaw to tip of snout), head width (HW, at level of angle of jaw), horizontal eye diameter (ED), interorbital distance (IOD), upper eyelid width (EW), internarial distance (IND), eye–nostril distance (E–N, straight line distance between anterior corner of orbit and posterior margin of external nares). Fingers and toes are numbered preaxially to postaxially from I–IV and I–V, respectively. We compared the lengths of Toes III and V by adpressing both toes against Toe IV; lengths of

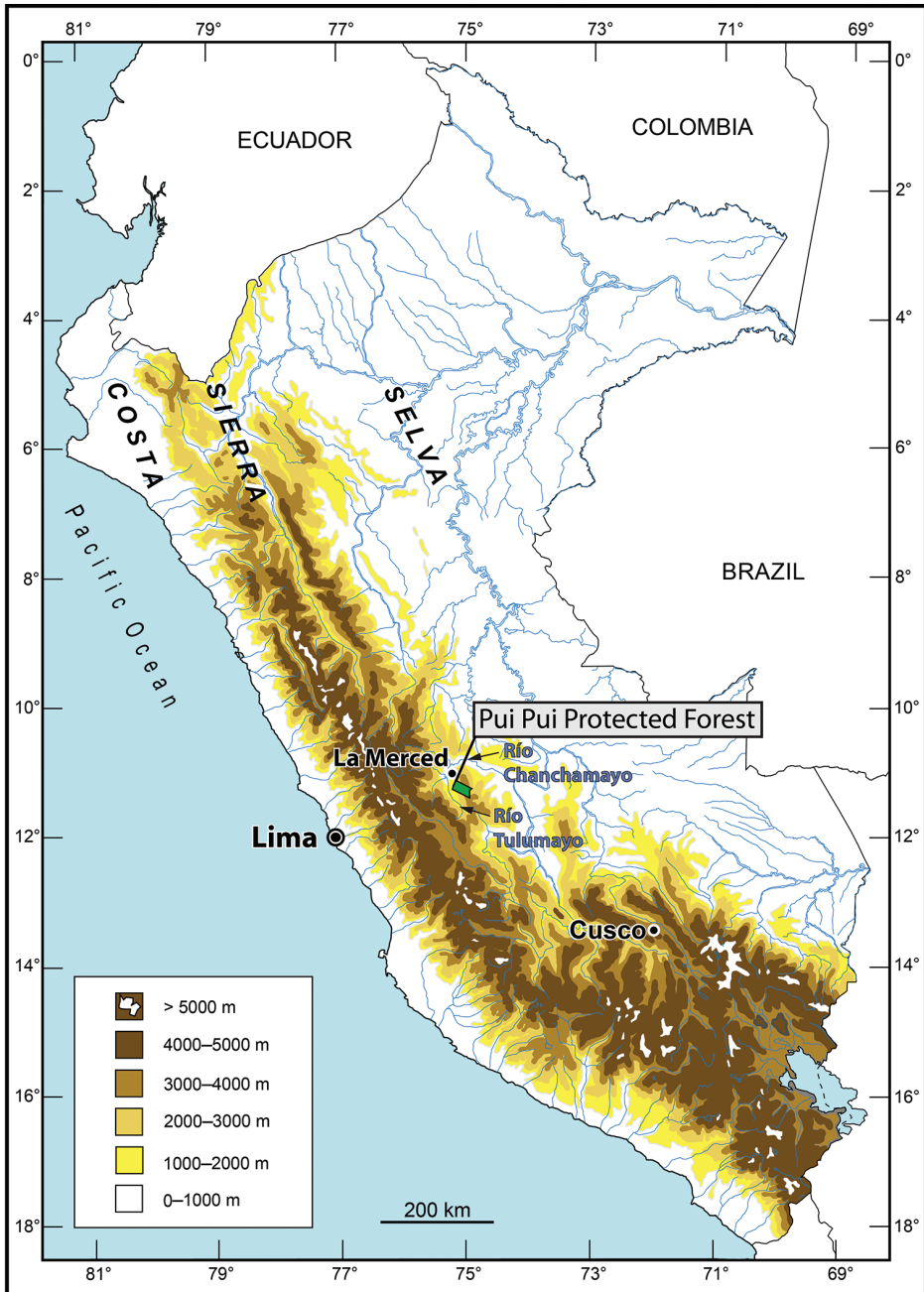


Figure 1. Map of Peru with the Pui Pui Protected Forest indicated in green.

Fingers I and II were compared by addressing the fingers against each other. Drawings were made by EL using a stereomicroscope and a camera lucida. Photographs taken by EL and JM were used for descriptions of coloration in life. The 16S DNA barcoding

was used for comparison of the new species with morphologically similar *Pristimantis* species from Ecuador and Peru (Lehr et al. in press). Comparisons of congeners focused on Andean and Amazonian lowland species from Ecuador and Peru with similar morphology and genetically close species as recovered in our tree (Lehr et al. in press). Information on species for comparative diagnoses was obtained from Duellman and Lehr (2009) and from original species descriptions. For specimens examined see Appendix. Codes of collections are: **MUSM** = Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru; **NMP6V** = National Museum Prague, Prague, Czech Republic. Field number code is: **IWU** = Illinois Wesleyan University. Threat status was evaluated using the IUCN criteria (2016).

Results

Pristimantis ashaninka sp. n.

<http://zoobank.org/30AFB9BB-BCF4-4129-B63D-A7744E479E72>

Pristimantis sp. Pui Pui in Lehr et al. (in press, Fig. 2)

Suggested English name: Asháninka Rubber Frog

Suggested Spanish name: Rana cutín asháninka

Holotype (Figs 2–3). MUSM 36517 (IWU 361), an adult female from the border of the Pui Pui Protected Forest (11°12'38.5"S, 74°57'28.9"W), 1700 m elevation, Distrito Pichanaqui, Provincia Chanchamayo, Región Junín, Peru, collected on 15 May 2014 by Edgar Lehr and Jiří Moravec.

Paratypes (Figs 4–5). Fourteen: four females: MUSM 32736, 32742, NMP6V 75063 (GenBank accession number KY006110), 75064 (KY006111); ten juveniles: NMP6V 75553–75555, MUSM 32721, 32722, 32728, 32730, 32734, 32741, NMP6V 75065 (KY006112), all collected at the type locality: on 13 May 2014 at 1700 m a.s.l. (NMP6V 75553, 75554, MUSM 32721, 32722, NMP6V 75065), on 14 May 2014 at 1700 m a.s.l. (MUSM 32728), on 15 May 2014 at 1700 m a.s.l. (MUSM 32730), on 17 May 2014 at 1700 m a.s.l. (MUSM 32734, NMP6V 75555), on 18 May 2014 at 1800 m a.s.l. (MUSM 32736, NMP6V 75064), on 20 May 2014 at 1750 m (MUSM 32741), on 21 May 2014 at 1800 m a.s.l. (MUSM 32742, NMP6V 75063).

Generic placement. We assign this species to *Pristimantis* based on general morphological similarity to other members of the genus and our molecular data (Lehr et al. in press).

Diagnosis. A new species of *Pristimantis* not assigned to any species group having the following combination of characters: (1) Skin on dorsum shagreen with many conical tubercles, skin on venter areolate; discoidal, thoracic, and dorsolateral folds absent; (2) tympanic membrane and tympanic annulus absent; (3) snout moderate, subacuminate in dorsal view, rounded in lateral view; (4) upper eyelid with enlarged conical tubercles; EW slightly shorter than IOD; cranial crests absent; (5) dentigerous processes of vomers present; (6) condition of vocal slits and nuptial pads unknown;



Figure 2. Life holotype (MUSM 36517, SVL 24.0 mm) of *Pristimantis ashaninka* sp. n. in lateral (A), dorsal (B), dorsolateral (C), ventral (D) views, and (E) posterior surface of thighs. Photos by E. Lehr.

(7) Finger I shorter than Finger II; discs of digits broadly expanded, rounded; (8) fingers with narrow lateral fringes; (9) small conical ulnar and tarsal tubercles present; (10) heel with small conical tubercles; inner tarsal fold absent; (11) inner metatarsal tubercle ovoid, 4 times as large as outer; outer metatarsal tubercle small, rounded; low, numerous supernumerary plantar tubercles; (12) toes with narrow lateral fringes; basal toe webbing absent; Toe V longer than Toe III; toe discs slightly smaller than those on fingers; (13) in life, dorsal coloration consists of a reddish-brown blotch in shape of a hourglass with dark grayish-brown markings, bordered laterally by creamish brown, a dark grayish-brown sinusoidal or W-mark on scapular region, extremities with dark grayish-brown bars; flanks usually paler than dorsum, creamish brown with broad diagonal dark grayish-brown stripes; dark grayish-brown bars on upper lip, dark grayish-brown canthal and supratympanic stripes present; groin, anterior and posterior surfaces of thighs uniformly grayish brown; venter pale gray and grayish brown mottled; iris pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris; (14) SVL in adult females 23.1–26.7 mm ($n = 5$).

Comparisons. *Pristimantis ashaninka* differs from its congeners by having the skin on dorsum shagreen with many conical tubercles giving it a spinose appearance, lacking a tympanum, having groin, anterior and posterior surfaces of thighs uniformly grayish brown, and a pale bronze iris with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris.

Seventeen species of *Pristimantis* from Peru lack a tympanum. These are *P. academicus* Lehr, Moravec & Gagliardi Urrutia, 2010, *P. altamazonicus* (Barbour & Dunn, 1921), *P. colodactylus* (Lynch, 1979), *P. coronatus* Lehr & Duellman, 2007, *P. croceinguinis* (Lynch, 1968), *P. cruciocularis* (Lehr, Lundberg, Aguilar & von May, 2006), *P. flavobracatus* (Lehr, Lundberg, Aguilar & von May, 2006), *P. imitatrix* (Duellman, 1978), *P. lirellus* (Dwyer, 1995), *P. leucorrhinus* Boano, Mazzotti & Sindaco, 2008, *P. martiae* (Lynch, 1974), *P. minutulus* Duellman & Hedges, 2007, *P. rhabdocnemus* (Duellman & Hedges, 2005), *P. simonsii* (Boulenger, 1900), *P. tantanti* (Lehr, Torres-Gastello & Suárez-Segovia, 2007), *P. ventrimarmoratus* (Boulenger, 1912), and *P. vilcabambae* Lehr, 2007.

Of these species, *P. lirellus* from 470–1200 m a.s.l. (Dwyer 1995) on the eastern slopes of the Cordillera Central in northern Peru, *P. martiae* from Colombia, Ecuador to central Peru up to 1330 m a.s.l. (Lynch 1974), and *P. rhabdocnemus* from the Cordillera Oriental in central Peru between 230–2900 m a.s.l. (Duellman and Hedges 2005) are most similar regarding morphology and coloration to *P. ashaninka*. However, the 16S DNA barcoding revealed clear distinctions between all three species (sequences of *P. rhabdocnemus* include specimens from its type locality) and *P. ashaninka* (Lehr et al. in press). Furthermore, the new species can be distinguished from them as follows (characters of *P. ashaninka* in parentheses unless otherwise stated): *Pristimantis lirellus* is smaller (SVL in females 19.4–24.5 mm vs. 23.1–26.7 mm [$n = 5$] in *P. ashaninka*, Dwyer 2005), has skin on dorsum shagreen with small, scattered tubercles (many conical

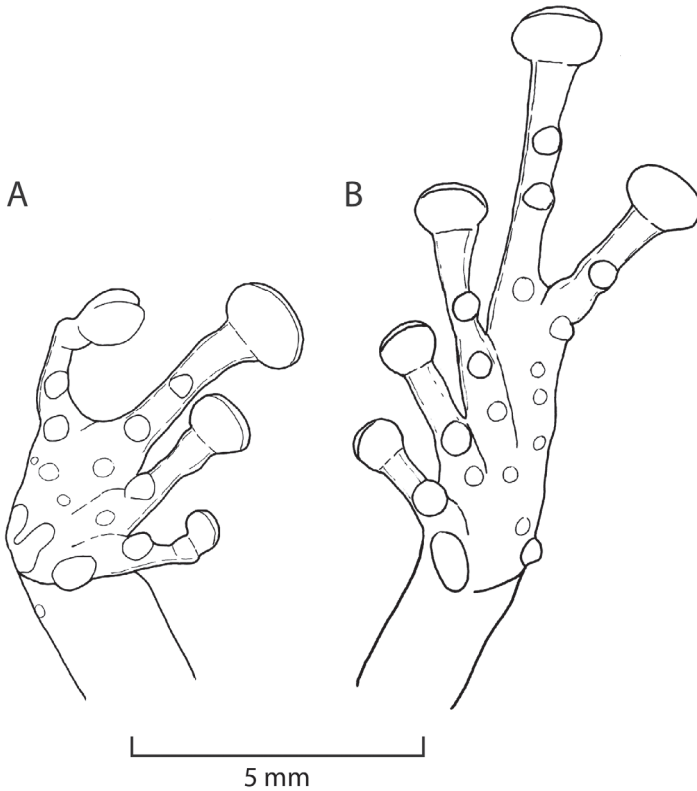


Figure 3. Ventral views of right hand (**A**) and left foot (**B**) of *Pristimantis ashaninka* sp. n. (holotype, MUSM 36517). Drawings by E. Lehr.

cal tubercles) and low longitudinal dermal ridge (absent), prominent discoidal fold (absent), groin with pale yellow to yellow-orange spot (uniformly grayish brown), and iris bronze to reddish brown with median horizontal red streak (pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris). *Pristimantis martiae* and *P. ashaninka* have the dorsum brown with darker brown middorsal blotch in shape of a hourglass and pale brown flanks, but *P. martiae* is smaller (SVL in females 18.3–23.00 mm, Lynch 1974), has skin on dorsum shagreen with low, flat warts (shagreen with many conical tubercles), groin, anterior and posterior surfaces of thighs dull cream or pale orange with brown to black bars or mottling (uniformly grayish brown), and a bronze iris with median horizontal brown streak (pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris). *Pristimantis rhabdocnemus* is of similar size (SVL in females 23.2–27.0 mm, Duellman and Hedges 2005), has skin on dorsum shagreen with or without scattered small tubercles (shagreen with many conical tubercles), posterior surfaces of thighs tan (uniformly grayish brown), and iris grayish

tan (pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris).

Pristimantis platydactylus (Boulenger, 1903) and *P. wiensi* (Duellman and Wild, 1993) look superficially similar with *P. ashaninka*, but can be distinguished as follows. *Pristimantis platydactylus* from the eastern Andes of central Peru to central Bolivia at elevations between 930 and 3470 m a.s.l. (Duellman and Lehr 2009) contains several unnamed species (Padial et al. 2009), is much larger (SVL in females 23.8–35.3 mm, Duellman and Lehr 2009), has a tympanum (absent), and is genetically different (Lehr et al. in press) based on sequences from 14 specimens of *P. platydactylus* from Peru and Bolivia obtained from Padial et al. (2009). *Pristimantis wiensi* from northern Peru (western slopes of Cordillera Huancabamba between 1600 and 1735 m a.s.l., Duellman and Wild 1993) is much larger (SVL in single female 37.0 mm, Duellman and Wild 1993), has discoidal and dorsolateral folds (both absent), and has a tympanum (absent).

Furthermore, *P. ashaninka* differs from other Andean *Pristimantis* (in alphabetical order) that lack a tympanum as follows: *Pristimantis colodactylus* from southern Ecuador and northern Peru between 2195–3140 m a.s.l. (Lynch 1979) has short and stocky fingers (of normal length) with small discs (broad). *Pristimantis coronotus* from northern Peru at 2850 m a.s.l. (Lehr and Duellman 2007) has posterior half of flanks, groin and proximal anterior surfaces of thighs red (uniformly grayish brown). *Pristimantis cruciocularis* from 1330–1850 m a.s.l. in the Cordillera Oriental in central Peru is smaller (SVL in females 18.7–21.8 mm, Lehr et al. 2006), has groin and anterior surfaces of thighs orange to red (uniformly grayish brown), and iris golden with fine black reticulations and dark brown horizontal and vertical streaks forming a cross (pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris). *Pristimantis flavobracatus* from the Amazonian slopes of the Cordillera Oriental at 1770 m a.s.l. in central Peru is smaller (SVL in females 21.5–23.4 mm, Lehr et al. 2006), has groin and anterior and posterior surfaces of thighs yellow (uniformly grayish brown), and golden iris with fine black reticulations with a brown median horizontal streak across iris and a black vertical streak downward from pupil (pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris). *Pristimantis leucorrhinus* from the Cordillera Oriental in central Peru at 2500 m a.s.l. (Boano et al. 2008) has the upper eyelid with one large conical tubercle (enlarged conical tubercles, but not as large as in *P. leucorrhinus*), a black groin (uniformly grayish brown) and anterior surfaces of thighs black (uniformly grayish brown) with two large white spots on each. *Pristimantis minutulus* from the Cordillera Oriental and lowland forests in central Peru between 250 and 1200 m a.s.l. (Duellman and Hedges 2007) has the groin with a large yellow blotch (uniformly grayish brown). *Pristimantis simonsii* from the Cordillera Occidental in northern Peru at elevations of 3050–3760 m a.s.l. (Duellman and Lehr 2009) has dorsolateral folds (absent). *Pristimantis vilcabambae* from the Cordillera de Vilcabamba at 2050 m a.s.l. (Lehr 2007) in southern Peru has fingers and toes with distinct lateral fringes (narrow), outer fringes of Finger IV and Toe V often continuing



Figure 4. Female paratypes of *Pristimantis ashaninka* sp. n. in dorsolateral (upper row), dorsal (middle row), and ventral (lower row) views. **A–C** (MUSM 32736, SVL 23.1 mm) **D–F** (NMP6V 75063, SVL 26.7 mm) **G–I** (MUSM 32742, SVL 23.3 mm). Photos by E. Lehr (**A, B, D–F, H**) and J. Moravec (**C, G, I**).

as discontinuous fold to outer edge of palm or plantar (absent), and venter cream with dark brown blotches (venter pale gray and grayish brown mottled).

Furthermore, *P. ashaninka* differs from the Amazonian lowland *Pristimantis* (in alphabetical order) that lack a tympanum as follows: *Pristimantis academicus* from lowlands of northern Peru is smaller (20.0–22.0 mm, Lehr et al. 2010), and has a yellow groin (uniformly grayish brown). *Pristimantis altamazonicus* from southern Colombia, Ecuador, Peru, and western Brazil has the groin, anterior and posterior surfaces of thighs red to salmon color with black mottling (uniformly grayish brown). *Pristimantis croceoinguinis* from southern Colombia, Ecuador, and extreme northeastern Peru is smaller (SVL in females 17.4–23.0 mm, Lynch 1968), has skin on dorsum tuberculate (shagreen with many conical tubercles), supernumerary plantar tubercles absent (present), canthal and postorbital stripes absent (present), deep yellow to orange spot in groin (groin uniformly grayish brown), and iris dull bronze with dense brown reticulations (pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower and upper half of iris). *Pristimantis imitatrix* from central and southern Peru and *P. ashaninka* have skin on dorsum shagreen with conical tubercles and bronze iris with median horizontal red

streak, but *P. imitatrix* is much smaller (SVL in females 14.6–20.2 mm, Duellman 1978), has dentigerous processes of vomers absent (present), supernumerary plantar tubercles absent (present), and groin, anterior and posterior surfaces of thighs mottled black and creamy white (uniformly grayish brown). *Pristimantis tantanti* from southern Peru has short triangular snout (subacuminate), and green dorsum with white spots (reddish brown with dark grayish-brown markings). *Pristimantis ventrimarmoratus* from the upper Amazon Basin and slopes of the Andes to 1740 m in Ecuador, Peru, and Bolivia (Duellman and Lehr 2009) has prominent discoidal fold (absent), and chest and belly white with bold black mottling (venter pale gray and grayish brown mottled).

Description of holotype. Head slightly narrower than body, slightly longer as wide; head length 39% of SVL; head width 38% of SVL; cranial crests absent; snout moderately long, subacuminate in dorsal view, rounded in lateral view (Fig. 2A, B); eye-nostril distance 77% of eye diameter; nostrils slightly protuberant, directed dorso-laterally; canthus rostralis moderately long, rounded in lateral view, weakly concave in dorsal view; loreal region concave; lips rounded; upper eyelid each with several tubercles, an enlarged conical tubercle at its center and one enlarged conical tubercle at its posterior end; upper eyelid width 90% of IOD; several enlarged conical tubercles on occipital and scapular region (see photos in life Fig. 2B, C); supratympanic fold short and broad, extending from posterior margin of upper eyelid slightly curved to insertion of arm; tympanic membrane and annulus absent; two conical postrictal tubercles present bilaterally. Choanae small, ovoid, not concealed by palatal shelf of maxilla; dentigerous processes of vomers small, widely separated; tongue discoidal, covering entire floor of mouth, posterior and lateral parts free.

Skin on dorsum and flanks shagreen with many conical tubercles (denser on dorsum than on flanks), dorsolateral folds absent; skin on throat and chest smooth, on belly areolate; discoidal and thoracic folds absent; cloacal sheath short.

Outer ulnar surface with minute low tubercles; palmar tubercle partially divided distally; thenar tubercle ovoid; subarticular tubercles well defined, round in ventral view, conical in lateral view; supernumerary tubercles distinct, ovoid, subconical, approximately half the size of subarticular tubercles; fingers with narrow, weakly defined lateral fringes; Finger I shorter than Finger II; discs on digits of fingers widely expanded, truncate (Fig. 3A).

Hind limbs moderately long, slender, tibia length 54% of SVL; foot length 46% of SVL; upper surfaces of hind limbs smooth with scattered tubercles; inner surface of thighs smooth, posterior and ventral surfaces of thighs weakly areolate; heels each with two prominent conical tubercles; outer surface of tarsus with scattered minute low tubercles; inner tarsal fold absent; inner metatarsal tubercle prominent, ovoid, four times the size of round outer metatarsal tubercle; subarticular tubercles well defined, round in ventral view, conical in lateral view; plantar supernumerary tubercles distinct, about quarter the size of subarticular tubercles; toes with narrow, weakly defined lateral fringes; basal webbing absent; discs expanded, oval, less expanded than those on fingers; relative length of toes: $1 < 2 < 3 < 5 < 4$; disc on Toe III not reaching distal subar-

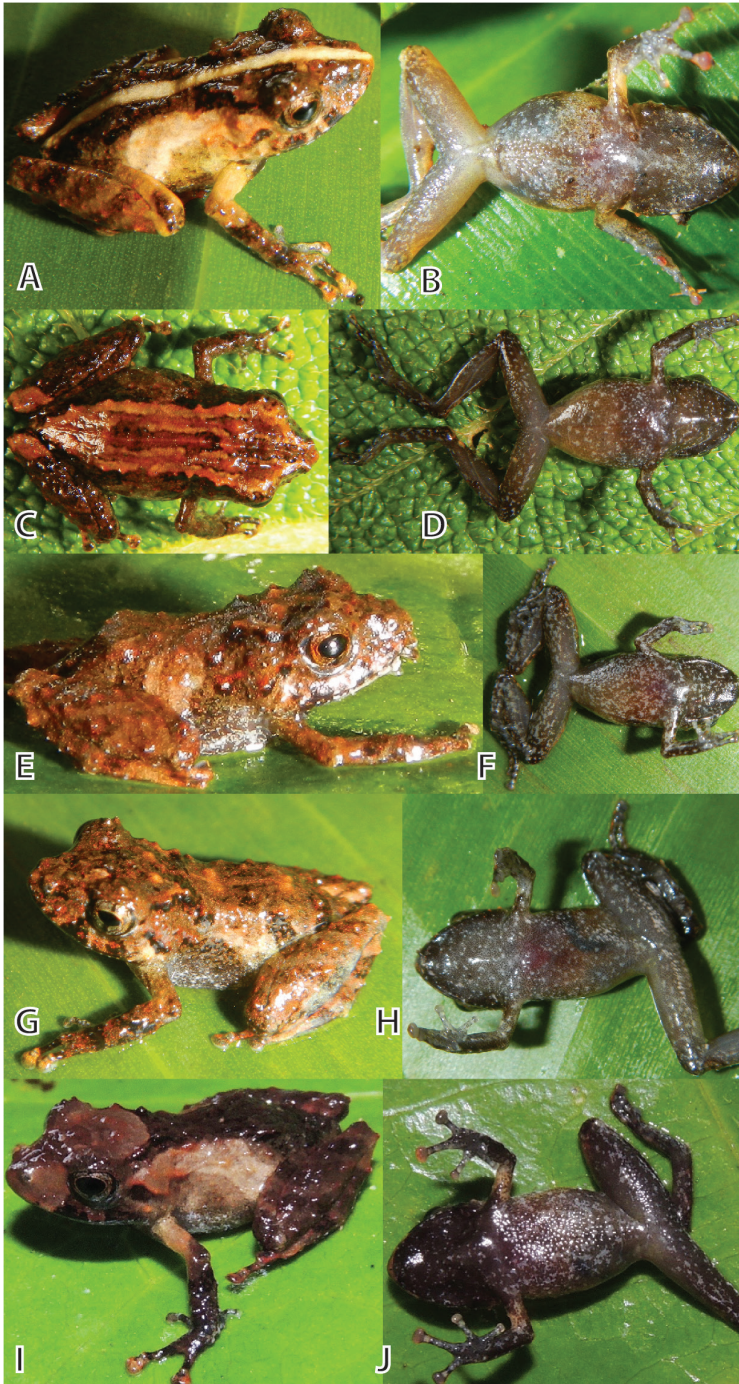


Figure 5. Juvenile paratypes of *Pristimantis ashaninka* sp. n. in dorsolateral/dorsal (left column) and ventral (right column) views. **A, B** (MUSM 32721, SVL 13.4 mm) **C, D** (MUSM 32730, SVL 12.6 mm) **E, F** (MUSM 32728, SVL 11.0 mm) **G, H** (MUSM 32722, SVL 12.5 mm), **I, J** (NMP6V 75555, SVL 12.1 mm). Photos by E. Lehr (**A–H**) and J. Moravec (**I, J**).

ticular tubercle on Toe IV, disc on Toe V extends distal subarticular tubercle on Toe IV; Fig. 3B.

Measurements of the holotype (in mm). SVL 24.0; TL 13.0; FL 11.0; HL 9.4; HW 9.2; ED 3.1; IOD 3.0; EW 2.7; IND 1.9; E-N 2.4.

Coloration of the holotype in life (Fig. 2). Dorsum covered by a large reddish-brown blotch in shape of a hourglass bordered laterally in its narrow portion by creamish brown coloration; a dark grayish-brown W-mark on scapular region covering the anterior portion of the hourglass; two longitudinal oblique dark grayish-brown markings on sacral region bordering the posterior portion of the hourglass; a small dark grayish-brown marking on intercanthal region; a diffuse dark grayish-brown to reddish-brown interorbital stripe; a diffuse dark grayish-brown blotch on canthus rostralis; upper lip with two reddish-brown subocular bars bordered by dark grayish-brown on each side of the head; dark grayish-brown spots in front of and in between subocular bars; a dark grayish-brown supratympanic bar; upper arm creamish brown dorsally; lower arm and hand reddish-brown dorsally except for creamish-brown discs; hind legs creamish brown dorsally with pale reddish-brown diagonal bars; toes creamish brown; flanks creamish brown with pale reddish-brown diagonal blotches; axilla, groin, and anterior and posterior surfaces of thighs uniformly grayish brown; throat, chest, belly, and ventral surfaces of thighs, hands, and feet pale gray and grayish brown mottled; iris pale bronze with fine black reticulations, a median reddish hint horizontally across iris, and a black narrow vertical streak from pupil across lower half of iris and thin dark gray streak across upper half of iris.

Coloration of the holotype in preservative. General coloration pattern is as described for the holotype in life, except for reddish brown which is dark brown and creamish brown which is pale tan. Groin and axilla are pale gray, anterior and posterior surfaces of thighs are pale brown; ventral surfaces except for brown thighs are pale brown and pale tan mottled; iris is silver with fine black reticulations with black narrow vertical streak from pupil across lower half of iris and thin dark gray streak across upper half of iris.

Variation. All female paratypes (Fig. 4, SVL 23.1–26.7, 24.1 ± 1.5 , $n = 4$) and juveniles (Fig. 5, SVL 10.6–13.4, 12.1 ± 1.0 , $n = 10$) are similar to the holotype regarding morphology (Tables 1, 2) and coloration pattern. Two females (MUSM 32742, NMP6V 75063, Fig. 4E, H) have dorsally an hourglass shape blotch as seen in the holotype with the hourglass dark brown in MUSM 32742, two other females (MUSM 32736, NMP6V 75064) lack the coloration contrast of dark dorsum and pale flanks. One female (NMP6V 75064) has the anterior interorbital and snout region creamish brown, three females (MUSM 32736, 32742, NMP6V 75063) have the interorbital area with two creamish brown or reddish brown blotches (Fig. 4B, E, H). Finger and toe discs are dorsally creamish brown (Fig. 4A, D, G).

The juveniles have the conical tubercles on the dorsal skin more pronounced (MUSM 32722, 32741, Fig. 5G) than the females. Conical tubercles form ridges on scapular region (MUSM 32722, 32728, 32741, Fig. 5E, G) and laterally on head and flanks (NMP6V 75555, Fig. 5I). The coloration pattern is similar, but juveniles seem

Table 1. Measurements (in mm) of female type specimens of *Pristimantis ashaninka* sp. n. For abbreviations see methods.

Character	NMP6V 75063	MUSM 36517	MUSM 32742	NMP6V 75064	MUSM 32736
SVL	26.7	24.0	23.3	23.2	23.1
TL	13.6	13.0	12.5	12.8	13.0
FL	11.6	11.0	9.9	10.6	10.9
HL	10.7	9.4	9.4	9.4	9.6
HW	10.4	9.2	9.3	9.0	8.9
ED	3.6	3.1	2.8	3.3	2.7
IOD	3.2	3.0	2.5	2.7	2.8
EW	2.8	2.7	2.2	2.6	2.7
IND	2.5	1.9	1.8	2.1	2.0
E–N	2.7	2.4	2.3	2.4	2.3

Table 2. Measurements (in mm) and proportions of female type specimens of *Pristimantis ashaninka* sp. n.; ranges followed by means and one standard deviation in parentheses. For abbreviations see methods.

Characters	Females (n = 5)
SVL	23.1–26.7 (24.1 ± 1.4)
TL	12.5–13.6 (13.0 ± 0.4)
FL	9.9–11.6 (10.8 ± 0.6)
HL	9.4–10.7 (9.7 ± 0.5)
HW	8.9–10.4 (9.4 ± 0.5)
ED	2.7–3.6 (3.1 ± 0.3)
IOD	2.5–3.2 (2.8 ± 0.2)
EW	2.6–2.8 (2.6 ± 0.2)
IND	1.8–2.5 (2.1 ± 0.2)
E–N	2.3–2.7 (2.4 ± 0.1)
TL/SVL	0.51–0.56
FL/SVL	0.42–0.47
HL/SVL	0.39–0.42
HW/SVL	0.38–0.40
HW/HL	0.9–1.0
E–N/ED	0.73–0.85
EW/IOD	0.88–0.96

to have a more reddish hint especially dorsally and laterally on head and scapular region (MUSM 32722, 32728, 32730, 32741, NMP6V 75554, Fig. 5E, G). One specimen (MUSM 32721, Fig. 5A) has a pale tan middorsal stripe dividing the dark reddish brown hourglass blotch. One specimen (MUSM 32730, Fig. 5C) has heels and dorsum pale reddish brown with dark reddish-brown flecks on dorsum. One specimen (NMP6V 75555, Fig. 5I) has the head dorsally and snout dorsally and laterally pale grayish brown. Ventral coloration of juveniles (Fig 5, right column) is darker than in females with pale

gray flecks, with the throat often black (NMP6V 75555) to dark grayish brown (MUSM 32728, 32730) with pale gray flecks. Finger and toe discs are dorsally creamish brown.

Etymology. The species epithet *ashaninka* is used in reference to the indigenous people Asháninka who inhabit forests in the Peruvian Regions Huánuco, Junín, Pasco, and Ucayali.

Distribution, natural history, and threat status. *Pristimantis ashaninka* is only known from the type locality, which is located at the northeastern border of the Pui Pui Protected Forest, ca. 18 km (straight airline) NW of the town of Satipo, Distrito de Pichanaqui, Provincia de Chanchamayo, Región Junín, Peru (Fig. 1). The type locality lies in the valley of a tributary of the Rio Bravo at an elevation between 1700 and 1800 m a.s.l. and can be reached by walking in one and a half day starting at the village of Ayte (11°09′46.7″S, 74°55′14″W, 1295 m a.s.l.), which serves as a control station for the administration of the PPPF. The valley is surrounded by steep mountain slopes, which gives it a narrow character. The surrounding mountains are covered by a primary mountain rainforest characterized by 15–20 m high canopy and frequent occurrence of bromeliads, ferns, and epiphytic mosses. The adult specimens of *P. ashaninka* were collected at night on vegetation up to 150 cm above the ground whereas juveniles occupied lower positions in the vegetation. Other craugastorid species found at the type locality in sympatry with *P. ashaninka* included *Pristimantis* cf. *albertus*, *P. bipunctatus* (Duellman and Hedges, 2005), *P. cruciocularis*, *P. cf. platydactylus*, and *Pristimantis* sp. nov. According to the sparse data available, we here classify *P. ashaninka* as “Data Deficient” according to the IUCN red list criteria.

Discussion

Despite high species diversity and endemism, many areas throughout the Tropical Andes remain unexplored because montane forests are difficult to reach. Consequently, biodiversity in these areas is poorly known compared to other ecoregions at lower elevations. This was true for the Pui Pui Protected Forest, which is located in the eastern Andes of the Region Junín. Because of its remote location and difficult access no biological surveys have been conducted inside the PPPF prior to our expeditions. The borders of the PPPF are covered by primary mountain forests interrupted with scattered coffee plantations and a few houses along the larger rivers. New road constructions to support expanding villages in the region are a latent threat to these cloud forests, which are considered valuable for some of their timber trees or as new land for agricultural crops such as passionfruit (*granadilla*) and chili pepper (*rocoto*). Contemporary plans to construct a dam with a power station on Rio Huatziroki in the northern border of the PPPF are an additional threat. This example points to the great importance of the existing buffer zone of PPPF, which primary role to preventing habitat destructions around the PPPF borders should be maximally respected. Given that the only known locality of *P. ashaninka* lies outside the boundary of the PPPF, the long-term protection of this species will depend on the type of land use in the area. This is especially relevant

considering that large areas of potentially suitable habitat have been converted to agriculture outside the preserve. Given that many amphibian species, including dozens of threatened species, in Peru are known to occur only outside natural protected areas (von May et al. 2008), is it essential to carry out additional field surveys focusing on target species to determine if their populations occur inside protected areas.

With the description of *P. ashaninka*, the number of *Pristimantis* known from Peru rises to 128 species (AmphibiaWeb 2016). Further new species of *Pristimantis* and *Phrynopis* from the PPPF and its surroundings will be described in the near future.

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Appendix

Comparative specimens examined

Pristimantis cf. *albertus* (1 specimen): PERU: JUNÍN: PPPF, 11°12'38.5"S, 74°57'28.9"W, 1700 m, MUSM 32729.

Pristimantis *bipunctatus* (2 specimens): PERU: JUNÍN: PPPF, 11°12'38.5"S, 74°57'28.9"W, 1700 m, MUSM 32723, 32724.

Pristimantis *cruciocularis* (2 specimens): PERU: PASCO: Yanachaga-Chemillén National Park (Sector San Daniel), Distrito de Huancabamba, Provincia de Oxapampa, ca. 2900 m, MUSM 31140, 31146.

Pristimantis *imitatrix* (2 specimens): PERU: MADRE DE DIOS: Cusco Amazónico, 15 km E Puerto Maldonado, 200 m, MUSM 7348, 14605.

Pristimantis *lirellus* (1 specimen): PERU: HUÁNUCO: Dantas, MUSM 11326.

Pristimantis *minutulus* (10 specimens): PERU: HUÁNUCO: Panguana, SMNS 13017–22; PASCO: 0.0–1.5 km W Cacazu, 900 m, KU 291677, 291679, 291680, 308608.

Pristimantis cf. *platydactylus* (3 specimens): PERU: JUNÍN: PPPF, 11°12'38.5"S, 74°57'28.9"W, 1800 m, MUSM 32735; Peru, 11°05'44.2"S, 75°13'39.8"W, 1550 m, MUSM 31929, 31930.

Pristimantis *rhabdocnemus* (3 specimens): PERU: PASCO: ca. 10°23'.718 S, 75°28'.919 W, 2350 m, MUSM 31110, 31112, 31114.

Checklist and distribution maps of the blow flies of Venezuela (Diptera, Calliphoridae, Mesembrinellidae)

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Abstract

A checklist of the 39 species of blow flies (Calliphoridae and Mesembrinellidae) so far known to occur in Venezuela is provided, based on a thorough literature review and the examination of ca. 500 specimens deposited in the main entomological collections of the country. Data from the literature and museum collections were used to generate distribution maps for 37 species. Three species are recorded from Venezuela for the first time: *Chrysomya putoria* (Wiedemann, 1830), *Mesembrinella spicata* Aldrich, 1925 and *Mesembrinella umbrosa* Aldrich, 1922.

Keywords

Calliphorids, diversity, Neotropical Region, South America

Introduction

Blow flies (also known as bluebottles, greenbottles, cluster flies and generically referred to as carrion flies) is the vernacular name traditionally used for the para/polyphyletic family Calliphoridae *sensu lato*. Historically, the taxonomic composition and phylogenetic relationships within this group of flies, belonging to the superfamily Oestroidea,

have been controversial. During the last two decades, a division of Calliphoridae into 14 subfamilies has been widely accepted (Rognes 1997, Norris 1999, Kutty et al. 2010), even though some of these subfamilies are considered by many authors as independent families. This is the case of Mesembrinellidae (Kutty et al. 2010, Singh and Wells 2013, Marinho et al. 2016) and Rhiniidae (Kutty et al. 2010, Pape et al. 2011, Marinho et al. 2016), two taxa now widely ranked at the family level. However, not all studies support the same family/subfamily ranks, and Polleniinae have been recently proposed as a family based on their phylogenetic position as sister group of Tachinidae (Singh and Wells 2013). Another group, Bengaliinae, has also been suggested as an independent family (Lehrer 2003), but further studies are required to support this controversial proposal and it currently remains widely accepted as a subfamily closely related to Auchmeromyiinae (Rognes 2005, Marinho et al. 2012). In this paper, we use the common name of blow flies to designate the traditional, non-monophyletic concept of Calliphoridae s. l., whereas the term Calliphoridae is used to refer to a less inclusive taxon not containing Mesembrinellidae and Rhiniidae, which are nowadays generally accepted as separate families.

A single species of Rhiniidae, *Stomorphina lunata* (Fabricius, 1805), is present in the New World where it is found only on the island of Bermuda (Rognes 1991). Mesembrinellidae are a relatively small family of Neotropical blow flies occurring from southern Mexico to northern Argentina (Peris and Mariluis 1984). Three subfamilies of Mesembrinellidae have been proposed (Guimarães 1977) and are widely accepted: Souzalopesiellinae and Laneellinae with a brown, non-metallic abdomen, and Mesembrinellinae with a metallic abdomen (Vargas and Wood 2012, Marinho et al. 2016). On the other hand, six subfamilies of Calliphoridae occur in the Neotropics: Calliphorinae, Chrysomyinae, Luciliinae, Melanomyinae, Polleniinae, and Toxotarsinae (Rognes 1991, 1997, Whitworth 2010).

Blow flies include more than 150 genera and approximately 1500 species worldwide (Rognes 1991, Pape et al. 2011). The adults of some species can impact human health, acting as vectors of pathogens by searching for and settling on feces, fresh and cooked meat, dairy products and wounds (Rognes 1991). The larvae of other species, e.g. *Cochliomyia hominivorax* (Coquerel, 1858), produce myiasis, invading and feeding on the tissues of live vertebrates, including humans (Zumpt 1965, Guimarães and Papavero 1999, Stevens et al. 2006). There are also blood-sucking species ectoparasitic on birds or mammals, e.g., *Protophormia* Hough, 1899 on nestling birds and *Auchmeromyia* Brauer & Bergenstamm, 1891 on humans (Rognes 1991). Blow flies are significant in forensic medicine because they are among the first insects to colonize animal remains (Smith 1986). Some species have been suggested as an effective tool for assessment of vertebrate biodiversity, representing an indirect source of DNA from the vertebrate carcasses on which they have fed (Calvignac-Spencer et al. 2013). They are also considered potential environmental indicators in tropical areas since many species, e.g., *Mesembrinella bellardiana* Aldrich, 1922, are non-synanthropic and therefore strongly related to natural habitats (Gadelha et al. 2009).

Many authors have contributed to reviewing the taxonomy of Neotropical Calliphoridae *sensu lato* (i.e., Shannon 1926, Aubertin 1933, Hall 1948, Mello 1961, 1962, 1967, James 1970, Guimarães 1977, Dear 1979, 1985, Mariluis and Peris 1984, Peris and Mariluis 1984, Peris 1990, 1992, Mariluis et al. 1994a, 1994b, Mello 1996, Peris et al. 1998, Mello 2003, Peris and González-Mora 2005). In more recent taxonomic studies from the region, Vargas and Wood (2012) provided a comprehensive review and key to Central American genera; Whitworth (2010) studied the species present in the West Indies, providing keys and reviewing some species, as well as describing a new one; the same author carried out a complete revision of the six species of *Calliphora* Robineau-Desvoidy, 1830 from the Neotropical Region (Whitworth 2012) and a revision of 23 species of the genus *Lucilia* Robineau-Desvoidy, 1830 found in the Neotropics, where he provided an identification key and described six new species (Whitworth 2014). The recent revisions of some genera of Mesembrinellidae, including descriptions of new species (Wolff et al. 2012, Wolff 2013, Wolff et al. 2013, 2014) and the first phylogenetic study of this family (Marinho et al. 2016), have been significant. There are also a list of valid blow fly names from the Americas south of Mexico provided by Kosmann et al. (2013) and a catalogue of Calliphoridae and Mesembrinellidae of Colombia (Wolff and Kosmann 2016).

Furthermore, lists of species, identification keys and ecological studies can be found for Nicaragua (Maes et al. 1994), Panama (Bermudez 2007), Colombia (Pape et al. 2004, Amat et al. 2008, Amat 2009), Brazil (Carvalho and Ribeiro 2000), Peru (Baumgartner and Greenberg 1983, 1984, 1985) and Argentina (Mariluis 1981, 1983, 2002, Mariluis and Mulieri 2003). Background information regarding blow flies in Venezuela is more limited. A first list of Venezuelan blow flies was published by Cova (1964). Other studies have focused on a few species that can cause myiasis (Moissant et al. 2004a, 2004b, Coronado and Kowalski 2009, Pulgar et al. 2009) and on forensically important species (Liria 2006, Magaña et al. 2006, Velásquez 2008, Vásquez and Liria 2012, Capote et al. 2014, Nuñez and Liria 2014).

In this paper, for the first time, a checklist is presented of valid species names of Calliphoridae and Mesembrinellidae so far known to occur in Venezuela, as well as distribution maps of each species in the country.

Materials and methods

The checklist is based on the examination of adult blow flies deposited in Venezuela's main entomological collections, combined with our own data and a detailed bibliographic review. We examined specimens housed in the following museums and institutions:

BMNH The Natural History Museum, London, United Kingdom.

CEUA Colección Entomológica de la Universidad de Alicante, Alicante, Spain.

- IVIC** Colecciones Biológicas del Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.
- MIZA** Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez, Universidad Central de Venezuela, Maracay, Venezuela.
- MJMO** Museo Entomológico “Dr. José Manuel Osorio”, Universidad Centroccidental Lisandro Alvarado, Barquisimeto, Venezuela.

Some of the specimens deposited in CEUA and IVIC were collected by the authors using Wind Oriented Traps (WOT) baited with fish and pig liver (see Vogt et al. 1985). The classification used in the checklist follows Rognes (1986, 1991, 1997) and Marinho et al. (2016). The material examined was identified on the basis of specific keys for each subfamily of Calliphoridae, i.e. Mariluis and Peris (1984) and Whitworth (2012) for Calliphorinae; Mariluis and Peris (1984), Mariluis et al. (1994b), Rognes (1994) and Whitworth (2010, 2014) for Luciliinae; Dear (1985), González-Mora et al. (1998), Mariluis et al. (1994a), Rognes and Paterson (2005), Whitworth (2010) and Grella et al. (2015) for Chrysomyinae, and Dear (1979) for Toxotarsinae. In the case of Mesembrinellidae the keys of Guimarães (1977), Bonatto and Marinoni (2005) and Wolff et al. (2014) were used. The dissection and study of male terminalia were carried out following Whitworth (2006, 2010). The identity of all specimens was confirmed by Dr Terry Whitworth from Washington State University (USA).

Localities of occurrence of both the examined material and records taken from the literature were georeferenced using Google Earth (v7.1.5.1557). Distribution maps were created with ArcView GIS 10.2 (Environmental Systems Research Institute, Inc., USA). Each point plotted on the maps represents a locality of occurrence. Distributions of species do not follow any alphabetic or taxonomic criterion but are instead represented in such a way as to avoid, as far as possible, the overlapping of dots.

Results

Table 1 lists a total of 39 species of blow flies for Venezuela, of which 25 are Calliphoridae and 14 Mesembrinellidae. We examined a total of 498 specimens, the subfamily Chrysomyinae being the most abundant (302), followed by the Luciliinae (166). From the material examined we identified 26 species, including one Calliphoridae and two Mesembrinellidae newly recorded for the country: *Chrysomya putoria* (Wiedemann, 1830), *Mesembrinella spicata* Aldrich, 1925 and *Mesembrinella umbrosa* Aldrich, 1922.

Doubtful records found in the literature were excluded from the list when there was no indication of how the species were identified or when the accuracy of the identifications was uncertain. Distribution maps showing the records obtained from the material examined and the literature are provided for 37 species (Figs 1–14). *Eumesembrinella randa* (Walker, 1849) and *Lucilia sericata* (Meigen, 1826) were cited for Venezuela by Peris and Mariluis (1984), Mariluis et al. (1994b), Kosmann et al. (2013) and Wolff and Kosmann (2016), but no locality information was provided.

Table 1. Checklist of the blow flies of Venezuela, including reviewed references and the depositories of examined specimens.

Species	References	Material examined
FAMILY CALLIPHORIDAE		
Subfamily CALLIPHORINAE		
<i>Calliphora nigribasis</i> Macquart, 1851	Cova (1964), Whitworth (2012), Kosmann et al. (2013), Wolff and Kosmann (2016)	MIZA
Subfamily CHRYSOMYINAE		
<i>Chloroprocta idioidea</i> (Robineau-Desvoidy, 1830)	Hall (1948), Cova (1964), Dear (1985), Kosmann et al. (2013), Wolff and Kosmann (2016)	BMNH
<i>Chrysomya albiceps</i> (Wiedemann, 1819)	Baumgartner (1988), Kosmann et al. (2013), Wolff and Kosmann (2016)	CEUA, IVIC, MJMO, MIZA
<i>Chrysomya megacephala</i> (Fabricius, 1794)	Baumgartner (1988)	CEUA, IVIC, MJMO
<i>Chrysomya putoria</i> (Wiedemann, 1830)	New record	MJMO
<i>Cochliomyia hominivorax</i> (Coquerel, 1858)	Moissant et al. (2004a, 2004b), Coronado and Kowalski (2009), Pulgar (2009)	MJMO
<i>Cochliomyia macellaria</i> (Fabricius, 1775)	Cova (1964), Dear (1985), Kosmann et al. (2013), Wolff and Kosmann (2016)	CEUA, IVIC, MIZA, MJMO
<i>Comptosyiops fulvicrura</i> (Robineau-Desvoidy, 1830)	Hall (1948), Cova (1964)	-
<i>Comptosyiops verena</i> (Walker, 1849)	Dear (1985), Kosmann et al. (2013), Wolff and Kosmann (2016)	MIZA
<i>Hemilucilia benoisti</i> Séguy, 1925a	Shannon (1926), Dear (1985), Peris and Mariluis (1989), Kosmann et al. (2013), Wolff and Kosmann (2016)	-
<i>Hemilucilia segmentaria</i> (Fabricius, 1805)	Shannon (1926), Hall (1948), Cova (1964), Peris and Mariluis (1989)	CEUA, IVIC, MIZA, MJMO
<i>Hemilucilia semidiaphana</i> (Rondani, 1850)	Dear (1985), Cova (1964), Peris and Mariluis (1989), Kosmann et al. (2013), Wolff and Kosmann (2016)	CEUA, IVIC, MIZA
<i>Paralucilia fulvinota</i> (Bigot, 1877)	Aldrich (1925), Shannon (1926), Dear (1985), Mariluis et al. (1994a), Kosmann et al. (2013), Wolff and Kosmann (2016)	MIZA

Species	References	Material examined
<i>Paralucilia paraensis</i> (Mello, 1969)	Dear (1985), Mariluis et al. (1994a), Kosmann et al. (2013), Wolff and Kosmann (2016)	-
Subfamily LUCILIINAE		
<i>Blepharicnema splendens</i> Macquart, 1843	Cova (1964), Mariluis and Peris (1984), Amat and Wolff (2007), Kosmann et al. (2013), Wolff and Kosmann (2016)	MIZA, MJMO
<i>Lucilia albofusca</i> Whitworth, 2014	Whitworth (2014)	-
<i>Lucilia cluvia</i> (Walker, 1849)	Mariluis et al. (1994b)	-
<i>Lucilia cuprina</i> (Wiedemann, 1830)	Cova (1964), Kosmann et al. (2013), Wolff and Kosmann (2016)	CEUA, IVIC, MIZA, MJMO
<i>Lucilia eximia</i> (Wiedemann, 1819)	Cova (1964), Mariluis et al. (1994b), Kosmann et al. (2013), Whitworth, (2014), Wolff and Kosmann (2016)	IVIC, MIZA, MJMO
<i>Lucilia nitida</i> Whitworth, 2014	Whitworth (2014)	CEUA
<i>Lucilia purpurascens</i> (Walker, 1836)	Cova (1964), Mariluis et al. (1994b), Kosmann et al. (2013), Whitworth (2014), Wolff and Kosmann (2016)	CEUA, MIZA, MJMO, IVIC
<i>Lucilia rognesi</i> Whitworth, 2014	Whitworth (2014)	CEUA
<i>Lucilia sericata</i> (Meigen, 1826)	Mariluis et al. (1994b), Kosmann et al. (2013), Wolff and Kosmann (2016)	-
<i>Lucilia vulgata</i> Whitworth, 2014	Whitworth (2014)	-
Subfamily TOXOTARSINAE		
<i>Sarconesia roraima</i> (Townsend, 1935)	Dear (1979), Mariluis and Peris (1984), Wolff and Kosmann (2016)	MIZA
FAMILY MESEMBRINELLIDAE		
<i>Eumesebrinella benoisti</i> (Séguy, 1925b)	Guimarães (1977), Kosmann et al. (2013), Wolff and Kosmann (2016)	MIZA
<i>Eumesebrinella quadrilineata</i> (Fabricius, 1805)	Aldrich (1922), Guimarães (1977), Peris and Mariluis (1984), Kosmann et al. (2013), Wolff and Kosmann (2016)	-
<i>Eumesebrinella randa</i> (Walker, 1849)	Peris and Mariluis (1984), Kosmann et al. (2013), Wolff and Kosmann (2016)	-
<i>Giovanella bolivar</i> Bonatto, 2005	Bonatto and Marinoni (2005), Kosmann et al. (2013)	-
<i>Huascaromusca decrepita</i> (Séguy, 1925b)	Kosmann et al. (2013), Wolff and Kosmann (2016)	CEUA, MIZA, MJMO
<i>Huascaromusca lara</i> Bonatto, 2005	Bonatto and Marinoni (2005), Kosmann et al. (2013)	IVIC, MIZA

Species	References	Material examined
<i>Huascaromusca vogelsangi</i> Mello, 1967	Guimarães (1977), Kosmann et al. (2013), Wolff and Kosmann (2016)	-
<i>Mesembrinella bellardiana</i> Aldrich, 1922	Peris and Mariluis (1984), Kosmann et al. (2013), Wolff and Kosmann (2016)	-
<i>Mesembrinella bicolor</i> (Fabricius, 1805)	Aldrich (1922), Guimarães (1977), Peris and Mariluis (1984)	MIZA, MJMO
<i>Mesembrinella spicata</i> Aldrich, 1925	New record	MJMO
<i>Mesembrinella umbrosa</i> Aldrich, 1922	New record	MJMO
<i>Mesembrinella xanthorrhina</i> (Bigot, 1887)	Hall (1948), Cova (1964)	-
<i>Souzalopesiella facialis</i> (Aldrich, 1922)	Guimarães (1977), Kosmann et al. (2013), Wolff and Kosmann (2016)	CEUA, MJMO
<i>Thompsoniella anomala</i> Guimarães, 1977	Guimarães (1977), Kosmann et al. (2013)	CEUA

Material examined

Family Calliphoridae

Subfamily Calliphorinae

Calliphora nigribasis Macquart, 1851 (Fig. 1)

Material examined (1 male): **Táchira State:** Betania, 2325m, 7.VIII.1972, J.B. Terán J. Salcedo leg. (MIZA).

Subfamily Chrysomyinae

Chloroprocta idioidea (Robineau-Desvoidy, 1830) (Fig. 3)

Material examined (1 male, 1 female): **Aragua State:** Maracay, 29.VIII.1943, [no collector] (BMNH).

Chrysomya albiceps (Wiedemann, 1819) (Fig. 9)

Material examined (38 males, 78 females): **Aragua State:** 2 males, Parque Nacional Henri Pittier, Portachuelo, 1152m, 26.I.2007, A. Martínez-Sánchez leg. (CEUA); 21 males, 57 females, Maracay, Universidad Central de Venezuela campus, 10°16'24.83"N, 67°35'37.05"W, approx. 400m, on dead chicken, various dates: 1 male, 10 females, 17.VII.2012; 1 male, 4 females, 18.VII.2012; 1 male, 1 female, 19.VII.2012; 1 male, 5 fe-

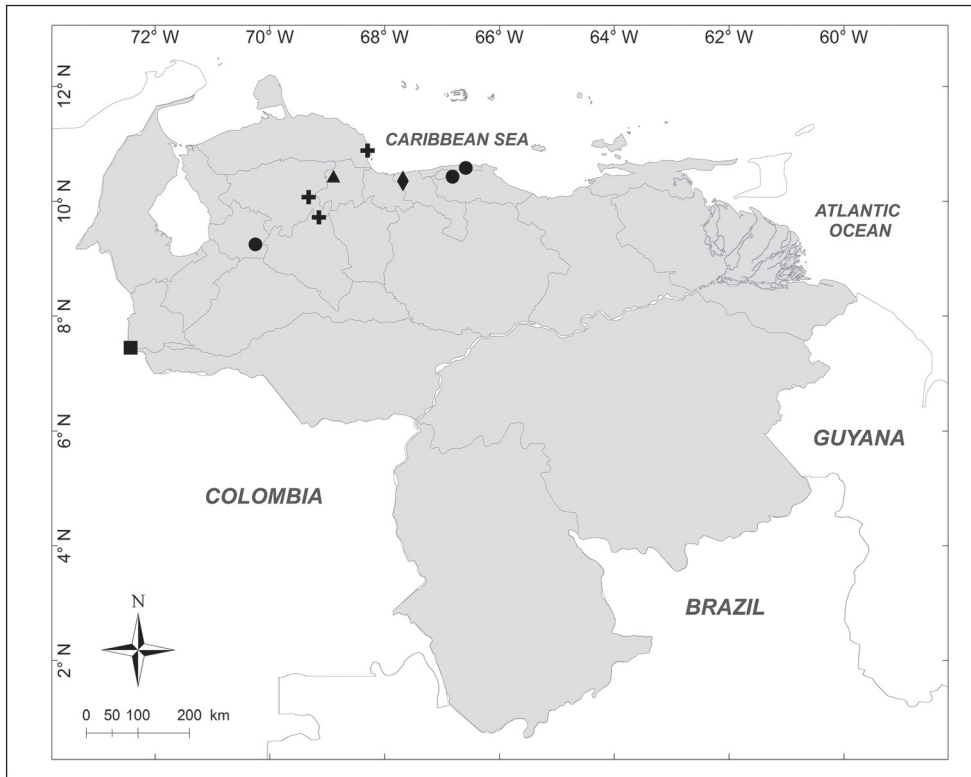


Figure 1. Known distributions of ■ *Calliphora nigribasis* Macquart, + *Cochliomyia hominivorax* (Cokerell), ● *Comptosyriops verena* (Walker), ▲ *Paralucilia fulvinota* (Bigot) and ◆ *Lucilia rognesi* Whitworth in Venezuela.

males, 20.VII.2012; 5 males, 36 females, 23.VII.2012; 12 males, 1 female, 27.VII.2012; all A. Thomas leg. (IVIC); 8 females, Maracay, Universidad Central de Venezuela campus, 24.I.2007, A. Martínez-Sánchez leg. (CEUA). **Lara State:** 5 males, 3 females, El Cercado, 500m, 25.VII.2002, from larva in dead common opossum, E. Arcaya leg. (MJMO); 9 males, Tarabana, 500m, XII.1989, A. Chávez leg. (MJMO). **Miranda State:** 1 male, 4 females, Altos de Pipe, Instituto Venezolano de Investigaciones Científicas, 10°24'5"N, 66°58'37"W, 1600m, 29.VII–2.VIII.2010, on dead rat, A. Thomas leg. (IVIC); 5 females, Macaracuay, Residencia Los Cien, 10°27'43.47"N, 66°48'34.71"W, 900m, on mango, 4.IX.2012, A. Thomas leg. (IVIC). **Trujillo State:** 1 female, La Cira, nr Betijoque, 500m, 4–9.XII.1996, J. Clavijo, J. de Marmels, J.L. García, A. Chacón leg. (MIZA).

Chrysomya megacephala (Fabricius, 1794) (Fig. 10)

Material examined (60 males, 162 females): **Aragua State:** 1 female, Parque Nacional Henri Pittier, Portachuelo, 1152m, 26.I.2007, A. Martínez-Sánchez leg. (CEUA); 3

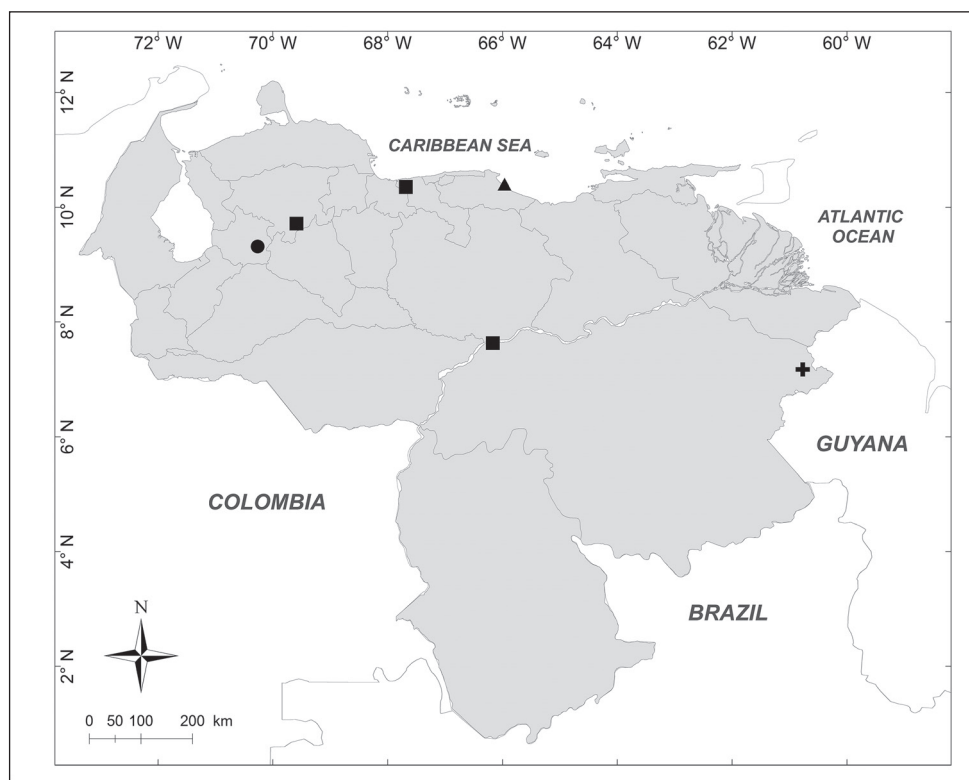


Figure 2. Known distributions of ■ *Mesembrinella bicolor* (Fabricius), ● *Mesembrinella umbrosa* Aldrich, + *Eumesembrinella benoisti* (Séguy) and ▲ *Thompsoniella anomala* Guimarães in Venezuela.

females, Maracay, Universidad Central de Venezuela campus, 7.IX.2006, from larva in chicken, students leg. (IVIC); 48 males, 138 females, Maracay, Universidad Central de Venezuela campus, 10°16'24.83"N, 67°35'37.05"W, approx. 400m, on dead chicken, various dates: 15 males, 81 females, 17.VII.2012; 4 males, 11 females, 18.VII.2012; 4 males, 13 females, 19.VII.2012; 4 males, 13 females, 23.VII.2012; 21 males, 20 females, 27.VII.2012; all A. Thomas leg. (IVIC); 1 male, 10 females, Maracay, Universidad Central de Venezuela campus, 24.I.2007, A. Martínez-Sánchez leg. (CEUA). **Lara State:** 1 male, 1 female, Barquisimeto, Museo Entomológico "Dr. José Manuel Osorio", 564m, VI.1989, on trunk of Acacia plagued by scale insect, [no collector] (MJMO); 2 males, 2 females, Tarabana, 500m, VI.1989, Acht leg. (MJMO); 1 female, 21.XII.1993–10.I.1994, Malaise trap [no collector] (MJMO). **Miranda State:** 1 male, Caucagua, 74m, 18–20.VII.2000, E. Carrasquero leg. (MJMO); 1 male, 1 female, Altos de Pipe, Instituto Venezolano de Investigaciones Científicas, 10°24'5"N, 66°58'37"W, 1600m, 29.VII–2.VIII.2010, on dead rat, A. Thomas leg. (IVIC); 6 males, 5 females, Macaracuay, Residencia Los Cien, 10°27'43.47"N, 66°48'34.71"W, 900m, 4.IX.2012, on mango, A. Thomas leg. (IVIC).

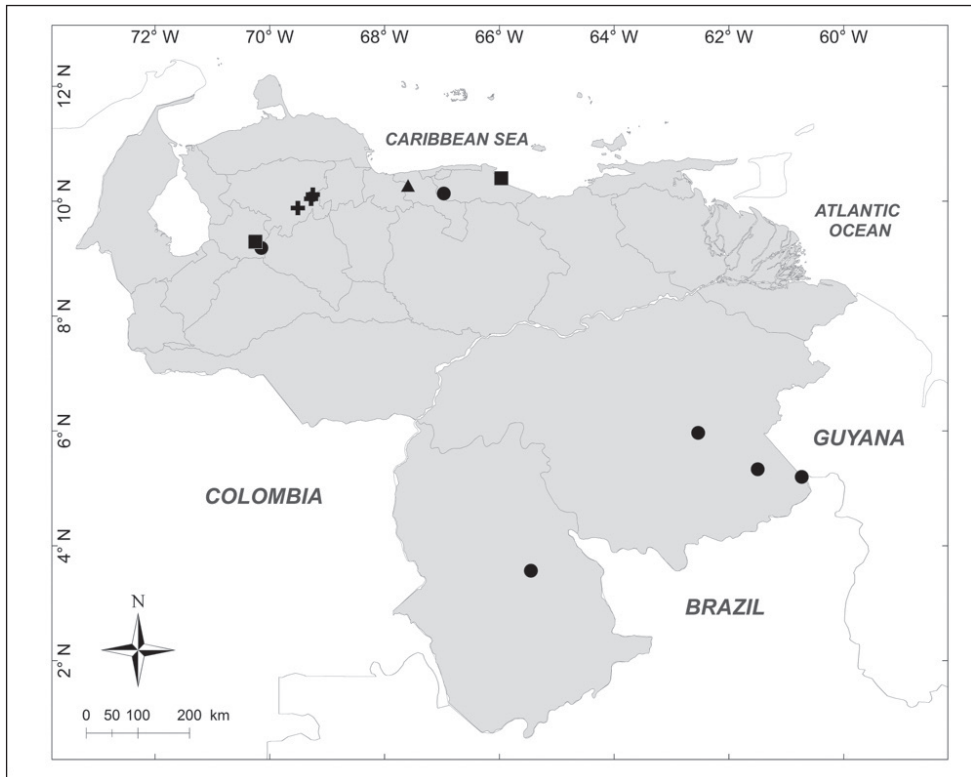


Figure 3. Known distributions of + *Chrysomya putoria* (Wiedemann), ■ *Souzalopesiella facialis* (Aldrich), ● *Sarconesia roraima* (Townsend) and ▲ *Chloroprocta idioidea* (Robineau-Desvoidy) in Venezuela.

***Chrysomya putoria* (Wiedemann, 1830) (Fig. 3)**

Material examined (2 males, 3 females): **Lara State:** 1 female, El Cercado, 500m 17.XII.1996, E. Arcaya leg. (MJMO); 1 male, San Miguel, 680m, 17.VI.1993, H. Chávez, R. Hernández leg. (MJMO); 1 male, 2 females, Tarabana, XII.1989, A. Chavez leg. (MJMO).

***Cochliomyia hominivorax* (Coquerel, 1858) (Fig. 1)**

Material examined (2 males, 2 females): **Falcón State:** 1 female, Parque Nacional Morrocoy, 20.III–IV.1999, H. Chávez leg. (MJMO). **Lara State:** 1 male, Barquisimeto, 564m, VI.1980, myiasis in *Canis familiaris*, C. Zambrano leg. (MJMO); 1 male, 1 female, Sanare, El Torrellero, 268m, 20.IV.1982, Malaise trap, [no collector] (MJMO).



Figure 4. Known distributions of ▲ *Huascaromusca decrepita* (Séguy), ● *Huascaromusca lara* Bonatto, + *Lucilia nitida* Whitworth and ■ *Mesembrinella bellardiana* Aldrich in Venezuela.

***Cochliomyia macellaria* (Fabricius, 1775) (Fig. 8)**

Material examined (3 males, 6 females): **Aragua State:** 1 male, El Limón, 450m, 22.II.1973, J.C. Marín leg. (MIZA); 1 female, Parque Nacional Henri Pittier, Portachuelo, 1152m, 26.I.2007, A. Martínez-Sánchez leg. (CEUA); 1 female, Maracay, Universidad Central de Venezuela campus, 7.IX.2006, students leg. (IVIC); 1 male, Villa del Cura, Estación Experimental Cataurito, 1000m, 9.IV.1981, J.L. García leg. (MIZA). **Carabobo State:** 1 female, Mariara, 12.VII.1979, F. Alarcón leg. (MIZA). **Falcón State:** 1 female, Cabure, 7.VI.1980, light trap, R. Casales, E. Zambrano leg. (MIZA). **Guárico State:** 1 female, Distrito Rivas, Carretera El Palmar km 133, La Smith, 4.VIII.1980, J. Valdivieso leg. (MIZA). **Lara State:** 1 female, Sanare, El Torrellero, 268m, 20.IV.1980, Malaise trap, [no collector] (MJMO). **Miranda State:** 1 male, Distrito Federal, El Valle, 10.XI.1949, on trunk of Bucare, F. Fernández Yépez leg. (MIZA).

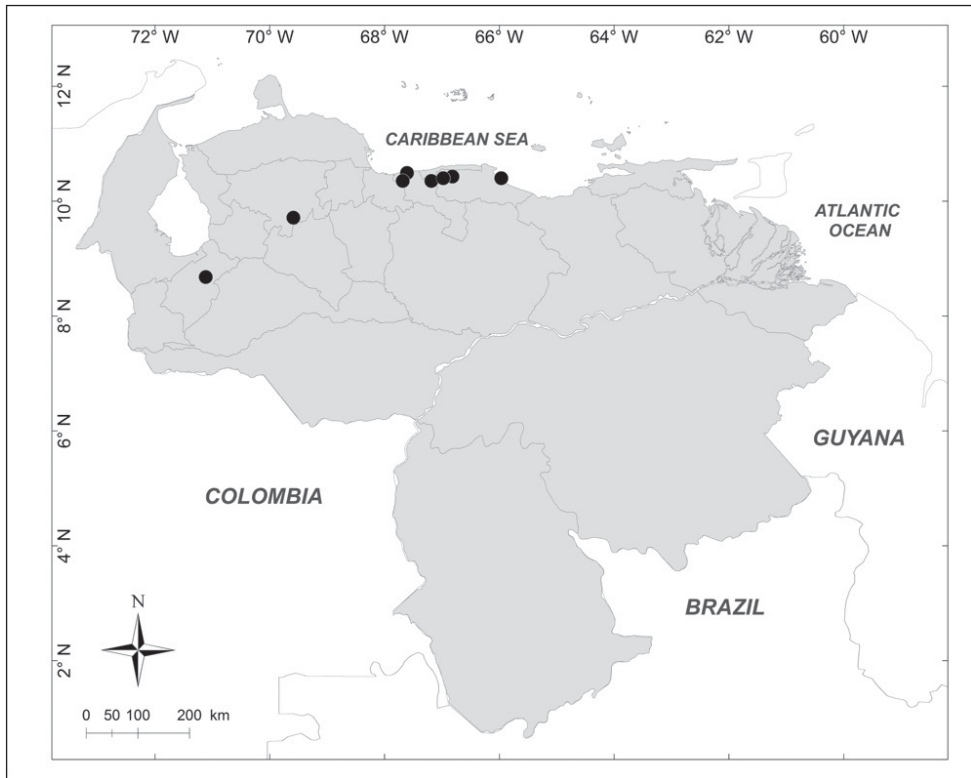


Figure 5. Known distributions of ● *Lucilia purpurascens* (Walker) in Venezuela.

***Compsomyiops verena* (Walker, 1849) (Fig. 1)**

Material examined (1 male, 2 females): **Miranda State:** 1 female, Distrito Federal, Serranía El Avila, Los Castillitos, 1300m, 24.III.1950, F. Fernández Yépez leg. (MIZA); 1 female, El Hatillo, Las Marías, 1350m, 5.II.1976, F. Kaletta leg. (MIZA). **Trujillo State:** 1 male, Carretera Boconó, La Negrita, 1850m, 29.X.1976, J. Salcedo & J. Clavijo leg. (MIZA).

***Hemilucilia segmentaria* (Fabricius, 1805) (Fig. 12)**

Material examined (2 males, 3 females): **Aragua State:** 1 female, Parque Nacional Henri Pittier, Rancho Grande, 1183m, 25.I.2007, A. Martínez-Sánchez leg. (CEUA). **Miranda State:** 1 male, Altos de Pipe, Instituto Venezolano de Investigaciones Científicas, 10°24'5"N, 66°58'37"W, 1600m, 7.II.2012, A. Thomas leg. (IVIC). **Trujillo State:** 1 male, 1 female, La Gira, nr Betijoque, 500m, 4–9.XII.1996, J. Clavijo, J. de Marmels, J.L. García, A. Chacón leg. (MIZA). **Yaracuy State:** 1 female, Cocorote, Sector El Candelo, 1650m, 17–20.X.2001, interception trap, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, E. Arcaya, L. Joly leg. (MJMO).

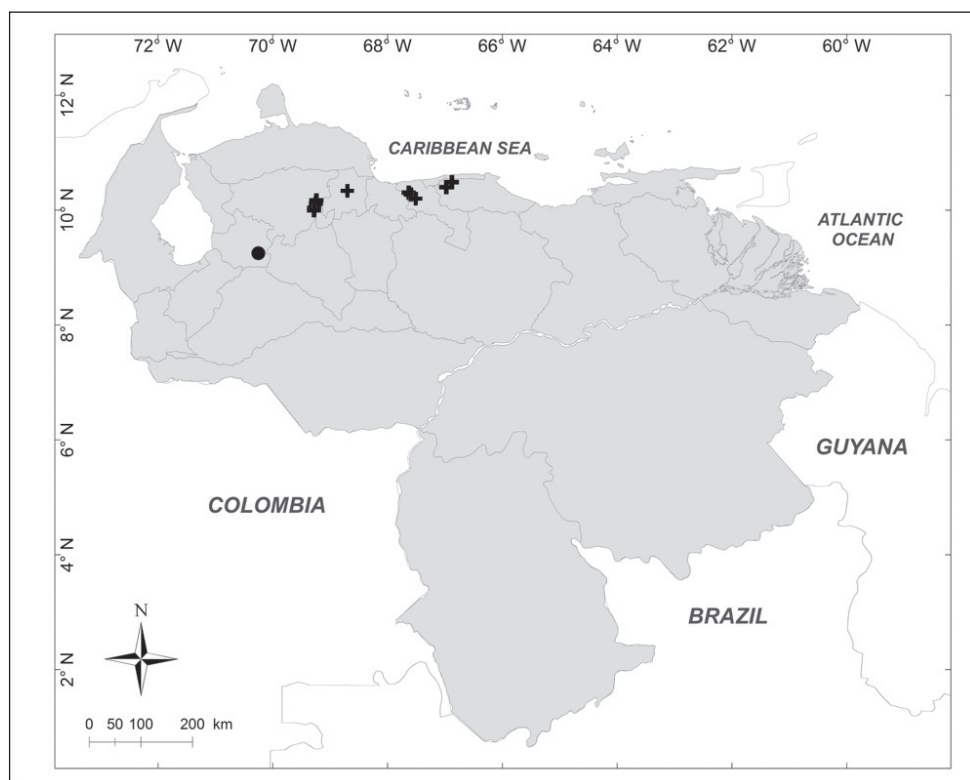


Figure 6. Known distributions of + *Lucilia eximia* (Wiedemann) and ● *Mesembrinella spicata* Aldrich in Venezuela.

***Hemilucilia semidiaphana* (Rondani, 1850) (Fig. 11)**

Material examined (5 males, 48 females): **Aragua State:** 4 males, 7 females, Parque Nacional Henri Pittier, Portachuelo, 1152m, 26.I.2007, A. Martínez-Sánchez leg. (CEUA); 13 females, Parque Nacional Henri Pittier, Rancho Grande, 1183m, 24–25.I.2007, WOT, A. Martínez-Sánchez leg. (CEUA); 19 females, 1183m, 25.I.2007, WOT, A. Martínez-Sánchez leg. (CEUA); 1 female, 1100m, 17.V.1973, J. Salcedo, J. Clavijo leg. (MIZA). **Miranda State:** 1 male, Guatopo (Agua Blanca), 8.X.1980, F. Fernández Yépez, A. Chacón leg. (MIZA); 1 female, San Antonio de los Altos, Instituto Venezolano de Investigaciones Científicas, 1680m, IV.2003, Y. Velásquez leg. (IVIC); 7 females, Altos de Pipe, Instituto Venezolano de Investigaciones Científicas, 10°24'5"N 66°58'37"W, 1600m, 7.II.2012, A. Thomas leg. (IVIC).

***Paralucilia fulvinota* (Bigot, 1877) (Fig. 1)**

Material examined (1 male): **Yaracuy State:** Aroa, 12.VIII.1975, E. Dietz leg. (MIZA).

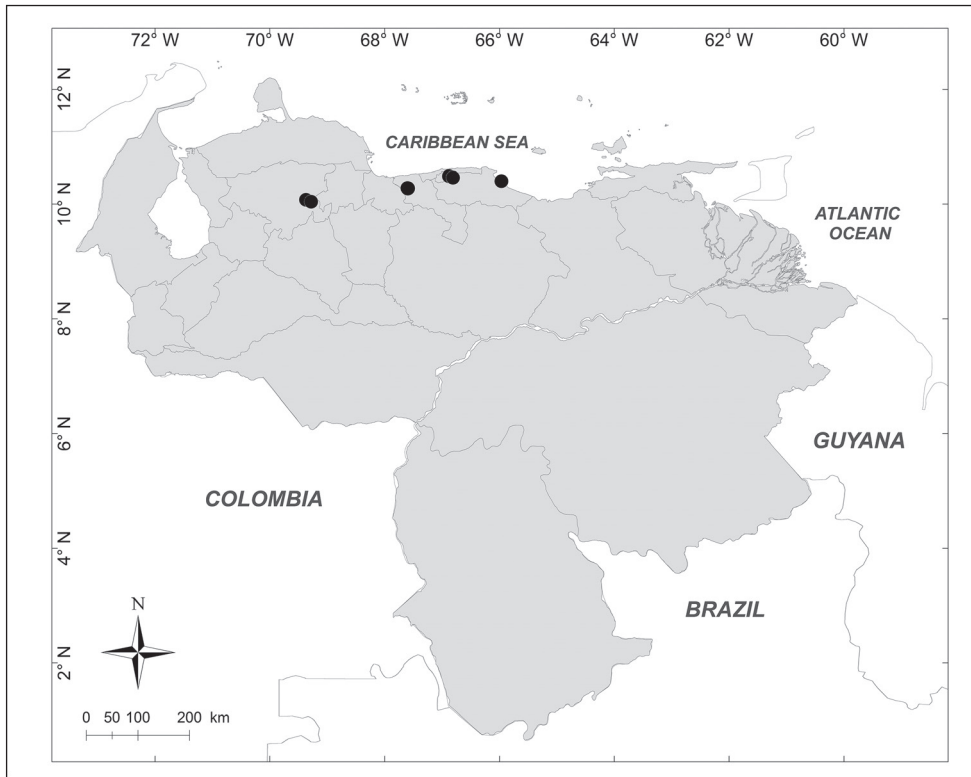


Figure 7. Known distributions of ● *Lucilia cuprina* (Wiedemann), ■ *Paralucilia paraensis* (Mello) and ▲ *Lucilia cluvia* (Walker) in Venezuela.

Subfamily Luciliinae

Blepharicnema splendens Macquart, 1843 (Fig. 11)

Material examined (4 males, 3 females): **Aragua State:** 1 female, Choroní, 1600m, 4.XI.1971, C.J. Rosales leg. (MIZA). **Lara State:** 2 males, Parque Nacional Yacambú, 15.X.1982, F. Gutiérrez, F. Martínez leg. (MIZA); 2 males, Piedra del Tigre, 1300m, 19.XI.2002, F. Díaz, F. Sosa, N. Valera leg. (MJMO). **Táchira State:** 1 female, Betania, on the route to the Páramo El Tamá, 2425m, 16–20.III.1983, “Excursión Instituto de Zoología Agrícola” leg. (MIZA). **Trujillo State:** 1 female, Parque Nacional Guaramacal, 1480m, 11–16.VI.2002, yellow pan trap, R. Briceño, J. Clavijo, R. Paz, F. Díaz, L. Joly, A. Chacón leg. (MJMO).

Lucilia cuprina (Wiedemann, 1830) (Fig. 7)

Material examined (5 males, 11 females): **Aragua State:** 1 male, 4 females, Maracay, Universidad Central de Venezuela campus, 10°16'24.83"N, 67°35'37.05"W, approx.

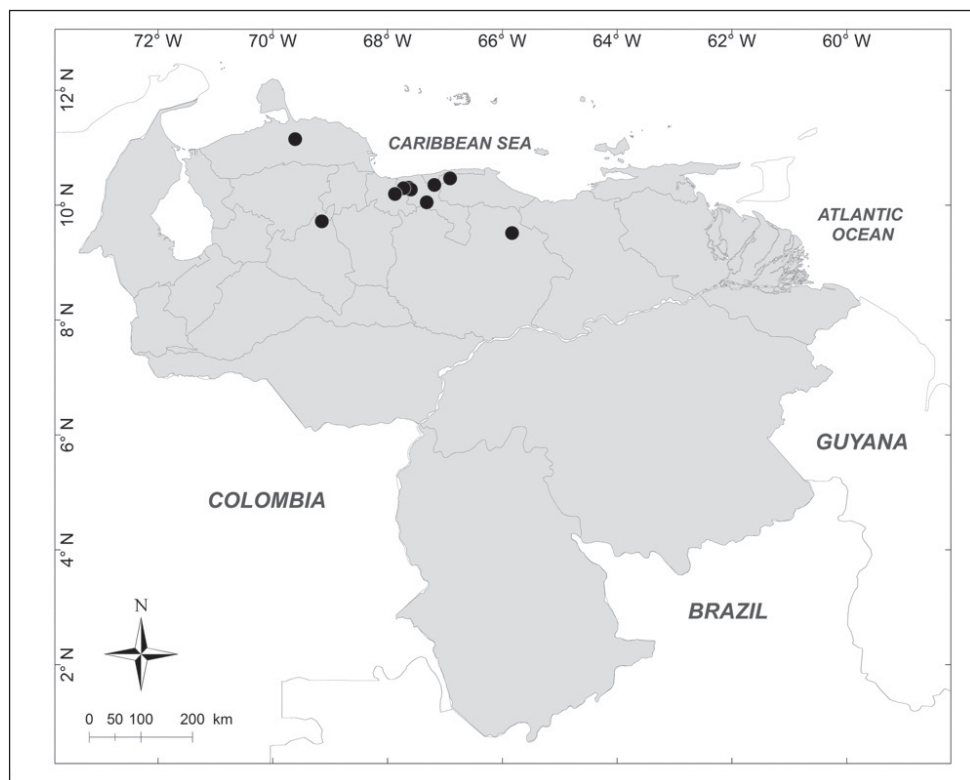


Figure 8. Known distribution of ● *Cochliomyia macellaria* (Fabricius) in Venezuela.

400m, on dead chicken, various dates: 1 male, 2 females, 17.VII.2012; 2 females, 17–26.VII.2012; all A. Thomas leg. (IVIC). **Lara State:** 3 females, Los Crespúsculos, 500m, 16.VII.1999, J. Nieto leg. (MJMO); 1 female, Tarabana, 500m, V.1990, myiasis on *Canis familiaris*, C. Zambrano leg. (MJMO). **Miranda State:** 1 female, Distrito Federal, Caracas, 1.II.1974, from larva on dead fish, F. Kaletta leg. (MIZA); 1 female, San Antonio de los Altos, Instituto Venezolano de Investigaciones Científicas, 1680m, 22.I.2007, A. Martínez-Sánchez leg. (CEUA); 4 males, 1 female, Macaracuay, Residencia Los Cien, 10°27'43.47"N, 66°48'34.71"W, 900m, 29.VII.2012, on mango, A. Thomas leg. (IVIC).

***Lucilia eximia* (Wiedemann, 1819) (Fig. 6)**

Material examined (19 males, 15 females): **Aragua State:** 1 female, El Limón, 480m, 27.V.1973, Malaise trap, C.J. Rosales leg. (MIZA); 1 female, Maracay, Universidad Central de Venezuela campus, 6.IX.2006, students leg. (IVIC). **Lara State:** 2 females, Cordero, 600m, 27–30.VI.1992, interception trap, [no collector] (MJMO); 4 males, 1 female, El Cercado, 500m, Malaise trap, various dates: 1 male, 17–21.VI.1999; 1 male,

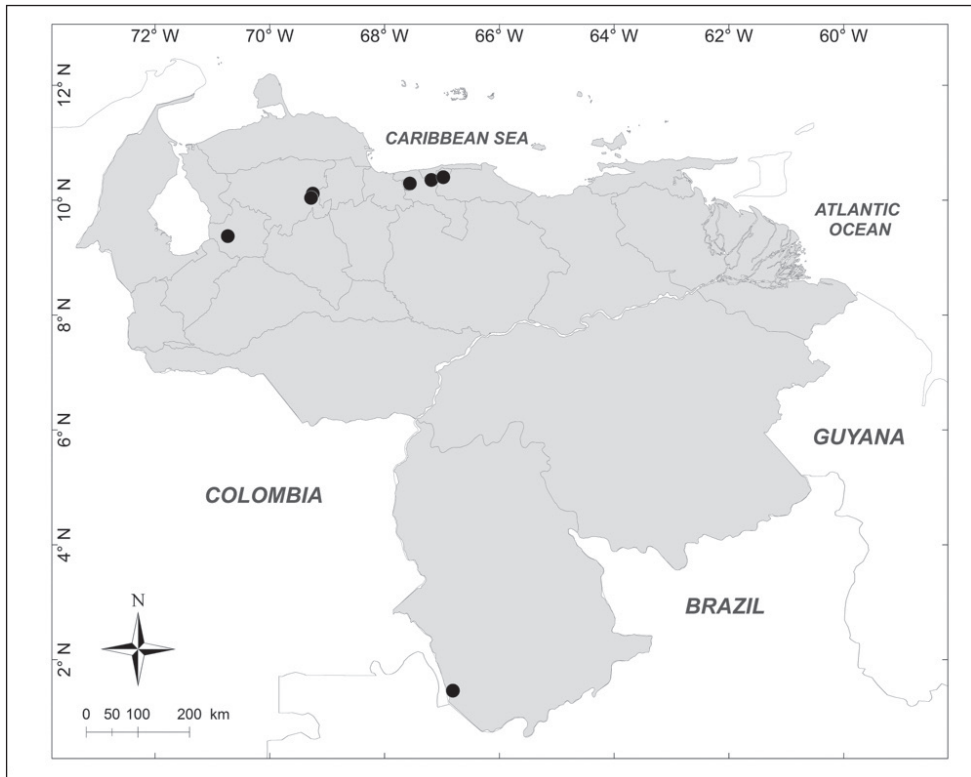


Figure 9. Known distribution of ● *Chrysomya albiceps* (Wiedemann) in Venezuela.

1 female, 24.VI–5.VII.1999; 2 males, 5–11.VII.1999; [all no collector] (MJMO); 5 males, El Cercado, 500m, 13.V.2002, on dead fish, E. Arcaya leg. (MJMO); 1 male, El Cercado, V.2002, [no collector] (MJMO); 2 males, 2 females, La Mora, 400m, 17.VI.2012, on *Stapelia gigantea*, T. Capote leg. (MJMO); 5 males, 3 females, Tarabana, 500m, various dates: 3 males, 1 female, 1.VI.2002; 1 male, 1 female, VII.2002; 1 male, 1 female, 14.II.2003; all E. Arcaya leg. (MJMO); 1 male, Tarabana, 17.VI.2002, on liver bait, E. Arcaya leg. (MJMO). **Miranda State:** 3 females, Altos de Pipe, Instituto Venezolano de Investigaciones Científicas, 10°24'5"N, 66°58'37"W, 1600m, 29.VII–2.VIII.2010, on dead rat, A. Thomas leg. (IVIC); 1 male, 1 female, Distrito Federal, Caracas, Jardín Botánico, 9.III.1966, A. Díaz leg. (MIZA). **Yaracuy State:** 1 male, 1 female, Cocorote, Sector El Candelo, 1600m, 4–10.XI.2002, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, E. Arcaya, L. Joly leg. (MJMO).

***Lucilia nitida* Whitworth, 2014 (Fig. 4)**

Material examined (2 females): **Aragua State:** Parque Nacional Henri Pittier, Rancho Grande, 1183m, 25.I.2007, WOT, A. Martínez-Sánchez leg. (CEUA).



Figure 10. Known distribution of ● *Chrysomya megacephala* (Fabricius) in Venezuela.

***Lucilia purpurascens* (Walker, 1837) (Fig. 5)**

Material examined (13 males, 58 females): **Aragua State:** 1 female, Choroní, 1400m, 10.X.1952, F. Kern leg. (MIZA); 4 females, Parque Nacional Henri Pittier, Portachuelo, 1152m, 26.I.2007, A. Martínez-Sánchez leg. (CEUA); 1 female, Parque Nacional Henri Pittier, Rancho Grande, 1100m, 30.IX.1974, J.L. García leg. (MIZA); 1 female, Parque Nacional Henri Pittier, Rancho Grande, 1183m, 25.I.2007, A. Martínez-Sánchez leg. (CEUA). **Lara State:** 1 female, Parque Nacional Yacambú, El Blanquito, 1480m, 14–21.IX.2001, Malaise trap, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, E. Arcaya leg. (MJMO); 1 female, Parque Nacional Yacambú, El Blanquito, 11–16.III.2002, yellow pan trap, R. Briceño, J. Clavijo, F. Díaz, R. Paz, E. Arcaya, A. Chacón leg. (MJMO). **Mérida State:** 1 female, Mérida, Hotel Valle Grande, 2000m, 2.IX.1980, C.J. Rosales leg. (MIZA). **Miranda State:** 1 male, El Hatillo, Las Marías, 1350m, 16.VI.1975, F. Kaletta leg. (MIZA); 12 males, 40 females, San Antonio de los Altos, Instituto Venezolano de Investigaciones Científicas, 1680m, 13.II.2007, reared from larva, mother collected on fish, A. Martínez-Sánchez leg. (CEUA); 2 females, Altos de Pipe, Instituto Venezolano de Investigaciones Científicas, 10°24'5"N, 66°58'37"W, 1600m, 29.VII–2.VIII.2010, on dead rat, A. Thomas leg. (IVIC); 2 females, Altos

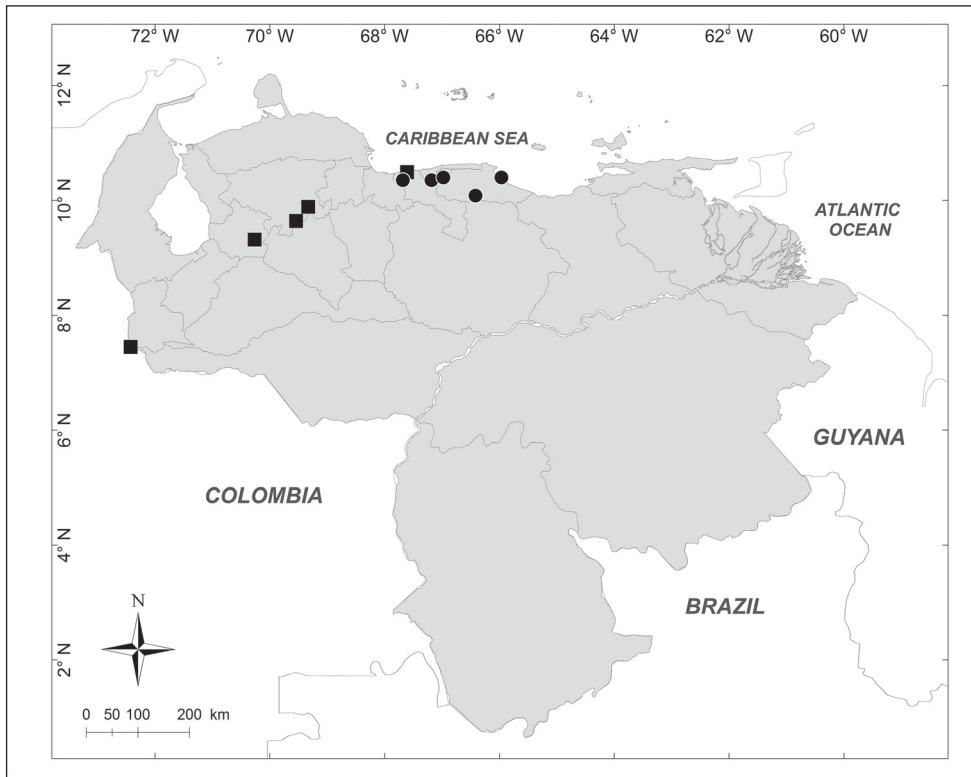


Figure 11. Known distributions of ■ *Blepharicnema splendens* Macquart and ● *Hemilucilia semidiaphana* (Rondani) in Venezuela.

de Pipe, Instituto Venezolano de Investigaciones Científicas, 10°24'5"N, 66°58'37"W, 1600m, 7.II.2012, A. Thomas leg. (IVIC). **Yaracuy State:** 4 females, Cocorote, Sector El Candelo, 1650m, 17–20.X.2001, interception trap, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, E. Arcaya, L. Joly leg. (MJMO).

***Lucilia rognesi* Whitworth, 2014 (Fig. 1)**

Material examined (2 females): **Aragua State:** Parque Nacional Henri Pittier, Rancho Grande, 1183m, 25.I.2007, WOT, A. Martínez-Sánchez leg. (CEUA).

Subfamily Toxotarsinae

***Sarconesia roraima* (Townsend, 1935) (Fig. 3)**

Material examined (5 males, 3 females): **Amazonas State:** 1 male, Parque Nacional Duida, Cerro Marahuaka, 2470m, 3–6.XI.1992, “Expedición Terramar”, J. Clavijo, A. Chacón leg. (MIZA). **Bolívar State:** 1 female, Auyentepui, 2150m, 26.II.1978, L. Joly leg. (MIZA); 1 male,

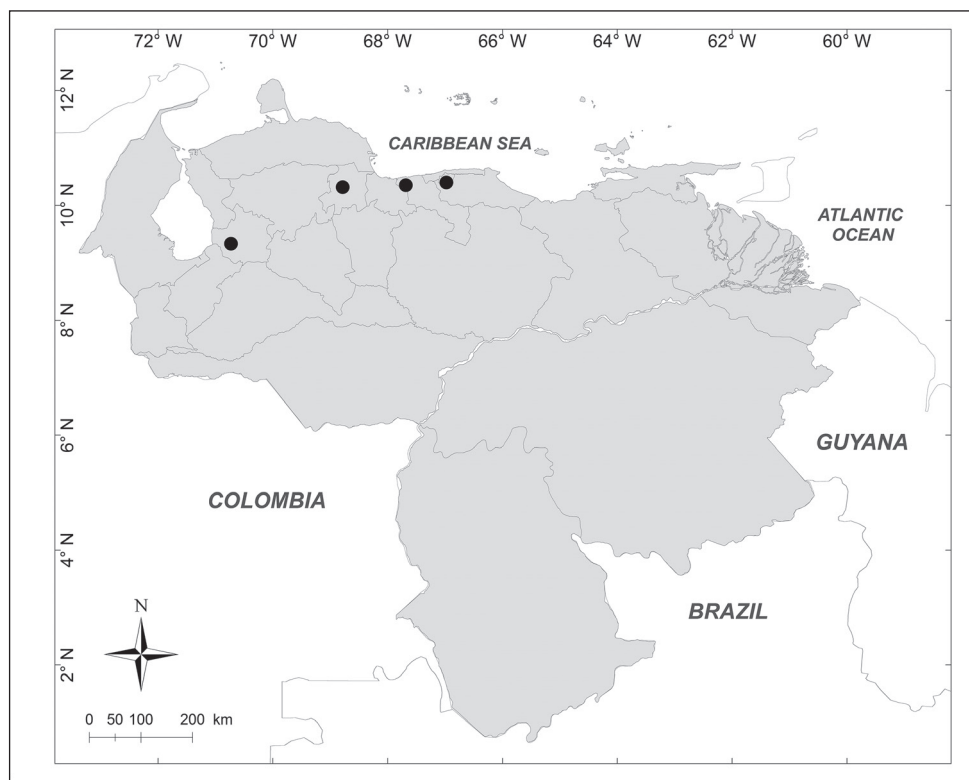


Figure 12. Known distribution of ● *Hemilucilia segmentaria* (Fabricius) in Venezuela.

Gran Sabana, Cerro Kukenan, 2700m, 12–17.IV.1988, A. Chacón, C. Andara leg. (MIZA); 1 female, Gran Sabana, Cerro Roraima, 2700m, 12–21.I.1991, “Expedición Terramar”, A. Chacón leg. (MIZA). **Miranda State:** 2 males, 1 female, Distrito Federal, El Junquito, Estación Experimental Bajo Seco, 1900m, 17.IV.1976, C.J. Rosales leg. (MIZA). **Trujillo State:** 1 male, Carretera Boconó, Guaramacal, 2300m, 29.X.1976, C.J. Rosales, J.L. García leg. (MIZA).

Family Mesembrinellidae

Eumesebrinella benoisti (Séguy, 1925b) (Fig. 2)

Material examined (1 female): **Bolívar State:** Reserva Forestal Imataca, El Bochinche, 200m, 6–18.XII.1974, “Expedición IZT–UCV” leg. (MIZA).

Huascaromusca decrepita (Séguy, 1925b) (Fig. 4)

Material examined (6 males, 1 female): **Lara State:** 1 male, 1 female, Parque Nacional Yacambú, El Blanquito, 29.I.2007, A. Martínez-Sánchez leg. (CEUA). **Trujillo State:**

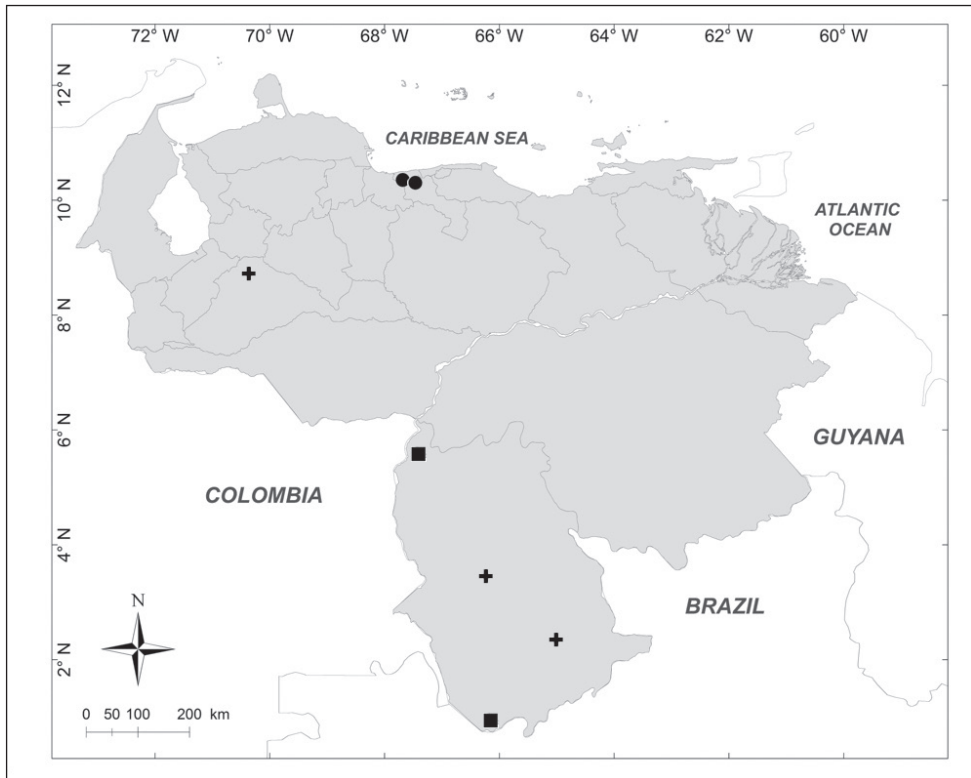


Figure 13. Known distributions of ● *Compsomyiops fulvicrura* (Robineau-Desvoidy), ■ *Lucilia albofusca* Whitworth and + *Hemilucilia benoisti* (Séguy) in Venezuela

2 males, San Isidro, 14 km Sur, La Soledad, 1500m, 30–31.V.1975, Malaise trap, R.E. Dietz leg. (MIZA); 2 males, Parque Nacional Guaramacal, 1480m, 19–25.V.2001, yellow pan trap, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz leg. (MJMO). **Yaracuy State:** 1 male, Cocorote, Sector El Candeló, 1650m, 17–20.X.2001, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, E. Arcaya, L. Joly leg. (MJMO).

***Huascaromusca lara* Bonatto in Bonatto & Marinoni, 2005** (Fig. 4)

Material examined (2 females): **Miranda State:** 1 female, El Hatillo, Las Marías, 1350m, 26.V.1976, F. Kaletta leg. (MIZA); 1 female, San Antonio de los Altos, Instituto Venezolano de Investigaciones Científicas, 1680m, IV.2003, Y. Velásquez leg. (IVIC).

***Mesembrinella bicolor* (Fabricius, 1805)** (Fig. 2)

Material examined (3 males): **Aragua State:** 1 male, Parque Nacional Henri Pittier, Rancho Grande, 1100m, 1.VI.1981, A. Field leg. (MIZA). **Bolívar State:** 1 male,

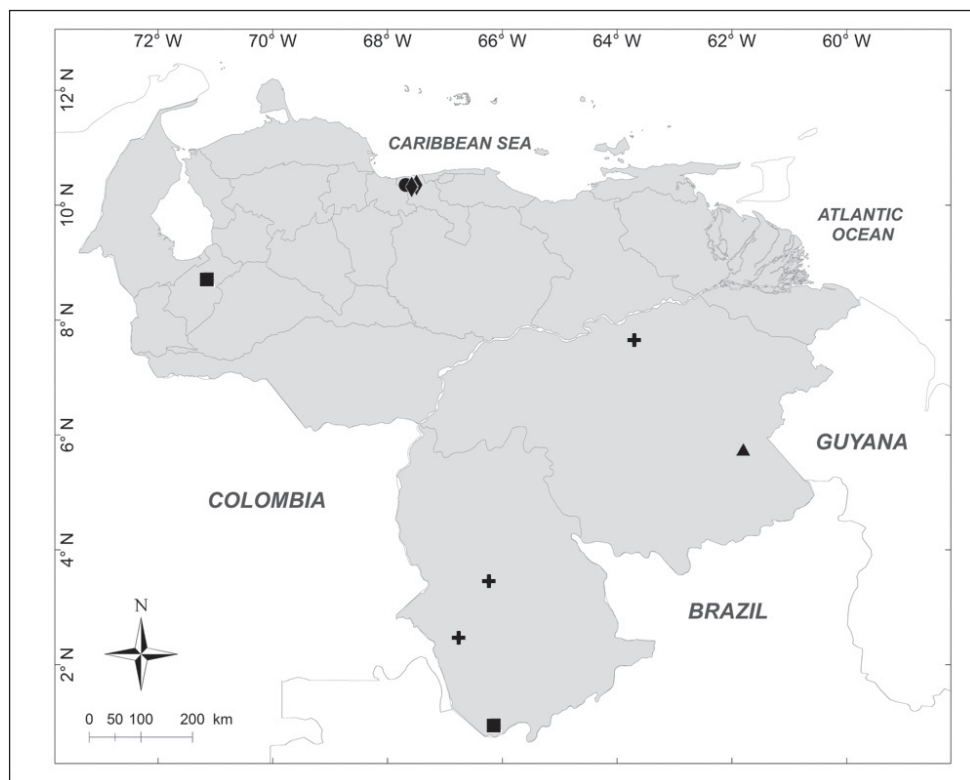


Figure 14. Known distributions of ● *Huascaromusca vogelsangi* Mello, ■ *Lucilia vulgata* Whitworth, ▲ *Giovannella bolivar* Bonatto, + *Eumesembrinella quadrilineata* (Fabricius) and ◆ *Mesembrinella xanthorrhina* (Bigot) in Venezuela.

Carretera Caicara, San Juan de Manapiare, 300m, 21–30.VII.1973, J.L. García leg. (MIZA). **Lara State:** 1 male, El Blanquito, 1480m, 11–16.III.2002, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, L. Joly leg. (MJMO).

***Mesembrinella spicata* Aldrich, 1925 (Fig. 6)**

Material examined (2 females): **Trujillo State:** Parque Nacional Guaramacal, 1480m, 14–20.II.2002, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, L. Joly leg. (MJMO).

***Mesembrinella umbrosa* Aldrich, 1922 (Fig. 2)**

Material examined (1 male): **Trujillo State:** Parque Nacional Guaramacal, 1480m, 14–20.II.2002, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, L. Joly leg. (MJMO).

***Souzalopesiella facialis* (Aldrich, 1922) (Fig. 3)**

Material examined (3 males, 1 female): **Aragua State:** 1 female, Parque Nacional Henri Pittier, Rancho Grande, 1183m, 25.I.2007, A. Martínez-Sánchez leg. (CEUA). **Lara State:** 1 male, Parque Nacional Yacambú, El Blanquito, 1480m, 11–16.III.2002, R. Briceño, J. Clavijo, R. Paz, F. Díaz, E. Arcaya, A. Chacón leg. (MJMO). **Trujillo State:** 2 males, Parque Nacional Guaramacal, 1480m, 14–20.II.2002, yellow pan trap, R. Briceño, A. Chacón, J. Clavijo, F. Díaz, R. Paz, L. Joly leg. (MJMO).

***Thompsoniella anomala* Guimarães, 1977 (Fig. 2)**

Material examined (1 female): **Miranda State:** San Antonio de los Altos, Instituto Venezolano de Investigaciones Científicas, 1680m, 22.I.2007, A. Martínez-Sánchez leg. (CEUA).

Discussion

This study is the first to determine the diversity of Calliphoridae and Mesembrinellidae in Venezuela. The checklist contains a total of 39 species of Calliphoridae, with 25 species distributed in ten genera, and Mesembrinellidae, with 14 species distributed in six genera. Twenty-six species were identified from examined material, while 13 species are listed based exclusively on records found in the literature. Compared to neighbouring countries that have been relatively well-studied, the known Venezuelan blow fly fauna is equivalent to that of Brazil (39 species: 24 Calliphoridae in eight genera and 15 Mesembrinellidae in seven genera) (Kosmann et al. 2013), but less diverse than that of Colombia (52 species: 31 Calliphoridae in 12 genera and 21 Mesembrinellidae in seven genera) (Wolff and Kosmann 2016).

Three species are newly recorded for the country: *Chrysomya putoria*, *Mesembrinella spicata* and *M. umbrosa*. These records were not unexpected, as these species have been found in other South American countries: *C. putoria* in Argentina, Bolivia, Brazil, Colombia, Ecuador, Paraguay and Peru (Baumgartner 1988, Wolff and Kosmann 2016), *M. spicata* in Costa Rica and Colombia (Bonatto and Marinoni 2005, Kosmann et al. 2013), and *M. umbrosa* in Bolivia, Colombia and Ecuador (Guimarães 1977, Peris and Mariluis 1984, Wolff and Kosmann 2016).

The absence from the examined material of species previously recorded in Venezuela or in neighbouring countries reflects the lack of study of these flies in this region. As an example, *Chloroprocta idioidea*, the only species of the genus *Chloroprocta* Wulp, 1896 (Calliphoridae), was recorded in Venezuela by Hall (1948), Cova (1964), Dear (1985) and Kosmann et al. (2013) and was the most abundant (66.3% of the total sampled specimens) species collected in a recent survey of necrophagous flies in the North Brazilian Amazon (Amat et al. 2016). However, it was not found in any

Venezuelan museum and only two specimens from Venezuela were examined, from BMNH. In his recent revision, Whitworth (2014) reported *Lucilia albofusca* and *L. vulgata* for Venezuela, but we did not find these two species in our field sampling or in entomological collections. Some authors reported *L. cluvia* and *L. sericata* in Venezuela (Mariluis et al. 1994b, Kosmann et al. 2013), but neither was found during this study. It is unlikely that *L. cluvia* occurs in the country, since Whitworth (2014) argued that reports of this species in South America are incorrect. On the other hand, *Lucilia sericata* has been reported as abundant in neighbouring countries such as Colombia and Brazil (Carvalho and Ribeiro 2000, Pape et al. 2004, Amat et al. 2008), so its absence during this study was surprising. This was also the case of other species previously cited for Venezuela, such as *Comptosomyiops fulvicrura*, *Hemilucilia benoisti*, *Paralucilia paraensis*, *Eumesembrinella quadrilineata*, *Eumesembrinella randa*, *Giovanella bolivar*, *Huascaromusca vogelsangi*, *Mesembrinella bellardiana* and *Mesembrinella xanthorrhina* (Aldrich 1922, Shannon 1926, Hall 1948, Cova 1964, Guimarães 1977, Peris and Mariluis 1984, Dear 1985, Peris and Mariluis 1989, Mariluis et al. 1994a, Bonatto and Marinoni 2005, Kosmann et al. 2013). The absence of these species in our samples could be related to non-exhaustive field sampling and/or to the heterogeneous composition of the examined entomological collections.

During this study, some specimens of *Lucilia* and Mesembrinellidae could not be identified as any known species. These specimens may represent undescribed species and for this reason were not included in the checklist. Some species of these groups are morphologically highly variable and further studies are required to determine whether they are separate species or examples of intraspecific variation (Whitworth, pers. comm.). If possible, we strongly recommend rearing specimens from live females collected in the field in order to obtain enough specimens to study the intraspecific variability of both these groups of blow flies.

The distribution maps obtained from the data available (Figs 1–14) show that the current distribution of Venezuelan blow flies is clearly determined by an unequal sampling effort around the country. Most records are from the predominantly mountainous north, where protected areas such as natural parks were the main collection sites. This sampling effort bias makes it difficult to draw any conclusions on the habitat preferences of these species, hence the necessity of further studies. In any case, the presence of some species in areas with less human disturbance adds value to these flies as indicators of the state of habitat conservation. This, in addition to the interest in these species in medicolegal and veterinary fields, will hopefully provide incentive to perform further studies on Venezuelan blow flies.

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On a desmitracheate “micronetine” *Nippononeta alpina* (Li & Zhu, 1993), comb. n. (Araneae, Linyphiidae)

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Abstract

The phylogenetic analyses based on molecular data demonstrate that all “micronetine” species of a desmitracheate system form a monophyly. *Macrargus* Dahl, 1886 is a “micronetine” genus, the species of which have a haplotracheate system in general, while *Macrargus alpinus* Li & Zhu, 1993 was found to have a desmitracheate system; this makes its generic placement problematic. According to the results of phylogenetic analysis, we transfer *M. alpinus* to another genus as *Nippononeta alpina* (Li & Zhu, 1993), **comb. n.**, and provide a redescription of its genital characters and somatic features. Comparisons with other “micronetine” species with a desmitracheate system are provided. Putative synapomorphies for *Nippononeta*, the clade *Nippononeta* + *Agyneta*, and for the “desmitracheate micronetines” clade, as well as their relationship with *Helophora*, are provided and discussed.

Keywords

Epigynal scape, genital morphology, phylogenetic placement, tracheal system

Introduction

Linyphiidae Blackwall, 1859 is a species-rich family of spiders which has species-specific genitalia but more conservative somatic features in general. The tracheate system in linyphiids consists of two pairs of trachea; “desmitracheate” and “haplotracheate” are the two terms referring to the two main tracheal conditions having the median

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pair highly branched and unbranched, respectively (Blest 1976, Millidge 1984). The seven subfamilies currently accepted in Linyphiidae are delimited largely based on genital characters, except for Erigoninae Emerton, 1882, which was originally defined by having a simple type male palp (Merrett 1963, Millidge 1977), and then redefined on the basis of its desmitracheate system (Blest 1976, Millidge 1984). Such a classification for “erigonines” has long been puzzled by the observations that some “erigonine” species have genitalia of simple type, but possess haplotracheate systems (Hormiga 2000, Miller and Hormiga 2004), while some “micronetine” species have genitalia of complex type, but possess desmitracheate systems (Millidge 1984, Dupérré 2013, Yan et al. 2015). Results of phylogenetic analyses based on molecular data recovered the monophyly of the Linyphiidae, in which the taxa of “micronetines” and “erigonines” nest (Arnedo et al. 2009, Wang et al. 2015); however, all “micronetine” species with a desmitracheate system form a monophyly (addressed as “desmitracheate micronetines” clade hereafter). Ancestral state reconstructions indicate that both tracheal features and genital characters used in subfamily classification are homoplastic. Furthermore, conversions between haplotracheate and desmitracheate conditions have taken place multiple times, and usually at the generic level. Until now, no evidence indicated that both tracheal conditions co-occur among congeneric species.

Macrargus Dahl, 1886 is a typical “micronetine” genus with a haplotracheate system (Blest 1976). However, *Macrargus alpinus* Li & Zhu, 1993, occurring in China, was found to have a desmitracheate system, different from its congeners. The “desmitracheate micronetines” clade resulting from phylogenetic analyses is distantly related to *Macrargus* (Wang et al. 2015). Some putative synapomorphies for the desmitracheate “micronetine” genus *Nippononeta* Eskov, 1992 and for the clade *Nippononeta*+*Agyneta* proposed by Yan et al. (2015) can also be found in *M. alpinus*, yet none of them is present in other *Macrargus* species. This implies that the generic placement of *M. alpinus* is questionable.

To test the phylogenetic placement of *Macrargus alpinus* and its relationships with other desmitracheate “micronetines”, we added the newly sequenced DNA sequence data of *M. alpinus* into the dataset of Wang et al. (2015). In the present study, we propose a new generic placement for *M. alpinus* based on the result of phylogenetic analysis of the new dataset. We present a redescription of *M. alpinus* and comparisons with closely related groups. Putative synapomorphies for *Nippononeta* and the desmitracheate “micronetine” groups proposed by Yan et al. (2015) are revised for further studies.

Materials and methods

Phylogenetic analysis

Two mitochondrial genes, cytochrome c oxidase subunit I (CO1) and 16S rRNA (16S), and two nuclear genes, 18S rRNA (18S), and 28S rRNA (28S) were amplified and sequenced for *Macrargus alpinus* and added to the dataset of Wang et al. (2015) to test its placement in Linyphiidae. Given that the primary analysis resulted

in *Macrargus alpinus* as a sister group to *Nippononeta coreana*, the four genes data of one additional *Nippononeta* species was downloaded from GenBank and added to test the monophyly of the genus *Nippononeta* and its relationship with *M. alpinus*. Based on the dataset of Wang et al. (2015), a total of 132 taxa was included in our matrix: 130 linyphiid species including the type species of *Macrargus*, seven species from four genera as representatives of desmitracheate “micronetines”, and two representative species of *Nippononeta*. Most outgroup taxa of Wang et al. (2015) were removed; only two representatives of Pimoidae, the sister group of Linyphiidae, were included as outgroups to root the tree.

Molecular protocols for amplification and sequencing follow that of Wang et al. (2015). Taxa sampled and sequence accession numbers are presented in Wang et al. (2015), and those of the two new taxa are presented in Suppl. material 1. Every sequence was first aligned using CLUSTAL X version 1.81 (Thompson et al. 1997) independently, and then the sequences of four genes were concatenated by MESQUITE (Version 2.75, Maddison and Maddison, 2009). The genes were unpartitioned. The gaps were considered as missing data. Maximum Likelihood analysis of the concatenated dataset was conducted by RAxML v. 7.2.7 as implemented on the Cipres Gateway (Miller et al. 2010), using GTR+I+R model, which was the best fitting model for the matrix by JModeltest examination. Bootstrap analysis was obtained with 1000 replicates to assess nodal support.

Morphological methods

Specimens were examined and illustrated by using a Leica M205A stereomicroscope and a Leica DM5500B compound microscope. The male palp and female epigynum were examined after they were dissected from the body. The embolic division was excised by breaking the membranous column connecting between the supratégulum and radix. For microscopic examination and illustration, the male palp and epigynum were cleared in methyl salicylate. Illustrations were made using a drawing tube. Scanning Electron Microscopy (SEM) images were taken by using a LEO 1430VP at the Department of Biological Sciences at George Washington University. For SEM examination the specimens were prepared following Álvarez-Padilla and Hormiga (2008). SEM images of the embolic division taken from the right palp were mirrored to match those taken from the left palp. All specimens examined here are deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (IZCAS), and the College of Life Sciences, Capital Normal University, China (CNU). Terminology for the genital and somatic characters follows Hormiga (2000), Tu and Hormiga (2010, 2011), Saaristo and Tanasevitch (1996) and Wang et al. (2015).

Results

With the data on *Macrargus alpinus* and an additional *Nippononeta* species added to their dataset, the Maximum Likelihood analysis recovered the general topology of

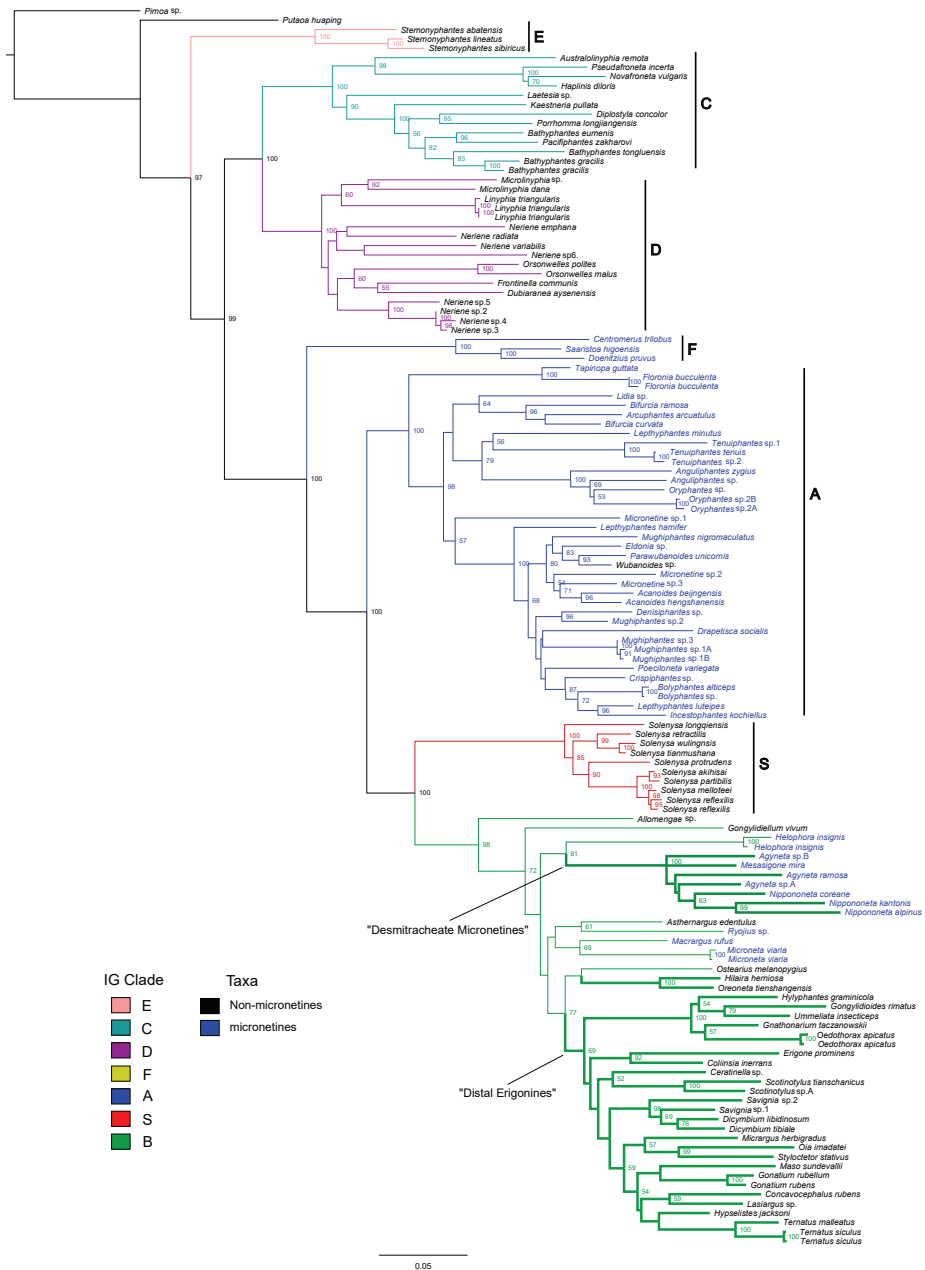


Figure 1. Linyphiid phylogeny resulting from the Maximum Likelihood analysis based on DNA sequence data. Numbers at nodes indicate bootstrap support above 50%. Branches in color represent seven robustly supported main clades within Linyphiidae. Branches in bold indicate the desmitracheate type. Taxa in blue are currently placed in Micronetinae.

Wang et al. (2015): the monophyly of Linyphiidae, its sister relationship with Pimoidae, the seven main clades and relationships among them within linyphiids (all with bootstrap > 95%), with variations on the placement of some weakly supported lineages within these clades (Fig. 1). “Micronetines” remain paraphyletic, forming clades A and F, as well as some basal lineages of clade B, nesting with “erigonines”. Three desmitracheate clades are included within clade B. Besides the “distal erigonines” clade (bootstrap = 69%) and one other “desmitracheate erigonines” lineage (bootstrap = 100%), all those “micronetine” species of a desmitracheate system form a well-supported clade, the “desmitracheate micronetines” clade (bootstrap = 100%), sister to the *Helophora* clade (bootstrap = 91%). *Macrargus rufus* and *Microneta viaria* form one of the “haplotracheate micronetines” lineages within clade B (bootstrap = 69%). Meanwhile, *Macrargus alpinus* falls into the *Nippononeta* clade (bootstrap = 63%), sister to *N. kantonis* (bootstrap = 99%), but distantly related to *Macrargus rufus*. The monophyly of *Agyoneta* and its relationship with the *Nippononeta* clade remain unresolved.

Taxonomy

Linyphiidae Blackwall, 1859

Nippononeta Eskov, 1992

Type species. *Nippononeta kurilensis* Eskov, 1992.

Nippononeta alpina (Li & Zhu, 1993), comb. n.

Macrargus alpinus Li & Zhu, in Song et al. 1993: 863, f. 21A–I (D♂♀); Li et al. 1994: 81, f. 31–33 (♀); Li and Zhu 1995: 41, f. 2a–i (♂♀); Song et al. 1999: 186, f. 104D, G, J (♂♀).

Type material examined. ♂ holotype (IZCAS), China, Hubei Province, Shennongjia Natural Conservation, Panlong County, 26 June 1986; 1♂ and 2♀ paratypes (IZCAS), same data as the holotype.

Additional material examined. 4♂ and 4♀ (CNU), China, Sichuan Province, Lushan County, Fenghuo town, Sanyou village, 7 July 2004, L. Tu leg; 5♂ and 4♀ (CNU), China, Sichuan Province, Tianquan County, Mt. Erlangshan Natural Forest Park, 8 July 2004, L. Tu leg; 3♂ and 4♀ (CNU), China, Zhejiang Province, Mt. Yandangshan, 28°35.78' N, 121°04.30' E, alt. ca 420m, 15 Aug. 2010, F. Wang leg.

Diagnosis. The male of *Nippononeta alpina* comb. n. can be distinguished from all other *Nippononeta* species by the proximal tibial process (Fig. 2A) and the paracymbial median branch (Fig. 3D), both absent in other *Nippononeta* species. The female epigynum is distinguished by the epigynal cavity fully filled by the sigmoid folded scape, with

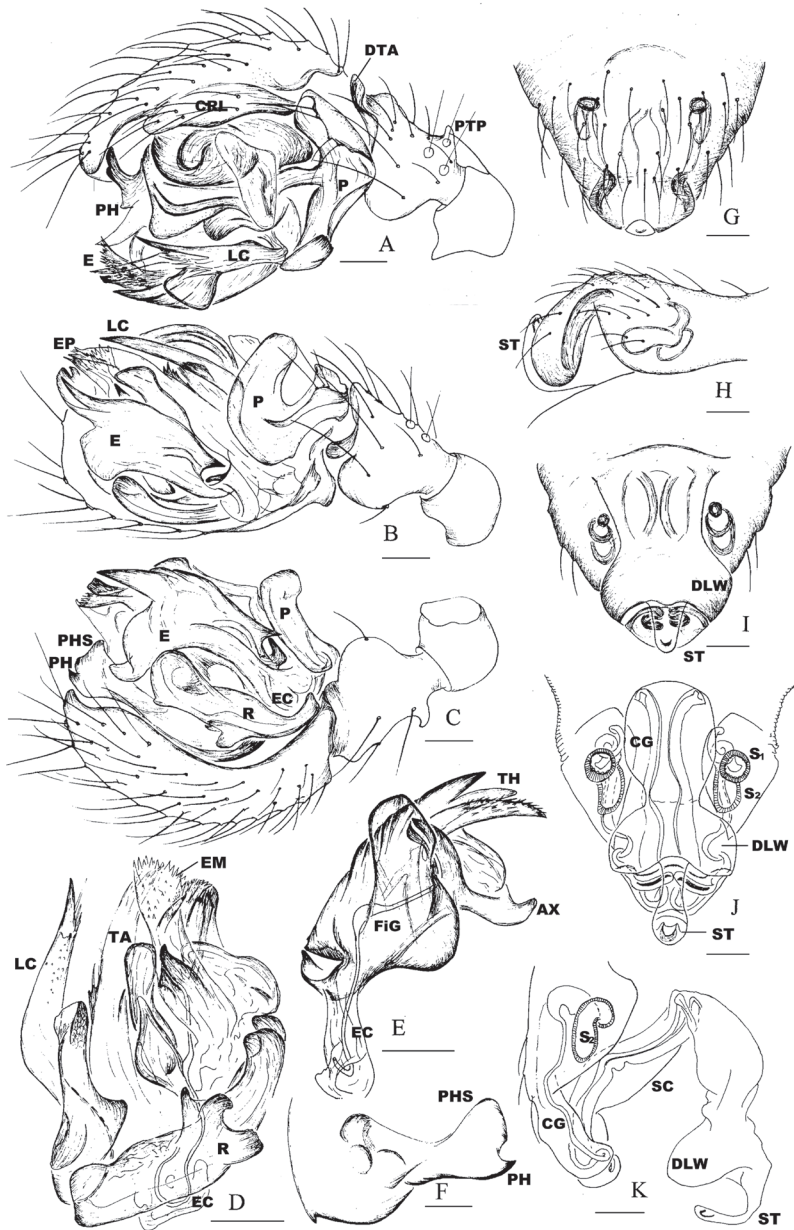


Figure 2. *Nippononeta alpina* comb. n. (A–F) male palp **A** retrolateral **B** ventral **C** prolateral **D** embolic division **E** embolus **F** distal suprategular apophysis (G–K) epigynum **G** ventral **H** lateral **I** dorsal **J** dorsal, cleared **K** lateral, cleared. Abbreviations: AX apex of embolus; CG copulatory groove; CRL cymbial retrolateral lobe; DLW lateral wing on distal part of scape; DTA distal tibial apophysis; E embolus; EC embolus column; EM embolic membrane; EP embolus proper; FiG Fickert's gland; LC lamella characteristic; P paracymbium; PH pit hook; PHS pit hook sclerite; PTP proximal tibial process; R radix; S₁ upper chamber of spermatheca; S₂ lower chamber of spermatheca; ST stretcher; TA terminal apophysis; TH thumb of embolus.

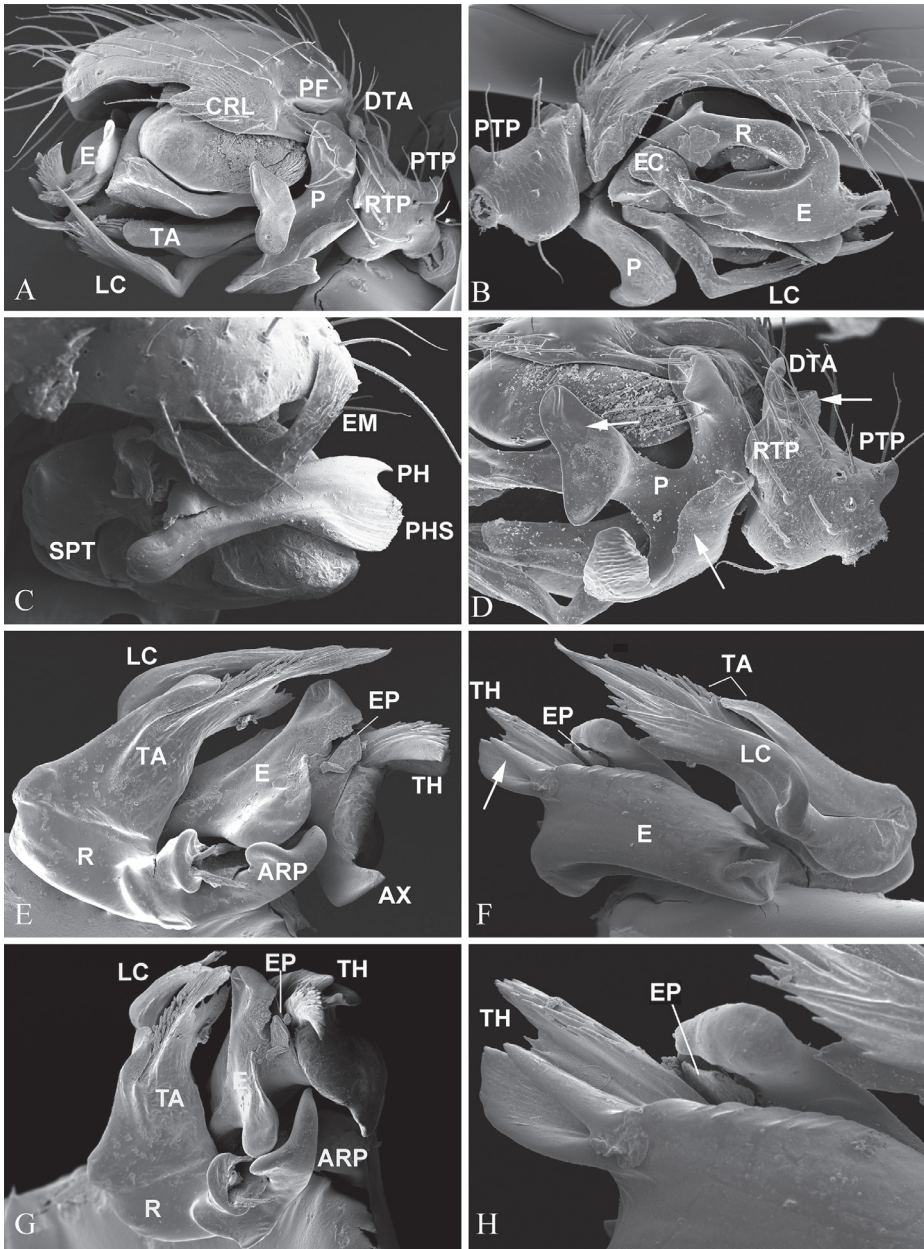


Figure 3. *Nippononeta alpina* comb. n., male palp. **A** retrolateral **B** prolateral **C** prolateral with embolic division removed **D** detail of A, arrows indicate serrated surface of DTA (upper right), median branch of paracymbium (upper left) and outer margin fold (lower) (**E–H**) embolic division **E** dorsal **F** ventral, arrow indicates embolic spine **G** anterior **H** detail of F, shows hid EP. Abbreviations: ARP anterior radical process; AX apex of embolus; CRL cymbial retrolateral lobe; DTA distal tibial apophysis; E embolus; EM embolic membrane; EP embolus proper; LC lamella characteristica; P paracymbium; PF posterior fold; PH pit hook; PHS pit hook sclerite; PTP proximal tibial process; R radix; SPT supratégulum; TA terminal apophysis; TH thumb of embolus.

a pair of lateral wings on the scape proximal part wrapping downward (Fig. 4A) and another pair of lateral wings on the scape distal part wrapping upward (Fig. 4B), while in most other *Nippononeta* species the epigynum usually diamond-shaped, with a dorsally opened epigynal cavity and a ventrally exposed scape (Yan et al. 2015: fig. 2A–B).

Description. Chelicerae of normal size, with narrower fang base and denser stridulatory ridges in the male than those in the female (Fig. 5C–D). Female palp without distal claw (Fig. 4H). Tracheal system having median trunk wider than the lateral pair, highly branched and extending into prosoma (Fig. 4G), tracheoles with taenidia. Epiandrous gland spigots absent in the male (Fig. 4F). Spinnerets (Fig. 4E–H): PLS in females having the mesal cylindrical gland spigot base enlarged (Fig. 5F), the triplet formed by one flagelliform and two aggregate gland spigots presented in the male PLS (Fig. 5H). For other somatic features, see description for the genus by Eskov (1992).

Male palp (Figs 2A–F, 3). Tibia short, as long as wide, with three apophyses: one distal, one retrolateral, one proximal; distal tibia apophysis with serrated surface. Cymbium with small retrolateral lobe and proximal fold above paracymbial base. Paracymbium U-shaped, distal arm shorter than proximal one, with well-developed median branch and outer margin fold. Distal suprategular apophysis modified as pit hook with hook sclerite. Embolic membrane furnished with many papillae. Embolic division: boat-shaped radix with ear-like anterior process. Embolus extremely complex, modified with multiple free ends; embolus proper covered by one of embolic sclerites; embolus thumb modified as spine-like projections; and apex triangular; Fickert's gland located within embolus. Lamella characteristica unbranched, sigmoid ribbon-like in ventral view, with thread-like projections distally. Terminal apophysis divided into two parts: the posterior strongly sclerotized with a rounded end, the anterior membranous part with thread-like projections distally.

Epigynum (Figs 2G–K, 4A–E). Epigynal plate protruding out, with wide epigynal basal part. Median plate absent on dorsal surface, but the tegument of epigynal basal part forming transverse dorsal fold. Epigynal cavity fully filled by sigmoid folded scape, covered by a pair of lateral wings on scape proximal part wrapping downward, and another pair of lateral wings on scape distal part wrapping upward; stretcher lifting up. Copulatory tracts in groove state; fertilization tracts changing from groove to duct state and extending towards epigastric furrow.

Remarks. *Nippononeta alpina* comb. n. originally was placed in the genus *Micrargus* Dahl, 1886, whose type species *M. rufus* Wider, 1834 has a typical haplotracheate system (Blest 1976). In addition to tracheal characters, the genitalia of *N. alpina* are of a different type from that of *M. rufus* (see descriptions by Saaristo in Marusik and Koponen 2008, Millidge 1977, 1984, Gnelitsa and Koponen 2010). Some genital characters of *N. alpina* are consistent with the putative synapomorphies for the genus *Nippononeta* and for the “desmitracheate micronetines” clade discussed bellow, but not shared by *M. rufus*.

In addition, *Micrargus* is masculine in gender, while *Nippononeta* is feminine. As *M. alpinus* is being transferred to *Nippononeta*, the species name has to be changed to *alpina*. However, Ono and Saito (2001) already described a species also named as *N.*

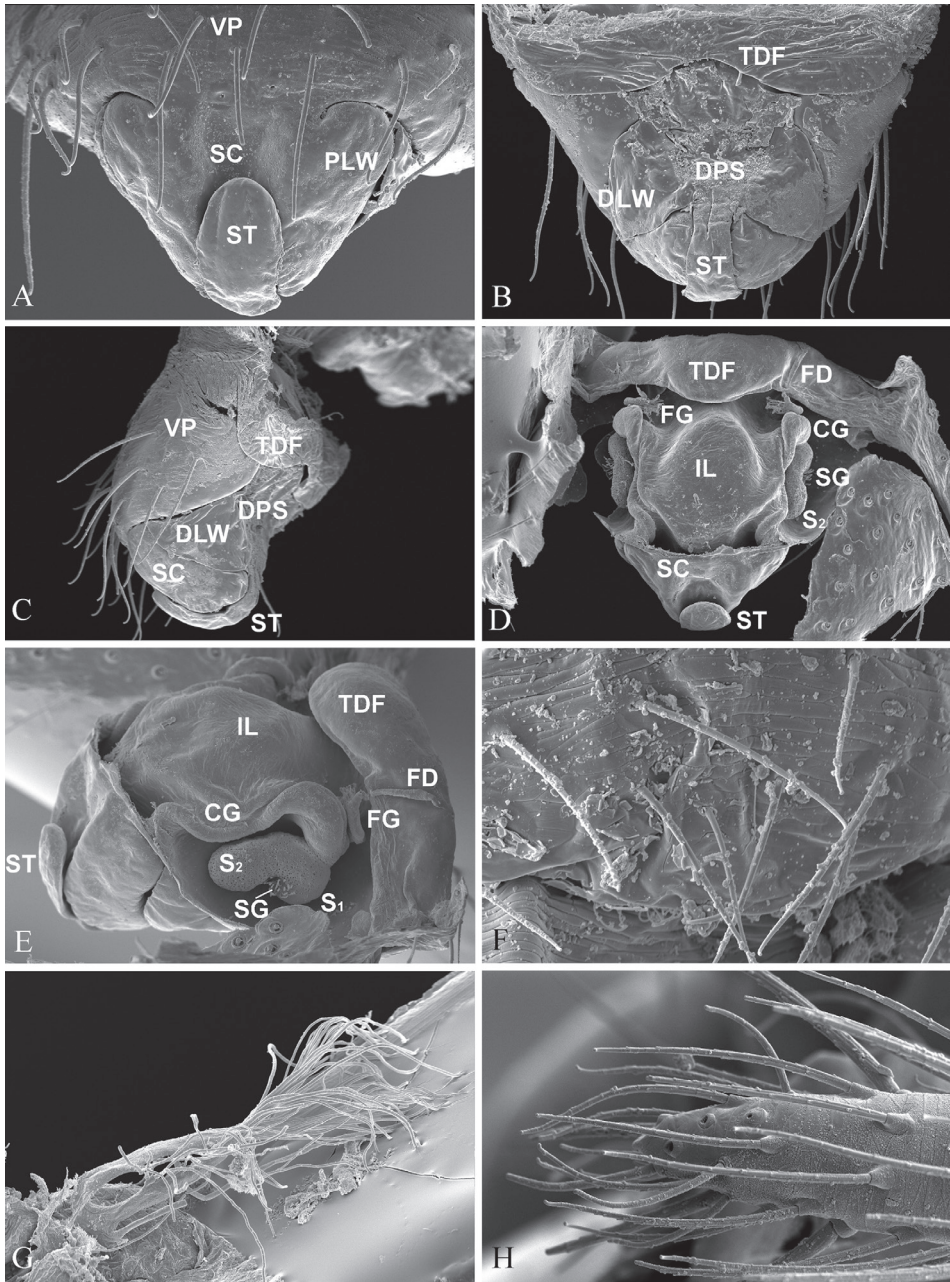


Figure 4. *Nippononeta alpina* comb. n. (A–E) epigynum **A** ventral **B** dorsal **C** lateral **D** ventral, cleared with ventral plate removed **E** lateral, cleared with ventral plate removed **F** male abdomen, ventral, shows epiandrous gland spigots absent **G** tracheal system, cleared **H** female palp, shows distal claw absent. Abbreviations: CG copulatory groove; DLW lateral wing on distal part of scape; DPS distal part of scape; FG fertilization groove; IL inner lobe; PLW lateral wing on proximal part of scape; S_1 upper chamber of spermatheca; S_2 lower chamber of spermatheca; SC scape; SG special gland; ST stretcher; TDF transversal dorsal fold; VP ventral plate.

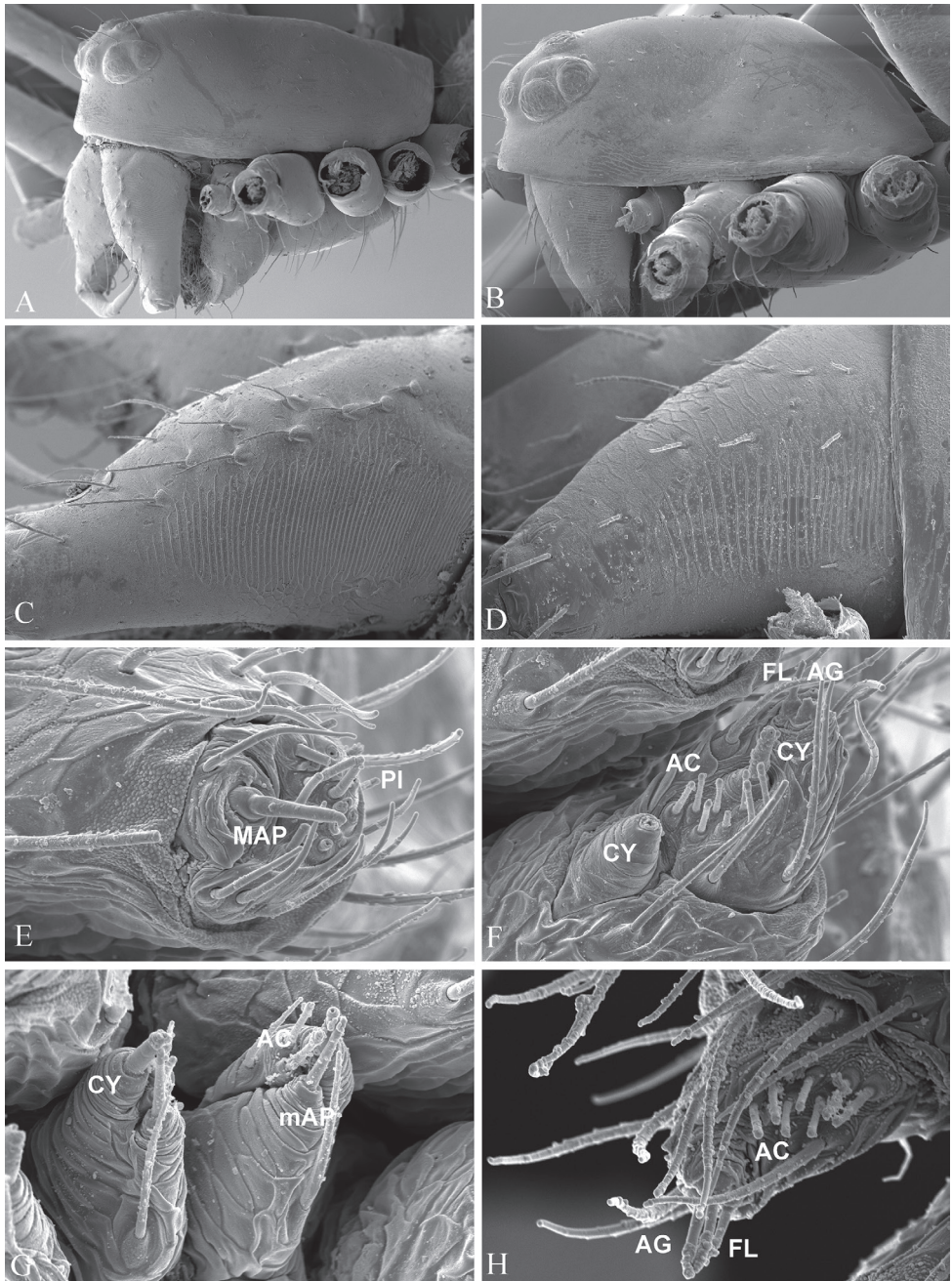


Figure 5. *Nippononeta alpina* comb. n. **A** male prosoma, lateral **B** female prosoma, lateral **C** male chelicerae, ectal **D** female chelicerae, ectal **E** female ALS **F** female PLS **G** female PMS **H** male PLS. Abbreviations: AC acini-form gland spigots; AG aggregate gland spigots; ALS anterior lateral spinneret; CY cylindrical gland spigot; FL flagelliform gland spigot; MAP major ampullate gland spigot; mAP minor ampullate gland spigot; PI piriform gland spigot; PLS posterior lateral spinneret; PMS posterior median spinneret.

alpina Ono & Saito, 2001, which is not a junior synonym of *M. alpinus*. Therefore we propose a replacement name as *onoii*, after one author’s name, for the species of Ono and Saito to avoid homonymy.

Discussion

Our results show that all desmitracheate “micronetines” form a monophyly, and *Macrargus alpinus* falls into the *Nippononeta* clade, distantly related to *Macrargus rufus* (Fig. 1). These suggest a new generic placement for this species, *Nippononeta alpina* comb. n. The two “micronetine” species, *N. alpina* and *M. rufus* grouped into clade B is consistent with the putative synapomorphies based on somatic characters proposed for the seven-clade division (Wang et al. 2015). It is also in accord with the tracheal characters to group *N. alpina* with other desmitracheate “micronetines”, rather than with the haplotracheate genus *Macrargus*.

According to Wang et al. (2015) and Yan et al. (2015), some putative synapomorphies based on genital characters support the relationships among the desmitracheate “micronetines” and other linyphiids. The presence of a median plate is a synapomorphy for all linyphiids, but is secondarily lost in *Helophora*, *Nippononeta*, *Agyneta* and *Mesasisigone*, as well as in *N. alpina* (Fig. 4B). Therefore, the absence of a median plate as a putative synapomorphy supports the sister relationship between the “desmitracheate micronetines” clade and *Helophora* (Fig. 1). The highly branched median pair of the tracheae, the protruding epigynum modified into a scape and epigynal cavity (Fig. 4A–D), and male palp with complex embolus (Fig. 1E) are putative synapomorphies for the former (Fig. 4G), while the moderately branched (Arnedo et al. 2009), the protruding epigynal plate without forming a scape and epigynal cavity (Tu and Hormiga 2010: fig. 7), and by the flagelliform embolus of the male palp (Chamberlin and Ivie 1933: fig. 85, Tao et al. 1995: fig. 69) are putative synapomorphies for the latter. Furthermore, the monophyly of the clade including *Nippononeta* and *Agyneta* (unknown for *Mesasisigone*) is supported by four putative synapomorphies: by the presence of a serrated distal tibial apophysis and a retrolateral tibial process on the male palp (Fig. 3D, see also Yan et al. 2015: fig. 1D); by the dorsally opened epigynal cavity resulting from a secondary loss of the median plate (Fig. 4B, see also Yan et al. 2015: fig. 2B); and by the fertilization tracts changing from groove to duct state (Fig. 4E, see also Tu and Hormiga 2010: fig. 6c, Yan et al. 2015: fig. 5D).

The monophyly of the *Nippononeta* clade is supported by the following four putative synapomorphies: the pointed apophysis on the paracymbial proximal arm (Fig. 3D, Yan et al. 2015: fig. 1D); the embolus thumb modified into spine-like projections on the male palp (Fig. 3H, Yan et al. 2015: fig. 1G); and the presence of a transverse dorsal fold and finger-like stretcher (Fig. 1K, Yan et al. 2015: fig. 2A). Furthermore, the new placement of *Nippononeta alpina* implies that several putative synapomorphies previously proposed for *Nippononeta* by Yan et al. (2015) have to be reviewed: the narrowed epigynal basal part and the expanded lateral epigynal shoulder (Yan et al. 2015: fig. 2A–D) are not present in *N. alpina*. Meanwhile, *N. alpina* also has some

apomorphies not shared with other *Nippononeta* species: the two pairs of scape lateral wings and the uplifting stretcher (Fig. 4A–D).

The monophyly of *Agyneta* is not supported and its relationships with other desmitracheate “micronetines” remain unresolved. Nevertheless, morphological studies show that *Agyneta* species are easily distinguished by some genital characters: e.g. the presence of a conical cymbial elevation and a sickle-shaped embolus with a large thumb, and the scaped epigynum with a pair of well-developed lateral lobes (see the review of Dupérré 2013). The uncertain phylogenetic relationship between *Agyneta* and other desmitracheate “micronetines” is largely due to a limited species-level sampling for such a diverse group. Clearly, more comprehensive sampling is needed for future studies.

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Supplementary material I

GenBank accession numbers

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