CHECKLIST



# Photosymbiotic ascidians from Pari Island (Thousand Islands, Indonesia)

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# Abstract

Photosymbiotic ascidian fauna were surveyed in the subtidal zone off Pari Island in the Thousand Islands (Java Sea, Indonesia). Nine species were recorded: *Didemnum molle, Trididemnum miniatum, Lissoclinum patella, L. punctatum, L. timorense, Diplosoma gumavirens, D. simile, D. simileguwa*, and *D. virens*. All of these species have been previously recorded in the Ryukyu Archipelago, Japan. *Diplosoma gumavirens* and *D. simileguwa* were originally described from the Ryukyu Archipelago in 2009 and 2005, respectively, and all of the observed species are potentially widely distributed in Indo-West Pacific coral reefs.

# Keywords

Algal symbiosis, Colonial ascidian, Biogeography, Coral reefs, Didemnidae

# Introduction

In tropical waters, some colonial ascidians harbor cyanobacterial symbionts such as *Prochloron* (reviewed by Lewin and Cheng 1989; E. Hirose et al. 2009a; Hirose 2014).

The host ascidians always belong to the family Didemnidae, which is likely the largest family in the class Ascidiacea (e.g., Kott 2004; Shenkar et al. 2011). Although photosymbiotic didemnids are sometimes more common than any other ascidians in shallow coral reefs, they are often overlooked because of their small size and cryptic habitats. In contrast, they have been attractive sources of bioactive compounds for researchers in the biochemical and pharmaceutical sciences (e.g., Schmidt et al. 2012). To date, about 30 species in the four didemnid genera (*Didemnum, Diplosoma, Lissoclinum,* and *Trididemnum*) have been recorded as host species worldwide (e.g., Kott 2001). However, the ranges of distribution of individual species are less understood, as few faunal records of photosymbiotic ascidians exist (e.g., Kott 2001; Monniot and Monniot 2001). To identify these species, it is often necessary to examine zooid morphology under a microscope. Therefore, reexamination may be necessary for some records in older publications.

The Pulau Pari Technical Management Unit for Human Resources Development on Oceanography Competency is a marine laboratory located on Pari Island (Thousand Islands, Indonesia). This laboratory of the Indonesian Institute of Sciences (LIPI) is one of the key stations for marine science in the Java Sea. Therefore, acquiring biodiversity data in this area is essential. Here, we report the photosymbiotic ascidian fauna observed in the shallow coral reef in the vicinity of this laboratory.

#### Materials and methods

Samples were collected by snorkeling in the shallow subtidal zone down to approximately 2 m or less at low tide in the back reef, reef flat, and reef crest off Pari Island (5°52'S, 106°36'40"E) on 28–30 November 2013 (Fig. 1). Ascidian colonies were photographed *in situ* prior to collection. Specimens were anesthetized using menthol and 0.37 M MgCl<sub>2</sub> for approximately 2 h and then fixed with 10% formalin–seawater. Fixed colonies were dissected under a binocular stereomicroscope. Zooids and spicules were photographed using a microscope equipped with differential interference contrast optics. In some photomicrographs of the thoraxes, several images were combined to increase the depth of field using the post-processing image software Helicon Focus Pro 4.2.8 (Helicon Soft, Ltd., Kharkov, Ukraine). Cyanobacterial symbionts were identified based on the colour in live specimens and the cytomorphology under a light microscope. Ascidian taxa were mainly identified following Kott (2001) and Hirose and Su (2011). The work by Shenkar et al. (2014) was also consulted for synonyms. Specimens were deposited in the Museum Zoologicum Bogoriense, Research Institute for Biology, Indonesian Institute of Science (LIPI), Indonesia.

#### Results

Nine photosymbiotic ascidian species were found in the subtidal zone of the coral reef off Pari Island. Symbiont cyanobacteria within all ascidian species were identified as



Figure 1. Location of Thousand Islands, Indonesia (A) and the surveyed area off Pari Island (B).

*Prochloron didemni* that is the only taxonomically valid species. Depending on the host species, *Prochloron* cells were distributed in the common cloacal cavities, in the tunic, or in both the common cloacal cavity and the tunic. Although the *Prochloron* cells in the cavity are morphologically different from those in the tunic (Cox 1986), they are indistinguishable genetically (Münchhoff et al. 2007; Hirose et al. 2012).

# Didemnum molle Herdman, 1886

Fig. 2A

Diplosomoides molle Herdman, 1886 Leptoclinum molle (Herdman, 1886) Lissoclinum molle (Herdman, 1886) Didemnum sycon Michaelsen, 1920 Specimen. MZB. Asc. 00001

Habitat. Coral limestone at reef crest.

**Remarks.** Colonies were dome-shaped. Several morphotypes in colony shape and color exist in this species (i.e., brown, gray, white, large, and small type). These morphotypes can also be distinguished by the partial sequence of the cytochrome oxidase subunit I (COI) gene (M. Hirose et al. 2009; Hirose et al. 2010a). Brown-type colonies were found in the present survey. *Prochloron* cells were distributed within the common cloacal cavity. Testis and/or oocyte were found in some zooids.

# Trididemnum miniatum Kott, 1977

Fig. 2B

Specimens. MZB. Asc. 00002

Habitat. Dead coral skeletons and macroalgae in shallow back reef.



**Figure 2.** Photosymbiotic ascidians with tunic spicules. Colonies *in situ* and tunic spicules (inset) of *Didemnum molle* (**A**), *Trididemnum miniatum* (**B**), *Lissoclinum patella* (**C**), *L. punctatum* (**D**), and *L. timorense* (**E**). Tunic cells contain *Prochloron* cells in the tunic of *L. punctatum* (**F**). Scale bars = 20  $\mu$ m.

**Remarks.** Thin sheets of colonies were white in exposed habitat and pale green in shaded habitat, depending on the amount of calcareous spicules in the tunic. *Prochloron* cells were distributed within the tunic.

#### Lissoclinum patella (Gottschaldt, 1898)

Fig. 2C

Didemnoides patella Gottschaldt, 1898 Didemnoides sulcatum Gottschaldt, 1898 Didemnoides ternatanum Gottschaldt, 1898 Didemnum meandrium Sluiter, 1909 Didemnum patella (Gottschaldt, 1898) Didemnum ternatanum (Gottschaldt, 1898) Leptoclinum patella (Gottschaldt, 1898) Lissoclinum patellum (Gottschaldt, 1898)

#### Specimens. MZB. Asc. 00003

Habitat. Dead coral skeletons in back reef.

**Remarks.** Colonies were thick cushions attaining about 10 mm in thickness. Tunic contains both stellate and globular spicules (Fig. 1C, inset). *Prochloron* cells were distributed within the common cloacal cavity. Some zooids had testes. Because of the large size, this species has been thoroughly studied for its natural compounds (e.g., Schmidt et al. 2012).

# Lissoclinum punctatum Kott, 1977

Fig. 2D, F

#### Specimens. MZB. Asc. 00004

Habitat. Shaded side of dead coral skeletons in reef flat.

**Remarks.** Colonies were irregularly shaped sheets. Globular spicules (Fig. 1D, inset) form a capsule-like aggregation enveloping each zooid. *Prochloron* was distributed within the common cloacal cavities and tunic. As reported in Hirose et al. (1996), algal cells in the tunic were contained in the tunic cells of the host ascidian (Fig. 1F).

#### Lissoclinum timorense (Sluiter, 1909)

Fig. 2E

Didemnum timorensis Sluiter, 1909 Didemnum voeltzkowi Michaelsen, 1920 Lissoclinum timorensis (Sluiter, 1909) Lissoclinum voeltzkowi (Michaelsen, 1920)

#### Specimens. MZB. Asc. 00005

Habitat. Dead coral skeletons and clefts between coral limestones in back reef and shallow reef flat.

**Remarks.** Colonies had linguiform projections of the tunic around the colony periphery and sometimes on the colony surface. Tunic contains both stellate and globular spicules (Fig. 1E, inset). *Prochloron* cells were distributed within the common cloacal cavity.

Because the zooids of *Lissoclinum bistratum* and *L. timorense* are very similar in morphology, Monniot and Monniot (2001) proposed that *L. timorense* is a junior synonym of *L. bistratum*. Typical colonies of the two species are easily distinguishable by the presence or absence of linguiform projections on the colony surfaces, although intermediate forms between the two exist. Kott (2001) discriminated the two species based on the presence or absence of stellate spicules. However, the two species defined by spicule type could not be discriminated by molecular phylogeny based on partial COI gene sequences (Hirose et al. 2010b). We did not find *L. bistratum*-type colonies in the present survey, although they are common in reef crests of the Ryukyus.

#### Diplosoma gumavirens Hirose & Oka, 2009

Fig. 3A, B

#### Specimens. MZB. Asc. 00006

Habitat. Shaded side of dead coral branches in reef flat.

**Remarks.** Colonies were oval cushions and entirely green due to *Prochloron* cells distributed within the common cloacal cavities. A blue ring of structural color encircled each branchial siphon. Retractor muscle emerged from halfway along esophageal neck of a zooid. On each of the right and left halves of the branchial sac, there were five stigmata in the first (top), second, and third stigmatal rows and four stigmata in the fourth row (bottom). Here, we describe the pattern of stigma number as 5-5-5-4. This record is the first of this species from outside of the Taiwan–Ryukyu area.

#### Diplosoma simile (Sluiter, 1909)

Fig. 3C, D

*Diplosoma midori* (Tokioka, 1954) *Leptoclinum midori* Tokioka, 1954 *Leptoclinum simile* Sluiter, 1909

#### Specimens. MZB. Asc. 00007

Habitat. Dead coral branch and coral limestone in reef flat and reef crest.

**Remarks.** Colonies were irregularly shaped sheets and entirely green due to *Prochloron* cells distributed within the common cloacal cavities. Retractor muscle emerged from underside of thorax. The numbers of stigmata were 6–6–6–5. Some zooids had testes. Embryos were brooded in some colonies.

#### Diplosoma simileguwa Oka & Hirose, 2005

Fig. 3E, F

#### Specimens. MZB. Asc. 00008

Habitat. Shaded side of dead coral branches in reef flat

**Remarks.** Colonies were irregularly shaped sheets and entirely green due to *Prochloron* cells distributed within common cloacal cavities. Retractor muscle emerged from underside of thorax. The numbers of stigmata were 4–5–4–3. This record is the first of this species from outside of the Taiwan–Ryukyu area.

#### Diplosoma virens (Hartmeyer, 1909)

Fig. 3G, H

Diplosoma viride Herdman, 1906 Leptoclinum calificiforme Sluiter, 1909 Leptoclinum varium Sluiter, 1909 Leptoclinum virens Hartmeyer, 1909

#### Specimens. MZB. Asc. 00009

Habitat. Basal parts on branching corals in back reef and reef flat.

**Remarks.** Colonies were irregularly shaped sheets and entirely green due to *Prochloron* cells distributed within common cloacal cavities. Retractor muscle emerged from halfway along esophageal neck. The numbers of stigmata were 6–6–6–5. Some zooids had testes.

# Discussion

All photosymbiotic ascidians described here have also been recorded in the Ryukyu Archipelago, Japan (Hirose 2013 and references therein). Among the nine species, *D. simileguwa* and *D. gumavirens* were originally described from the Ryukyus in 2005 and 2009, respectively (Oka et al. 2005; E. Hirose et al. 2009b), and this report is the first to record these species outside of the Taiwan–Ryukyu area. The present records significantly expand our understanding of their range of distribution. The other seven species have also been described from the Great Barrier Reef (Kott 2001); thus, these species are widely distributed in the Indo–West Pacific. Among the five photosymbiotic ascidians recorded from Singapore, i.e., *Diplosoma simile, Lissoclinum bistratum*, *L. punctatum*, *L. timorense* and *Trididemnum cyclops*, (Su et al. 2013), two species, *L. bistratum* and *T. cyclops* were not recorded in the present survey. These species are likely distributed in the Java Sea, considering that they are commonly found in West Pacific coral reefs. The recognition and identification of species are often important in field



**Figure 3.** Photosymbiotic ascidians without tunic spicules (*Diplosoma* species). Colonies *in situ* and thorax of zooid of *Diplosoma gumavirens* (**A**, **B**), *D. simile* (**C**, **D**), *D. simileguwa* (**E**, **F**), and *D. virens* (**G**, **H**). **en** endostyle; **es** esophagus; **rt** retractor muscle. Scale bars = 0.1 mm.

studies dealing with biocoenosis, and we hope that the present report will be helpful in future surveys and field courses in this area. Additional species, including undescribed species, are potentially distributed around Pari Island, considering its location within a biodiversity hot spot. Therefore, additional extensive surveys are necessary to characterize the photosymbiotic ascidian fauna in this area.

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RESEARCH ARTICLE



# A new genus and species in the mite family Eupodidae (Acari, Eupodoidea) from Crimea

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#### Abstract

A new genus *Pseudoeupodes* Khaustov, **gen. n.** and new species *Pseudoeupodes porosus* **sp. n.** are described from moss in Crimea. The taxonomy of the Eupodidae and some other families and genera of Eupodoidea is reviewed. The genus *Turanopenthalodes* Barilo, 1988 is transferred from Penthalodidae to Penthaleidae. The family Cocceupodidae Jesionowska, 2010 and the genus *Filieupodes* Jesionowska, 2010 are considered as junior synonyms of Eupodidae Koch, 1842 and *Cocceupodes* Thor, 1934, respectively. A key to genera of the family Eupodidae is provided.

#### **Keywords**

Acarina, Eupodoidea, Eupodidae, Pseudoeupodes, systematics, key, Crimea

# Introduction

Mites of the cosmopolitan superfamily Eupodoidea Koch, 1842 are fungivorous, phytophagous and predatory. The classification of the superfamily lacks stability (Baker and Lindquist 2002). The superfamily Eupodoidea currently includes nine families: Eupodidae Koch, 1842, Penthaleidae Oudemans, 1931, Penthalodidae Thor, 1933, Rhagidiidae Oudemans, 1922, Strandtmanniidae Zacharda, 1979, Eriorhynchidae Qin & Halliday, 1997, Pentapalpidae Olivier & Theron, 2000, Dendrochaetidae Olivier, 2008 and Cocceupodidae Jesionowska, 2010 (Jesionowska 2010; Walter et al. 2009). The validity of the latter two families is problematic in my opinion (see Discussion). The family Eupodidae includes two subfamilies: Benoinyssinae Fain, 1958 and Eupodinae Koch, 1842, although this subdivision is not followed by most workers. The genera *Cocceupodes* Thor, 1934 and *Linopodes* Koch, 1835, which were previously placed in the family Eupodidae, were recently transferred to the separate family Cocceupodidae (Jesionowska 2010); and the genus *Hawaiieupodes* Strandtmann & Goff, 1978 was transferred to the family Penthalodidae (Jesionowska 2008).

This paper presents a description of a new genus and species of eupodid mite, *Pseudoeupodes porosus* gen. n., sp. n., collected from moss in Crimea, and discusses the taxonomy of some families and genera of Eupodoidea.

#### Materials and methods

Mites were collected from moss using Berlese funnels and mounted in Hoyer's medium. Notations for the prodorsal and leg setae follow Lindquist and Zacharda (1987) and Baker (1995), and the remaining nomenclature is as applied to eupodoids by Baker (1990). All measurements are given in micrometres ( $\mu$ m) for the holotype and for five paratypes (in parentheses). In descriptions of leg setation the number of solenidia is given in parentheses. Photographs were taken with a digital camera Tucsen 3.0 via the ocular of light microscope MBI-11 with phase contrast device. The type material is deposited in the mite collection of the Tyumen State University, Tyumen, Russia.

#### **Systematics**

Family Eupodidae Koch, 1842

# Genus Pseudoeupodes Khaustov, gen. n. http://zoobank.org/BABD612A-51F7-4111-A1D5-31760CE6208A

#### Type species. Pseudoeupodes porosus Khaustov, sp. n. Monotypic.

**Description. Female**. *Idiosomal dorsum* (Figs 1, 11–13). Idiosoma oval. Cuticle soft and striated. Sejugal furrow well developed. Prodorsum with three pairs of tactile setae  $(v_1, v_2, sc_2)$  and a pair of filiform trichobothria  $(sc_1)$ . Naso (epivertex) folded downward onto ventral surface of prodorsum, setae  $v_1$  situated on dorsal part of naso near anterior margin of prodorsum; naso defined by different pattern of striation from surrounding prodorsum (Fig. 11). Hysterosoma with eight pairs of dorsal setae  $(c_1, c_2, d_1, e_1, f_1, f_2, h_1, h_2)$  and three pairs of large round lyrifissures (*ia*, *im*, *ip*). Hysterosoma dorsally with two transverse furrows, between setae  $c_1$  and  $d_1$ , and between  $e_1$  and  $f_1$ . Setae  $f_1$  not trichobothrium-like.

*Idiosomal venter* (Figs 2, 3, 14–15). Coxisternal setal formula 3-1-4-2; six pairs of eugenital setae; six pairs of genital setae; five pairs of aggenital setae; two pairs of pseudanal setae; one pair of lyrifissures (*ih*), same form as dorsals.

*Gnathosoma* (Figs 4–6). Palp setal formula 0-2-3-8( $\varpi$ ), tarsus ovoid. Chelicerae: typical for eupodid mites, movable digit slender and acuminate distally, fixed digit distinctly shorter than movable digit and truncated distally; seta *cha* present.

*Legs* (Figs 7–10). All legs shorter than body. Soft cuticle separating coxisternal plates and trochanters of all legs with distinct pore-like structure (Fig. 14). Rhagidial organ I with two longitudinally arranged solenidia; rhagidial organ II with three longitudinally arranged rhagidial solenidia. Tarsus I with famulus (stellate setae) situated in shallow depression; tarsus II with spine-like famulus. Tibiae I and II with one distal rhagidial solenidion; tibiae I-III with proximal erect solenidion; genua I and II with one erect solenidion. Femur IV not enlarged. Trochanteral setal formula 1-1-1.

Male and immatures. Unknown.

**Etymology.** The genus name is derived from the related genus *Eupodes* and prefixed pseudo- (from Greek  $\psi \epsilon \upsilon \delta \eta \varsigma$ ) meaning false.

Differentiation of the genus. The new genus is most similar to *Caleupodes* Baker, 1987. Both genera are characterized by the naso folded down to the ventral surface of the prodorsum, the same palpal chaetotaxy, six pairs of eugenital and five pairs of aggenital setae, the presence of only two pairs of pseudanal setae, femur IV not enlarged, trochanteral setal formula 1-1-1-1, and legs I-IV shorter than idiosoma. The new genus differs from *Caleupodes* by having striated dorsal cuticle (reticulated in *Caleupodes*), the absence of a transverse furrow between segments D and E (all hysterosomal segments are clearly separated by transverse furrows in *Caleupodes*), coxisternal setal formula 3-1-4-2 (3–1–4–3 in *Caleupodes*), and genua I and II with a solenidion (absent in *Caleupodes*). The new genus is also closely related to Niveupodes Barilo, 1991 in the naso folded down to the ventral surface of the prodorsum, femur IV not enlarged, trochanteral setal formula 1–1–1–1, legs I-IV shorter than idiosoma, and only two pairs of pseudanal setae. It differs from Niveupodes by the presence of dorsal transverse furrows between segments C -D and E - F (dorsal hysterosoma without transverse furrows in *Niveupodes*), coxisternal setal formula 3-1-4-2 (3-1-4-3 in Niveupodes), five pairs of aggenital and six pairs of eugenital setae (four aggenital and five eugenital in Niveupodes), the absence of scapular lyrifissure isc (present in Niveupodes, according to Barilo 1991), and the ovoid palptarsus (cone-shaped in Niveupodes). The new genus differs from all other known eupodoid genera by the presence of pore-like structures of unknown origin and function situated on the soft cuticle between the coxisternal plates and the trochanters of all legs. These pore-like structures are unknown in any other group of trombidiform mites.

#### Pseudoeupodes porosus Khaustov, sp. n.

http://zoobank.org/3897531B-758D-4AC5-B0F0-F9D85048964C Figs 1–15

Description. Female. Length of idiosoma 288 (280-300), width 163 (157-180).

*Idiosomal dorsum* (Figs 1, 11–13). Idiosoma with striae bearing microtubercles (Figs 11–13). All dorsal setae densely pilose, setae  $v_1$  slightly widening apically. Prodorsum with



Figures 1-2. Pseudoeupodes porosus Khaustov, gen. n., sp. n., female: 1 idiosomal dorsum 2 idiosomal venter.

pair of longitudinal lines between the bases of setae  $sc_2$  and anterior margin of prodorsum near setae  $v_1$ . Setae  $sc_1$  with large barbs, a weak reticulate subcuticular ornamentation visible posteriorly to bases of  $sc_1$  (Fig. 11). Lyrifissures *ia* situated posterolaterally to bases of setae  $c_1$ ; *im* situated posterolaterally to bases of setae  $d_1$ ; *ip* situated between setae  $e_1$  and  $f_1$ . Length of dorsal setae:  $v_1$  9 (98-10),  $v_2$  16 (15–19),  $sc_1$  50 (47–55),  $sc_2$  15 (14–17),  $c_1$  13 (12–16),  $c_2$  21 (19–25),  $d_1$  16 (14–19),  $e_1$  15 (14–18),  $f_1$  40 (37–45),  $f_2$  25 (24–28),  $h_1$  23 (21–26),  $h_2$  17 (15–21). Setae  $f_1$  longest of dorsal hysterosomal setae but not of trichobothrial form.

*Idiosomal venter* (Figs 2, 3, 14–15). All ventral setae densely pilose. Setae 1*a*, 3*a*, 4*a* (10–13) slightly widened distally; setae 1*c* the shortest on coxal fields. Genital setae arranged in one longitudinal row, anterior five pairs  $(g_1-g_5)$  situated on non-striated genital covers bearing only microtubercles, posterior pair  $(g_6)$  situated outside genital covers on striated cuticle (Figs 3, 15). Anterior two pairs of genital setae distinctly longer (10–11) than other genitals (6–7). Aggenital setae increasing in length from  $ag_5$  (7) to  $ag_1$  (10–11). Eugenital setae situated on protuberances and arranged in three groups: two anterior  $(eu_1, eu_2)$ , one medial  $(eu_3)$  and three posterior  $(eu_4-eu_6)$  (Fig. 3). Pseudanal setae  $ps_1$  15 (14–18) distinctly longer than  $ps_3$  11 (10–14). Lyrifissure *ih* located anterolaterally to bases of setae  $ps_3$ .



Figures 3-6. *Pseudoeupodes porosus* Khaustov, gen. n., sp. n., female: 3 genital area 4 subcapitulum 5 chelicera, antiaxial aspect 6 palp, antiaxial aspect.

*Gnathosoma* (Figs 4–6). Integument papillate. Subcapitulum (Fig. 4) roughly triangular, with two pairs of minute smooth adoral setae  $(or_1, or_2)$ , located subapically; subcapitular setae  $sbc_2$  densely pilose,  $sbc_1$  smooth, located laterally at level of proximal margin of palp trochanters, about one-third as long as  $sbc_2$ ,  $sbc_2$  inserted ventrally one third to one quarter of distance between  $sbc_1$  and tip of subcapitulum, labrum acuminate. Chelicera (Fig. 5) 60 in length. Palps (Fig. 6) with supracoxal seta *ep* minute, brush-like, femorogenual and tibial setae densely pilose, tarsal setae *acm* and sl''smooth, other tarsal setae pilose, p "bifurcate distally.

*Legs* (Figs 7–10). Relative lengths of legs: I>IV>III>II. All leg setae densely pilose. Leg I (Fig. 7): Supracoxal setae *ep* of the same shape and length as palpal supracoxal setae



Figures 7-10. Pseudoeupodes porosus Khaustov, gen. n., sp. n., female: 7-10 legs I-IV, respectively.

*ep.* Femur incompletely divided into basi- and telofemur. Setal formula: Tr - 1, Fe - 6+5,  $Ge - 7(1\sigma)$ ,  $Ti - 7(2\varphi)$ , Ta - 18 (2 $\omega$ , 1 $\varepsilon$ ). Famulus  $\varepsilon$  (stellate seta) located in a shallow depression clearly posterolaterally to basal part of rhagidial solenidion  $\omega_1$  and anterior to seta *ft*". Rhagidial solenidion  $\varphi_1$  obliquely oriented toward anterior lateral margin of leg, located anterodorsally and situated in shallow depression; solenidion  $\varphi_2$  located postero-dorsally in the space between setae  $l'_1$  and  $l''_1$ ; solenidion  $\sigma$  located anterodorsally, about 1.5 times as long as  $\varphi_2$ . All setae on tarsus, *d* and *l* on tibiae are eupathidia (as applied for Eupodoidea by Lindquist and Zacharda 1987). Leg II (Fig. 8): Femur incompletely divided into basi- and telofemur. Setal formula: Tr - 1, Fe - 5+5,  $Ge - 4(1\sigma)$ ,  $Ti - 5(2\varphi)$ , Ta - 12 (3 $\omega$ , 1 $\varepsilon$ ). Famulus  $\varepsilon$  spine-like, located just posterolaterally to base of rhagidial



**Figures 11–15.** *Pseudoeupodes porosus* Khaustov, gen. n., sp. n., female: **11** prodorsum **12** striation in the area of setae  $c_1$  and  $d_1$  **13** striation in the area of setae  $d_1$  and  $e_1$  **14** venter of propodosoma, arrows point to pore-like structures **15** genital area.

solenidion  $\omega_1$ . Rhagidial solenidion  $\varphi_1$  obliquely oriented toward anterior lateral margin of leg, located anterodorsally and situated in shallow depression; solenidion  $\varphi_2$  located posterodorsally; solenidion  $\sigma$  located anterodorsally, subequal to  $\varphi_2$ . Tarsal setae (*u*), (*p*), (*it*) and *tc*' are eupathidia. Leg III (Fig. 9): Setal formula: Tr – 1, Fe – 4+4, Ge – 4, Ti – 5(1 $\varphi$ ), Ta – 10; femur completely divided into basi- and telofemur; solenidion  $\varphi$  located posterodorsally; all setae on tarsus and *d* on tibia are eupathidia. Leg IV (Fig. 10): Setal formula: Tr – 1, Fe – 2+3, Ge – 4, Ti – 5, Ta – 11; femur completely divided into basiand telofemur; tarsal setae (*u*), *p*', *ft*' and *d* on tibia are eupathidia.

**Type material.** Holotype female, slide № AK210494, **CRIMEA**: Yalta mountainforest Nature Reserve, moss on soil, 21 April 1994, coll. A.A. Khaustov. Paratypes: five females, same data as holotype; seven female paratypes, **CRIMEA**: Yalta, moss on soil, 5 March 1994, coll. A.A. Khaustov.

**Etymology.** The name of the new species refers to the presence of unusual pore-like structures in the soft cuticle between the coxisternal plates and the trochanters of all legs.

# Discussion

The present systematic organisation of the family Eupodidae and the superfamily Eupodoidea is highly unstable. Preliminary morphological cladistic analyses (Qin 1996, Qin and Halliday 1997) suggested that only two families (the Eriorhynchidae and Penthalodidae) are monophyletic. Of the other four, the Rhagidiidae plus Strandtmanniidae formed a monophyletic group, the Penthaleidae was paraphyletic, and the status of the Eupodidae was not resolved (Baker and Lindquist 2002). Jesionowska (1989) described the genus Protopenthalodes Jesionowska, 1989 in the family Penthalodidae and moved Hawaiieupodes Strandtmann & Goff, 1978 from Eupodidae to Penthalodidae (Jesionowska 2008). According to Jesionowska (2008), this new concept of the Penthalodidae will be published in a separate paper. Qin (1997) reconsidered the taxonomic position of Protopenthalodes and suggested that this genus is more appropriately placed in the family Eupodidae because of soft body integument. Barilo (1988) described Turanopenthalodes Barilo, 1988, which is another problematic genus in the family Penthalodidae. The key characters of the family Penthalodidae are the fully sclerotized body and the presence of an epirostrum projecting over the gnathosoma (Qin and Halliday 1997; Olivier 2008; Walter et al. 2009), but other characters such as the idiosomal setation have never been used to separate Penthalodidae from other families of Eupodoidea. In my opinion the genera Hawaiieupodes and Protopenthalodes are more closely related to Penthalodidae than to Eupodidae, in agreement with the suggestion by Jesionowska (2008). Like Penthalodes Murray, 1877, the type genus of the family Penthalodidae, both Hawaiieupodes and Protopenthalodes have the following synapomorphies: sejugal furrow not developed, lens-like eyes present near the setae  $sc_2$ , setae  $h_2$  absent, naso usually very small, oval with minute setae  $v_1$ . This combination of characters is not found in the closely related families Penthaleidae and Eupodidae, and following Jesionowska (2008), I retain Hawaiieupodes and Protopenthalodes in the Penthalodidae. The position of the genus *Turanopenthalodes* in the family Penthalodidae is doubtful. Barilo (1988) placed Turanopenthalodes in Penthalodidae based on a single character, the presence of epirostral processes lateral to the naso, similar to those found in the penthalodid genus Stereotydeus Berlese, 1901, which he considered the main differential character of the family Penthalodidae. Other apomorphic characters of this genus, such as neotrichy of the idiosoma, a small anal opening situated dorsally, short and truncated palptarsus and very characteristic "trident" at the distal end of the fixed digit of the chelicera, are similar to those found in Penthaleus Dugès, 1834, the type genus of the family Penthaleidae, and I currently place Turanopenthalodes in the family Penthaleidae. Undoubtedly some characters that are now used to separate some families in the Eupodoidea should be re-evaluated. Such an attribute as more conspicuously sclerotized dorsal body surfaces, which is characteristic of the family Penthalodidae (Walter et al. 2009), is highly variable. There is an undescribed species of *Protopenthalodes* in my collection with

a soft body, but having subcuticular reticulate ornamentation throughout the body surface, which I consider as intermediate in the extent of body sclerotization.

The monotypic family Dendrochaetidae (Olivier 2008, 2009), which includes only the genus *Dendrochaetus* Olivier, 2009, shares some synapomorphic characters with *Hawaiieupodes* and *Protopenthalodes*. All these genera lack setae  $h_2$ , the sejugal furrow is absent, the naso is small, almost round, and well separated from the anterior margin of the prodorsum. The only difference between Dendrochaetidae and soft-bodied Penthalodidae (*Hawaiieupodes* and *Protopenthalodes*) is the presence of an additional transverse furrow at the level of setae  $v_1$ . *Dendrochaetes acarus* (Olivier, 2008) needs to be restudied and redescribed to clarify the status of the family Dendrochaetidae.

Jesionowska (2010) erected the family Cocceupodidae, in which she included three genera: Cocceupodes Thor, 1934, Linopodes Koch, 1835 and Filieupodes Jesionowska, 2010. According to Jesionowska (2010) the family Cocceupodidae differs from Eupodidae by two main characters: setae  $v_1$  situated posterior to naso and the presence of only two pairs of circumanal setae ( $p_{s_1}$  and  $p_{s_2}$ ). In my opinion, the decision to create the family Cocceupodidae is groundless. The similar location of setae  $v_1$  on dorsal part of naso near anterior margin of prodorsum is also found in the eupodid genera Caleupodes, Niveupodes and Pseudoeupodes, but in these genera the naso is not directed anteriorly, but folded to the ventral side of the prodorsum. The presence of only two pairs of pseudanal setae (ps, absent, according to Baker 1990) is a variable character in the family Eupodidae. Setae  $ps_2$  are absent in the genera Caleupodes, Niveupodes, Pseudoeupodes and Benoinyssus Fain, 1958. The absence of setae  $ps_{1}$  is a reduction and could happen independently in different lineages of eupodoid mites (homoplasy). On the other hand, Jesionowska (2010) did not mention some synapomorphic characters of *Cocceupodes* and *Eupodes* Koch, 1842. Both genera have characteristic swollen femora IV adapted for jumping. Another synapomorphic attribute is the relatively long and thin legs I, which are usually subequal to or longer than the idiosoma, and much longer than legs II. In early derivative genera of Eupodidae, such as Neoprotereunetes Fain & Camerik, 1994, Caleupodes and *Niveupodes*, femora IV are not swollen and legs I are not so long and thin. Thus, the characters separating Cocceupodidae and Eupodidae proposed by Jesionowska (2010) are variable within the family Eupodidae. I therefore consider the family Cocceupodidae as a junior synonym of Eupodidae.

Jesionowska (2010) created a new genus *Filieupodes*, which differs from *Cocceupodes* by a single character, the filiform setae  $v_1$  (clavate or capitate in *Cocceupodes*). Filiform setae  $v_1$  is a plesiomorphic character state and should not be used for recognition of a new taxon. I therefore consider *Filieupodes* as a junior synonym of *Cocceupodes*.

Currently I recognize 11 genera in the family Eupodidae: *Xerophiles* Jesionowska, 2003, *Benoinyssus*, *Claveupodes* Strandtmann & Prasse, 1976, *Eupodes*, *Aethosolenia* Baker and Lindquist, 2002, *Neoprotereunetes*, *Linopodes*, *Cocceupodes*, *Niveupodes*, *Caleupodes*, and *Pseudoeupodes* gen. n.

# Key to the genera of the family Eupodidae

1	Setae <i>f</i> <sub>1</sub> trichobothrium-like <b>2</b>
_	Setae $f_1$ not trichobothrium
2	Setae <i>ps</i> , present
_	Setae ps, absentBenoinyssus
3	Setae <i>ps</i> , present
_	Setae <i>ps</i> , absent7
4	Trichobothria (sc,) filiform
_	Trichobothria (sc,) clavate
5	Femur IV not swollen
_	Femur IV swollen, adapted for jumpingEupodes
6	Setae $h_{1}$ trichobothrium-like, adanal setae present, tibia and tarsus I much
	thicker than other leg segments
_	Setae $h_1$ not trichobothrium, adanal setae absent, tibia and tarsus I not en-
	larged
7	Leg I shorter or slightly longer than idiosoma, solenidia in rhagidial organs
	not T-shaped
_	Leg I more than 3 times longer than idiosoma, solenidia in rhagidial organs
	T-shaped Linopodes
8	Leg I distinctly shorter than idiosoma, sejugal furrow well developed, femur
	IV not enlarged, naso folded to ventral surface of prodorsum
_	Leg I usually longer than idiosoma, sejugal furrow absent or poorly devel-
	oped, femur IV enlarged, naso directed anteriorly
	Соссепродея
9	Hysterosoma dorsally with 3 pairs of lyrifissures
_	Hysterosoma dorsally with 4 pairs of lyrifissures (scapular lyrifissure present)
	Niveupodes
10	Idiosoma dorsally reticulated, all hysterosomal segments delineated by dis-
	tinct transverse furrows, solenidia on genua I and II absent
-	Idiosoma dorsally striated, transverse furrows present only between segments
	C–D and E–F, solenidia on genua I and II present
	Pseudaeutades gen n.

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RESEARCH ARTICLE



# First fossil record of Discocephalinae (Insecta, Pentatomidae): a new genus from the middle Eocene of Río Pichileufú, Patagonia, Argentina

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# Abstract

A new genus and species of Discocephalini, *Acanthocephalonotum martinsnetoi* gen. n. et sp. n. is described from Río Pichileufú, middle Eocene of Patagonia, Argentina at palaeolatitude ~ 46°S. The new species is the first fossil representative of the Discocephalinae. This taxon is extant in equatorial to subtropical America, and some species reach warm temperate latitudes (Buenos Aires province). The new genus is distinguished from the other genera of Discocephalini by the combination of these characters: interocular width greater than head length; head massive and quadrangular with the anterior margin almost straight; juga touching each other; labrum thick and curved; triangular ante-ocular process extending beyond the eye; broad spine-like antero-lateral process of the pronotum; pronotum explanate and bean shaped; scutellum triangular with a circular tongue reaching the anterior side of abdominal segment 7; and wings well developed with membrane just surpassing end of abdomen.

#### Keywords

Acanthocephalonotum gen. n., Heteroptera, Discocephalini

# Introduction

Pentatomidae is a diverse and globally distributed family of insects with nearly 900 described genera and 4722 living species (Schuh and Slater 1995; Rider 2006, 2011). Their fossil record is less rich, including about 136 species restricted to the Cenozoic (Mitchell 2013) of Asia, Europe, and Central and North America. This distribution is of course due to the presence of palaeoentomologists and intensive collection of fossil insects in these regions. Our fossil belongs to Discocephalinae (Petrulevičius 2008), whose distribution is restricted to the Western hemisphere like three other pentatomoid Neotropical subfamilies: Cyrtocorinae, Edessinae and Stirotarsinae. Until now, the Discocephalinae had no fossil record. The extant forms are restricted to the Neotropical region and represented by 44 genera in Discocephalini and 32 in Ochlerini (Campos and Grazia 2006; Garbelotto et al. 2013; Grazia et al. 2012).

The single specimen comes from the Patagonian locality of Río Pichileufú, Río Negro, Argentina (Petrulevičius 2008). The locality was dated using <sup>40</sup>Ar/<sup>39</sup>Ar analyses in Wilf et al. (2005) and recalculated in Wilf (2012), giving an age of 47.74 ± 0.05 Ma (million years ago). The locality is renowned for its very high plant diversity (Berry 1938; Wilf et al. 2005, Wilf 2012). Previously reported insects from the same site is the Myrmeciinae ant, *Archimyrmex piatnitzkyi* (Viana and Haedo Rossi 1957; Dlussky and Perfilieva 2003). Other species of *Archimyrmex, A. smekali* (Rossi de García 1983; Dlussky and Perfilieva 2003) comes from the close but of unknown age locality of Confluencia (Petrulevičius 1999, 2001; Petrulevičius and Martins-Neto 2000). Patagonian Eocene localities are exponentially increasing their known plant and insect diversity in recent years through sustained efforts to collect and describe their fossils (Wilf et al. 2005), giving interesting results in, e.g., Odonata (Frenguelliidae, Austroperilestidae and Aeshnidae; Petrulevičius and Nel 2003, 2005, 2013; Petrulevičius et al. 2010; Petrulevičius 2013) and Mecoptera (Petrulevičius 2005, 2009).

#### Material and methods

The fossil is housed at the Museo Asociación Paleontológica Bariloche (repository prefix MAPBAR), San Carlos de Bariloche, Río Negro, Argentina. Recent specimens of Discocephalinae are housed in the Entomological collection (Box 1895) of the Museo de La Plata (MLP), La Plata, Argentina. The holotype of *Glyphuchus sculpturatus* Stål, 1858 is housed in the Naturhistoriska riksmuseet, Stockholm, Sweden.

The fossil and recent specimens from Argentina were photographed with a Nikon D5000 digital camera. The new species was drawn with a camera lucida attached to a Wild M8 stereomicroscope.

# Systematic palaeontology

Hemiptera Linnaeus, 1758 Heteroptera Latreille, 1810 Pentatomomorpha Leston, Pendergrast & Southwood, 1954 Pentatomoidea Leach, 1815 Pentatomidae Leach, 1815 Discocephalini Fieber, 1860

# *Acanthocephalonotum* gen. n. http://zoobank.org/C0D67A2B-DDB6-4AC0-86DB-98E23E1D6304

#### Type species. Acanthocephalonotum martinsnetoi sp. n.

**Diagnosis.** Pronotum with the humeral and posterior angles developed; origin of the labium caudad of the anterior limit of the eyes; head wider than long, anterior margin of head almost straight; labrum thick and curved; juga touching each other before clypeus; interocular width greater than head length  $(1.16 \times)$ ; triangular ante-ocular process extending beyond the eye and perpendicular to the sagittal plane; pronotum with an antero-lateral process (broad spine-like), parallel to the sagittal plane; scutellum triangular with a developed and circular tongue; wings well developed with membrane just surpassing end of abdomen; costal margin bending acutely before end of basal half (boomerang shaped); apex of the scutellum not reaching the apex of corium.

Included species. Type species: Acanthocephalonotum martinsnetoi sp. n.

**Etymology.** From the Latin *acanthus*, meaning spiny, the Greek κεφαλή, meaning head and the Greek νώτος, meaning dorsal and signalling dorsal part of prothorax. "After the head and pronotum with broad spine-like processes".

#### Acanthocephalonotum martinsnetoi sp. n.

http://zoobank.org/53C87349-C765-463A-B96D-EA1E5A9896C4 Figs 1, 2

**Diagnosis.** Same as for the genus, by monotypy.

**Description.** The specimen is mainly complete and articulated in dorsal position with a composite view of dorsal and ventral structures.

Body: 4.7 mm long and 3.6 mm wide at pronotum; width (at the base of the hemelytra) / specimen length ratio, 0.78; antennae and legs not visible; head broad, almost rectangular with numerous punctures, wider than long; anterior margin of head almost straight in almost all its width; head 1.15 mm wide in its anterior margin, 0.8 mm long; eyes, 0.24 mm wide, 0.11 mm long; anteocular length 0.36 mm; inter-ocular width 0.95 mm; inter-ocular width / head length ratio, 0.84; distance between



**Figure 1.** Habitus of *Acanthocephalonotum martinsnetoi* gen. n. et sp. n. Holotype specimen MAPBAR 4137 **A** Photograph **B** line drawing. Scale bars represent 1 mm.



**Figure 2.** Photograph of detail of head and thorax of *Acanthocephalonotum martinsnetoi* gen. n. et sp. n. Holotype specimen MAPBAR 4137. Scale bar represents 1 mm.

ocelli 0.48 mm; distance between eyes and ocellus 0.2 mm; juga (= mandibular plates) touching each other before clypeus; apex of juga contiguous about 0.11 mm; lateral margins of juga deeply concave; clypeus bullet shaped; ante-ocular process extending beyond the eye and perpendicular to the sagittal plane, subtriangular shaped, 0.23 mm

long, with its anterior margin convex and posterior margin concave and beside the eye; labrum thick and curved (ventral structure); origin of the labium caudad of the anterior limit of the eyes (ventral structure); pronotum with a broad spine-like anterolateral process, stout and acute, parallel to the sagittal plane, 0.2 mm long; head length / pronotal width ratio, 0.87; pronotum with numerous punctures, strongly explanate and bean-shaped, 3.6 mm wide, 1 mm long; lateral margins rounded and irregular; scutellum triangular with a developed and circular tongue; scutellum about 2.8 mm wide at base, 1.9 mm long; tongue, 1.2 mm wide and 0.75 mm long; apex of tongue surpassing the corium; apex of scutellum reaching the anterior side of abdominal segment 7; posterior margin of abdominal segment 7 with three straight sides; gonocox-ites 8 (ventral structure) with sub-triangular truncate shape, outer lateral margins ob-liques, posterior ones straights; laterotergites 8 large, sub-triangular, truncate in inner lateral margins.

Wings: well developed membrane just surpassing end of abdomen; corium with punctures; costal margin bending acutely before end of basal half (boomerang shaped); costal angles of corium above ante-penultimate tergum; R slightly curved and followed by punctures by both sides; M slightly zigzagged; CuA almost straight and followed by punctures by both sides; venation not visible in membrane.

Material. holotype specimen MAPBAR 4137.

**Type locality and horizon.** Volcanic caldera-lake beds, Río Pichileufú, quarry RP3 (Wilf et al. 2005), province of Río Negro, Patagonia Argentina, palaeolatitude ~ 46°S; middle Eocene (47.7 Ma) (Wilf et al. 2005; Wilf 2012).

**Etymology.** Dedicated to the memory of Rafael Gioia Martins-Neto, outstanding palaeoentomologist and "irmão de alma", who unexpectedly and prematurely passed away in 2010 at age 56.

#### Discussion

The specimen is a female in dorsal position albeit some ventral structures of head and genitalia are visible resulting in a composite view. Females of Discocephalinae are recognized by having external genital structures as laterotergites and gonocoxites (Fig. 3A, C). Laterotergites 8 are joined by a transverse band visible from the dorsal side (Fig. 3A), but this structure is not visible in the fossil specimen (Figs 1, 2). Males of recent representatives of the group have a pygophore that is easily lost, leaving the free posterior face of the seventh segment with three sides (Fig. 3B, D).

The specimen can be attributed to a species of Pentatomoidea by the presence of several characters (Grazia et al. 2008): pronotum with the humeral and posterior angles developed, scutellum long, general outline of the body ovoid, head dorso-ventrally flattened and laterally carinate, and mandibular plates well developed, reaching or surpassing the clypeus. The first character is considered a synapomorphy of the group by Grazia et al. (2008) (character also present in some Coreidae; Pavel Štys rev. comm.). The new species can be attributed to Pentatomidae: Discocephalinae



**Figure 3.** Photographs of habitus of extant genera of Discocephalini **A-D** specimens of *Dryptochephala lurida* Erichson, 1848 **A** female specimen, dorsal view, Tucumán, Argentina **B** male specimen without terminalia, ventral view, Loreto, Misiones, Argentina **C** detail of female abdomen in ventral view, Tucumán, Argentina, Gc8: gonocoxite 8, La8: laterotergite 8, La9: laterotergite 9 **D** detail male abdomen with pygophore in dorsal view, Iguazú, Misiones, Argentina **E** holotype of *Glyphuchus sculpturatus* Stål, 1858, female specimen, dorsal view "Rio Janeiro" (Stål, 1872), Brazil. Scale bars represent 2 mm.

by having the origin of the labium caudad to the anterior limit of the eyes (Rolston and McDonald 1979; Rolston 1981). It has the inter-ocular width greater (1.2) than the head length. This character is shared with 14 genera of the tribe Discocephalini (Rolston, 1990) called "broadheaded discocephalines". Rolston (1990) explicitly excluded from this group two genera, *Parvamima* Ruckes, 1960 and *Dryptocephala* Laporte, 1833, because they differ vastly from each other and the other broad-headed genera (Rolston 1990). There are other five broad-headed genera of Discocephalini not discussed by Rolston (1990): *Dentocephala* Ruckes, 1960, *Alcippus* Stål, 1867, *Paralcippus* Becker and Grazia, 1986, *Glyphuchus* Stål, 1860, and *Oncodochilus* Fieber, 1851. Our species is not related to the "broadheaded discocephalines" sensu Rolston (1990) because of its explanate pronotum, quadrangular head and ante-ocular processes. However, the new species shares these characters with Dryptocephala (Ruckes, 1966a) and Glyphuchus (Stål, 1860). Parvamima (Ruckes, 1960) is quite different from our specimen with an almost triangular head and no explanate pronotum. Dryptocephala (Figs 3A-D) shares with the described species a strongly explanate bean-shaped pronotum and the ante-ocular processes well developed (Figs 3A, B). Differences between our species and Dryptocephala concern the head, which in the fossil is: 1) more massive, 2) quadrangular shape, 3) juga touching each other before the clypeus, 4) anterior margin almost straight in almost all its width, 5) ante-ocular processes perpendicular to the sagittal plane; concerning the pronotum: 6) anterolateral processes parallel to the sagittal plane; concerning the scutellum: 7) tongue well developed and circular in shape. Glyphuchus (Fig. 3d) shares with the described species the massive head, with quadrangular shape, with juga touching each other, and its anterior margin almost straight in almost all its width. Nevertheless, they differ in several characters, i.e., 1) head wider (head length / pronotal width ratio, 0.87 contra 0.79 in Glyphuchus sculpturatus Stål, 1858), 2) ante-ocular processes strongly developed, 3) scutellum bean-shaped, and 4) tongue circular shaped. Glyphuchus (Fig. 3D) also possesses some special characters, like scutellum explanate but with straight margins (anterior, antero-lateral, lateral, postero-lateral and posterior margins), and laterotergites strongly serrated making a stair-shaped abdomen. Stål (1872) related Oncodochilus to Glyphuchus because they share a thick and curved labrum which seems to be the case in the described species. Oncodochilus also shares with this latter the well developed tongue but with a different shape. Dentocephala (Ruckes, 1960) and Oncodochilus (Stål, 1860) differ in possessing the ante-ocular processes less developed and lacking an explanate pronotum. Our species could be differentiated from Oncodochilus, Alcippus (Stål, 1867), and Paralcippus (Becker & Grazia, 1986) because it has the head massive, and quadrangular, with the anterior margin almost straight in almost all its width, the ante-ocular processes perpendicular to the sagittal plane, the antero-lateral processes of the pronotum parallel to the sagittal plane, and the explanate pronotum surrounding the head. The last two features are quite similar in Paralcippus but the pronotum of that genus is narrower than the abdomen (wider in our specimen) and does not reach the anterior half of the head (reaching the anterior third in our specimen). Paralcippus and the new species differ also in the shape of the body (our specimen is much broader) and the tongue (almost quadrangular and incipiently bilobed in *Paralcippus* contra rounded in our specimen).

Other genera that are not broad-headed discocephalines but share other similarities with the new species are *Sympiezorhincus* Spinola, 1837, *Psorus* Bergroth, 1914, *Pelidnocoris* Stål, 1867, *Abascantus* Stål, 1864, and *Coriplatus* White, 1842. They all share a developed tongue (Becker 1977; Becker and Ruckes 1969; Ruckes 1966b; Ruckes and Becker 1970), but the shape is different, being much longer than in the new species. *Psorus* also has an explanate pronotum like the new species but has pedunculate eyes more posteriorly placed and the anterior margin of the head not straight (Fernandes et al. 2008). Considering the differences and unique characters of the new species with respect to the other genera of Discocephalini, we establish a new genus, *Acanthocephalonotum* gen. n.

The Discocephalinae are considered a tropical to subtropical taxon with some species reaching a warm temperate latitudes (Campos and Grazia 2006; Llosa et al. 1990; Grazia et al., submitted; Rider 2011). The southern distribution was Buenos Aires, Argentina (Becker and Grazia 1985; Berg 1884; Grazia et al., submitted) reaching with our discovery the Río Negro province at a palaeolatitude of ~ 46°S.

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RESEARCH ARTICLE



# Then there were five: a reexamination of the ant genus *Paratrechina* (Hymenoptera, Formicidae)

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#### Abstract

The ant genus *Paratrechina* is reexamined based on the discovery of two new species from Madagascar (*P. ankarana* **sp. n.** and *P. antsingy* **sp. n.**). *Paratrechina kohli*, a species known from central Africa, is transferred to *Paratrechina* from *Prenolepis* based on a new morphological interpretation of the genus and an updated morphological diagnosis of the genus is provided. This means that other than the widespread *P. longicornis*, whose origins remain uncertain, all *Paratrechina* are restricted either to the Afrotropical or Malagasy regions. It would also appear that of the five *Paratrechina* species now known, three are from dry forest habitats. With this reexamination of the genus, the possible origins of *P. longicornis* are discussed. A key to the genera of the *Prenolepis* genus-group is provided, as is a key to the workers of *Paratrechina*. In addition, we also designate a lectotype for *Paratrechina kohli*.

#### Keywords

Afrotropics, Madagascar, new species, Paratrechina, Prenolepis, taxonomy

# Introduction

During the course of revising the ant genera *Paraparatrechina* (LaPolla et al. 2010c) and Nylanderia (LaPolla and Fisher, in prep) in Madagascar, two species were discovered that were initially thought to belong to the genus Prenolepis. This was particularly interesting because the genus is unknown from the Malagasy region. Despite recent definition changes to *Prenolepis*, following the removal of the Neotropical species to their own genus, Zatania (LaPolla et al. 2012), the genus remains poorly understood taxonomically. There were two features of these two new species from Madagascar that gave us pause as to their potential placement in Prenolepis: they did not possess the characteristic mesonotal constriction immediately behind the pronotum that is present in most Prenolepis, and the eyes were towards the midline of the head, whereas most Prenolepis have high eyes relative to the midline of the head (LaPolla et al. 2010a). Given that these same characters are variable within Zatania, it was assumed Prenolepis was displaying a similar level of variability. However, to be certain, the DNA of one of the new Malagasy species was sequenced (due to sample's old age this was impossible for the second species) and compared against a large dataset of other formicine ants (Ward, unpublished data). The molecular data strongly suggested that the species from Madagascar was not a Prenolepis but rather belonged in Paratrechina. This in retrospect makes morphological sense because it renders the mesosomal constriction coupled with high eyes and long palps an unequivocal diagnostic feature of Prenolepis. It also provides for a more complete understanding of morphological variation observed within Paratrechina.

Here we describe those two species in the genus *Paratrechina* and make some definition changes to the genus, including moving a species from *Prenolepis* to *Paratrechina*, based on the discovery of these new species. We also provide an updated key to the genera of the *Prenolepis* genus-group.

# Materials and methods

Specimens examined for this study are deposited in the following institutions:

BMNH	Natural History Museum, London, U.K.
CASC	California Academy of Sciences, San Francisco, USA
PBZT	Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar
USNM	National Museum of Natural History, Washington, DC, USA

All measurements were taken at 80× power with a Leica M125 microscope using an orthogonal pair of micrometers, recorded to the nearest 0.001 mm, and rounded to two decimal places for presentation. Multiple specimens were measured for each species, and minimum and maximum measurements and indices are presented. All measurements are given in millimeters. Digital color images were created using a Lei-
ca DFC425 digital camera. Leica Application Suite software (ver. 3.8) was used for images. Each imaged specimen is uniquely identified with a specimen-level unique identifier (e.g. CASENT0454372). Morphological terminology for measurements and indices employed throughout are defined as (following LaPolla et al. 2011a, LaPolla et al. 2011b):

- EL (Eye Length): maximum length of compound eye in full-face view.
- **GL** (Gaster Length): the length of the gaster in lateral view from the anteriormost point of the first gastral segment (third abdominal segment) to the posteriormost point.
- **HL** (Head Length): the length of the head proper, excluding the mandibles; measured in full-face view from the midpoint of the anterior clypeal margin to a line drawn across the posterior margin from its highest points.
- **HW** (Head Width): the maximum width of the head in full-face view.
- **MMC** (Mesonotal Macrosetal Count): the number of erect macrosetae on mesonotum to one side of sagittal plane.
- **PW** (Pronotal Width): the maximum width of the pronotum in dorsal view.
- **PFL** (Profemur Length): the length of the profemur from its margin with the trochanter proximally to its margin with the tibia distally.
- **PMC** (Pronotal Macrosetal Count): the number of erect macrosetae on pronotum to one side of sagittal plane.
- **SL** (Scape Length): the maximum length of the antennal scape excluding the condylar bulb.
- **SMC** (Scape Macrosetal Count): the number of erect macrosetae on the scape visible in full frontal view. This count does not include the terminal cluster of setae often found around the joint of the scape and the funiculus.
- TL (Total Length): HL+WL+GL
- **WL** (Weber's Length): in lateral view, the distance from the posteriormost border of the metapleural lobe to the anteriormost border of the pronotum, excluding the neck.
- CI (Cephalic Index): (HW/HL) × 100
- **REL** (Relative Eye Length Index): (EL/HL) × 100
- **REL2** (EL/HW) × 100
- **SI** (Scape Index): (SL/HW) × 100

# **Results and discussion**

LaPolla et al. (2013) provided a diagnosis for *Paratrechina*, which was based on the only two species known from the genus at that time: *P. longicornis* (Latreille, 1802) and *P. zanjensis* LaPolla, Hawkes & Fisher, 2013. Based on both morphological similarity and molecular data (Ward, unpublished data), it would appear that those two species are sister taxa, so the earlier diagnosis provided for the genus was

morphologically restrictive compared to what we now know is a morphologically more diverse genus. This emphasizes that there is a need to keep documenting new species in this genus-group, in particular in some of the smaller genera (i.e. *Euprenolepis, Zatania*) where new species might cause some additional morphological definition changes. In more speciose genera, such as *Nylanderia* and *Paraparatrechina*, this appears to be less of a concern, as the morphological definitions provided (LaPolla et al. 2010a) have not needed to be amended despite the recent discovery of dozens of new species in each genus (Kallal and LaPolla 2012; LaPolla et al. 2010b; LaPolla et al. 2011b).

The addition of three species to *Paratrechina* not only allows for the more complete morphologically based definition provided, but it also simplifies the morphological diagnosis of *Prenolepis*. With the removal of *P. kohli* from *Prenolepis*, the mesonotal constriction immediately behind the pronotum becomes a characteristic of all *Prenolepis* species. Such a constriction is also seen in the Southeast Asian genus *Euprenolepis* (La-Polla 2009), as well as in the Caribbean and Mesoamerican genus *Zatania*, but only in three of the five known species (LaPolla et al. 2012).

With our placement of *P. kohli* into *Paratrechina* and the description of two endemic Malagasy species, the center of *Paratrechina* diversity is now squarely in the Afrotropical and Malagasy regions, which raises some interesting questions as to the origin of the now pantropical P. longicornis (LaPolla et al. 2013). A review of the argument that P. longicornis is Asian in origin is provided by Wetterer (2008) and LaPolla et al. (2013) (i.e., on the observation that P. longicornis has only been found in undisturbed habitats in tropical Asia). However, since P. zanjensis appears to be a miombo woodland specialist and its sister taxon is almost certainly P. longicornis, it raises the possibility that *P. longicornis* might be an African woodland specialist as well. Given the fact that most African woodland habitat has been impacted to some extent by humans, it might be difficult to prove that *P. longicornis* is in fact native there. It is worth noting that the two new Malagasy species described here are native to dry forest habitats on limestone outcrops. While certainly P. kohli is a rainforest species, we cannot dismiss the possibility that it is the only species native to rainforests in the genus. There is one report of P. longicornis from native forest in Cameroon (Dejean et al. 1996). However, only one specimen of P. longicornis (out of 62,708 specimens) was collected from 15 forest sites in Tanzania (P. Hawkes, pers. comm.), so the conclusions of the previous study remain equivocal. Clearly, the question of the native range of *P. longicornis* remains an open one, but with the discovery of multiple *Paratrechina* species in the Afrotropical and Malagasy regions, a more complete survey is needed, and an Asian origin for the species now seems questionable.

#### Diagnosis of the genus

For only one species are all castes known (*P. longicornis*); therefore we provide only a worker-based diagnosis for the genus.

Monomorphic, medium sized (2.1–3.2 mm in total length); ranging from almost black to brownish-yellow, with lighter mandibles, antennae (especially funicular segments towards tips) and legs (especially distal portion of tibiae and tarsi). Head with medially erect macrosetae roughly paired, extending through the medial portion of clypeus. Antennae 12 segmented; scapes long, with scape index above 140, in most species around or above 200 (SI range 143–226). Scapes with a dense layer of pubescence. Head is usually distinctly longer than wide, with cephalic index below 100 (CI range 71–94); posterolateral corners rounded, with straight posterior margin. Eyes large relative to head width (REL2 greater than 25); eyes distinctly convex, extending beyond head margin in full-face view. Mandibles in all species, except P. kohli, with 5 teeth; in P. kohli 8 teeth present, with 7th tooth on basal angle of mandible and 8th tooth on inner mandibular margin; mandalus large and anteriorly placed; palps very long; palp formula 6:4. Mesosoma elongated, most robust in P. kohli; most gracile in P. longicornis; propodeal dorsal face variable from either nearly flat (*P. longicornis*) or distinctly convex (*P. antsingy*); propodeum without macrosetae, anteriorly occasionally with a sparse layer of pubescence; pronotal setal count 6–12 (both sides of notum); mesonotal setal count 4–8 (both sides of notum). In lateral view, petiole cuneate, broadly rounded dorsally, with much longer posterior face and not surpassing the height of the propodeum. Legs distinctly long (profemur length 0.6–1.0 mm). Gaster robust, covered in abundant erect macrosetae.

#### Updated worker-based key to the genera of the Prenolepis genus-group

This key is modified from that found in LaPolla et al. (2012).

1	Maxillary palps with five or fewer segments; species often strongly polymor-
	phic, with a major and minor caste expressed2
_	Maxillary palps with six segments; species monomorphic to slightly polymor-
	phic, with no discernable major or minor caste expressed4
2	Polymorphic Afrotropical species
	Paraparatrechina (weissi species-group)
_	Monomorphic or polymorphic Australasian/Indoaustralian species
3	Eyes large, typically REL ≥ 20 (one exception: <i>Euprenolepis negrosensis</i> ); la-
	bial palps typically with four segments; mesothorax constricted immediately
	behind pronotum; mandalus large and conspicuous, usually visible without
	dissection of mandible (This character can be difficult to determine unless the
	mandible is dissected. It is only required if specimens have four-segmented
	labial palps AND five mandibular teeth.) Euprenolepis
_	Eyes small, REL < 20, typically 15 or less; labial palps typically with two
	or three segments; mesothorax typically not constricted immediately behind
	pronotum; mandalus small and inconspicuous, usually not visible without
	dissection of mandible Pseudolasius
4	Mesothorax constricted immediately behind pronotum

_	Mesothorax not constricted immediately behind pronotum
5	Head round in general appearance, with rounded, indistinct posterolateral
	corners; mesothorax always constricted immediately behind pronotum, typi-
	cally with distinctly convex eyes (if eyes not distinctly convex, then head is
	distinctly round)Prenolepis
_	Head rectangular in general appearance, with more angular, distinct postero-
	lateral corners; eyes relatively flat
6	Mandibles with five or eight teeth (one species, Paratrechina kohli, has eight
	teeth, one on basal angle and one on the inner mandibular margin, all others
	have five teeth)7
_	Mandibles with 6 teeth (a few species of Nylanderia have seven teeth, but
	never five or eight teeth as above)
7	Erect setae (one pair) present on propodeum; erect setae on head form a pat-
	tern of four setae along posterior margin and six or seven rows from posterior
	margin to clypeal margin; femora and tibiae lacking large erect setae
_	Erect setae absent on propodeum; erect setae on head scattered across surface;
	femora and tibiae with large erect setae Paratrechina
8	Scapes with either pubescence or very short abundant erect macrosetae (mac-
	rosetae no longer than width of scapes); profemur length greater than 0.8
	mmZatania (in part)
_	Scapes usually with macrosetae that are much longer than width of scapes; in
	a few species no macrosetae present, but profemur length is always less than
	0.8 mm
	•

# Synopsis of Paratrechina species

Paratrechina ankarana sp. n. Madagascar
Paratrechina antsingy sp. n. Madagascar
Paratrechina kohli (Forel, 1916), comb. rev. DR Congo
Paratrechina longicornis (Latreille, 1802) Pantropical tramp, origin uncertain
=Paratrechina currens Motschoulsky, 1863. Junior synonym of longicornis by Emery, 1892: 166. Neotype designated by LaPolla et al. 2010b: 1
=Paratrechina gracilescens (Nylander, 1856). Synonymy with longicornis by Roger 1863: 10
= Paratrechina longicornis hagemanni (Forel, 1901). Junior synonym of longicornis by Wheeler 1922: 942. Revived from synonymy by Emery 1925: 217. Junior synonym of longicornis by LaPolla et al. 2010a: 128.
= Paratrechina vagans (Jerdon, 1851). Junior synonym of longicornis by Dalla Torre 1893: 179; Forel 1894: 408

*Paratrechina zanjensis* LaPolla, Hawkes & Fisher, 2013 Angola, Mozambique and Tanzania

40

#### Worker-based key to Paratrechina species

1	Mandibles with eight teeth; six teeth on masticatory margin, one tooth on
	basal angle, another tooth on inner mandibular margin; head cuticle densely
	rugorecticulate
_	Mandibles with five teeth; head cuticle smooth and shiny2
2	Scapes without macrosetae; dorsum of propodeum almost flat to very shal-
	lowly domedlongicornis
_	Scapes with macrosetae; dorsum of propodeum rounded, dome-like
3	Macrosetae dark brown, occasionally with lighter tips; SI greater than 195;
	Afrotropical regionzanjensis
_	Macrosetae yellow to whitish; SI less than 195; Malagasy region4
4	Dark brown, with cuticle giving a faint greenish-blue reflection; propodeum
	with fine striations across surface ankarana
_	Brown with patches of lighter cuticle across body, giving species a mottled
	appearance; propodeum smooth and very shiny antsingy

#### Species descriptions

*Paratrechina ankarana* sp. n. http://zoobank.org/484B4368-33CB-4BFB-B387-C6F6A0D49A0D Figs 1–3

**Holotype worker.** MADAGASCAR: Province Antsiranna, Rés. Spéc. Ankarana; 22.9 km 224° SW Antivorano Nord; 80 m; 12°55'S, 49°7'E; 10-16.ii.2001; Fisher et al.; CASENT0454372 (CASC). 6 paratype workers with the same locality information as the holotype (CASC, USNM); 9 paratype workers, Antsiranna, Rés. Spéc. Ankarana; 22.9 km 224° SW Antivorano Nord; 210 m; 12°52'S, 49°14'E; 16-21.ii.2001; Fisher et al. (BMNH, CASC, PBZT, USNM).

**Worker diagnosis.** Dark brown, with cuticle giving a faint greenish-blue reflection; propodeum with fine striations across surface; SI less than 195.

Worker. *Measurements (n=6)* TL: 2.60–2.84; HW: 0.60–0.63; HL: 0.66–0.72; EL: 0.18–0.20; SL: 1.10–1.17; PW: 0.42–0.46; WL: 1.01–1.07; GL: 0.90–1.14; PFL: 0.80–0.90; CI: 85–92; REL: 27–29; REL2: 31–33; SI: 184–191; SMC: 24–30; PMC: 3–6; MMC: 3–4.

Dark brown; antennae lighter with trochanters yellow to white; cuticle smooth and shiny, except on propodeum which posseses fine striations across surface; under microscrope view cuticle has a faint greenish-blue reflection; abundant macrosetae across scapes, head, pronotum, mesonotum, and gaster; scapes with short pubescence. Head ovate; posterolateral corners rounded with complete posterior margin; midpoint of eyes at approximately midline of head; eyes convex; 3 small ocelli present; mandibles with 5 teeth; apical tooth the longest, 3<sup>rd</sup> tooth from apical shortest, remainder about the same size; outer



Figures 1–3. Lateral, full face and dorsal view of body. *Paratrechina ankarana* holotype worker CASENT0454372.

mandibular surface with slight striations across surface. In lateral view, pronotum rises in a straight margin towards mesonotum with only slight convexity towards mesonotal margin; propodeum large and bulbous, making division between dorsal and declivitous faces difficult; metanotal suture with distinct impression that extends down along the mesopleural/metapleural margin; mesopleuron with darkened ridge along impression.

**Etymology.** The specific epithet is derived from the name of the reserve where the species was found.



Figures 4–6. Lateral, full face and dorsal view of body. *Paratrechina antsingy* holotype worker CASENT0906916.

# Paratrechina antsingy sp. n.

http://zoobank.org/49E90C54-69FC-4D3B-91D0-9C5EACAFC875 Figs 4–6

Holotype worker. MADAGASCAR: Province Antsiranna, Montagne Français, 7.2 km 142° SE Diego Suarezi, 180m, 12°19'S, 49°20'E, 22–28.ii.2001, Fisher et al.

CASENT0906916 (CASC). 12 paratype workers with same locality information as holotype (BMNH, CASC, USNM).

**Worker diagnosis.** Cuticle smooth and very shiny; brown with patches of lighter cuticle across body, giving species a mottled appearance; SI less than 195.

Worker. *Measurements (n=6)* TL: 2.07–2.69; HW: 0.58–0.60; HL: 0.67–0.70; EL: 0.17–0.19; SL: 1.02–1.08; PW: 0.40–0.44; WL: 0.97–1.02; GL: 0.76–1.05; PFL: 0.74–0.78 CI: 84–89; REL: 26–28; REL2: 29–32; SI: 177–180; SMC: 24–30; PMC: 4–5; MMC: 3–4.

Brown with lighter areas on mandibles, antennae, lateral portions of head, mesonotum, pronotum, mesonotum, trochanters and joints of femora; first gastral tergite with mottled lighter and darker areas; cuticle smooth and very shiny, except for mesonotum, metanotal area and propodeum being slightly rugolose; scapes, head, pronotum, mesonotum and gaster with abundant macrosetae; scapes with short, appressed pubescence. Head ovate; posterolateral corners rounded with complete posterior margin; midpoint of eyes at approximately midline of head; eyes convex; 3 small ocelli present; mandibles with 5 teeth; apical tooth the longest, 3<sup>rd</sup> tooth from apical shortest, remainder about the same size; outer mandibular surface with slight striations across surface. In lateral view, pronotum convex; pronotal/mesonotal margin with mesonotum raised slightly above margin; propodeum convex with dorsal face rising steeply to a rounded peak with steep declivitous face; metanotal suture with distinct impression that extends down along the mesopleural/metapleural margin.

**Etymology.** The specific epithet is derived from the name of the province where the species was found.

#### Paratrechina kohli (Forel, 1916), comb. rev.

Figs 7-9

Prenolepis kohli Forel, 1916: 438 (worker and queen decribed). Syntype worker and queen, Democratic Republic of the Congo (Kohl) (MHNG) [examined]. Combination in *Paratrechina (Nylanderia*): Emery 1925: 218; in *Prenolepis*: LaPolla, Brady and Shattuck 2010a: 129. Lectotype [designated here], pinned worker, Congo (Kohl) (MHNG: CASENT0907121) [examined]; Paralectotype [designated here], pinned queen with same data as lectotype (MHNG: CASENT0907122) [examined].

**Worker diagnosis.** Head and mesosoma light brown to yellow with slightly darker gaster; mesosoma and head densely rugorecticulate; mandible with 8 teeth, one tooth on basal margin, another on inner mandibular margin.

Worker. *Measurements (n=6)* TL: 2.9–3.0; HW: 0.78–0.90; HL: 0.93–1.03; EL: 0.21–0.25; SL: 1.22–1.43; PW: 0.54–0.6; WL: 1.3–1.61; GL: 1.12–1.37; PFL: 0.84–1.04 CI: 79–85; REL: 22–25; REL2: 27–30; SI: 143–169; SMC: 0; PMC: 3–5; MMC: 1–3.



Figures 7-9. Lateral, full face and dorsal view of body. Paratrechina kohli worker CASENT0249635.

Light brown with darker gaster; head and mesosomal cuticle densely rugorecticulate; head, pronotum, mesonotum and gaster with scattered macrosetae; scapes without macrosetae, with layer of dense pubscence. Head subrectangular; posterolateral corners with slight angles; most of eyes above midline of head; eyes convex; no ocelli apparent; mandibles with with 8 teeth, one tooth on basal margin, another on inner mandibular margin; outer mandibular surface with slight striations across surface. In lateral view, pronotal margin nearly straight, at less than 45° angle rise towards mesonotum; pronotal/mesonotal margin with mesonotum raised slightly above margin; propodeum convex with rounded dorsal face and longer, steep declivitous face; metanotal suture with distinct impression.

#### Notes on Paratrechina species

Workers of *Paratrechina* are relatively easy to identify to species based on morphology. In addition to the color and sculpture differences listed in the key and descriptions, the two Malagasy species have shorter scapes than *P. longicornis* and *P. zanjensis* (SI less than 195) relative to their head widths. The widespread *P. longicornis* has the lowest profile of any *Paratrechina* with an almost flat pronotum, mesonotum, and propodeum. Scape macrosetae are variable within the genus with both *P. longicornis* and *P. kohli* lacking them altogether, but *P. ankarana, P. antsingy* and *P. zanjensis* than in the other species, typically well into the upper thirties and low forties. For the other three species the REL2 is typically in the upper twenties and low thirties, reflecting an overall wider head relative to eye length.

Only *P. longicornis* and *P. zanjensis* workers come closest to resembling one another and these can be easily separated based on the present or absence of scape macrosetae. Workers of all species, except *P. kohli*, have three small, but distinct, ocelli present. The number of mandibular teeth on *P. kohli* is particularly interesting. The tooth on the inner mandibular margin is separated from the basal angle tooth by a short diastema. It would not be surprising to occasionally find a *P. kohli* worker with more than 8 teeth because the single queen specimen known in collections has a tooth that is divided with two sets of cusps, implying more teeth might occasionally be expressed in individuals. In all other *Paratrechina* workers only five mandibular teeth are present. Males are known only from *Paratrechina longicornis* (LaPolla et al. 2013), so it is impossible at this time to discuss general male characteristics for this genus.

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CHECKLIST



# New records of bee flies (Diptera, Bombyliidae) from Cuatro Ciénegas, Coahuila, Mexico

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#### Abstract

Forty one new records of species of Bombyliidae are reported for Coahuila in northeastern Mexico. Nine of these species are reported for the first time for the country. The specimens were collected in the Cuatro Ciénegas Basin and Sierra La Madera mountains during 2007–2013. The modified distributions of species are discussed. The gaps in the distribution of many species suggest an undersampling of this group of insects in the north of Mexico.

#### **Keywords**

Biodiversity, distribution expansion, Nearctic region, desert fauna

#### Introduction

The bee flies (Bombyliidae) belong to the superfamily Asiloidea and are the eighth most diverse family within Diptera with 5382 described species (Pape et al. 2011). All species of Bombyliidae are parasitoids, hyperparasitoids or predators of immature stages of Coleoptera, Hymenoptera, Lepidoptera, Orthoptera, Neuroptera, and

Diptera (Yeates and Greathead 1997, Boesi et al. 2009). Unlike most other taxa, bee flies are most abundant and diverse in arid and semiarid portions of the world (Hull 1973, Evenhuis 1989). In the immature stages these insects function as a natural control for populations of other insects and as adults are efficient pollinators (Motten et al. 1981, Kearns 2001).

Some faunistic studies have been completed including Bombyliidae in Mexico (Rodríguez-Ortuño 1989, Ávalos-Hernández 2007), but the northern region of the country is poorly known for this family. Although Evenhuis and Greathead (1999) list 15 species of Bombyliidae for Coahuila, species richness in this state is probably higher as suggested by the richness of surrounding Mexican states with similar or even smaller size and similar ecosystems (e.g., Nuevo León, 37 species; Durango, 41 species) and of Texas (171 species), the nearest USA state.

Cuatro Ciénegas Basin in the northeast of Coahuila is especially interesting because of its geological history and the presence of water ponds and gypsum dunes, which create a different environment from the surrounding areas. The basin was a shallow sea from the Pangea breakup until the Eocene, 40 Ma, when the Sierra Madre Oriental in the east of Mexico rose isolating the Basin from the Atlantic Ocean (Souza et al. 2012). The physiology of Cuatro Ciénegas bacteria is similar to that of marine species, with which they are closely related (Souza et al. 2006). According to Moreno-Letelier et al. (2012) this evidence indicates that some water was kept trapped in the Basin when the ocean retreated giving the basin unique characteristics. These characteristics produced a high number endemism for vertebrates and prokaryotes in Cuatro Ciénegas (Souza et al. 2006, 2012).

The present study is the first known long-term systematic sampling of Diptera in Cuatro Ciénegas. The objective of this project is to complete the list of species of Bombyliidae in the basin and surrounding mountains. In this paper, 41 new specieslevel records for Coahuila from Cuatro Ciénegas are presented, including nine new records for Mexico. The modified distributions of the species are discussed.

# Methods

Beeflies were collected at nine sites from Cuatro Ciénegas Basin and Sierra La Madera within the Municipality of Cuatrociénegas (Figure 1). Abbreviations for study sites (Table 1) are used throughout. Samplings were performed during 2007-2013, using aerial net and a Malaise trap. The Malaise trap had white polyester netting, was square in configuration, 210 cm tall and 120 cm wide and the collecting head located at the top. Trap was set from 9:00 to 17:00 when weather conditions allowed it. To avoid damage to the specimens no killing agent was used, insects were extracted at the end of the day. Specimens were pinned and labeled. Generic identification was carried out under a stereomicroscope according to the keys by Hall (1981b) and Kits et al. (2008). Species were identified by the first and second authors with specialized keys for each genus and comparison with museum specimens, keys used for identification of each



**Figure 1.** Field work sites. Cuatro Ciénegas basin is located in Coahuila at northeast of Mexico. Sierra La Madera is located at northwest of the basin.

genera are specified below. Taxonomic classification and distribution data are based on Evenhuis and Greathead (1999) and host data are based on Hull (1973), if not indicated otherwise. Distribution gaps are suggested as disjunct distribution patterns or the result of under sampling by comparing the location of records in Mexico with those in the southern states of the USA. All specimens are deposited in the Colección Nacional de Insectos (Instituto de Biología, Universidad Nacional Autónoma de México; CNIN-IBUNAM).

# Results

A total of 41 new species-level records are presented for the state of Coahuila. Nine of these 41 species are recorded for the first time in Mexico, being their most southern records. Of the 15 species previously listed for Coahuila, two were collected during this study: *Heterostylum robustum* (Osten Sacken, 1877) (Material collected: CHU: Apr (1 M), Sep (1 M); EAM: Mar (2 M), Sep (1 F), Jun (2 F), Jul (1 F), Oct (1 F); ROR: Apr (1 F, 3 M), May (1 F, 2 M), Jul (3 F), Sep (1 F); RPA: Apr (1 F, 1 M), Jul (2 F, 2 M), Sep (1 M), Oct (2 M)); and *Anastoechus melanohalteralis* Tucker, 1907 (Material collected: EAM: Oct (1 M); ECA: Oct (1 F, 1M); ROR: Oct (7 F, 6 M); RPA: Sep (1 M)).

	Site (Code)	Location	Altitude (m)	Vegetation		
1	Churince (CHU)	N26°50'30", W102°08'10"	770	Gypsum dunes; sedges and marshes; mezquital, halophile		
2	Rancho Orozco (ROR)	N26°52'18", W102°05'17"	740	Sedges and marshes; mezquital; halophile		
3	Rancho Pozas Azules (RPA)	N26°49'39", W102°01'24"	710	Sedges and marshes; mezquital; halophile		
4	Ejido Antiguos Mineros (EAM)	N26°46'58", W102°00'20"	725	Sedges and marshes; mezquital; halophile		
5	El Cañón (ECA)	N27°00'34", W102°04'42"	780	Mezquital; desert scrub		
6	Ejido El Oso (EEO)	N27°03'08", W102°13'35"	1085	Desert scrub; chaparral		
7	Rancho El Espejo (REE)	N27°13'19", W102°30'19"	1425	Desert scrub; chaparral		
8	Rancho El Chupadero (REC)	N27°10'07", W102°34'26"	1790	Desert scrub; chaparral; Pine-Oak forest		
9	Rancho La Casita (RLC)	N27°06'45", W102°23'40"	1630	Desert scrub; chaparral; Pine-Oak forest		

Table 1. Field work sites in Cuatro Ciénegas. Vegetation according to Pinkava (1979).

New records of the species included in this paper are from 17 genera for which modern revisions are available. Six taxa of *Hemipenthes* (3), *Lordotus* (1), *Paravilla* (1) and *Rhynchanthrax* (1) could not be identified accurately, being probably undescribed species. Identification of species in another 10 genera found in the study (e.g. *Villa, Chrysanthrax*, and *Exoprosopa*) is difficult and unreliable. The number of morphospecies and specimens collected of these genera are presented in Table 2. Six species of *Tmemophlebia* (1), *Geron* (1), *Exoprosopa* (3) and *Villa* (1) previously listed for Coahuila were probably collected but specimens of these genera are still being identified. Taxonomic work will continue, updates of the species list and descriptions of the new taxa will be published in subsequent papers.

A total of 28 genera were found during this study, of which 21 are new records for the state. Two genera previously listed for Coahuila (*Neacreotrichus* and *Relictiph-thiria*) were not found in Cuatro Ciénegas area. With the new records presented here, the list of bee fly species in Coahuila increases to 56 (Table 2).

#### Subfamily Toxophorinae

#### Genus Toxophora Meigen

**Remarks.** *Toxophora* is distributed worldwide, being more diverse in the Afrotropical and Palearctic regions. Mexico's fauna includes three Neotropical species and five Nearctic species. All Nearctic species of Mexico were distributed in the western half of

**Table 2.** Updated list of genera and species of Bombyliidae in Coahuila (\* species not collected in this study, but recorded previously in Coahuila; \*\* species most likely collected in this study, but identification not yet certain).

Subfamily, genus and species name	New record	Unidentifiable material	
PHTHRIINAE			
Neacreotrichus Cockerell			
*Neacreotrichus consors (Osten Sacken, 1887)			
Poecilognathus Jaennicke	Coahuila	1 morphospecies, 3 specimens	
Relictiphthiria Evenhuis			
*Relictiphthiria psi (Cresson, 1919)			
Timemophlebia Evenhuis		1 morphospecies, 21 specimens	
** Tmemophlebia coquilletti (Johnson, 1902)			
TOXOPHORINAE			
Geron Meigen		2 morphospecies, 194 specimens	
** Geron holosericeus Walker, 1849	_		
Systropus Wiedemann	Coahuila	1 morphospecies, 5 specimens	
Toxophora Meigen	Coahuila		
Toxophora maxima Coquillett, 1886	Coahuila		
Toxophora virgata Osten Sacken, 1877	Coahuila		
BOMBYLIINAE		1	
Anastoechus Osten Sacken			
Anastoechus melanohalteralis Tucker, 1907			
Bombylius Linnaeus			
Bombylius (Bombylius) frommerorum Hall & Evenhuis, 1980	Coahuila		
* Bombylius (Bombylius) sylphae Evenhuis, 1984			
* Bombylius (Parabombylius) aleophilus (Hall & Evenhuis, 1981)			
* Bombylius (Parabombylius) coahuilensis (Hall & Evenhuis, 1981)			
* Bombylius (Parabombylius) paradoxus (Hall & Evenhuis, 1981)			
* Bombylius (Parabombylius) syndesmus (Coquillett, 1894)			
Conophorus Meigen	Coahuila	1 morphospecies, 3 specimens	
Heterostvlum Macquart			
Heterostylum croceum Painter, 1930	Mexico		
Heterostylum robustum (Osten Sacken, 1877)			
		1 morphospecies.	
Lordotus Loew	Coahuila	38 specimens	
Lordotus diplasus Hall, 1954	Coahuila		
Lordotus divisus Cresson, 1919	Coahuila		
Lordotus perplexus Johnson & Johnson, 1959	Coahuila		
Triploechus Edwards	Coahuila		
Triploechus novus (Williston, 1893)	Coahuila		
LOMATIINAE	1	l.	
Ogcodocera Macquart	Coahuila		
Ogcodocera analis Williston, 1901	Coahuila		
TOMOMYZINAE			
Paracosmus Osten Sacken	Coahuila		
Paracosmus (Paracosmus) morrisoni Osten Sacken, 1887	Coahuila		
ANTHRACINAE			
Anthrax Scopoli	Coahuila		
Anthrax atriplex Marston, 1970	Coahuila		

Subfamily, genus and species name	New record	Unidentifiable material
Anthrax cybele (Coquillett, 1894)	Mexico	
Anthrax georgicus Macquart, 1834	Coahuila	
Anthrax irroratus Say, 1823	Coahuila	
Anthrax oedipus Fabricius, 1805	Coahuila	
Anthrax pauper (Loew, 1869)	Mexico	
Anthrax seriepunctatus (Osten Sacken, 1886b)	Coahuila	
Aphoebantus Loew	Coahuila	4 morphospecies, 236 specimens
Chrysanthrax Osten Sacken	Coahuila	6 morphospecies, 240 specimens
Dipalta Osten Sacken	Coahuila	
Dipalta serpentina (Osten Sacken, 1877)	Coahuila	
Exoprosopa Macquart		9 morphospecies, 395 specimens
** Exoprosopa aztec Painter & Painter, 1969		
** Exoprosopa butleri Johnson & Johnson, 1958		
** Exoprosopa dorcadion Osten Sacken, 1877		
Hemipenthes Loew	Coahuila	3 morphospecies, 146 specimens
Hemipenthes jaennickeana (Osten Sacken, 1886a)	Coahuila	
Hemipenthes lepidota (Osten Sacken, 1886b)	Coahuila	
Hemipenthes scylla (Osten Sacken, 1887)	Coahuila	
Hemipenthes sinuosa (Wiedemann, 1821)	Coahuila	
Lepidanthrax Osten Sacken	Coahuila	
Lepidanthrax arizonensis Hall, 1976	Mexico	
Lepidanthrax disiunctus (Wiedemann, 1830)	Coahuila	
Lepidanthrax hesperus Hall, 1976	Coahuila	
Lepidanthrax hyposcelus Hall, 1976	Coahuila	
Lepidanthrax proboscideus (Loew, 1869)	Coahuila	
Ligyra Newman	Coahuila	1 morphospecies, 2 specimens
Neodiplocampta Curran	Coahuila	
Neodiplocampta (Neodiplocampta) miranda Hull & Martin, 1974	Coahuila	
Paravilla Painter	Coahuila	1 morphospecies, 48 specimens
Paravilla edititoides (Painter, 1933)	Coahuila	
Paravilla flavipilosa (Cole, 1923)	Coahuila	
Paravilla parvula Hall, 1981a	Coahuila	
Paravilla separata (Walker, 1852)	Mexico	
Poecilanthrax Osten Sacken	Coahuila	
Poecilanthrax effrenus (Coquillett, 1887)	Coahuila	
Poecilanthrax fasciatus Johnson & Johnson, 1957	Mexico	
Poecilanthrax hyalinipennis Painter & Hall, 1960	Mexico	
Poecilanthrax poecilogaster (Osten Sacken, 1886b)	Coahuila	
Rhynchanthrax Painter	Coahuila	1 morphospecies, 70 specimens
Rhynchanthrax capreus (Coquillett, 1887)	Mexico	
Rhynachantrax texanus (Painter, 1933)	Coahuila	
Thyridanthrax Osten Sacken	Coahuila	
Thyridanthrax pallidus (Coquillett, 1887)	Mexico	
Thyridanthrax selene (Osten Sacken, 1886b)	Coahuila	
Villa Lioy		9 morphospecies, 115 specimens
** Villa fumicosta Painter & Painter, 1962	1	, <u> </u>
Xenox Evenhuis	Coahuila	
Xenox xylocopae (Marston, 1970)	Coahuila	

the country. These two new records represent the first of this genus in Coahuila and the most eastern distribution of the Nearctic species in the country. The New World species of this genus were keyed using Cunha et al. (2011).

#### Toxophora maxima Coquillett, 1886

Figure 2a, b

#### Material examined. CHU: Jul (1 M); EEO: Jul (2 F, 2 M), Oct (1 F, 3 M).

**Known Nearctic records.** Mexico (Baja California, Baja California Sur, Coahuila); USA (Arizona, California, Idaho, Kansas, New Mexico, Oklahoma, Oregon, Texas).

**Comments.** In Mexico *T. maxima* was only known from Baja California Peninsula and now Coahuila. This apparent gap in its distribution is probably due to undersampling. Sampling of the intermediate zones is necessary to know if these populations form a continuous unit as they do in the southern states of USA.

#### Toxophora virgata Osten Sacken, 1877

Figure 2c, d

**Material examined.** EAM: Jun (1 F, 1 M), Jul (1 F); CHU: Aug (1 M), Oct (1 M); EEO: Jul (1 M), Oct (1 F, 1 M); RLC: Jun (1 M); ROR: Apr (1 F, 2 M); RPA: Oct (1 F).

**Known Nearctic records.** Mexico (Baja California Sur, Coahuila, Sonora); USA (Arizona, California, Colorado, Georgia, Idaho, Nevada, New Mexico, Oklahoma, Texas, Utah).

Known hosts. Odynerus sp. (Vespidae); Stenodynerus toltecus Saussure (Vespidae).

**Comments.** This species is present in the all southwestern states of the USA and northwest of Mexico. This is the first record in the northeast of Mexico. The species is probably also present in Chihuahua, between Sonora and Coahuila.

#### Subfamily Bombyliinae

#### Genus Bombylius Linnaeus

**Remarks.** With 278 described species, *Bombylius* is the second most diverse genus of Bombyliidae. It has a worldwide distribution being especially diverse in the Palearctic and Nearctic regions. One endemic species is present in Coahuila: *B. (Parabombylius) coahuilensis* (Hall & Evenhuis, 1981). Four other species are reported for the state: *B. sylphae* Evenhuis, 1984, *B. aleophilus* (Hall & Evenhuis, 1981), *B. paradoxus* (Hall & Evenhuis, 1981), *B. syndesmus* (Coquillett, 1894). A review with identification keys for Nearctic species is presented in Hall and Evenhuis (1980), later Evenhuis (1984) revised and present keys for the *comanche* group of America.



**Figure 2.** *Toxophora. T. maxima*, male (CNIN 1115) **a** dorsal view **b** lateral view; *T. virgata*, male (CNIN 1109) **c** lateral view **d** posterior view. All scale bars: 3 mm.

# **Bombylius (Bombylius) frommerorum Hall & Evenhuis, 1980** Figure 3

#### Material examined. EEO: Aug (1 M), Oct (1 F).

**Known Nearctic records.** Mexico (Chihuahua, Coahuila); USA (Arizona, New Mexico, Texas).

**Comments.** This species is restricted to the southwest of the USA and north of Mexico.

#### Genus Heterostylum Macquart

**Remarks.** The genus is only present in Nearctic and Neotropical regions. Although not as diverse as other genera (only 12 species), specimens from some species are abun-



Figure 3. Bombylius (B.) frommerorum, female (CNIN 772) a dorsal view b lateral view. All scale bars: 3 mm.

dant in the field. *Heterostylum robustum* was previously known from Coahuila and was collected during this study. This species is distributed from Canada to central Mexico. There are two revisions for this genus that contains identification keys, one by Hall and Evenhuis (1980) and the more recent by Cunha et al. (2007).

# Heterostylum croceum Painter, 1930

Figure 4

# Material examined. REE: Apr (1 F).

**Known Nearctic records.** Mexico (Coahuila); USA (Colorado, Kansas, Missouri, New Mexico, Texas).

**Comments.** *Heterostylum croceum* is recorded for the first time in Mexico; previously known from the southern-central United States. Hall and Evenhuis (1980) suggest that *H. croceum* may be related to *H. engelhardti* Painter, 1930 or even be a subspecies of that taxon, *Heterostylum croceum* is the eastern form and *H. engelhardti* the western form (Arizona, California, Texas, Utah) although both species are present in Texas. Cunha et al. (2007) comment that *H. engelhardti* can be distinguished by the presence of white to very pale yellow hair and brown-tipped hairs on the abdomen compared with the darker yellow hairs in *H. croceum*.

# Genus Lordotus Loew

**Remarks.** Most of the 29 species in this exclusively Nearctic genus are distributed in the southwest of the USA and north of Mexico, although eight species are present in the northwest of the USA (*L. apicula* Coquillet, 1887; *L. bipartitus* Painter, 1940; *L. diversus* Coquillett, 1891; *L. gibbus* Loew, 1863; *L. miscellus* Coquillett, 1887; *L.* 



Figure 4. Heterostylum croceum, female (CNIN 858) a dorsal view b lateral view. All scale bars: 3 mm.

*pulchrissimus* Williston, 1893; *L. striatus* Painter, 1940; *L. zona* Coquillett, 1887). The three species present in Coahuila are also found in California; their distribution probably includes all northern states of Mexico. Hall (1954) and Hall and Evenhuis (1982) present reviews of the genus and keys to the species.

Lordotus diplasus Hall, 1954

Figure 5a, b

Material examined. CHU: Sep (2 M); RLC: Sep (2 M); RPA: Sep (1 F).

**Known Nearctic records.** Mexico (Coahuila, Zacatecas); USA (Arizona, California, New Mexico).

*Lordotus divisus* Cresson, 1919 Figure 5c

**Material examined.** ECA: Mar (1 M), Apr (2 M); EEO: Apr (16 M); REE: Apr (4 M); ROR: Apr (1 M).

Known Nearctic records. Mexico (Coahuila, Baja California); USA (Arizona, California, Nevada, New Mexico, Texas).

# Lordotus perplexus Johnson & Johnson, 1959

Figure 5d, e

**Material examined.** CHU: Apr (1 H), ECA: Apr (1 H); EEO: Apr (4 F); REE: Apr (7 F); ROR: Apr (1 F).



Figure 5. Lordotus. L. diplasus, a female (CNIN 774) dorsal view b male (CNIN 861) dorsal view c L. divisus, male (CNIN 777) dorsal view; L. perplexus, female (CNIN 801) d dorsal view e lateral view. All scale bars: 3 mm.

**Known Nearctic records.** Mexico (Baja California, Coahuila, Sinaloa, Sonora); USA (Arizona, California, Nevada, Texas).

**Comments.** *Lordotus perplexus* has the most southern distribution in the genus, reaching Sinaloa on the Pacific coast.

#### Genus Triploechus Edwards

**Remarks.** Four species of *Triploechus* are present in Nearctic region: *T. luridus* Hall, 1975; *T. novus* (Williston, 1893); *T. sackeni* (Bigot, 1892); *T. stagei* Hall, 1975. Of these *T. stagei* is endemic to Mexico and *T. novus* has the widest distribution of this genus, being present in the south of the USA and center of Mexico. Hall and Evenhuis (1981) present a revision and key for species for this genus.

#### Triploechus novus (Williston, 1893)

Figure 6

Material examined. CHU: Apr (7 F, 6 M); REE: Apr (1 M); RPA: Apr (1 M).

**Known Nearctic records.** Mexico (Coahuila, Durango, San Luis Potosí, Sonora); USA (Arizona, California, Nevada, New Mexico, Texas).

**Comments.** This is a widespread and apparently common species. All specimens were collected in April so it may have a short flight season.

#### Subfamily Lomatiinae

#### Genus Ogcodocera Macquart

**Remarks.** The only two species in this genus have been collected from the neotropical part of Mexico to north of the USA and Canada. *Ogcodocera leucoprocta* (Wiedemann, 1828), not sampled during this study, is present in the whole Nearctic region from Canada to south of Mexico.

Ogcodocera analis Williston, 1901

Figure 7

#### Material examined. EEO: Aug (2 M), Oct (1 M).

Known Nearctic records. Mexico (Coahuila, Guerrero, Morelos); USA (Arizona, Texas).

**Comments.** This record is the first of this species in the north of Mexico, but it has been previously collected in the south of Mexico and in the south of USA, and thus is probably distributed across the whole country. Unlike *O. leucoprocta*, *O. analis* has its most northern distribution in Arizona and Texas.



Figure 6. Triploechus novus, female (CNIN 1237) dorsal view. Scale bar: 3 mm.



Figure 7. Ogcodocera analis, male (CNIN 146) dorsal view. Scale bar: 3 mm.

### Subfamily Tomomyzinae

#### Genus Paracosmus Osten Sacken

**Remarks.** All five extant species of *Paracosmus* have Nearctic distributions, and all are present in California. Two of these species have been collected in the northwest of Mexico (*P. (Actherosia) rubicundus* Melander, 1950 and *P. (Paracosmus) morrisoni* Osten Sacken, 1887).

# Paracosmus (Paracosmus) morrisoni Osten Sacken, 1887

Figure 8

**Material examined.** EAM: Apr (1 F, 1 M); CHU: Apr (2 M), Jul (1 F), Aug (2 F); ECA: Apr (1 M); EEO: May (1 F); REE: Apr (1 F); ROR: Apr (2 M), May (1 F, 3 M); RPA: Apr (1 F).

**Known Nearctic records.** Mexico (Coahuila, Sonora); USA (Arizona, California, Nevada, Texas).

**Comments.** *Paracosmus (P.) morrisoni* has the widest distribution within this genus, but in Mexico had previously only been recorded in Sonora. This record represent the most eastern distribution for the genus in the country.

#### Subfamily Anthracinae

#### Genus Anthrax Scopoli

**Remarks.** This is a diverse genus with 248 species worldwide. Two old but complete revisions of the genus, including distribution maps and keys, were made by Marston (1963, 1970). Thanks to these *Anthrax* species can be easily identified. Some *Anthrax* species are widely distributed occupying two biogeographic regions. From the seven *Anthrax* species collected in this study in Coahuila, just *A. cybele* (Coquillett, 1894) has a restricted distribution. The other six species are widespread across the Nearctic region. Two species of *Anthrax* are reported for the first time for Mexico.

# Anthrax atriplex Marston, 1970

Figure 9a

Material examined. EAM Apr (1 F); ROR: Oct (2 M); RPA: Aug (1 M); Sep (1 M); Oct (1 F, 2 M).

**Known Nearctic records.** Mexico (Baja California Sur, Coahuila, Durango, Sonora, Tamaulipas); USA (Arizona, California, New Mexico, Oregon, Texas, Utah).



Figure 8. Paracosmus (P.) morrisoni, male (CNIN 832) a dorsal view b lateral view. All scale bars: 3 mm.



**Figure 9.** *Anthrax* part I. **a** *A. atriplex*, male (CNIN 1098) dorsal view **b** *A. cybele*, male (CNIN 1087) dorsal view **c** *A. georgicus*, female (CNIN 1071) dorsal view **d** *A. irroratus*, male (CNIN 1027) dorsal view. All scale bars: 3 mm.

Known host. Megachile gentilis Cresson (Megachilidae).

**Comments.** This species may be present in all the north of Mexico, including Chihuahua, Nuevo León and possibly Sinaloa.

#### Anthrax cybele (Coquillett, 1894)

Figure 9b

#### Material examined. ECA: Apr (2 F); EEO: Apr (1 M).

Known Nearctic records. Mexico (Coahuila); USA (Arizona, California).

**Comments.** This is a rare species flying in April. Its distribution is disjunct so far, present in the southwest of the USA and northeast of Mexico. It is probably also found in New Mexico and Texas in the USA and Sonora and Chihuahua in Mexico.

#### Anthrax georgicus Macquart, 1834

Figure 9c

**Material examined.** EAM: Mar (1 F), Apr (1 M), Jun (1 F, 2 M), Jul (2 F), Sep (2 M); ROR: Apr (1 F), Sep (1 M); RPA: Mar (1 M), Apr (1 F, 1 M), Jul (2 F, 1 M), Sep (6 F, 3 M), Oct (6 F, 3 M).

Known Nearctic records. Canada (Alberta, British Columbia, Manitoba, Northwest Territory, Ontario, Quebec, Saskatchewan); Mexico (Coahuila, Guerrero, Michoacán de Ocampo, Morelos, Nuevo León, Puebla, Sonora, Veracruz); USA (Arizona, Arkansas, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Georgia, Idaho, Illinois, Iowa, Kansas, Kentucky, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Tennessee, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming).

**Comments.** The range of *A. georgicus* includes all North America and Central America (Nicaragua, Costa Rica) covering a wide diversity of habitats and environmental conditions. Common in the rainy season and present in the dry season (March), this species is probably present in most if not all states of Mexico, but has only been collected in eight of them.

# Anthrax irroratus Say, 1823

Figure 9d

**Material examined.** EAM: Apr (1 M), Aug (2 M), Oct (1 M); ECA: Apr (1 F), May (1 F, 1 M); EEO: Apr (2 F), Jul (4 F), Aug (1 M); REC: Apr (3 F, 10 M), Aug (1 M); REE: Aug (1 M); RLC: Jul (6 F, 10 M); ROR: Feb (1 M), Aug (5 M), Sep (1 M); RPA: Apr (1 M), Aug (2 M).

Known Nearctic records. Canada (Alberta, British Columbia, Manitoba, Northwest Territory, Nova Scotia, Ontario, Quebec, Saskatchewan); Mexico (Baja California, Baja California Sur, Coahuila, Colima, Guerrero, Michoacán, Morelos, Nayarit, Puebla, San Luis Potosí, Sinaloa, Sonora, Veracruz, Zacatecas); USA (Alaska, Arizona, Arkansas, California, Colorado, Connecticut, Idaho, Illinois, Indiana, Kansas, Maryland, Massachusetts, Michigan, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New York, Oregon, Pennsylvania, Tennessee, Texas, Utah, Virginia, West Virginia, Wyoming).

Known hosts. Megachile gentilis Cresson (Megachilidae); M. mendica Cresson (Megachilidae); Dianthidium heterulkei fraternum Timberlake (Megachilidae); Aschmendiella bucconis denticulata Cresson (Megachilidae); Hylaeus asininus Cockrell and Casad (Colletidae). Scott and Strickler (1992) also reared A. irroratus from Megachile relativa Cresson (Megachilidae) and M. inermis Provancher (Megachilidae).

**Comments.** *Anthrax irroratus*, like *A. georgicus* (above), is present in all of North America and reaches Central America and Caribbean islands (Honduras, Puerto Rico). More abundant than its congener, this species has been collected in 15 states in Mexico (including Oaxaca of the Neotropical region not listed above) and all regions of the USA. *A. irroratus* should be collected in any systematic, long term Bombyliidae sample in Mexico and the USA.

#### Anthrax oedipus Fabricius, 1805

Figure 10a

**Material examined.** ECA: Apr (1 F, 1 M), Jul (1 F); EEO: Apr (2 F, 1 M), May (1 F, 4 M), Jul (1 M); REC: Apr (1 F); REE: Apr (2 M); RLC: Jul (2 F, 4 M), Sep (1 F); RPA: Apr (1 M), Aug (1 M).

Known Nearctic records. Mexico (Baja California, Coahuila, Nayarit, Morelos, Sinaloa, Sonora); USA (Nevada, Texas).

**Comments.** Apparently closely related to *A. irroratus*, *A. oedipus* has a narrow distribution in the Nearctic region but is widely distributed in all South America. In the USA it has been collected only in two southern states, while it occurs in most of the northern states of Mexico and one central state (Morelos); it may be present in most areas from Texas to Argentina.

Anthrax pauper (Loew, 1869)

Figure 10b

#### Material examined. CHU: Apr (1 F, 1 M).

Known Nearctic records. Canada (Ontario); Mexico (Coahuila); USA (Alabama, Colorado, Illinois, Indiana, Kansas, Maryland, Massachusetts, Michigan, Nebraska, New Jersey, New Mexico, New York, Oklahoma, Pennsylvania, Texas, Utah, Vermont, Virginia, Wisconsin).



**Figure 10.** *Anthrax* part II. **a** *A. oedipus*, female (CNIN 1055) dorsal view **b** *A. pauper*, female (CNIN 1085) dorsal view **c** *A. seriepunctatus*, female (CNIN 1089) dorsal view. All scale bars: 3 mm.

**Comments.** With just two specimens collected, *A. pauper* appears to be a rare species in this region. This population is the most southern recorded of this species, mostly present in the center and east of the USA. Presumably adapted to colder climates, it is no coincidence that it was collected in the most elevated site sampled.

#### Anthrax seriepunctatus (Osten Sacken, 1886b)

Figure 10c

**Material examined.** EAM: Jun (1 M); CHU: Apr (1 F), Aug (1 F), Sep (1 F); ECA: Jun (1 M); REE: Aug (1 F); RLC: Jun (1 F), Jul (1 F, 2 M).

**Known Nearctic records.** Mexico (Baja California Sur, Coahuila, Sonora, Puebla); USA (Arizona, Nevada, New Mexico, Texas).

**Comments.** This species is recorded mostly from the south of the USA and north of Mexico, but its presence in Puebla in central Mexico suggests a wider distribution within the country, at least in all northern states.

#### Genus Dipalta Osten Sacken

**Remarks.** *Dipalta* is a small genus with just two species. *Dipalta banksi* Johnson, 1921 is only present in eastern Canada and USA, while *D. serpentina* is distributed from Central America to the northern USA.

#### Dipalta serpentina (Osten Sacken, 1877)

Figure 11

#### Material examined. REC: Aug (1 M); RLC: Jul (1 M).

Known Nearctic records. Mexico (Coahuila, Guerrero, Hidalgo, México, Morelos, Puebla, San Luis Potosí, Sinaloa); USA (Arizona, Arkansas, California, Colorado, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, Montana, Nebraska, Nevada, New Mexico, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, South Dakota, Tennessee, Texas, Utah, Washington, Wisconsin, Wyoming).

Known host. Myrmeleon immaculatus De Geer (Myrmeleontidae).

**Comments.** This species is probably present in all of Mexico, but this is the only record in the north of Mexico.

#### Genus Hemipenthes Loew

**Remarks.** *Hemipenthes* is equally diverse in the Nearctic (29 species), Neotropical (26 species) and Palearctic (37 species) regions, with just six species in the Oriental region and one in the Afrotropical region. Four species of this genus were collected in Coahuila. All of these have broad distributions but apparently from poor sampling because records are not continuous, especially in Mexico. Ávalos-Hernández (2009) recently published a revision of *Hemipenthes*, with a key for Nearctic species.

#### Hemipenthes jaennickeana (Osten Sacken, 1886a)

Figure 12a

**Material examined.** REC: Apr (18 F), Aug (4 F); REE: Feb (3 F); RLC: Mar (7 F), Jul (23 F, 3 M), Sep (3 F).

Known Nearctic records. Mexico (Coahuila, Morelos, Sonora); USA (Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Texas, Utah).

**Comments.** Present mainly in the Pacific coast states of the USA and Mexico, from Oregon as far as Morelos in the center of Mexico. This record is the most eastern record in Mexico.



Figure 11. Dipalta serpentina, male (CNIN 215) dorsal view. Scale bar: 3 mm.



**Figure 12.** *Hemipenthes.* **a** *H. jaennickeana*, female (CNIN 1137) dorsal view **b** *H. lepidota*, female (CNIN 200) dorsal view **c** *H. scylla*, male (CNIN 725) dorsal view **d** *H. sinuosa*, female (CNIN 1134) dorsal view. All scale bars: 3 mm.

#### Hemipenthes lepidota (Osten Sacken, 1886b)

Figure 12b

**Material examined.** EAM: Apr (1 M), Aug (1 F); CHU: Apr (1 F, 3 M), Aug (1 M); EEO: Jul (1 F, 4 M), Aug (1 F); REC: (1 M); REE: Apr (11 F, 2 M), Aug (1 F); RLC: Jun (1 F), Jul (3 F); RPA: Apr (1 F), Sep (4 F), Oct (2 F).

**Known Nearctic records.** Canada (Alberta); Mexico (Baja California, Baja California Sur, Coahuila, Chihuahua, Guerrero, Morelos, Puebla, San Luis Potosí, Sonora, Tamaulipas); USA (Arizona, California, Colorado, Idaho, Louisiana, Nevada).

**Comments.** This species is abundant in the rainy season in most of the Nearctic region but has not been collected in many states of Mexico or the USA where it probably is present.

#### Hemipenthes scylla (Osten Sacken, 1887)

Figure 12c

**Material examined.** REC: Apr (23 M), Aug (7 M); REE: Feb (5 M), Apr (2 M); RLC: Mar (8 M), Jul (8 M), Sep (9 M).

Known Nearctic records. Mexico (Coahuila, Morelos, Guanajuato, Sonora); USA (Arizona, Texas).

**Comments.** Males of this species are abundant all year long but females are unknown. There is no explanation for this lack of females in the collections. Extreme sexual dimorphism and misidentification of females can be dismiss, since there is no *Hemipenthes* species from which only females are known. One possible explanation is that females life span is too short and therefor encounter probabilities are low. Distribution is discontinuous with populations present in central and northern Mexico and the southern USA; it is unknown whether this species is present in between these areas.

#### Hemipenthes sinuosa (Wiedemann, 1821)

Figure 12d

**Material examined.** REC: Apr (3 F); REE: (Feb (1 F), Apr (2 F); RLC: Jul (1 F, 1 M), Sep (1 M); RPA: Sep (2 F).

Known Nearctic records. Mexico (Coahuila, Morelos); USA (Alabama, Arizona, Arkansas, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, West Virginia, Wisconsin).

Known host. Neodiprion sertifer Geoff. (Diprionidae).

**Comments.** *Hemipenthes sinuosa* is only known from Morelos in the center of Mexico and Coahuila in the northeast, but can be found almost in all of the USA. It is clearly undersampled in Mexico.

#### Genus Lepidanthrax Osten Sacken

**Remarks.** Forty seven of the 52 species of *Lepidanthrax* are from the Nearctic region. Hall (1976) published a revision of this genus including keys for species.

#### Lepidanthrax arizonensis Hall, 1976

Figure 13a

#### Material examined. EEO: Mar (1 F); Oct (2 M).

Known Nearctic records. Mexico (Coahuila); USA (Arizona).

**Comments.** *Lepidanthrax arizonensis* has a restricted distribution, being present only in Arizona and Coahuila, but probably is also present in Chihuahua, Texas and New Mexico.

### Lepidanthrax disiunctus (Wiedemann, 1830)

Figure 13b

#### Material examined. REC: Aug (2 F, 1 M).

**Known Nearctic records.** Mexico (Coahuila, Distrito Federal, Guerrero, Veracruz); USA (Arizona).

**Comments.** The distribution of *L. disiunctus* has its northern extreme in Arizona and its southern extreme in Oaxaca, in the southeast of Mexico. It seems this species is rarely collected, but widely distributed.

#### Lepidanthrax hesperus Hall, 1976

Figure 13c

**Material examined.** EAM: Apr (2 M); CHU: Apr (2 F, 5 M); ROR: Apr (1 F, 3 M), May (1 F, 1 M); RPA: Apr (1 F, 14 M).

**Known Nearctic records.** Mexico (Baja California, Coahuila, Sinaloa, Sonora); USA (Arizona, California, New Mexico, Texas).

Comments. This record is the first in northeastern Mexico.

### Lepidanthrax hyposcelus Hall, 1976

Figure 13d

Material examined. RLC: Sep (4 F, 15 M).

Known Nearctic records. Mexico (Coahuila, Guerrero, Morelos, Puebla).



**Figure 13.** Lepidanthrax. **a** L. arizonensis, female (CNIN 1352) dorsal view **b** L. disiunctus female (CNIN 334) dorsal view **c** L. hesperus, male (CNIN 1339) dorsal view **d** L. hyposcelus, male (CNIN 369) dorsal view **e** L. proboscideus, male (CNIN 357) dorsal view. All scale bars: 3 mm.

**Comments.** *Lepidanthrax hyposcelus* is endemic to Mexico, previously only known from the southwest of the country; this record extends its distribution to the northeast of the country.

#### Lepidanthrax proboscideus (Loew, 1869)

Figure 13e

**Material examined.** ECA: Sep (1 F, 2 M); EEO: Apr (1 F), Aug (1 F, 1 M), Oct (4 F, 15 M); ROR: Sep (2 M); RPA: Sep (2 M), Oct (1 M).

**Known Nearctic records.** Mexico (Baja California, Baja California Sur, Coahuila, Durango, Guerrero, Morelos, Sonora); USA (Arizona, California, Nevada, New Mexico, Utah).

**Comments.** Lepidanthrax proboscideus, L. fuscipennis Hall, 1976 and L. disiunctus are the only species of this genus distributed in both the Nearctic and Neotropical regions. Of these L. proboscideus extends as far as El Salvador, the most southern distribution for a Nearctic species of this genus. This is the first record of this species in the northeast of Mexico.

#### Genus Neodiplocampta Curran

**Remarks.** *Neodiplocampta* is a small American genus, more diverse in the Neotropical than the Nearctic region. Hull and Martin (1974) described seven of the 16 species and published a key for all species of the genus.

# *Neodiplocampta (Neodiplocampta) miranda* Hull & Martin, 1974 Figure 14

**Material examined.** CHU : Aug (1 F); EEO: Aug (1 F, 1 M); ROR: Jul (1 F); Oct (1 M); RPA: Aug (1 F, 2 M).

**Known Nearctic records.** Mexico (Coahuila, Guerrero, San Luis Potosi, Sinaloa, Sonora); USA (Arizona, California, Florida, Texas).

**Comments.** Neodiplocampta (N.) miranda and N. (Agitonia) sepia Hull, 1966 are the only two species distributed in both biogeographic regions (Nearctic and Neotropical). This species is distributed from the south of the USA to Nicaragua, but has not been collected in most Mexican states. This lack of records is possibly due its low abundance.

#### Genus Paravilla Painter

**Remarks.** Fifty five of the 58 species of the genus are Nearctic. All species of *Paravilla* collected in Coahuila were exclusively collected in the summer months from April to July. Hall (1981a) reviewed this genus and presented a key for species and description of new species.


Figure 14. Neodiplocampta (N.) miranda, female (CNIN 225) dorsal view. Scale bar: 3 mm.

# Paravilla edititoides (Painter, 1933)

Figure 15a

**Material examined.** EAM: Jun (1 M); CHU: Apr (1 F), Jul (2 F, 1 M); ECA: Apr (1 F, 1 M), Jul (1 M); EEO: Apr (1 F, 10 M), May (1 F, 2 M), Jul (9 M); REE: Apr (1 M); RLC: Jun (2 F, 5 M), Jul (1 F); ROR: Jul (1 F, 3 M); RPA: Oct (1 M).

**Known Nearctic records.** Canada (Saskatchewan); Mexico (Chihuahua, Coahuila, Durango, Jalisco, México, Zacatecas); USA (Arizona, Colorado, Idaho, Kansas, Montana, Nebraska, New Mexico, Oklahoma, Utah, Texas, Wyoming).

**Comments.** This species is very abundant and present in most of North America, from Canada as far as Jalisco in central Mexico.

# Paravilla flavipilosa (Cole, 1923)

Figure 15b

**Material examined.** CHU: Apr (1 M); Jul (1 M); ECA: Apr (1 M); EEO: Apr (7 M), May (11 M); ROR: Apr (2 M); RPA: Apr (1 M).

**Known Nearctic records.** Mexico (Baja California Sur, Coahuila, Nuevo León); USA (Arizona, California, Colorado, Texas).

**Comments.** *Paravilla flavipilosa* is abundant and restricted to the south of the USA and north of Mexico.



**Figure 15.** *Paravilla.* **a** *P. edititoides*, male (CNIN 1272) dorsal view **b** *P. flavipilosa*, male (CNIN 1125) dorsal view **c** *P. parvula*, female (CNIN 884) dorsal view **d** *P. separata*, female (CNIN 898) dorsal view. All scale bars: 3 mm.

# Paravilla parvula Hall, 1981a

Figure 15c

Material examined. EAM: Apr (1 F); CHU: Apr (1 M); RPA: Apr (7 F, 13 M).

Known Nearctic records. Mexico (Chihuahua, Coahuila, Durango, Guanajuato, Hidalgo, Jalisco, México, Michoacán, Nuevo León, San Luis Potosí, Sonora, Zacatecas), USA (Arizona, New Mexico, Texas, Utah).

**Comments.** *Paravilla parvula* is relatively well collected in northern and central Mexico. Its distribution also includes the south of the USA but no farther north than Utah.

# Paravilla separata (Walker, 1852)

Figure 15d

**Material examined.** CHU: Apr (1 F); EEO: Apr (3 F); REE: Apr (5 F, 3 M); RPA: Apr (1 M).

**Known Nearctic records.** Canada (Ontario, Manitoba); Mexico (Coahuila); USA (Alabama, Florida, Georgia, Iowa, Kansas, Michigan, Minnesota, Mississippi, Nebraska, Ohio, South Dakota, Wisconsin).

**Comments.** *Paravilla separata* is present mainly in the eastern half of the USA, and southeastern Canada. This record in Coahuila represents the southern extreme of the distribution of this species, and is the first in Mexico. It may also be present in Tamaulipas and Nuevo León but doubtfully in the northwest of Mexico.

## Genus Poecilanthrax Osten Sacken

**Remarks.** Four species from this mainly Nearctic genus are recorded in Coahuila for the first time. Painter and Hall (1960) published a review of *Poecilanthrax* with a key and images of the species.

## Poecilanthrax effrenus (Coquillett, 1887)

Figure 16a

**Material examined.** EAM: Apr (1 F, 1 M), Jun (1 F, 1 M), Sep (1 F); CHU: Jun (1 F); ROR: May (10 F, 5 M), Jul (2 F, 3 M), Aug (1 M); RPA: Jun (4 F, 1 M), Jul (6 F, 6 M), Aug (1 F, 1 M), Sep (4 F), Oct (2 F, 1 M).

**Known Nearctic records.** Mexico (Baja California Sur, Chihuahua, Coahuila, Sonora, Tamaulipas); USA (Arizona, California, New Mexico, Oklahoma, Texas).

**Comments.** This record fills a gap in *P. effrenus* distribution between northwest and northeast populations of Mexico. This species is probably present in Baja California and Nuevo León, but has not yet been recorded.

# Poecilanthrax fasciatus Johnson & Johnson, 1957

Figure 16b

Material examined. EAM: Sep (1 M); CHU: Oct (1 M); ROR: Oct (1 M); RPA: Oct (1 M).
Known Nearctic records. Mexico (Coahuila); USA (Colorado, Kansas, Texas).
Known host. *Chorizagrotis auxiliaris* Grote (Noctuidae).

**Comments.** *Poecilanthrax fasciatus* is collected in Mexico for the first time, and this extends the southern limit of this species distribution.

## Poecilanthrax hyalinipennis Painter & Hall, 1960

Figure 16c

**Material examined.** EAM: Mar (3 M), Oct (1 F); CHU: Oct (1 M); ROR: Oct (4 M); RPA: Sep (1 F), Oct (2 F, 6 M).



**Figure 16.** *Poecilanthrax.* **a** *P. effrenus*, female (CNIN 1380) dorsal view **b** *P. fasciatus*, male (CNIN 218) dorsal view **c** *P. hyalinipennis*, female (CNIN 1365) dorsal view **d** *P. poecilogaster*, male (CNIN 1356) dorsal view. All scale bars: 3 mm.

**Known Nearctic records.** Mexico (Coahuila); USA (Arizona, California, Nevada, Utah).

**Comments.** This record extends the distribution of *P. hyalinipennis* into the northwest of Mexico. Considering its distribution in the USA, this species may also be present in the northeast of Mexico.

# Poecilanthrax poecilogaster (Osten Sacken, 1886b)

Figure 16d

## Material examined. REE: Apr (2 M).

**Known Nearctic records.** Canada (Alberta, Manitoba, Ontario, Saskatchewan); Mexico (Coahuila, Morelos, Nuevo León, Sonora); USA (Arizona, California, Colorado, Idaho, Nevada, New Mexico, Oregon, Utah).

**Comments.** Most of the records in the USA and Mexico of this rarely collected but widespread species are from Pacific Coast states, although, there are records from Nuevo Leon and Coahuila in northeast Mexico.

## Genus Rhynchanthrax Painter

**Remarks.** Of the seven species of this exclusively Nearctic genus, six are present in Mexico, with *R. maria* (Williston, 1901) and *R. nigrofimbriatus* (Williston, 1901) being endemic to this country. Only *Rhynchanthrax parvicornis* (Loew, 1869) has not been collected in Mexico, but it is distributed across the southern USA and may also occur in the north of Mexico.

## Rhynchanthrax capreus (Coquillett, 1887)

Figure 17a

**Material examined.** CHU: Apr (1 M), Aug (13 F, 6 M), Jul (12 F, 6 M), Sep (1 F, 3 M).

**Known Nearctic records.** Mexico (Coahuila); USA (Arizona, California, Colorado, Nebraska, Nevada, New Mexico, Utah).

**Comments.** This is the first record of this species in Mexico. *Rhynchanthrax capreus* is the only species occurring in the northwest of the USA, while the other species in the genus are present mainly in the south and east of the country. This species may also be present in the northwest of Mexico (Baja California, Sonora, Chihuahua).

#### Rhynachantrax texanus (Painter, 1933)

Figure 17b

#### Material examined. EEO: May (1 M); RLC: Jun (1 F, 11 M), Jul (3 M).

Known Nearctic records. Mexico (Coahuila, Sonora); USA (Kansas, New Mexico, Texas).

**Comments.** This is the most eastern record in Mexico for this species. In the USA it is distributed in the southern-center of the country, but in Mexico it has been collected in Sonora so it probably also occurs in Arizona.

#### Genus Thyridanthrax Osten Sacken

**Remarks.** *Thyridanthrax* has twice as many species in the Palearctic region as in the Nearctic and Neotropical regions combined. All 12 species in North America are present in the USA with five also in Mexico. These are the first records of this genus in Coahuila. The distribution of *T. selene* (Osten Sacken, 1886b) and *T. pallidus* (Coquillett, 1887) are very similar, being present in all of the southern USA and probably also in all of northern Mexico, although they have been only collected in Sonora and Coahuila to date. Both species are rare and were collected only in April.



**Figure 17.** *Rhynchanthrax.* **a** *R. capreus*, female (CNIN 940) dorsal view **b** *R. texanus*, male (CNIN 263) dorsal view. All scale bars: 3 mm.

# Thyridanthrax pallidus (Coquillett, 1887)

Figure 18b

## Material examined. REE: Apr (4 F, 1 M).

**Known Nearctic records.** Mexico (Coahuila); USA (Arizona, California, Nevada, Texas, Utah).

Comments. This represents the first record of this species in Mexico.

## Thyridanthrax selene (Osten Sacken, 1886b)

Figure 18a

Material examined. EAM: Apr (1 M); REE: Apr (2 F, 2 M).

**Known Nearctic records.** Mexico (Coahuila, Sonora); USA (Arizona, California, Texas).

**Comments.** This is the most eastern record in Mexico.

# Genus Xenox Evenhuis

**Remarks.** Of the five species that constitute this genus, four are present in Mexico.

# Xenox xylocopae (Marston, 1970)

Figure 19

## Material examined. ECA: Sep (1 M).



**Figure 18.** *Thyridanthrax.* **a** *T. selene*, male (CNIN 182) dorsal view **b** *T. pallidus*, female (CNIN 1162) dorsal view. All scale bars: 3 mm.



Figure 19. Xenox xylocopae, male (CNIN 1165) dorsal view. Scale bar: 3 mm.

Known Nearctic records. Mexico (Chihuahua, Coahuila, Sonora), USA (Arizona, New Mexico, Texas).

Known host. *Xylocopa micheneri micheneri* (Hurd) (Apidae) as reported by Minckley (1989).

**Comments.** *Xenox xylocopae* appears to be restricted to the northeast of Mexico and south of the USA. Three of the other species also have restricted and separate distributions: *X. delila* Loew, 1869 is present in the northwest of Mexico and California; *X. nigritus* (Schaeffer, 1768) occurs from the northeast of Mexico (Veracruz and Tamaulipas without overlap with *X. xylocopae*) to South America; and *X. tigrinus* (De Geer, 1776) is present in the eastern USA and southern Ontario. Only *X. habrosus* (Marston, 1970) has a distribution overlapping with the other four species, being present in all of Mexico and the southwest of the USA.

## Discussion

The data presented here increase the knowledge of Bombyliidae in Mexico but also reveals the deficiencies in sampling of the family in the country. The species list for the state increased three-fold, which demonstrates the lack of knowledge of the Bombyliidae fauna in this region. Almost all states of Mexico are in a similar situation but northern states appear to have higher diversity and should be priorities for sampling. Hull (1973) identified the northwest of Mexico as a species concentration area of Bombyliidae, but the northeast portion of the country may have the same species richness. Diversity of this family in the north of Mexico is probably much higher than recorded, as indicated by the richness in the south of USA which has similar environmental characteristics but much better sampling. Therefore northeast Mexico is possibly one of the most under sampled areas in the Nearctic region for Bombyliidae, given the great diversity of this family in the area, combined with the size of this part of the country. The study of Bombyliidae in the northern states of Mexico should be more of a priority than field work in the center or the southern states.

Most of the species collected in this study have a broad distribution in the USA but Mexican records are isolated. There are probably more species yet to be recorded from Coahuila and other Mexican states, especially species present in southern border states of the USA. Some species are recorded only from Coahuila in the northeast of Mexico but are also present in the northwest of the country. More studies are required to determine if these species have a disjunct distribution or if any are represented by distinct, cryptic eastern and western species.

Cuatro Ciénegas' biological and conservational importance has long been recognized for reptiles (McCoy 1984), birds (Contreras-Balderas 1984), plants (Pinkava 1984, Villarreal and Encina 2005), snails (Hershler 1984), Crustacea (Cole 1984) and particularly fishes (Minckley 1984), but little is known of other groups like insects. The insects contain 53% of the described species in the planet (Chapman 2009), so their distribution and diversity should be considered for conservation and natural reserve design. The diversity of insects, especially of Bombyliidae and similar arid-regions-diverse groups, increases the conservational value of Cuatro Ciénegas.

# Conclusions

The data presented here indicates the significance of Cuatro Ciénegas for Bombyliidae diversity. Comparison with other nearby areas should be undertaken to confirm whether this area really is richer for this family. Data also reveal that true species richness of Bombyliidae is much higher than previously recorded. This could also be true for other insect groups. More funding should be destined for faunistic studies of megadiverse groups with ecological importance such as Diptera, Coleoptera, Hymenoptera and Lepidoptera. The information obtained from these studies might be used first to quantify the species richness and species exchange between areas (beta diversity) (Whittaker 1972) and later to propose conservation management schemes.

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RESEARCH ARTICLE



# A Linnaeus NG<sup>™</sup> interactive key to the Lithocolletinae of North-West Europe aimed at accelerating the accumulation of reliable biodiversity data (Lepidoptera, Gracillariidae)

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#### Abstract

We present an interactive key that is available online through any web browser without the need to install any additional software, making it an easily accessible tool for the larger public. The key can be found at http://identify.naturalis.nl/lithocolletinae. The key includes all 86 North-West European Lithocolletinae, a subfamily of smaller moths ("micro-moths") that is commonly not treated in field guides. The user can input data on several external morphological character systems in addition to distribution, host plant and even characteristics of the larval feeding traces to reach an identification. We expect that this will enable more people to contribute with reliable observation data on this group of moths and alleviate the workload of taxonomic specialists, allowing them to focus on other new keys or taxonomic work.

#### **Keywords**

*Cameraria, Phyllonorycter, Macrosaccus, Triberta*, identification, monitoring, conservation, biodiversity, leafminers

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## Introduction

Taxonomic identification is the key to aggregating knowledge about species. We are increasingly aware that we live in a changing world and that different species respond differently to changes (Bellard et al. 2012; Butchart et al. 2010; Fernandez-Triana et al. 2011; Heller and Zavaleta 2009; Pereira et al. 2010; Thomas et al. 2004). Statistical models that elucidate the impact of environmental change on ecological communities in future scenarios depend on appropriate underlying faunistic data (Ellis et al. 2013; Soberon and Peterson 2009; ter Steege et al. 2013). Current biodiversity indicators that have potential to be included in such models often rely on monitoring data of relatively restricted systematic groups. Commonly these are aesthetically attractive, such as birds (Schmeller et al. 2012), butterflies (Dover et al. 2011) and dragonflies (Parr 2010) or have a societal impact such as bees (Schindler et al. 2013). For these groups momentum can be generated amongst the general public to gather and contribute faunistic data to large databases. There are, however, new opportunities arising to expand to other groups of organisms that may strengthen biodiversity models. The accessibility of information as well as possibilities to submit data through the internet enables a new generation of observers to contribute to databases, such as www.observado.org or www.lepiforum. de. A genuine concern with this method of data collection is the reliability of the identifications. Although most databases strongly suggest including photographs with each observation, this recommendation is not always followed, and may not even be feasible with, for instance, trap samples consisting of numerous specimens. Furthermore, even when a photograph is present it may not show the relevant characters or there are simply not enough taxonomic specialists available to verify all sightings. Instead of using an approach of 'damage control' by attempting to verify all sightings, it may therefore be more efficient for taxonomic specialists to provide better *a priori* information through identification tools that are tailored to these growing groups of enthusiasts.

For the past two centuries identifications of organisms have usually been carried out with dichotomous keys. One of the governing issues with dichotomous keys is that they "are compiled by those who do not need them for those who cannot use them" (http://www.zin.ru/Animalia/Coleoptera/eng/syst8.htm). Although useful dichotomous keys certainly exist, they always have the disadvantage of being static and cannot be adjusted easily with new taxonomic insights. Furthermore, it is impossible to skip certain couplets when a required character is missing or not visible. Often, several keys are needed for a single group to target different developmental stages, sexes or character sets (e.g. external characters, genitalia). The total amount of couplets in a key is the amount of taxa included, minus one. The more questions a key contains, the less likely there will be an accurate identification (Osborne 1963). As the taxa in a key are 'fixed', a species not included in it may nevertheless easily end up in a couplet, thus giving a wrong identification. Different alternatives have been proposed for dichotomous keys, with more recently computer aided ones, usually called 'interactive keys' (Penev et al. 2012). Although a review of all present methods is beyond this paper (but see Dallwitz 2000; Dallwitz et al. 2000 onwards; Farr 2006; Walter and Winterton 2007), the vast

majority of them have issues which make them unsuitable for a larger public. These issues include the necessity to install and possibly purchase software, which may or may not be available for all operating systems, and the need for training due to the complexities of the particular software package.

Butterflies are often used as biodiversity indicators, but only represent a small proportion of the order Lepidoptera (Mutanen et al. 2010; van Nieukerken et al. 2011; Regier et al. 2013). Most Lepidoptera are herbivores of higher plants with different degrees of specialization (Menken et al. 2010; Powell et al. 1998). Relationships with higher trophic levels include a wide variety of parasitoids and insectivores. These insectivores include mammals with protected conservation status, such as some species of bats. The diversity of Lepidoptera in an area thus relays information about a whole ecosystem. Among enthusiasts that go night-collecting to observe moths there is a tendency to only identify species of a selection of families that mainly contain larger moths ("macro-moths") which have traditionally been treated in field guides. For the interactive key presented here we therefore selected the subfamily Lithocolletinae in a family with smaller moths (Gracillariidae), limiting ourselves for practical reasons to all 86 species from North-West Europe (Buszko 2013) (see Table 1 for names and authorities). Lithocolletinae in Europe are predominantly represented by the species rich genus *Phyllonorycter* Hübner, 1822, with 128 known species (Buszko 2013). The subfamily is further represented by three genera each with one species in the area: Cameraria Chapman, 1902 with the well-known horse-chestnut pest Cameraria ohridella, and the recently established genera Macrosaccus Davis & De Prins, 2011 and Triberta De Prins, 2013 (see Davis and De Prins 2011, De Prins et al. 2013). All species within the subfamily have comparable wing pattern elements and are similar in size and morphology. This makes them superficially very similar, but most species can be identified using a combination of several wing pattern elements. Furthermore, searching for larval feeding traces on plants is an effective alternative to collecting adults for gathering faunistic data. The majority of Lithocolletinae larvae feed inside leaves, where they consume parenchyma but leave the epidermis intact. This results in damage that is usually referred to as a leafmine. The high degree of monophagy (Lopez-Vaamonde et al. 2003) allows for a reliable identification in most cases through a combination of an identified host plant and several characters of the larval feeding method.

Keys that currently exist for Lithocolletinae or *Phyllonorycter* treat restricted geographic regions and are mostly for adults only. Examples include a key for the British Isles (Emmet et al. 1985), for France and the British isles (Bradley et al. 1969) and for Fennoscandia and Denmark (Bengtsson and Johansson 2011). Specifically for the larval feeding traces on plants there is a dichotomous web-key covering the whole of Europe (Ellis 2014). For the British Isles there is also an interactive key available for the genus *Phyllonorycter*, using the Delta-Intkey platform (Watson and Dallwitz 2003 onwards). To address the need for accessible identification methods targeting groups of animals that have potential to be important for biodiversity estimates, we present in this paper an initial step in this direction through a key using the Linnaeus NG platform that includes all North-West European species of Lithocolletinae.

This paper is formatted following the guidelines for interactive keys (Penev et al. 2012).

#### Software technical specification

Linnaeus NG is a web-based species information management system. Linnaeus NG comprises several modules, such as a species description module, a module for plotting distribution, and two types of keys. For this study, the multi-entry key was employed. The data underlying the key are managed in a spreadsheet and can be uploaded to a Linnaeus NG project as comma separated value (.CSV) files by project administrators. Two different files need to be uploaded. One contains a matrix with species data, characters, states and the relation between species and states. A second file contains image links for all character states. Alternatively, these values can be added and edited directly through the web-based multi-entry key management interface. This interface also contains an upload facility for supplying images for states and, optionally, species. Linnaeus NG is developed using open source techniques (PHP, MySQL) and is hosted in a Linux environment. On the client-side, project administrators interact with the program solely through a web browser. Recent versions of all major browsers are supported, for regular platforms and tablets. Currently, Linnaeus NG is proprietary software; updates and changes can only be made in agreement with Naturalis Biodiversity Center. However, access to Linnaeus NG is not limited to employees or associates of Naturalis Biodiversity Center and can be granted on request.

# User interface

Users can access the key at: http://identify.naturalis.nl/lithocolletinae and fully use the key online through any web browser. No additional software is required. The interface was designed to be intuitive and graphic, using detail images to explain different character states and directly showing the effects of each choice by only displaying images of the remaining possible outcomes. This combination of character state selection and general visual recognition of candidate species prevents users from having to select character states until only a single option remains.

Each possible outcome of the key is represented by a thumbnail photograph of a mounted adult specimen in the main section (Figure 1: 3). Photographs were taken using stacking photography with a motorized Zeiss V20 with MRc5 camera and Axiovision software. During post-processing, photographs were sharpened, reduced in size to 800×600 pixels and backgrounds were homogenized in Adobe Photoshop CS5<sup>®</sup>. The main section of the key starts with all 96 possible outcomes, of which only the first 15 will be shown initially to prevent long loading times. Scrolling down and clicking the "show more results" button will show more options with increments of 15. Photographs can be enlarged by clicking on the thumbnail. By using the forward and previous buttons in the image overlay, the user can navigate through all remaining search results. There are three buttons below each thumbnail. The left symbol (Figure 1: a) provides additional information through an external link to the corresponding species page on Lepiforum.de. The centre symbol (Figure 1: b) is activated when less



**Figure 1.** Screenshot of the user interface with different sections indicated. **I** Search by name **2** search by character **3** main window with resulting selection, **a** more information, links to respective species page on fauna europaea **b** distinctive characters, becomes visible with a selection of 8 or less **c** displays species with a similar appearance.

than eight options remain and lists the differences in character states. The symbol on the right (Figure 1: c) shows which outcomes are highly similar, when applicable. The selection of highly likely outcomes is based on indications in literature or personal experience of the authors, there is no automated algorithm involved.

A user can directly search for a species they suspect they might be trying to identify by using the search box (Figure 1: 1). Alternatively, identification may be reached by selecting character states. The section beneath the search option (Figure 1: 2) lists the character groups, which will expand upon clicking and show all characters within that group. As with any interactive key the user is free to choose which character to begin with. There are three morphological character groups for adult specimens: forewing, thorax and antenna. Of these, only the forewing is further subdivided, into 11 characters. Once a character has been selected, a pop-up will appear on the screen with a brief description and 200×150 pixel detail images representing the different character states. After making a choice the resulting selection is shown in the main section (Figure 1: 3). Instead of just focussing on adults, the key can also be used to identify larvae or leafmines. The characters in the sections biology, distribution and systematics are not morphological, but may help with the identification nonetheless. Alternatively, these options may be used to quickly get a graphic overview of a given species group in a certain country, or all species found on a particular family of host plants.

#### Taxonomic coverage

There are 97 possible outcomes of the key, representing 86 species (Table 1). The majority of outcomes coincide with species, but some species that exhibit intraspecific variation are subdivided. There are species with clearly different forms, such as the aestival and autumnal form of *Ph. issikii* (Huisman et al. 2013) or the *irmella* "form" of *Ph. lautella*, which was at one point regarded a different species (van Nieukerken et al. 2001). In other cases it is less obvious, with a darker and lighter form, sexual dimorphism or the variable presence or absence of a fascia. Because highly variable forms will persist in any selection and hamper an efficient identification, it has often been decided to split these into different outcomes. A positive side effect of this is that the images of several forms give the user some indication on the intraspecific variation.

The 86 species (Table 1) that are included make the key complete for all species found in North-West Europe, more specifically those reported as present in Fauna Europaea (Buszko 2013) in: Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Great Britain, Ireland, Luxembourg, The Netherlands, Norway, Poland, Slovakia, Sweden and Switzerland. In practice, the key will also be functional in the northern half of France. Although sometimes disputed (Baryshnikova 2012), we treat *Ph. distentella* and *Ph. mannii* as distinct species based on a difference in wing pattern and DNA barcode data (Lopez-Vaamonde et al. in prep.). On the other hand, there are no clear morphological, life history or molecular characters to support a split between *Ph. pyrifoliella* and *Ph. gerasimowi*. We therefore treat *Ph. pyrifoliella* as a junior synonym (Zdeněk Laštůvka, pers. comm.).

# Metadata

The selection of morphological characters (Table 2) was initially based on existing dichotomous keys and we follow the terminology used therein. The white ground colour of the forewings for example is the starting point in both the NationalNyckeln (Bengtsson and Johansson 2011) and Bradley's key (1969). Only characters that are applicable to virtually all taxa were included. All previously used characters mainly relied on forewing pattern. We added several characters that can be found on the head, thorax and antennae of adults. After beta-testing a preliminary version of the key with a varied group of novice and advanced identifiers, we found that several characters were unsuitable. The main character leading to faulty identifications was the colour of the head tuft. Judging a colour proved too subjective in general, especially when identifying from photographs. Moreover, the character was variable in many species.

Cameraria ohridella Deschka & Dimić, 1986	Ph. lantanella (Schrank, 1802)
Macrosaccus robiniella (Clemens, 1859)	Ph. lautella (Zeller, 1846)
Phyllonorycter abrasella (Zeller, 1846)	Ph. leucographella (Zeller, 1850)
Ph. acaciella (Duponchel, 1843)	Ph. maestingella (Müller, 1764)
Ph. acerifoliella (Zeller, 1839)	Ph. mannii (Zeller, 1846)
<i>Ph. aemula</i> Triberti, Deschka & Huemer, 1997	Ph. medicaginella (Gerasimov, 1930)
Ph. agilella (Zeller, 1846)	Ph. mespilella (Hübner, 1805)
Ph. alpina (Frey, 1856)	Ph. messaniella (Zeller, 1846)
Ph. anderidae (W. Fletcher, 1885)	Ph. millierella (Staudinger, 1871)
Ph. apparella (Herrich-Schäffer, 1855)	Ph. monspessulanella (Fuchs, 1897)
Ph. blancardella (Fabricius, 1781)	Ph. muelleriella (Zeller, 1839)
Ph. brevilineatella (Benander, 1944)	Ph. nicellii (Stainton, 1851)
Ph. cavella (Zeller, 1846)	Ph. nigrescentella (Logan, 1851)
Ph. cerasicolella (Herrich-Schäffer, 1855)	<i>Ph. oxyacanthae</i> (Frey, 1855)
Ph. cerasinella (Reutti, 1853)	Ph. parisiella (Wocke, 1848)
Ph. comparella (Duponchel, 1843)	Ph. pastorella (Zeller, 1846)
Ph. connexella (Zeller, 1846)	Ph. platani (Staudinger, 1870)
Ph. coryli (Nicelli, 1851)	Ph. populifoliella (Treitschke, 1833)
Ph. corylifoliella (Hübner, 1796)	Ph. quercifoliella (Zeller, 1839)
Ph. cydoniella (Denis & Schiffermüller, 1775)	Ph. quinqueguttella (Stainton, 1851)
Ph. delitella (Duponchel, 1843)	Ph. rajella (Linnaeus, 1758)
Ph. deschkai Triberti, 2007	Ph. roboris (Zeller, 1839)
Ph. distentella (Zeller, 1846)	Ph. rolandi (Svensson, 1966)
Ph. dubitella (Herrich-Schäffer, 1855)	Ph. sagitella (Bjerkander, 1790)
Ph. emberizaepenela (Bouché, 1834)	Ph. salicicolella (Sircom, 1848)
Ph. esperella (Goeze, 1783)	Ph. salictella (Zeller, 1846)
Ph. eugregori Laštůvka & Laštůvka, 2006	Ph. scabiosella (Douglas, 1853)
Ph. fraxinella (Zeller, 1846)	Ph. schreberella (Fabricius, 1781)
Ph. froelichiella (Herrich-Schäffer, 1855)	Ph. scitulella (Duponchel, 1843)
Ph. geniculella (Ragonot, 1874)	Ph. scopariella (Zeller, 1846)
Ph. gerasimowi (Hering, 1930)	Ph. sorbi (Frey, 1855)
Ph. harrisella (Linnaeus, 1761)	Ph. spinicolella (Zeller, 1846)
Ph. heegeriella (Zeller, 1846)	Ph. staintoniella (Nicelli, 1853)
Ph. heringiella (Grønlien, 1932)	Ph. stettinensis (Nicelli, 1852)
Ph. hilarella (Zetterstedt, 1839)	Ph. strigulatella (Zeller, 1846)
Ph. hostis Triberti, 2007	Ph. tenerella (de Joannis, 1915)
Ph. ilicifoliella (Duponchel, 1843)	Ph. trifasciella (Haworth, 1828)
Ph. insignitella (Zeller, 1846)	Ph. trifoliella (Gerasimov, 1933)
Ph. issikii (Kumata, 1963)	Ph. tristrigella (Haworth, 1828)
Ph. joannisi (Le Marchand, 1936)	Ph. ulicicolella (Stainton, 1851)
Ph. junoniella (Zeller, 1846)	Ph. ulmifoliella (Hübner, 1817)
Ph. klemannella (Fabricius, 1781)	Ph. viminetorum (Stainton, 1854)
Ph. kuhlweiniella (Zeller, 1839)	Triberta helianthemella (Herrich-Schäffer, 1860)

**Table 1.** The 86 species included in the key in alphabetical order.

Character group	Character	States		
Forewing	Forewing ground colour	White; other		
Forewing	Forewing mottling	Mottled; no mottling		
Forewing	Forewing basal streak	Present; absent		
Forewing	Forewing basal streak contour	None; costal; bilateral		
Forewing	Forewing fascia	0; 1; 2; 3		
Forewing	Forewing costals	0; 1; 2; 3; 4		
Forewing	Forewing dorsals	0; 1; 2; 3; 4		
Forewing	Forewing markings contour	Unilateral basal; unilateral proximal; bilateral; absent		
Forewing	Forewing apical marking	Dot; stripe; mottled; absent		
Forewing	Forewing cilia line	Present; absent		
Forewing	Forewing apical fringe	With markings; uninterrupted		
Thorax	Thorax pattern	Striped; uniform; silver or golden		
Antenna	Antenna colour pattern	Even or chequered; black tip; white tip		
Leafmine	Leafmine orientation	Tentiform underside; Tentiform upperside; Full depth blotch; Epidermal upperside blotch; Stem-mine		
Leafmine Leafmine location		Along secondary veins; Along main vein; Leaf base; Leaf margin; Leaf lobe; Whole leaf; Stem; Rachis wings		
Leafmine	Leafmine ribs	None; 1; Several; Many		
Leafmine	Leafmine frass	Linear; Aggregated; Scattered; Attached to cocoon		

Table 2. Morphology characters and states.

Head tuft colour was therefore removed from the key. The forewing ground colour is traditionally vividly described in the literature, with states including shining ochreous, pale golden brown, shining pale golden ochreous, dark brown to golden brown etc. (Emmet et al. 1985). Because of the subjective nature and dependence on lighting conditions, we opted for a simplified colour scheme of 'white' and 'other' for this key.

Lithocolletinae are all herbivores in their larval life stage with commonly a high degree of monophagy. The host plant is therefore often important for the identification. The key includes data on: host family, host genus and host genus/species (Table 3). Host genus and host genus/species partly overlap. The list 'host genus/species' includes all 303 known host species for the 86 Lithocolletinae (De Prins and De Prins 2014), but also includes records where there is only a generic identification of the host. Generic host associations are repeated in the separate list 'host genus' because this results in a significantly shorter list of options and may be preferred by the user. Characters of the larva and feeding method are included under "Leafmine" (Table 2). Early larval instars are sap-feeding only, but during later instars plant tissue is consumed and excreted in small pellets called "frass". The positioning of the frass in the mine is often diagnostic. The relative place and orientation of the mine on the leaf is also of importance. Mines can be "full depth", where everything except the two epidermal layers is eaten, or "epidermal" at the upper or lower side of the leaf, where the parenchyma is partly left intact. The epidermis of either mine type may contain ribs. The mines' position on the leaf, for example along a vein or in a leaf lobe, is often diagnostic and can be helpful with plants that are host to many species of Lithocolletinae. Further information that can be valuable for narrowing the selection

Character group	Character
Biology	Host family
Biology	Host genus
Biology	Host genus/species
Distribution	Country
Systematics	Species group

Table 3. Non-morphological characters.

is distribution information and the taxonomic species group. All character states in Table 2 are represented by images, the different states for the characters in Table 3 are presented in an alphabetical list. All lists can be searched by using the browsers' search function, commonly accessed through CTRL+F (Windows) or Command+F (Apple).

# Discussion

The key presented here for the Lithocolletinae of North-West Europe includes more species, covers a larger area and can be used for more life stages than any existing key for this subfamily. As such, it enables a large potential user group to identify Lithocolletinae through different approaches with minimized specialist effort involved, including future effort regarding updates. However, accomplishing this has not been without challenges. Most existing keys treat the species of a relatively restricted region, often a single country. A larger area holds more species and thus more candidate species that have to be ruled out. This can be circumvented by first selecting a country under distribution. However, inherent to an interactive key, the user has to make this choice actively. On the other hand, having more species in the key than just those already known and published for a country may enable recognition of introduced, migrating or previously overlooked species. A second challenge posed by covering North-West Europe involves the differences in voltinism at different altitudes. Species that are strictly univoltine in northern Sweden may be bivoltine in a warmer climate in Belgium. Adult flight period(s) or larval feeding period(s) could therefore not be included in our key. An advantage that dichotomous keys have over interactive keys in general is that they can include characters that are specific for a selection of taxa. An interactive key is based on a character matrix where for each taxon all character states need to be filled out; in a dichotomous key, there may be certain species pairs or groups that have distinguishing characters that are lacking in all other taxa (e.g. Laštůvka et al. 2013; Triberti 2007). In other cases, neither external adult characters, larval feeding characters, nor distribution data is sufficient to separate similar species and genitalia need to be examined. Genitalia characters are not included in the key because they are difficult to describe in a quantitative manner. There are plans to include an option in a future version of the key to switch from images of mounted adults to views of different stages or parts, such as the leafmines or genitalia, but this has not yet been implemented.

Aside from the challenges, this key also contains many advantages over existing keys. Perhaps one of the most crucial is the ability to combine quantitative characters with the human brain's ability to recognize subjective visual patterns (DiCarlo et al. 2012). In a dichotomous key, there is little indication of the remaining taxa and a user can only hope that the final outcome, usually just a species name, matches the candidate. Taxonomic specialists often rely on their extensive exposure to species for identifying them just as much as memorizing the distinguishing characters. The general impression of size and shape can be used in our key as a leading method for the identification by browsing images, or combined with selecting character options and visually verifying that the selection still might contain the species that is attempted to be identified. Optimally, a fast and reliable identification is reached by selecting several characters and visually selecting the best candidate from the remaining selection. Prerelease testing on photographs of live specimens from a regional observers databases by a group composed of novice and experienced identifiers (in a fashion that did not allow for statistical testing) indicated that an identification can be reached for most specimens by selecting three or four states only, with up to nine states for taxonomically difficult species or worn specimens. Over time, a user will become more experienced and will know which characters will be most useful when using the key.

Data on the larval life stage, host plant and distribution were included to take further advantage of all the benefits an interactive key has to offer. Several cautionary notes need to be taken into consideration with these characters. The list of host species is not cross-referenced with distribution. For example, a species may be recorded to feed on Acer pseudoplatanus in Germany, but not in Great Britain. If a user in Great Britain thus selects Acer pseudoplatanus as host species and Great Britain as country, they may end up with a selection of Lithocolletinae that includes false positives. On the other hand it can broaden the view of the user and allow for earlier recognition of new host records for a country, similar to how new species records for a country can be enabled by not narrowing down to a country first. When using the key, it should be advised to consult regional literature or websites on the resulting identification to see if this may be the case. Using a combination of several fairly easy characters of the larval feeding traces and an identified host should in most cases provide a reliable identification. The key can thus be used to record Lithocolletinae not only during their flight period, but also during the larval feeding period and greatly expand on the amount of faunistic data.

The target audience for this key is limited by the requirement that the insect first has to be recognised or identified as belonging to the Lithocolletinae. However, Lithocolletinae is a species rich subfamily with between 33 (Luxembourg) and 84 species (Austria) per country with distinct adult and larval features that separate them from other Lepidoptera. This makes them generally recognizable by professional lepidopterists and enthusiasts alike. The connection of the 86 Lithocolletinae in this key with 303 host plant species further indicates that the subfamily is an important component of most ecosystems in North-West Europe. Collecting faunistic data on Lithocolletinae has potential to contribute to biodiversity studies, and hopefully more interactive keys with this objective for other Lepidoptera groups will be created by taxonomic specialists to enable more enthusiasts to contribute their data to databases.

## Conclusion

We expect that the key presented here for Lithocolletinae of North-West Europe enables more people to contribute faunistic data with reliable identifications. The key has been designed to allow easy access for inexperienced users, yet still be an efficient tool for advanced users. This publication marks the release of version 1.0. Future changes will be noted under the version history at the website. We will greatly appreciate feedback from users and we hope to further expand and improve the key. Ultimately, we hope to include all European Lithocolletinae, and develop databases with reliable faunistic knowledge that can be useful for biodiversity estimates.

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RESEARCH ARTICLE



# New species of *Eriopeltastes* Burmeister & Schaum, 1840 (Coleoptera, Scarabaeidae, Cetoniinae, Trichiini) from South Africa

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#### Abstract

Both male and female of a new species of *Eriopeltasttes* Burmeister & Schaum, 1840, *E. (E.) ornatus* Ricchiardi, **sp. n.**, are described from the mistbelt grassland of KwaZulu-Natal. Sexual dimorphism is extreme in this genus, with females being brachypterous, fossorial and entirely black to dark brown in general body colour. This is only the fifth out of 12 known species in this genus for which the female is known. The species appears to be restricted to areas within or in the immediate vicinities of two marginal nature reserves, Karkloof and Blinkwater, in a grassland habitat that is regarded as one of the most threat-ened in the Province of KwaZulu-Natal. Despite its acknowledged importance as centre of endemism the area is currently in a precarious state of large-scale degradation.

#### **Keywords**

Trichiini, new species, KwaZulu-Natal, mistbelt grassland, Karklook Nature Reserve, Blinkwater Nature Reserve

# Introduction

As investigations progress in the more remote and previously neglected areas of the KwaZulu-Natal Province of South Africa, new taxa are being discovered on a regular basis. Often this occurs in places earmarked for conservation that are under threat

of land use change, with potentially irreversible environmental impact. A satisfactory biodiversity census of this region is, unfortunately, still lacking, but is currently being pursued by the provincial conservation authority, Ezemvelo KwaZulu-Natal (EKZN) Wildlife. Within this programme, it is of fundamental importance that new species be described and their distribution mapped speedily in order to avoid compromising the viability of minor or secondary reserves where micro-endemic and threatened species may occur. The rapid reporting of previously unknown species will contribute to assessing the renewal of the protected status and potential consolidation of these reserves, under the auspices of the integrated Biodiversity Conservation Planning of EKZN Wildlife (Ricchiardi and Perissinotto 2013).

Since January 2000, a number of specimens of an undescribed species of *Eriopeltastes* Burmeister & Schaum, 1840 have been collected during investigations in and around two minor reserves with an uncertain future, Karklook and Blinkwater. Both are situated in the Midlands region of KwaZulu-Natal, which has undergone massive transformation during the past century, mainly as a result of afforestation for the timber and paper industry (Mucina and Rutherford 2006). Several invasive plant species have taken over large areas previously supporting indigenous vegetation, thus compounding the problem of habitat deterioration. The genus *Eriopeltastes* is endemic to South Africa and virtually all its known species are micro-endemic, mountain relicts extremely susceptible to habitat disturbance/destruction (Ricchiardi et al. 1999). A description of this new species is presented here, to provide among other things, support for the renewal of the protected status and consolidation of these secondary reserves.

## **Methods**

In the Karkloof Reserve, field collections were undertaken from January 2000 to January 2001 in the main section (R. Perissinotto & L. Clennell *legit*), and during November 2012 in the "Melmoth" section (A. Armstrong *legit*). At Blinkwater, a one time collection was made on 5 January 2012, on the main ridge just below its summit (A. Armstrong *legit*). On all occasions, specimens were either collected in flight using a standard net, or picked by hand while perched on grass blades or crawling on the ground. No special trapping devices were used.

The description of morphological characters follows the terminology used by Krikken (1984) and Ricchiardi et al. (1999). Specimen length was measured from the anterior margin of the clypeus to the apex of the pygidium. Specimen width represents the maximum width of the elytra. Photos of the holotype were taken with a Nikon Coolpix P7700 fixed to one of the eyepieces of a Wild dissecting microscope. Photos were processed with photo stacking technique, using Combine ZP (free software by Alan Hadley, http://www.hadleyweb.pwp.blueyonder.co.uk). Finally, the background was removed from the photos using Adobe Photoshop, in order to increase clarity of resolution.

The first author wrote the taxonomic part of this study, including the description of the new species. The second author contributed all natural history and ecological observations. Collections are abbreviated as follows: DMSA – Natural Science Museum, Durban, South Africa; ISAM – Iziko South African Museum, Cape Town, South Africa; SANC – South African National Collection of Insects, Pretoria, South Africa; TMSA – Ditsong National Museum of Natural History (formerly Transvaal Museum), Pretoria, South Africa; PCER– Private Collection Enrico Ricchiardi, Turin, Italy; PCRP – Private Collection R. Perissinotto & L. Clennell, Port Elizabeth, South Africa. Geographic abbreviations: ECA – Eastern Cape Province; FRS – Free State Province; KZN – Kwa-Zulu Natal Province; MPU – Mpumalanga Province.

#### Taxonomic account

*Eriopeltastes (Eriopeltastes) ornatus* Ricchiardi, sp. n. http://zoobank.org/20E676E6-4586-4476-AFE1-7824F1BF2467 Figures 1–3

**Type series.** Holotype (HT)  $\Diamond$ : South Africa, KZN, Karkloof Nature Reserve, 6.II.2000, R Perissinotto & L Clennell *legit* (ISAM). Paratypes: 6  $\heartsuit$  same data as HT, but 22-23.I.2000 (ISAM, PCRP); 6  $\Diamond$  2  $\heartsuit$ : same data as HT (TMSA, PCER, PCRP); 16  $\Diamond$  1  $\heartsuit$ , same data as HT but 26-27.II.2000 (DMSA, PCER, PCRP); 1  $\Diamond$ , same data as HT but 28.I.2001 (PCRP); 1  $\Diamond$ , Karkloof NR Melmoth Section, 5.XI.2012, A Armstrong *legit* (SANC); 3  $\Diamond$ , Blinkwater Ridge, 30.I.2012, A Armstrong *legit* (DMSA, PCER).

**Diagnosis.** Eriopeltastes (E.) ornatus Ricchiardi, sp. n. is closest to E. (E.) lineatus Ricchiardi 1997. Both species have in common long and strongly curved (c-shaped) antennal clubs. They can, however, be easily separated as E. (E.) ornatus not only is much smaller in size than E. (E.) lineatus, but also exhibits the discal-lateral costa raised in the posterior half only. In E. (E.) lineatus, on the other hand, this costa is visibly raised throughout its length. The raised part of the discal-lateral costa is black in both species. The parameres are very similar in the two species, but differ significantly at the apex, which is slightly more expanded and curved downwards in E. (E.) ornatus Ricchiardi, sp. n. (Figure 1c; Ricchiardi 1997, Figures 3d & 3e).

**Etymology.** The species is named after the prominent J-shaped black band it exhibits on the sutural margin and on the apical raised part of the costal disc of each elytron.

**Description, HT**  $\circlearrowleft$ . Length 9.6 mm; width 4.6 mm. Body black; elytra light brown, with sutural margins black, black band continuing around posterior margin of elytral disk crossing apical umbone and extending anteriorly about  $\frac{1}{2}$  length of elytra (Figure 1a).

*Head.* Black, slightly shiny; wider than long; with dense punctuation on frons and vertex but poorly developed on clypeus; with scattered, testaceous, erected setae; eye canthus with long, scattered, testaceous setae; clypeus deeply concave, transverse, with front and side margins sharply raised, anterior margin rounded; antennae testaceous; club strongly C-shaped, more than twice as long as clypeus.



**Figure 1.** *Eriopeltastes (E.) ornatus* Ricchiardi, sp. n. Holotype  $\mathcal{O}$ : **a** habitus in dorsal view **b** habitus in ventral view **c** parameres in frontal view **d** parameres in lateral view (Photo & drawing: Enrico Ricchiardi).



**Figure 2.** *Eriopeltastes* (*E.*) *ornatus* Ricchiardi, sp. n. Paratype  $\mathcal{Q}$ : **a** habitus in dorsal view **b** habitus in ventral view (Photo: Enrico Ricchiardi).



**Figure 3.** Male specimen of *Eriopeltastes (E.) ornatus* Ricchiardi, sp. n. in its grassland habitat on the slopes of Blinkwater Ridge (Photo: Adrian Armstrong).

*Pronotum*. Black, shiny, glabrous; shape trapezoidal, with sides ridged but not crenulated; basal corners rounded, basal margin laterally sinuate, ridged basolaterally only; sides with white tomentum band approximating basal corner; midline grooved and covered internally with white tomentum, particularly at base; additional small tomentose area present on each side of disc; surface entirely covered with dense, long, testaceous, erect setae.

*Scutellum*. Black, glabrous, semicircular, enlarged; shiny, with scattered, round punctures.

*Elytra*. Testaceous, glabrous, shiny, with a round opaque area on the posterior margin of disc; apex rounded; with juxtasutural area black and posterior half of discolateral costa raised and black; behind disc a C-shaped black band joins juxtasutural and disco-lateral costae; striae poorly indicated; interstrie with large but shallow and scattered round punctures.

*Pygidium*. Semicircular, much wider than long, slightly constricted at sides; black, with erect, scattered black setae on disc; white tomentum covering entire surface, except area from middle of disc to apex.

*Ventral surface.* Black, covered with very scattered, long, flattened testaceous setae; abdominal sternites covered in white tomentum, except anal; tergites covered with white tomentum except at posterior edge; mesosternal protrusion absent (Figure 1b).

*Legs.* Protibiae testaceous, mesotibiae and metatibiae dark brown; with scattered, locally denser, long, erect, testaceous setae; protibiae with two external denticles; first protarsomere longer than second.

**Description, PT**  $\bigcirc$ . Length 10.1 mm; width 5.6 mm. Body black to dark brown, shiny, glabrous (Figure 2a).

*Head.* Black, poorly punctured, shiny, glabrous; clypeus concave, apex rounded, deflected and somewhat enlarged; antennae completely testaceous; club rounded, shorter than clypeal length.

*Pronotum*. Black, shiny, glabrous, trapezoidal, with sides parallel at centre and non-crenate; hind corners strongly rounded; hind margin centrally rounded, not ridged at middle and diverging smoothly towards lateral margins; with distinguishable rounded lateral impression at middle of lateral margins; covered with large but shallow, scattered punctures.

*Scutellum*. Black, glabrous, shiny, semicircular, wider than long, covered with deep, very scattered punctures.

*Elytra*. Apex rounded, dark brown, glabrous, shiny; striae marked with large, shallow, punctures; interstriae almost flat, with very scattered, small, shallow punctures.

*Pygidium*. Black, narrowing toward apex, laterally ridged, with large depression at middle of each side to apex; glabrous, shiny, black, with scattered shallow punctures.

*Ventral surface*. Black to dark brown; with very few, short and scattered testaceous setae; without any white tomentum (Figure 1b).

*Legs.* Generally as in male, with all tarsi slightly shorter; protibia broader than in male and exhibiting three denticles, with proximal smaller than other two.

**Remarks.** The male paratypes are similar to the holotype in general appearance, but there is a range of variability in the extent of white tomentum markings on the
pronotum and the width/length of the black band on the disco-lateral elytral costae. In extreme cases, the pronotum can be entirely black, without visible tomentose areas, while the elytra can appear completely pale testaceous, with black band virtually obsolete. All females are morphologically very similar, with ground colour from black to dark brown (Figure 2).

## Eriopeltastes updated species list

- E. (Parapeltastes) clarki Ricchiardi, 2004: ECA
- E. (Eriopeltastes) clennelli Ricchiardi, 1999: ECA
- E. (E.) evansi Ricchiardi, 1997: MPU
- E. (E.) leucoprymnus Burmeister & Schaum, 1840: FRS, KZN, MPU
- E. (E.) lineatus Ricchiardi, 1997: KZN
- E. (E.) maculatus Ricchiardi, 1999: KZN
- E. (E.) modestus (Péringuey, 1907): MPU
- E. (E.) montanus Ricchiardi, 1997: KZN
- E. (E.) natalensis (Péringuey, 1907): KZN
- E. (E.) ntinini Ricchiardi, 2013: KZN
- E. (E.) ornatus Ricchiardi, sp. n.: KZN
- E. (E.) perissinottoi Ricchiardi, 1999: ECA

#### Updated key to the males of Eriopeltastes

	1 Clypeus oval, elongate; el
Ricchiardi, 2004	
ltastes)2	- Clypeus oval, widened; el
	2 Antennal clubs straight
7	<ul> <li>Antennal clubs curved, at</li> </ul>
1	3 Antennal clubs not longe
Ricchiardi, 1999	
	<ul> <li>Antennal club 1.8–2.3 tin</li> </ul>
	– Antennal club at least 2.5
Véringuey, 1907)	
Ricchiardi, 1997	4 Front margin of clypeus s
5	<ul> <li>Front margin of clypeus of</li> </ul>
Ricchiardi, 2013	5 Front margin of clypeus sli
6	<ul> <li>Front margin of clypeus n</li> </ul>
	6 Clypeal surface covered in
Ricchiardi, 1997	
	<ul> <li>Clypeal surface covered in</li> </ul>
& Schaum, 1840	••••••

7	Antennal clubs C-shaped
_	Antennal clubs curved at apex
8	Discolateral costa of elytron black and elevated on entire length
	<i>E. lineatus</i> Ricchiardi, 1997
_	Discolateral costa of elytron elevated and black in posterior half only
	<i>E. ornatus</i> Ricchiardi, sp. n
9	Antennal clubs 1.8–2.3 times longer than clypeal length
	<i>E. maculatus</i> Ricchiardi, 1999
_	Antennal clubs at least 2.5 times longer than clypeal length
	<i>E. clennelli</i> Ricchiardi, 1999

#### Discussion

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There are currently 12 species described in the genus *Eriopeltastes* (Ricchiardi 1997, Ricchiardi et al. 1999, 2004; Ricchiardi and Perissinotto 2013) and 50% of these are endemic to the Province of KwaZulu-Natal. As females are brachypterous and unable to fly, species are generally very restricted in their distribution range, occupying elevations such as mountain slopes, hilltops and ridges (Ricchiardi et al. 1999). They are therefore of special biodiversity value, but unfortunately also very vulnerable to land use change and habitat degradation.

Eriopeltastes (E.) ornatus Ricchiardi, sp. n., here described, is most closely related to E. (E.) lineatus, which is currently only known from few high altitude localities in the southern and central Drakensberg, namely Cobham, Giants Castle and Mdedelelo (Ricchiardi 1997, Ricchiardi et al. 1999). Eriopeltastes (E.) ornatus Ricchiardi, sp. n. occurs in the Midlands of KwaZulu-Natal, at altitudes ranging from approximately 1460 to 1664 m asl and in humid grassland habitats in the proximity of wetlands or streams. All specimens were collected during late morning to early afternoon hours (between 10:00 and 14:00) of sunny or partly cloudy days, immediately after major rainfall events. Typically, males fly fast and low just above the grass cover searching for pheromonal signals emitted by females on the ground (Figure 3). Females are generally occupied lying eggs, burrowing just below the surface of wet soil or crawling among grass tufts while searching for new suitable breeding areas. Occasionally, they have been observed also climbing up tall grass stems to bask in the late morning sun (R.P. pers. observ.). But it is not clear yet precisely what function this behaviour may play or facilitate. It seems likely that it may have something to do with their thermophysiological balance, or with facilitating the emission of stronger and clearer pheromonal signals to guide searching males. It is also possible that this may even provide a take-off platform for short flights, although no female has been observed in flight yet.

Unlike the Drakensberg range, which is currently adequately protected through the Maloti Drakensberg Transfrontier World Heritage Site and includes a Ramsar Wetland of International Importance, the KZN Midlands has been exposed to large-scale degradation, mainly through agroforestry, inappropriate cultivation, uncontrolled fires, over-



**Figure 4.** View of Karkloof, with grassland patches at the margin of mistbelt forest in the vicinities of the local colony of *Eriopeltastes (E.) ornatus* Ricchiardi, sp. n. (Photo: Kelson Camp).

grazing by livestock and invasion of alien vegetation (Low and Rebelo 1996). The natural vegetation types where Eriopeltastes (E.) ornatus Ricchiardi, sp. n. is found are classified as Mooi River Highland Grassland (type Gs 8) and Midlands Mistbelt Grassland (type Gs 9), which are part of the Sub-Escarpment Grassland Bioregion of the Grassland Biome (Mucina and Rutherford 2006). In the Karkloof Nature Reserve, rainfall averages over 1000 mm pa, with most occurring in summer. As a result, the western section of the reserve consists mainly of Southern Mistbelt Forest (type FOz 3), with patches of sourveld grassland interspersed with Protea tree species (Figure 4). The lower reaches of the Karkloof Nature Reserve and all of the Blinkwater Ridge exhibit predominantly Midlands Mistbelt Grassland, dominated by species such as Themeda triandra and Aristida junciformis, but also a large diversity of flowering plants (Figure 5) (Mucina and Rutherford 2006). This vegetation type is currently classified as "Endangered", being regarded as one of the most threatened habitats in the Province of KwaZulu-Natal (Mucina and Rutherford 2006). The higher elevations of the Karkloof Nature Reserve are Mooi River Highland Grassland, which is classified as 'Vulnerable' because these grasslands are also under threat of extinction. According to the 2008 landcover map of KwaZulu-Natal (Ezemvelo KZN Wildlife 2011), only 24.5% of the original extent of the Midlands Mistbelt Grassland remained, much of it in a degraded condition, while 54.8% of the original extent of the Mooi River Highland Grassland remained, regardless of its condition.

Of the two localities where *Eriopeltastes* (*E*.) *ornatus* Ricchiardi, sp. n. is currently known to occur, one is part of provincial areas under statutory protection, the Karkloof



Figure 5. View of typical mistbelt grassland across the Blinkwater Ridge (Photo: Adrian Armstrong).

Nature Reserve, while the other falls just outside the current borders of the Blinkwater Nature Reserve. The first is located approximately 19 km north of Howick, while the second lies 20 km southwest of Greytown. The two are less than 50 km apart, but the areas in between, as well as on each side, have undergone drastic land use changes. The Karkfloof Nature Reserve was formally proclaimed in July 1980 (Ordinance No. 76, Provincial Gazette of Natal No. 4185), while its eastern extensions and the Blinkwater Reserve were only proclaimed in August 2012 (Notice No. 83, Provincial Gazette of KwaZulu-Natal Vol. 6 No. 799). Challenges to the management of both nature reserves remain, due to a shortage of operational budgets.

The Mistbelt area of KwaZulu-Natal is increasingly being recognised as an important centre of endemism (van Wyk and Smith 2001), yet it is in a precarious state of large-scale degradation. The discovery of a Trichiini species new to science within or in the immediate vicinities of the Karkloof and Blinkwater reserves should contribute significantly to the biodiversity value of the region. It is thus important that the two nature reserves maintain their current statutory protection and possibly be earmarked for further expansion and consolidation. In particular, it would be important at this stage to secure the incorporation of the slope where the colony of *E*. (*E*.) *ornatus* occurs on the Blinkwater Ridge (Figure 5) into the adjacent Nature Reserve.

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RESEARCH ARTICLE



# Two new species of *Mediomastus* (Annelida, Capitellidae) from Tokyo Bay, Japan

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#### Abstract

Two undescribed species of polychaetes in *Mediomastus* (Annelida: Capitellidae) were collected from intertidal to shallow habitats in Tokyo Bay, Japan. These are *M. duobalteus* **sp. n.** and *M. hanedaensis* **sp. n.** *Mediomastus duobalteus* **sp. n.** is distinguishable from all congeners by the following characters: 1) segments 3, 4, 8–11 stainable with methyl green, 2) thoracic capillary chaetae unilimbate, 3) abdominal capillary chaetae absent, 4) paddle-like chaetae in the thorax absent, and 5) abdominal hooded hooks not flared. *Mediomastus hanedaensis* **sp. n.** is similar to *M. warrenae* Green, 2002, but differs from the latter in the shapes of the thoracic capillary chaetae and the abdominal hooded hooks, and the staining pattern with methyl green. In addition, a key to all *Mediomastus* species is provided.

#### Keywords

Taxonomy, morphology, polychaete, Pacific

#### Introduction

Capitellids in the genus *Mediomastus* Hartman, 1944 are benthic polychaete worms that occur in marine and brackish water. *Mediomastus* is distinguishable from other genera in Capitellidae by the following characters: 1) peristomium (segment 1) without chaetae, 2) thorax with 10–12 segments, 3) segments 2–5 with capillary chaetae in both



**Figure 1.** Map showing the distribution of *Mediomastus* records in Japan. Open circles, previous studies; closed circle, this study. Sources: Rishiri Island (Kato et al. 2003, Tomioka et al. 2014); Sarufutsu (Imajima 1992); Monbetsu (Tomioka et al. 2014); Abashiri (Tomioka et al. 2013); Oshoro (Tomioka et al. 2014); Tokyo Bay (Nishi and Tanaka 2007, Nishi et al. 2009); Sagami Bay (Imajima 2006); Ariake Bay (Suyama et al. 2003, Niki et al. 2006).

rami, and 4) remaining thoracic and abdominal segments with hooded hooks (Hartman 1944, Warren et al. 1994, Green 2002). The genus was originally established for *M. californiensis* Hartman, 1944 (Hartman 1944) and now contains 14 species (Tomioka et al. 2013). In Japan, species in the genus have been reported from eight localities (Fig. 1, references therein), but only two of these records were identified to species: *M. californiensis* from Sagami Bay (Imajima 2006) and *M. opertaculeus* from Hokkaido (Abashiri, Monbetsu, Rishiri Island, and Oshoro) (Tomioka et al. 2013, 2014). Nishi and Tanaka (2007) reported the occurrence of representatives of *Medio-mastus* in Tokyo Bay but did not identify their material to species. Our capitellid specimens from Tokyo Bay were collected during an environmental assessment for the Haneda Airport re-expansion project (Nishi et al. 2009). They turned out to comprise two undescribed species, which we describe and illustrate in this paper. In addition, we provide a key to all species in *Mediomastus*.

## Materials and methods

Worms were collected from sandy mud sediment off Haneda, at the mouth of the Tamagawa River, Tokyo Bay, Japan. All specimens were fixed in 10% formalin in seawater and were later transferred to 70% ethanol after rising in deionized water. Morphological observation and methyl-green staining were performed as described by Tomioka et al. (2013). All specimens have been deposited in the Natural History Museum and Institute, Chiba, Japan. Morphological terminology follows that of Warren et al. (1994).

## **Systematics**

#### Mediomastus duobalteus sp. n.

http://zoobank.org/BD73215A-6470-4BE1-A439-A3F6ED585513 Figs 2–6

**Material examined.** Holotype: CBM–ZW 1088, Haneda, Tokyo Bay, St. L3e-2-1 (35.52783203°N, 139.7884979°E, sandy mud bottom, incomplete, collected May 2012. Paratypes (six specimens): CBM-ZW 1089, Haneda, Tokyo Bay, St. L4e-1-1, 35.52949905°N, 139.7836609°E, incomplete; mounted on SEM stub, collected spring 2007; CBM-ZW 1090, Haneda, Tokyo Bay, St. L2b-2, 35.52531815°N, 139.7931824°E, sandy-mud bottom, 5 m depth, incomplete, some parts of body mounted on slides, remaining parts preserved in 70% ethanol, collected May 2012; CBM-ZW 1091, Haneda, Tokyo Bay, St. 07s-L4e-1-2, 35.52949905°N, 139.7836609°E, sandy mud bottom, incomplete, collected spring 2007; CBM-ZW 1092, St. 07s-L4e-1-3, 35.52949905°N, 139.7836609°E, sandy mud bottom, incomplete, collected spring 2007; CBM-ZW 1093, St. 07s-L4e-1-4, 35.52949905°N, 139.7836609°E, incomplete, collected spring 2007; CBM-ZW 1094, Haneda, Tokyo Bay, St. L4e-1-2, sandy mud bottom, incomplete, collected May 2012.

**Description.** Holotype, anterior fragment with 73 segments; 18.0 mm in length; 0.51 mm in maximum width. Body color white in ethanol. Epithelium smooth. Nephridiopores lacking. Branchiae absent. All segments cylindrical. Sex uncertain.

Prostomium (Fig. 2A) conical, with short palpode; eversible proboscis with numerous minute papillae. Peristomium twice as long as chaetiger 1, without eyespots, achaetigerous.



**Figure 2.** *Mediomastus duobalteus* sp. n., holotype, CBM-ZW 1088. **A** Anterior end of body, left lateral view **B** abdominal segments, left lateral view.



Figure 3. *Mediomastus duobalteus* sp. n., paratype, CBM-ZW 1089, SEM images. A Capillary chaetae on segment 3 B notopodial hooded hooks on segment 6 C neuropodial hooded hooks on segment 6 D notopodial hooded hooks on segment 13.

Capillary chaetae unilimbate, with narrow wing (Figs 3A, 4A), present on chaetigers 1–4; noto- and neurochaetae each 8–12 in number per fascicle (Fig. 3A). Chaetigers 5–10 with hooded hooks, but without paddle-like chaetae. Notopodial hooded



**Figure 4.** *Mediomastus duobalteus* sp. n., paratype, CBM-ZW 1090. **A** Capillary chaeta from segment 2 **B** notopodial hooded hook from segment 6 **C** neuropodial hooded hook from segment 6 **D** notopodial hooded hook from segment 20.

hooks (Figs 3B, 4B) with short, stout fang and 3 fine teeth (Fig. 4B); hood with opening (Fig. 3B); shaft without constriction (Fig. 4B); shoulder indistinct (Fig. 4B); 6–10 hooks per fascicle. Neuropodial hooded hooks (Figs 3C, 4C) with short, stout fang and 3 fine teeth (Fig. 4C); hood with small opening (Fig. 3C); shaft without constriction (Fig. 4C); shoulder indistinct (Fig. 4C); 5–10 hooks per fascicle (Fig. 4C).

Abdominal segments twice as long as wide (Fig. 2B); with hooded hooks only. Hooded hooks (Figs 3D, 4D) with stout, pointed fang and 3 coarse teeth (Fig. 4D); hood with opening (Fig. 3D); shaft without constriction (Fig. 4D); shoulder indistinct; 2–6 hooks per fascicle.

Transition from thorax to abdomen marked by change in shape of hooded hooks; hooded hooks in thorax have small fang with fine, small teeth (Fig. 4B, C), while those in abdomen have stout, pointed fang; coarse and large teeth (Fig. 4D).

**Methyl-green staining.** Among seven specimens observed, methyl-green staining resulted in three patterns (Fig. 5). All patterns showed two bands of numerous, dense, minute spots: one band on segments 2 and 3, or 3 and 4; the other extending from segments 8 or 10 to segment 11. Figure 6 shows a stained worm having the pattern diagrammed in Fig. 5C.

**Etymology.** The specific name is a noun in the nominative singular, from the Latin numeral *duo* ("two") and the Latin noun *balteus* ("belt"), referring to the species' diagnostic staining pattern, in which the staining pattern is two belt-like bands.

**Remarks.** Among 14 congeners, *M. duobalteus* most closely resembles *M. warrenae*, but differs from the latter in the staining pattern with methyl green (segments 5



**Figure 5.** Diagram showing the three methyl-green staining patterns observed in the thorax (consisting of 11 segments) among seven specimens of *Mediomastus duobalteus* sp. n. **A** Paratype, CBM-ZW 1089 **B** holotype, CBM-ZW 1088 **C** paratype, CBM-ZW 1094.



Figure 6. Photograph of the thorax of *Mediomastus duobalteus* sp. n., holotype, CBM-ZW 1088, showing the methyl-green staining pattern, with the segment numbers and segmental boundaries labeled.

and 6 lack spots in *M. duobalteus* but are darkly stained post-chaetally in *M. warrenae*), the shape of the thoracic capillary chaetae (unilimbate in *M. duobalteus* vs. bilimbate in *M. warrenae*), and the shape of the abdominal hooded hooks (constriction absent in *M. duobalteus* but present in *M. warrenae*).

#### Mediomastus hanedaensis sp. n.

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http://zoobank.org/CE8D8CDA-3450-4E4D-86C6-AC40DD6EA18B
Figs 7–11
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**Material examined.** Holotype: CBM-ZW 1095, Haneda, Tokyo Bay, St. 07s-L4e-1-c, 35.52949905°N, 139.7836609°E, incomplete. Paratypes (two specimens): CBM-ZW 1096, same collection site as holotype, incomplete, mounted on SEM stub; CBM-ZW 1097, same collection site as holotype, incomplete, cut into 5 portions, all mounted on two slides. All specimens collected spring 2007.

**Description.** Holotype incomplete, with 27 segments, sex uncertain; 16.2 mm in length; 1.33 mm in maximum width. Body color whitish yellow in ethanol. Epithelium smooth. Nephridiopores lacking. Branchiae absent. All segments cylindrical.

Prostomium (Fig. 7A) conical, with short palpode; eversible proboscis with numerous minute papillae. Peristomium 1.2 times as wide as long, slightly longer than chaetiger 1, without eyespot, achaetigerous.

Thoracic chaetigers biannulate. Thin, unilimbate, capillary chaetae with narrow wing (Figs 8A, 9A) present on chaetigers 1–4; noto- and neurochaetae each 8–13 in number per fascicle (Fig. 8A). Chaetigers 5–10 with hooded hooks, but without paddle-like chaetae. Notopodial hooded hooks (Figs 8B, 9B) with short, blunt fang and 6 teeth (Fig. 9B); hood with small opening (Fig. 8B); shaft not constricted (Fig. 9B); shoulder indistinct (Fig. 9B); 6–8 hooks per fascicle (Fig. 8B). Neuropodial hooded hooks (Figs 8C, 9C) with short, stout fang and 7 teeth (Fig. 9C); hood with small opening (Fig. 8C); shaft without constriction (Fig. 9C); shoulder indistinct (Fig. 9C); 4–7 hooks per fascicle (Fig. 8C).

Abdominal segments 2.5 times as wide as long (Fig. 7B), with hooded hooks only. Hooded hooks with long fang and 3 teeth (Figs 8D, 9D); fangs in abdominal hooks longer and sharper than those in thoracic hooks; opening of hood wider than that of thoracic hooks; shaft with distinct constriction (Fig. 9D); 2–5 hooks per fascicle (Fig. 8D).



**Figure 7.** *Mediomastus hanedaensis* sp. n., holotype, CBM-ZW 1095. **A** Anterior end of body, left lateral view **B** abdominal segments, left lateral view.



Figure 8. *Mediomastus hanedaensis* sp. n., paratype, CBM-ZW 1096, SEM images. A Capillary chaetae on segment 3 B notopodial hooded hooks on segment 6, with an arrowhead indicating hood opening C neuropodial hooded hooks on segment 6 D notopodial hooded hooks on segment 33.

Transition from thorax to abdomen marked by alteration in shape of segments (longer in abdomen), shape of shaft of hooded hooks (with constriction in abdominal hooks), and length of fang of hooded hooks (longer in abdominal hooks).

**Methyl-green staining.** Among the three specimens observed, methyl-green staining resulted in three patterns (Fig. 10). Numerous minute spots sparsely and uniformly covered segments 5–9, 7–10, or 8–10; segment 10 (Fig. 10A) or 11 (Fig. 10B, C) with denser spots. Figure 11 shows the stained worm diagrammed in Fig. 10A.

Etymology. The specific name is an adjective, referring to the type locality.

**Remarks.** Mediomastus hanedaensis is similar to M. warrenae in the shape of the thoracic hooded hooks, but differs from the latter in the staining pattern with methyl green (segments 8 and 9 uniformly spotted in M. hanedaensis vs. post-chaetally spotted in M. warrenae) and in the shape of the thoracic capillary chaetae (unilimbate in M. hanedaensis vs. bilimbate in M. warrenae).



**Figure 9.** *Mediomastus hanedaensis* sp. n., paratype, CBM-ZW 1097. **A** Capillary chaeta from segment 2 **B** notopodial hooded hook from segment 9 **C** neuropodial hooded hook from segment 7 **D** notopodial hooded hook from segment 18; arrow indicates constriction.



**Figure 10.** Diagram showing methyl-green staining patterns observed in the thorax (consisting of 11 segments) among three specimens of *Mediomastus hanedaensis* sp. n. **A** Paratype, CBM-ZW 1097 **B** paratype, CBM-ZW 1096 **C** holotype, CBM-ZW 1095.



Figure 11. Photograph of the thorax of *Mediomastus hanedaensis* sp. n., holotype, CBM-ZW 1095, showing the methyl-green staining pattern, with the segment numbers and segmental boundaries labeled.

## Key to species of genus Mediomastus

Data compiled from Hartman (1944, 1947, 1969), Hartmann-Schröder (1959, 1962), Day (1961), Pillai (1961), Rasmussen (1973), Ben-Eliahu (1976), Warren et al. (1994), Green (2002), Tomioka et al. (2013), and this study.

1	Abdominal capillary chaetae present2
_	Abdominal capillary chaetae absent
2	Spine-like hooded hooks present <i>Mediomastus ambiseta</i> (Hartman, 1947)
_	Spine-like hooded hooks absent
3	Thorax with unilimbate capillary chaetae
_	Thorax with bilimbate capillary chaetae
4	Paddle-like chaetae present
_	Paddle-like chaetae absent
5	Thorax does not stain with methyl green6
_	Thoracic segments 1–4 stain with methyl green7
_	Thoracic segments 5–11 stain with methyl green
6	Eye spots present
_	Eye spots absent
7	Thorax with whip-like capillary chaetae
_	Thorax with unilimbate capillary chaetae Mediomastus duobalteus sp. n.
_	Thorax with bilimbate capillary chaetae Mediomastus warrenae Green, 2002
8	Thorax with whip-like capillary chaetae
	Mediomastus thomassini Warren et al., 1994

_	Thorax with hook-tipped capillary chaetae Mediomastus capensis Day, 1961
_	Thorax with unilimbate capillary chaetae9
9	Nephridiopores present
_	Nephridiopores absent
10	Thoracic segments 6–10 do not stain uniformly with methyl green, resulting
	in striped pattern Mediomastus australiensis Warren et al., 1994
_	Thoracic segments 6–10 stain uniformly with methyl green
	Mediomastus californiensis Hartman, 1944
11	Capillary chaetae with broad wing Mediomastus cirripes Ben-Eliahu, 1976
-	Capillary chaetae with narrow wing12
12	Parapodial ridge present <i>Mediomastus calliopensis</i> Warren et al., 1994
_	Parapodial ridge absent

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