

# Taxonomic review and phylogenetic analysis of fifteen North American *Entomobrya* (Collembola, Entomobryidae), including four new species

Aron D. Katz<sup>1</sup>, Rosanna Giordano<sup>1,2</sup>, Felipe Soto-Adames<sup>1,2</sup>

**1** Department of Entomology, University of Illinois, 320 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801, USA **2** Department of Biology, University of Puerto Rico, San Juan, PR 00931, USA

Corresponding author: Aron D. Katz ([aronkatz@illinois.edu](mailto:aronkatz@illinois.edu))

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## Abstract

The chaetotaxy of 15 species of eastern North American *Entomobrya* is redescribed in order to determine potential characters for the diagnosis of cryptic lineages and evaluate the diagnostic and phylogenetic utility of chaetotaxy. As a result, four new species (*E. citrensis* Katz & Soto-Adames, **sp. n.**, *E. jubata* Katz & Soto-Adames, **sp. n.**, *E. neotenica* Katz & Soto-Adames, **sp. n.** and *E. unifasciata* Katz & Soto-Adames, **sp. n.**) are described, and new diagnoses are provided for *E. asuta* Folsom, *E. atrocincta* Schött, *E. decemfasciata* (Packard), *E. ligata* Folsom, *E. multifasciata* (Tullberg), and *E. quadrilineata* (Bueker). Furthermore, previously undocumented levels of intraspecific variation in macrosetal pattern are reported, tempering the exclusive use of chaetotaxy for species delimitation. Phylogenetic relationships, estimated using both morphological and molecular data, indicate that *Entomobrya* is likely paraphyletic. The phylogenies also suggest that unreliable character homology, likely fostered by *Entomobrya*'s profusion of macrosetae, may limit the phylogenetic utility of chaetotaxy in groups characterized by an abundance of dorsal macrosetae.

## Keywords

Chaetotaxy, cryptic species, phylogeny, species diagnosis, *Willowsia*

## Introduction

Studies concerning species delimitation and taxonomy of Collembola have traditionally relied on comparative morphology. For some groups, however, uncertain homology, intraspecific variation, and characters of difficult observation have limited the utility of morphological characters as tools for species diagnosis and phylogenetic inference. Recent advances in DNA sequencing technology have provided scientists with additional ways to delimit groups that lack informative morphology, such as cryptic species complexes (Fрати et al. 1994, 1995, 2000; Carapelli et al. 1995, 2005; Simonsen et al. 1999; Soto-Adames 2002; Burkhardt and Filser 2005; Cicconardi et al. 2010, 2013; Felderhoff et al. 2010; Porco et al. 2012; Zhang et al. 2014c; Katz et al. 2015), but in practice most species diagnosis remains dependent on fixed characters, only obtained by rigorous morphological examination (Will and Rubinoff 2004).

Taxonomy and species delimitation of the genus *Entomobrya* Rondani, 1861 has been especially problematic due to intraspecific morphological variation and a general lack of informative taxonomic characters (Christiansen 1958b; South 1961). Conspicuous dorsal color patterns exhibited by members of this genus originally served as a practical means for species diagnosis (Bonet 1934). Other characters such as claw structure, antennal sense organs, setae types, and chaetotaxy of the male genital plate were introduced in Christiansen's (1958b) revision of North American *Entomobrya*, who attributed many color forms to intraspecific variants. These new characters marginalized color pattern as an exclusive diagnostic tool, but some are difficult to observe. Szeptycki's (1979) publication on the phylogenetic significance of dorsal chaetotaxy, introduced standard nomenclature for dorsal setae, providing a practical system to assess element homology between species. This system established chaetotaxy as the most important tool for species diagnosis in the family Entomobryidae and in theory, provided many additional characters to infer phylogenetic relationships (Soto-Adames 2008). Christiansen and Bellinger (1980, 1998) later combined traditional morphology with chaetotaxy in order to clarify species boundaries. Jordana and Baquero (2005) then simplified Szeptycki's (1979) system of body chaetotaxy and incorporated additional nomenclature for elements on the head later formalized by Soto-Adames (2008). Following this new nomenclature system, Jordana (2012) was able to delimit approximately 270 Palearctic species in subfamily Capbryinae and tribe Entomobryini using chaetotaxy.

Chaetotaxy has surely proven to be a valuable tool for springtail taxonomists, but not without complications. For some groups, especially those characterized by large numbers of setae, such as *Entomobrya*, the homology of macrosetae is not always clear. Intraspecific variation, apparent differences in setae arrangements, and differences of setae types make it difficult to determine homology between species (Soto-Adames 2008). Despite these complications, chaetotaxy still provides fixed observable differences that have been widely applied to successfully delimit species boundaries (Christiansen and Bellinger 1998; Carapelli et al. 2001; Jordana and Baquero 2005; Soto-Adames 2010; Jordana 2012). However, its utility as diagnostic and phylogenetic

characters is clearly dependent on a detailed assessment of intraspecific variation and homology between species of interest.

Christiansen and Bellinger's (1998) "The Collembola of North America" is the current authority for Nearctic Collembola species identification. However, some of their descriptions of *Entomobrya* express the need for re-examination due to a lack of informative characters for clear delineation of species boundaries, and high levels of observed intraspecific chaetotaxy and color pattern variation that suggest the presence of cryptic species complexes. In addition, their descriptions of chaetotaxy are limited and often unclear. Therefore, the primary goal of this study is to examine and document *Entomobrya* chaetotaxy to provide detailed descriptions and figures in order to clarify species boundaries and to simplify the diagnosis of eastern North American *Entomobrya* species. A total of 15 species of North American *Entomobrya* that occur east of the Mississippi River are examined and described with special emphasis on chaetotaxy and color pattern, including three cryptic species lineages identified by Katz et al. (2015) and two new species. Additionally, phylogenies, incorporating both the morphology described in this study and molecular COI sequences from Katz et al. (2015), are presented in order to explore how chaetotaxy and other morphological characters affect phylogenetic estimation.

## Methods

### Specimen collection and preparation

Approximately 146 specimens, representing 15 *Entomobrya* species (11 previously reported, 4 new), were examined in detail throughout the course of this study. Historical collections of *Entomobrya* from the Illinois Natural History Survey were also examined, but were not useful for the present study. Specimens preserved in 70% EtOH are old (e.g., collected prior to 1980) and in poor condition; although color pattern is often preserved, chaetotaxy and other small, but diagnostic, characters are extremely difficult to observe with confidence. Therefore, more recent material was needed to study details of chaetotaxy and other characters. Most specimens examined were collected by the senior author between 2011 and 2012 or otherwise provided by colleagues from localities throughout the USA, east of the Mississippi River. In the Material Examined sections it is assumed that the senior author collected all material, unless otherwise noted. Specimens were usually collected from leaf litter and extracted using a Berlese funnel or hand collected from bark and vegetation with an aspirator. Table 1 lists all *Entomobrya* species reported from North America.

Individuals sampled were sorted under a dissecting microscope to morphospecies according to color pattern and photographed to record dorsal thoracic and abdominal color patterns prior to slide mounting. All specimens were cleared with Nesbitt's solution and mounted on Hoyer's medium (Mari Mutt 1979) in preparation for light microscopy. The heads of specimens generally take longer to clear so they were dissected and mounted

**Table 1.** List of all *Entomobrya* species reported from North America. Of the 31 species, 15 were examined for this study and 16 were not included.

Species examined and described for this study	Species not included in study	
	eastern species <sup>1</sup>	western species <sup>2</sup>
<i>E. assuta</i>	<i>E. comparata</i>	<i>E. arnaudi</i>
<i>E. atrocincta</i>	<i>E. confusa</i>	<i>E. arula</i>
<i>E. bicolor</i>	<i>E. gisini</i>	<i>E. erratica</i>
<i>E. citrensis</i> sp. n.	<i>E. griseoolivata</i>	<i>E. kincaidi</i>
<i>E. clitellaria</i>	<i>E. sinelloides</i>	<i>E. nigriceps</i>
<i>E. decemfasciata</i>		<i>E. suzanna</i>
<i>E. intermedia</i>		<i>E. triangularis</i>
<i>E. jubata</i> sp. n.		<i>E. troglodytes</i>
<i>E. ligata</i>		<i>E. troglophila</i>
<i>E. multifasciata</i>		<i>E. washingtonia</i>
<i>E. neotenica</i> sp. n.		<i>E. zona</i>
<i>E. nivalis</i>		
<i>E. quadrilineata</i>		
<i>E. unifasciata</i> sp. n.		
<i>E. unostrigata</i>		

<sup>1</sup>Species reported east of the Mississippi River that were not obtained for this study

<sup>2</sup>Species with western North American distributions that have not been reported east of the Mississippi River. These species were not obtained for this study.

separately. Heads and bodies were both mounted dorsal-side up; the optimal position for observing important morphological characters. All illustrations were hand-drawn under a camera lucida then scanned and digitized with Adobe Illustrator CS6 software. Previously described species included in this study were identified based on morphological characters as delimited in Collembola of North America (Christiansen and Bellinger 1998).

All type specimens of *E. assuta*, *E. decemfasciata*, *E. ligata*, and *E. quadrilineata* deposited at the Illinois Natural History Survey, Champaign, IL, were examined. Types were either unavailable or their repository was unknown for most other species included in this study: *E. atrocincta*, unknown; *E. bicolor* Guthrie, unknown; *E. clitellaria* Guthrie, type stored at the Department of Animal Biology at the University of Minnesota (unavailable); *E. intermedia* Brook, unknown; *E. multifasciata*, unknown; *E. nivalis* (Linnaeus), unknown; and *E. unostrigata* Stach, type stored at the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, Krakow, Poland (not examined). Additional types for *E. decemfasciata* are stored at the Museum of Comparative Zoology, Harvard (unavailable), and *E. ligata* and *E. assuta* at the American Museum of Natural History, New York (unavailable).

Four new species are described and named based on morphological and molecular differentiation: *Entomobrya unifasciata* sp. n., *Entomobrya citrensis* sp. n., *Entomobrya neotenica* sp. n. and *Entomobrya jubata* sp. n., originally identified as *Entomobrya ligata*

color form B, *Entomobrya assuta* color form D, *Entomobrya* sp. n. 1 and *Entomobrya* sp. n. 2 in Katz et al. (2015). The cryptic lineages of *Entomobrya quadrilineata* color forms B, C, and D in Katz et al. (2015) are identified as *Entomobrya decemfasciata*.

## Discussion of characters

*Color pattern:* *Entomobrya* is characterized by distinct, but often complex and variable, color patterns. Given this diversity of color forms, color pattern was given a secondary diagnostic role partly due to confusion in the taxonomic literature caused by presumed occurrences of intra- and inter-population color pattern variation (Christiansen 1958b; South 1961). However, recent molecular phylogenetic studies have supported the resurgence of color pattern as a valid tool for species delimitation in some springtail groups (Carapelli et al. 1995, 2005; Frati et al. 1994, 1995, 2000; Simonsen et al. 1999; Soto-Adames 2002) including *Entomobrya* (Katz et al. 2015). For some species, color pattern serves as an easily observable and valid diagnostic tool when combined with traditional characters. Therefore, this study is mainly concerned with dorsal chaetotaxy and color pattern for species descriptions and diagnosis.

*Apical bulb of 4<sup>th</sup> antennal segment:* The apical bulb of the 4<sup>th</sup> antennal segment is present in all species of North American *Entomobrya* (except *E. sinelloides* Christiansen, 1958b) and has a number of different forms (e.g., single lobe, bi-lobed, tri-lobed). Christiansen and Bellinger (1980, 1998) incorporate these different character states into their species descriptions and diagnostic tables. However, apical bulbs are prone to misinterpretation of character states, being absent or irregular due to antennal regrowth, and moderate intraspecific variation. Therefore, this character is described here, but not considered useful for species diagnosis.

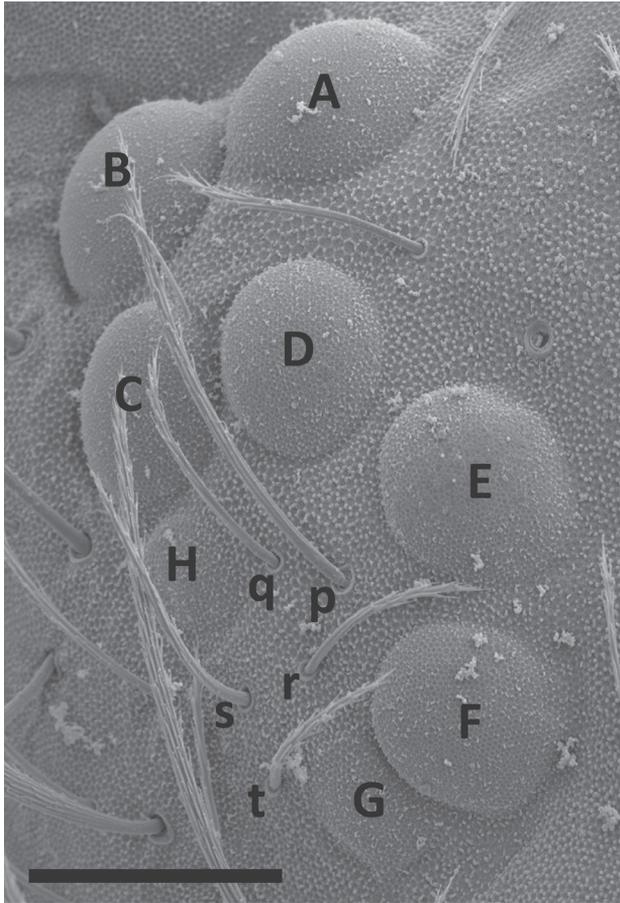
*Apical sense organ of 3<sup>rd</sup> antennal segment:* This setal complex consists of two sense pegs, “guard setae”, and a number of differentiated setae (Chen and Christiansen 1993). These characters are very difficult to observe and lack useful variation for *Entomobrya* species diagnosis.

*Differentiated setae on ventral side of 1<sup>st</sup> antennal segment:* Several small, spine-like setae occur on the ventral side of the 1<sup>st</sup> antennal segment. However, no useful variation was observed for the diagnosis of species treated here.

*Eye patch setae:* The number of setae in the eye patch (Fig. 1A) is important for distinguishing some species of *Entomobrya*. The number of setae vary from three to six, but five is most common. Eye patch setae nomenclature in this study follows Mari Mutt (1986).

*Prelabral and labral chaetotaxy:* The number of setae in the prelabral and three labral rows is 4,5,5,4, typical of Entomobryidae. However, the prelabral setae (row basal to labrum), which are usually ciliate, may appear to be smooth under low magnification for some species of *Entomobrya*.

*Labral papillae:* The morphology of the labral papillae (Fig. 2A–D) varies considerably among species of *Entomobrya* (Christiansen 1958b; South 1961) and is relatively easy to observe, thus, descriptions of the papillae are also included in the present study.

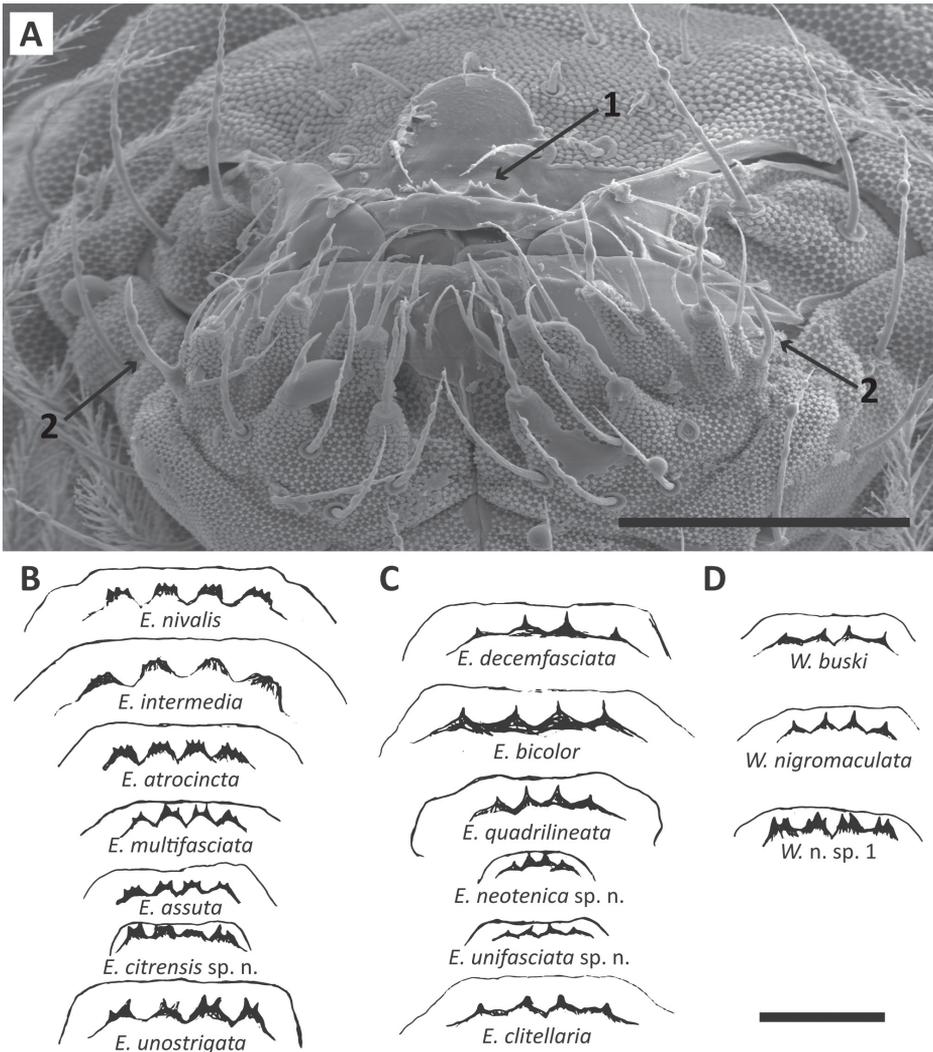


**Figure 1.** *Entomobrya atrocincta*. SEM photograph of right eye patch, eyes (A–H) and eye patch setae (p–t). Scale bar = 20  $\mu$ m.

*Labial appendage:* Christiansen (1958b) incorporated the ratio of labial appendage on papilla E (Fig. 2A) to papilla length, even after recognizing the potential limitations of relative ratios (Christiansen 1954). The deformation of these soft body parts after slide mounting and pronounced variation between instars makes these measurements unreliable and not practical for diagnostic purposes (South 1961).

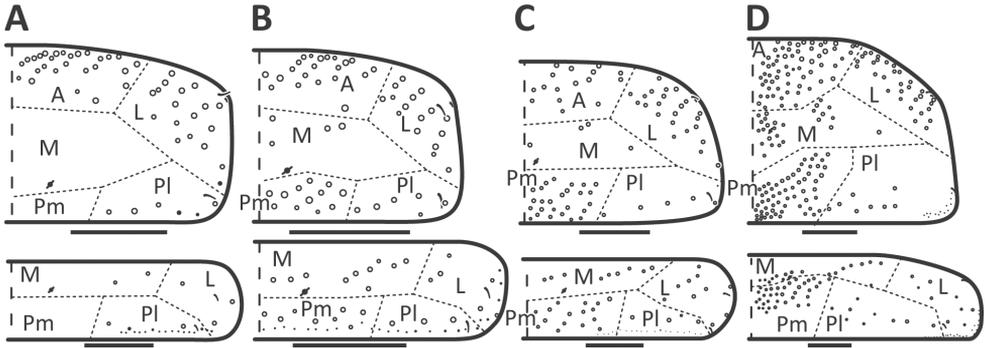
*Labial chaetotaxy:* Labial palp proximal setae, labial triangle setae, and post-labial setae have been shown to be taxonomically informative for other genera (Chen and Christiansen 1993, 1997; Soto-Adames 2010), but generally lack any useful variation in North American *Entomobrya*. For this study, all descriptions of labial chaetotaxy follow nomenclature developed by Chen and Christiansen (1993).

*Dorsal chaetotaxy:* The introduction of “stable” character systems for dorsal macrosetae (Szeptycki 1979; Jordana and Baquero 2005; Soto-Adames 2008) have provided a large set of characters that are relatively easy to observe and compare among different



**Figure 2.** **A** *Entomobrya atrocincta*. SEM photograph of mouth cone, four labral papillae with multiple projections (1) (arrow points to 3<sup>rd</sup> papilla from left to right) and labial appendage (2) **B–D** drawings of labral papillae of *Entomobrya* and *Willowsia* species: *Entomobrya* with multiple projections per papilla (column B); *Entomobrya* with a single projection per papillae (column C); labral papillae of all *Willowsia* species occurring in the Eastern United States (column D). Note *Willowsia* n. sp. 1 can be differentiated by the presence of multiple projections per papilla. *E. jubata* sp. n. and *E. ligata* are not included in figure but are similar to *E. clitellaria* and *E. unifasciata* sp. n. respectively. Scale bars = 20  $\mu$ m.

species. *Entomobrya* are extremely setaceous (polychaetotic), providing many diagnostic characters. However, the abundance of macrosetae and observed variation among instars (including among adult instars) makes homology assessment of each element difficult. The macrosetae inserted external to the sensilla on abdominal segments (Abd.) 1-3 and



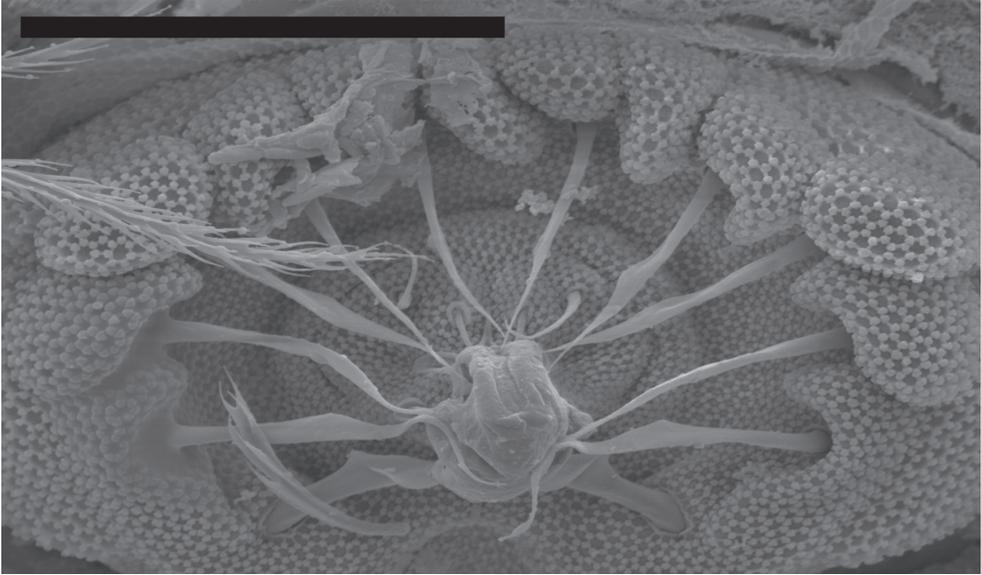
**Figure 3.** Differences of macrosetae abundance within thoracic chaetotaxy zones A, L, M, Pm, and Pl for Th. 2 (top) and Th. 3 (bottom): **A** *E. assuta* with the lowest number of macrosetae **B** *E. atrocincta* with a moderate number of macrosetae **C** *E. unostriata* with a moderate number of macrosetae **D** *E. decemfasciata* with the most macrosetae. Marked differences in zone Pm for both segments clearly differentiate between some groups of *Entomobrya*. Scale bars = 100 µm.

external to the lateral bothriotricha on Abd. 4 were not included in descriptions or analysis due to extensive variation and difficulty of observation. The dorsal chaetotaxy of the head and along the dorsal median line on the mesothorax (Th. 2), metathorax (Th. 3), Abd. 1–3 and macrosetae internal to the lateral bothriotricha on Abd. 4 are easy to observe and relatively stable, thus are emphasized in the descriptions and analysis. The chaetotaxy of Abd. 5 lacks useful variation in specimens observed in this study. Thoracic zones (Fig. 3) originally described by Szeptycki (1979), differ in number of macrosetae among *Entomobrya* species and may provide some initial direction in the identification process.

**Trochanteral organ:** Differences in number and arrangement of small, spine-like setae on the trochanter, termed the trochanteral organ, have also been used to support the identification of some species (Christiansen 1958b). These setae are not only difficult to observe, but intraspecific variation limits their use for *Entomobrya* species delimitation (South 1961). However, the setal pattern, rather than the presences/absence or specific seta, seems to separate some species.

**Male genital plate:** Differences in chaetotaxy of the male genital plate can accurately delineate many North American *Entomobrya* species (Christiansen 1958a, 1958b). However, males with a well-developed plate are uncommon in the samples examined and when present, plates are difficult to observe under light microscopy, requiring electron microscopy in order to easily discern characters with certainty (Fig. 4).

**Mucro and tarsal claw:** Ratios of relative positions of mucronal and unguinal teeth do not usually deform by the mounting process, but they depend heavily on the angle or position of the slide mount in order to properly standardize relative measurements between individuals. Additionally, these characters present few discernable differences between *Entomobrya* species and have been noted to be of little taxonomic value for European *Entomobrya* (South 1961). There are marked differences in the distance between dorsal tooth and lateral teeth on the unguis between *Entomobrya* species.



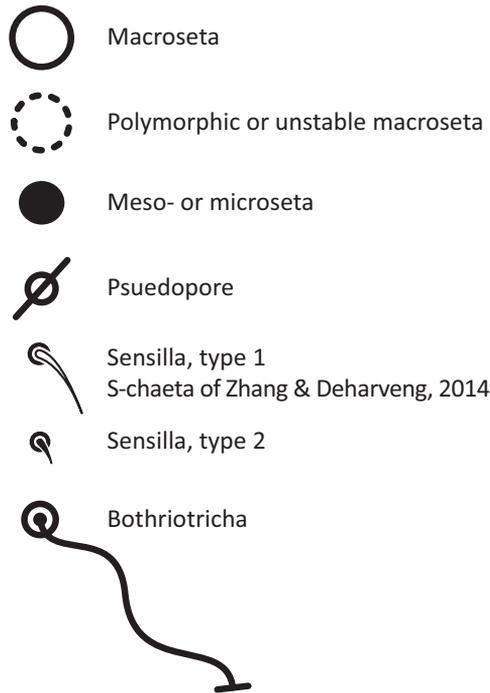
**Figure 4.** *Entomobrya atrocincta*. SEM photograph of papillate male genital plate. Scale bar = 20  $\mu$ m.

However, these measurements are difficult to quantify due to variation and mounting inconsistencies.

*Anatomical measurement ratios:* Some authors have used relative anatomical length or distance ratios for *Entomobrya* species separation (Christiansen and Bellinger 1998; Jordana 2012). Although measurement ratios may provide some level of diagnostic utility, high levels of variation in ratios (Christiansen 1954, 1958b), deformation of soft tissues, and variable final position of mounted specimens reduce character consistency and reliability, thus ratio measurements are not used here as diagnostic characters.

### Character nomenclature, abbreviations, and symbols

Descriptions of adult dorsal macrosetae provided in this study follow the dorsal trunk chaetotaxy nomenclature established by Szeptycki (1979) and the dorsal head chaetotaxy from Jordana and Baquero (2005) and Soto-Adames (2008). References to thoracic and abdominal segments are abbreviated as Th. (2-3) and Abd. (1-6) respectively. Symbols used in chaetotaxy descriptions are presented in Figure 5. There are seven general morphological structures, including three types of setae, recognized for the purpose of this study. Macrosetae are the primary setae, characterized by a large socket, long shaft, and are usually apically truncate or blunt (Fig. 6A). Microsetae are common type 5 (Christiansen 1958b), ciliate, short, thin, acuminate setae with very small sockets (Fig. 6A). Mesosetae are morphologically similar to microsetae, but are ostensibly longer, with larger sockets (Fig. 6A). They tend to be smaller and thinner



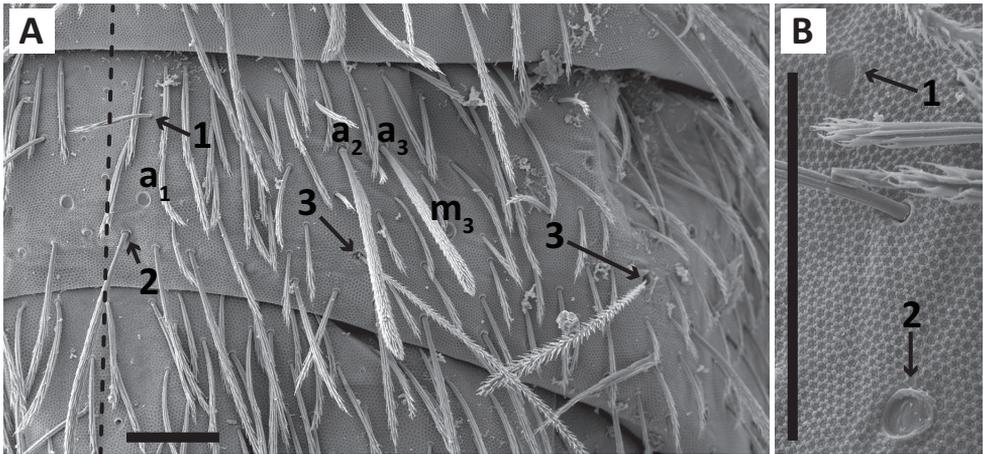
**Figure 5.** Symbol legend for diagrammatic figures of chaetotaxy presented in the species descriptions.

than macrosetae, but substantial variation in length and size of both setae types cause overlap. Bothriotricha (Fig. 6A) are specialized setae characterized by unique morphology; long, thin, with conspicuous ciliation. There are also two types of short, smooth, acuminate, spine-like sensilla: type 1 (S-chaeta of Zhang and Deharveng 2015) is long and more common and occur on all thoracic and abdominal segments; while type 2 (S-microchaeta of Zhang and Deharveng 2015) is short, sometimes slightly blunted, usually paired with type 1, and is only present on Th. 2, Abd. 1, and Abd. 3. Pseudopores are relatively difficult to observe and resemble sockets of macroseta, but are generally shallower and lack a thickened socket wall (Fig. 6B).

### Phylogenetic analysis

In order to investigate the effects chaetotaxy and other morphological characters have on phylogenetic relationships, Bayesian and maximum likelihood phylogenetic analyses were conducted using MrBayes v. 3.2.1 (Ronquist et al. 2012) and RAxML v. 8.1.20 (Stamatakis 2014); one based only on morphological characters and an additional analysis based on combined morphology and molecular datasets.

The morphological analysis of 22 taxa, including 14 species of *Entomobrya* and 8 additional species, was based on 179 morphological characters. *Pseudosinella violenta*



**Figure 6.** *Entomobrya atrocincta*. SEM photographs of selected morphological characters: **A** close-up view of 3<sup>rd</sup> abdominal segment, macroseta (socket of  $a_3$  and  $m_3$  are labeled), type 5 microseta (1), mesoseta (2), bothriotricha (3), the dotted line represents the medial division of Abd. 3 **B** comparison between a pseudopore (1) and macroseta socket (2). Scale bars = 20  $\mu$ m.

(Folsom) was selected as the outgroup. Character state assignments (Suppl. material 1) were attained through observation of material collected for this study, except for *P. violenta* and *Seira dowlingi* (Wray), which were obtained from Soto-Adames (2010) and Soto-Adames (2008) respectively. Morphological characters were analyzed under the Mk model of evolution (Lewis 2001), with rate variation among characters (gamma distribution), four independent runs, starting from random trees, four Markov chains (temp=.5), and 25,000,000 generations, sampling every 1000<sup>th</sup> generation. Default values were used for all other parameters.

The combined analysis incorporating complete COI sequences (1539 bp) for 89 exemplars (See Katz et al. 2015 for specific details regarding specimens, GenBank accession numbers, gene choice, DNA extraction, amplification, sequencing, and primer development) with *P. violenta* as the outgroup. The appropriate model of sequence evolution (GTR+I+G), was selected using jModeltest (Posada 2008), whereas the Mk model (Lewis 2001) was implemented for the morphology dataset. Morphology was not examined for sequenced individuals due to the destructive process of DNA extraction; therefore character states were attained from other individuals of the same species or from descriptions in the literature (Soto-Adames 2008, 2010) and were added to corresponding OTU's in the matrix. Bayesian analyses were conducted using MrBayes on the CIPRES Science Gateway (Miller et al. 2010), convergence was assessed by observation of average standard deviation of split frequencies values below  $p < 0.003$ , a 25% burn-in was used, and all posterior probabilities and consensus trees were computed in MrBayes. The maximum likelihood analyses were conducted using RAxML's rapid bootstrap algorithm incorporating 1000 bootstrap replicates.

### ***Character state assignments***

Dorsal setae were identified by their relative positions to bothriotricha, sensilla, pseudopores, and to neighboring setae, following descriptions provided by Szeptycki (1979), Jordana and Baquero (2005), and Soto-Adames (2008). All macrosetae labeled in figures for species descriptions were included as characters in the phylogenetic analysis (See Suppl. material 1 for morphological character matrix). Macrosetae external to sensillum on Abd. 1-3 and external to bothriotricha on Abd. 4 were not included in descriptions or analysis due to uncertain homology. Character states for setae were defined as present, absent, or polymorphic (i.e., variable between individuals of the same species). Macrosetae were considered absent if meso- or microsetae were present in corresponding position. Polymorphic states are indicated in the figures as dotted circles. Other morphological characters included in the phylogenetic analysis are number of eye patch setae, labral setae (smooth or ciliate), labral papillae (smooth, single projection, or multiple projections), setae within labial triangle (ciliate or smooth), antennal bulb (absent or present), scales (absent or present), and dental spines (absent or present). See Suppl. material 1 for a complete list of all 179 morphological characters and character states circumscription used in this analysis.

## **Results**

### **Species descriptions and taxonomy**

#### **Family Entomobryidae Schäffer, 1896**

#### **Entomobryini Schäffer, 1896 sensu Soto-Adames et al. 2008**

#### ***Entomobrya* Rondani, 1861**

This genus characterized by having 8+8 eyes within black or dark blue patches of pigment, a bidentate mucro with a smooth basal spine, basic chaetotaxy formed by type 5 microsetae and the absence of antennal sub-segmentation, scales, dental spines, and differentiated “smooth” setae on the inner surface of the hind tibiotarsus.

In addition, all species treated here have an apical antennal bulb; main sensilla on 3<sup>rd</sup> antennal segment sense organ thin, smooth, blunt and peg-like; differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment of two types, short and spine-like and long and seta-like; labral setae 5,5,4 and smooth; outer maxillary lobe of maxilla with subapical and apical setae smooth and subequal, and sublobal plate with three smooth seta-like appendages; lateral appendage of labial papilla E slightly curved, relatively thick, blunt; all post labial setae type 5; unguis with one outer, two lateral and four inner teeth, and a lanceolate unguiculus; and mucronal spine smooth; and most have all posterior setae of labial triangle ciliate, as M1, r, E, L1, L2, with r significantly smaller than other setae and A1-A5 smooth.

More general morphological descriptions of this genus are provided in Christiansen (1958b), Stach (1963), Christiansen and Bellinger (1998), and Jordana (2012). Chaetotaxy provided in the following descriptions follows the nomenclatural systems established by Szeptycki (1979) and Jordana and Baquero (2005) with some modifications.

Informative diagnostic adult chaetotaxy characters of all *Entomobrya* species treated here are listed in Table 2. These characters were specifically chosen for their ease of observation, stability (lack of polymorphisms), and confident homology.

**Table 2.** Informative diagnostic adult chaetotaxy for the separation of all *Entomobrya* species in this study<sup>5,6</sup>. These characters were specifically chosen for their ease of observation, stability, and lack of polymorphic states. Refer to species remarks for more specific diagnostic characters. Parentheses indicate a rarely observed state.

Species	Head macrosetae <sup>1</sup>					Th. 2 macrosetae <sup>1</sup>		Abd. 2 macrosetae <sup>1</sup>			Abd. 3 macrosetae <sup>1</sup>		Prelabral setae <sup>2</sup>	Number of eye patch setae <sup>3</sup>	Labral papilla <sup>4</sup>
	M <sub>3i</sub>	S' <sub>0</sub>	S <sub>4i</sub>	Ps <sub>3</sub>	Ps <sub>5</sub>	m <sub>2</sub>	m <sub>5</sub>	a <sub>2</sub>	a <sub>3</sub>	m <sub>3ep</sub>	a <sub>1</sub>	a <sub>2</sub>			
<i>E. assuta</i>	0	0	1	0	1	0	0	0	0	0	0	1	0	5	0
<i>E. atrocincta</i>	0(1)	0	1	0	1	1	0	1	1(0)	0(1)	1	1	1	5	0
<i>E. bicolor</i>	1	0	1	0	0	1	1	1	1	1	0	1	1	5	1
<i>E. citrensis</i> sp. n.	0	0	1	1	1	0	0	0	1	0	0	1	0	5	0
<i>E. clitellaria</i>	0	0	1	0	1	1	1	1	1	1	0	1	1	5	1
<i>E. decemfasciata</i> <sup>5</sup>	1	0	1	0	1	1	1	1	1	1	0	1	1	5	1
<i>E. intermedia</i>	0	0	0	0	1	1	1	1	1	0	1	0	1	5	0
<i>E. jubata</i> sp. n.	0	1	1	0	0	1	1	1	1	1	0	1	1	5	1
<i>E. ligata</i> <sup>6</sup>	0	0	1	0	1	1	0	1	0	1	0	0	0	3	1
<i>E. multifasciata</i>	0	0	0	0	1	1	0	1	1	0	1	1	1	5	0
<i>E. neotonica</i> sp. n.	0	0	0(1)	0	1	1	0	1	0	0	0	0	0	3	1
<i>E. nivalis</i>	0	0	1	P	1	1	1	1	1	1	1	0	1	5(6)	0
<i>E. quadrilineata</i> <sup>5</sup>	1	0	1	0	1	1	1	1	1	1	0	1	1	5(6)	1
<i>E. unifasciata</i> sp. n. <sup>6</sup>	0	0	1	0	1	1	0	1	0(1)	1	0	0	0	3	1
<i>E. uostrigata</i>	0	0	1	0	1	0	1	1	1	1	1	0	1	5	0

<sup>1</sup> Character states: absent (0), present (1), polymorphic (P).

<sup>2</sup> Character states: smooth or finely ciliate (0), ciliate (1).

<sup>3</sup> Refer to Figure 1 for SEM photograph of eye patch setae.

<sup>4</sup> Character states: multiple projections (0), single projection (1). Refer to Figure 2 for SEM photograph and drawings of labral papillae.

<sup>5</sup> *E. decemfasciata* and *E. quadrilineata* cannot be separated by these diagnostic characters. Color pattern is critical for diagnosis.

<sup>6</sup> *E. ligata* and *E. unifasciata* sp. n. cannot be separated by these diagnostic characters. Color pattern is critical for diagnosis.

***Entomobrya assuta* Folsom, 1924**

Figs 2, 3A, 7, 8, 39

**Description.** *Body shape and color pattern.* Body dorso-ventrally flattened. Dorsal color pattern highly variable, and in many cases, without clear discrete forms (Fig. 7). Patterns usually consisting of black or dark blue pigment on a white, yellow, orange, or light purple background. Thorax pigmentation variable. Sometimes Th. 2 and Abd. 3 entirely dark, forming two strong transverse bands. All forms studied have a dark transverse band across the posterior margin of Abd. 2. Abd. 3 entirely dark or with two or three pale spots. Abd. 4 and 5 also with 2-3 pale spots each. Posterior margin of Abd. 4 usually lacking pigment, forming an irregular pale transverse band. Antennae usually entirely purple, but 1<sup>st</sup> antennal segment at times considerably lighter.

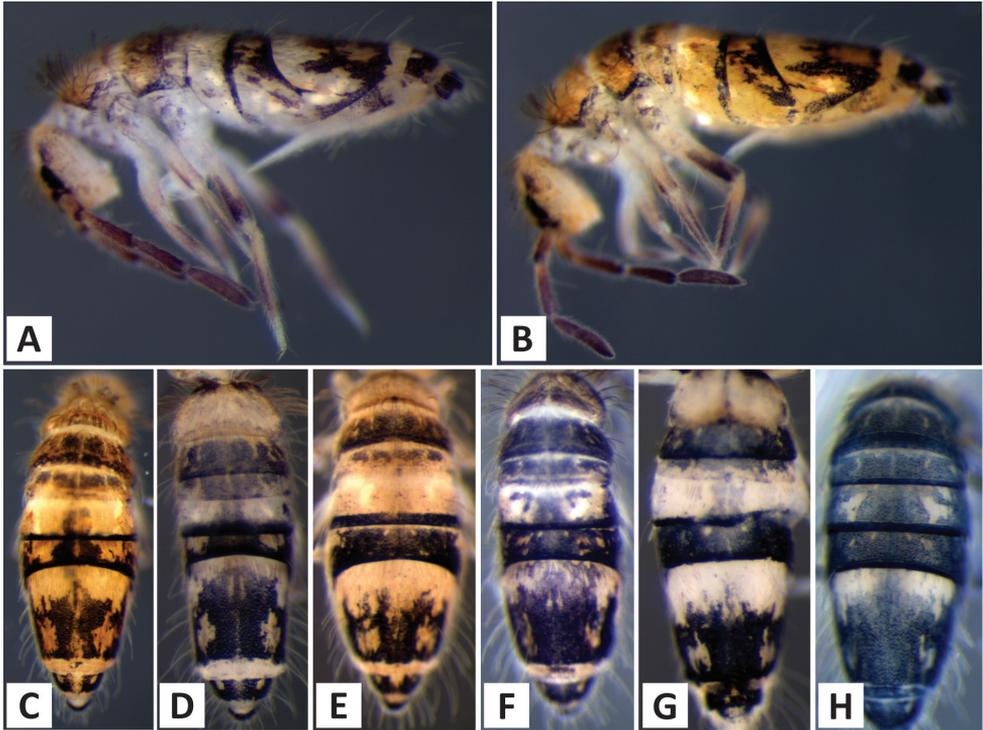
*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually bilobed, sometimes simple or, rarely, trilobed. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 3\times$  short setae. Prelabral setae finely ciliated, seemingly smooth at low magnification under light microscopy. Distal margin of labral papillae with 2-3 small spine-like projections (Fig. 2). Dorsal head chaetotaxy reduced in comparison with other species (Fig. 8A); macrosetae An<sub>0</sub>, A<sub>6</sub>, M<sub>3</sub>, S<sub>0</sub>, S<sub>1</sub>, Pi<sub>1</sub>, Pa<sub>2</sub>, Pa<sub>3</sub>, Pm<sub>2</sub>, and Pm<sub>11</sub> always absent; An<sub>3a3</sub> seen in one individual; M<sub>3</sub> present in 1/4 of individuals observed; S<sub>0</sub> and Pa<sub>1</sub> usually present. Eyes G and H small and subequal. Eye patch with 5 setae.

*Thorax.* Thoracic chaetotaxy extremely reduced but stable, without macrosetae variation in specimens studied. A row of microsetae present along entire posterior margin of Th. 2 and Th. 3 (not displayed in figures). Th. 2, with a<sub>5</sub> and 5 posterior macrosetae (Fig. 8B): p<sub>4</sub>, p<sub>5</sub>, p<sub>6</sub>, p<sub>6e</sub>, and p<sub>6ep</sub>. Th. 3, with 7 macrosetae (Fig. 8C): a<sub>5</sub>, a<sub>6</sub>, m<sub>5</sub>, m<sub>6</sub>, m<sub>7</sub>, p<sub>4</sub>, and p<sub>6</sub>; macrosetae a<sub>1</sub>, a<sub>2</sub>, and a<sub>3</sub> absent. Chaetotaxy of zone Pm extremely reduced for both thoracic segments, without macrosetae (Figs 3A; 8B,C).

*Legs.* Trochanteral organ with triangular setal pattern and up to 23 setae. Unguis with 4 inner teeth; basal teeth located approximately middle of inner claw length.

*Abdomen.* Abdominal chaetotaxy reduced but stable, no macrosetae variation observed. Abd. 1 with 1 macroseta only (Fig. 8D); row of microsetae along entire posterior margin present (not displayed in figure). Abd. 2 with 3 macrosetae: m<sub>3</sub>, m<sub>3e</sub>, and m<sub>5</sub> (Fig. 8E). Abd. 3 with 4 macrosetae: a<sub>2</sub>, m<sub>3</sub>, pm<sub>6</sub>, and p<sub>6</sub>. Mesosetae a<sub>2a</sub>, inserted slightly anterior and exterior to a<sub>2</sub>, sometimes with relatively large socket resembling socket of macroseta a<sub>3</sub>, but due to mesoseta a<sub>2a</sub>'s close proximity to a<sub>2</sub>, it is most likely a duplicate of a<sub>2</sub> rather than homologous to a<sub>3</sub> (Fig. 8F). Abd. 4 with 4 inner macrosetae (Fig. 8G). Mucronal teeth subequal.

**Remarks.** *Entomobrya assuta* is the only species with the color pattern as described above in combination with the absence of all the following macrosetae: head Ps<sub>3</sub>, Th. 2 m<sub>2</sub> and m<sub>3</sub>, Abd. 2 a<sub>2</sub>, a<sub>3</sub> and m<sub>3ep</sub>, and Abd. 3 a<sub>1</sub> (see Table 2 for additional diagnostic characters). Among all species observed in this study, only *E. assuta* and *E. citrensis* sp. n. share this unique pattern of substantially reduced dorsal chaetotaxy. *Entomobrya assuta* and *E. citrensis* sp. n. share very similar chaetotaxy and color pattern. In fact, *E.*

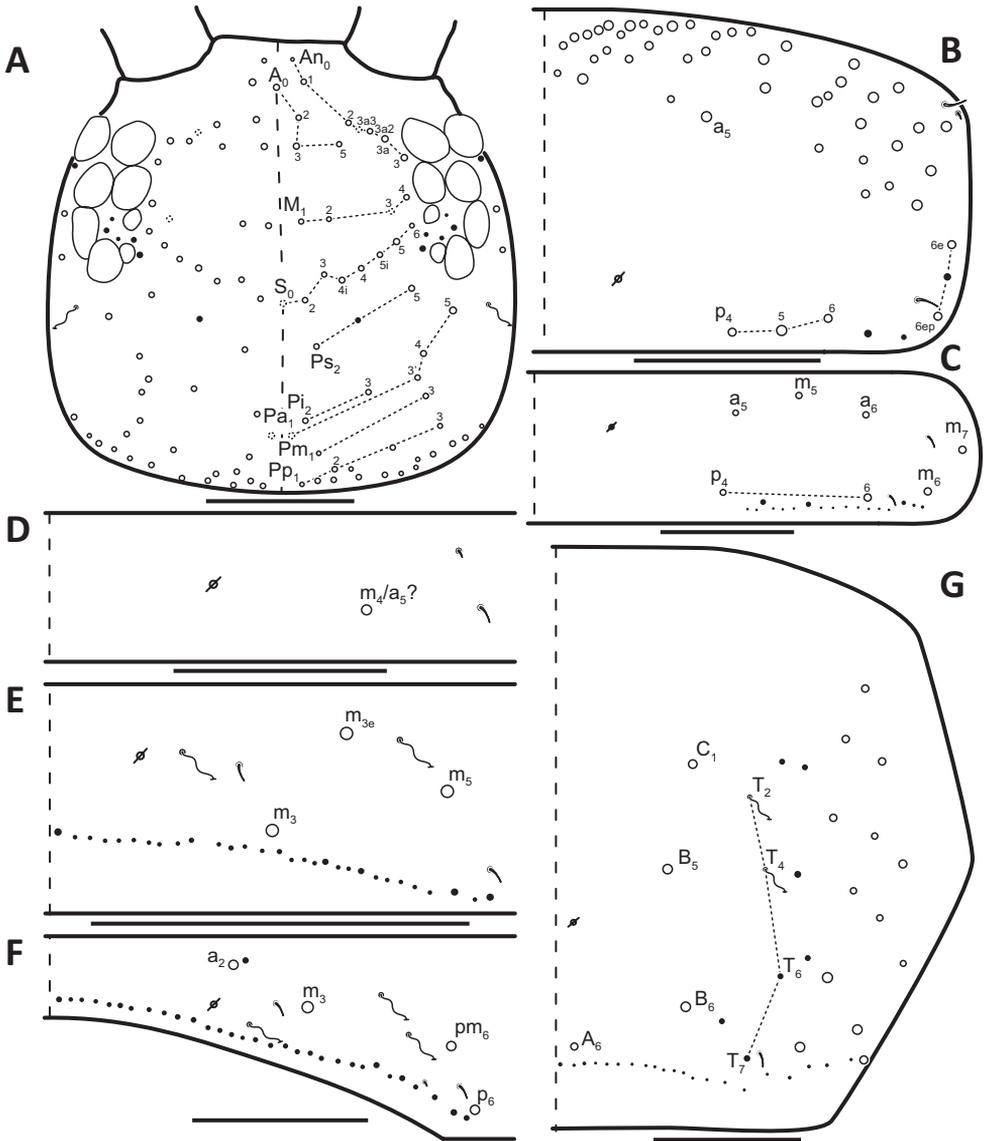


**Figure 7.** Color patterns of *Entomobrya assuta*. Photographed specimens are from the following localities: **A** Champaign Co., IL **B** Champaign Co., IL **C** Champaign Co., IL **D** Knox Co., TN **E** Knox Co., TN **F** Mason Co., IL **G** Knox Co., TN **H** Iroquois Co., IL.

*citrensis* sp. n. was at first considered to be an undescribed color form of *E. assuta*, but molecular data provides evidence for their separation (Katz et al. 2015).

Subsequent comparative morphological observations between the two forms show that head macroseta  $Ps_3$  and Abd. 2 macroseta  $a_3$  are both absent in *E. assuta*, but present in *E. citrensis* sp. n.; additionally, *E. citrensis* sp. n. has a complete, dark transverse band located medially across Abd. 4, whereas this band is absent in *E. assuta*. Labral morphology also separates these species, *E. assuta* has relatively uniform labral papillae, each with two to three seta or spine-like projection, whereas *E. citrensis* sp. n. has up to five minute bumps or serrations on the two internal papillae and only two larger spine-like projections on the two external papillae (Fig. 2). It should be pointed out that the last character may be variable and should be used in combination with chaetotaxy and color pattern for diagnosis.

Christiansen and Bellinger (1998) reported seven different color forms of *E. assuta*, each occurring in separate localities across North America. Many of these color forms were not sampled for this study, and in view of the discovery of *E. citrensis* sp. n. it is possible that some of them may represent distinct species. Future determinations of *E. assuta* will have to be based on analysis of chaetotaxy and other morphological characters outlined in the present description and not just color pattern.



**Figure 8.** Dorsal chaetotaxy of *Entomobrya assuta*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

*Entomobrya assuta* appears to be an intrusion of a southern subtropical or tropical *Entomobrya* lineage into the Nearctic region. Christiansen and Bellinger (1998) noted that the dorsal and genital chaetotaxy of *E. assuta* is more similar to tropical rather than Nearctic species. In fact, the reduction in chaetotaxy approaches that seen in *E. longiseta* Soto-Adames and *E. linda* Soto-Adames from the Caribbean, more than other Nearctic forms.

**Distribution.** Endemic to North America (Suppl. material 2: A).

**Material examined.** USA: *Cotypes* (J. W. Folsom), 2 on slide, Vermont, Clarendon, under bark Mar-April 1898 (O. W. Barrett), INHS Cat. No. 528,332; *Cotypes* (J. W. Folsom), 5 in vial, same information as above; *Cotypes* (J. W. Folsom), 4 in vial, Geneva, N. Y., June 18, 1917, beneath apple bark (H. Glasgow). Other material examined: 2 in vial, Alabama, Covington Co., Conecuh National Forest, off Co. Rd. 11 (31.07900,-86.61203), under bark, 2.i.2012 (A. Katz & M. DuBray), AK12-6; 3 on slides, 20 in vial, Florida, Taylor Co., Econfina State Park (30.0656,-83.91066), under bark, 9.viii.2011, AK11-116; 2 in vial, Illinois, Champaign Co., Champaign, Kaufman Lake Park (40.11514,-88.29000), in bird nest, 8.v.2011, AK11-26; 3 on slides, 5 in vial, Illinois, Champaign Co., Urbana, Brownfield Woods (40.14462,-88.16543), on low-lying vegetation, 7.vii.2011, AK11-60; 1 on slide, Illinois, Champaign Co., Urbana, bird nest, 28.ix.1957 (R. Hurley); 5 on slides, Illinois, Iroquois Co., Iroquois County Forest Preserve (40.99279,-87.59734), under bark, 19.viii.1989 (F. Soto-Adames); 1 in vial, Illinois, Jasper Co., Sam Parr State Fish and Wildlife Area (39.03293,-88.12516), under bark, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-73; 2 in vial, Illinois, Jo Davies Co., South Blanding Rd., Stevenson Property (42.29895,-90.36967), under bark, 27.viii.2011, A11-148; 1 on slide, 6 in vial, Illinois, Jo Davies Co., Princess Mine 1 (42.30565,-90.39740), under bark, 26.viii.2011, AK11-142; 1 in vial, Illinois, Jo Davies Co., Princess Mine 1 (42.30565,-90.39740), beating vegetation, 26.viii.2011, AK11-143; 4 in vial, Illinois, Jo Davies Co., South River Rd., Asgard Vent 1 (42.30170,-90.40345), under bark, 27.viii.2011, AK11-153; 1 on slide, 7 in vial, Illinois, Jo Davies Co., South River Rd., Asgard Vent 2 (42.30378,-90.40108), under bark, 27.viii.2011, AK11-157; 1 on slide, Illinois, Kankakee Co., Kankakee River State Park (41.19482,-87.96875), from bird nest, 10.iv.2011, AK11-6; 1 on slide, Illinois, Kankakee Co., Kankakee River State Park (41.19482,-87.96875), from squirrel nest, 10.iv.2011, AK11-8; 1 in vial, Illinois, Mason Co, Revis Hill Nature Prairie Reserve (40.15246,-89.85330), beating vegetation, 18.vii.2011 (A. Katz & F. Soto-Adames), AK11-86; 2 on slides, 4 in vial, Illinois, Mason Co., Sand Ridge State Park (40.40892,-89.87590), beating branches, 18.vii.2011 (A. Katz & F. Soto-Adames), AK11-82; 2 on slides, 8 in vial, Illinois, Piatt Co., Lodge Park (40.06709,-88.56596), under bark, 23.vii.2011, AK11-100; 1 in vial, Illinois, Pike Co., Lincoln's New Salem State Park (39.96868,-89.83386), beating grasses, 18.vii.2011 (A. Katz & F. Soto-Adames), AK11-90; 1 on slide, 3 in vial, Illinois, Pope Co., Lake Glendale (37.41350,-88.65982), under bark, 24.ix.2011, AK11-160; 1 on slide, Illinois, Vermilion Co., University of Illinois Observatory, Nixon Fork, leaf litter, 25.iv.2009 (F. Soto-Adames & L. Deem), FD09-25; 1 in vial, Illinois, Vermilion Co., Kennekuk Cover County Park, Windfall Prairie Nature Preserve (40.20995,-87.74181), aspirated from bushes, 16.vi.2011 (A. Katz & F. Soto-Adames), AK11-59; 3 on slides, Illinois, Will Co., Braidwood (41.25118,-88.19494), soil and leaf litter, 6-8.ix.2011 (F. Soto-Adames); 2 on slides, Michigan, Ingham Co., Michigan State University, Baker Wdlt. (42.66527,-84.36264) under bark of standing dead pine, 24.vii.2008 (E. C. Bernard), BW-11; 1 on slide, Michigan, St. Clair Co., Algonac State Park (42.65447,-82.52430), under

bark of recently fallen maple, 25.vii.2008 (E. C. Bernard), ASP-15; 8 in vial, Pennsylvania, Chester Co., Wayne, sweep of *Forsythia* sp., 29.vi.2012, AK12-50; 7 on slides, 30+in vial, Tennessee, Knox Co., University of Tennessee, Ag. Campus, Morgan Hall (36.01023,-83.93829), on moist fallen bark, April 2010 (E. C. Bernard).

### ***Entomobrya atrocincta* Schött, 1896**

Figs 1, 2, 3B, 4, 6, 9, 10, 39

**Description.** *Body shape and color pattern.* Sexually dimorphic in color pattern and body shape. Males and females with variable but characteristically different color patterns (Fig. 9). Male body relatively cylindrical, slender, with bright orange background with black pigment usually forming a thick and complete transverse dorsal band covering posterior margin of Th. 2 and all of Th. 3 and Abd. 1, band sometimes absent. Male light form without dark pigment on Th. 2 through Abd. 6 (except sometimes along anterior margin of Th. 2). Male dark form with band covering Th. 2 through Abd. 2, irregular pigment patterns sometimes forming 1+1 orange spots on Abd. 3, and two narrow longitudinal stripes connected by transverse band on posterior margin of Abd. 4 (Fig. 9A–D). Females with slightly dorso-ventrally flattened body and slightly larger than males. Female color pattern strikingly different from males, white or light yellow background with black, dark blue or purple pigment forming transverse bands across the posterior margins of Th. 2 through Abd. 4. All females with two longitudinal stripes or triangular extensions connected by two transverse bands on Abd. 4; one incomplete medial band and another complete band on posterior margin of Abd 5 (Fig. 9E–I). Mesonotum white in both sexes, lacking pigment except for a small irregular band across anterior margin. Medial area of Th. 2 relatively transparent and fat bodies visible through cuticle under a dissecting microscope. Both males and females with purple pigment usually extending from apical end of 2<sup>nd</sup> antennal segment through apex of 4<sup>th</sup> antennal segment.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment located in deep pit, usually simple, sometimes with up to four distinct lobes. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 2.5x$  short setae. Prelabral setae ciliate. Ornamentation of distal margin of labral papillae with 3–4 small seta or spine-like projections (Fig. 2). Dorsal head chaetotaxy variable (Fig. 10A); macrosetae  $An'_0$ ,  $An_{3a2}$ ,  $An_{3a3}$ ,  $S'_0$ ,  $S_0$ ,  $Ps_3$ ,  $Pi_1$ ,  $Pa_3$ , and  $Pm_{1i}$  always absent;  $S_0$  usually present,  $M_{3i}$  usually absent,  $A_6$  present in roughly half of observed specimens. Eyes G and H small and subequal; eye patch with 5 setae.

*Thorax.* Chaetotaxy of Th. 2 stable, without variation in number of macrosetae. A row of microsetae occurs along entire posterior margin of Th. 2 (not displayed in figure);  $a_5$ ,  $m_1$ ,  $m_2$ ,  $m_4$ ,  $m_{4p}$ , and all posterior macrosetae (series  $Pi$ ,  $Pa$ ,  $Pm$ , and  $Pp$ ) present (Fig. 10B). Chaetotaxy of Th. 3 variable: 8 macrosetae always present in zone M, 5 in zone L, and 3 in zone Pl (Fig. 3B); macroseta  $a_{5e}$ ,  $m_4$ ,  $a_{6i}$ , and  $p_{1i}$  usually present (Fig. 10C).

*Legs.* Trochanteral organ with triangular setal pattern and up to 30 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

*Abdomen.* Abdominal chaetotaxy variable. Abd. 1, with 4-8 macroseta (Fig. 10D), row of microsetae present along entire posterior margin (not displayed in figure). Abd. 2 with 4-6 macrosetae (Fig. 10E):  $a_2$ ,  $m_3$ ,  $m_{3e}$ , and  $m_5$  always present;  $a_3$  and  $m_{3ep}$  polymorphic. Abd. 3 chaetotaxy stable, with 7 macrosetae:  $a_1$ ,  $a_2$ ,  $a_3$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$  (Fig. 10F). Abd. 4 inner macrosetae varying from 8-11 (Fig. 10G). Basal mucronal tooth slightly larger.

**Remarks.** *Entomobrya atrocincta* can be distinguished by the male or female color patterns as described above combined with the presence of macrosetae head  $S_{4i}$  and Abd. 3  $a_1$  and  $a_2$  and the absence of macrosetae head  $ps_3$  and Th. 2  $m_5$  (see Table 2 for additional diagnostic characters). The color pattern of female *E. atrocincta* is virtually indistinguishable from that of *E. multifasciata* and very similar to *E. intermedia*, and *E. nivalis*. Though these species may exhibit slight differences in color pattern, it is easier to differentiate them with the characters outlined in Table 3. Female *E. multifasciata* and *E. atrocincta* can be separated by the presence of head macroseta  $S_{4i}$  in *E. atrocincta* and its absence in *E. multifasciata*, and morphology of the labral papillae; *E. multifasciata* has 2-3 large seta or spine-like projections per papillae, whereas *E. atrocincta* has 3-4 small, seta or spine-like projections per papillae (Fig. 2).

The male form always has a unique orange color, but the distribution of purple pattern is variable. Christian and Bellinger (1998) report four discrete color forms. An additional male color form was collected during this study. The different color forms can commonly be found together within the same population. There are no significant morphological or COI DNA sequence differences between male forms and variation in color pattern (male and female) is attributed to intraspecific variation (Katz et al. 2015). The most common male form collected in this study (Fig. 9A) is somewhat similar to *E. clitellaria*. However, the two forms can be easily separated by chaetotaxy (Table 3).

Sexual dimorphism in this species has caused serious taxonomic confusion due to the similarity of female pattern to *E. multifasciata* and *E. nivalis*. Ramel et al. (2008) first described the sexual dimorphism of *E. atrocincta* from Greece and even noted that records of *E. multifasciata* may be misidentified *E. atrocincta* females. However, Jordana (2012) separated most European specimens and classified them as *E. nigrocincta* Denis based on chaetotaxy, synonymizing Ramel et al.'s (2008) descriptions with *E. nigrocincta*. Jordana (2012) attributes the sexual dimorphism (the same displayed by the specimens collected in North America and included in this study) to *E. nigrocincta* only, keeping the description by Christiansen (1958b) valid for *E. atrocincta*. Molecular data confirm that different color forms represent different sexes of the same species (Katz et al. 2015), demonstrating the presence of sexual dimorphism in North American *E. atrocincta*. However, species diagnosis remains unclear. Chaetotaxy outlined for both *E. atrocincta* and *E. nigrocincta* by Ramel et al. (2008) and Jordana (2012) do not match the specimens examined in this study (Table 4). The excessive intraspecific variation in chaetotaxy observed in these specimens raises concern about basing species diagnosis strictly on discrete chaetotaxic characters. Further molecular analysis of European populations is needed in order to elucidate the correct taxonomic status and distribution of these two species.

**Table 3.** Diagnostic characters to separate species within the nivalis complex: *E. atrocincta*, *E. multifasciata*, *E. intermedia*, and *E. nivalis*. Diagnostic characters of *E. clitellaria* are also included in relation to *E. atrocincta* due to similarity in color pattern.

Species	Head macroseta S <sub>4i</sub> <sup>1</sup>	Th. 2 macroseta m <sub>5</sub> <sup>1</sup>	Abd. 2 macroseta m <sub>3ep</sub> <sup>1</sup>	Abd. 3 macroseta a <sub>1</sub> <sup>1</sup>	Abd. 3 macroseta a <sub>2</sub> <sup>1</sup>	Labral papillae <sup>2</sup>
<i>E. atrocincta</i>	1	0	0(1) <sup>3</sup>	1	1	0
<i>E. multifasciata</i>	0	0	0	1	1	0
<i>E. intermedia</i>	0	1	0	1	0	0
<i>E. nivalis</i>	1	1	1	1	0	0
<i>E. clitellaria</i>	1	1	1	0	1	1

<sup>1</sup> Character states: absent (0), present (1).

<sup>2</sup> Character states: multiple projections (0), single projection (1).

<sup>3</sup> Parentheses indicate a rarely observed state.

**Table 4.** Comparison between descriptions of *E. atrocincta* (and *E. nigrocincta*) provided by this study with those provided by Jordana (2012), Ramel et al. (2008), and Christiansen and Bellinger (1998). Characters represent the number of dorsal macrosetae present in fields (H1-A10) outlined by Jordana and Baquero (2005) and Jordana (2012). Clear differences between descriptions are highlighted in bold and underlined. Parentheses indicate a rarely observed state. Question marks indicate the characters were not included in the description.

Species description	Sexual dimorphism	H1	H2	H3	H4	H5	T1	T2	A1	A2	A3	A4	A5	A6–A10
<i>E. atrocincta</i> <sup>1</sup>	Yes	3	2(1)	0	3	2	2	3	2(1)	2(3)	1	2	1	8-11
<i>E. nigrocincta</i> <sup>2</sup>	Yes	3	1	0	<u>2</u>	2	2	3	1	2	1	<u>1</u>	1	9(8?)
<i>E. atrocincta</i> <sup>3</sup>	<b>No</b>	<u>2</u>	1	0	3	2(3)	2	3	2	3	1	2	1	<b>13</b>
<i>E. atrocincta</i> <sup>4</sup>	<b>No</b>	?	?	?	?	?	?	?	2	2	1	2	1	?

<sup>1</sup> Description of *E. atrocincta* specimens observed in the present study.

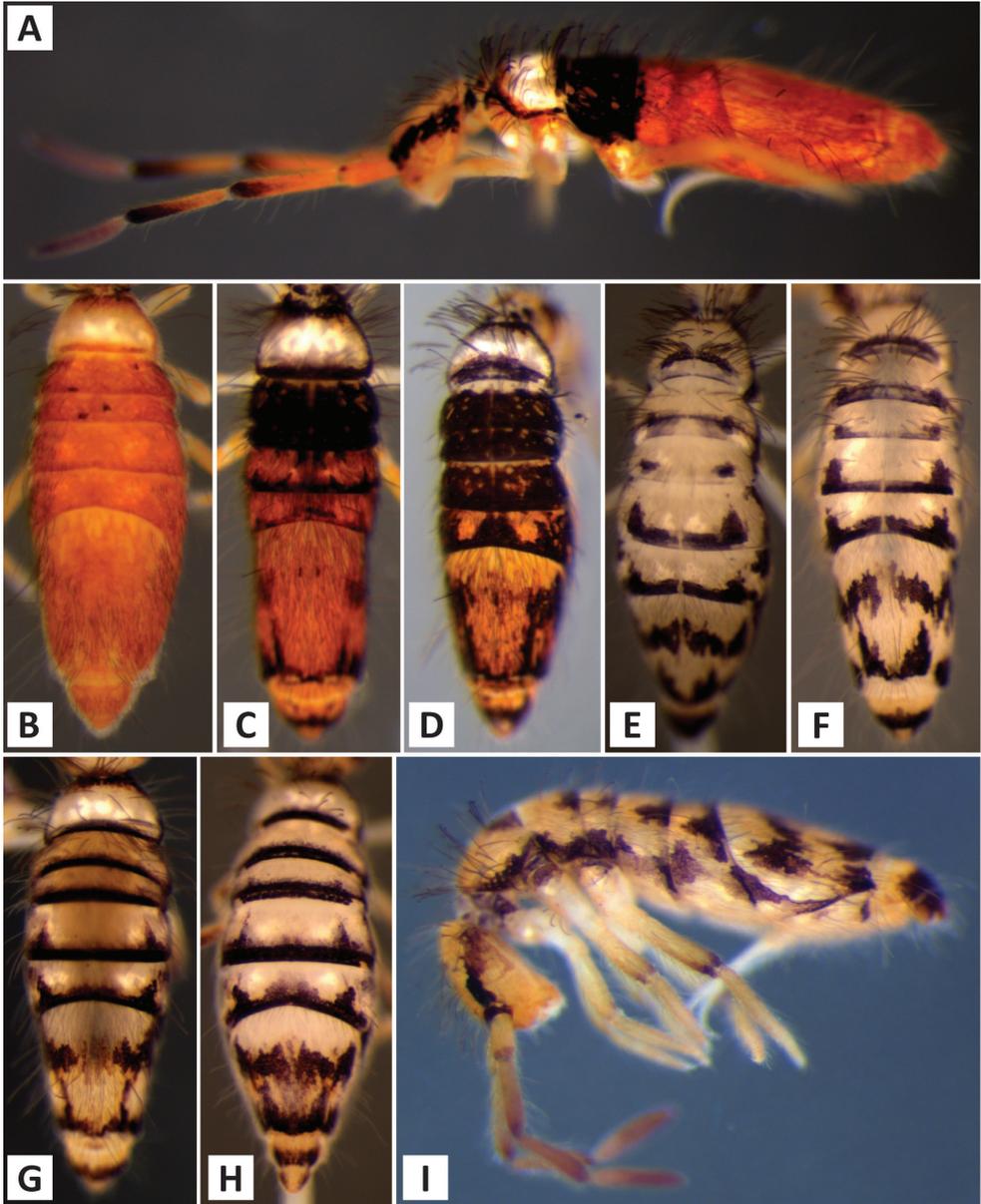
<sup>2</sup> Description of *E. nigrocincta* provided by Jordana (2012) and Ramel et al. (2008).

<sup>3</sup> Description of *E. atrocincta* provided by Jordana (2012).

<sup>4</sup> Description of *E. atrocincta* provided by Christiansen and Bellinger (1998).

The original description of *E. atrocincta* by Schött (1896) was based on the male form collected from California. Christiansen's (1958b) descriptions and methods for species delimitation placed heavy emphasis on the male genital plate, which may have led to his inadvertent omission of the female form. Given that the combination *E. atrocincta* Schött, 1896 has priority over *E. nigrocincta* Denis, 1923, and also because the holotype designated for *E. atrocincta* was collected in North America, the specimens along with the descriptions outlined in this study have been assigned to *E. atrocincta*.

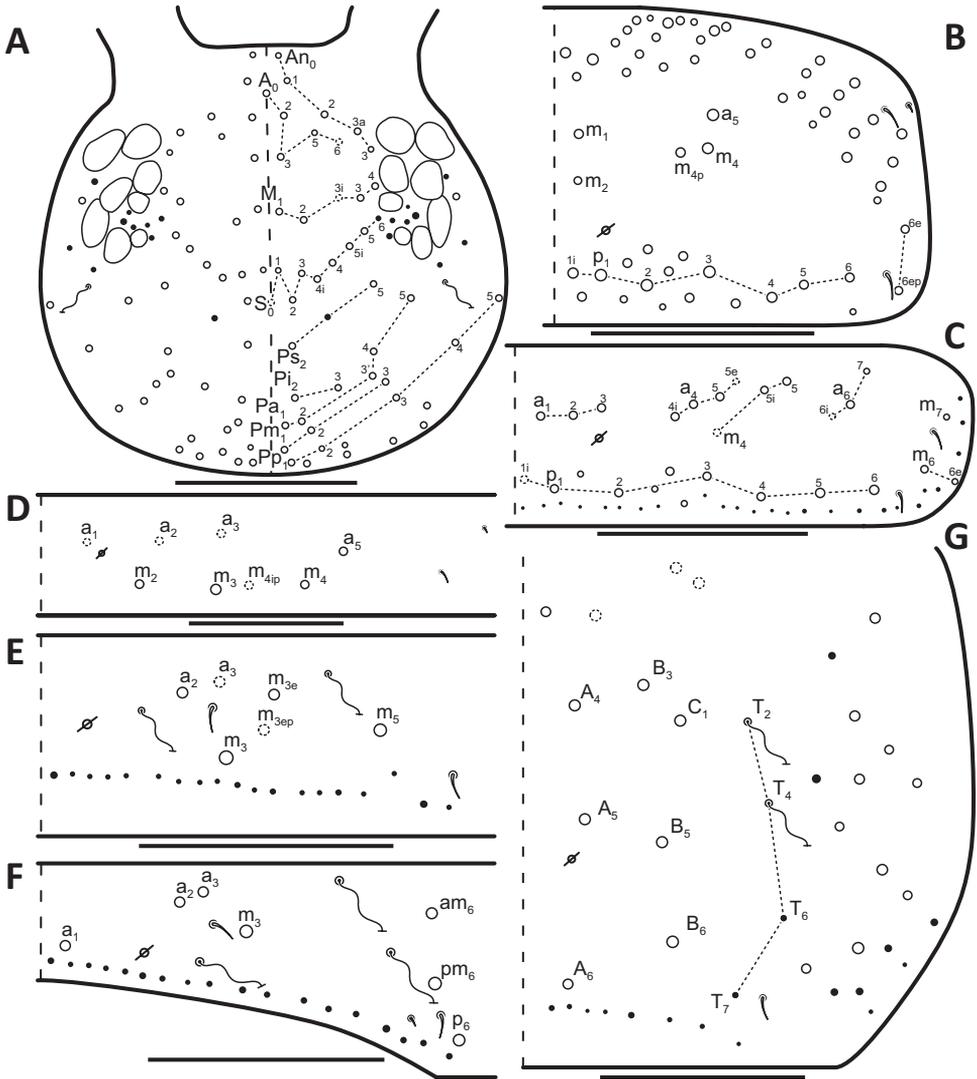
**Distribution.** North America, Hawaii and possibly Europe. Records of *E. multifasciata* in North America and Hawaii (Christiansen and Bellinger 1992) are suspect due to their similarity to the female *E. atrocincta* color form. A considerable number of collections of *E. atrocincta* from Hawaii also include *E. multifasciata*, indicating the species is also sexually dimorphic in the Pacific Islands (Christiansen and Bellinger 1992).



**Figure 9.** Color patterns of *Entomobrya atrocincta*: **A–D** Male color forms **E–I** Female color forms. All photographed specimens were collected from Champaign Co., IL.

Palaearctic records of *E. atrocincta* may be *E. nigrocincta* provided by Jordana (2012). The distribution of *E. atrocincta* in North America is shown in Suppl. material 2: B.

**Material examined.** USA: 1♂ in vial, Alabama, Clay Co., Talladega National Forest, on CR6000-1 off of Hwy148 (33.19723,-86.06325), in moss on forest floor, 2.i.2012 (A. Katz & M. DuBray), AK12-5; 6♂ & 2♀ in vial, Illinois, Champaign



**Figure 10.** Dorsal chaetotaxy of *Entomobrya atrocincta*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

Co., Champaign, Kaufman Lake Park (40.11514,-88.29000), in bird nest, 8.v.2011, AK11-26; 2♂ on slides, 4♂ & 6♀ in vial, Illinois, Champaign Co., Champaign, Kaufman Lake Park (40.11514,-88.29000), squirrel nest, 8.v.2011, AK11-27; 7♂ & 7♀ on slides, 50+♂ & 50+♀ in vial, Illinois, Champaign Co., Urbana, Natural Resource Building (40.10071,-88.22812), in leaf litter under bush by parking lot, 22.iv.2011, AK11-17; 1♂ on slides, Illinois, Champaign Co., Urbana, Meadowbrook Park (40.08063,-88.20828), in leaf litter, 14.iv.2012, AK12-24; 3♀ in vial, Illinois, Champaign Co., Urbana, South side of Natural Resource Building (40.10110,-88.22963),

in sweet gum seed pods and leaf litter, 23.iv.2011 (A. Katz & M. DuBray), AK11-15; 3♀ on slides, 20♂ & 10♀ in vial, Illinois, Champaign Co., Urbana, Natural Resource Building (40.10047,-88.22840), in leaf litter under bush along Pennsylvania Ave, 5.v.2011, AK11-25; 1♂ & 1♀ on slides, 50+♂ & 50+♀ in vial, Tennessee, Knox Co., University of Tennessee, Ag. Campus, Morgan Hall (36.01023,-83.93829), on moist fallen bark, April 2010 (E. C. Bernard); 1♂ & 1♀ in vial, Tennessee, Knox Co., Farragut, 12108 Ridgeland Drive (36.01023,-83.93829), in old bald-faced hornet nest, 2.iii.2008 (E. C. Bernard).

### *Entomobrya bicolor* Guthrie, 1903

Figs 2, 11, 12, 13A, 39

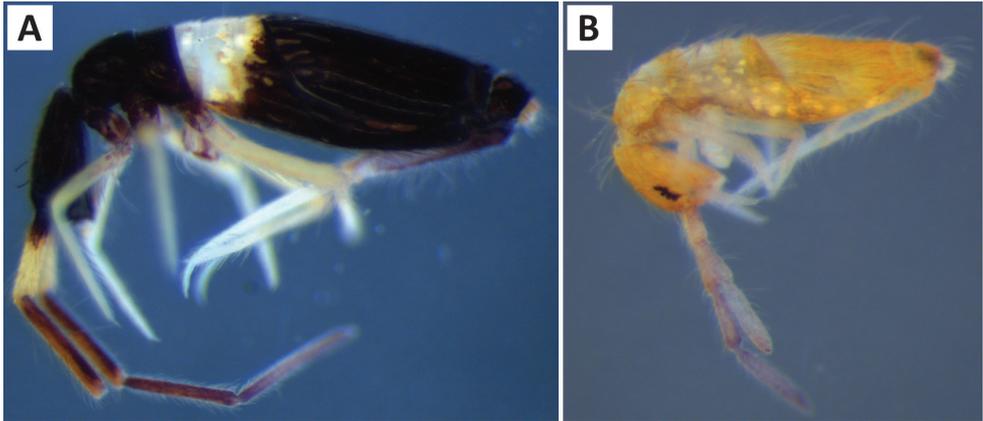
**Description.** *Body shape and color pattern.* Body elongate and cylindrical. Only one reported color form in adults (Fig. 11A), dark brown or black pigment covering whole body except for a white band across Abd. 1, 2 and medial area of Abd. 3; Abd. 5 sometimes with 1+1 pale spots; legs, furcula, Abd. 6 pale, lacking all dark pigmentation; antennae lightly colored with brown or purple pigment, usually with a white area on distal half of 1<sup>st</sup> antennal segment. Juvenile pattern distinct from adult, yellow background without dark pigment except for eye patch; legs, furcula, Abd. 6 pale, lacking all dark pigmentation; antennae with light purple pigment. (Fig. 11B)

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually bilobed, rarely simple. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx$  3–4 $\times$  short seta. Prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with a single seta or spine-like projection (Fig. 2). Labial papilla E with lateral appendage reaching just above tip of papilla. Labial triangle in one individual with 2 small supplementary ciliate microsetae internal to M1. Dorsal head chaetotaxy as in Figure 12A: macrosetae An<sub>0</sub>, A<sub>6</sub>, S<sub>0</sub>, S<sub>6</sub>, Ps<sub>3</sub>, Ps<sub>5</sub>, Pi<sub>1</sub>, Pi<sub>3</sub>, and Pm<sub>11</sub> always absent; An<sub>3a3</sub> usually absent (present on one side in one specimen). Eyes G and H small and subequal. Eye patch with 5 setae.

*Thorax.* Thoracic chaetotaxy abundantly developed, highly variable with many supplemental macrosetae. Th. 2 macrosetae p<sub>6e</sub> and p<sub>6ep</sub> absent and macrosetae m<sub>4i2</sub>, m<sub>4i3</sub> polymorphic (Fig. 12B). Th. 3 macrosetae a<sub>5e3</sub>, m<sub>4</sub>, m<sub>5p</sub>, a<sub>6i</sub>, a<sub>7</sub>, and m<sub>7</sub> are absent (Fig. 12C). If present, m<sub>7</sub> is always a mesoseta. Chaetotaxy of zone Pm of both thoracic segments densely packed with many supplemental macrosetae, forming wing-like patches of posterior setae extending near anterior row, typical for species within the *E. bicolor* complex (Fig. 3D). Position of pseudopores on Th. 3 atypical for *Entomobrya*, displaced anteriorly and closer to macrosetae a<sub>1</sub>, a<sub>2</sub>, and a<sub>3</sub> than in other species.

*Legs.* Trochanteral organ with rectangular setal pattern and up to 37 setae. Unguis with 4 internal teeth; basal teeth enlarged and located approximately middle of inner claw length.

*Abdomen.* Abdominal chaetotaxy highly developed. Row of microsetae along entire posterior margin present in all segments (not displayed in figure). Abd. 1 with 12

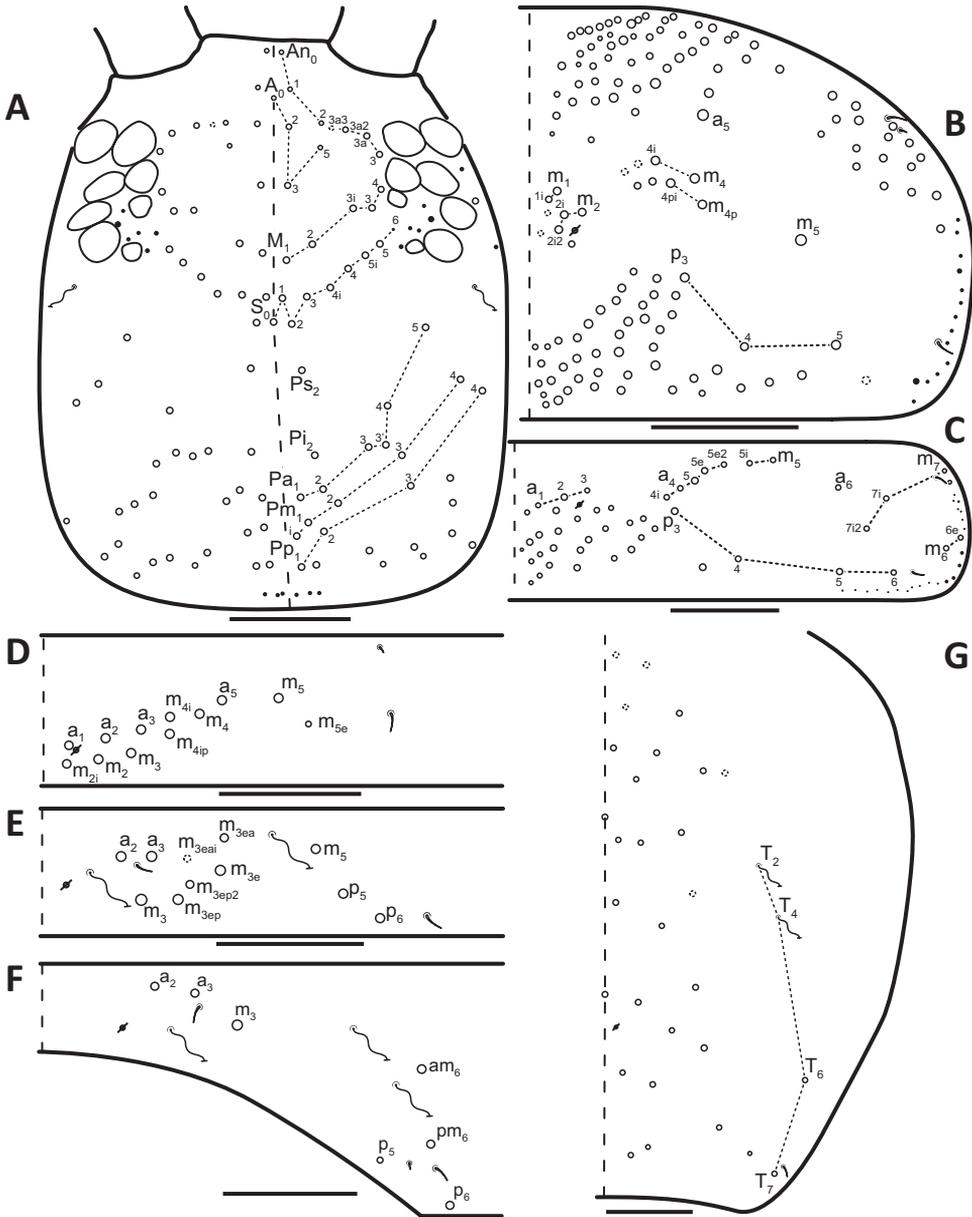


**Figure 11.** Color patterns of *Entomobrya bicolor*: **A** adult **B** young immature instar. Both photographed specimens were collected from Henderson Co., IL.

macrosetae (Fig. 12D). Abd. 2 with 10-11 macrosetae:  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3e}$ ,  $m_{3ep}$ ,  $m_{3ep2}$ ,  $m_{3ea}$ ,  $m_5$ ,  $p_5$ , and  $p_6$  always present. Abd. 2 macroseta  $m_{3eai}$  sometimes present,  $m_{3ei}$  always absent (Fig. 12E). Abd. 3 macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ ,  $p_5$ ,  $p_6$  present;  $a_1$  always absent (Fig. 12F). Abd. 4 elongated, with at least 22 inner macrosetae (Fig. 12G), number of macrosetae extremely variable between individuals and within individuals (Fig. 13A). Position of pseudopores on Abd. 4 unstable, even varying with respect to macroseta and bothriotracha between sides on same individual (Fig. 13A). Mucronal teeth subequal; mucronal spine enlarged.

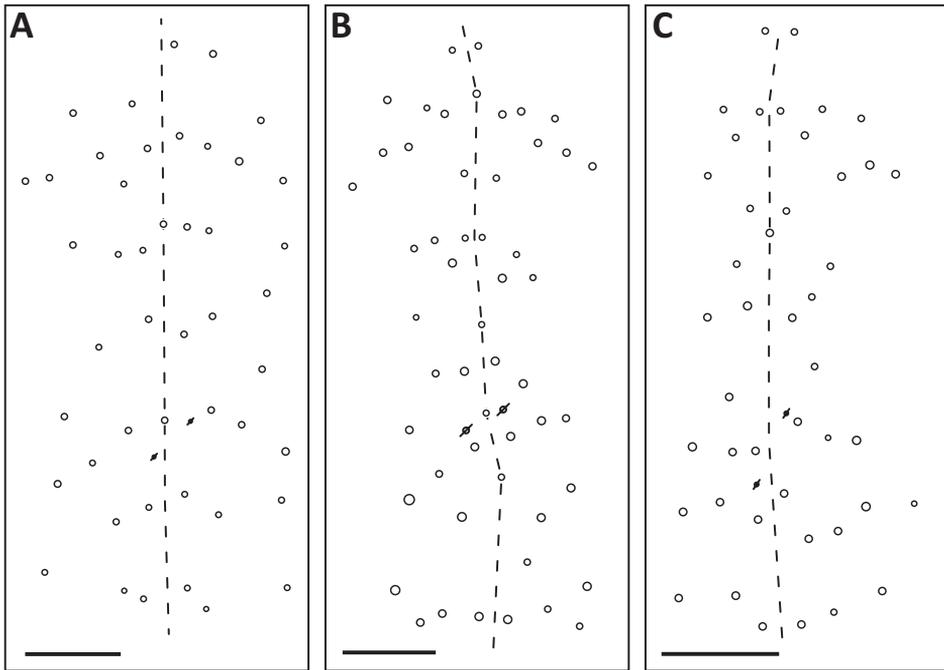
**Remarks.** *Entomobrya bicolor* adults can be easily distinguished by the unique color pattern described above combined with the absence of head macroseta  $Ps_5$  (see Table 2 for additional diagnostic characters). Adults have a single, highly stable color form that is easily recognizable and perfectly acceptable for diagnosis (Fig. 11A). Molecular data from Katz et al. (2015) revealed that a small specimen found with *E. bicolor*, which was completely yellow and lacking all dark pigment, is the juvenile form of *E. bicolor* (Fig. 11B). The *E. bicolor* juvenile color form has never been reported in the literature. Unfortunately, it may be difficult to diagnose juvenile members of this species if collected without adults present in the sample.

Christiansen (1958b) placed this species in what he termed “the *E. bicolor* group”; a complex of three closely related species; *E. quadrilineata*, *E. decemfasciata*, and *E. bicolor*. This group is characterized by a highly elongate, cylindrical body, a relatively long furcula, antennae and legs, and by their abundant and highly variable chaetotaxy; many duplicate, supplementary macrosetae, distinctive and augmented setal patterns in thoracic zone Pm, expanded chaetotaxy of Abd. 4, and high levels of asymmetry (Fig. 13A). The extreme setal variation obscures potentially informative characters and results in a lack of discrete, useful diagnostic chaetotaxy between species in this complex. The absence of head macroseta  $Ps_5$  is the only character (other than color pattern)



**Figure 12.** Dorsal chaetotaxy of *Entomobrya bicolor*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

that differentiates *E. bicolor* from *E. decemfasciata* and *E. quadrilineata*. Color pattern is critical for species diagnosis within this complex. See Table 5 for a summary of the important diagnostic characters to separate species in this complex.



**Figure 13.** Macrosetae variation and asymmetrical polymorphism of Abd. 4 in the *E. bicolor* complex: **A** *E. bicolor* **B** *E. decemfasciata* **C** *E. quadrilineata*. Only medial macrosetae (those which occur between bothriotricha  $T_2$  and  $T_4$ ) are included in diagram. The dotted line represents the medial division of Abd. 4. Scale bars = 100  $\mu$ m.

**Table 5.** Important diagnostic characters for the separation of species within the bicolor complex: *E. bicolor*, *E. decemfasciata*, and *E. quadrilineata*. Character states: absent (0), present (1).

Species	Head macrosetae $Ps_5$	Parallel, longitudinal bands on thorax
<i>E. bicolor</i>	0	0
<i>E. decemfasciata</i>	1	0
<i>E. quadrilineata</i>	1	1

**Distribution.** Endemic to North America (Suppl. material 2: C).

**Material examined.** USA: 9 on slides, 6 in vial, Illinois, Henderson Co., Big River State Forest (41.03435,-90.91620), vacuum sand prairie, 8.vi.2011 (C. H. Dietrich).

***Entomobrya citrensis* Katz & Soto-Adames, sp. n.**

<http://zoobank.org/4C33A32D-EFF4-4B53-8880-2C9C3844076C>

Figs 2, 14, 15, 16A

**Etymology.** This species is named after the locality it was collected in: Citrus County, Florida. Citrensis is Latin for “from the place of citrus”.



**Figure 14.** Color pattern of *Entomobrya citrensis* sp. n. Lateral (A) and dorsal (B) views of specimen collected from Citrus Co., FL.

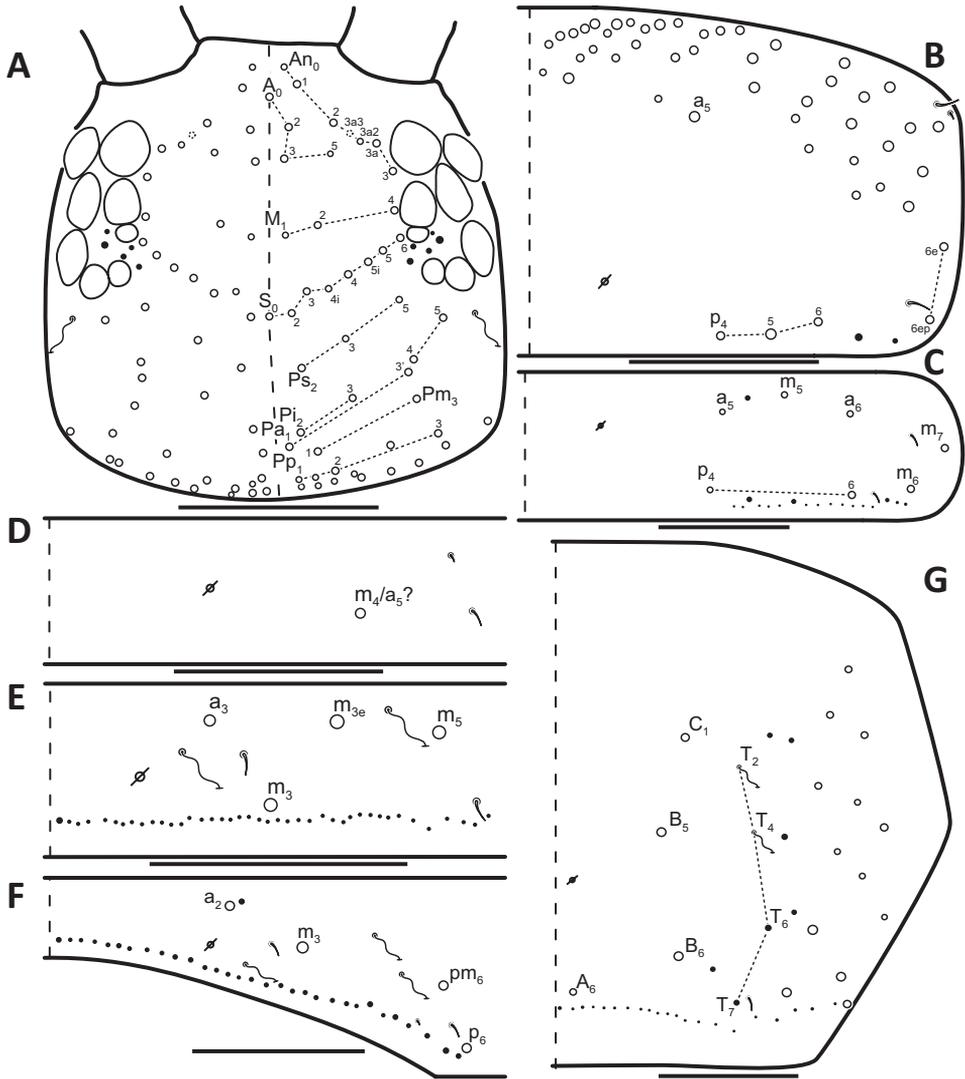
**Type material.** *Holotype*, ♂, USA: Florida, Citrus County, Chassahowitzka National Wildlife Refuge (28.75997,-82.57583), beating vegetation, 12.viii.2011 (A. Katz & J. Cech), AK11-134.

*Paratypes*, USA: 1 on slide, Florida, Citrus Co., Chassahowitzka National Wildlife Refuge (28.75997,-82.57583), under bark, 12.viii.2011 (A. Katz & J. Cech), AK11-136; 1 on slide, Florida, Citrus Co., Chassahowitzka National Wildlife Refuge (28.75997,-82.57583), beating vegetation and in leaf litter, 5.i.2014 (A. Katz & M. DuBray), AK14-1.

**Description.** *Body shape and color pattern.* Body dorso-ventrally flattened. Length up to 1.4 mm. Color pattern monomorphic (Fig. 14): background white, or slightly yellow, with black and traces of dark brown pigmentation forming transversal bands and spots. Thorax dorsal pigmentation patchy and irregular. Abd. 2 usually with 2 dark lateral spots. Posterior margin Abd. 2 and the anterior margin of Abd. 4 with a dark transverse band. Abd. 4 with a conspicuous irregular dark transverse band medially. The antennae are completely pigmented purple.

*Head.* Apical bulb of 4<sup>th</sup> antennal simple. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 2.5\times$  short setae. Prelabral setae with very fine ciliations that look smooth at low magnification under light microscopy. Ornamentation of the distal margin of the labral papillae with 3-4 spine-like projections on the inner papillae and 2 spine-like projections on the external papillae. Lateral appendage of labial papilla E slightly curved, relatively thin, reaching just below tip of papilla. Dorsal head chaetotaxy slightly reduced (Fig. 15A): macrosetae  $An'_0$ ,  $A_6$ ,  $M_3$ ,  $M_{3i}$ ,  $S'_0$ ,  $S_1$ ,  $P_1$ ,  $Pa_2$ ,  $Pa_3$ ,  $Pm_2$ , and  $Pm_{1i}$  always absent;  $An_{3a3}$  present, asymmetrically absent in one specimen. Eyes G and H small and subequal. Eye patch with 5 setae.

*Thorax.* Thoracic chaetotaxy reduced and fixed, no macrosetae variation observed. Row of microsetae along entire posterior margin of Th. 2 and Th. 3 (not displayed in figures). Th. 2, with  $a_5$  and posterior macrosetae  $p_4$ ,  $p_5$ ,  $p_6$ ,  $p_{6c}$ , and  $p_{6ep}$  (Fig. 15B). Th.

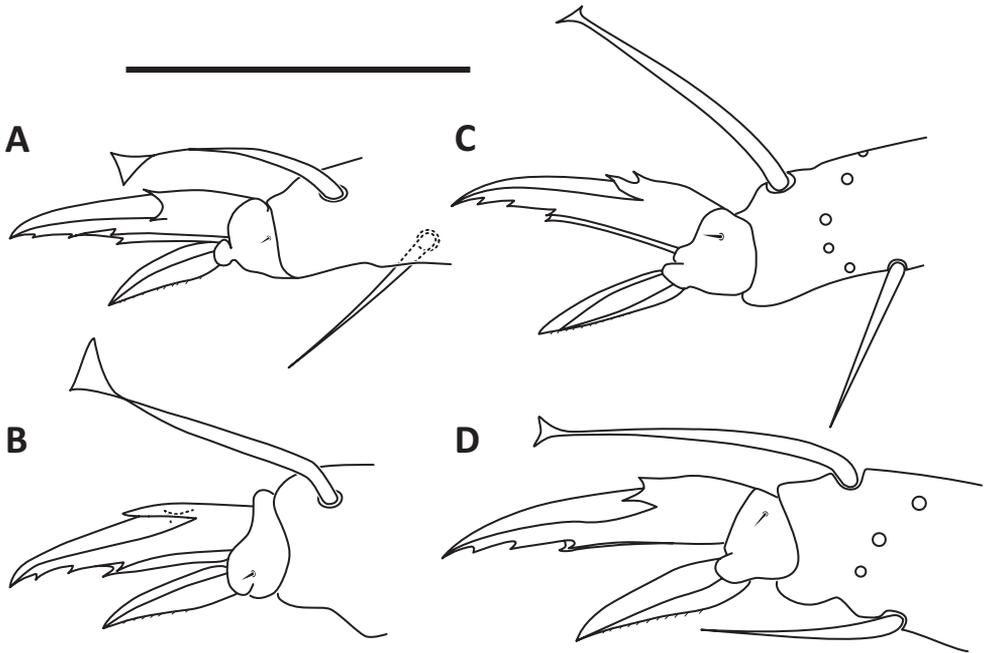


**Figure 15.** Dorsal chaetotaxy of *Entomobrya citrensis* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

3, with 7 macrosetae present:  $a_5$ ,  $a_6$ ,  $m_5$ ,  $m_6$ ,  $m_7$ ,  $p_4$ , and  $p_6$ ; macrosetae  $a_1$ ,  $a_2$ , and  $a_3$  absent (Fig. 15C). Zone Pm (Fig. 3) without macrosetae.

*Legs.* Trochanteral organ with triangular setal pattern and up to 14 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length (Fig. 16A).

*Abdomen.* Abdominal chaetotaxy reduced and stable; no macrosetae variation observed. Abd. 1 with 1 macroseta only (Fig. 15D). Abd. 1 row of microsetae along



**Figure 16.** Unguis (top claw) and unguiculus (bottom claw) on 3<sup>rd</sup> pair of legs. **A** *Entomobrya citrensis* sp. n. **B** *Entomobrya jubata* sp. n. **C** *Entomobrya neotenica* sp. n. **D** *Entomobrya unifasciata* sp. n.

posterior margin is present (not displayed in figure). Abd. 2 with 4 macrosetae:  $a_3$ ,  $m_3$ ,  $m_{3e}$ , and  $m_5$  (Fig. 15E). Abd. 3 with 4 macrosetae:  $a_2$ ,  $m_3$ ,  $pm_6$ , and  $p_6$ ; mesosetae  $a_{2a}$  sometimes present, inserted slightly anterior and exterior to  $a_2$ ; socket of  $a_{2a}$  large, resembling that of macroseta  $a_3$ , but its close proximity to  $a_2$  suggests a duplication of  $a_2$  rather than homologous to  $a_3$  (Fig. 15F). Abd. 4 with 4 inner macrosetae (Fig. 15G). Mucronal teeth subequal.

**Remarks.** *Entomobrya citrensis* sp. n. can be distinguished by its color pattern in combination with the absence of Th. 2 macrosetae  $m_2$  and  $m_5$ , Abd. 2  $a_2$  and  $m_{3ep}$ , and the presence of Abd. 2  $a_3$  (see Table 2 for additional diagnostic characters).

This species is closely related to *E. assuta*; both have highly compressed, or dorso-ventrally flattened bodies, reduced chaetotaxy, and similar color patterns. Head macroseta  $Ps_3$  and Abd. 2 macroseta  $a_3$  are both present in *E. citrensis* sp. n., but are absent in *E. assuta*. These species can also be separated by color pattern and morphology of the labral papillae (Fig. 2).

See remarks for *E. assuta* for additional diagnosis information. Only a few specimens of *E. citrensis* sp. n. were observed from one locality. Additional sampling may reveal more variation in color pattern or chaetotaxy.

**Distribution.** Endemic to North America. Reported from a single locality: Chasahowitzka National Wildlife Refuge in Citrus County, Florida (Suppl. material 2: D).

***Entomobrya clitellaria* Guthrie, 1903**

Figs 2, 17, 18, 39

**Description.** *Body shape and color pattern.* Body dorso-ventrally flattened. Dimorphic color pattern, unrelated to sex (Fig. 17): dark blue, purple, or black pigment covers Th. 3 and Abd. 1-3. Abd. 4 usually white, yellow, or orange with variable levels of pigment ranging from an irregular medial transverse band, randomly distributed irregular patches, or completely pigmented. Th. 2 always white, with a dark band along the anterior margin; medial area of Th. 2 almost transparent in some specimens and internal fat bodies can be observed through the cuticle under a dissecting microscope. Darker specimens with head mostly covered by dark pigment. Antennae usually entirely covered by purple pigment, but some specimens have a mixture of orange, brown, and purple coloration.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually simple, sometimes bilobed. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 3\times$  short setae. Prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with a single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E slightly curved, relatively thin, nearly reaching tip of papilla. Labial triangle in one individual with 1 small supplementary ciliate microsetae internal to M1. Dorsal head chaetotaxy (Fig. 18A) with macrosetae  $An'_0$ ,  $A_{3a3}$ ,  $M_{3i}$ ,  $S'_0$ ,  $S_6$ , and  $Ps_3$  always absent;  $An_{3a2}$  usually present;  $Pi_1$ ,  $Pm_1$ ,  $Pm_{1i}$ , and  $Pp_2$  present or absent. Eyes G and H small and subequal. Eye patch with 5 setae.

*Thorax.* Thoracic chaetotaxy well-developed, with some slight variation. Th. 2 macrosetae  $m_{4i3}$  absent,  $m_{1i}$  usually present (Fig. 18B). Th. 3 macrosetae  $a_{5e3}$ ,  $m_4$ ,  $m_{5p}$ , and  $a_7$  absent (Fig. 18C). Both thoracic segments with many supplemental macrosetae in zone Pm (18B,C).

*Legs.* Trochanteral organ with triangular setal pattern and up to 26 setae. Unguis with 4 inner teeth; basal teeth located approximately middle of inner claw length.

*Abdomen.* Abdominal chaetotaxy highly developed. Abd. 1 with 8-13 macrosetae (Fig. 18D). Abd. 2 macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3ep}$ ,  $m_{3e}$ , and  $m_5$  present;  $M_{3ei}$  and  $M_{3ea}$  usually present and  $M_{3eai}$  usually absent (Fig. 18E). Abd. 3 macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$  present;  $a_1$  always absent (Fig. 18F). Abd. 4 chaetotaxy stable, with 7 macrosetae between bothriotrichal complexes (Fig. 18G). Mucronal teeth subequal.

**Remarks.** *Entomobrya clitellaria* can be distinguished by the combination of color pattern, absence of macroseta head  $S'_0$  and Abd. 3  $a_1$  and presence of head  $Ps_5$ , Th. 2  $m_5$ , Abd. 2  $m_{3ep}$ , and Abd. 3  $a_2$  (see Table 2 for additional diagnostic characters). This species has a relatively conspicuous and diagnostic color pattern, but may be confused with the male *E. atrocincta*. However, there are obvious differences in their morphology outlined in Table 3. Christiansen and Bellinger (1998) described six different color forms, but after extensive examination of material collected for this study, it was determined that many (but not all) of the color forms they described were most likely variants within a continuous gradient of color pattern variation, without clear genetic isolation (Katz et al. 2015). The two lighter



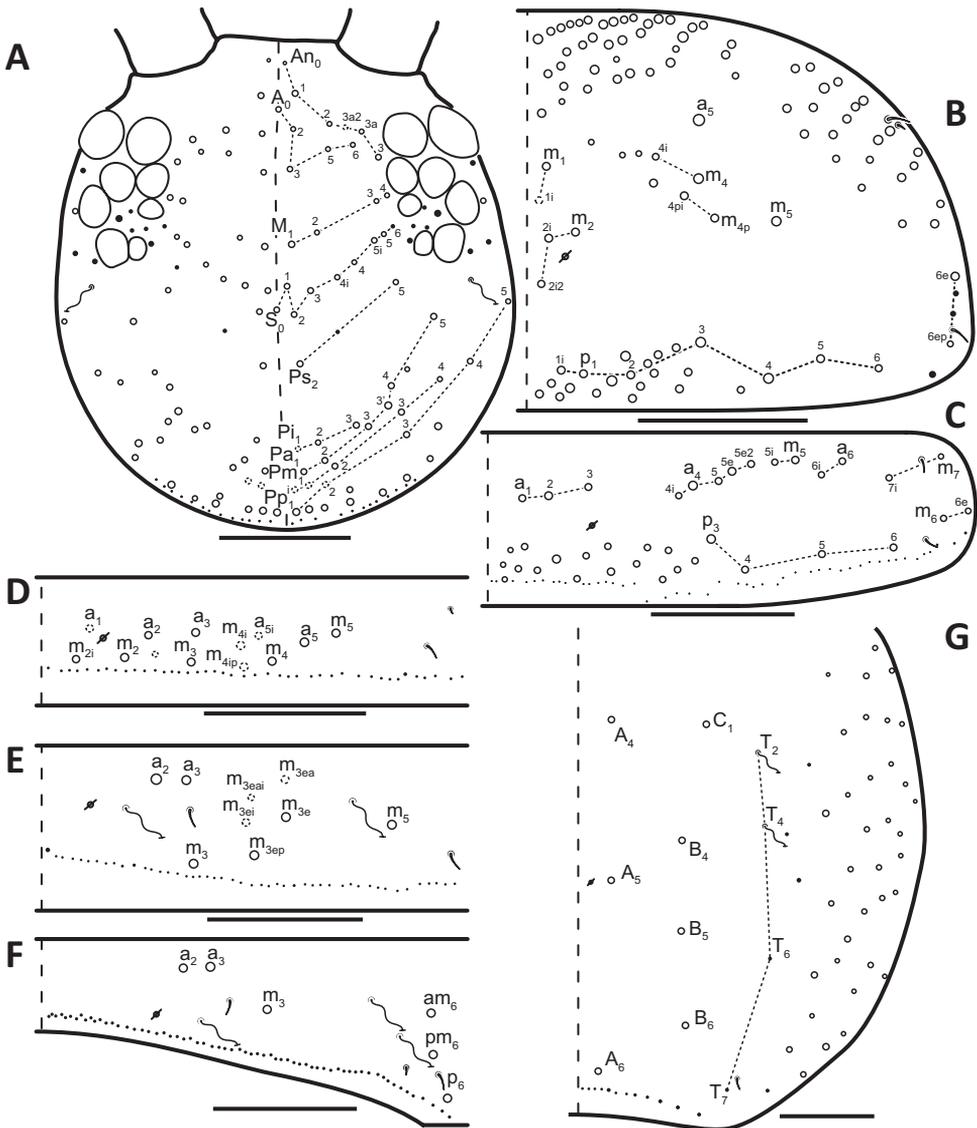
**Figure 17.** Color patterns of *Entomobrya clitellaria*. Photographed specimens are from the following localities: **A** Monroe Co., IL **B** Jasper Co., IL **C** Kankakee Co., IL **D** Monongalia Co., WV.

forms (labeled E and F in Christiansen and Bellinger 1998) proved to be elusive and were not collected during this study.

This species is closely related to *E. jubata* sp. n., but can be easily separated by color pattern, chaetotaxy, and morphology of the labral papillae. The absence of head macrosetae  $S'_0$  and the presence of head macrosetae  $Ps_3$  separate *E. clitellaria* from *E. jubata* sp. n. Labral papillae morphology also differs between these species: *E. jubata* sp. n. has two or three seta or spine-like projection on each papilla, while *E. clitellaria* only has one seta or spine-like projection per papilla (Fig. 2).

**Distribution.** Endemic to North America (Suppl. material 2: E).

**Material examined.** USA: 1 on slide, Florida, Taylor Co., Econfina State Park (30.0656,-83.91066), under bark, 9.viii.2011, AK11-116; 1 in vial, Illinois, Champaign Co., Urbana, Brownfield Woods (40.14462,-88.16543), on bushes and low-lying shrubs, 7.vii.2011, AK11-60; 1 on slide, Illinois, Champaign Co., Urbana, Brownfield Woods (40.14391,-88.16468), under bark, 10.ix.2009 (F. Soto-Adames); 1 in vial, Illinois, Coles Co., Fox Ridge State Park (39.40248,-88.14893), under bark, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-81; 3 on slides, 20 in vials, Illinois, Jasper Co.,



**Figure 18.** Dorsal chaetotaxy of *Entomobrya clitellaria*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

Sam Parr State Fish and Wildlife Area (39.03275,-88.12474), under bark, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-73, AK11-74 & AK11-75; 1 in vial, Illinois, Jasper Co., Sam Parr State Fish and Wildlife Area (39.03275,-88.12474), in leaf litter, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-77; 1 on slide, 1 in vial, Illinois, Jo Davies Co., Princess Mine 1 (42.30565,-90.39740), under bark, 26.viii.2011, AK11-142; 1 in vial, Illinois, Jo Davies Co., S Blanding Rd, Stevenson Property (42.29895,-

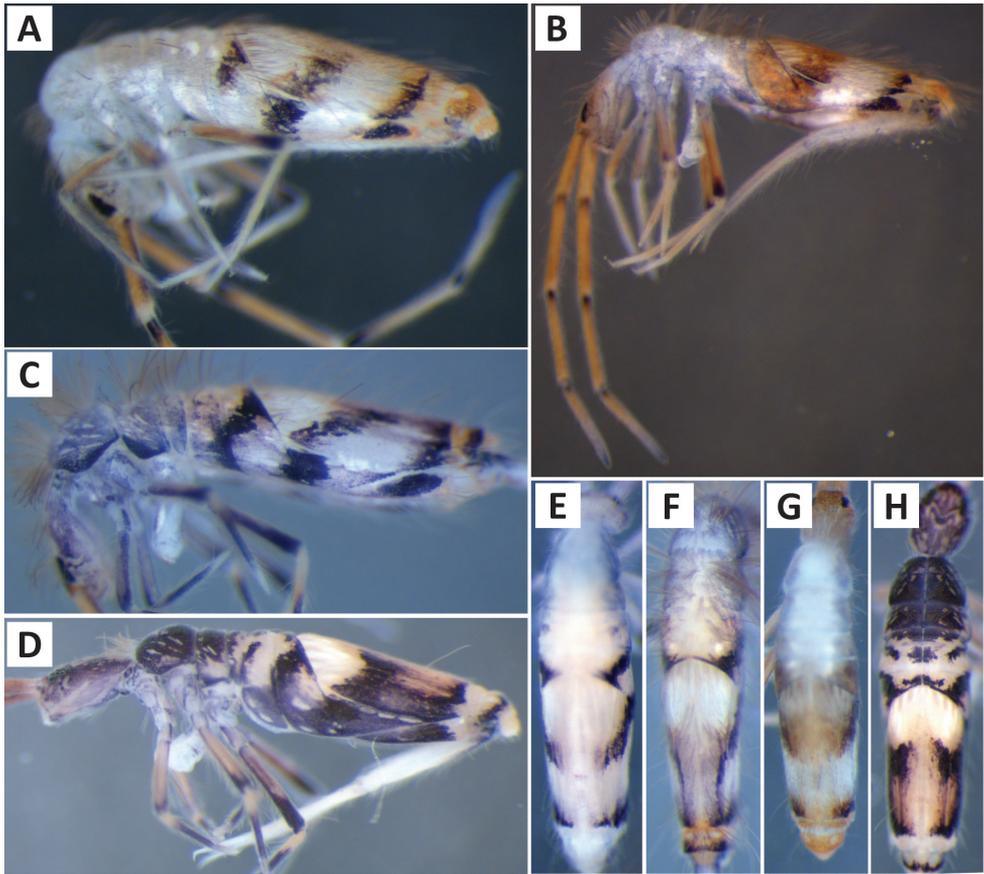
90.36967), under bark, 27.viii.2011, AK11-148; 1 on slide, Illinois, Kankakee Co., Kankakee River State Park (41.19482,-87.96875), from bird nest, 10.iv.2011, AK11-6; 4 on slides, Illinois, Monroe Co., Bat Sump Cave, Berlese of bark, 1-3.xi.2009 (S. Taylor & F. Soto-Adames), sjt09-130; 4 on slides, 50+ in vial, Illinois, Piatt Co., Lodge County Forest Preserve Park (40.06709,-88.56596), dead logs, cavity in tree, and under bark, 23.vii.2011, AK11-100; 2 on slides, 10 in vial, Illinois, Pope Co., Bell Smith Springs (37.51927,-88.65738), leaf litter, 24.ix.2011, AK11-166; 2 on slides, Illinois, Vermilion Co., Kickapoo State Park (40.16576,-87.74746), on rotten log, 30.xi.1989 (F. Soto-Adames); 1 in vial, Illinois, Vermilion Co., Kennekuk Cover County Park, Windfall Prairie Nature Preserve (40.20995,-87.74181), aspirated from bushes, 16.vi.2011 (A. Katz & F. Soto-Adames), AK11-59; 1 in vial, Michigan, Ingham Co., Michigan State University, Baker Wdlt. (42.66527,-84.36264) under bark of standing dead pine, 24.vii.2008 (E. C. Bernard), BW-11; 1 on slide, 3 in vial, Michigan, St. Clair Co., Algonac State Park (42.65447,-82.52430), under bark of recently fallen maple, 25.vii.2008 (E. C. Bernard), ASP-15; 1 on slide, West Virginia, Monongalia Co., Tyronne (39.62746,-79.86567), under collected on the lower branches of eastern hemlock, 19.vii.2005 (R. M. Turcotte), ID#44-GE-21-5-4-A.

### ***Entomobrya decemfasciata* (Packard), 1873**

Figs 2, 3D, 13B, 19, 20, 39

**Description.** *Body shape and color pattern.* Body very elongate and cylindrical with mesothorax forming a slight hump behind head. Color pattern remarkably variable with continuous variation and many intermediate forms (Fig. 19). Typical pattern without thoracic bands but with 2-4 irregular, angled bands on lateral margins of abdomen. Color also variable ranging from white, yellow, orange, or sometimes light blue or purple background with black, dark blue, or brown pigment forming bands. Dark bands outlining posterior and lateral margins of Th. 2 and Th. 3 sometimes present. Apex of femora usually with a dark patch. Head either entirely blue or purple, or lacking all pigment except for the eye patches. Dark patches of pigment usually occur on distal end of antennal segments 2-4. Juveniles usually with light blue pigment background and faint brown abdominal banding.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually bilobed or simple, but with up to 6 distinct lobes. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 3\times$  short setae. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E slightly curved, almost twice as long as papilla. Labial triangle chaetotaxy slightly irregular and atypical for this genus: M1, r, E, L1, L2 ciliate; r significantly smaller than other setae; A1-A5 smooth; sometimes 2 additional ciliate setae inserted internal to M1 and A1, respectively, often relatively difficult to observe. Post labial setae abundant, all type 5. Dorsal head macrosetae (Fig. 20A) An'<sub>0</sub>, An'<sub>3a3</sub>, S'<sub>0</sub>, S<sub>6</sub>, Ps<sub>3</sub>, and Pm<sub>11</sub> absent; A<sub>6</sub> sometimes present. Eyes G and H small and subequal. Eye patch with 5 setae.

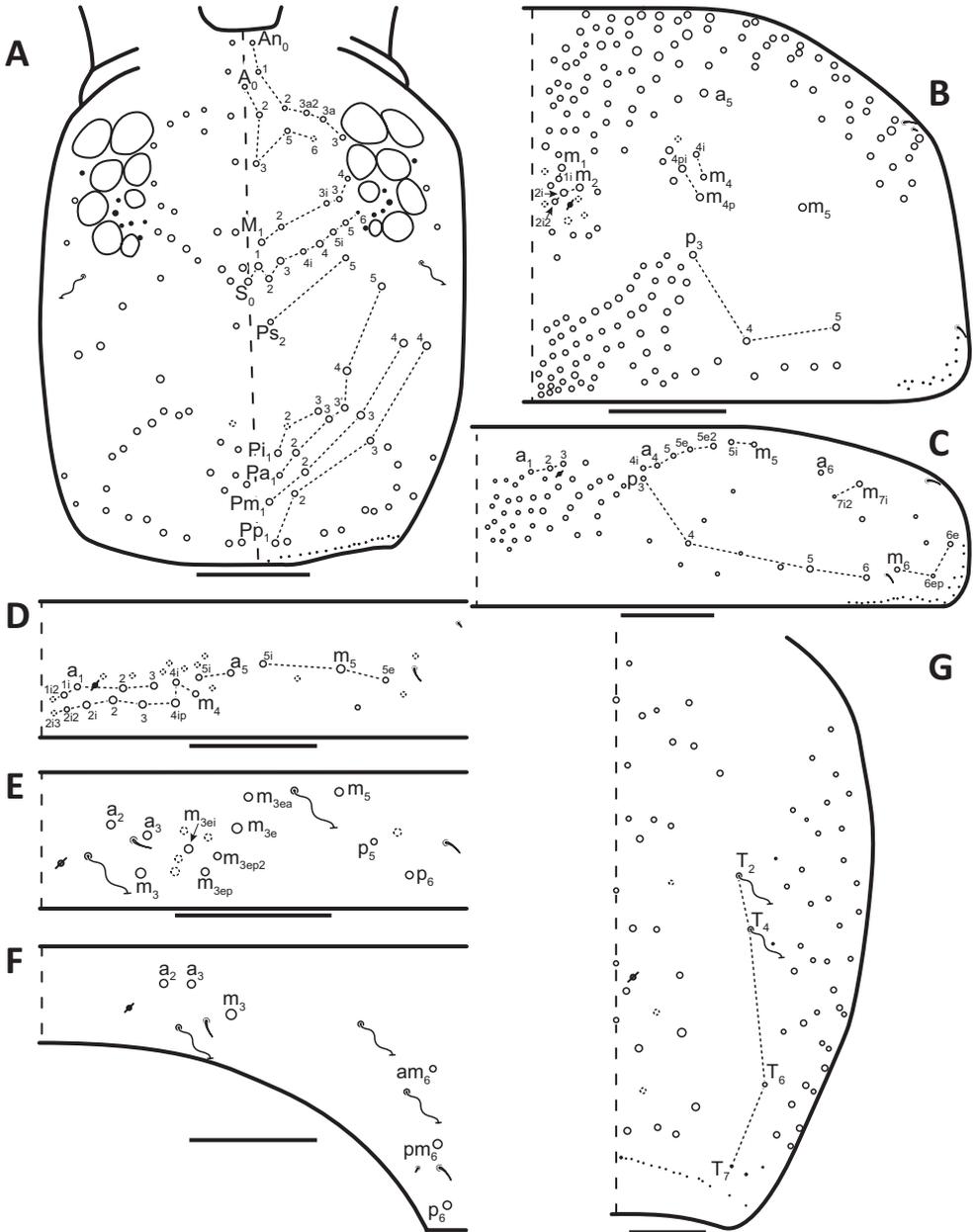


**Figure 19.** Color patterns of *Entomobrya decemfasciata*. Photographed specimens are from the following localities: **A** Stewart Co., TN **B** Stewart Co., TN **C** Clay Co., AL **D** Sevier Co., TN **E** Sequatchie Co., TN **F** Clay Co., AL **G** Union Co., IL **H** Sevier Co., TN.

*Thorax.* Thoracic chaetotaxy developed, highly variable, with many supplemental macrosetae. Th. 2 macrosetae  $p_6$ ,  $p_{6e}$  and  $p_{6ep}$  absent. Macrosetae in Zone A so abundant that usually merge with medial macrosetae, forming a single, large patch of setae (Figs 3D; 20B). Th. 3 macrosetae  $a_{5e3}$ ,  $m_4$ ,  $m_{5p}$ ,  $a_{6i}$ ,  $a_7$ , and  $m_7$  absent (Fig. 20C). Macrosetae in Zone Pm also very abundant on both thoracic segments, with many supplemental macrosetae present forming wing-like patches of posterior setae extending near the anterior row (Fig. 3D). Insertion of pseudopore on Th. 3 different from most *Entomobrya*, displaced anteriorly, very close to macrosetae  $a_1$ ,  $a_2$ , and  $a_3$ . Additional duplicate or supplementary setae often form columns internal to  $a_1$ .

*Legs.* Trochanteral organ with rectangular setal pattern and up to 86 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

*Abdomen.* Abdominal chaetotaxy extensively developed. Row of microsetae along entire posterior margin present in all segments (not displayed in figure). Abd. 1 with



**Figure 20.** Dorsal chaetotaxy of *Entomobrya decemfasciata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

16-30 macrosetae (Fig. 20D). Abd. 2 macroseta  $M_{3ei}$  always present, with up to 4 additional supplementary macrosetae (possibly including  $M_{3ei}$ ) internal to  $m_{3e}$ ; macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3e}$ ,  $m_{3ep}$ ,  $m_{3ep2}$ ,  $m_{3ea}$ ,  $m_5$ ,  $p_5$ , and  $p_6$  present (Fig. 20E). Abd. 3 with

macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$ ;  $a_1$  absent (Fig. 20F). Abd. 4 very elongated, with at least 25 inner macrosetae (Fig. 20G), but number of macrosetae extremely variable between individuals and even within a single individual (Fig. 13B). Insertion of pseudopores on Abd. 4 varying with respect to macroseta and bothriotricha even in same individual (Fig. 13B). Basal mucronal tooth enlarged.

**Remarks.** *Entomobrya decemfasciata* can be distinguished by the absence of parallel, longitudinal bands on the thorax, the presence of 2–4 irregular, angled bands on lateral margins of abdomen, and the presence of head macroseta  $Ps_5$  (see Table 2 for additional diagnostic characters). This species belongs to the *E. bicolor* group (see remarks for *E. bicolor*), and exhibits high levels of variation in both color pattern and chaetotaxy. *E. decemfasciata* is perhaps the most setaceous species of *Entomobrya* reported for North America, clothed in hundreds of macrosetae. This abundant and hyper-variable chaetotaxy provides few characters to differentiate *E. decemfasciata* from *E. bicolor* and *E. quadrilineata*. However, clear differences in color pattern can be observed between these species; *E. bicolor* lacks band or stripes, *E. quadrilineata* always has two parallel longitudinal stripes extending from the thorax through Abd. 2 and *E. decemfasciata* never has bands or stripes on the thorax. Table 5 details important diagnostic characters to separate species within this species complex.

The considerable variation in chaetotaxy and color pattern and relatively high molecular divergences between *E. decemfasciata* color forms suggest the presence of a cryptic species complex (Katz et al. 2015). However, the lack of diagnostic morphological characters between color forms does not allow the circumscription of new species at this time. More specimens and additional molecular and morphological analyses are needed for further action.

This species has a long history of taxonomic issues (Christiansen 1958b) and its separation from *E. quadrilineata* only became evident after a thorough molecular analysis (Katz et al. 2015). Christiansen (1958b) separated *E. decemfasciata* from *E. quadrilineata* using color pattern, male genital plate, antennal ratios, and later added chaetotaxy in *The Collembola of North America* (Christiansen and Bellinger 1998). High variability in color pattern and chaetotaxy, deformation of antennae after slide mounting, and the difficulty of discerning the morphology of setae on the genital plate, lessens the utility of these characters for diagnosis. Christiansen and Bellinger (1998) even suggested *E. quadrilineata* may be a variant form of *E. decemfasciata* and seemed uncertain regarding differences in chaetotaxy. Furthermore, Christiansen (1958b) reported three distinct color forms for *E. decemfasciata* (all of which had a V-shape or two angled lateral bands on Abd. 2 and lacking thoracic stripes) and three distinct color forms for *E. quadrilineata*, one of which also lacks thoracic stripes. During the course of this study, many color forms were collected, including forms both with and without thoracic stripes occurring together in the same sample. These sympatric forms lacked a V-shape or two lateral angled bands on the 2<sup>rd</sup> abdominal segment, and, following Christiansen and Bellinger's (1998) concept, were diagnosed as *E. quadrilineata*. However, large molecular distances made it apparent that forms with and without thoracic stripes were separate species (Katz et al. 2015), warranting further diagnostic inquiry. Type specimens from the Illinois Natural His-

tory Survey were attained for both *E. quadrilineata* and *E. decemfasciata*. Though both types, preserved in alcohol, were in relatively poor condition, two longitudinal thoracic stripes were clearly observed on the *E. quadrilineata* specimen (Suppl. material 3: A, B). Thoracic banding was not observed on the type specimen of *E. decemfasciata* (Suppl. material 3: C). Based on molecular evidence (Katz et al. 2015) and the observations of types, specimens with the morphology corresponding to both *E. quadrilineata* and *E. decemfasciata* can be diagnosed by the presence or absence of parallel thoracic longitudinal stripes. Christiansen's (1958b) report of an *E. quadrilineata* color form lacking thoracic stripes may have been a case of misidentification of *E. decemfasciata*. If individuals with and without longitudinal bands were collected together, similar morphology and abdominal pigmentation may have led him to conclude that they were both *E. quadrilineata*. However, we now know that both species can occur in sympatry.

**Distribution.** Endemic to North America (Suppl. material 2: F).

**Material examined.** USA: "*Type material*", 1 on slide, Knoxville, Tennessee (Dr. Curtis), INHS Cat. No. 528,321; *Cotype*, 1 on slide, no locality information; 1 on slide, 17 in vial, Alabama, Clay Co., Talladega National Forest, CR-7 & Hwy148 (33.19723,-86.06325), leaf litter, 2.i.2012, AK12-2; 3 on slides, 20 in vial, Illinois, Union Co., Anna, Shawnee National Forest, Rich's Cave, vestibule of back high entrance, moist leaf litter along the wall and some dry litter from the center of the vestibule, 21.vi.2012 (F. Soto-Adames, S. Taylor & A. Katz); 16 in vial, Illinois, Union Co., Anna, Shawnee National Forest, Rich's Cave, humid and cool litter in side niche near main entrance, 21.vi.2012 (F. Soto-Adames, S. Taylor & A. Katz); 4 on slides, 2 in vial, Tennessee, Sequatchie Co., Moonshadow (35.64167,-83.76359), forest leaf litter, 14.i.1997 (M. M. Gibbs); 2 on slides, Tennessee, Sevier Co., Great Smoky Mountains National Park, ATBI plot, Goshen Prong (35.67961,-83.50021), malaise 22 83 32 34 35 36 38, 12.xi.2001-5.xii.2001 (Parker, Stocks & Peterson); 2 on slides, 3 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, Fox Ridge Rd (36.66392,-87.98596), leaf litter, 7.viii.2011, AK11-105; 2 on slides, 50+ in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, Fox Ridge Rd. (36.66392,-87.98596), leaf litter, 7.viii.2011, AK11-107; 2 on slides, 11 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area (36.53830,-87.91428), leaf litter, 7.viii.2011, AK11-108.

### *Entomobrya intermedia* Brook, 1883

Figs 2, 21, 22, 39

**Description.** *Body shape and color pattern.* Body oval and cylindrical. Color pattern monomorphic (Fig. 21): yellow background with black, dark brown or purple pigment forming two incomplete, broken, longitudinal bands from Th. 2 through Abd. 3 and a conspicuous W-shaped mark on Abd. 4. Usually with dark pigment covering the lateral margins of the head. Antennae light brown or purple becoming increasingly dark towards the apex.



**Figure 21.** Color pattern of *Entomobrya intermedia*. Lateral (A) and dorsal (B) views of specimen collected from Chester Co., PA.

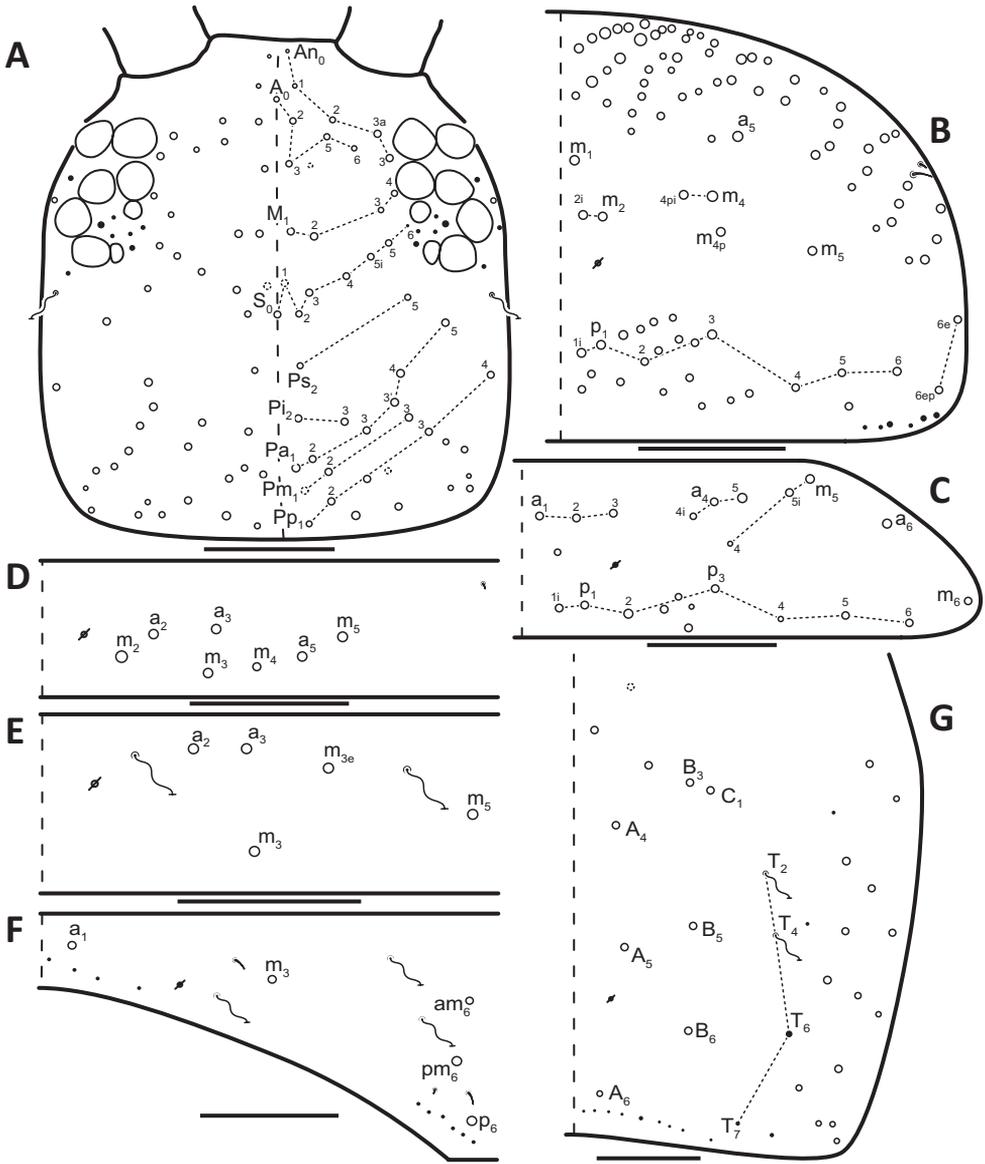
**Head.** Apical bulb of 4<sup>th</sup> antennal segment simple. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 5\times$  short setae. Prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with 2–4 small spine-like projections (Fig. 2). Lateral appendage of labial papilla E slightly curved, thin, nearly reaching tip of papilla. Dorsal head chaetotaxy (Fig. 22A) with macrosetae  $An'_0$ ,  $A_{3a2}$ ,  $A_{3a3}$ ,  $M_{3i}$ ,  $S'_0$ ,  $S_{4i}$ ,  $S_6$ ,  $Ps_3$ ,  $Pi_1$ , and  $Pm_{1i}$  absent; an additional macroseta external to  $A_{3a3}$  present in some specimens;  $S_1$  and  $Pm_1$  usually present, but may be asymmetrical. Eyes G and H small and subequal. Eye patch with 5 setae.

**Thorax:** Thoracic chaetotaxy well-developed and relatively stable. Th. 2 macrosetae  $a_5$ ,  $m_1$ ,  $m_2$ ,  $m_{2p}$ ,  $m_4$ ,  $m_{4p}$ ,  $m_{4i}$ , and  $m_5$  present (Fig. 22B). Lateral sensilla obscured in all specimens examined. Posterior macrosetae (series P) present. Th. 3 macrosetae  $a_1$ ,  $a_2$ ,  $a_3$ ,  $a_4$ ,  $a_{4i}$ ,  $a_5$ ,  $a_6$ ,  $m_4$ ,  $m_5$ ,  $m_{5i}$ , and  $m_6$  present (Fig. 22C). Posterior macrosetae (series P) present. The chaetotaxy of zone Pm with a moderate number of supplemental macrosetae in both thoracic segments (Fig. 22B,C). Typical sensilla present (not shown in figure).

**Legs.** Trochanteral organ with triangular setal pattern and up to 31 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

**Abdomen.** Abdominal chaetotaxy stable. Abd. 1 with 7 macrosetae (Fig. 22D). Abd. 2 macroseta  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3cp}$ , and  $m_5$  present (Fig. 22E). Abd. 3 with macrosetae  $a_1$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$  (Fig. 22F). Abd. 4 with 9–10 inner macrosetae (Fig. 22G). Mucronal teeth subequal. Typical sensilla present (not shown in figure).

**Remarks.** *Entomobrya intermedia* can be easily identified by the presence of two longitudinal stripes, a W-shaped mark on Abd. 4 combined with the presence of Th. 2 macrosetae  $m_5$  and Abd. 3  $a_1$ , and the absence of head macrosetae  $S_{4i}$ , Abd. 2  $m_{3cp}$ , and Abd. 3  $a_2$  (see Table 2 for additional diagnostic characters). Historically, this species was considered a synonym of *E. nivalis*. However, the clear differences in chaetotaxy (see Table 3) and color pattern separate *E. intermedia* from *E. nivalis* and other similar forms such as *E. multifasciata* and female *E. atrocincta*. Further-



**Figure 22.** Dorsal chaetotaxy of *Entomobrya intermedia*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

more, molecular data supports the separation of this species from *E. nivalis* (Katz et al. 2015). The combination of chaetotaxy outlined in Table 3 should be used in conjunction with color pattern characters to differentiate this species. Note that observation of chaetotaxy was only examined for specimens collected from Chester County, Pennsylvania. It is likely that specimens from additional localities may re-

veal more variation in chaetotaxy than described here, especially since the chaetotaxy reported in Palearctic specimens (Jordana 2012) is different than those observed in North American specimens.

**Distribution.** North America and Europe. The actual distribution of *E. intermedia* in North America is unclear, as before Christiansen and Bellinger's (1998) monograph the species was included within *E. nivalis* (Christiansen 1958b). In fact, some records of *E. nivalis*, *E. multifasciata* and female *E. atrocincta*, regardless of date, may actually refer to *E. intermedia*. Suppl. material 1: G shows the distribution of *E. intermedia* in North American as currently understood.

**Material examined.** USA: 2 on slides, Pennsylvania, Chester Co., Wayne, sweep of *Forsythia* sp., 23.v.2011, AK11-32; 8 on slides, 12 in vial, Pennsylvania, Chester Co., Wayne, sweep of *Forsythia* sp., 29.vi.2012, AK12-50.

***Entomobrya jubata* Katz & Soto-Adames, sp. n.**

<http://zoobank.org/B178F030-EA9E-43C6-99B3-98C89487F3EB>

Figs 16B, 23, 24, 39

**Etymology.** The word jubatus is Latin for maned, or crested, and refers to the abundance of dorsal macrosetae on the thoracic segments.

**Type material.** *Holotype*, ♀, USA: Alabama, Covington County, Conecuh National Forest (31.07900,-86.61203), under bark, 2.i.12 (A. Katz & M. DuBray), AK12-9 & AK12-6.

*Paratypes*, USA: 7 on slides, 20 in vials, Alabama, Covington Co., Conecuh National Forest (31.07900,-86.61203), under bark, 2.i.12 (A. Katz & M. DuBray), AK12-9 & AK12-6.

**Description.** *Body shape and color pattern.* Body cylindrical, slightly dorso-ventrally flattened. Length up to 2 mm. Color pattern monomorphic (Fig. 23): light brown background with black pigment forming dark transverse bands across the posterior margins of Abd. 4, Abd. 5, and Abd. 6; dark pigment present along lateral margins of Th. 2 through Abd. 2, forming two lateral stripes. Two patches of dark pigment usually present medially on Abd. 4 and may appear to form an incomplete irregular transverse band; Th. 2 entirely white except for black pigment lining anterior and lateral margins; legs range in color from white to light brown to purple near the apex.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually simple, rarely bilobed. Long differentiated smooth seta on ventral side of 1<sup>st</sup> antennal segment 3× as long as short setae. Prelabral setae ciliate. Distal margin of the labral papillae with 2-3 seta or spine-like projections. Labial papilla E with lateral appendage almost straight, reaching tip of papilla. Dorsal head chaetotaxy as in Figure 24A: macrosetae An<sub>0</sub>, A<sub>3a2</sub>, A<sub>3a3</sub>, M<sub>3i</sub>, S<sub>6</sub>, Ps<sub>3</sub>, and Ps<sub>5</sub> always absent; Pi<sub>1</sub> and Pm<sub>1i</sub> present or absent; S<sub>0</sub> always present. Eyes G and H small and subequal. Eye patch with 5 setae.



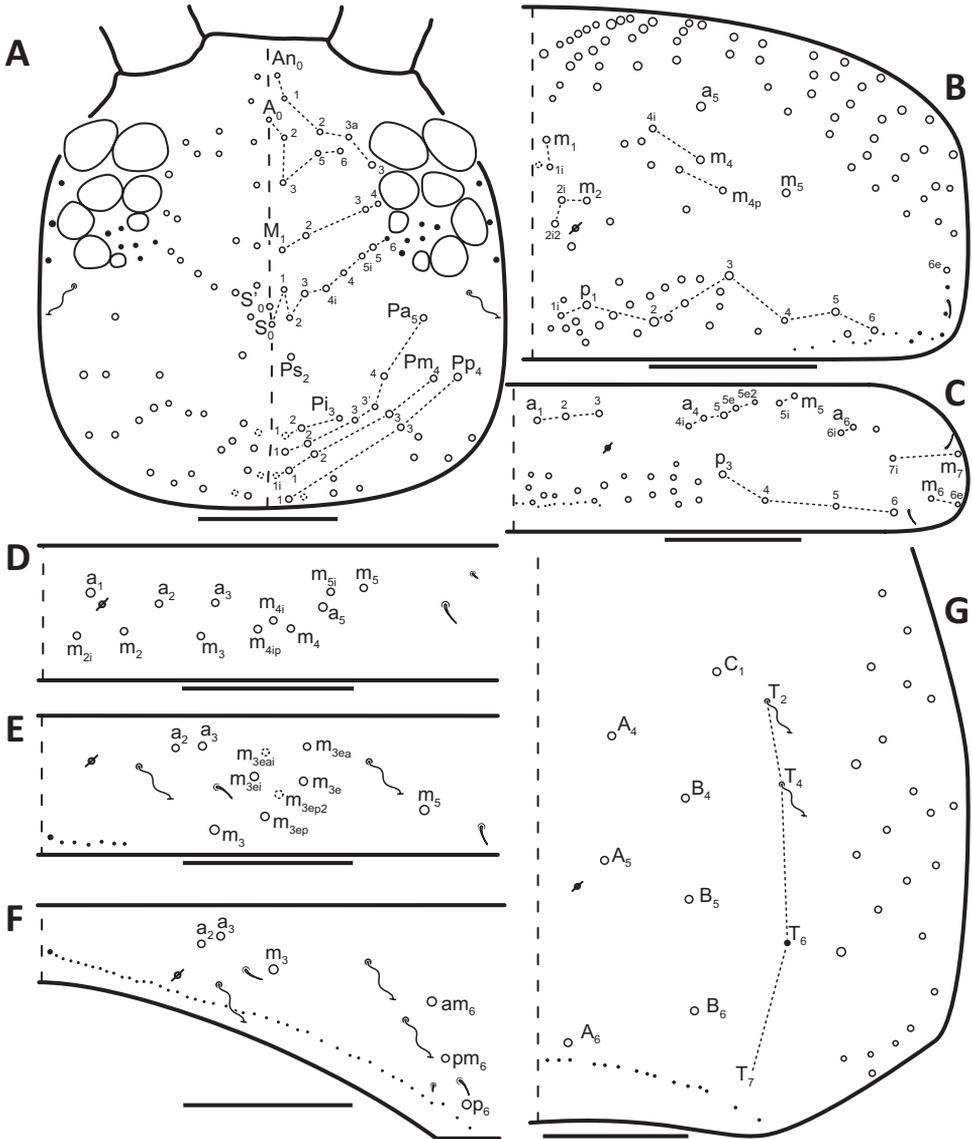
**Figure 23.** Dorsal color patterns of *Entomobrya jubata* sp. n. collected for this study. Lateral and dorsal views for two individuals (**A, B** and **C, D**) collected from Covington Co., AL.

*Thorax.* Thoracic chaetotaxy well-developed (Fig. 24B). Th. 2 with all described macrosetae present, except  $m_{4i3}$ . Th. 3 macrosetae  $a_{5e3}$ ,  $m_4$ ,  $m_{5p}$ , and  $a_7$  absent (Fig. 24C). Zone Pm with many supplemental macrosetae present in both thoracic segments.

*Legs.* Trochanteral organ with triangular setal pattern and up to 25 setae. Unguis with 4 inner teeth; basal teeth located approximately middle of inner claw length (Fig. 16B).

*Abdomen.* Abdominal chaetotaxy abundantly developed. Abd. 1 with 12 macrosetae (Fig. 24D). Abd. 2 macrosetae variable:  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3ep}$ ,  $m_{3e}$ ,  $m_{3ei}$ ,  $m_{3ea}$ , and  $m_5$  always present;  $m_{3ep2}$  and  $m_{3eai}$  sometimes present (Fig. 24E). Abd. 3 macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$  present;  $a_1$  always absent (Fig. 24F). Abd. 4 chaetotaxy stable, with 7 inner macrosetae (Fig. 24G). Mucronal teeth subequal.

**Remarks.** *Entomobrya jubata* sp. n. can be easily distinguished by the unique color pattern described above combined with the absence of head macroseta  $Ps_5$ , the



**Figure 24.** Dorsal chaetotaxy of *Entomobrya jubata* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment (Abd. 2) **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

presence of head macroseta  $S'_0$  and Th. 2 macrosetae  $m_2$  and  $m_5$  (see Table 2 for additional diagnostic characters). This species is closely related to *E. clitellaria* and both share similar chaetotaxy. However, these species can be easily separated by color pattern alone; *E. jubata* sp. n. does not have dark pigment on Th. 3 through Abd.

3. The presence of head macrosetae  $S'_0$  and the absence of head macrosetae  $Ps_5$  also separate *E. jubata* sp. n. from *E. clitellaria*. The color pattern and chaetotaxy exhibited by this species have not been reported in the literature.

**Distribution.** Endemic to North America. *E. jubata* sp. n. was collected from a single locality in Covington County, Alabama (Suppl. material 2: H).

### *Entomobrya ligata* Folsom, 1924

Figs 25, 26, 39

**Description.** *Body shape and color pattern.* Body oval and cylindrical. Color pattern stable, monomorphic (Fig. 25), always with four transverse bands; two thin regular bands along the posterior margin of Th. 2 and Th. 3 respectively, an irregular, patchy, thick band covering most of Abd. 3, and a highly irregular and sometimes broken band across the medial section of Abd. 4. A small patch of pigment covers Abd. 5 and Abd. 6. Patterns usually consisting of black or dark blue pigment with a yellow background. Dark pigment usually in patches along lateral margins of Th. 2 through Abd. 4. Antennae with purple pigment, darken near apex. Legs usually white, with small purple patches on apical end of femora.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually bilobed, sometimes multilobed. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 3x$  short setae. Prelabral setae finely ciliated, seemingly smooth at low magnification under light microscopy. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projections. Lateral appendage of labial papilla E slightly curved, relatively thick and short, extending only  $\frac{3}{4}$  papilla length. Dorsal head macrosetae (Fig. 26A)  $An_{3a2}$ ,  $An_{3a3}$ ,  $A_6$ ,  $M_{3i}$ ,  $S'_0$ ,  $S_6$ ,  $Ps_3$ ,  $Pi_1$ ,  $Pm_{1i}$ , and  $Pp_2$  absent;  $An'_0$ , a short mesoseta located medially between both  $An_0$  present. Eyes G and H small and subequal. Eye patch with 3 setae.

*Thorax.* Th. 2 macrosetae  $a5$ ,  $m_1$ ,  $m_2$ ,  $m_{2i}$ ,  $m_4$ ,  $m_{4p}$ , and  $m_{4i}$  present (Fig. 26B). Th. 3, macrosetae  $a_1$ ,  $a_2$ ,  $a_3$ ,  $a_4$ ,  $a_{4i}$ ,  $a_5$ ,  $a_{5e}$ ,  $a_6$ ,  $a_{6i}$ ,  $m_5$ ,  $m_{5i}$ ,  $m_6$ ,  $m_{6e}$ , and  $m_7$  present (Fig. 26C); all posterior macrosetae (series P) present. Zone Pm in both thoracic segments with a moderate number of supplemental macrosetae (Fig. 26B,C).

*Legs.* Trochanteral organ with triangular setal pattern and up to 17 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length.

*Abdomen.* Abdominal chaetotaxy reduced; no macrosetae variation observed. Abd. 1 with 4 macrosetae:  $a_5$ ,  $m_2$ ,  $m_3$ , and  $m_4$  (Fig. 26D). Abd. 2 with 5 macrosetae:  $a_2$ ,  $m_3$ ,  $m_{3e}$ ,  $m_{3ep}$ , and  $m_5$  (Fig. 26E). Abd. 3 with 3 macrosetae:  $m_3$ ,  $pm_6$ , and  $p_6$  (Fig. 26F). Abd. 4 with 6 inner macrosetae (Fig. 26G). Mucronal teeth subequal.

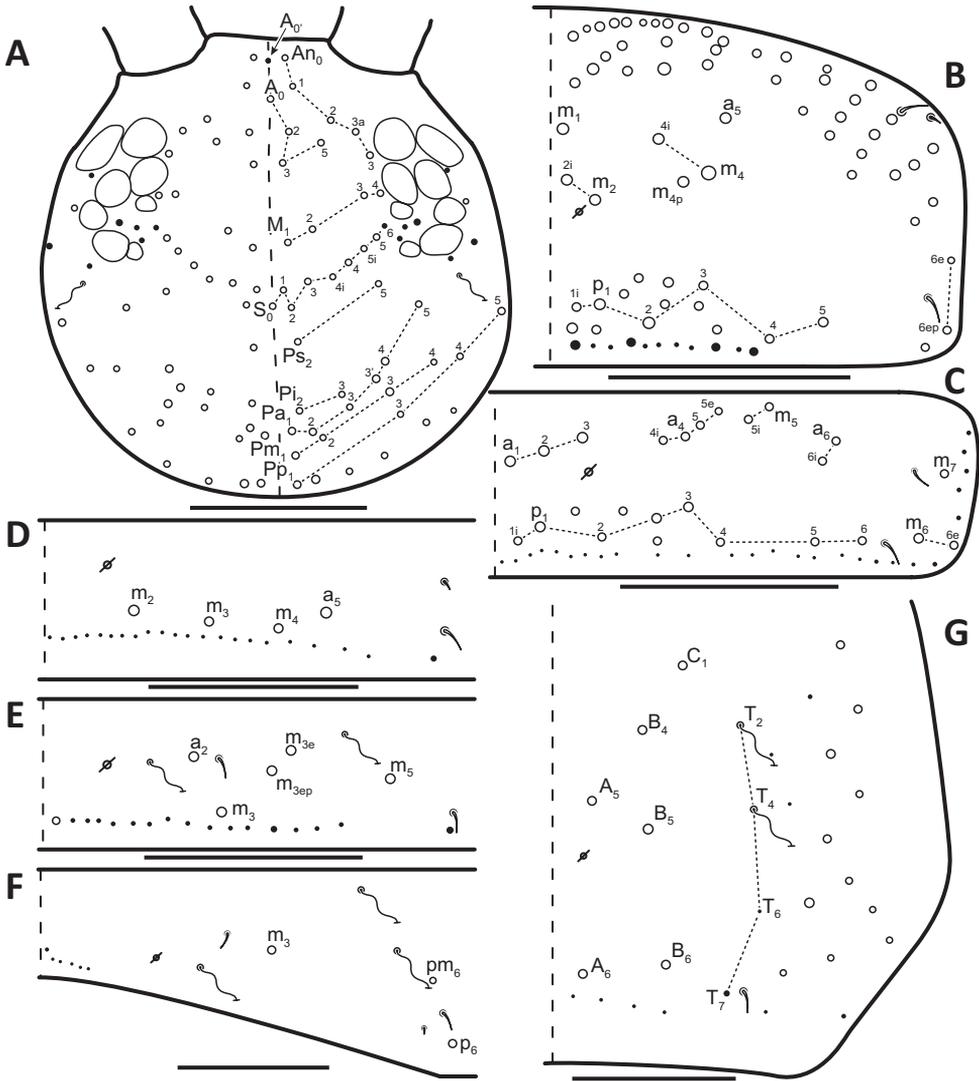
**Remarks.** This species can be identified by the presence of four transverse bands, head mesoseta  $An'_0$  present, four macrosetae on Abd. 1, and only three eye patch setae (see Table 2 for additional diagnostic characters). *Entomobrya unifasciata* sp. n. and *E. neotenica* sp. n. are closely related to this species and form the *E. ligata* complex, char-



**Figure 25.** Color pattern of *Entomobrya ligata*. Lateral (A) and dorsal (B) views of specimen collected from Chester Co., PA.

acterized by the presence of only three microsetae in the eye patch, four macrosetae on Abd. 1, and six macrosetae on Abd. 4. *Entomobrya ligata* can be separated from *E. unifasciata* sp. n. and *E. neotenica* sp. n. by characters outlined in Table 6.

*E. ligata* was described by Folsom (1924) and redescribed by Christiansen (1958b). Both descriptions depict *E. ligata* with four dorsal transverse bands (Folsom described five bands; he considered the pigment on Abd. 5 and 6 an additional band), two of which occur on the posterior margins of the Th. 2 and Th. 3, respectively. Samples were originally diagnosed as *E. ligata* based on chaetotaxy described by Christiansen and Bellinger (1998). However, Katz et al. (2015) showed that a population from Chester Co., Pennsylvania was highly divergent and genetically isolated from other populations. The Pennsylvania population differs from all other populations by the presence of a dark transverse band along the posterior margin of Th. 2. This dark band is present in all individuals collected in Pennsylvania and absent in individuals from all other localities. The original descriptions by Folsom (1924) and Christiansen (1958b) described *E. ligata* as having this band present and noted a lack of additional variations in color form. Multiple type specimens deposited at the INHS were examined; all collected in the state of New York, and all carry a dark band along the posterior margin of Th. 2 (Suppl. material 3: D–G). Therefore, the combination *E. ligata* is reserved for populations in which individuals carry the posterior band on Th. 2, whereas populations without this band are referred to *E. unifasciata* sp. n. (see below).



**Figure 26.** Dorsal chaetotaxy of *Entomobrya ligata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

**Distribution.** Endemic to North America. The species has been reported as having a wide distribution, occurring east of the Mississippi River to the Atlantic coast (Suppl. material 2: I). However, in light of the new circumscription provided above and the possible confusion with *E. unifasciata* sp. n., most historical reports are questionable, especially those between the western Smokey Mountains and the Mississippi River. The syntypic series of *E. ligata* was collected at different localities in New York State

**Table 6.** Diagnostic characters to separate species within the ligata complex: *E. ligata*, *E. unifasciata* sp. n., and *E. neotenica* sp. n. Character states: absent (0), present (1).

Species	Head mesoseta An' <sub>0</sub>	Abd. 3 macroseta m <sub>3ep</sub>	1 dark transverse band on posterior margin of Th. 2	2 dark triangular patches on Abd. 3
<i>E. ligata</i>	1	1	1	0
<i>E. unifasciata</i> sp. n.	1	1	0	0
<i>E. neotenica</i> sp. n.	0	0	0(1) <sup>1</sup>	1

<sup>1</sup> Parentheses indicate a rarely observed state.

and all fresh material was collected in Chester Co., Pennsylvania, suggesting the species may be restricted to the northeast section of the country.

**Material examined.** USA: *Syntypes*, 1 on slide, Karner, N.Y., 7-14-23 (A. Wolf) INHS Cat. No. 528,351; *Cotypes*: 1 in vial, Karner, N.Y., July, 14 1923 (A. Wolf); *Cotypes*: 2 in vial, N.Y., July 8, 1923 (A. Wolf); *Syntypes*, 1 on slide, Mineola, L. I., N.Y., July 8, 1923 (O. W. Barrett) INHS Cat. No. 528,350; *Cotypes*, 1 in vial, Roorhesville, N. Y., Aug 30, 1923 (M. S. Leonard); *Cotypes*: 2 in vial, Roorhesville, N. Y., Aug 30, 1923 (M. S. Leonard). Other material: 1 on slide, 1 in vial, Pennsylvania, Chester Co., Wayne, McKaig Nature Education Center (40.06923,-75.37903), leaf litter, 23.v.2011, AK11-33; 1 on slide Pennsylvania, Chester Co., Wayne, McKaig Nature Education Center (40.06923,-75.37903), bark, 23.v.2011, AK11-34.

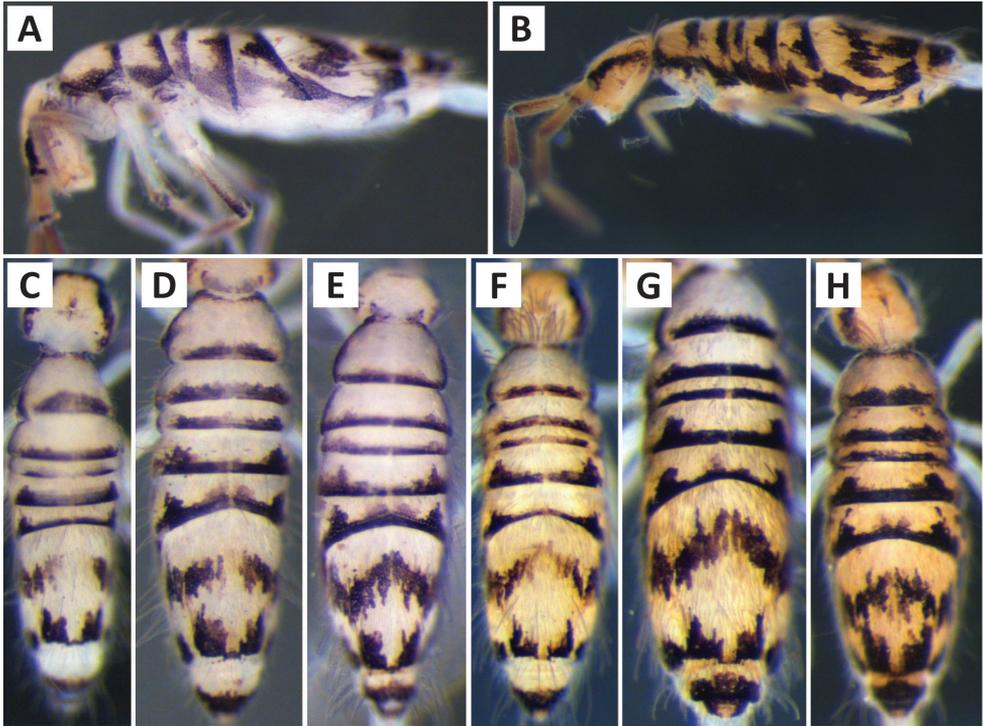
### *Entomobrya multifasciata* (Tullberg), 1871

Figs 2, 27, 28, 39

**Description.** *Body shape and color pattern.* Body oval and cylindrical. One primary color form, with slight variations (Fig. 27); yellow background with black, dark brown or purple pigment forming 5 transverse bands along posterior margins of Th. 2 through Abd. 3. Abd. 4 pattern variable, but usually with 2 triangular patches of pigment along posterior margin of segment that point anteriorly toward a W-shaped mark or broken and irregular transverse band. Abd. 5 and Abd. 6 mostly covered with dark pigment. Dark pigment present along lateral margins of all segments, sometimes in broken patches. Antennae light brown or purple pigment increasingly dark towards the apex.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually bilobed, sometimes simple. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment ≈2–3× short setae. Four prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with 2-3 seta or spine-like projections (Fig. 2). Labial lateral appendage slightly curved, relatively thin, reaching just beyond tip of papilla. Dorsal head macrosetae (Fig. 28A) An'<sub>0</sub>, A<sub>3a2</sub>, A<sub>3a3</sub>, M<sub>3i</sub>, S'<sub>0</sub>, S<sub>4i</sub>, S<sub>6</sub>, Ps<sub>3</sub>, Pi<sub>1</sub>, and Pm<sub>1i</sub> absent; S<sub>1</sub> and Pm<sub>1</sub> usually present, but may be asymmetrical. Eyes G and H small and subequal. Eye patch with 5 setae.

*Thorax.* Th. 2 macrosetae a<sub>5</sub>, m<sub>1</sub>, m<sub>2</sub>, m<sub>4</sub>, and m<sub>4p</sub> present (Fig. 28B); m<sub>1i</sub> and m<sub>2i</sub> sometimes present; posterior macrosetae (series P) present. Th. 3 macrosetae a<sub>1</sub>, a<sub>2</sub>, a<sub>3</sub>,



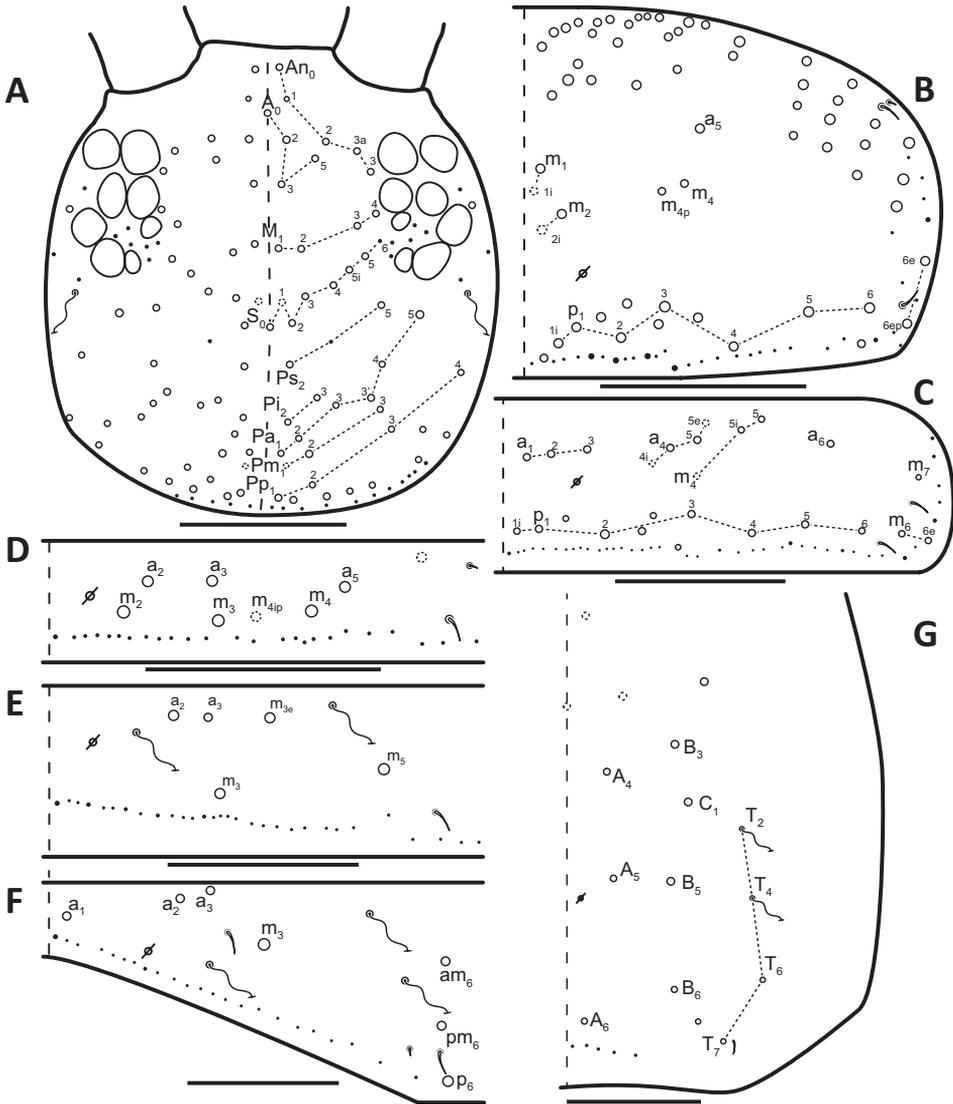
**Figure 27.** Color patterns of *Entomobrya multifasciata*. All photographed specimens (A–H) are from São Miguel Island, Azores, Portugal.

$a_4$ ,  $a_5$ ,  $a_6$ ,  $m_5$ ,  $m_{5i}$ ,  $m_6$ ,  $m_{6c}$ , and  $m_7$  present (Fig. 28C);  $a_{4i}$ ,  $a_{5c}$ , and  $m_4$  sometimes present; posterior macrosetae (series P) present. Zone Pm of both thoracic segments with moderate number of supplemental macrosetae (Figs 3; 39B,C).

*Legs.* Trochanteral organ with triangular setal pattern and up to 18 setae.

*Abdomen.* Abd. 1 with 6–8 macrosetae (Fig. 28D). Abd. 2 macroseta  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3c}$ , and  $m_5$  are present (Fig. 28E). Abd. 3 macroseta  $a_1$ ,  $a_2$ ,  $a_3$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$  present (Fig. 28F). Abd. 4 with 8–12 inner macroseta (Fig. 28G). Mucronal teeth subequal.

**Remarks.** *Entomobrya multifasciata* can be distinguished by the color pattern described above combined with the presence of Abd. 3 macrosetae  $a_1$  and  $a_2$  and the absence of macrosetae head  $S_{4i}$ , Th. 2  $m_5$ , and Abd. 2  $m_{3ep}$  (see Table 2 for additional diagnostic characters). Chaetotaxy and color pattern observed in this species is almost indistinguishable from that in female *E. atrocincta*. Furthermore, variation in chaetotaxy displayed in *E. atrocincta* obscures most potentially diagnostic characters needed to distinguish *E. multifasciata* from *E. atrocincta*. However, *E. multifasciata* can be recognized by the presence of head macroseta  $S_{4i}$  and the morphology of labral papillae; *E. multifasciata* has two to three larger seta or spine-like projections per papillae, while *E. atrocincta* has three to four smaller seta or spine-like projections per papillae (Fig. 2). Table 3 provides additional diagnostic characters separating *E. multifasciata* from



**Figure 28.** Dorsal chaetotaxy of *Entombrya multifasciata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

*E. nivalis* and *E. intermedia*, which share superficially similar color patterns but can be separated by chaetotaxy.

We were unable to obtain North American samples of *E. multifasciata* and the description and diagnosis provided above are based on specimens from São Miguel Island, Azores, Portugal. The Nearctic distribution of this species remains unclear in light of the sexual dimorphism of *E. atrocincta* described in this study (see remarks for *E. atrocincta*). Christiansen and Bellinger (1998) report a widespread distribution,

with localities found across the United States, but they also note that their records may be questionable as a result of likely misidentification. Christiansen and Bellinger (1998) describe the labral papillae of *E. multifasciata* as having three to four small seta or spine-like projections, a condition that resembles those in *E. atrocincta*, possibly indicating an identification error. In the Collembola of North America, Christiansen and Bellinger (1998) report a male genital plate, presumably from an individual with the *E. multifasciata* pattern collected in Massachusetts, which eliminates the possibility that the specimen could have been a female *E. atrocincta*. Since a number of samples were collected in the Northeastern United States (Christiansen and Bellinger 1998), the presence of *E. multifasciata* in North America cannot be ruled out. However, in the course of the present study, all specimens collected bearing the banded color pattern were female *E. atrocincta* and were usually accompanied by male *E. atrocincta*.

**Distribution.** North America (Christiansen and Bellinger 1998), Hawaii (Christiansen and Bellinger 1992), Europe and Russia (Jordana 2012). Records from North America and Hawaii are questionable due to likely misidentification of *E. atrocincta* females. See Suppl. material 2: J for a distribution map and below for a list of material examined with collection and locality information.

**Material examined.** PORTUGAL: 2 on slides, 10 in vial, Azores, São Miguel Island, (J. Marcelino), vial #119; 6 on slides, 10 in vial, Azores, São Miguel Island, (J. Marcelino), vial #86; 5 in vial, Azores, São Miguel Island, (J. Marcelino), vial #55; 4 in vial, Azores, Terceira Island, (J. Marcelino), vial #9; 4 in vial, Madeira Island, (J. Marcelino), vial #2.

***Entomobrya neotenica* Katz & Soto-Adames, sp. n.**

<http://zoobank.org/C70B2A90-547A-4A0F-9A4E-E5F33B83BF8A>

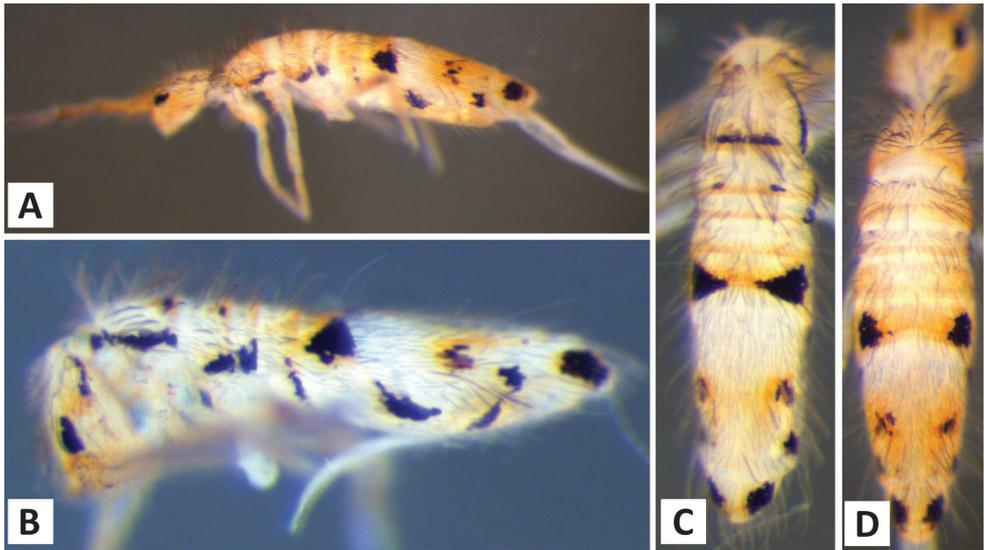
Figs 2, 16C, 29, 30, 39

**Etymology.** This species is named for its apparent neoteny; small size and reduced chaetotaxy.

**Type material.** *Holotype*, ♂, USA: Alabama, Lawrence County, William B. Bankhead National Forest (34.3369,-87.3461), leaf litter, 9.viii.2011, AK11-112.

*Paratypes*, USA: 1 on slide, 1 in vial, Alabama, Clay Co., Talladega National Forest (33.19723,-86.06325), moist leaf litter, 2.i.12 (A. Katz & M. DuBray), AK12-2; 1 in vial, Alabama, Lawrence Co., William B. Bankhead National Forest (34.3369,-87.3461), leaf litter, 9.viii.2011, AK11-112; 1 on slide, Florida, Taylor Co., Econfina River State Park (30.0656,-83.9107), under bark, 9.viii.2011, AK11-116; 2 on slides, 18 in vial, Illinois, Union Co., Anna, Shawnee National Forest, Rich's Cave, moist leaf litter under bark on fallen tree, 21.vi.2012 (F. Soto-Adames, S. Taylor & A. Katz); 1 on slide, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area (36.5354,-87.9214), forest floor leaf litter, 3.v.2011, AK-43.

**Description.** *Body shape and color pattern.* Body oval and cylindrical. Length up to 1.15 mm. One primary color form (Fig. 29): white and yellow or orange background



**Figure 29.** Color pattern of *Entomobrya neotenica* sp. n. Lateral and dorsal views of specimens collected from Lawrence Co., AL (**A** and **D**) and Clay Co., AL (**B** and **C**).

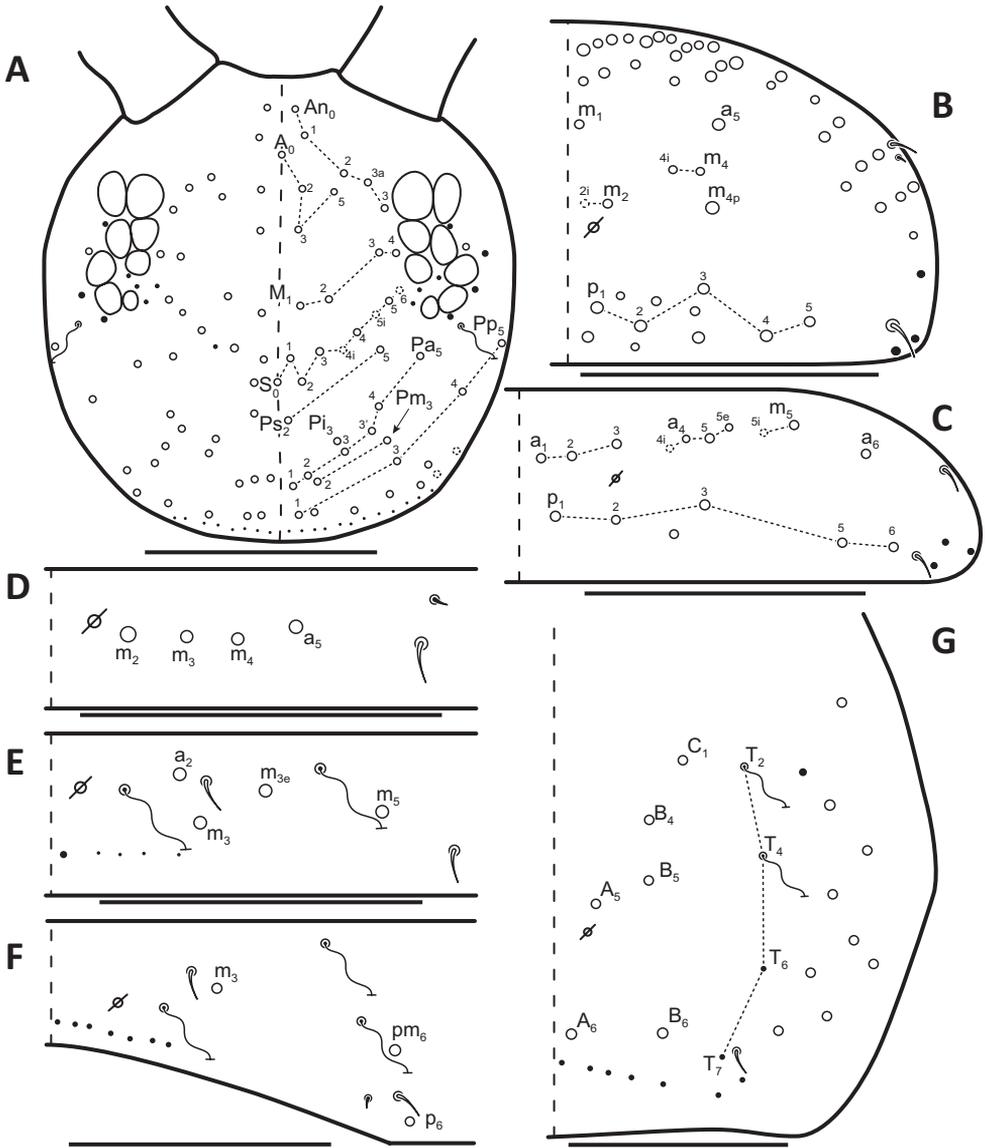
with black or dark purple pigments forming two lateral triangles on the sides Abd. 3; triangles sometimes reduced to irregular patches. Additional irregular patches of pigment usually on lateral margins of all segments. A band sometimes present along posterior margin of Th. 2. Abd. 5 with 2 dark spots, sometimes forming irregular triangular shapes. Antennae usually light purple near apex and relatively long. Legs usually white, with small purple patches on apical end of femora.

**Head.** Apical bulb of 4<sup>th</sup> antennal segment simple or bilobed. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 2\text{--}2.5\times$  short setae. Four prelabral setae finely ciliate, appearing smooth under light microscopy. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projections (Fig. 2). Labial lateral appendage slightly curved, relatively thick, not reaching tip of papilla. Dorsal head chaetotaxy reduced (Fig. 30A), macrosetae  $An'_0$ ,  $An_{3a2}$ ,  $An_{3a3}$ ,  $A_6$ ,  $M_{3i}$ ,  $S'_0$ ,  $P_{s3}$ ,  $P_{i1}$ ,  $P_{i2}$ ,  $P_{m1}$ ,  $P_{m_{ii}}$ , and  $P_{p2}$  absent;  $S_{5i}$  usually present;  $S_{4i}$  and  $S_6$  usually absent. Eyes G and H smaller than A-F but enlarged; G slightly larger than H. Eye patch with 3 setae.

**Thorax.** Thoracic chaetotaxy reduced, with relatively few supplementary setae. Th. 2 macrosetae  $a_5$ ,  $m_1$ ,  $m_2$ ,  $m_4$ ,  $m_{4p}$ , and  $m_{4i}$  present (Fig. 30B); all posterior macrosetae (series P) present. Th. 3 macrosetae  $a_1$ ,  $a_2$ ,  $a_3$ ,  $a_4$ ,  $a_{4i}$ ,  $a_5$ ,  $a_{5e}$ ,  $a_6$ ,  $a_{6i}$ ,  $m_5$ ,  $m_{5i}$ ,  $m_6$ ,  $m_{6e}$ , and  $m_7$  present (Fig. 30C). Both thoracic segments with few supplemental macrosetae present in zone Pm (Fig. 30B,C).

**Legs.** Trochanteral organ with triangular setal pattern and up to 20 setae. Unguis with 4 inner teeth (Fig. 16C). Unguiculus acuminate with small serrations on inner edge.

**Abdomen.** Abdominal chaetotaxy reduced; no macrosetae variation observed. Abd. 1 with 4 macrosetae;  $a_5$ ,  $m_2$ ,  $m_3$ , and  $m_4$  (Fig. 30D). Abd. 2 with 4 macrosetae:  $a_2$ ,  $m_3$ ,



**Figure 30.** Dorsal chaetotaxy of *Entomobrya neotenica* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

$m_{3e}$ , and  $m_5$  (Fig. 30E). Abd. 3 with 3 macrosetae:  $m_3$ ,  $pm_6$ , and  $p_6$  (Fig. 30F). Abd. 4 with 6 inner macrosetae (Fig. 30G). Mucronal teeth subequal; mucronal spine smooth.

**Remarks.** *Entomobrya neotenica* sp. n. can be diagnosed by the presence of two lateral dark triangular shaped or irregular spots on Abd. 3, only 3 setae in eye patch, and the absence of head mesoseta  $An'_0$  and Abd. 3 macroseta  $m_{3ep}$  (see Table 2 for ad-

ditional diagnostic characters). This species is included in the *E. ligata* complex (see remarks for *E. ligata*) and has a unique color pattern and chaetotaxy never before reported in the literature. *E. neotenica* sp. n. is exceptionally small compared to most Nearctic *Entomobrya*. In fact, most individuals were thought to be juvenile forms of *E. ligata* prior to the observation of the male genital plate. *Entomobrya neotenica* sp. n. is very similar to *E. ligata* and *E. unifasciata* sp. n., but can be separated by characters outlined in Table 6.

**Distribution.** Endemic to North America (Suppl. material 2: K)

### *Entomobrya nivalis* (Linnaeus), 1758

Figs 2, 31, 32, 39

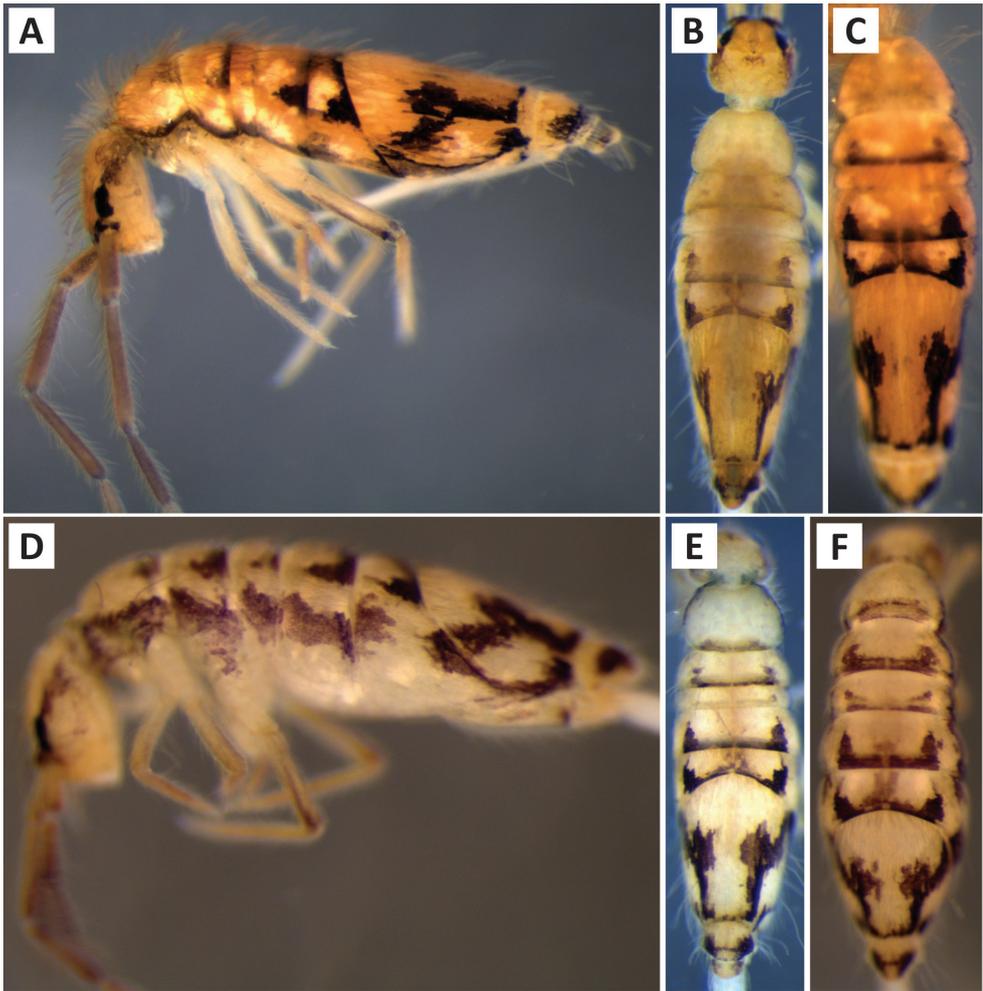
**Description.** *Body shape and color pattern.* Body cylindrical. One primary, but variable, color form (Fig. 31): yellow or white background with black, dark brown or purple pigment always forming thin transverse bands along the posterior margin of Th. 3, and Abd. 2 through Abd. 6. Additional transverse bands present or absent on Th. 2 and Abd. 1. Abd. 4 usually with U-shaped or “11”-shaped pattern connecting basally with band along posterior margin. Antennae usually lack dark pigmentation, sometimes with light brown or purple pigment, darkening near the apex.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually bi- or trilobed. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 3\times$  short setae. Ornamentation of the distal margin of the labral papillae with 3–4 small seta or spine-like projections per papilla (Fig. 2). Labial lateral appendage slightly curved, relatively thick, nearly reaching tip of papilla. Labial triangle chaetotaxy normal, one specimen with a supplemental ciliate seta internal to M1 (one side only). Dorsal head chaetotaxy (Fig. 32A) with macrosetae An'<sub>0</sub>, A<sub>3a2</sub>, A<sub>3a3</sub>, M<sub>3i</sub>, S'<sub>0</sub>, Ps<sub>3</sub>, Pi<sub>2</sub>, Pi<sub>3</sub>, Pm<sub>1i</sub>, and Pm<sub>2</sub> absent; S<sub>1</sub> usually present, A<sub>6</sub>, S<sub>6</sub>, Ps<sub>3</sub>, and Pa<sub>2</sub> sometimes present. Eyes G and H small and subequal. Eye patch with 5 or 6 setae.

*Legs.* Trochanteral organ with triangular setal pattern and up to 34 setae. Unguis with 4 internal teeth; basal teeth located approximately 60% of inner claw length. Unguiculus acuminate with small serrations on internal edge.

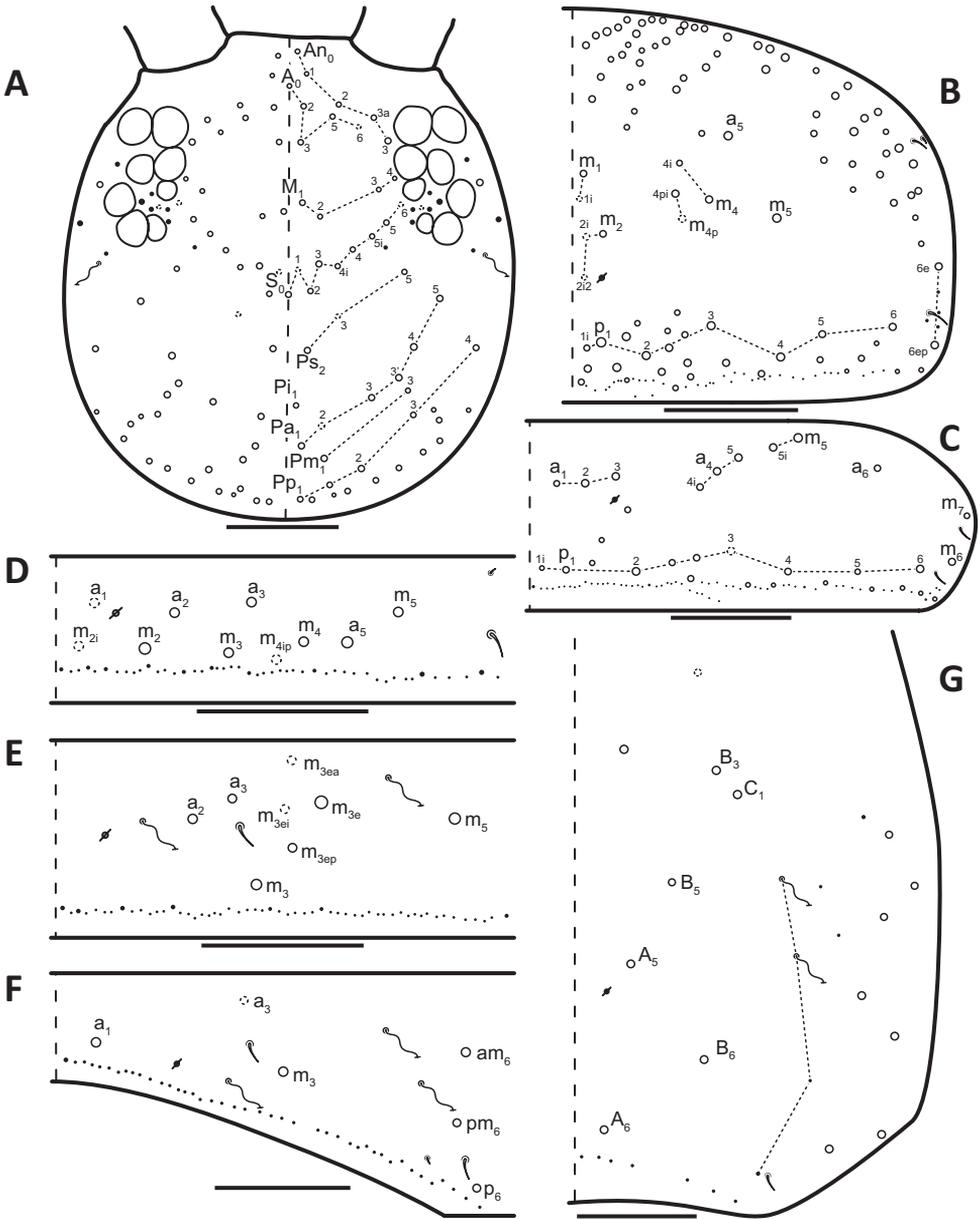
*Thorax.* Th. 2 macrosetae a<sub>5</sub>, m<sub>1</sub>, m<sub>2</sub>, m<sub>4</sub>, m<sub>4i</sub>, m<sub>4pi</sub>, and m<sub>5</sub> present (Fig. 32B); m<sub>2i</sub> and m<sub>2i2</sub> usually present; m<sub>1i</sub> and m<sub>4p</sub> sometimes present; all posterior macrosetae (series P) are present. Th. 3 macrosetae a<sub>1</sub>, a<sub>2</sub>, a<sub>3</sub>, a<sub>4</sub>, a<sub>4i</sub>, a<sub>5</sub>, a<sub>6</sub>, a<sub>7</sub>, m<sub>5</sub>, m<sub>5i</sub>, m<sub>6</sub>, and m<sub>7</sub> present (Fig. 32C); most posterior macrosetae (series P) present, p<sub>3</sub> present or absent. Both thoracic segments with a moderate number of supplemental macrosetae in zone Pm (Fig. 32B,C).

*Abdomen.* Abd. 1 with 7–10 macrosetae (Fig. 32D). Abd. 2 macroseta a<sub>2</sub>, a<sub>3</sub>, m<sub>3</sub>, m<sub>3e</sub>, m<sub>3ep</sub>, and m<sub>5</sub> present; m<sub>3ei</sub> and m<sub>3ea</sub> usually absent (Fig. 32E). Abd. 3 macroseta a<sub>1</sub>, m<sub>3</sub>, am<sub>6</sub>, pm<sub>6</sub>, and p<sub>6</sub> present (Fig. 32F); a<sub>3</sub> usually absent. Abd. 4 with 7–8 inner macrosetae (Fig. 32G). Mucronal teeth subequal.



**Figure 31.** Color pattern of *Entomobrya nivalis* collected from: **A** Lamoille Co., VT **B** Sauk Co., WI **C** Hancock Co., ME **D** Lamoille Co., VT **E** Hancock Co., ME **F** Sauk Co., WI.

**Remarks.** *Entomobrya nivalis* can be diagnosed by the presence of a U-shaped or “11” shaped pattern on Abd. 4 combined with the presence of macrosetae head  $S_{4i}$ , Th. 2  $m_5$ , Abd. 2  $m_{3ep}$ , and Abd. 3  $a_1$ , and the absence of Abd. 3  $a_2$  (see Table 2 for additional diagnostic characters). This species has a highly variable color pattern with many intermediate forms that intergrade with *E. atrocincta* females, *E. intermedia*, and *E. multifasciata*. However, *E. nivalis* can be separated from these species by chaetotaxy (Table 3) and, with careful consideration, color pattern; the presence of a U-shaped or “11” shaped pattern on Abd. 4 is unique to this species. Therefore, it is critical to evaluate chaetotaxy in addition to color pattern when making a species diagnosis.



**Figure 32.** Dorsal chaetotaxy of *Entomobrya nivalis*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

It is important to note that the large genetic distances between presumably conspecific individuals (Katz et al. 2015; Feng Zhang, personal communication), differences in chaetotaxy between populations in North America and Europe (Jordana 2012), and

variable color pattern among populations across its world-wide distribution, suggests that *E. nivalis* likely represents a cryptic species complex.

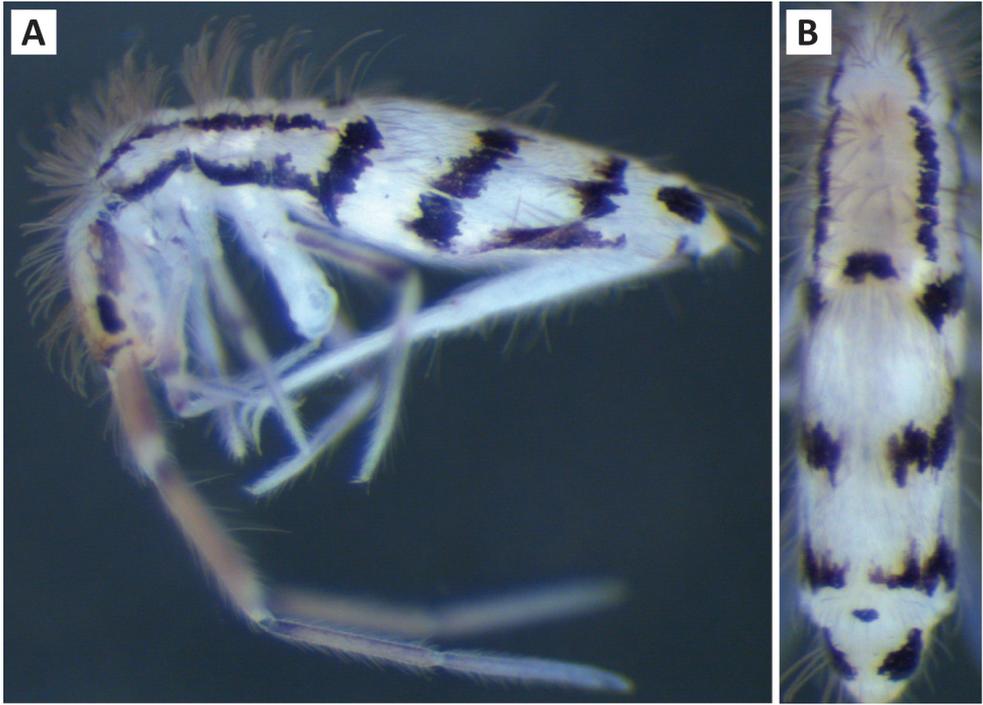
**Distribution.** North America and Europe. Records of *E. nivalis* in North America are suspect if diagnosed without considering chaetotaxy given the superficial similarities in color form expressed by *E. atrocincta* females (See Fig. 9G,H). See Suppl. material 2: L for a distribution map and below for a list of material examined with collection and locality information.

**Material examined.** USA: 2 on slides, 50+ in vial, Maine, Hancock Co., Acadia National Park (44.353823,-68.224754), moss, veg. sweep (blueberry, juniper, populus), 17.viii.2011 (E. C. Bernard), #2009-37; 3 on slides, 5 in vial, Pennsylvania, Allegheny Co., Allegheny National Forest, Dewdrop campground (41.83092,-7895937), leaf litter, 8.vii.2008 (S. M. Shreve); 1 on slide, Vermont, Chittenden Co., Red Rock, Locality I (44.44493,-73.23040), leaf litter, 6.vi.2011 (J. Fisher); 2 on slides, Vermont, Chittenden Co., Farrell Park, Locality II (44.44454,-73.20178), leaf litter, 13.vi.2001 (J. Fisher); 1 on slide, Vermont, Chittenden Co., Farrell Park, Locality II (44.44454,-73.20178), leaf litter, 4.vii.2001 (J. Fisher); 2 on slides, Vermont, Chittenden Co., Farrell Park, Locality II (44.44454,-73.20178), leaf litter, 26.vii.2001 (J. Fisher); 1 on slide, Vermont, Lamoille Co., Stowe (44.48377,-72.69859), leaf litter, 24.vii.2001 (J. Fisher); 1 on slide, Vermont, Lamoille Co. (44.54858,-72.79393), DNA ID#: 12-FSVTlam-ni-1; 1 on slide, Vermont, Washington Co., Locality II, Barre (44.19968,-72.50135), leaf litter, 8.vi.2001 (J. Fisher); 2 on slides, Vermont, Washington Co., Locality II, Barre (44.19968,-72.50135), leaf litter, 24.vii.2001 (J. Fisher); 2 on slides, 6 in vial, Vermont, Rutland Co., Green Mountain National Forest, Greendale Recreation Area (43.35112,-72.82225), leaf litter, 10.vii.2008 (S. M. Shreve); 1 on slide, 6 in vial, Wisconsin, Dodge Co., Horicon Marsh National Wildlife Refuge, end of Dike Rd (43.52736,-88.64381), 12.vi.2011, AK11-47; 2 on slides, 7 in vial, Wisconsin, Sauk Co., Devil's Lake State Park, 0.5mi down Steinke Basin Loop trail (43.4255,-89.71039), 12.vi.2011, AK11-50.

### *Entomobrya quadrilineata* Bueker, 1939

Figs 2, 13C, 33, 34, 39

**Description.** *Body shape and color pattern.* Body very elongate and cylindrical. Color pattern monomorphic (Fig. 33); white or yellow background with black or dark blue or purple pigment forming two dark parallel longitudinal stripes extending from anterior margin of Th. 2 through posterior margin of Abd. 2. Dark pigment present along lateral margins of Th. 2 through Abd. 2, forming 2 additional lateral longitudinal bands. Abd. 3 and Abd. 4. with 2 angled bands. A small patch of dark pigment sometimes occurs medially on Abd. 3. An irregular and (and sometimes incomplete) transverse band present along posterior margin of Abd. 4. Abd. 5 with 2 small lateral patches of pigment sometimes forming 2 triangles. Abd. 6 usually pale, without dark pigment. Apex of femora usually with a patch of dark pigmentation. Antennae usually

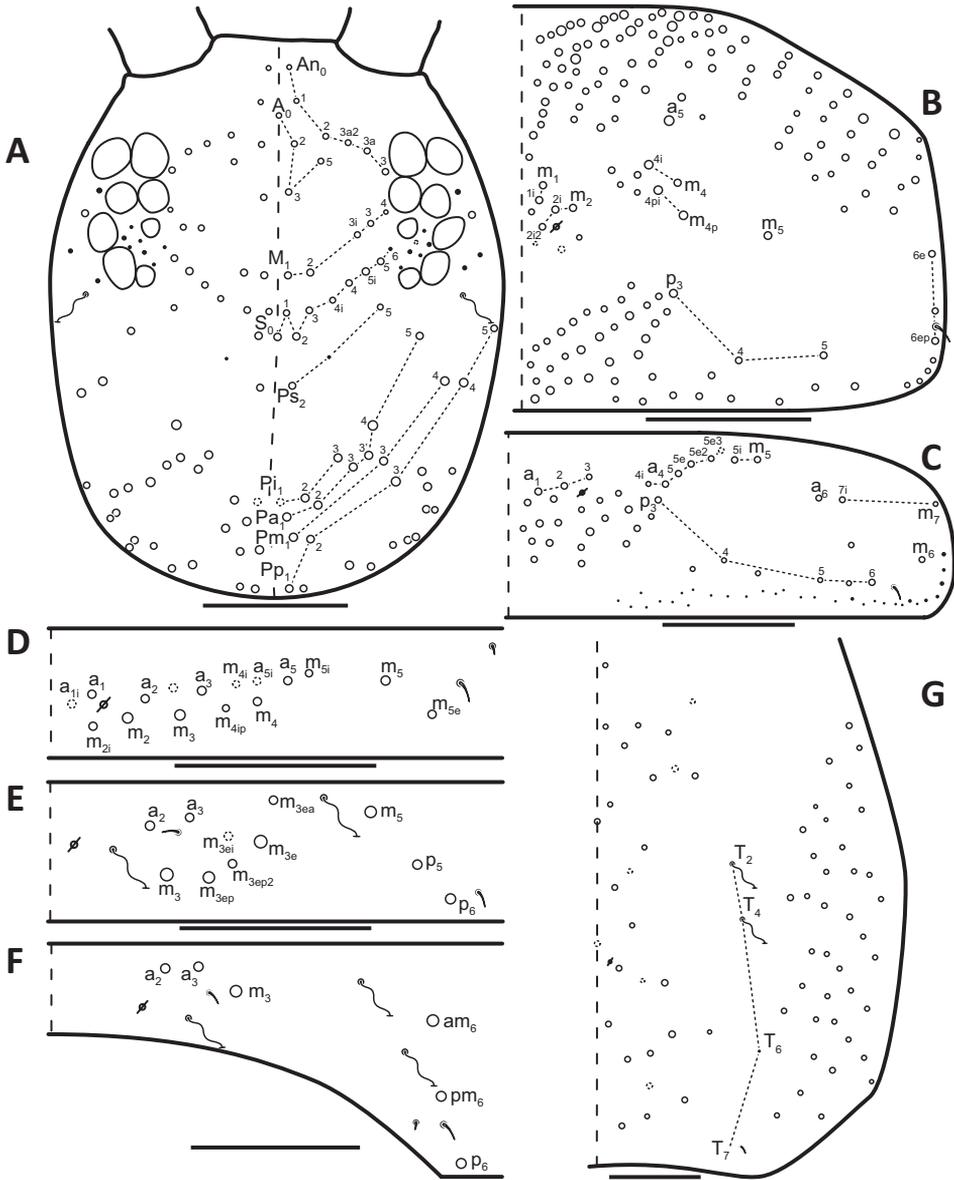


**Figure 33.** Color pattern of *Entomobrya quadrilineata*. Lateral (A) and dorsal (B) views of specimen collected from Stewart Co., TN.

with some light brown or purple pigment, darkening near apex. Longitudinal bands usually present in juveniles.

**Head.** Apical bulb of 4<sup>th</sup> antennal segment usually bilobed. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 4\times$  short setae. Four prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E almost twice as long as papilla. Labial triangle chaetotaxy slightly irregular and atypical: M1, r, E, L1, L2, all ciliate; r significantly smaller than other setae; a supplementary ciliate seta sometimes present internal to M1, and relatively difficult to observe; A1-A5 smooth. Dorsal head chaetotaxy (Fig. 34A) with macrosetae An'<sub>0</sub>, An<sub>3a3</sub>, A<sub>6</sub>, S'<sub>0</sub>, S<sub>6</sub>, Ps<sub>3</sub>, and Pm<sub>1i</sub> absent; Pi<sub>1</sub> sometimes present. Eyes G and H small and subequal. Eye patch with 5 or 6 setae.

**Thorax.** Thoracic chaetotaxy greatly developed, with high levels of variation and many supplemental macrosetae. Th. 2 zone A enlarged and sometimes merging with medial macrosetae forming a single, large patch of setae (Fig. 34B). Th. 3 macrosetae m<sub>4</sub>, m<sub>5p</sub>, a<sub>6i</sub>, and a<sub>7</sub> absent (Fig. 34C). Both thoracic segments with zone Pm enlarged, with many supplemental macrosetae present forming wing-like patches and extending near anterior row (Fig. 34B,C). Th. 3 pseudopore displaced anteriorly, very close to macrosetae a<sub>1</sub>, a<sub>2</sub>, and a<sub>3</sub>.



**Figure 34.** Dorsal chaetotaxy of *Entomobrya quadrilineata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

*Legs.* Trochanteral organ with rectangular setal pattern and up to 41 setae.

*Abdomen.* Abdominal chaetotaxy highly developed. Row of microsetae along posterior margin present in all segments (not displayed in figure). Abd. 1 with 12-16 macrosetae (Fig. 34D). Abd. 2 macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3e}$ ,  $m_{3ep}$ ,  $m_{3ep2}$ ,  $m_{3ea}$ ,  $m_5$ ,  $p_5$ , and

$p_6$  present (Fig. 34E);  $m_{3ei}$  usually present. Abd. 3 macrosetae  $a_2$ ,  $a_3$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$  present (Fig. 34F);  $a_1$  absent. Abd. 4 elongate, with at least 25 macrosetae internal to bothriotricha  $T_2$  and  $T_4$  (Fig. 34G), but number of macrosetae extremely variable between individuals and even within single individuals (Fig. 16C). Pseudopores on Abd. 4 with asymmetric relative insertions with respect to macroseta and bothriotricha (Fig. 13C). Mucronal teeth subequal

**Remarks.** *Entomobrya quadrilineata* can be identified by the presence of 2 parallel longitudinal thoracic stripes combined with the presence of head macrosetae  $M_{3i}$  and  $ps_5$  and the absence of Abd. 3 macroseta  $a_1$  (see Table 2 for additional diagnostic characters). This species is part of the *E. bicolor* complex. Many supplemental macrosetae and extreme setal variation make it difficult to separate species in this group using chaetotaxy and other traditional morphological characters. However, there are clear differences in color pattern which can be used to differentiate *E. quadrilineata* from *E. decemfasciata* and *E. bicolor* (Table 5); *E. quadrilineata* has a relatively stable and easily distinguishable color pattern and can be diagnosed by the presence of two parallel longitudinal stripes extending from the anterior margin of Th. 2 through the posterior margin of Abd. 2 (Fig. 33). See remarks for *E. decemfasciata* for more information regarding species diagnosis and delimitation. Also, see Suppl. material 3: A,B for photographs of the type specimen.

**Distribution.** Endemic to North America (Suppl. material 2: M). Many records may be misidentifications due to similarity in color pattern and chaetotaxy with *E. decemfasciata*. Records of *E. quadrilineata* without thoracic stripes are most likely *E. decemfasciata*.

**Material examined.** USA: *Neo-holotype* (Christiansen 1951), 1 on slide, Fountain Bluff, Ill. 5-15-32, Coll. Ross + Mohr, INHS Cat. No. 529,188; *Neo-paratype* (Christiansen 1951), 1 on slide, Fountain Bluff, Ill. 5-15-32, Coll. Ross + Mohr, INHS Cat. No. 529,189; *Neo-paratype* (Christiansen 1951), 1 in vial, Fountain Bluff, Ill. 5-15-32, Coll. Ross + Mohr; 4 on slides, 13 in vial, Illinois, Monroe Co., Kidd Lake Marsh State Natural Area (37.97211,-89.80135), leaf litter #42, 29.ix.2009 (S. Taylor & F. Soto-Adames), sjt09-114; 1 in vial, Illinois, Pope Co., Bell Smith Springs (37.51882,-88.65782), bare sandstone bedrock above canyon in mosses and lichens, 28.viii.2011 (J. Cech); 1 on slide, Illinois, Vermilion Co., Kennekuk Cove County Park, Windfall Prairie Nature Preserve (40.20995,-87.74181), vacuum hill prairie, 16.vi.2011, AK11-59a; 1 in vial, Illinois, Wayne Co., County Road 580N (38.34122,-88.23992), leaf litter from north side of road, 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-61; 4 in vial, Illinois, Wayne Co. (38.32500,-88.25016), 15.vii.2011 (A. Katz & F. Soto-Adames), AK11-63; 1 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, .25mi down Neville Bay Rd. (36.60757,-87.93457), leaf litter on forest floor, 31.v.2011, AK11-44; 1 on slide, 2 in vial, Tennessee, Stewart Co., Land Between the Lakes National Recreation Area, Fox Ridge Rd (36.66392,-87.98596), leaf litter, 7.viii.2011, AK11-105.

***Entomobrya unifasciata* Katz & Soto-Adames, sp. n.**

<http://zoobank.org/A68AD8F0-1545-471C-812D-2F066A08858F>

Figs 2, 16D, 35, 36, 39

**Etymology.** From the Latin words *uno* and *fasciatus*, which translates to “one band”. This species has only one band found along the posterior margin of the metathorax, a character that distinguishes it from *E. ligata*, which has two bands; one along each posterior margin of the meta- and mesothorax.

**Type material.** *Holotype*, ♂, USA: Kentucky, Laurel County, Levi Jackson State Park (37.08247,-84.04528), leaf litter collected at night, 28.v.2011, AK11-37.

*Paratypes*, USA: 2 on slides, 1 in vial, Georgia, Union Co., Brasstown Bald Rd., tiny water trickle near road surrounded by dryish leaves (34.86040,-83.80193), leaf litter, 26.v.2011 (E. C. Bernard) #2011-28; 1 on slide, Kentucky, Laurel Co., Levi Jackson State Park (37.08247,-84.04528), leaf litter collected at night, 28.v.2011, AK11-37; 1 on slide, 2 in vial, North Carolina, Henderson Co., Blue Ridge Parkway, Mill River Overlook (35.4482,-82.71963), under bark on logs, 4.vi.2007 (E. C. Bernard), 07031EB; 10 in vial, North Carolina, Swain Co., Great Smoky Mountains National Park, Balsom Mountain, Heintooga Ridge Rd. (35.57030,-83.16917), leaf litter along road, 29.v.2011, AK11-38; 1 on slide, 35 in vial, North Carolina, Swain Co., Great Smoky Mountains National Park, Balsom Mountain, Heintooga Ridge Rd. (35.57030,-83.16917), under bark, 29.v.2011, AK11-39; 11 in vial, North Carolina, Swain Co., Great Smoky Mountains National Park, Balsom Mountain, Heintooga Ridge Rd. (35.57030,-83.16917), leaf litter by river, 29.v.2011, AK11-40; 1 on slide, 1 in vial, Tennessee, Sevier Co., Great Smoky Mountains National Park, 1 mi down greenbrier Rd. (35.72640,-83.40173), leaf litter by stream, 30.v.2011, AK11-41; 1 in vial, Tennessee, Sevier Co., Great Smoky Mountains National Park, 1 mi down greenbrier Rd. (35.72640,-83.40173), leaf litter stuck in nook of tree, 30.v.2011, AK11-42.

**Description.** *Body shape and color pattern.* Body oval and cylindrical. Length up to 1.85 mm. Color pattern stable (Fig. 35), always with 3 transverse bands, 1 thin regular band along posterior margin of Th. 3, an irregular, patchy, thick band covering most of Abd. 3, and a highly irregular and sometimes broken band across medial section of Abd. 4. A small patch of pigment covers Abd. 5 and 6. Patterns usually consisting of black or dark blue pigment on a yellow background. Dark pigment usually occurring in patches along lateral margins of Th. 2 through Abd. 4. Small, rectangular black patches may occur in pairs on posterior margin of Th. 2, Abd. 1, and Abd. 2. A faint transverse band sometimes on posterior margin of Th. 2, but if present, always much lower in opacity compared to transverse band along posterior margin of Th. 3. Antennae with purple pigment, darker near apex. Legs usually white, with small purple patches on apical end of femora. A medial ring of purple pigment also occurs on tibiotarsus of hind legs.

*Head.* Apical bulb of 4<sup>th</sup> antennal segment usually bilobed, sometimes simple. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 3\times$  short setae. Four prelabral setae finely ciliate, seemingly smooth at low magnification under light micros-



**Figure 35.** Color pattern of *Entomobrya unifasciata* sp. n. collected from: **A** Sevier Co., TN **B** Union Co., GA **C** Laurel Co., KY.

copy. Ornamentation of the distal margin of the labral papillae with single seta or spine-like projection (Fig. 2). Lateral appendage of labial papilla E short, extending only  $\frac{3}{4}$  papilla length. Dorsal head chaetotaxy (Fig. 36A) with macrosetae  $A_6$ ,  $M_{3i}$ ,  $S_6$ ,  $Ps_3$ ,  $Pi_1$ ,  $Pm_{1i}$ , and  $Pp_2$ , absent;  $S'_0$  usually absent, but observed in 2 individuals;  $An'_0$ , a short mesoseta present medially between both  $An_0$ . Eyes G and H small and subequal. Eye patch with 3 setae.

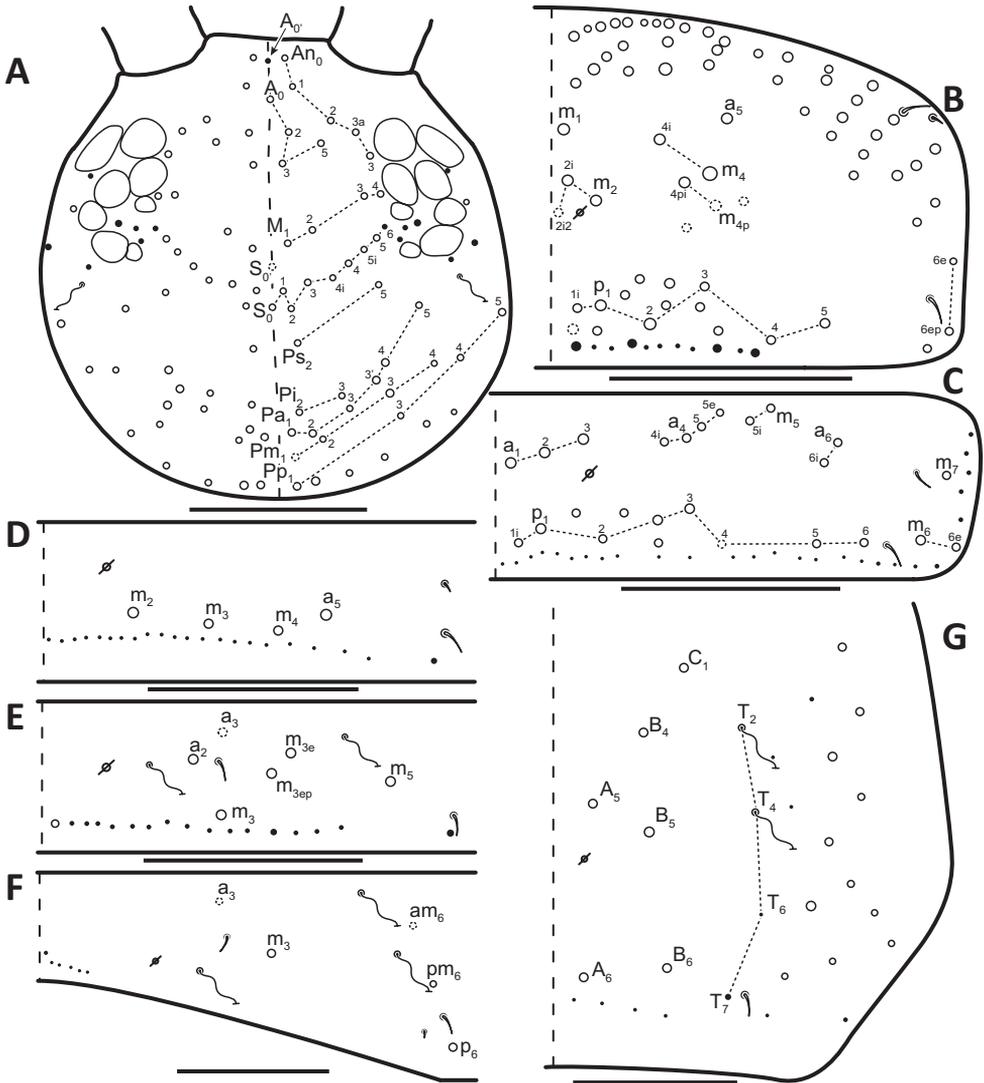
**Thorax.** Th. 2 macrosetae  $a_5$ ,  $m_1$ ,  $m_2$ ,  $m_4$ ,  $m_{4pi}$ , and  $m_{4i}$  present (Fig. 36B). Macrosetae  $m_{2i2}$ ,  $m_{4p}$ , and  $m_5$  sometimes present; 2 additional macrosetae sometimes present on either side of  $m_{4p}$ . Th. 3 macrosetae  $a_1$ ,  $a_2$ ,  $a_3$ ,  $a_4$ ,  $a_{4i}$ ,  $a_5$ ,  $a_{5e}$ ,  $a_6$ ,  $a_{6i}$ ,  $m_5$ ,  $m_{5i}$ ,  $m_6$ ,  $m_{6e}$ , and  $m_7$  present (Fig. 36C); most posterior (series P) macrosetae present,  $p_4$  present or absent. Both thoracic segments with moderate number of supplemental macrosetae on zone Pm (Fig. 36B,C).

**Legs.** Trochanteral organ with triangular setal pattern and up to 22 setae. Unguis with 4 internal teeth; basal teeth located approximately middle of inner claw length (Fig. 16D). Unguiculus acuminate with small serrations on internal edge.

**Abdomen.** Abdominal chaetotaxy reduced and slightly variable. Abd. 1 with 4 macrosetae:  $a_5$ ,  $m_2$ ,  $m_3$ , and  $m_4$  (Fig. 36D). Abd. 2 with 5 macrosetae:  $a_2$ ,  $m_3$ ,  $m_{3e}$ ,  $m_{3ep}$ , and  $m_5$  (Fig. 36E);  $a_3$  usually absent. Abd. 3 with 3 macrosetae:  $m_3$ ,  $pm_6$ , and  $p_6$  (Fig. 36F);  $a_3$  and  $am_6$  usually absent. Abd. 4 with 6 inner macrosetae (Fig. 36G).

**Remarks.** *Entomobrya unifasciata* sp. n. can be diagnosed by the presence of only three dark transverse bands (no band across the posterior margin of Th. 2), presence of head mesoseta  $An'_0$ , 4 macrosetae on Ab. 1, and 3 eye patch setae (see Table 2 for additional diagnostic characters). This species is part of the *E. ligata* complex (see remarks for *E. ligata*) and is very similar to *E. ligata* and *E. neotenica* sp. n., but can be separated by characters outlined in Table 6. Though identical in chaetotaxy, molecular evidence (Katz et al. 2015) and the absence of a dark transverse band on the posterior margin of Th. 2 separate this species from *E. ligata*.

**Distribution.** Endemic to North America (Suppl. material 2: N). Many records of *E. ligata*, especially those collected from the Smokey Mountain region west to the Mississippi River, are likely to be *E. unifasciata* sp. n.



**Figure 36.** Dorsal chaetotaxy of *Entomobrya unifasciata* sp. n.: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100 µm. See Figure 5 for symbol legend.

***Entomobrya unostrigata* Stach, 1930**

Figs 2, 3C, 37, 38, 39

**Description.** *Body shape and color pattern.* Body relatively robust and cylindrical. Color form largely monomorphic (Fig. 37): white, pale green or yellow background with purple or black pigment forming a thin medial longitudinal stripe from anterior margin of Th. 2 to posterior margin of Abd. 5; band ostensibly thicker on Th. 3 through Abd. 3. Patches of pigment forming a lateral line along margins of Th. 2



**Figure 37.** Color patterns of *Entombrya unostrigata*. Lateral (A) and dorsal (B) views of specimen collected from Chittenden Co., VT.

through Abd. 5, with variable spots of pigment present throughout. Abd. 5 and Abd. 6 usually lack dark pigment. Legs and furcula white and/or with light purple pigment. Antennae with light purple pigment.

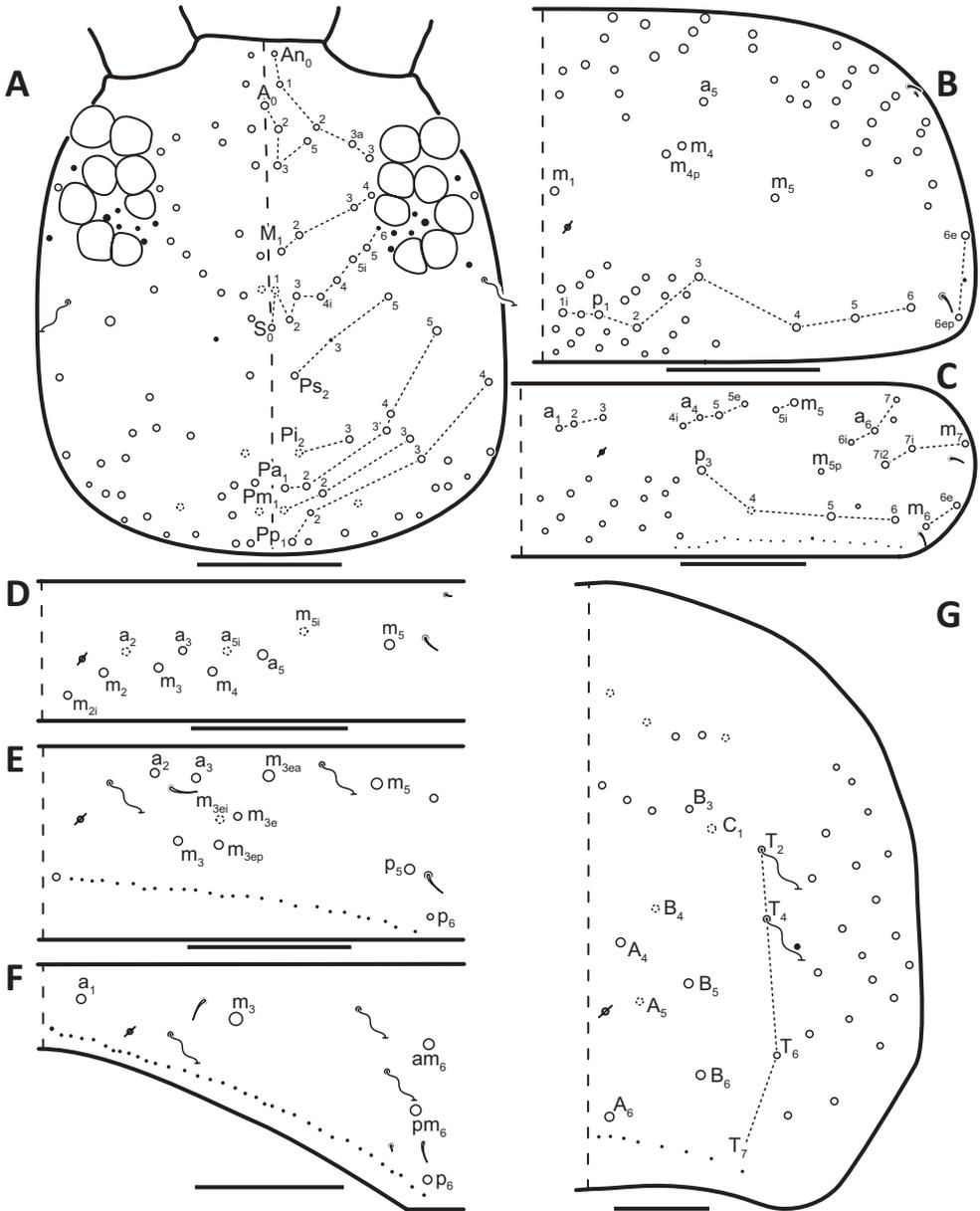
**Head.** Apical bulb of 4<sup>th</sup> antennal segment usually bilobed. Long differentiated smooth setae on ventral side of 1<sup>st</sup> antennal segment  $\approx 3\times$  short setae. Four prelabral setae ciliate. Ornamentation of the distal margin of the labral papillae with 2 seta or spine-like projections (Fig. 2). Lateral appendage of labial papilla E extending just past tip of papilla. Dorsal head chaetotaxy (Fig. 38A) with macrosetae  $An'_0$ ,  $A_{3a2}$ ,  $A_{3a3}$ ,  $A_6$ ,  $M_{3i}$ ,  $S'_0$ ,  $S_6$ ,  $Ps_3$ ,  $Pi_1$ ,  $Pa_3$ , and  $Pm_{1i}$  absent;  $S_1$ ,  $Pi_2$ , and  $Pm_1$  usually present. Eyes G and H enlarged and similar in size to eyes C-F. Eye patch with 5 setae.

**Thorax.** Thoracic chaetotaxy well-developed and relatively stable. Th. 2 macrosetae  $a_5$ ,  $m_1$ ,  $m_4$ ,  $m_{4p}$ , and  $m_5$  present (Fig. 38B); posterior macrosetae (series P) present. Th. 3 macrosetae  $a_{5e2}$ ,  $a_{5e3}$ ,  $m_4$ ,  $m_{5p}$ , and  $a_7$  absent (Fig. 38C);  $P_4$  usually present. Both thoracic segments with many supplemental macrosetae on zone Pm (Figs 3C; 38B,C).

**Legs.** Trochanteral organ with triangular setal pattern and up to 21 setae.

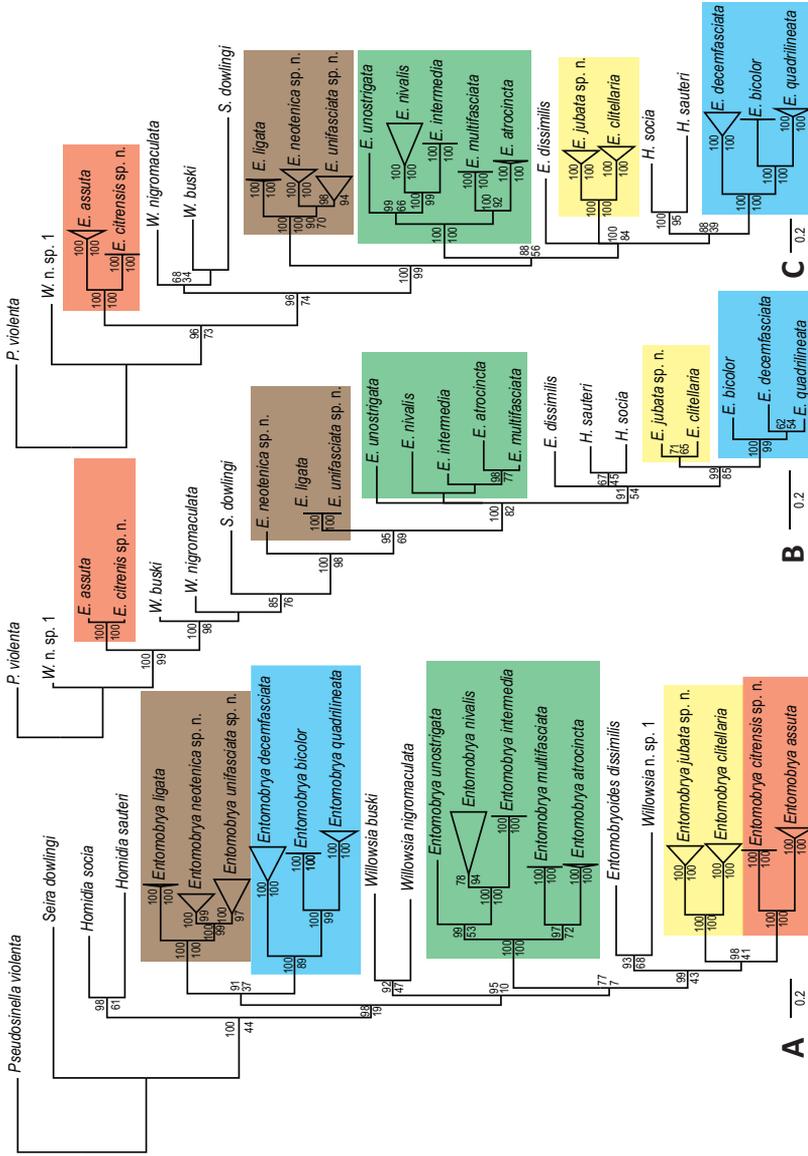
**Abdomen.** Abdominal chaetotaxy highly developed and variable. Abd. 1 with 7-10 macrosetae (Fig. 38D). Abd. 2 macroseta  $a_2$ ,  $a_3$ ,  $m_3$ ,  $m_{3ep}$ ,  $m_{3e}$ ,  $m_{3ea}$ , and  $m_5$  present (Fig. 38E);  $m_{3ei}$  usually present. Abd. 3 macroseta  $a_1$ ,  $m_3$ ,  $am_6$ ,  $pm_6$ , and  $p_6$  present (Fig. 38F). Abd. 4 with 10-16 macrosetae present interior to bothriotricha  $T_2$  and  $T_4$  (Fig. 38G). Anterior half of Abd. 4 usually with 1 or 2 conspicuous rows of macrosetae. Apical mucronal tooth enlarged.

**Remarks.** *Entombrya unostrigata* can be easily diagnosed by color pattern, a thin medial longitudinal stripe from the anterior margin of Th. 2 to the posterior margin of Abd. 5, combined with the absence of macrosetae Th. 2  $m_2$  and Abd. 3  $a_2$  and the presence of macrosetae  $m_5$  on Th. 2 and  $a_1$  on Abd. 3 (see Table 2 for additional diagnostic



**Figure 38.** Dorsal chaetotaxy of *Entomobrya unostriolata*: **A** Head **B** Mesothorax **C** Metathorax **D** 1<sup>st</sup> abdominal segment **E** 2<sup>nd</sup> abdominal segment **F** 3<sup>rd</sup> abdominal segment **G** 4<sup>th</sup> abdominal segment. Scale bars = 100  $\mu$ m. See Figure 5 for symbol legend.

characters). This species exhibits atypical morphology for a Nearctic *Entomobrya*. Eyes G and H are greatly enlarged and similar in size to eyes C-F, an uncommon trait unobserved in other Nearctic *Entomobrya*. Additionally, macrosetae usually form 2 rows on the anterior half of Abd. 4; a pattern shared with many Palearctic *Entomobrya* and



**Figure 39.** Bayesian 50% majority rules consensus trees with *Pseudosinella violenta* as the outgroup: **A** phylogeny resulting from the analysis of cytochrome *c* oxidase I DNA sequences (complete gene, 1539 bp) from Katz et al. (2015) **B** phylogeny resulting from the analysis of 179 morphological characters **C** combined phylogeny (COI and morphology). *Entomobrya* species groups are highlighted with colored boxes. Branches of representing taxa from the same species were collapsed into triangles (triangle length represents branch lengths between collapsed branches) for simplicity. Branch labels include posterior probabilities (above) and maximum likelihood bootstrap support (below). Scale bars represent 0.2 base substitutions.

also with members in the genus *Homidia*, but not with other *Entomobrya* treated here. *Entomobrya unostrigata* is a recently introduced species, now with a widespread Nearctic distribution (Christiansen and Bellinger 1998), which may explain its distinction from other North American *Entomobrya* species included in the present study. The *E. unostrigata* specimens observed for this study have only two large seta or spine-like projections on each labral papillae (Fig. 2). However, multiple variations of the labral papillae have been reported (Christiansen 1958b; Christiansen and Bellinger 1992; Christiansen and Bellinger 1998; Jordana 2012).

**Distribution.** Nearctic, Palearctic, and Australia (Suppl. material 2: O).

**Material examined.** USA: 2 on slides, Vermont, Chittenden Co., South Burlington, Vegetable garden on south side of Swift St. at intersection with Spear St. (44.4433,-73.1893), 3.viii.2003 (F. Soto-Adames); 1 on slide, Wisconsin, Dodge Co., Horicon Marsh National Wildlife Refuge, end of Dike Rd. (43.52736,-88.64381), 12.vi.2011, AK11-47.

### Morphological phylogeny

Bayesian analysis of 179 morphological characters from 23 taxa produced a single consensus tree with high support (Fig. 39B). The most likely tree inferred by the maximum likelihood analysis is congruent with the Bayesian tree and bootstrap values were added to the Bayesian consensus tree (Fig. 39B). When *Pseudosinella violenta* is designated as the outgroup, the topology of the tree assumes a ladder-like evolutionary progression that is associated with an increase in the number of dorsal macrosetae. *Entomobrya assuta* and *E. citrensis* sp. n., the two species with the smallest number of macrosetae, form a monophyletic group at the base of the tree, whereas *Entomobrya bicolor*, *E. decemfasciata*, and *E. quadrilineata*, which are characterized by an abundance of dorsal macrosetae, form the most derived monophyletic group. This tree indicates that *Entomobrya* is paraphyletic, as the genera *Seira*, *Homidia*, *Entomobryoides*, and most representatives of *Willowsia* are interspersed among clades of *Entomobrya* species. The two species in the genus *Homidia* form a monophyletic group, but the genus *Willowsia* is not monophyletic. Other clades identified by the molecular COI analysis in Katz et al. (2015) (Fig. 39A) were not resolved as monophyletic groups based on morphological characters.

### Combined phylogeny (COI and morphology)

Bayesian analysis based on combined morphology and COI (Katz et al. 2015) datasets yielded a highly supported tree (Fig. 39C). The most likely tree inferred by the maximum likelihood analysis is congruent with the Bayesian tree and bootstrap values were added to the Bayesian consensus tree (Fig. 39C). The addition of molecular characters resolves all monophyletic species groups/clades of *Entomobrya* observed in

the COI only tree (Fig. 39A). However, the deeper relationships among clades follow the progressive evolution towards an increased number of macrosetae supported by the morphological analysis. Both analyses (morphology only and combined) indicate that *Entomobrya* is paraphyletic.

## Discussion

### Diagnostic and phylogenetic utility of chaetotaxy

Detailed examination of the adult chaetotaxy of 15 species of North American *Entomobrya* suggests that the exclusive use of chaetotaxy for species diagnosis and as phylogenetic characters can potentially cause serious confusion. This study uncovered high levels of previously undocumented intraspecific variation and asymmetry of dorsal macrosetae (Fig. 13), and has made it clear that examination of many individuals is critical to properly identify variable chaetotaxy in order to choose appropriate characters for species delimitation. For example, Christiansen and Bellinger (1998) separate many species based on the chaetotaxy of Abd. 2 and Abd. 3, but in some species groups both regions contain significant intraspecific variation. In the Collembola of North America (Christiansen and Bellinger 1998), species within the *nivalis* group (*E. nivalis*, *E. atrocincta*, *E. multifasciata*, and *E. intermedia*) are separated by a combination of color pattern and by the presence or absence of four macrosetae:  $m_{3ep}$  on Abd. 2 and  $a_1$ ,  $a_2$ , and  $a_3$  on Abd. 3. This study shows that these characters must be used in combination to provide sufficient separation of these species due to overlapping intergrades of color forms and the presence macrosetae polymorphisms. Species in the *bicolor* group (*E. bicolor*, *E. decemfasciata*, and *E. quadrilineata*) present the most obvious example of the failure of chaetotaxy to provide an adequate means for species separation. Large numbers of macrosetae, extreme levels of inter-individual variation, and the common occurrence of asymmetries, cause significant overlap and obscure the homology of macrosetae otherwise considered important in species separation. The present study shows that color pattern is absolutely critical for the identification of species within this group.

These problems are cause for concern considering the important role of dorsal chaetotaxy in diagnosis and delimitation of species in the family Entomobryidae. Many studies are based on chaetotaxy as primary (or sole) evidence for species separation, following Szeptycki's (1979) nomenclatural system (Chen and Christiansen 1993; Jordana 2012). The homology of macrosetae as defined by Szeptycki (1979) assumes that a fixed number of macrosetae occur in strict, predefined positions rather than randomly distributed within a given area. Homologies are easier to determine between species in genera characterized by small numbers of macrosetae, and chaetotaxy likely provides accurate phylogenetic estimation in these groups. However, in groups such as *Entomobrya*, intraspecific variation, differences in setae arrangements, asymmetries, and large numbers of supplemental setae, render homology assessment a subjective and arbitrary process (Potapov and Kremenista 2008). Incorrect homology assignments obscure any useful phylogenetic information provided by chaetotaxy.

Post-embryonic studies that test Szeptycki's (1979) hypotheses have been successful in identifying and refining setae homologies in some pluri- and polychaetotic species and groups (Soto-Adames 2008; Zhang et al. 2011), but these studies are laborious and are only conclusive for species and/or groups examined. Information regarding the post-embryonic development of *Entomobrya* chaetotaxy is very limited and mainly concerned with Palearctic species (Szeptycki 1979). Explicit hypotheses concerning the underlying molecular mechanisms governing macrosetae development and position, to our knowledge, have only been tested in Diptera. These mechanisms are generally very complex and highly regulated processes, controlled by multiple genes that ensure macrosetae do occur in strictly defined locations (Simpson 1990; Heitzler et al. 1996; Leyns et al. 1996; Simpson et al. 1999; Furman and Bukharina 2008). However, it is uncertain (and unlikely) that Collembola macrosetae, and/or the genes that regulate them, are homologous to those of Diptera.

The phylogenies based on morphological and molecular data illustrate the effects that chaetotaxy has on phylogeny estimation. The trend of progressive evolution towards an increased number of macrosetae presented in the morphology and combined phylogenies is suspicious and may be driven by outgroup choice and character coding strategy. *Entomobrya assuta* and *Entomobrya citrensis* sp. n., sister species characterized by reduced chaetotaxy, are basal to all other *Entomobrya*, together with all scaled species included in the analysis (*Pseudosinella*, *Willowsia*, and *Seira*), which also have few macrosetae. This association is not reflected in the analysis of COI alone (Katz et al. 2015; Fig. 39A) and is likely due to the accumulation of characters (each seta in a multiplet was defined and scored as a separate character, see Suppl. material 1) present in polychaetotic species but absent in the *E. assuta* group and the outgroup. Evaluation of coding strategies for chaetotaxy, and the effect of outgroup choice is clearly needed to exact the maximum amount of phylogenetic information while minimizing homoplasy. Despite the conflict in branching pattern for deep nodes estimated by morphology and COI, the combined analysis retains the species composition and relationship of the five species groups identified by the COI analysis. These relationships are quite obvious and were proposed by Christiansen (1958b) before modern sequencing and dorsal chaetotaxy systems were available.

### Phylogenetic relationships within Entomobryini and *Willowsia*

The result of the combined phylogenetic analysis (COI and morphology) of 15 species of North American *Entomobrya* in addition to 6 species representing 3 closely related genera (*Entomobryoides*, *Homidia* and *Willowsia*) and 2 outgroup species (*P. violenta* and *S. dowlingi*), generated a highly supported phylogeny of the North American Entomobryini and *Willowsia*. Several interesting results were observed concerning relationships among some currently recognized *Entomobrya* species. *Entomobrya nivialis*, *E. intermedia*, *E. multifasciata*, and *E. atrocincta* were resolved as a monophyletic clade; all are closely related but distinct species diagnosable using morphology and COI sequences. Overlapping intraspecific variation in color pattern and chaetotaxy

has caused many to consider these species synonymous (See Christiansen 1958b and Jordana 2012 for revision history). This study has, for the first time, combined substantial molecular (Katz et al. 2015) and morphological evidence in support of their separation. It is also worth noting that these were the only *Entomobrya* species included in the analysis with Holarctic distribution, all commonly reported throughout North America (Christiansen and Bellinger 1998) and Europe (Jordana 2012). The monophyly of the group and separation from species endemic to North America suggest a common origin in Europe or Palearctic region. It is possible that the group is either part of a shared, relictual Laurasian fauna or that the species were introduced from Europe in historical times. Evaluation of these two hypotheses will require molecular analysis of extensive samples of North American and European populations.

The resulting phylogeny also raises questions regarding the generic relationships within the tribe Entomobryini (here represented by the genera *Entomobrya*, *Entomobryoides* and *Homidia*) and *Willowsia*, a closely related genus. Research concerning the systematics of these groups is limited, and most phylogenetic studies have focused on suprageneric relationships by utilizing morphology, allozymes, and/or ribosomal markers that provided limited resolution and support of generic relationships (Lee and Park 1991; Lee et al. 1995; D’Haese 2002; Xiong et al. 2008). However, more recent work by Zhang et al. (2014b) indicated the non-monophyly of Entomobryini and Willowsiini based on molecular phylogeny. Zhang et al. (2014a) also questioned the monophyly of Willowsiini. Further analyses of S-chaetae (dorsal sensilla) dismissed Willowsiini as monophyletic (Zhang and Deharveng 2015).

This study supports Zhang et al.’s (2014a, 2014b) and Zhang and Deharveng’s (2015) findings regarding the non-monophyly of Entomobryini and Willowsiini. Our phylogenetic analysis places both *Willowsia nigromaculata* (Lubbock) and *W. buski* (Lubbock) within the *Entomobrya* clade, further supporting the paraphyly of *Entomobrya*. This is not entirely unexpected considering the means by which these genera are differentiated. The genus *Entomobrya* is considered the most morphologically generalized group of Entomobryinae without distinct apomorphies while morphologically similar species with autapomorphic characters are separated into different genera (e.g., *Willowsia* with scales, *Entomobryoides* with smooth tibiotarsal setae and *Homidia* with dental spines). In light of the relationships inferred from the phylogeny, the classification of *Entomobrya*, along with some other genera within Entomobryidae, may need to be reevaluated in the future.

Results also indicate that the genus *Willowsia* may be polyphyletic. A new *Willowsia* species (*Willowsia* n. sp. 1) collected in Citrus Co., Florida, is resolved as a sister species to the *Entomobrya* clade, while *W. buski* and *W. nigromaculata* seem to have evolved from lineages within the *Entomobrya* clade. Considering their world-wide distribution, presumably spread by humans, and their similarity to Asian species (Zhang et al. 2011), *W. buski* and *W. nigromaculata* most likely were introduced into North America from Asia. However, *Willowsia* n. sp. 1 shares characteristics (i.e., scale type; see Zhang et al. 2011) with *W. mexicana* Zhang, Palacios-Vargas & Chen, the only other *Willowsia* species known to be endemic to North America (Zhang et al. 2007). *Willowsia* n. sp. 1’s morphological similarity to *W. mexicana* may support an inde-

pendent origin of New World *Willowsia*. Further exploration by utilizing additional markers and more complete taxon-sampling among closely related genera is needed in order to establish appropriate generic relationships and classifications.

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## References

- Bonet F (1934) Colémbolos de la República Argentina. EOS 9: 123–194.
- Brook G (1883) A revision of the genus *Entomobrya*, Rond. (Degeeria, Nic.). Journal of the Linnean Society of London (Zoology) 17: 270–283. doi: 10.1111/j.1096-3642.1884.tb02023.x
- Bueker ED (1939) Springtails (Collembola) of the St. Louis Area. Transactions of the Academy of Science of Saint Louis 30: 3–30.
- Burkhardt U, Filser J (2005) Molecular evidence for a fourth species within the *Isotoma viridis* group (Insecta, Collembola). Zoologica Scripta 34: 177–185. doi: 10.1111/j.1463-6409.2005.00181.x
- Carapelli A, Fanciulli PP, Frati F, Dallai R (1995) The use of genetic markers for the diagnosis of sibling species in the genus *Isotomurus* (Insecta, Collembola). Italian Journal of Zoology 62: 71–76. doi: 10.1080/11250009509356053
- Carapelli A, Frati F, Fanciulli PP, Dallai R (2001) Taxonomic revision of 14 south-western European species of *Isotomurus* (Collembola, Isotomidae), with description of four new species and the designation of the neotype for *I. palustris*. Zoologica Scripta 30: 115–143. doi: 10.1046/j.1463-6409.2001.00055.x

- Carapelli A, Frati F, Fanciulli PP, Nardi F, Dallai R (2005) Assessing species boundaries and evolutionary relationships in a group of south-western European species of *Isotomurus* (Collembola, Isotomidae) using allozyme data. *Zoologica Scripta* 34: 71–79. doi: 10.1111/j.1463-6409.2005.00174.x
- Chen J-X, Christiansen K (1993) The genus *Sinella* with special reference to *Sinella s.s.* (Collembola: Entomobryidae) of China. *Oriental Insects* 27: 1–54. doi: 10.1080/00305316.1993.10432236
- Chen J-X, Christiansen K (1997) Subgenus *Coecobrya* of the genus *Sinella* (Collembola: Entomobryidae) with special reference to the species of China. *Annals of the Entomological Society of America* 90: 1–19. doi: 10.1093/aesa/90.1.1
- Christiansen K (1954) Ratios as a means of specific differentiation in Collembola. *Entomological News* 65: 177–178.
- Christiansen K (1958a) The Entomobryiform male genital plate. *Proceedings of the Iowa Academy of Science* 65: 474–476.
- Christiansen K (1958b) The Nearctic members of the genus *Entomobrya* (Collembola). *Bulletin of the Museum of Comparative Zoology* 118: 439–594.
- Christiansen K, Bellinger P (1980) The Collembola of North America north of the Rio Grande; A taxonomic analysis. Grinnell College, Grinnell, IA.
- Christiansen K, Bellinger P (1992) *Insects of Hawaii: A manual of the Insects of the Hawaiian Islands, including an enumeration of the species and notes on their origin, distribution, hosts, parasites, etc.* Volume 15. Collembola. University of Hawaii Press, Honolulu, 445 pp.
- Christiansen K, Bellinger P (1998) The Collembola of North America north of the Rio Grande; A taxonomic analysis. 2<sup>nd</sup> Edition. Grinnell College, Grinnell, IA.
- Cicconardi F, Fanciulli PP, Emerson BC (2013) Collembola, the biological species concept and the underestimation of global species richness. *Molecular ecology* 22: 5382–5396. doi: 10.1111/mec.12472
- Cicconardi F, Nardi F, Emerson BC, Frati F, Fanciulli PP (2010) Deep phylogeographic divisions and long-term persistence of forest invertebrates (Hexapoda: Collembola) in the North-Western Mediterranean basin. *Molecular Ecology* 19: 386–400. doi: 10.1111/j.1365-294X.2009.04457.x
- D’Haese CA (2002) Were the first springtails semi-aquatic? A phylogenetic approach by means of 28S rDNA and optimization alignment. *Proceedings of the Royal Society of London. Series B, Biological sciences* 269: 1143–1151. doi: 10.1098/rspb.2002.1981
- Denis JR (1923) Sur la faune française des Aptérygotes, IV. Note préliminaire. – *Bulletin de la Société Entomologique de France* 92: 53–58.
- Felderhoff KL, Bernard EC, Moulton JK (2010) Survey of *Pogonognathellus* Börner (Collembola: Tomoceridae) in the Southern Appalachians based on morphological and molecular data. *Annals of the Entomological Society of America* 103: 472–491. doi: 10.1603/AN09105
- Folsom JW (1924) New species of Collembola From New York State. *American Museum Novitates* 108: 1–12.
- Frati F, Carapelli A, Fanciulli PP (1995) The genus *Isotomurus*: where molecular markers help to evaluate the importance of morphological characters for the diagnosis of species. *Polskie Pismo Entomologiczne* 64: 41–51.

- Frati F, Dell'Ampio E, Casasanta S, Carapelli A, Fanciulli PP (2000) Large amounts of genetic divergence among Italian species of the genus *Orchesella* (Insecta, collembola) and the relationships of two new species. *Molecular Phylogenetics and Evolution* 17: 456–461. doi: 10.1006/mpev.2000.0854
- Frati F, Fanciulli PP, Dallai R (1994) Further acquisitions on systematic relationships within the genus *Orchesella* (Collembola, Entomobryidae) using allozymes. *Acta Zoologica Fennica* 195: 35–43.
- Furman DP, Bukharina TA (2008) Genetic control of macrochaetae development in *Drosophila melanogaster*. *Russian Journal of Developmental Biology* 39: 195–206. doi: 10.1134/S1062360408040012
- Guthrie JE (1903) The Collembola of Minnesota. (Vol. 4). Geological and Natural History Survey of Minnesota, Minneapolis, Minnesota, 110 pp. doi: 10.5962/bhl.title.1701
- Heitzler P, Haenlin M, Ramain P, Calleja M, Simpson P (1996) A genetic analysis of pannier, a gene necessary for viability of dorsal tissues and bristle positioning in *Drosophila*. *Genetics* 143: 1271–1286.
- Jordana R (2012) Synopses of Palaearctic Collembola: Capbryinae & Entomobryini. *Soil Organisms* 84: 1–390.
- Jordana R, Baquero E (2005) A proposal of characters for taxonomic identification of *Entomobrya* species (Collembola, Entomobryomorpha), with description of a new species. *Abhandlungen und Berichte des Naturkundemuseums Görlitz* 76: 117–134.
- Katz AD, Giordano R, Soto-Adames FN (2015) Operational criteria for cryptic species delimitation when evidence is limited, as exemplified by North American *Entomobrya* (Collembola: Entomobryidae). *Zoological Journal of the Linnean Society* 173: 810–840. doi: 10.1111/zoj.12220
- Lee B-H, Hwang U-W, Kim W, Park K-H, Kim J-T (1995) Phylogenetic study of the suborder Arthropleona (Insecta: Collembola) based on morphological characters and 18S rDNA sequence analysis. *Polskie Pismo Entomologiczne* 64: 261–277.
- Lee B-H, Park K-H (1991) A systematic study of Korean Entomobryidae (Collembola, Insecta) based on cladistic analysis of phenotypic and allozyme data. *The Korean Journal of Zoology* 34: 265–288.
- Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic biology* 50: 913–925. doi: 10.1080/106351501753462876
- Leyns L, Gómez-Skarmeta JL, Dambly-Chaudière C (1996) Iroquois: A prepatterning gene that controls the formation of bristles on the thorax of *Drosophila*. *Mechanisms of Development* 59: 63–72. doi: 10.1016/0925-4773(96)00577-1
- Linnaeus C (1758) *Systema Naturae*, Ed. 10: 608–609.
- Mari-Mutt JA (1979) A revision of the genus *Dicranocentrus* Schött (Insecta: Collembola: Entomobryidae). *Bulletin of the University of Puerto Rico* 259: 1–79.
- Mari-Mutt JA (1986) Puerto Rican species of *Lepidocyrtus* and *Pseudosinella* (Collembola: Entomobryidae). *Caribbean Journal of Science* 22: 1–48.
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop (GCE), 1–8. doi: 10.1109/GCE.2010.5676129

- Packard AS (1873) Synopsis of the Thysanura of Essex County, Mass., with descriptions of a few extralimital forms. Annual Report of the Trustees of the Peabody Academy of Science 5: 23–51.
- Porco D, Potapov M, Bedos A, Busmachiu G, Weiner WM, Hamra-Kroua S, Deharveng L (2012) Cryptic diversity in the ubiquitous species *Parisotoma notabilis* (Collembola, Isotomidae): a long-used chimeric species? PLoS ONE 7: e46056. doi: 10.1371/journal.pone.0046056
- Posada D (2008) jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25: 1253–1256. doi: 10.1093/molbev/msn083
- Potapov M, Kremenitsa A (2008) Comments on the chaetotaxy of the genus *Orchesella* (Collembola, Entomobryomorpha) with a redefinition of the ‘*spectabilis*’ group and description of a new species of *Orchesella* from the Caucasus. Soil Organisms 80: 99–115.
- Ramel G, Baquero E, Jordana R (2008) Biodiversity of the Collembola Fauna of Wetland Kerkini (N. Greece), with description of the sexual dimorphism of *Entomobrya atrocincta* Schött 1896 (Collembola: Entomobryomorpha). Annales de la Société Entomologique de France 44: 113–128. doi: 10.1080/00379271.2008.10697548
- Rondani C (1861) Dipterologiae Italicae Prodromus. 4:40. A. Stochi, Parma.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. doi: 10.1093/sysbio/sys029
- Schäffer C (1896) Die Collembolen der Umgebung von Hamburg und benachbarter Gebiete. Mitteilungen aus dem Naturhistorischen Museum in Hamburg 13: 149–216.
- Schött H (1896) North American Apterygogenea. Proceedings of the California Academy of Sciences, Series 2 6: 169–196.
- Simonsen V, Krogh PH, Filser J, Fjellberg A (1999) Three species of *Isotoma* (Collembola, Isotomidae) based on morphology, isozymes and ecology. Zoologica Scripta 28: 281–287. doi: 10.1046/j.1463-6409.1999.00025.x
- Simpson P (1990) Lateral inhibition and the development of the sensory bristles of the adult peripheral nervous system of *Drosophila*. Development 109: 509–519.
- Simpson P, Woehl R, Usui K (1999) The development and evolution of bristle patterns in Diptera. Development 126: 1349–1364.
- Soto-Adames FN (2002) Molecular phylogeny of the Puerto Rican *Lepidocyrtus* and *Pseudosinella* (Hexapoda: Collembola), a validation of Yoshii’s “color pattern species”. Molecular phylogenetics and evolution 25: 27–42. doi: 10.1016/S1055-7903(02)00250-6
- Soto-Adames FN (2008) Postembryonic development of the dorsal chaetotaxy in *Seira dowlingi* (Collembola, Entomobryidae); with an analysis of the diagnostic and phylogenetic significance of primary chaetotaxy in *Seira*. Zootaxa 1683: 1–31.
- Soto-Adames FN (2010) Two new species and descriptive notes for five *Pseudosinella* species (Hexapoda: Collembola: Entomobryidae) from West Virginian (USA) Caves. Zootaxa 2331: 1–34.
- Soto-Adames FN, Barra J-A, Christiansen K, Jordana R (2008) Suprageneric classification of Collembola Entomobryomorpha. Annals of the Entomological Society of America 101: 501–513. doi: 10.1603/0013-8746(2008)101[501:SCOCE]2.0.CO;2

- South A (1961) The taxonomy of the British species of *Entomobrya* (Collembola). Transactions of the Royal Entomological Society of London 113: 387–416. doi: 10.1111/j.1365-2311.1961.tb00798.x
- Stach J (1963) The Apterygotan Fauna of Poland in relation to the World-fauna of this group of Insects: Tribe: Entomobryini. Acta monographica Musei Historiae naturalis, Polska Akademia Nauk. Inst. Zool. Karakow, 125 pp.
- Szeptycki A (1979) Chaetotaxy of the Entomobryidae and its phylogenetical significance. Morpho-systematic studies on Collembola (Vol. 4). Państwowe Wydawnictwo Naukowe, Warsaw, 1–218.
- Tullberg T (1871) Forteckning ofver Svenska Podurider. Öfversigt af Kongliga Vetenskapsakademiens förhandlingar 28: 143–145.
- Will KW, Rubinoff D (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. Cladistics 20: 47–55. doi: 10.1111/j.1096-0031.2003.00008.x
- Xiong Y, Gao Y, Yin W, Luan Y (2008) Molecular phylogeny of Collembola inferred from ribosomal RNA genes. Molecular Phylogenetics and Evolution 49: 728–735. doi: 10.1016/j.ympev.2008.09.007
- Zhang F, Bedos A, Deharveng L (2014a) Disjunct distribution of *Szeptyckiella* gen. n. from New Caledonia and South China undermines the monophyly of Willowsiini (Collembola: Entomobryidae). Journal of Natural History 48: 1299–1317. doi: 10.1080/00222933.2013.859317.
- Zhang F, Chen Z, Dong RR, Deharveng L, Stevens MI, Huang YH, Zhu CD (2014b) Molecular phylogeny reveals independent origins of body scales in Entomobryidae (Hexapoda: Collembola). Molecular Phylogenetics and Evolution 70: 231–239. doi: 10.1016/j.ympev.2013.09.024
- Zhang F, Deharveng L (2015) Systematic revision of Entomobryidae (Collembola) by integrating molecular and new morphological evidence. Zoologica Scripta 44: 298–311. doi: 10.1111/zsc.12100
- Zhang F, Palacios-Vargas JG, Chen J-X (2007) The genus *Willowsia* and its Mexican species (Collembola: Entomobryidae). Annals of the Entomological Society of America 100: 36–40. doi: 10.1603/0013-8746(2007)100[36:TGWAIM]2.0.CO;2
- Zhang F, Yu D, Luo Y, Ho SYW, Wang B, Zhu C (2014c) Cryptic diversity, diversification and vicariance in two species complexes of *Tomocerus* (Collembola, Tomoceridae) from China. Zoologica Scripta 43: 393–404.
- Zhang F, Yu D, Xu G (2011) Transformational homology of the tergal setae during postembryonic development in the *Sinella-Coecobrya* group (Collembola: Entomobryidae). Contributions to Zoology 80: 213–230.

## Supplementary material 1

### Morphology matrix

Authors: Aron D. Katz, Rosanna Giordano, Felipe Soto-Adames

Data type: Nexus file optimized for Mesquite

Explanation note: Alignment nexus file for all morphological characters used for phylogenetic analysis. This file is optimized for viewing in mesquite and includes all character state information.

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## Supplementary material 2

### Distribution maps

Authors: Aron D. Katz, Rosanna Giordano, Felipe Soto-Adames

Data type: PDF file

Explanation note: Distribution maps: **A**, *Entomobrya assuta*; **B**, *Entomobrya atrocincta*; **C**, *Entomobrya bicolor*; **D**, *Entomobrya citrensis* sp. n.; **E**, *Entomobrya clitellaria*; **F**, *Entomobrya decemfasciata*; **G**, *Entomobrya intermedia*; **H**, *Entomobrya jubata* sp. n.; **I**, *Entomobrya ligata*; **J**, *Entomobrya multifasciata*; **K**, *Entomobrya neotenica* sp. n.; **L**, *Entomobrya nivalis*; **M**, *Entomobrya quadrilineata*; **N**, *Entomobrya unifasciata* sp. n.; **O**, *Entomobrya unostrigata*.

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### Supplementary material 3

#### Photographs of type specimens

Authors: Aron D. Katz, Rosanna Giordano, Felipe Soto-Adames

Data type: PDF file

Explanation note: Photographs of type specimens stored at the Illinois Natural History Survey, Champaign, IL. Lateral (**A**) and dorsal (**B**) views of a neo-paratype specimen of *Entomobrya quadrilineata* Bueker, 1939, Fountain Bluff, IL., V.15.1932, Ross & Mohr. Lateral view of a co- type specimen of *Entomobrya decemfasciata* Packard 1873 (**C**), no locality information. *Entomobrya ligata* Folsom 1924: Co-type specimens, Roorheesville?, NY 30Aug1923, M. J. Leonard (**D, E, F**); Co-type specimen, NY, July 1923, A. Wolf (**G**).

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# Two new phragmotic ant species from Africa: morphology and next-generation sequencing solve a caste association problem in the genus *Carebara* Westwood

Georg Fischer<sup>1</sup>, Frank Azorsa<sup>2</sup>, Francisco Hita Garcia<sup>3</sup>,  
Alexander S. Mikheyev<sup>1</sup>, Evan P. Economo<sup>1,4</sup>

**1** Okinawa Institute of Science and Technology Graduate University, 1919–1 Tancha, Onna-son 904–0495, Japan **2** División de Entomología, Centro de Ecología y Biodiversidad, CEBIO. Lima, PERU **3** Department of Natural History – Zoology, Hessisches Landesmuseum Darmstadt, Friedensplatz 1, 64283 Darmstadt, Germany **4** Department of Ecology and Evolutionary Biology, Museum of Zoology, University of Michigan, 830 N University Street, Ann Arbor, MI 48701, USA

Corresponding author: *Georg Fischer* ([georgf81@gmail.com](mailto:georgf81@gmail.com))

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## Abstract

Phragmotic or “door head” ants have evolved independently in several ant genera across the world, but in Africa only one case has been documented until now. *Carebara elmenteitae* (Patrizi) is known from only a single phragmotic major worker collected from sifted leaf-litter near Lake Elmenteita in Kenya, but here the worker castes of two species collected from Kakamega Forest, a small rainforest in Western Kenya, are studied. Phragmotic major workers were previously identified as *Carebara elmenteitae* and non-phragmotic major and minor workers were assigned to *C. thoracica* (Weber). Using evidence of both morphological and next-generation sequencing analysis, it is shown that phragmotic and non-phragmotic workers of the two different species are actually the same and that neither name – *C. elmenteitae* or *C. thoracica* – correctly applies to them. Instead, this and another closely related species from Ivory Coast are both morphologically different from *C. elmenteitae*, and thus they are described as the new species *Carebara phragmotica* **sp. n.** and *Carebara lilith* **sp. n.**

## Keywords

Phragmosis, new species descriptions, Hymenoptera, Formicidae, *Carebara lilith*, *Carebara phragmotica*, worker polymorphism, RAD-seq, Afrotropics, Kenya, Ivory Coast

## Introduction

The ant genus *Carebara* is highly diverse with about 250 named taxa to date (Bolton 2014), while the true diversity is probably much higher due to a large number of undescribed species (Fischer et al. 2014). For the vast majority of this diversity virtually nothing is known about their respective ecologies, and data about species' biogeographic distributions is still incomplete. Apart from the conspicuous, mass-raiding marauder ants of the former genus *Pheidologeton* (now *Carebara*, see Fischer et al. 2014), most of the species are minute in size, often with very cryptic lifestyles, making field observation difficult.

Due to a lack of comprehensive revisions and identification keys for Old World *Carebara*, identifications are challenging. On a regional level, however, taxonomic treatments exist for the Arabian Peninsula (Sharaf and Aldawood 2013), Taiwan (Terayama, Lin and Eguchi 2012), India (Bharti and Kumar 2013), and Fischer et al. (2014) revised the newly defined and mostly Afrotropical *C. polita* group. Weber's (1950) revision for the Afrotropical *Oligomyrmex* species is outdated and, as it does not contain a key, is also of very limited use for identifications of the treated species. For the New World, Fernández (2004) published a valuable revision of *Carebara* with a provisional key, where he synonymized the former genera *Oligomyrmex* (Mayr), *Paedalgus* (Forel), and *Afroxyidris* (Belshaw & Bolton) with *Carebara* and defined species complexes based on worker morphology. As several previous studies showed (e.g. Ettershank 1966, Fernández 2004, Fischer et al. 2014, and Azorsa and Fisher in revision) all of the synonymized genera were morphologically poorly delimited from *Carebara* and thus treated as polyphyletic units. Bharti and Kumar (2013) also pointed out the necessity to restructure Fernández' New World species group definitions in order to incorporate the much more species-rich but poorly studied Old World fauna. Undersampling in many tropical and sub-tropical areas and especially in non-epigeaic strata is still a major issue for *Carebara* taxonomy and biogeography, and contributes to major gaps in our knowledge. Hence, more ecological and taxonomic studies in these areas are needed in order to better understand the evolution and biology of this interesting and diverse genus.

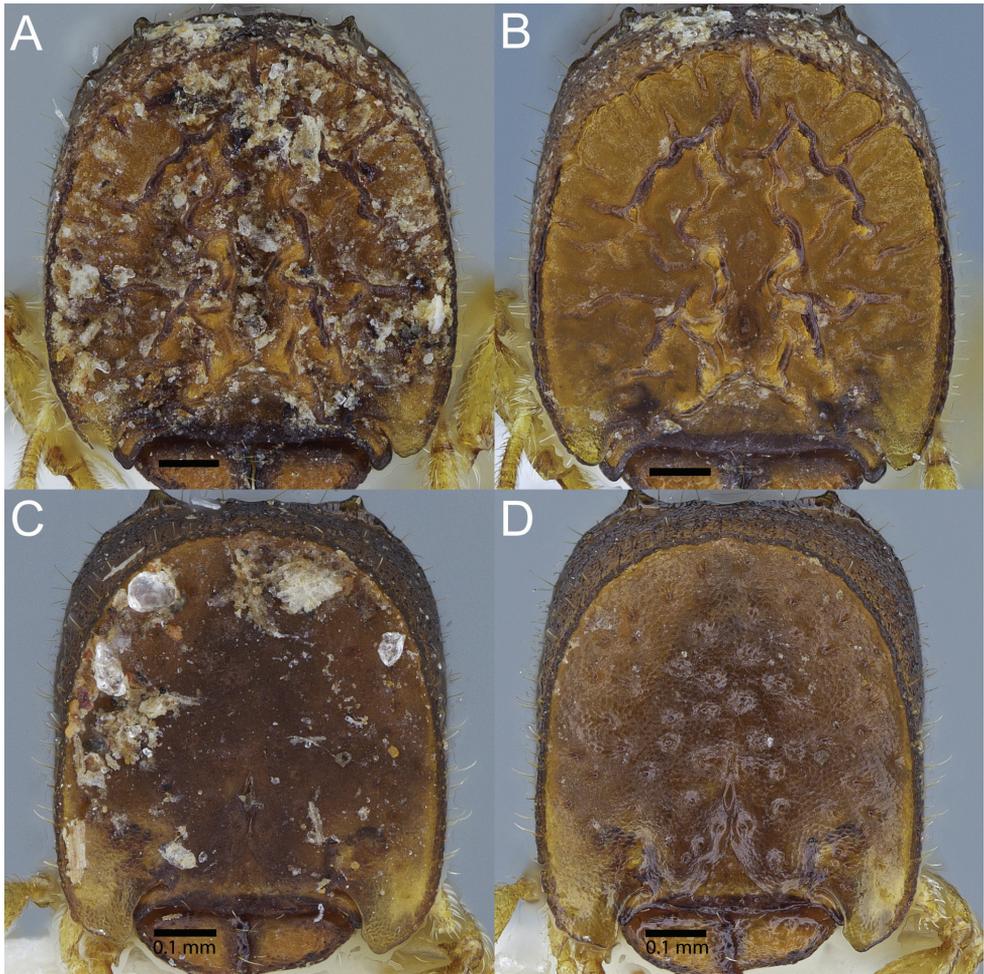
Taxonomic research in ants heavily depends on dry specimens in entomology collections and associated collection-based data, but field observations can contribute valuable insights, with the potential to improve species boundaries. The main obstacle from ecological surveys using standardized, passive collection methods (e.g. leaf-litter extraction and pitfall trapping), are disassociated specimens from different castes or subcastes. Especially in genera with distinct worker di- and polymorphism, this can create problems of inflated diversity counts. As in the hyperdiverse genus *Pheidole* Westwood, workers of many *Carebara* species are divided into two distinct subcastes, minor and major workers (or soldiers), with additional subcastes and intermediates present in several species (Azorsa and Fisher in revision, Fischer et al. 2014). While the major workers' most important tasks are chopping and transportation of larger prey and the defence of foraging trails and the nest, the main function of phragmotic workers is blocking nest entrances against intrusion of other predatory ants and invertebrates (Hölldobler and Wilson 1990).

Phragmosis in ants (truncated body parts – usually the head – used for plugging nest entrances) has evolved independently in the diverse ant genera *Camponotus* Mayr (*Colobopsis*, *Hypercolobopsis*), *Cephalotes* Latreille, *Colobostruma* Wheeler (*C. leae*), *Crematogaster* Lund (*Colobocrema*), *Pheidole* Westwood (*P. colobopsis*, *P. lamia*), but also in other genera, such as *Blepharidatta* Smith, (*B. conops*), *Tetraponera* Smith (*T. phragmotica*) and *Carebara* Westwood (Brandão et al. 2001, Hölldobler and Wilson 1990). Phragmosis is most strongly developed in the New World arboreal genus *Cephalotes*, where usually all castes (queens, and major and minor workers) have highly adapted shield-like head morphologies that enable them to plug their nest entrance without exposing eyes, antennae or mandibles to any would-be intruders. Wheeler and Hölldobler (1985) discussed phragmosis in *Cephalotes* and reported the discovery of “glandular openings” on the cephalic shield that supposedly excrete fibrous material, covering the head in a dense layer of organic material and most likely serving as camouflage of the head that plugs the nest entrance. A very similar phragmotic head shape has evolved independently in some Old World species of *Carebara*, where a special major worker subcaste occurs in addition to regular major and minor workers.

Heads in the shape of a saucer or a concave shield protecting eyes, antennae and mandibles from possible injury by attackers may have evolved convergently in major workers of the Southeast Asian *C. butteli* (Forel), *C. nayana* (Sheela & Narendran) and in the subsequently treated Afrotropical *Carebara* species. The cephalic shields in the two newly described species were found to be covered by a layer of debris (soil, maybe organic material; see Fig. 1), which may serve as camouflage to make the ant blend in with the soil around the nest entrance.

During field work between 2005 and 2009 in Kakamega Forest, Western Kenya, phragmotic *Carebara* workers have been collected from seven leaf-litter samples (out of 300+), along with workers of four other species of the genus (Hita Garcia et al. 2009, Hita Garcia et al. 2013). Since most leaf-litter samples contained multiple species, their various worker subcastes were often intermixed and had to be re-associated during sorting. Because of this association problem and because of their morphological isolation due to the highly derived head shapes, the phragmotic workers were first identified as *C. elmenteitae* (Patrizi). The sympatrically occurring workers of the other four species were identified as *C. GF4*, *C. GF5*, *C. polita* (Santschi), and *C. thoracica* (Weber) (for updated species IDs see Table 1). At first, there was no evidence for a relationship between the phragmotic workers and those of any other species.

However, more recently we inferred that they were most likely an additional subcaste to non-phragmotic major and minor workers which were falsely identified as *C. thoracica*. Our assumption was based primarily on the specimens (described here as *C. lilith* sp. n.) found during a visit to the MHNG ant collection in Geneva during November 2013. For *C. lilith*, a phragmotic worker had been collected together with two minor workers. These specimens, with morphologies highly similar to the phragmotic workers and minor workers found in Kakamega Forest leaf-litter samples, were the initiator of our following investigations. Morphological key features such as antennal segmentation, petiole and postpetiole morphology, sculpture patterns, and pilosity were



**Figure 1.** **A, B** full-face view of phragmotic major workers of *C. phragmotica* sp. n. and **C, D** *C. lilith* sp. n. **A, C** on the left side the head is depicted in the state that it was found in the samples, with debris sticking to cephalic shield **B, D** right view: same specimens with debris removed in ultrasonic bath.

examined of all Kakamega species co-occurring with the phragmotic workers. That way it was possible to infer the relationship between specimens identified as *C. elementitae* (phragmotic workers) and *C. thoracica* (non-phragmotic workers with 10 antennal segments) and to exclude the other *Carebara* species with non-matching morphologies as likely conspecifics. For a more rigorous test of our hypotheses de-novo DNA sequencing (RAD-seq) and analysis of the relevant material was used. As outgroup material the sympatric and morphologically related, yet distinct, *C. alluaudi*, was selected as well as the more distantly related *C. sylvestrii*, and two undetermined Chinese *Carebara* morphospecies (collected by Liu et al.).

**Table 1.** Updated species IDs of Kakamega Forest *Carebara* specimens used in this study, compared to old IDs in Hita Garcia et al. (2009, 2013).

Updated species ID	Old species ID	Worker subcaste
<i>C. phragmotica</i> sp. n.	<i>C. elmenteitae</i>	phragmotic
<i>C. phragmotica</i> sp. n.	<i>C. thoracica</i> (10 ant. segments)	minor & major
<i>C. thoracica</i>	<i>C. thoracica</i> (9 ant. segments)	minor & major
<i>C. silvestrii</i>	<i>C. GF4</i>	minor & major
<i>C. alluaudi</i>	<i>C. GF5</i>	minor & major
<i>C. polita</i>	<i>C. polita</i>	minor & major

As a result of these morphological and genetic studies, two new phragmotic *Carebara* species, *C. lilith* sp. n. from Ivory Coast and *C. phragmotica* sp. n. from Kenya are described, that both are likely related to *Carebara elmenteitae* (Fig. 2). All three species have the phragmotic major worker subcaste and although *C. elmenteitae* is known from a phragmotic worker only, we assume the missing workers to be morphologically related to the workers of our newly described species. In addition to detailed descriptions and high-resolution composite images of the two new species, a species-level identification key is provided for the three phragmotic species, as well as a discussion for their taxonomic placement within the Afrotropical *Carebara* fauna.

## Material and methods

All specimens in this study were examined with a Leica MZ165x stereo microscope (up to maximum magnification of 120×), and measured with an orthogonal pair of micrometers.

Morphological characters and measurements used in this publication are the same as in Fischer et al. (2014), which are mostly derived from Bolton (1994, 2003), Bolton and Belshaw (1993), Belshaw and Bolton (1994), Ettershank (1966), Fernández (2004, 2006, 2010). For sculpture characters we refer to Harris (1979) and for different pilosity patterns we use the five inclination types described by Wilson (1955).

High-resolution images were created using Leica DFC 425 and DFC 450 cameras in combination with the Leica Application Suite software (version 3.8) and Helicon Focus 6 software. All images were individually edited using Photoshop and combined into plates with Adobe Illustrator software. Images can be viewed and downloaded at [www.AntWeb.org](http://www.AntWeb.org).

Most of the material studied in this publication is located in ZFMK in Bonn, the holotype of *C. phragmotica* is deposited in ZFMK, the material of *C. lilith* sp. n. belongs to the MHNG ant collection in Geneva. Paratypes of *C. phragmotica* sp. n. will be deposited in the NMK (Nairobi), in the MCZC (Cambridge, MA), and in the MHNG (Geneva).

## Abbreviations of depositories

- BMNH** British Museum of Natural History, London, UK  
**DCZU** University of Calicut, Department of Zoology  
**IEGG** Istituto di Entomologia “Guido Grandi” Università di Bologna, Bologna, Italy  
**MHNG** Muséum d’Histoire Naturelle de la Ville de Genève, Geneva, Switzerland  
**MCZC** Museum of Comparative Zoology, Cambridge, Mass. U.S.A.  
**NMK** The National Museums of Kenya, Nairobi  
**ZFMK** Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

## DNA sequencing and analysis

Phylogenetic analysis of restriction site-associated DNA (RAD-seq) was used (Baird et al. 2008) to test the relationship of phragmotic workers previously identified as *C. elementitae* with non-phragmotic major and minor workers identified as *C. thoracica* and containing specimens with both, nine and ten antennal segments (results in Fig. 2). Outgroup taxa are two species from Kakamega Forest occurring in sympatry with the above mentioned ingroup taxa (*C. sylvestrii* and *C. alluaudi*) and two other *Carebara* species from Yunnan, China (*C. clm001* and *C. clm009*). The 19 specimens included in the analysis (Table 2) comprise focal taxa plus outgroups for phylogenetic context.

DNA was non-destructively extracted from each specimen following Tin et al. (2014) by soaking it overnight in a chaotropic buffer. The DNA was then bound to magnetic beads and washed prior to library preparation. RAD-tag libraries were then prepared as in Tin et al. (2015) using a Biomek<sup>®</sup> FXP Laboratory Automation Workstation (Beckman Coulter) to perform all of the liquid handling steps up to PCR. Sequencing was performed on an Illumina Hi-Seq platform. The barcodes were designed following Bystrykh (2012). Trimmomatic (Bolger et al. 2014) was used to filter by quality and trim the sequences to 55bp (parameters SLIDINGWINDOW:8:10 MINLEN:41 CROP:41). The FASTq files containing DNA sequence reads were uploaded to the DNA Data Bank of Japan (DDBJ, <http://www.ddbj.nig.ac.jp/>, bioproject\_id: PRJDB3919). We used PyRAD v3.0.4 (Eaton 2014) for *de novo* assembly of RAD loci (parameters: *Mindepth*=6, *NQual*=5, *Wclust*=0.88, *MinCov*=4, *MaxSH*=3, otherwise default) and performed a maximum likelihood (ML) phylogenetic analysis on the full alignment (1.78 million bp) with ExaML v3.0.14 (Kozlov et al. 2015). The GTR+G nucleotide substitution model was chosen, as it was the only option implemented in ExaML suitable for a dataset of this size. To evaluate support for the ML topology, 1000 bootstraps were performed in a combination of ExaML and RaxML v8.0.0 (Stamatakis 2014), following the procedure described in the ExaML manual. The alignment, inferred topology, and further details on the procedure for the ML search are available on datadryad.org (<http://datadryad.org/review?doi=doi:10.5061/dryad.1jc33>).

**Table 2.** List of *Carebara* specimens used for DNA sequencing (\* phragmotic workers, previously identified as *C. elmenteitae*; \*\* minor workers, previously identified as *C. thoracica* (Hita Garcia et al. 2009, Hita Garcia et al. 2013)).

Species ID [number of antennal segments]	Specimen code	Basepairs analysed	DDBJ experiment ID	Country
<i>C. phragmotica</i> sp. n.* [10]	CASENT0738556	333886	DRX032389	Kenya
<i>C. phragmotica</i> sp. n.* [10]	CASENT0738559	948331	DRX032390	Kenya
<i>C. phragmotica</i> sp. n.** [10]	CASENT0738560	783551	DRX032399	Kenya
<i>C. phragmotica</i> sp. n.** [10]	CASENT0738561	621109	DRX032400	Kenya
<i>C. thoracica</i> [9]	CASENT0738564	454125	DRX032401	Kenya
<i>C. thoracica</i> [9]	CASENT0738565	456134	DRX032402	Kenya
<i>C. alluaudi</i> [9]	CASENT0738554	780239	DRX032395	Kenya
<i>C. alluaudi</i> [9]	CASENT0738555	665163	DRX032396	Kenya
<i>C. alluaudi</i> [9]	CASENT0738566	880370	DRX032397	Kenya
<i>C. alluaudi</i> [9]	CASENT0738567	884559	DRX032398	Kenya
<i>C. silvestrii</i> [11]	CASENT0738557	303910	DRX032391	Kenya
<i>C. silvestrii</i> [11]	CASENT0738558	414766	DRX032392	Kenya
<i>C. silvestrii</i> [11]	CASENT0738562	525888	DRX032393	Kenya
<i>C. silvestrii</i> [11]	CASENT0738563	428646	DRX032394	Kenya
<i>C. clm001</i> [9]	CASENT0735929	155549	DRX032384	China
<i>C. clm001</i> [9]	CASENT0735930	26192	DRX032385	China
<i>C. clm009</i> [9]	CASENT0735913	220323	DRX032386	China
<i>C. clm009</i> [9]	CASENT0735914	78455	DRX032387	China
<i>C. clm009</i> [9]	CASENT0735915	70339	DRX032388	China

## Measurements and indices

The following measurements are illustrated in Figure 1 in Fischer et al. (2014):

- HL** *head length*: maximum distance from midpoint of anterior clypeal margin to midpoint of posterior margin of head, measured in full-face view; in majors, measured from midpoint of tangent between anterior-most position of clypeus to midpoint of tangent between posterior-most projection of the vertex.
- HW** *head width*: measured at widest point of head, in full-face view behind eye level.
- SL** *scape length*: maximum scape length, excluding basal condyle and neck.
- EL** *eye length*: maximum diameter of compound eye measured in oblique lateral view.
- MFL** *metafemur length*: measured from junction with trochanter to junction with tibia.
- MTL** *metatibia length*: measured from junction with femur to junction with first tarsal segment.
- MDL** *mandible length*: maximum length, measured in oblique frontolateral view, from apex to lateral base.
- PNW** *pronotal width*: maximum width of pronotum measured in dorsal view.

- WL** *Weber's length*: diagonal length of mesosoma in profile from anterior point of pronotal slope and excluding neck, to posteroventral margin of propodeum.
- PSL** *propodeal spine length*: in dorsocaudal view, with apex of measured spine, its base, and center of propodeal concavity between both spines in focus: measurement is taken from apex to base along one axis of a dual-axis micrometer, which is aligned along length of spine, while second axis crosses base of measured spine, and connects base with center of propodeal concavity.
- PTL** *petiole length*: maximum diagonal length of petiole, measured in profile, from most anteroventral point of peduncle, at or below propodeal lobe, to most posterodorsal point at junction to first helcial tergite.
- PTH** *petiole node height*: maximum height of petiolar node measured in lateral view from highest (median) point of node, orthogonally to ventral outline of node.
- PTW** *petiole node width*: maximum petiolar node width, measured in dorsal view.
- PPL** *postpetiole length*: maximum length of postpetiole, measured in profile, from anterior beginning of dorsal slope to posterior juncture of postpetiole and second helcial tergite.
- PPH** *postpetiole height*: maximum height of postpetiole, measured in profile, from the highest (median) point of node to lowest point of ventral face, often in an oblique line.
- PPW** *postpetiole width*: maximum width of postpetiole, measured in dorsal view.

## Indices

- CI** *cephalic index*:  $HW / HL \times 100$
- SI** *scape index*:  $SL / HW \times 100$
- MDI** *mandible index*:  $MDL / HW \times 100$
- EI** *eye index*:  $EL / HW \times 100$
- FI** *metafemur index*:  $MFL / HW \times 100$
- PSLI** *propodeal spine index*:  $PSL / HW \times 100$
- LPpI** *lateral postpetiole index*:  $PPL / PPH \times 100$
- DPpI** *dorsal postpetiole index*:  $PPW / PPL \times 100$
- PpWI** *postpetiole width index*:  $PPW / PTW \times 100$
- PpLI** *postpetiole length index*:  $PPL / PTL \times 100$
- PpHI** *postpetiole height index*:  $PPH / PTH \times 100$

## Results

### Species synopsis

*Carebara elmenteitae* (Patrizi)

*Carebara lilith* Fischer, Azorsa & Hita Garcia, sp. n.

*Carebara phragmotica* Fischer, Azorsa & Hita Garcia, sp. n.

### Other *Carebara* species with phragmotic workers

*Pheidologeton (Lecanomyrma) butteli* Forel, 1913: 56, fig. S (s.w.) SRI LANKA, Peradeniya, Experiment Station (*v. Butteli*) (MHNG) [examined]. Combination in *Aneleus*: Emery 1924: 215; in *Oligomyrmex*: Ettershank 1966: 123; in *Carebara*: Fernández 2004: 235.

*Neoblepharidatta nayana* Sheela & Narendran, 1997: 89, figs 1-4 (s., not q. as stated) INDIA, Kerala, Iritty Forest near Aaralam farm, 16.xii.1995 (*Sheela*) (DCZU) [not examined]. Combination in *Oligomyrmex*: Bolton 2003: 273. Combination in *Carebara*: Fernández 2004: 196 (by implication).

### Preliminary definition of the *Carebara phragmotica* clade

The three species treated are loosely defined here as a clade based on the presence of and morphological similarity between the phragmotic workers. We do not claim that these species form a monophyletic or exclusive clade within the genus *Carebara*. Although we think that a sister-species relationship between them is the most likely hypothesis, it is nevertheless possible that morphological similarities are due to convergence and that they are not closely related. Another hypothesis is that they are indeed very closely related, but forming a monophyletic group with other species that do not possess phragmotic workers. As highly visible in the systematic history of *Carebara* and its constituent species and synonymous genera, the definition of species groups or even genera based on morphology alone can be both, a tedious and sometimes frustrating approach with taxonomic group definitions changing frequently (see Ettershank 1966, Fernández 2004, Fischer et al. 2014).

Since the emergence of increasingly affordable DNA-sequencing methods generating more comprehensive data-output as compared to Sanger sequencing (e.g. genome and next-generation sequencing), higher emphasis should be placed on combined taxonomic and genetic analyses in order to reduce discrepancies between both approaches. For the *phragmotica* clade and the majority of *Carebara* species, only a large-scale taxonomic treatment and/or a near-comprehensive phylogenetic analysis of the whole genus would be able to provide the level of confidence needed for definition of exclusive and monophyletic species groups. However, future studies are necessary to close these gaps in our taxonomic understanding of the genus *Carebara* Westwood.

### Shared characters of *C. phragmotica* clade species (all worker subcastes)

(the characters listed below may not be autapomorphic, since the majority of Afrotropical taxa remain poorly characterized and because of possible convergent evolution)

Phragmotic major workers present, with oval cephalic shield and anterolateral lobes covering the lateral base of the mandible. Antennae with 10 segments and 2-segmented club, the apical segment between combined length of antennal segments 3 to 9 and

length of remainder of funiculus (antennal segments 2-9). Antennal scape relatively short, in minor workers failing to reach the posterior head margin by about length of 9th antennal segment, in majors of *C. phragmotica* ending at about midlength of head (SI 46-49), in phragmotic workers distinctly shorter and reduced (SI 21-34). Mandibles triangular and masticatory margin with five teeth, mandibles of phragmotic workers reduced and very small, about half as long as those of major workers in *C. phragmotica* (MDI 24-28). Anterior margin of clypeus in phragmotic workers very wide and straight to medially concave. Eyes minute and consisting of one ocellus, in phragmotic workers reduced and almost invisible, single median ocellus often present in major workers of *C. phragmotica*, but invisible or absent in phragmotic workers. Metanotal groove in profile impressed and propodeum higher than long. Propodeal teeth developed, relatively small and apically rounded to short-triangular and acute. Petiole quite massive in profile, with moderately long peduncle, a small, anteriorly pointing anteroventral tooth, and often with conspicuously convex ventral bulge, in dorsal view almost as wide as (minor workers) to wider than propodeal dorsum (majors and phragmotic workers). Postpetiole roundly subrectangular in dorsal view, between 1.2 and 1.5 times wider than petiole. In minor workers (of *C. phragmotica* and *C. lilith*) sculpture absent from head, promesonotum, dorsum of postpetiole and gaster.

### Delimitation from other *Carebara* groups and species in the Afrotropical region

Here a general account of the Afrotropical *Carebara* fauna is given as well as information on how to differentiate species belonging to the *phragmotica* clade from the remainder of *Carebara* species that were found and described for the region, not including Madagascar. They can be divided into several groups of morphologically related species, some of which correspond to the preliminary groups defined by Fernández (2004): *lignata* complex for *Carebara sensu stricto* (before synonymization of *Oligomyrmex* Mayr), *escherischi* complex for former *Paedalgus* species, and with the former *Oligomyrmex* species roughly corresponding to the *concinna* complex, although it was defined for New World species, which have eleven antennal segments, contrasting to mostly 9- and 10-segmented Old World species. Two of these New World *concinna* complex species, *C. brevipilosa* Fernández and *C. urichi* (Wheeler), are now included in the *polita* group, but exact phylogenetic relationships within and between the different faunas are still unresolved. Because we want to avoid creating polyphyletic species groups, we leave the definition of systematic species groups to larger-scale studies in the future.

Afrotropical *Carebara* species belonging to the former genus *Pheidologeton* are: *C. aberrans* (Santschi) (queen), *C. diversa standfussi* (Forel), *C. hammoniae* (Stitz), *C. hostilis* (Smith), *C. kunensis* (Ettershank), *C. mayri* (Forel), *C. solitaria* (Stitz) (queen), and *C. volsatella* (Santschi) (male). They are mainly characterized by possessing eleven antennal segments, a markedly polymorphic worker caste with several intermediate worker subcastes, comparatively large, multi-faceted eyes, minor workers with antennal scapes usually surpassing the posterior head margin, and large major work-

ers, usually with one to several large ocelli present. Morphologically, this group is closest to some species of the *polita* group, e.g. *C. nicotiana* (Arnold) and *C. polita* (Santschi). The *polita* group can be distinguished from other *Carebara* by antennae with eleven segments (but only nine in *C. madibai* Fischer & Azorsa), eyes reduced, in minor workers usually consisting of a single ocellus, in majors sometimes larger and multi-faceted, but smaller than in former *Pheidologeton* species, major workers usually with high, weakly squamiform petiole node, and minor workers with postpetiole significantly longer than high in profile (Fischer et al. 2014). In Africa the *polita* group includes: *Carebara madibai* Fischer & Azorsa, *C. perpusilla* (Emery), *C. polita* (Santschi), *C. nicotianae* Arnold, *C. silvestrii* (Santschi), and *C. villiersi* (Bernard). Species described in or assigned to the former genus *Paedalgus* (Forel) (= *escherischi* complex in Fernández 2004) are: *C. distincta* (Bolton & Belshaw), *C. octata* (Bolton & Belshaw), *C. pisinna* (Bolton & Belshaw), *C. rara* (Bolton & Belshaw), *C. robertsoni* (Bolton & Belshaw), *C. sarita* (Bolton & Belshaw), *C. sudanensis* (Weber) (queen), and *C. termitolestes* (Wheeler). They all share morphological characters that distinguish them from other *Carebara* species, i.e. nine antennal segments, mandibles with four teeth, metanotal groove not impressed, propodeum oblique in profile and declining towards posterior end without distinct angle, and propodeal teeth absent. Species of *Carebara sensu stricto* (definition before Fernández 2004, = *lignata* complex) are characterized by small workers and usually much larger queens, the workers usually with nine antennal segments, mandibles with three to four teeth, eyes and propodeal teeth absent, the propodeal dorsum often rounding into the posterior declivity without any angle. Species with matching morphologies are *C. arnoldi* (Forel), *C. guineana* Fernández, *C. junodi* Forel, *C. osborni* Wheeler, *C. vidua* Smith, *C. vidua* var. *fur* Santschi, *C. wheeleri* Ettershank (replacement name for *C. silvestrii* Santschi). *Carebara ampla* Santschi and its subspecies, *C. bartrumi* Weber, *C. langi* Wheeler, *C. sicheli* Mayr, and *C. sudanica* Santschi are all known from queens and/or males only, but their queens are usually very large and are morphologically close to *C. vidua*. It is unclear, however, how many species described by alates are synonymous with worker-based species. This has to be investigated in future studies, but colony collections with associated workers and alates are rare and difficult to achieve in a systematic way. Workers of *Carebara fayrouzae* Sharaf, which occurs in Saudi Arabia, also have nine antennal segments and minor worker and queen morphologies closely match those of the above listed species in *Carebara s. str.* If they they should turn out to be closely related, then *C. fayrouzae* would be the first species in this group of which major workers have been found and described. In that case, it would not be unlikely that other species of *Carebara s. str.* and former *Paedalgus* are not monomorphic, but di- or even polymorphic as well. As Fernández (2004) pointed out, the currently available material is insufficient to answer this question. Workers of *Carebara crigenensis* (Belshaw & Bolton) – described originally in its own genus *Afroxyidris* – which are morphologically similar to *Carebara s. str.*, are characterized by antennae with ten segments, mandibles with two apical teeth plus one small basal tooth, eyes absent, and propodeum unarmed and rounded posteriorly.

Species from the *phragmotica* clade are part of a larger group of morphologically related species, which includes many taxa belonging to the former genus *Oligomyrmex* (Mayr). Before its synonymisation under *Carebara* by Fernández (2004), *Oligomyrmex* was defined by possessing nine to eleven antennal segments (rarely eight), a markedly dimorphic worker caste, a well-developed metasternal process, anterior subpetiolar process present and radial cell of wing closed (Ettershank 1966). Including the two newly described species, workers of 30 valid species and subspecies of Afrotropical *Carebara* match this character combination. Twelve of them have nine antennal segments: *C. alluaudi* (Santschi), *C. alluaudi* var. *cataractae* (Santschi), *C. angolensis* (Santschi), *C. angolensis* r. *congolensis* (Forel), *C. convexa* (Weber), *C. donisthorpei* (Weber), *C. frontalis* (Weber), *C. jeanneli* (Santschi), *C. latro* (Santschi), *C. pumilia* (Fischer, Azorsa, & Fisher; replacement name for *Carebara nana* (Santschi)), *C. santschii* (Weber), and *C. thoracica* (Weber). It has to be noted though, that Weber (1952) later found specimens of *C. thoracica* with both, nine and ten antennal segments, as well as specimens that had nine segments on one antenna and ten on the other one. This character polymorphism seems to be not uncommon and can be observed in a few other species as well, calling to attention the relatively high plasticity in some of the characters that are usually used for taxonomic delimitation.

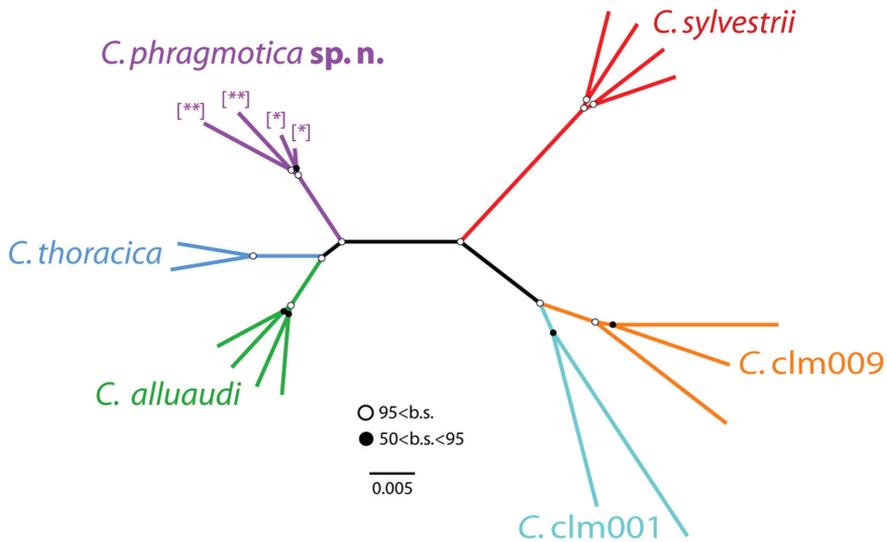
One species, *C. diabola* (Santschi), which was originally described in the genus *Aneleus*, has eleven antennal segments. Workers of the remaining 17 species have ten antennal segments, including *C. elementitae*, *C. lilith* sp. n. and *C. phragmotica* sp. n. All of the latter three species are probably polymorphic, with the highly derived phragmotomic majors as a distinct third subcaste, but the other two worker subcastes share many morphological characters with the other 14 species of this group. Some of them are easily distinguishable from the *phragmotica* clade, but several are strikingly similar in their outer morphologies and only a complete taxonomic treatment will be able to draw more definitive species boundaries. In the following paragraph, is a short account of possibly related taxa, listing some supposedly stable characters that may be useful for their identification and delimitation.

Major workers of *C. acuta* (Weber) are characterized by reticulate-punctate sculpture on head dorsum, with striae anteriorly, and propodeal teeth long and acute, minor workers without visible sculpture except for striae on anterior head (Weber 1952). The majors of *C. africana* (Forel) (minors not described) without longitudinal rugulae on head, mandibles with six teeth, and propodeal teeth absent or reduced. *Carebara arabica* (Collingwood & Van Harten) is morphologically very similar to *phragmotica* clade specimens, but its major workers are characterized by oblique posterior head corners with a distinct angle towards the median emargination, moderately large horns on the distal part of the posterior margin, and longitudinal rugulae moderately abundant, evenly spaced; the minor workers with reduced sculpture on meso- and metapleurae and absent or reduced propodeal teeth. Major and minor workers of *C. arnoldiella* (Santschi) are also lacking distinct propodeal teeth and head sculpture in major workers is strongly reduced and consists of only a few weakly developed rugulae. Head shape and sculpture of *Carebara debilis*

(Santschi) major workers is very similar to *C. phragmotica* majors, but the type specimen possesses a very large median ocellus, minute, rounded propodeal teeth, petiole longer than high and petiole node in profile widely convex, postpetiole in dorsal view very broadly elliptical, and some majors with only nine antennal segments; minor workers with very reduced propodeal teeth and only nine antennal segments. *Carebara erythraea* (Emery) major worker's head with very shallow posterior emargination, relatively few, short, longitudinal rugulae, frons and posterior sides almost smooth, propodeal teeth in major and minor workers not defined, but posterolateral lamella present. Major workers of *C. incerta* (Arnold), *C. khamiensis* (Arnold), and *C. lucida* (Santschi) are not described; their minors are characterized by absent or reduced propodeal teeth, short, in profile subtriangulate petiole with very short peduncle, mandibles in *C. incerta* and *C. khamiensis* with only four teeth, but five in *C. lucida*. The holotype of *Carebara petulca* (Wheeler), of which only the major worker is described, is characterized by densely rugulose head sculpture, a large median ocellus, small horns on the posterior head margin, relatively large eyes with six ommatidia, a distinct scutellum, and a high, posteriorly bluntly angled propodeum with two distinct teeth. Major workers of *Carebara semilaevis* (Mayr), described in its junior synonym *C. hewitti* (Santschi), are characterized by head densely rugulose, except for smooth anteromedian spot on frons, posterior head margin with very shallow emargination, horns absent, propodeal teeth in major and minor absent or reduced to rounded angles; minor workers petiole very short-pedunculate and about as high as long in profile. *Carebara traeghordi* (Santschi), *C. ugandana* (Santschi), and *C. vorax* (Santschi) are described only from minor workers, the former two are defined by head and promesonotal dorsum mostly smooth and shiny, propodeal teeth absent or reduced to blunt angles, the petiole in *C. traeghordi* being more compact than in *C. ugandana*, with shorter peduncle, and petiole node in profile more broadly convex and petiole ventrally convex. The head and body of *C. vorax* minors are covered with punctate-reticulate sculpture except for smooth spot anteromedially on frons and the anterior of pronotum, propodeum with well-developed lamella and teeth at its posterior corners, petiole relatively long-pedunculate.

## Results from DNA sequencing

Phylogenetic analysis with the RAD-seq data produced a resolved, highly supported topology (Figure 2). The phragmotic workers previously identified as *C. elmenteitae* (in Hita Garcia et al. 2009, Hita Garcia et al. 2013; type drawings in Fig. 3) are in a species clade together with minor worker specimens previously identified as *C. thoracica* (type drawings in Fig. 4). Both have ten antennal segments and belong to the new species *C. phragmotica*. In a separate clade, *Carebara thoracica* specimens with nine antennal segments cluster together with *Carebara alluaudi*, which also possesses 9-segmented antennae. The African *C. silvestrii* and the Chinese *Carebara* taxa are more distantly related (which is also reflected in different morphologies).



**Figure 2.** Maximum likelihood tree of sequenced *Carebara* specimens reconstructed with ExaML v3.0.14. Most nodes are supported with more than 0.95% of bootstraps (represented by open circles) except for five, which have between 0.5 and 0.95% support (black circles). The tree shows the division between *C. phragmotica* sp. n. specimens with ten antennal segments (previous IDs: [\*] *C. elmenteitae*, [\*\*] *C. thoracica*) and all other sampled specimens, including *C. thoracica* with nine antennal segments, which are closer related to 9-segmented *C. alluaudi*.

### Notes on phragmotic *Carebara* species

Contradicting the description of *Carebara elmenteitae* as a dealate female (queen) by Patrizi (1948), we agree with Bolton (1995) in his opinion that the described specimen is actually a major worker. As in all other examined phragmotic major workers of this group, ocelli are absent (or vestigial) and the mesosoma is clearly not built for flight, as the sclerites necessary for flying are too small and also they are fused. The drawing of the type specimen shows “scars” where in an alate queen wings would have been attached. Yet, these scars are most likely vestigial and they can be observed in the majors of many other *Carebara* species, including the two species that we describe here. However, it is possible that those specimens are ergatoid queens and that winged queens are either non-existent in this group or haven’t yet been collected or associated. In favor of this explanation could be the observation from the collection of *C. butteli* Forel from Sri Lanka, another, possibly convergently evolved, species with a phragmotic head. The collector, Prof. von Buttel-Reepen, noted that he found eggs, larvae, minor and major workers but no queen in the walnut-sized nest inside a termite mound (Forel 1913). The *C. butteli* type specimen does have a much enlarged gaster (see at AntWeb.org: CASENT0908888), suggesting that it

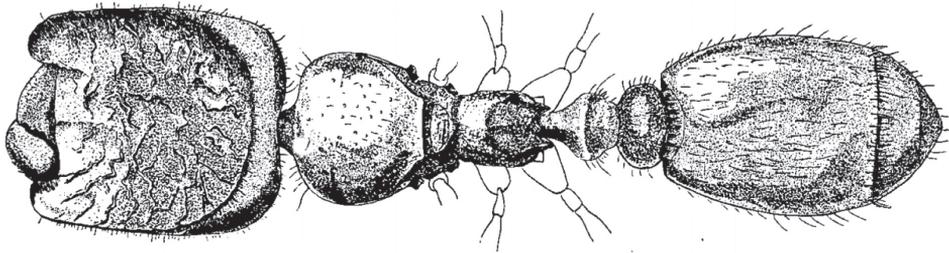


FIG. I.

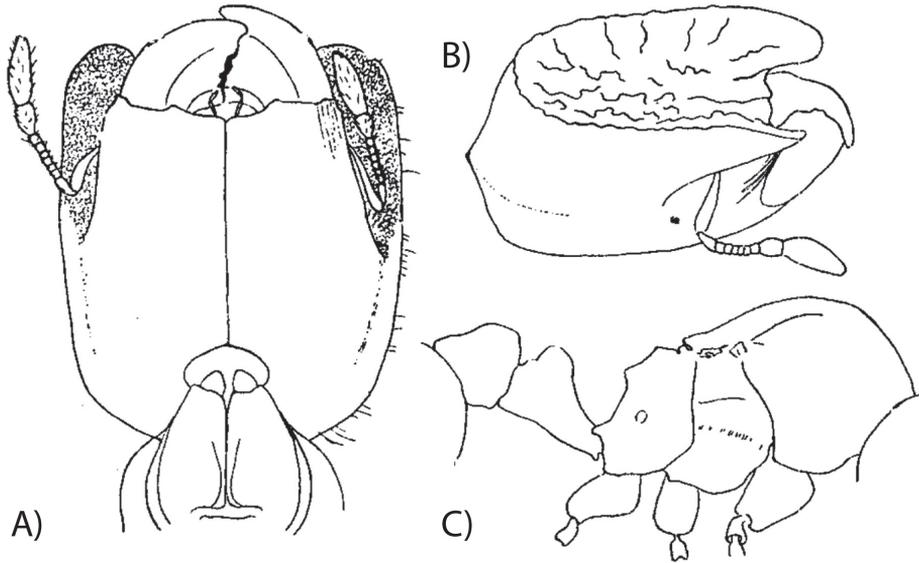
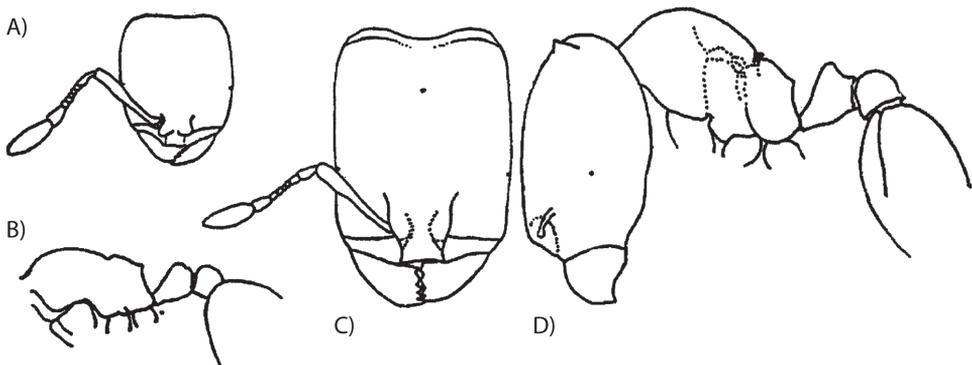


FIG. II.

**Figure 3.** *Carebara elementitae* (Patrizi, 1948) – original drawings. Holotype phragmotic worker (I), dorsal view (II): **A** head in ventral view **B** head in oblique dorsolateral view **C** mesosoma and waist in profile.



**Figure 4.** *Carebara thoracica* (Weber, 1950) – original drawings. **A** minor worker full-face view **B** minor worker profile of body **C** major worker full-face view **D** major worker profile.

is either physogastric, or that the majors are functioning as repletes. More collections and direct observations will be necessary, however, to draw solid conclusions. In order to learn more about species’ caste and worker evolution and their respective behaviours and functions within the colony, future field and laboratory studies ought to make an effort and investigate the ecology of these and other cryptic ant species more closely.

**Identification key for species of the *C. phragmotica* species clade**

**Phragmotic major workers:**

- 1 Head with distinct horns at posterior margin, cypeal margin with anterolateral lobes partly hidden under cephalic shield. Center of cephalic shield either with two highly raised, subparallel ridges, or flat with punctures and cone-shaped, gland-like structures (Figs 5A, 6A) .....2
- Horns on posterior border of head and anterolateral lobes of clypeal margin lacking or invisible in full-face view. Sculpture in center of cephalic shield irregularly rugose, neither flat nor with two raised ridges (Fig. 3I, II) .....  
..... *C. elmenteitae* (Patrizi) (Kenya) [major & minor workers unknown].
- 2 Cephalic shield lobes longer than and covering most of anterolateral lobes of clypeus. Center of cephalic shield flat, punctate and with cone-shaped, gland-like structures (Fig. 5A).....  
..... *C. lilith* sp. n. (Ivory Coast) [major workers unknown]
- Anterolateral lobes of clypeus longer than those of cephalic shield and anteriorly surpassing them. Center of cephalic shield with two highly raised, subparallel ridges (Fig. 6A,B) ..... *C. phragmotica* sp. n. (Kenya)

**Minor workers** (not known for *C. elmenteitae*):

- 1 Head weakly subquadratic to subrectangular (CI 90-93), hind femur short (FI 68–69), postpetiole slightly higher than long (LPpI 76–94) and on average about 1.35 times wider than petiole (PpWI 133-137) (Fig. 5) .....  
..... *C. lilith* sp. n. (Ivory Coast)
- Head subrectangular (CI 84-88), hind femur moderately short (FI 72-78), postpetiole in profile as long as high or slightly longer (LPpI 100-120) and on average about 1.45 times wider than petiole (PpWI 136-150) (Fig. 7).....  
..... *C. phragmotica* sp. n. (Kenya)

## Species accounts

### *Carebara elmenteitae* (Patrizi, 1948)

Fig. 3

*Solenopsis* (*Crateropsis*) *elmenteitae* Patrizi, 1948: 176, figs I, II (s.) KENYA. Holotype (IEGG) (Lake) Elmenteita, 20.xii.1945 (Patrizi) [not examined]. Combination in *Oligomyrmex*: Ettershank 1966: 123; in *Carebara*: Fernández 2004: 235.

**Diagnosis. Phragmotic worker** (minor and major worker unknown): Head with strongly defined oval cephalic shield, anterolaterally with lobes covering antennae when in repose, mandibles small, clypeus with straight anterior margin and median carina, and anterolateral clypeal lobes either absent or hidden under cephalic shield lobes. Dorsal face of cephalic shield concave, with irregular rugulae or shallow ridges.

**Distribution.** This species has not been recorded from any locality other than from the type collection near Lake Elmenteita in central Kenya's Rift Valley.

**Discussion.** We were not able to examine the holotype specimen from the Patrizi collection in Bologna. Thus, we refrain from a detailed re-description of this species within the present publication and defer to larger-scale future *Carebara* revisions. Therefore, we would like to encourage myrmecologists to collect at or near the type locality in Kenya, which will hopefully lead to findings of additional phragmotic specimens and of the undescribed major and minor worker subcastes. It is also unclear if winged queens exist within this specific clade or if maybe the phragmotic workers are actually ergatoid queens. From the drawings phragmotic workers of *C. elmenteitae* can be easily differentiated from those of the new species because of the sculpture inside the cephalic shield: *Carebara elmenteitae* with irregular rugulae or shallow ridges, *C. lilith* punctate and with cone-shaped, gland-like structures present, and *C. phragmotica* with two subparallel, conspicuously elevated ridges in center of cephalic shield (see also *C. lilith* and *C. phragmotica* diagnoses and discussions).

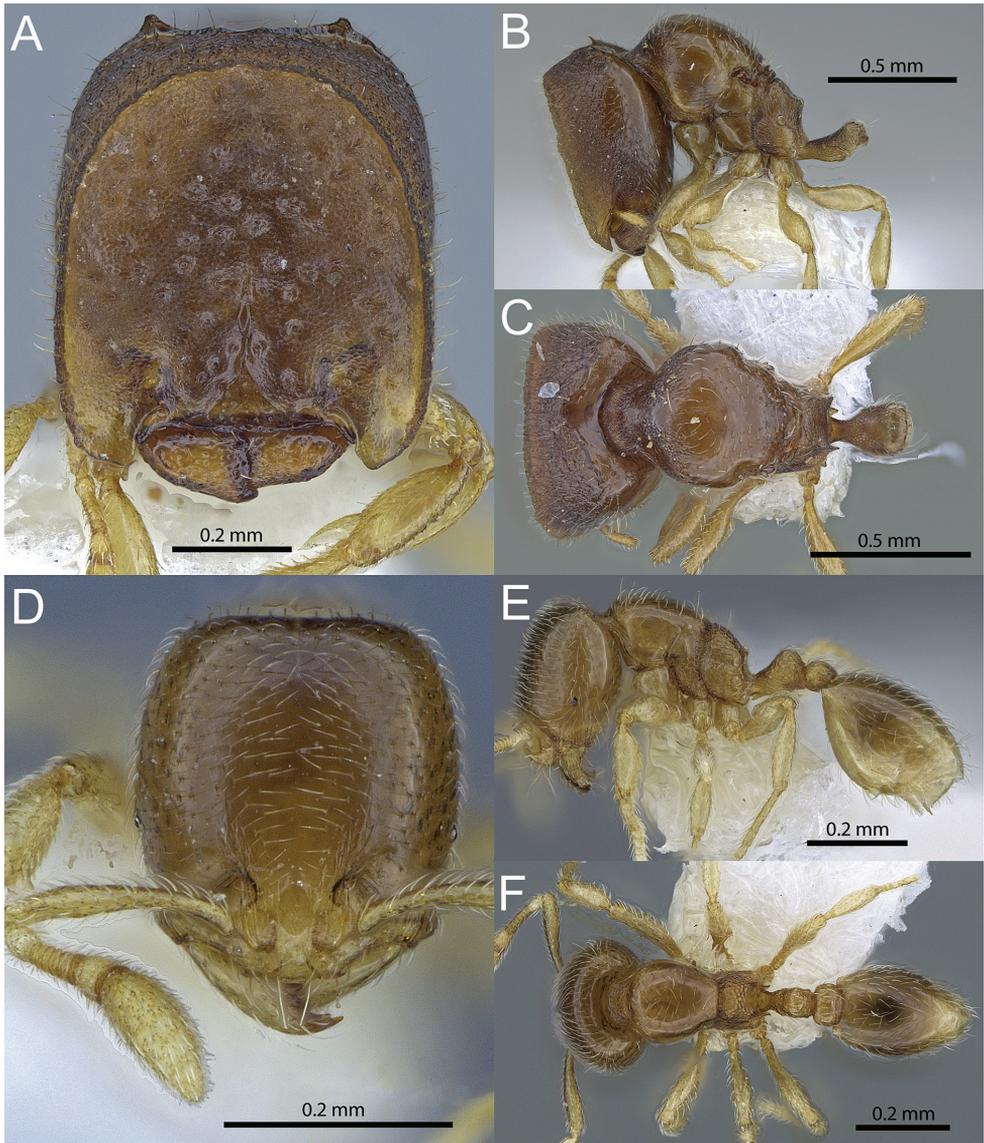
### *Carebara lilith* Fischer, Azorsa & Hita Garcia, sp. n.

<http://zoobank.org/F797ED46-6335-4C4B-AD19-82CD354BF6CA>

Figs 1C, D, 5

**Holotype.** (major worker), IVORY COAST, Grégbeu, 06.8°, -006.717°, 06.x.1980 (*V. Mahnert* & *J.-L. Perret*) (CASENT0709545), (MHNG). **Paratypes:** 2 minor workers (CASENT0709546, CASENT0709547), same data as holotype (MHNG).

**Diagnosis. Phragmotic worker:** Anterolateral lobes of clypeus small, shorter than and ending well before anterior margin of lateral shield lobes, sculpture on cephalic shield simple, punctate and with cone-shaped, gland-like structures present. **Major worker:** unknown. **Minor worker:** Head weakly subquadrate to subrectangular (CI 90-93), hind femur short (FI 68-69), postpetiole slightly higher than long (LPpI 76-94) and on average about 1.35 times wider than petiole (PpWI 133-137).



**Figure 5.** *Carebara lilith* sp. n. Phragmotic worker (holotype: CASENT0709545). **A** head in full-face view **B** body in profile view **C** body in dorsal view. Minor worker (paratype: CASENT0709546) **D** head in full-face view **E** body in profile view **F** body in dorsal view.

**Description of phragmotic major worker.** Measurements (n=1): HW 0.65, HL 0.75, SL 0.18, MDL 0.23, EL 0.01, WL 0.73, PNW 0.47, PTL 0.25, PPL -/-, PTH 0.17, PPH -/-, PTW 0.17, PPW -/-, PSL 0.06, MFL 0.36, MTL 0.29, CI 86, SI 28, MDI 35, EI 1, FI 56, PS LI 9.

Head in full-face view modified, phragmotic with a distinct, concave and oval cephalic shield with two forward-extending, semi-transparent anterolateral lobes, in-

side of cephalic shield in oblique frontolateral view deeply concave and with a sharply raised margin. Head shape in full-face view subrectangular, longer than wide (CI 86), posterior of cephalic shield with rounded posterolateral corners and small horns lateral of shallowly V-shaped posterior emargination. Mandibles reduced and compact (MDI 35). Anterior margin of clypeus straight, widely emarginate and with short anterior lobes lateral of mandibles, which are considerably shorter than and ending before anterior margin of anterolateral cephalic shield lobes. In profile, head anteriorly straight at the truncated cephalic shield margin, short antennal scrobe present ventrally, shielding scrobe and funiculus. Antennae ten-segmented, short, with reduced scape (SI 28), apical funicular segment about as long as the remaining segments combined. Eyes strongly reduced, consisting of one small ommatidium (EI 1), situated at the posterior end of scrobe.

In profile view, promesonotum high and convex, posteriorly roundly sloping towards a very short, partly fused scutellum, both together in dorsal view remotely resembling a diamond-shaped shield. Promesonotal suture absent or inconspicuous, posterior of scutellum a similar-sized, isolated, metanotum present. Propodeal dorsum in profile moderately short, weakly concave towards the short-triangular posterior teeth, posterior declivity almost vertical, with very narrow lamella, propodeal lobe well developed. Propodeal lobes weakly triangular. Propodeal spiracle circular, situated centrally at lateropropodeum.

Petiole in profile with long peduncle, ventrally weakly convex, posterior of small anterior, tooth-like subpetiolar process, the node weakly nodiform or very broadly wedge-shaped, dorsally rounded, anteriorly and posteriorly very weakly concave, in anterodorsal view very weakly convex, almost transverse. Holotype with postpetiole and gaster missing.

Mandibles, clypeus and most of the face finely shagreened, the interior of cephalic shield with many small, cone-shaped, gland-like structures present, posterior portion of clypeus with a short longitudinal carina. Sides of head, lateral to cephalic shield, with weakly reticulate-punctate sculpture, posterior of cephalic shield, towards posterior head margin, longitudinal, weakly reticulate, rugulae present, posterior head margin, between horns, weakly carinate. Ventral side of head smooth and shiny. Promesonotum, anepisternum, katepisternum, propodeal declivity and dorsum of petiole node mostly smooth and shiny; punctures present only at anterolateral promesonotum, at sides and dorsum of propodeum and remainder of petiole, lateropropodeum, below spiracle and near its base, with few longitudinal rugulae.

Lateral and posterior portions of head mostly with short and relatively stout, erect-suberect hairs, no hairs on cephalic shield visible. Mesosoma and petiole node dorsum with abundant, fine, relatively short and mostly decumbent pilosity, plus some longer, subdecumbent to suberect hairs on mesosoma and petiole. Scape and tibia pilosity short, appressed to decumbent. Color light reddish brown, antennae and legs lighter colored.

**Description of minor workers.** Measurements (n=2): HW 0.29–0.30, HL 0.32–0.33, SL 0.21–0.22, MDL 0.18–0.19, EL 0.02, WL 0.32, PNW 0.19, PTL 0.10–0.13, PPL 0.06, PTH 0.09–0.10, PPH 0.06–0.08, PTW 0.07, PPW 0.09–0.10,

PSL 0.04–0.05, MFL 0.20, MTL 0.16–0.17, CI 90–93, SI 73–73, MDI 62–63, EI 5, FI 68–69, PSLI 13–15, LPpI 76–94, DPpI 150–163, PpWI 133–137, PpLI 47–62, PpHI 71–81.

Head longer than wide (CI 90–93), in full-face view weakly subquadrate to subrectangular, with convex sides, posterior head margin straight or very weakly concave medially. Clypeus faintly bicarinate, anterior margin medially very weakly convex or almost transverse. Frontal carinae inconspicuous. Antennae with ten segments, scapes ending before posterior head margin (SI 72–73). Eyes present, consisting of one ommatidium and situated anterior of cephalic midline (EI 5).

In profile view, promesonotum convex, metanotal groove impressed. Propodeum in profile higher than long, weakly convex and declining towards short, acute, weakly triangular posterior teeth, posterior declivity oblique with a narrow lamella and well developed, triangular propodeal lobes. Propodeal spiracle circular, situated just below posterior teeth and very close to posterolateral border of propodeum.

In profile, petiole with moderately short peduncle, ventrally with convex bulge and acute anterior tooth, dorsal face of petiole node more or less convex to weakly wedge-shaped. Postpetiole about as long as high, distinctly lower than petiole (PpHI 71–81), convex dorsally, weakly convex ventrally. In dorsal view petiole node slightly wider than long, postpetiole on average 1.3 times wider than petiole (PpWI 133–137) with sides tapering anteriorly.

Mandibles and clypeus smooth and shiny. Face smooth and shiny, near antennal insertion with few weak, concentric rugulae. Promesonotum, postpetiole dorsum and gaster smooth and shiny, metapleuron, propodeum and petiole with large, partly e-faceted areolae, propodeal declivity largely smooth and shiny.

Whole body with abundant, relatively short, decumbent pilosity. Clypeus and mesosoma with few longer, suberect hairs present. Scapes and tibiae with short, decumbent pilosity. Color light brown with yellowish antennae and legs.

**Distribution.** So far, this species is only known from the type locality, although it's most likely present in unsorted and/or unidentified material in other collections with African ants, possibly collected without phragmotic workers.

**Discussion.** No ecological or collection data exist for this species. Without the phragmotic major worker, *Carebara lilith* can easily be confused with similar *Carebara* species from the former genus *Oligomyrmex*, as for example *C. thoracica*, from which it can be distinguished by possessing ten instead of nine antennal segments. Phragmotic workers of *C. lilith* are differentiated from those of *C. phragmotica* and *C. elmenteitae* by the character combination given in the diagnosis. Morphological differences between minor workers of *C. lilith* and *C. phragmotica* are not very significant and may decrease even more with larger sample sizes. Especially for the former species, more material is needed for a better resolution of intra- and interspecific variability. Phragmotic workers may be necessary for definitive identifications, but it seems likely that the three species do not co-occur biogeographically.

**Etymology.** This species is named after the Hebrew name Lilith, a female demon in Jewish mythology. The name is a noun in apposition and thus invariant.

***Carebara phragmotica* Fischer, Azorsa & Hita Garcia, sp. n.**

<http://zoobank.org/F36AF74C-CE29-4B5B-9338-DF39C10006CB>

Figs 1A, B, 6, 7

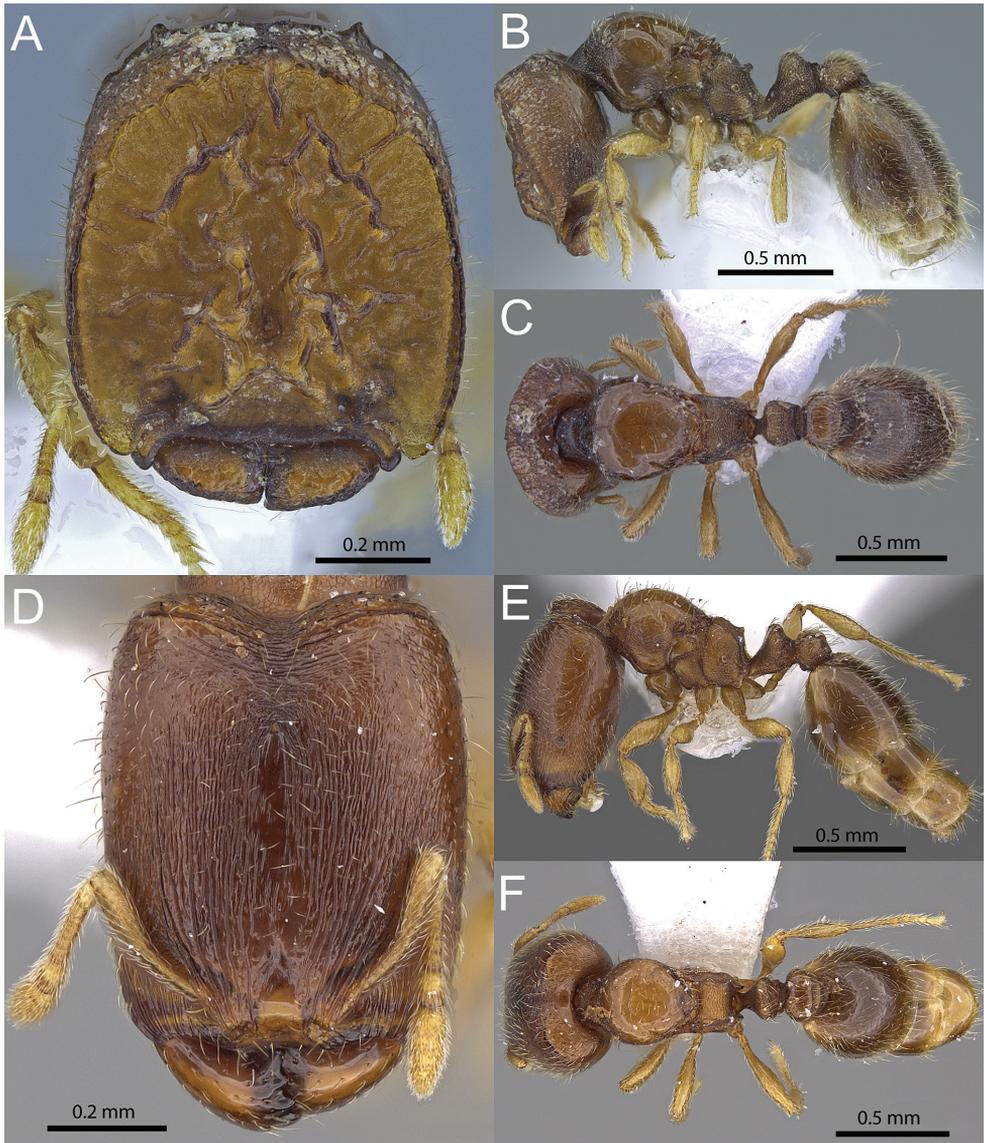
**Holotype.** phragmotic worker, KENYA, Kakamega Forest, Colobus trail, 000.3551389°, 34.8583611°, 1650m, rainforest, leaf litter, 14.vi.2007 (*M. Peters*) (ZFMK: CASENT0709551).

**Paratypes.** 3 major workers (same data as holotype) (CASENT0906158, CASENT0709548, CASENT0709549); 2 phragmotic workers, Kakamega Forest, Kaimosi fragment, 00.128°, 034.84°, 1600m, rainforest, leaf litter, 04.viii.2008 (*G. Fischer*) (CASENT0709550, CASENT0738556); 1 phragmotic worker, Kakamega Forest, Malawa fragment, 00.4617889°, 034.8587333°, 1650m, rainforest, leaf litter, viii.2007 (*F. Hita Garcia*) (CASENT0277301); 1 phragmotic worker, Kakamega Forest, Yala, 0.202°, 34.868°, 1650m, rainforest, leaf litter, v.2008 (*M. Peters*) (CASENT0738559); 2 major workers, Kakamega Forest, Malawa fragment, 00.4543611°, 034.8635556°, rainforest, leaf litter, 01.ix.2005 (*G. Fischer*) (CASENT0709552, CASENT0709553); 5 minor workers, 1 phragmotic worker, Kakamega Forest, Kisere fragment, 0.385278°, 34.892417°, 1650m, rainforest, leaf litter, 25.xi.2005 (*G. Fischer*) (CASENT0709554, CASENT0709555, CASENT0709556, CASENT0709557, CASENT0709558); 2 minor workers, Kakamega Forest, Salazar, 00.3266667°, 034.8707222°, 1650 m, rainforest, leaf litter, 09.iii.2009 (*M. Peters*) (CASENT0709559, CASENT0217819); 2 minor workers, Kakamega Forest, Isecheno, 00.235°, 34.869°, 1650m, rainforest, leaf litter, 28.viii.2007 (*F. Hita Garcia*) (CASENT0709560, CASENT0709561); 2 major, 2 minor workers, Kakamega Forest, Kisere fragment (CASENT0709594, CASENT0709595, CASENT0709596, CASENT0709597).

**Diagnosis. Phragmotic worker:** Cephalic shield with two subparallel, conspicuously elevated ridges in its center, in profile distinctly elevated above the rim of the shield. Anterolateral lobes of cephalic shield shorter than and ending before anterior border of clypeal lobes. **Major worker:** Frons and anterior sides of head with abundant, narrow longitudinal rugulae, near posterior head margin a few irregular, weakly defined, transverse rugulae present. **Minor worker:** Head subrectangular (CI 84–88), hind femur moderately short (FI 72–78), postpetiole as long as high or longer (LPpI 100–120) and on average about 1.45 times wider than petiole (PpWI 136–150).

**Description of phragmotic major workers.** Measurements (n=3): HW 0.68–0.71 (0.70), HL 0.76–0.79 (0.78), SL 0.15–0.23 (0.21), MDL 0.14–0.20 (0.17), EL 0.02, WL 0.78–0.79 (0.79), PNW 0.48–0.50 (0.49), PTL 0.32–0.33 (0.32), PPL 0.15–0.18 (0.17), PTH 0.21–0.22 (0.22), PPH 0.19–0.20 (0.19), PTW 0.20–0.21 (0.21), PPW 0.26–0.27 (0.26), PSL 0.10–0.12 (0.11), MFL 0.39–0.41 (0.40), MTL 0.31–0.32 (0.31), CI 89–90 (89), SI 21–34 (30), MDI 20–28 (24), EI 2, FI 57–59 (58), PSLI 14–17 (15), LPpI 80–96 (88), DPpI 142–170 (156), PpWI 126–129 (127), PpLI 48–55 (52), PpHI 86–90 (88).

Head in full-face view almost as wide as long (CI 89–90), with a phragmotic cephalic shield, outline of shield oval, sharply margined, anterolaterally with short,



**Figure 6.** *Carebara phragmotica* sp. n. Phragmotic worker (paratype: CASENT0709550). **A** head in full-face view **B** body in profile view **C** body in dorsal view. Major worker (paratype: CASENT0906158) **D** head in full-face view **E** body in profile view **F** body in dorsal view.

semi-transparent lobes. Anterior margin of clypeus widely transverse with anteriorly projecting lateral lobes, slightly surpassing the laterally overlapping lobes of the shield.

Cephalic shield with two wavy, sub-parallel, raised ridges centrally, surrounded by several radiating and irregular, shorter canyons and ridges. The inside of the cephalic shield is normally covered with a layer of dirt. Mandibles small, compact (MDI 20–28), and when tightly closed partly hidden under anteriorly projecting clypeus and ce-

phalic shield. Head-shape in profile anteriorly straight along border of cephalic shield, the head appearing like a thick, anteriorly flattened door or plug. Antennal scrobe hidden under anterolateral lobes of cephalic shield. Antennae ten-segmented, short, with reduced scape length as compared to other major workers (SI 21–34), in full-face view largely hidden under cephalic shield. Eyes strongly reduced, consisting of one small ommatidium (EI 2), situated at the posterior end of scrobe.

In profile view, promesonotum high and convex, posteriorly sloping linearly towards a short, separated or anteriorly fused, posteriorly sharply margined scutellum, in dorsal view comparable to a polished convex shield. Promesonotal suture absent or inconspicuous, scutellum small and weakly to not isolated in dorsal view, metanotum present as a small bump extending dorsally between propodeum and scutellum, metanotal groove narrowly impressed. Propodeal dorsum short, anteriorly convex, with a blunt angle halfway towards the short, bluntly triangular to rounded propodeal teeth, posterior declivity oblique, with short and narrow lamella and well developed, lamellate propodeal lobe. Propodeal spiracle circular, situated close to center of lateropropodeum.

Petiole in profile with relatively short peduncle, ventrally straight to weakly convex, with small to reduced anterior tooth, lateroventral margins posteriorly with very thin, elongate lamellae present, the node sub-triangular and dorsally rounded to very broadly wedge-shaped, postpetiole in profile higher than long (LPpI 80–96) and almost as high as petiole (PpHI 86–96), its dorsum convex and with a very short ventral face. In dorsal view, petiole node shape transversely oval, wider than long and posteriorly flattened, postpetiole wider than long, suboval, anterior margin concave and posterior margin almost straight, about 1.3 times wider than petiole (PpWI 126–129).

Mandibles, clypeus and most of the cephalic shield finely shagreened, highest areas on central ridges smooth and shiny. Posterior of cephalic shield, near head margin, shagreening overlain with weakly reticulate rugulae, posterior head margin with weakly raised carina and small horns present at posterolateral corners. Ventral side of head very finely and obliquely striate. Promesonotum, parts of anepisternum and katepisternum, postpetiole dorsum and gaster mostly smooth and shiny, remainder of body punctate.

Lateral and posterior portions of head with very short, erect to suberect hairs, no visible hairs on cephalic shield, mesosoma with relatively sparse, fine, relatively short, decumbent pilosity, and few longer, subdecumbent to suberect fine hairs. Waist segments and gaster covered with very abundant pilosity, apical segments of gaster also with many suberect, long standing hairs. Scape and tibiae pilosity abundant and decumbent. Color light or reddish brown, antennae, legs and parts of gaster, yellow.

**Description of major workers.** Measurements (n=5): HW 0.59–0.63 (0.61), HL 0.74–0.78 (0.76), SL 0.27–0.30 (0.29), MDL 0.32–0.34 (0.33), EL 0.03, WL 0.64–0.67 (0.65), PNW 0.36–0.39 (0.37), PTL 0.26–0.27 (0.26), PPL 0.14–0.17 (0.15), PTH 0.19–0.20 (0.21), PPH 0.17–0.19 (0.18), PTW 0.18–0.20 (0.19), PPW 0.23–0.25 (0.23), PSL 0.08–0.10 (0.09), MFL 0.34–0.38 (0.36), MTL 0.26–0.29 (0.28), CI 79–82 (80), SI 46–49 (47), MDI 53–55 (54), EI 5, FI 58–62 (59), PSLI 13–15 (14), LPpI 80–92 (87), DPpI 141–160 (152), PpWI 119–133 (126), PpLI 56–61 (58), PpHI 86–96 (90).



**Figure 7.** *Carebara phragmotica* sp. n. Minor worker (paratype: CASENT0709554). **A** head in full-face view **B** body in profile view **C** body in dorsal view.

Head in full-face view rectangular, about 1.25 times longer than wide (CI 79–82), sides subparallel, posterior margin with transverse carina present on either side of narrow, evenly concave median emargination, horns small and obtuse, posterolateral corners rounded. Mandibles triangular, about half as long as head width, masticatory margin with five teeth including basal tooth. Frontal carinae absent or inconspicuous. Anterior margin of clypeus concave medially and laterally on either side of median concavity. Frons sometimes with median ocellus present. Antennae with ten segments. Scapes short and when laid back not surpassing cephalic midlength (SI 46–49). Eyes present, consisting of one relatively large ommatidium (EI 5).

In profile, pronotum high and convex, posteriorly declining linearly, propodeum distinctly higher than long, dorsal face obliquely declining, posterior corners either edentate and angulate or with very small triangular teeth, posterior declivity nearly vertical with very shallow lamella, propodeal lobes relatively small. Pronotum in dorsal view strongly rounded and almost circular, pronotal suture on dorsum inconspicuous or present as weak impression, scutellum very small and often fused with pronotum, metanotal groove present, barely or not impressed, propodeal spiracle circular, situated almost at center of lateropropodeum.

Petiole in profile with short peduncle, almost subtriangular in shape, posteroventrally weakly convex, anteriorly with small tooth or subpetiolar process. Petiole node dorsally flat to weakly convex, in dorsal view much wider than long, its posterior end well defined to weakly marginate. Postpetiole in profile higher than long (LPpI 80–92), almost as high as petiole (PpHI 86–96), convex dorsally, and with a small, angulate

ventral process. In dorsal view postpetiole wider than long, suboval with rounded sides, on average 1.3 times wider than petiole (PpWI 119–133).

Mandibles smooth and shiny with scattered, short, appressed pilosity and weak, short rugulae laterally near their bases. Head with very fine and densely packed longitudinal striations, near posterior margin replaced by irregular transverse rugulae, with a conspicuously raised and transversely curved carina laterally of median concavity. Mesosoma and petiole mostly weakly punctate, except for smooth and shiny promesonotal dorsum and parts of lateropronotum, with rugosities at junctions of pronotum, anepisternum, katepisternum and metapleuron. Postpetiole dorsally smooth and shiny, its remainder punctate. Gaster smooth and shiny.

Head and body with abundant, moderately long, decumbent to subdecumbent pubescence and with few suberect hairs. Scape and tibia pilosity abundant and decumbent. Color brown to light brown, antennae, legs and parts of gaster, slightly lighter.

**Description of minor workers.** Measurements (n=5): HW 0.32–0.34 (0.33), HL 0.38–0.39 (0.38), SL 0.23–0.25 (0.24), MDL 0.20–0.28 (0.23), EL 0.02, WL 0.38–0.39 (0.39), PNW 0.21–0.23 (0.22), PTL 0.14–0.16 (0.15), PPL 0.08–0.09 (0.08), PTH 0.11–0.12 (0.12), PPH 0.08–0.09 (0.08), PTW 0.08–0.09 (0.08), PPW 0.11–0.12 (0.11), PSL 0.05, MFL 0.24–0.26 (0.25), MTL 0.19–0.20 (0.19), CI 84–88 (86), SI 70–75 (73), MDI 60–82 (69), EI 4–7 (6), FI 72–78 (74), PSLI 13–15 (14), LPpI 100–120 (105), DPpI 133–150 (140), PpWI 136–150 (145), PpLI 53–61 (56), PpHI 67–74 (70).

Head longer than wide (CI 84–88), in full-face view weakly subrectangular, sides convex, posterior margin nearly straight to faintly convex. Anterior margin of clypeus straight medially, weakly bicarinate, and narrow between antennal insertions. Frontal carinae very weakly developed, ending at or before eye level. Antenna with ten segments, scapes, when laid back, ending well before posterior head margin (SI 70–75). Eyes small (EI 4–7), consisting of one ommatidium, situated slightly anterior to cephalic midline.

In profile, promesonotum convex, metanotal groove impressed, propodeal dorsum weakly convex, shorter than posterior declivity, declining posteriorly towards small, acute to bluntly triangular, lamellate propodeal teeth, declivity of propodeum oblique, with narrow lamella connecting the teeth and relatively large propodeal lobes. Propodeal spiracle circular and situated close to posterior border of propodeum, just below the propodeal teeth.

Petiole in profile view with a short peduncle, with a convex ventral bulge, anteriorly with a short, triangular tooth, petiole node dorsally roundly subtriangular. Postpetiole relatively short and low, as long as high or slightly longer (LpPI 100–120), lower than petiole (PpHI 67–74), dorsum convex. In dorsal view petiole node about as wide as long, postpetiole subrectangular with rounded corners, about 1.45 times wider than petiole (PpWI 136–150).

Mandibles and clypeus smooth and shiny. Face smooth and shiny, near antennal insertion with weak concentric carinae, frontal carinae very short and reduced. Promesonotum, postpetiole dorsum, most of propodeal declivity, and gaster smooth and shiny, metapleuron, propodeum and petiole areolate.

Whole body with abundant, relatively short, decumbent pilosity. Clypeus and body with very few erect to suberect hairs present. Scape pilosity short, decumbent to subdecumbent, tibia pilosity mostly decumbent. Color orange to light brown, with yellowish antennae and legs.

**Distribution.** This species is known only from Kakamega Forest and its smaller fragments in the north (Malawa and Kisere Forest) and in the south (Kaimosi Forest), in the Western Province of Kenya.

**Discussion.** *Carebara phragmotica* is different from *Carebara elmenteitae* (Patrizi), which was collected in leaf-litter on the banks of the river Kariandus near Lake Elmenteita in the Great Rift Escarpment in Kenya (Patrizi 1948), and that has been described from a single phragmotoc worker. Interestingly the specimen was identified as a queen, but according to its' description and the drawing it very closely resembles the phragmotoc major workers of *C. phragmotica* sp. n. and *C. lilith* sp. n. Despite their high morphological similarity, the phragmotoc majors of these three species can be separated from each other by the following head characters: horns at posterior head margin and anterolateral lobes of clypeus lacking or indistinct in *C. elmenteitae* versus horns distinct in *C. phragmotica* and *C. lilith*, and the clypeal lobes anteriorly surpassing the lateral lobes of the relatively small cephalic shield lobes in *C. phragmotica* versus the cephalic shield lobes significantly larger and anteriorly surpassing the clypeal lobes in *C. lilith*. Finally, the cephalic shield in *C. elmenteitae* contains low, irregular rugulae or ridges, versus the two highly raised, subparallel central ridges, that, in profile, are distinctly higher than the rim of the cephalic shield in *C. phragmotica*, whereas *C. lilith* is characterized by a mostly flat cephalic shield surface without ridges or canyons, instead with punctures and small, cone-shaped structures present.

Minor workers of *C. phragmotica* can be differentiated from those of *C. lilith* by the characters listed in the diagnosis (see discussion of *C. lilith*). Minor and major workers of *C. phragmotica* can be distinguished from those of *C. thoracica* by their antennal segmentation (ten segments in *phragmotica* versus nine in *thoracica*).

**Etymology.** This species' name is derived from the modified head morphology of the phragmotoc major workers.

## Acknowledgements

We would like to thank the subject editor Marek Borowiec and two anonymous reviewers for their valuable comments on an earlier version of the manuscript. We are very grateful to Dr. Giulio Cuccodoro and Dr. Bernard Landry, curators at MHNG, for the loan of *C. lilith* specimens and to Dr. Ralph Peters for loaning us material of *C. phragmotica* from the ZMFK ant collection. Furthermore, we acknowledge the help of John Deyrup, Jo Ann Tan, Masako Ogasawara, Mandy Tin, and Miguel Grau Lopez for help with specimen, molecular, or informatics work. This work was supported by subsidy funding to OIST and the first author's visit to MHNG was funded by an Ernst Mayr travel grant.

## References

- Azorsa F, Fisher BL (in revision) The ant genus *Carebara* Westwood (Hymenoptera: Formicidae) in the Malagasy Region. *Zootaxa*.
- Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresco WA, Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* 3(10): e3376. doi: 10.1371/journal.pone.0003376
- Belshaw R, Bolton B (1994) A new myrmicine ant genus from cocoa leaf litter in Ghana (Hymenoptera: Formicidae). *Journal of Natural History* 28: 631–634. doi: 10.1080/00222939400770291
- Bharti H, Kumar R (2013) Six new species of *Carebara* Westwood (Hymenoptera: Formicidae) with restructuring of world species groups and a key to Indian species. *Journal of Entomological Research Society* 15: 47–67.
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, btu170. doi: 10.1093/bioinformatics/btu170
- Bolton B (1994) Identification guide to the ant genera of the world. Harvard University Press, Cambridge, Mass., 222 pp.
- Bolton B (1995) New general catalogue of the ants of the world. Harvard University Press, Cambridge, Mass., 504 pp.
- Bolton B (2003) Synopsis and Classification of Formicidae. *Memoirs of the American Entomological Institute* 71: 1–370.
- Bolton B (2014) An online catalog of the ants of the world. <http://antcat.org> [accessed 06 April 2015]
- Bolton B, Belshaw R (1993) Taxonomy and biology of the supposedly lestobiotic ant genus *Paedalgus* (Hymenoptera: Formicidae). *Systematic Entomology* 18: 181–189. doi: 10.1111/j.1365-3113.1993.tb00661.x
- Brandão CRF, Diniz JLM, Silva PR, Albuquerque NL, Silvestre R (2001) The first case of intranidal phragmiosis in ants. The ergatoid queen of *Blepharidatta conops* blocks the entrance of the brood chamber. *Insectes Sociaux* 48: 251–258. doi: 10.1007/PL00001774
- Bystrykh LV (2012) Generalized DNA Barcode Design Based on Hamming Codes. *PLoS ONE* 7(5): e36852. doi: 10.1371/journal.pone.0036852
- Eaton DAR (2014) PyRAD: assembly of *de novo* RADseq loci for phylogenetic analyses. *Bioinformatics* 30 (13): 1844–1849. doi: 10.1093/bioinformatics/btu121
- Emery C (1924) (“1922”) Hymenoptera. Fam. Formicidae. Subfam. Myrmicinae. [concl.]. *Genera Insectorum* 174C: 207–397.
- Ettershank G (1966) A generic revision of the world Myrmicinae related to *Solenopsis* and *Pheidologeton*. *Australian Journal of Zoology* 14: 73–171. doi: 10.1071/ZO9660073
- Fernández F (2004) The American species of the myrmicine ant genus *Carebara* Westwood. *Caldasia* 26: 191–238.
- Fernández F (2006) A new species of *Carebara* Westwood and taxonomic notes on the genus. *Revista Colombiana de Entomología* 32: 97–99.
- Fernández F (2010) A new species of *Carebara* from the Philippines with notes and comments on the systematics of the *Carebara* genus group (Hymenoptera: Formicidae: Myrmicinae). *Caldasia* 32: 191–203.

- Forel A (1913) Ameisen aus Sumatra, Java, Malacca und Ceylon. Gesammelt von Herrn. Prof. Dr. v. Buttel-Reepen in der Jahren 1911–1912. Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere 36: 1–148.
- Fischer G, Azorsa F, Fisher BL (2014) The ant genus *Carebara* Westwood (Hymenoptera, Formicidae): synonymisation of *Pheidologeton* Mayr under *Carebara*, establishment and revision of the *C. polita* species group. ZooKeys 438: 1–57. doi: 10.3897/zookeys.438.7922
- Harris RA (1979) A glossary of surface sculpturing. California Department of Food and Agriculture, Bureau of Entomology 28: 1–31.
- Hita Garcia F, Fischer G, Peters MK, Snelling RR, Wägele JW (2009) A preliminary checklist of the ants (Hymenoptera: Formicidae) of Kakamega Forest (Kenya). Journal of East African Natural History 98: 147–165. doi: 10.2982/028.098.0201
- Hita Garcia F, Wiesel E, Fischer G (2013) The Ants of Kenya (Hymenoptera: Formicidae) – Faunal overview, first species checklist, bibliography, accounts for all genera, and discussion on taxonomy and zoogeography. Journal of East African Natural History 102: 127–222. doi: 10.2982/028.101.0201
- Hölldobler B, Wilson EO (1990) The ants. Cambridge, Mass.: Harvard University Press, xii + 732 pp. doi: 10.1007/978-3-662-10306-7
- Kozlov AM, Aberer AJ, Stamatakis A (2015) ExaML Version 3: A Tool for Phylogenomic Analyses on Supercomputers. Bioinformatics, btv184. doi: 10.1093/bioinformatics/btv184
- Patrizi S (1948) Contribuzioni alla conoscenza delle formiche e dei mirmecofili dell’Africa orientale. 6. *Crateropsis elmenteitae*, nuovo sottogenere aberrante di *Solenopsis* Westw. Bollettino dell’Istituto di Entomologia della Università di Bologna 17: 174–176.
- Sharaf MR, Aldawood AS (2013) The ant genus *Carebara* Westwood in the Arabian Peninsula (Hymenoptera, Formicidae). Zookeys 357: 67–83. doi: 10.3897/zookeys.357.5946
- Sheela S, Narendran TC (1997) A new genus and a new species of Myrmicinae (Hymenoptera: Formicidae) from India. Journal of Ecobiology 9: 87–91.
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. doi: 10.1093/bioinformatics/btu033
- Terayama M, Lin C, Eguchi K (2012) Additions to knowledge of the ant fauna of Taiwan (Hymenoptera, Formicidae, Solenopsidini): genera *Anillomyrma* and *Carebara*. Japanese Journal of Systematic Entomology 18: 1–6.
- Tin MMY, Economo EP, Mikheyev AS (2014) Sequencing Degraded DNA from Non-Destructively Sampled Museum Specimens for RAD-Tagging and Low-Coverage Shotgun Phylogenetics. PLoS ONE 9: e96793. doi: 10.1371/journal.pone.0096793
- Tin MMY, Rheindt FE, Cros E, Mikheyev AS (2015) Degenerate adaptor sequences for detecting PCR duplicates in reduced representation sequencing data improve genotype calling accuracy. Molecular Ecology Resources 15: 329–336. doi: 10.1111/1755-0998.12314
- Ward PS, Brady SG, Fisher BL, Schultz TR (2015) The evolution of myrmicine ants: phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera: Formicidae). Systematic Entomology 40: 61–81. doi: 10.1111/syen.12090
- Weber NA (1950) The African species of the genus *Oligomyrmex* Mayr (Hymenoptera, Formicidae). American Museum Novitates 1442: 1–19.

- Weber NA (1952) Studies on African Myrmicinae, I (Hymenoptera, Formicidae). American Museum Novitates 1548: 1–32.
- Wheeler DE, Hölldobler B (1985) Cryptic phragmosis: the structural modifications. *Psyche: A Journal of Entomology* 92: 337–353. doi: 10.1155/1985/76848
- Westwood JO (1840) Observations on the genus *Typhlopone*, with descriptions of several exotic species of ants. *Annals and Magazine of Natural History* 6: 81–89. doi: 10.1080/03745484009443610
- Wilson EO (1955) A monographic revision of the ant genus *Lasius*. *Bulletin of the Museum of Comparative Zoology* 113: 1–201.



# A new species of *Themus* (*Themus*) Motschulsky from Yunnan, China and a redescription of *T. (T.) testaceicollis* Wittmer, 1983 (Coleoptera, Cantharidae)

Junyan Su<sup>1</sup>, Limei Li<sup>1</sup>, Yuxia Yang<sup>1</sup>, Xingke Yang<sup>2</sup>

**1** *The Key Laboratory of Zoological Systematics and Application, College of Life Sciences, Hebei University, Baoding 071002, Hebei Province, China* **2** *Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China*

Corresponding author: *Yuxia Yang* ([yxyang@hbu.edu.cn](mailto:yxyang@hbu.edu.cn))

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## Abstract

A new species is described, *Themus* (*Themus*) *dimorphus* **sp. n.** from Yunnan, China. *Themus* (*Themus*) *testaceicollis* Wittmer, 1983 is redescribed and compared with the new species. The two species are illustrated with habitus and genitalia of both sexes and abdominal sternites VIII of female.

## Keywords

Taxonomy, Cantharidae, *Themus*, new species, China

## Introduction

The genus *Themus* Motschulsky, 1858 was divided into four subgenera (Wittmer 1973, 1997), whose diagnoses were redefined by Švihla (2008). The nominotypical subgenus includes more than 100 species widely distributed in the Oriental and eastern Palaearctic regions (Švihla 2008). In China, approximately 50 species of this subgenus are known, and most species were described or revised by Wittmer (1983).

In recent study, a new species was discovered from Yunnan, China and is described here under the name of *Themus* (*Themus*) *dimorphus* sp. n. It is similar to *T. (T.) testaceicollis* Wittmer, 1983, which is redescribed in the present study to make comparisons with the new species.

## Material and methods

The types or other specimens are deposited in the following collections:

<b>IZAS</b>	Institute of Zoology, Chinese Academy of Sciences, Beijing, China;
<b>MHBU</b>	Museum of Hebei University, Baoding, China;
<b>MNHN</b>	Muséum national d'Histoire naturelle, Paris, France;
<b>NHMB</b>	Naturhistorisches Museum Basel, Switzerland.

The studied method and description format follow that of Yang et al. (2014). Morphological terminology of female genitalia follows that of Brancucci (1980) and abbreviations in the figures are listed as follows: **ag** accessory gland; **co** coxite; **di** diverticulum; **tg9** abdominal tergite IX; **sp** spermatheca; **ov** median oviduct; **va** vagina; **sr** sclerotized bracket or ridge.

## Taxonomy

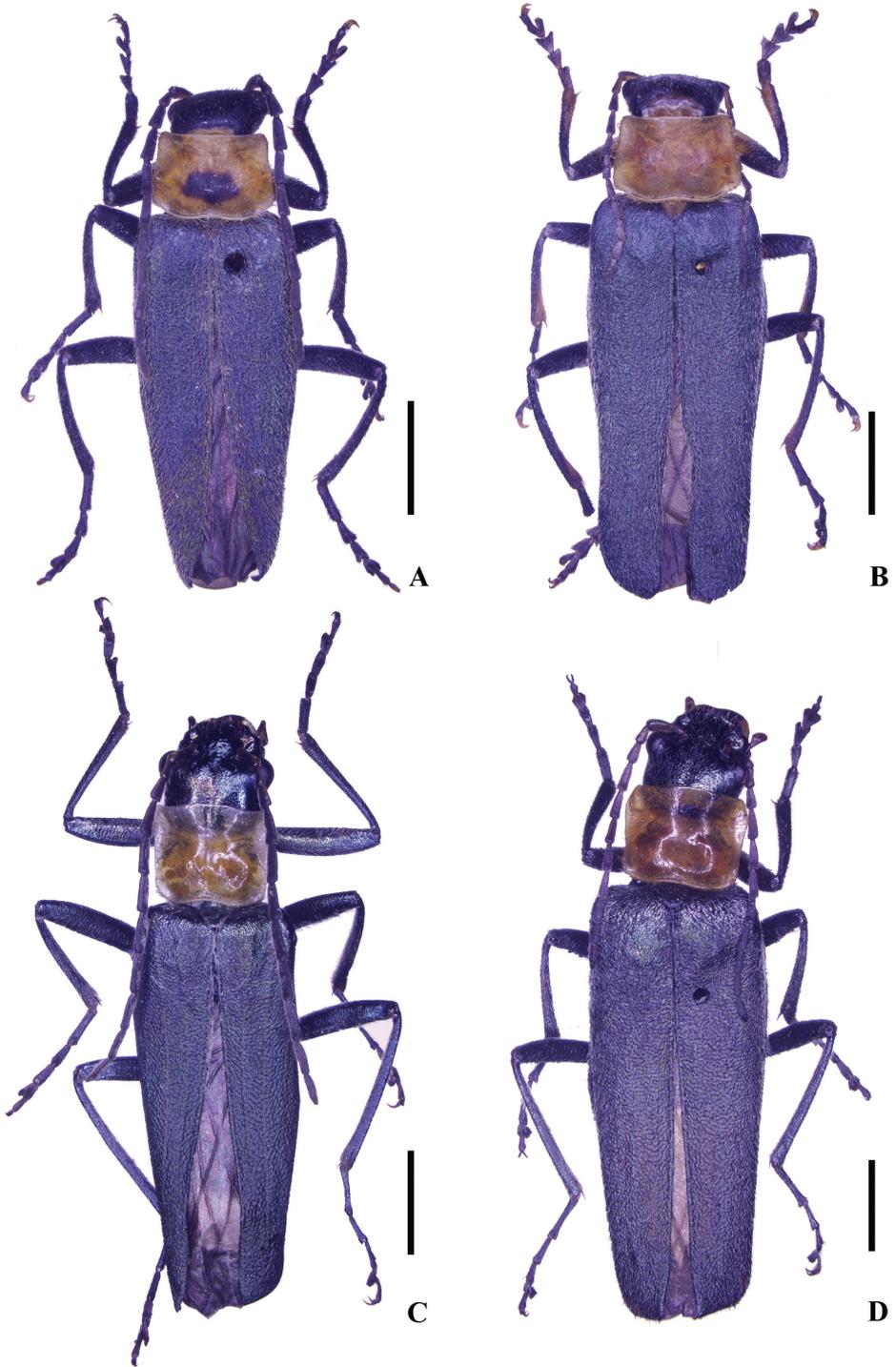
### *Themus* (*Themus*) *dimorphus* Y. Yang & X. Yang, sp. n.

Figs 1A–B, 2A–C, G, 3

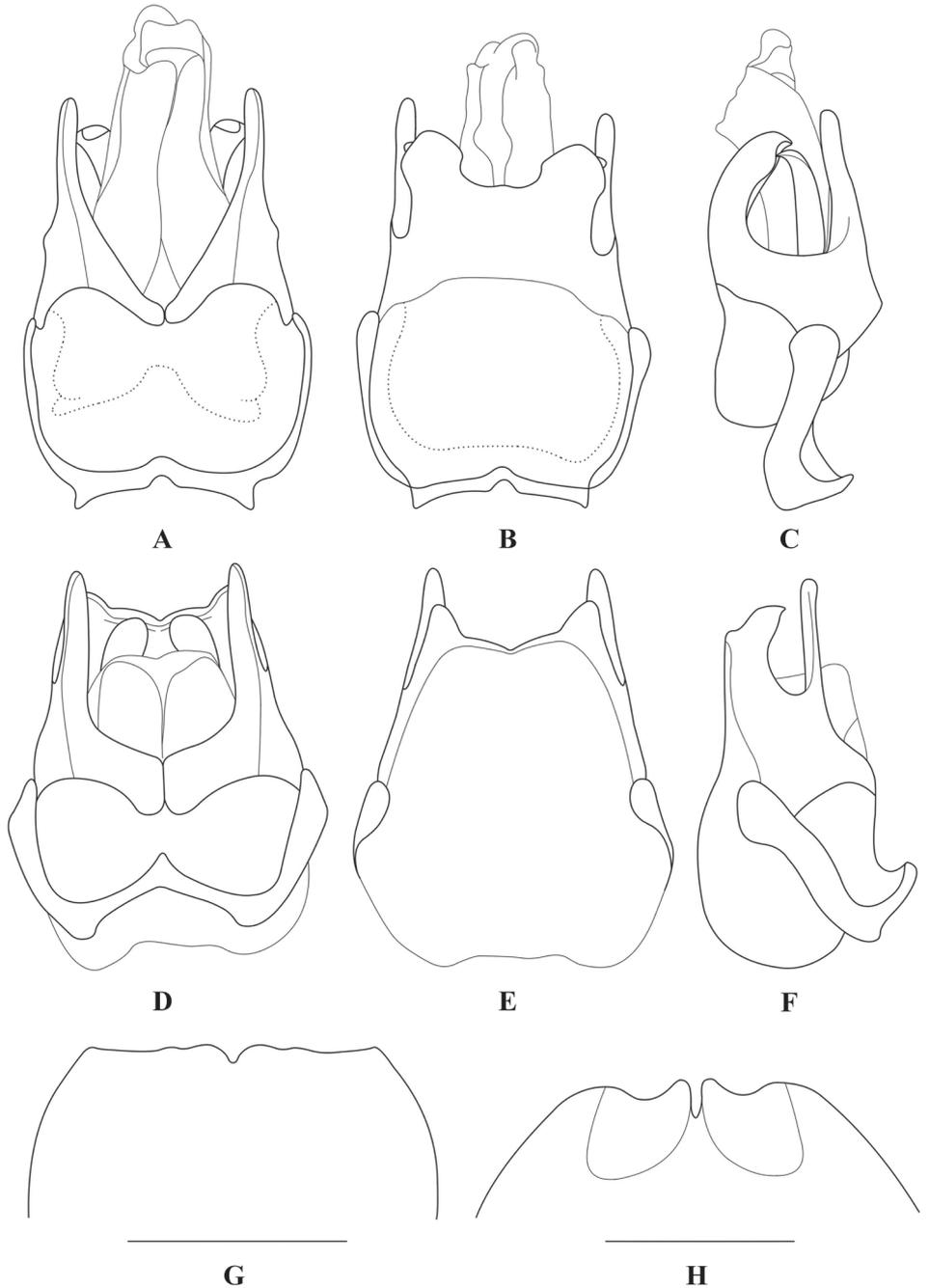
**Type material.** Holotype, male (MHBU): **CHINA:** Yunnan, Yiliang, Xiaocaoba, 25.VIII.2013, leg. Xun Bian & Guang-Lin Xie; Paratypes: 2 males (MHBU): same data to the holotype; 2 females (MHBU): same locality and collectors, 24.VIII.2013.

**Description.** Male (Fig. 1A). Head metallic dark blue and weakly shining, lateral parts of clypeus yellow, mouthparts yellow, maxillary and labial palpi and apices of mandibles dark brown; prothorax yellow, pronotum with a moderately large, irregular black marking in center of disc, antennae black, antennomeres I–III yellow on ventral sides, scutellum black, elytra metallic green and weakly shining, legs and meso- and metasterna metallic dark blue and weakly shining, abdomen yellow, slightly darkened on both sides of ventrites I–V. Body densely covered with decumbent brown pubescence, mixed with slightly long semierect pubescence on elytra and a few long hairs along anterior margin of clypeus.

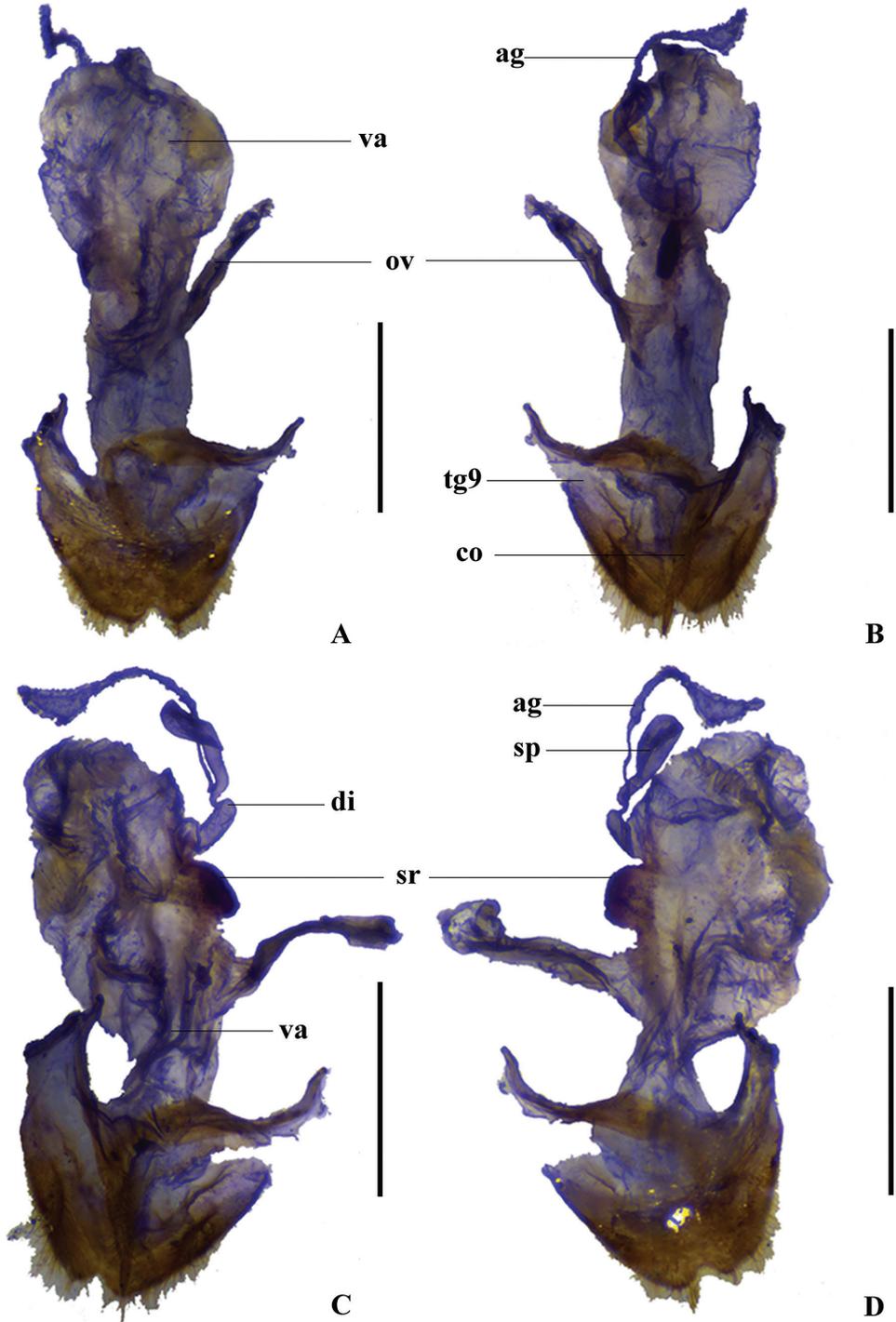
Head rounded, densely and finely punctate, surface lustrous; eyes slightly protruding, breadth across eyes slightly wider than anterior margin of pronotum; terminal maxillary palpomeres widest nearly in middle, arcuate and sharp at apical parts of inner margins, rounded at apices; antennae extending to basal two-fifth length of elytra, antennomeres II approximately twice as long as wide at apices, III–X slightly obliquely widened apicad, III



**Figure 1.** Habitus, dorsal view (A, C male B, D female): A–B *Themus* (*Themus*) *dimorphus* sp. n. C–D *T. (T.) testaceicollis* Wittmer, 1983. Scale bars = 2.0 mm.



**Figure 2.** A–F Aedeagus (A, D ventral view B, E dorsal view C, F lateral view); G–H. abdominal sternite VIII of female, ventral view: A–C, G *Themus (Themus) dimorphus* sp. n. D–F, H *T. (T.) testaceicollis* Wittmer, 1983. Scale bars = 1.0 mm.



**Figure 3.** Female genitalia of *Themus (Themus) dimorphus* sp. n.: **A** dorsal view **B** ventral view **C** right lateral view **D** left lateral view. Scale bars = 1.0 mm.

approximately twice as long as wide at apices and 1.5 times as long as II, IV approximately 1.5 times as long as III, IV–XI subequal in length, each with a small round to short narrow longitudinal impression in middle or basal part of outer margin, XI pointed at apices.

Pronotum approximately 1.40 times as wide as long, widest near base, anterior margin arcuate, lateral margins slightly sinuate at anterior part and distinctly diverging posteriorly, posterior margin bisinuate and narrowly bordered, anterior angles rounded, posterior angles nearly rectangular, disc slightly convex at posterolateral parts, surface lustrous, slightly finely and sparsely punctate than that on head.

Elytra approximately 3.0 times longer than pronotum, 2.6 times longer than humeral width, lateral margins distinctly converging posteriorly, apex of each elytron rounded, disc rugulose-lacunose and coarsely punctate, surface matt.

Aedeagus (Fig. 2A–C): ventral process of each paramere narrow and straight, rounded at apex; conjoint dorsal plate of parameres slightly shorter than ventral processes, largely subroundly emarginated in middle of apical margin, with lateroapical angles acute and bent ventrally; laterophyses widely separated on both sides of median lobe, with apices acute and pointed dorsally to lateroapical angles of conjoint dorsal plate.

Female (Fig. 1B). Similar to male, but clypeus uniformly yellow; antennae narrower and shorter, extending to basal one-fifth length of elytra; antennomeres III nearly as long as II, IV–XI without impressions, XI slightly longer than X; pronotum without any black marking (one specimens with two indistinct small dark brown markings on both sides of disc); elytra approximately 2.5 times longer than humeral width, with lateral margins slightly converging posteriorly; scutellum sometimes yellow (one specimen). Abdominal sternite VIII (Fig. 2G) moderately triangularly emarginated in middle and indistinctly emarginated on both sides of posterior margin. Internal reproductive organ of genitalia (Fig. 3): vagina stout, abruptly narrowed into a short tube at apical part of ventral side, below which present with a pair of strongly sclerotized, arcuate and conjoint ridges extending to median oviduct; diverticulum and spermatheca arising from the end of the short tube of vagina; diverticulum very short and rounded at apex; spermatheca sac-shaped and rounded at apex, distinctly expanded apicad and bent in middle, provided with a moderately long and thin accessory gland at base.

Body length: 8.0–11.0 mm; width: 3.0–4.5 mm.

**Distribution.** China (Yunnan).

**Etymology.** The specific name is derived from Latin *dimorphus* (dimorphic), referring to its different coloration of head and pronotum in both sexes.

**Diagnosis.** The new species can be distinguished from most of the species of *Themus* (*Themus*) Motschulsky by the smaller body and uniformly orange pronotum or at most with one or two small black markings, which characters also equipped by the following species in the key.

#### A key to *Themus* (*Themus*) *dimorphus* sp. n. and its similar species

- |   |                                      |   |
|---|--------------------------------------|---|
| 1 | Legs mixed with orange and blue..... | 2 |
| – | Legs uniformly blue.....             | 3 |

- 2 Pronotum distinctly wider than long, with lateral margins nearly parallel; aedeagus: laterophyses flattened and tapered apically, slightly longer than conjoint parameres of parameres..... *T. (T.) tumlonganus* (Pic, 1916)
- Pronotum slightly wider than long, with lateral margins slightly converging posteriorly; aedeagus: laterophyses unlike above, stout, slightly shorter than conjoint dorsal plate of parameres..... *T. (T.) foveicollis* (Fairmaire, 1900)
- 3 Pronotum distinctly wider than long, with lateral margins slightly diverging posteriorly; aedeagus: conjoint dorsal plate of parameres subroundly emarginated in middle of apical margin..... *T. (T.) dimorphus* sp. n.
- Pronotum slightly wider than long, with lateral margins slightly converging posteriorly; aedeagus: conjoint dorsal plate of parameres inverse-trapeziformly emarginated in middle of apical margin.... *T. (T.) testaceicollis* Wittmer, 1983

***Themus* (*Themus*) *testaceicollis* Wittmer, 1983**

Figs 1C–D, 2D–F, H, 4

*Themus* (s. str.?) *testaceicollis* Wittmer, 1983: 218, Fig. 97.

*Themus* (s. str.) *testaceicollis*: Wittmer 1995: 127.

**Type material examined.** Holotype: 1 female (MNHN): [h] “Tatsien Lu \ 1896” [CHINA, Sichuan, Kangding], [h] “*Themus* (s. str.?) \ *testaceicollis* \ Wittm. \ det. W. Wittmer”, [p] “HOLOTYPUS”. Paratype: 1 female (NHMB): [p] “Ta-tsien-Loû \ Chasseurs Thibétains \ 1896” [CHINA, Sichuan, Kangding], [h] “*Themus* (s.str.?) \ *testaceicollis* \ Wittm. \ det. W. Wittmer”, [p] “PARATYPUS”.

**Additional material examined.** CHINA: Shaanxi: 12 males, 7 females (IZAS): Taibai, Baiyuanxiang, Primary Forest, 33.814°N, 107.618°E, 1619 m, 19.VI.2012, leg. Sha Li; 4 males (IZAS): Liuba, Shangtaizi, Zibaishan, 22.VI.2012, leg. Yi Hua; 1 female (MHUB): Fengxian, Heigou, 13.VI.2005, leg. Yi-Bin Ba; Hubei: 1 female (MHUB): Badong, Lvcongpo, 1700 m, 16.VII.2006, leg. Li Cai; Sichuan: 1 female (MHUB): Jiuzhaigou, Xinyangerlin, 14.VII.2009, leg. Zhen-Hua Gao & Yi-Ping Niu.

**Redescription.** Male (Fig. 1C). Head metallic green and strongly shining, mouthparts metallic green, mandibles light yellow at bases and dark brown at apices, terminal maxillary and labial palpomeres dark brown, antennae black, antennomeres I–II metallic green on dorsal sides, I–VI or VIII orange on ventral sides, prothorax light yellow, pronotum orange in center of disc, scutellum, elytra and legs metallic green and strongly shining, meso- and metasterna and abdomen metallic dark blue and strongly shining. Head and elytra densely covered with decumbent light brown pubescence, pronotum with black pubescence, meso- and metasterna and abdomen with gray pubescence, sparsely mixed with long erect pubescence along anterior margin of clypeus.

Head rounded, densely and finely punctate, surface lustrous; eyes slightly protruding, breadth across eyes slightly narrower than anterior margin of pronotum; terminal maxillary palpomeres widest nearly in middle, arcuate and sharp at apical half parts of

inner margins, rounded at apices; antennae almost extending to mid-length of elytra, antennomeres II approximately twice as long as wide at apices, III–X slightly obliquely widened apicad, III about 1.5 times as long as wide at apices and slightly longer than II, IV approximately 1.5 times longer than III, IV–VIII subequal in length and slightly longer than the following antennomeres, IV–XI each with a short longitudinal impression nearly in middle of outer margin, XI slightly longer than X and pointed at apices.

Pronotum approximately 1.20 times as wide as long, widest before middle, anterior margin arcuate, lateral margins slightly sinuate and converging posteriorly, posterior margin bisinuate and narrowly bordered, anterior angles nearly rectangular, posterior angles rounded, disc slightly convex at posterolateral parts, finely and sparsely punctate, surface lustrous.

Elytra approximately 3.5 times longer than pronotum, 2.7 times longer than humeral width, lateral margins distinctly converging posteriorly, apex of each elytron rounded, disc rugulose-lacunose and coarsely punctate, surface matt.

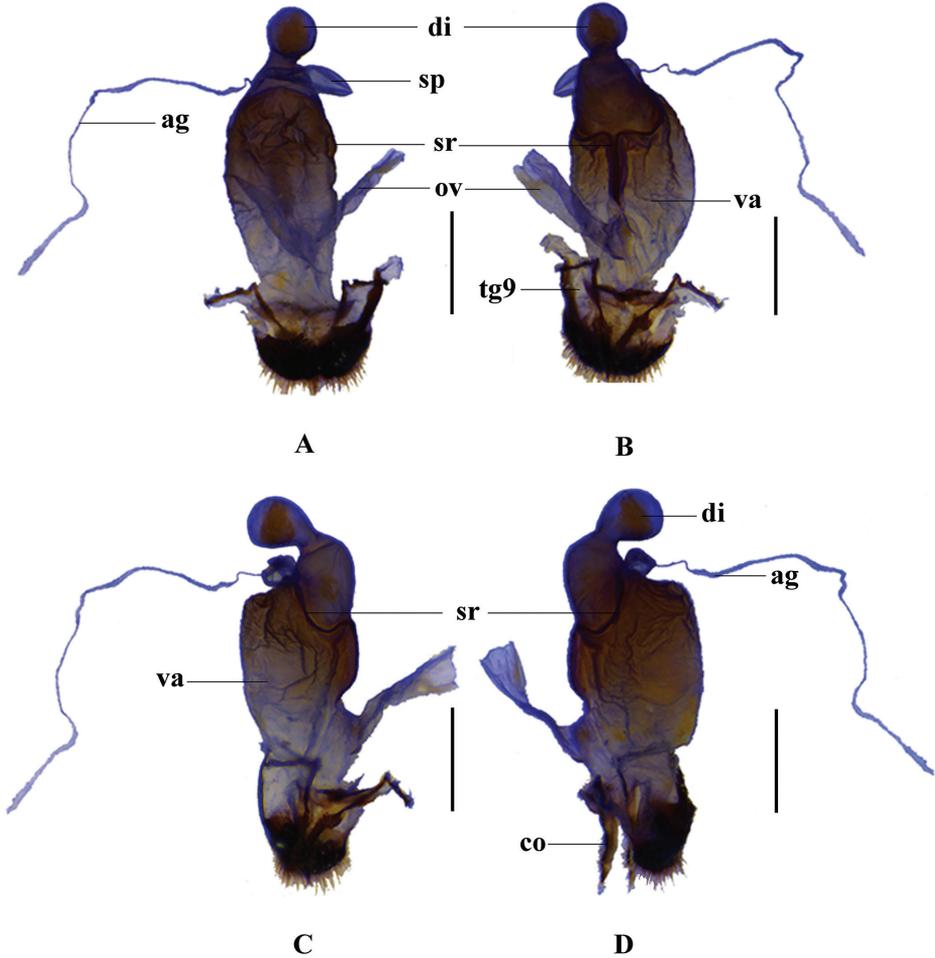
Aedeagus (Fig. 2D–F): ventral process of each paramere narrow and nearly straight, rounded at apex; conjoint dorsal plate of parameres distinctly shorter than ventral processes, largely inverse-trapeziformly emarginated in middle of apical margin, present with two narrow transverse ridges near bottom of middle emargination on inner surface, lateroapical angles acute and bent ventrally in lateral view; laterophyses separated on dorsal side of median lobe, slightly shorter than conjoint dorsal plate, acute at apices which pointed towards the lower transverse ridge of conjoint dorsal plate.

Female (Fig. 1D). Similar to male, but eyes less protruding; antennae narrower and shorter, extending to basal one-fourth length of elytra; pronotum approximately 1.25 times as wide as long; elytra with lateral margins nearly parallel. Abdominal sternite VIII (Fig. 2H) each side of with a large and nearly triangular concave on posterior part, narrowly and moderately emarginated in middle and widely roundly emarginated on both sides of posterior margin, the portions between middle and lateral emarginations narrowly rounded at apices. Internal reproductive organ of genitalia (Fig. 4): vagina stout, with diverticulum and spermatheca situated at apex and median oviduct in middle of ventral side; spermatheca sac-shaped and rounded at apex, distinctly expanded apicad and bent in middle, provided with a long and thin accessory gland at base; diverticulum nearly gourd-shaped, global at apical part, with apex bent dorsally; spermatheca and diverticulum surrounded with a strongly sclerotized bracket at base, which is confluent in middle and extending to median oviduct on ventral side, the opening of spermatheca arising from left end of the sclerotized bracket on dorsal side.

Body length: 11.5–15.0 mm; width: 3.0–4.0 mm.

**Distribution.** China (Hubei, Sichuan, Shaanxi, Gansu).

**Remarks.** In the original publication (Wittmer 1983), this species was described based on female types collected in Sichuan, China. Later some male characters were supplemented (Wittmer 1995). In the present study, it is redescribed and provided with illustrations of genitalia of both sexes for the first time. And it is newly recorded in Hubei province of China.



**Figure 4.** Female genitalia of *Themus (Themus) testaceicollis* Wittmer, 1983: **A** dorsal view **B** ventral view **C** right lateral view **D** left lateral view. Scale bars = 1.0 mm.

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## References

- Brancucci M (1980) Morphologie comparée, évolution et systématique des Cantharidae (Insecta: Coleoptera). *Entomologica Basiliensia* 5: 215–388.
- de Motschulsky V (1858) Entomologie spéciale. Insectes du Japon. *Études Entomologiques* 6(1857): 25–41.
- Švihla V (2008) Redescription of the subgenera of the genus *Themus* Motschulsky, 1858, with description of five new species (Coleoptera: Cantharidae). *Veröffentlichungen des Naturkundemuseums Erfurt* 27: 183–190.
- Wittmer W (1973) Zur Kenntnis der Gattung *Themus* Motsch. (Col. Cantharidae). *Entomologischen Arbeiten aus dem Museum G. Frey* 24: 186–228.
- Wittmer W (1983) Beitrag zur einer Revision der Gattung *Themus* Motsch. Coleoptera: Cantharidae. *Entomologischen Arbeiten aus dem Museum G. Frey* 31/32: 189–239.
- Wittmer W (1995) Neue Cantharidae (Col.) aus dem indo-malaiischen und palaearktischen Faunengebiet mit Mutationen. *Entomologica Basiliensia* 18: 109–169.
- Wittmer W (1997) Neue Cantharidae (Col.) aus dem indo-malaiischen und palaearktischen Faunengebiet mit Mutationen. 2. Beitrag. *Entomologica Basiliensia* 20: 223–366.
- Yang YX, Su JY, Kopetz A (2014) Description of three new cantharid species related to *Themus* (*Themus*) *senensis* (Pic, 1922) (Coleoptera, Cantharidae). *Annales Zoologici* 64(4): 655–666. doi: 10.3161/000345414X685938

# New species without dead bodies: a case for photo-based descriptions, illustrated by a striking new species of *Marleyimyia* Hesse (Diptera, Bombyliidae) from South Africa

Stephen A. Marshall<sup>1</sup>, Neal L. Evenhuis<sup>2</sup>

**1** Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada **2** J. Linsley Gressitt Center for Entomological Research, Bernice Pauahi Bishop Museum, Kaiwi'ula Campus, 1525 Bernice Street, Honolulu, Hawaii 96817-2704, USA

Corresponding author: Neal L. Evenhuis ([NealE@bishopmuseum.org](mailto:NealE@bishopmuseum.org))

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## Abstract

A new bombyliid species *Marleyimyia xylocopae* Marshall & Evenhuis, **sp. n.**, an apparent mimic of the carpenter bee *Xylocopa flavicollis* (De Geer), is described from South Africa on the basis of photographs only. The pros and cons of species descriptions in the absence of preserved type specimens are discussed.

## Keywords

South Africa, bee fly, mimicry, *Xylocopa*, type specimens, photography, taxonomy

“Collecting specimens is no longer required to describe a species or to document its rediscovery.”  
—Minteer et al. (2014: 260)

“Describing a new species without depositing a holotype when a specimen can be preserved borders on taxonomic malpractice.”—Krell and Wheeler (2014: 815)

## Introduction

A recent paper in *Science* by Minter et al. (2014) ignited a controversy by suggesting that specimens are no longer the ‘gold standard’ for species description, and that alternative methods such as high-resolution photography should be considered instead. The quotes above, from the Minter et al. (2014) paper and a response letter by Krell and Wheeler (2014), give some indication of the apparent polarization of the community on this issue. We take the opportunity here to contend that the apparently antipodal positions summarized in those quotes are both correct, and provide a description of a new species based only on photographs to illustrate and confirm our points.

First of all, let us reiterate the obvious reasons that collecting specimens is highly desirable, and briefly consider and reject arguments against collecting. Specimens are indeed the ‘gold standard’ for species descriptions. Not only do they allow for consideration of a full suite of characters including internal morphology, microscopic and genetic characters, they preserve data for future access with future technologies and future questions. Specimen collections are our greatest treasure trove of biodiversity information and continued collection development must remain a priority. Arguments against specimen collections usually pivot on the potential impact of collecting on fragile populations. Such arguments are weak, since there are vanishingly few examples of scientific collecting having a detrimental effect at the population or species levels and there are very few circumstances under which the removal of a few individuals from a population might seriously harm the ultimate survival of the species. Most, by orders of magnitude, of the animal species awaiting description are invertebrates, and it is especially difficult to make a case against invertebrate collecting on the basis of conservation biology. As invertebrate taxonomists, we therefore agree with the letter of Krell’s (2014) statement that “*Describing a new species without depositing a holotype when a specimen can be preserved borders on taxonomic malpractice*” (p. 815), but at issue here is the caveat of when a specimen can be preserved. We do not accept opposition to killing and preserving invertebrate specimens on moral or conservation biology grounds, but there are circumstances under which a type cannot be preserved. Furthermore, there is no doubt that collection of potential type specimens will become more and more difficult as restrictions on collecting and transporting specimens continue to increase. These difficulties, along with the rapidly increasing numbers of skilled ‘digital collectors’ who are building collections of images instead of specimens, will inevitably force the biodiversity community to adapt to growing numbers of new taxa recognized without benefit of dead, preserved type specimens. Fortunately, as Minter et al. (2014) put it, “*collecting specimens is no longer required to describe a species ....*” (p. 260). Collecting specimens is highly desirable, but it is indeed no longer required.

As explained by Wakeham-Dawson et al. (2002) and Polaszek et al. (2005), although Article 16.4 of the ICZN Code (ICZN 1999) requires all holotypes that are “extant” to be deposited in a collection, Article 73.1.4 allows for the description of new taxa without preserving a collected specimen by the following statement: “*Designation of an illustration of a single specimen as a holotype is to be treated as designation of the*

*specimen illustrated; the fact that the specimen no longer exists or can be traced does not of itself invalidate the designation*". Additionally, we interpret the wording of Article 16.4 to allow for description of a new species on the basis of a lost or escaped holotype, where the term "extant" means a physically "existing" specimen. Thus a lost, escaped, or purposefully released specimen is not "extant".

Even in the absence of a collected type specimen, current technologies such as high-resolution photography can often provide enough information for a proper description resulting in a readily recognizable and unequivocally distinct newly named species, and in some cases can provide more information (such as colour, soft parts, delicate structures, posture, behaviour) than could be extracted from a preserved specimen. The few previous descriptions of extant new species without a type (or part thereof) have for the most part been restricted to large vertebrates, for example primate species known from only small populations (Jones et al. 2005, Li et al. 2015, Mendes Pontes et al. 2006). We provide the first example of a new insect species described and named solely on the basis of field photographs of the type specimen.

In the example provided here, a highly distinctive fly species belonging to an extremely rare genus (only three other known specimens of two species) was photographed on two occasions and then collected in the field, but the captured individual escaped before it could be preserved as a traditional dead type specimen. Our description, based on photographs of two different living flies, is complete and adequate to identify this species and adds an interesting and easily recognized species to the literature. It not only increases our knowledge of the biodiversity of the area in which it was collected and of the genus in which it is placed but, as we explain below, also provides some interesting ecological and biological information.

The situation leading to this approach is a simple one to understand, since it pivots on an accidentally lost type specimen that might not be collected again due to its rarity. Although this description is by definition singular because it is the first of its kind (at least for Diptera), we predict that a growth in descriptions without physical type specimens is inevitable, and that this growth will result not as much from accidental loss of specimens as from increasing restrictions on collecting. Every taxonomist has been in the position of completing a revision that needs to be rounded out with species from places from which specimens are difficult or impossible to obtain, often because of laws preventing collection or export of specimens. For many of these required taxa, the solution to this problem is for the taxonomist to instead "collect" digital images that, in the case of new species, can represent type specimens. We are not arguing that this practice is generally desirable, only that it is inevitable and increasingly practical when diagnostic characters are distinct and discernable through photographs.

Another trend pushing us inexorably to a wider acceptance of species descriptions without physical type specimens is the rapid growth of extensive, high quality digital image collections dissociated from collections of physical specimens (Marshall, in press). As these image collections become curated just as dead specimens are curated today, these digital "specimens" will find their way into the work of practicing taxonomists, and they will need names. At a time when we need more than ever to identify the

biodiversity of this planet before it disappears, it is unrealistic to think that distinct and diagnosable new taxa known only from good photographs and appropriate associated metadata should be organized and referred to only as “undescribed species #nnn”, when they can and should be organized and named using the existing rules of nomenclature.

In recognizing the need to name species without dead type specimens we are not arguing for a loosening of taxonomic standards. In fact, we expect that descriptions unsupported by existing physical type specimens will be subject to especially critical scrutiny by skeptical editors and responsible reviewers. We expect that such descriptions that do not render new species unequivocally recognizable will be rejected, just as they should be if they were based on dead type specimens. Once published, digital representations of type specimens will be much more widely available for use and scrutiny than physical type specimens archived in distant museums.

Dubois and Nemésio (2007) argue specifically against the use of photographs as surrogates for type specimens, suggesting that digital photographs can be faked or misinterpreted. This is, of course true, but one could also alter or misinterpret a type specimen. We see no merit in impeding the documentation of biodiversity on the baseless assumption that there will be more errors, incompetency or dishonesty in descriptions based on photographs than currently exists in descriptions based on specimens or parts thereof.

## Material and methods

The species described below was photographed in nature using a Nikon D800 with a 105 macro lens and a hand-held flash. The holotype specimen was not captured, so the image presented here serves as representation of the holotype. Morphological terminology follows Greathead and Evenhuis (2001) and wing venation follows Saigusa (2006).

## Taxonomy

### Genus *Marleyimyia* Hesse

*Marleyimyia* Hesse, 1956: 521. Type species: *Marleyimyia natalensis* Hesse, 1956, by original designation.

**Remarks.** *Marleyimyia* Hesse, 1956 was originally described based on a single male specimen with vestigial mouthparts and bred from a log containing cossid larvae. The genus is currently known from only three specimens representing two described species from widely disjunct localities: *Marleyimyia goliath* (Oldroyd) from Peninsular Malaysia and *M. natalensis* Hesse from southern Africa. In proposing his new genus, Hesse (1956) distinguished *Marleyimyia* from *Oestranthrax* Bezzi, 1921 by the larger body, the head wider than the thorax, and the differently shaped, although reduced, proboscis (pointed apically in *Marleyimyia* and short with a small fleshy labellum in *Oestran-*

*thrax*). Hesse distinguished *Marleyimyia* from *Villoestrus* Paramonov, 1931 by the same body and head features as above, but also the presence of a reduced proboscis (proboscis totally absent in *Villoestrus*). Oldroyd (1951) described his new Malaysian species as *Oestranthrax goliath* based on a single male and female bred from the pupa of a cossid moth and claimed it to be the largest in bulk of any bee fly he had seen. Bowden (1975) transferred *O. goliath* to *Marleyimyia* and Bowden (1978) echoed Oldroyd's (1951) presumption that the species in the genus had the appearance of a crepuscular or nocturnal habit. If this nocturnal habit is proven to be true, then the new species described below differs in having been seen during the day (photographed at two separate localities), but it has the same unusual antennal shape as found in the male and female of *M. goliath* (a similar lanceolate shape but shorter and stouter is found only in one other bombyliid species, the Nearctic *Oestranthrax farinosus* Johnson & Maughan, and only in females of that species). The antennal shape in the new species described is not found in the male holotype of the type species, *Marleyimyia natalensis*, but it is found in the female of the undescribed species of *Marleyimyia* from Nigeria mentioned by Bowden (1978) and may be a female-specific character for species of *Marleyimyia*.

***Marleyimyia xylocopae* Marshall & Evenhuis, sp. n.**

<http://zoobank.org/02D005F8-59D4-4FD7-B0D2-FAA8BC017BC8>

Figs 1–4

**Type locality.** REPUBLIC OF SOUTH AFRICA: KwaZulu Natal: Ndumo Nature Preserve, Ndumo Campground, 26°54'31.07"S; 32°18'57.85"E.

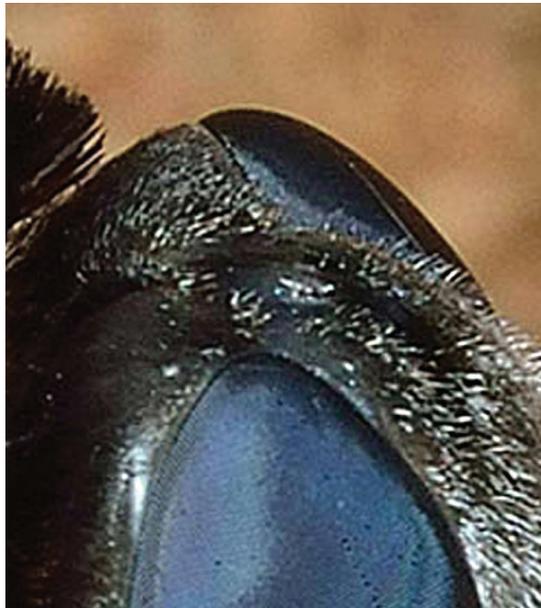
**Type specimen.** Holotype female from SOUTH AFRICA: KwaZulu Natal: Ndumo Nature Preserve, Ndumo Campground, 26°54'31.07"S; 32°18'57.85"E, 74.0 m elev., 1 Dec 2014, S.A. Marshall. Holotype represented in photograph No. 7007 (Fig. 1); other photos taken: Nos. 7002, 7003, 7004, 7005, 7006, 7008. Paratype female represented in photograph No. 7015. photographed at the following locality: SOUTH AFRICA: KwaZulu Natal: Ndumo Nature Preserve, Red Cliffs, 26°51'21.9"S; 32°12'26.3"E, 35.0 m elev., 27 November 2014, S.A. Marshall. Other photos taken: Nos. 7009, 7010, 7012, 7013, 7014, 7016, 7017. All photographs are archived with Morphobank (project P2277 : <http://morphobank.org/permalink/?P2277>, images M397297–M397315).

**Diagnosis.** Separated from its congeners by the all black infuscated wing (hyaline in *M. goliath* and *M. natalensis*), and the mesonotal pattern of black hairs anteriorly and yellow hairs posteriorly (entirely black-haired in *M. goliath* and predominantly yellowish brown-haired in *M. natalensis*).

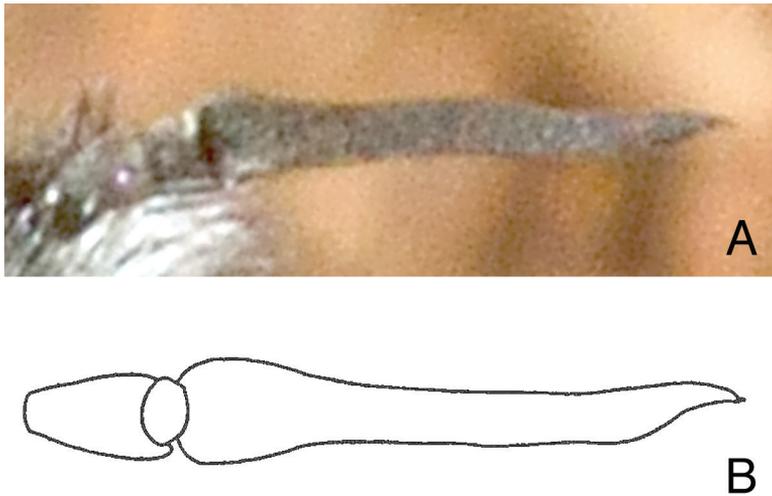
**Description.** Female. Body: ca. 18–20 mm in length (extrapolated from comparison of grass blade width of *Eremochroa* (centipede grass) in a larger habitus photograph [No. 7009]). *Head* (Fig. 2). As wide or wider than thorax, shining black in ground colour with some bluish highlights, clothed with silvery white hairs and tomentum. Frons width ca. 1/3 of head width at occiput; ocellar triangle ellipsoid, lateral ocelli slightly larger than anterior ocellus; occiput with short silvery white hairs dorsally, silvery white



**Figure 1.** Habitus of female *Marleyimyia xylocopae* Marshall & Evenhuis, sp. n. from Ndumo Game Preserve, South Africa, 1 December 2014. Fig. 1 derives from Photo no. 7007 (Campground site) and was adjusted for brightness and contrast. Photo: Steve Marshall.



**Figure 2.** *Marleyimyia xylocopae* Marshall & Evenhuis, sp. n., detail of head. Photo: Steve Marshall.



**Figure 3.** Right antenna of *Marleyimyia* species. **A** *M. xylocopae* Marshall & Evenhuis, sp. n. **B** *M. go-liath* (Oldroyd) [from Oldroyd 1951, fig. 5]. Fig. 3A derives from Photo no. 7015 (Red Cliffs site), was rotated slightly to match the illustration (Fig. 3B), and was adjusted for brightness and contrast. Photo: Steve Marshall.



**Figure 4.** *Marleyimyia xylocopae* Marshall & Evenhuis, sp. n., left wing. Fig. 4 derives from Photo no. 7007 (Campground site) and was adjusted for brightness and contrast. Photo: Steve Marshall.

tomentum along posterior eye margin, tomentum densest at medial eye indentation and on postgena. Eye dark bluish black, indented medially on posterior margin, with bisecting line length ca. half eye width. Frons short silvery white pilose and tomentose, bare medially below ocellar triangle, pile longest and densest at level of antennae. Face receding with dense silvery white hairs, oral margin narrowly brownish orange near eye margin. Antenna (Fig. 3A) cinereous; scape subcylindrical with admixed black and white hairs dorsally and laterally; pedicel subellipsoid, wider than long, bare; flagellomere long, ca.  $4 \times$  length of scape, linear-lanceolate, bare, slightly bulging basally, slightly tapering to apex; apical style minute.



**Figure 5.** *Marleyimyia natalensis* Hesse, male holotype. **A** habitus, dorsal view **B** Head, frontal view **C** habitus, lateral view **D** labels. Photos: Simon Van Noort, Iziko South African Museum.

*Thorax.* Mesonotum and pleura shining black in ground color (scutellum ground color obscured); mesonotum with dense short “clipped-looking” black pile anteriorly to level of wing base, yellow pile from wing base to posterior edge of mesonotal disc including postalar calli, long, shaggy laterally, short and “clipped-looking” on disc; scutellum densely shaggy yellow pilose; pleura thickly black haired, those hairs on anepisternum with dark brownish sheen. (Halter and pleural area under wing obscured in photos).

*Legs.* (Hind femur obscured in photographs). Fore and mid legs (and hind legs beyond femur) black with a shiny greasy appearance, some bluish highlights on femora and tarsi. Fore and mid femora short, stout, with long black hairs ventrally, longest basally, tapering to shorter apical hairs; tibiae shorter than femora, with short black spicules.

*Wing* (Fig. 4). Infuscated dark brownish black throughout except brownish infuscation in center of anal lobe and cell fourth posterior cell and subhyaline apex of wing, veins black; crossvein r-m just proximal to middle of cell dm (with anomalous second crossvein in left wing); veins  $R_{2+3}$  and  $R_4$  sinuous, subparallel to wing margin; origin or  $R_{2+3}$  just before r-m crossvein; first posterior cell open in wing margin; crossvein dm-m S-shaped, origin on vein  $M_4$  at basal one-fourth; crossvein m-m slightly wider than r-m; cell cua narrowly open in wing margin; anal lobe well developed; alula small.

*Abdomen.* Broad, ovular in shape, shining black in ground color with bluish highlights (sternites not visible); tergite II and III with admixed short silvery white hair



**Figure 6.** *Xylocopa flavicollis* (De Geer) from Ndumo Game Preserve, South Africa, 8 December 2014. Photo: Steve Marshall.

and tomentum dorsolaterally and sparse silvery tomentum with bluish highlights dorsomedially; tergites IV–VII with adpressed black tomentum and sparse silvery white tomentum dorsomedially. *Genitalia*. Not dissected.

**Remarks.** Two different specimens were photographed (one at each locality indicated above). That they are different is evidenced by the rubbed frons in the Red Cliffs paratype (photos taken on 27 November) and that the photos taken later at the Campground site (on 1 December) were of a specimen without a rubbed frons. This new species shares its unusual large body, wing shape, wing venation, and antennal flagellomere shape with *M. goliath*, which occurs in Peninsular Malaysia. These characters differ from the smaller and more slender type species, *Marleyimyia natalensis* (Fig. 5), from Sydenham, near Durban, South Africa (see map in Fig. 7; <http://www.simplemappr.net/map/4577>). Bowden (1978) mentioned an undescribed species of *Marleyimyia* from Nigeria which, from photographs of the specimen in the BMNH, appears to be more similar to *M. natalensis* in size and coloration than either *M. goliath* or *M. xylocopae* sp. n., but the Nigerian species has the same antennal shape as *M. goliath* and *M. xylocopae* sp. n. Because of the similarity of *M. xylocopae* to characters shown in *M. goliath*, we feel confident of its current generic placement. Further material of this genus should be secured in order to better assess the true generic limits.



**Figure 7.** Map (<http://www.simplemapp.net/map/4577>) showing the known localities of *Marleyimyia natalensis* Hesse and *M. xylocopae* sp. n., in Kwa-Zulu Natal, South Africa.

## Discussion

The striking yellow and black vestiture pattern on the thorax and abdomen, and the body shape, are unusual in bombyliids and show a remarkable similarity to xylocopid bees. The model for this possible case of Batesian mimicry appears to be *Xylocopa flavicollis* (De Geer), which was also photographed at around the same time in the Ndumo area (Fig. 6) and is fairly widespread throughout eastern and southeastern Africa. It is not known if *Marleyimyia xylocopae* is a parasite of the bee. Other bee fly parasites of *Xylocopa* are also unusually large-bodied and similarly shaped (e.g., *Xenox* Evenhuis and *Satyramoeba* Sack). Previous published records of African xylocopid biologies are few and the only recorded bee fly associate is in the genus *Anthrax* (Watmough 1974). More biological observations on xylocopid nesting behavior will hopefully lead to better knowledge of the full suite of their possible bee fly parasites.

## Acknowledgments

We thank and Nigel Wyatt (BMNH) for kindly providing photos of *M. goliath* and the undescribed Nigerian *Marleyimyia*. Burgert Muller kindly checked the KwaZulu-Natal Museum for possible collected specimens of *M. xylocopae*. The observations that made this paper possible were made during the KwaZulu Natal Expedition 2014, organized by A.H. Kirk-Spriggs (National Museum, Bloemfontein, South Africa) and V.R. Swart (University of the Free State, Bloemfontein, South Africa).

## References

- Bowden J (1975) Family Bombyliidae. In: Delfinado MD, Hardy DE (Eds) A catalog of the Diptera of the Oriental Region. Volume II. Suborder Brachycera through Division Aschiza, Suborder Cyclorrhapha. Univ. Press of Hawaii, Honolulu, 165–184.
- Bowden J (1978) Diptera. In: Werger MJA (Ed.) Biogeography and ecology of Southern Africa. Volume II. W. Junk, The Hague, 775–796. doi: 10.1007/978-94-009-9951-0\_25
- Li C, Zhao C, Fan PF (2015) White-cheeked macaque (*Macaca leucogenys*): a new macaque species from Medog, southeastern Tibet. *American Journal of Primatology* 77(7): 753–766. doi: 10.1002/ajp.22394
- Dubois A, Nemésio A (2007) Does nomenclatural availability of nomina of new species or subspecies require the deposition of vouchers in collections? *Zootaxa* 1409: 1–22.
- Greathead DJ, Evenhuis NL (2001) Annotated keys to the genera of African Bombylioidea (Diptera: Bombyliidae; Mythicomyiidae). *African Invertebrates* 42: 105–224.
- Hesse AJ (1956) A revision of the Bombyliidae (Diptera) of southern Africa. Parts II & III. *Annals of the South African Museum* 35: 1–972.
- ICZN [International Commission on Zoological Nomenclature] (1999) International code of Zoological Nomenclature. Fourth edition. International Trust for Zoological Nomenclature, London, xxix + 306 pp.
- Jones T, Ehardt CL, Butynski TM, Davenport TRB, Mpunga NE, Machaga SJ, De Luca DW (2005) The highland mangabey *Lophocebus kipunji*: a new species of African monkey. *Science* 308: 1161–1164. doi: 10.1126/science.1109191
- Krell FT, Wheeler QD (2014) Specimen collection: plan for the future. *Science* 344: 815–816. doi: 10.1126/science.344.6186.815
- Marshall SA (in press) Digital photography and the democratization of biodiversity information. In: Footitt RG, Adler PH (Eds) *Insect Biodiversity: Science and Society*, Volume II. John Wiley & Sons, Chichester, UK.
- Mendes Pontes AR, Malta A, Asfora PH (2006) A new species of capuchin monkey, genus *Cebus* Erxleben (Cebidae, Primates): found at the very brink of extinction in the Pernambuco Endemism Centre. *Zootaxa* 1200: 1–12.
- Minteer BA, Collins JP, Puschendorf R (2014) Avoiding (re)extinction. *Science* 344: 260–261. doi: 10.1126/science.1250953
- Oldroyd H (1951) A giant bombyliid (Diptera) bred from the pupa of a cossid moth. *Proceedings of the Royal Entomological Society (B)* 20: 49–50. doi: 10.1111/j.1365-3113.1951.tb01018.x
- Polaszek A, Grubb P, Groves C, Ehardt CE, Butynski TM (2005) What constitutes a proper description? Response. *Science* 309: 2164–2166.
- Saigusa T (2006) Homology of wing venation of Diptera. Fukuoka. [Privately published]
- Wakeham-Dawson A, Morris S, Tubbs P (2002) Type specimens? Dead or alive? *Bulletin of Zoological Nomenclature* 59: 282–284.
- Watmough RH (1974) The biology and behaviour of carpenter bees in Southern Africa. *Journal of the Entomological Society of Southern Africa* 37: 261–281.



# Two new species of the genus *Anisomysis* (*Anisomysis*) (Crustacea, Mysida, Mysidae) from coral reef waters in Thailand

Mitsuyasu Moriya<sup>1</sup>, Khwanruan Srinui<sup>2</sup>, Shozo Sawamoto<sup>3</sup>

**1** Atmosphere and Ocean Research Institute, University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8564, Japan **2** Institute of Marine Science, Burapha University, Muang, Chonburi 20131, Thailand **3** Department of Marine Biology, School of Marine Science and Technology, Tokai University 3-20-1, Orido, Shimizu, Shizuoka 424-8610, Japan

Corresponding author: Mitsuyasu Moriya ([moriyamaori@u-tokyo.ac.jp](mailto:moriyamaori@u-tokyo.ac.jp))

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## Abstract

Two new species of *Anisomysis* Hansen, 1910 (Mysida, Mysidae), *Anisomysis* (*Anisomysis*) *spinaintus* **sp. n.** and *A. (A.) phuketensis* **sp. n.**, from coral-reef waters in Thailand are described. *Anisomysis* (*A.*) *spinaintus*, collected in the Chaolao Beach, Chanthaburi Province, is distinguished from the closely allied species *A. (A.) incisa* Tattersall, 1936, and *A. (A.) hawaiiensis* Murano, 1995, by the presence of 6–9 spines on the apical cleft of telson, which are absent in the latter two allied species. The new species can also be distinguished from *A. (A.) aikawai* Ii, 1964, by the presence of a deep telson cleft and a large number of spines on the lateral margin of telson. *A. (A.) phuketensis* **sp. n.**, collected in Ko Lon, Phuket, is distinguished from the allied species *A. (A.) robustispina* Panampunnayil, 1984, by having a short telson and a pair of long spines on the apical part of the telson. Keys to the subgenera and species of *Anisomysis*, including the two new species, are presented.

## Keywords

*Anisomysis*, Chantaburi, new species, Phuket, taxonomy

## Introduction

The genus *Anisomysis* was established by Hansen (1910) to describe *Anisomysis laticauda* collected from Laiwui, Obi Island, Indonesia, during the Siboga Expedition.

Băcescu (1973) divided the genus into two subgenera, *Paranisomysis* and *Anisomysis*, mainly on the basis of the structure of mandibular palp: the subgenus *Paranisomysis* has flagellate tubercles on the inner margin of the second segment of palp, while such tubercles are lacking in the subgenus *Anisomysis*. Furthermore, Băcescu (1992) provisionally created the subgenus *Javanisomysis*, which is characterized by the non-segmented exopod and no endopod of the fourth male pleopod. As the peculiar morphological characteristics were different from the existent characteristics of the genus *Anisomysis*, Murano and Fukuoka (2003) proposed to establish a new genus *Javanisomysis*. The genus *Javanisomysis* is cited as a valid name (i.e. Wittmann et al. 2014), however, recently the genus is re-defined as a subgenus in the genus *Anisomysis* (Sawamoto, Srinui & Moriya, 2015) on the basis of examination of the paratypes of *Javanisomysis gutzui*.

Murano and Fukuoka (2003) carried out a systematic study of the genus *Anisomysis* and created the fourth subgenus, *Pseudanisomysis*, to accommodate a few species that have the eye divided into two parts by a groove based on *A. bipartoculata*. The genus *Anisomysis* is composed of the four subgenera, *Anisomysis*, *Paranisomysis*, *Pseudanisomysis* (Murano & Fukuoka, 2003) and *Javanisomysis* (Sawamoto et al., 2015), and most of which are known in tropical and subtropical waters of the Indian Ocean, the western and the central Pacific Ocean, and the marginal seas of these oceans (Murano and Fukuoka 2003). According to Mees (2015) the subgenus *Pseudanisomysis* is accepted as a junior synonym of the genus *Carnegieomysis*. However, the latter is insufficiently described by Tattersall (1943) and is re-defined correctly and is housed in the genus *Anisomysis* by Murano (1995).

Currently, the genus *Anisomysis* contains 36 nominal species in the subgenus *Anisomysis*, 18 species in the subgenus *Paranisomysis*, and four species in the subgenus *Pseudanisomysis* (Mees, 2015) and three species in the subgenus *Javanisomysis* (Sawamoto et al., 2015). In particular, 15 species in the four subgenera are currently reported from Southeast Asian waters (Sawamoto 2014; Sawamoto et al. 2015). *Anisomysis* (*Anisomysis*) *thurneysseni* is included in the subgenus *Javanisomysis*, but is excluded from the species list of the Southeast Asia. The other two species in the subgenus is added to the list (Sawamoto et al. 2015).

The present paper reports two new species of the subgenus *Anisomysis*, which were discovered during a study of the mysid diversity in Southeast Asia. Keys to the four subgenera and to the 38 species of the subgenus *Anisomysis* have been provided.

## Materials and methods

### Sample collection and morphological measurements

Mysid specimens were collected with a hand net by skin diving in a coral reef in Thailand (see “Systematics” section for details). The mysids from the net samples were immediately fixed in 5% seawater-buffered formalin for morphological analysis and 99% ethanol for genetic analysis, the results of which will be reported elsewhere.

Terminology was mainly based on Murano and Fukuoka (2003). The body length (BL) of the specimens was measured from the anterior end of rostrum to the posterior end of telson as the body was stretched. Illustrations were made with the aid of a camera lucida.

Type specimens are housed in the National Museum of Nature and Science, Japan (NSMT).

## Systematics

Order Mysida Boas, 1883

Family Mysidae Haworth, 1825

Subfamily Mysinae Haworth, 1825

Tribus Anisomysini Wittmann, Ariani & Lagardère, 2014

Genus *Anisomysis* Hansen, 1910

Subgenus *Anisomysis* Băcescu, 1973

*Anisomysis* (*Anisomysis*) *spinaintus* sp. n.

<http://zoobank.org/D61A9F38-853F-40D4-8EA4-00322F37D952>

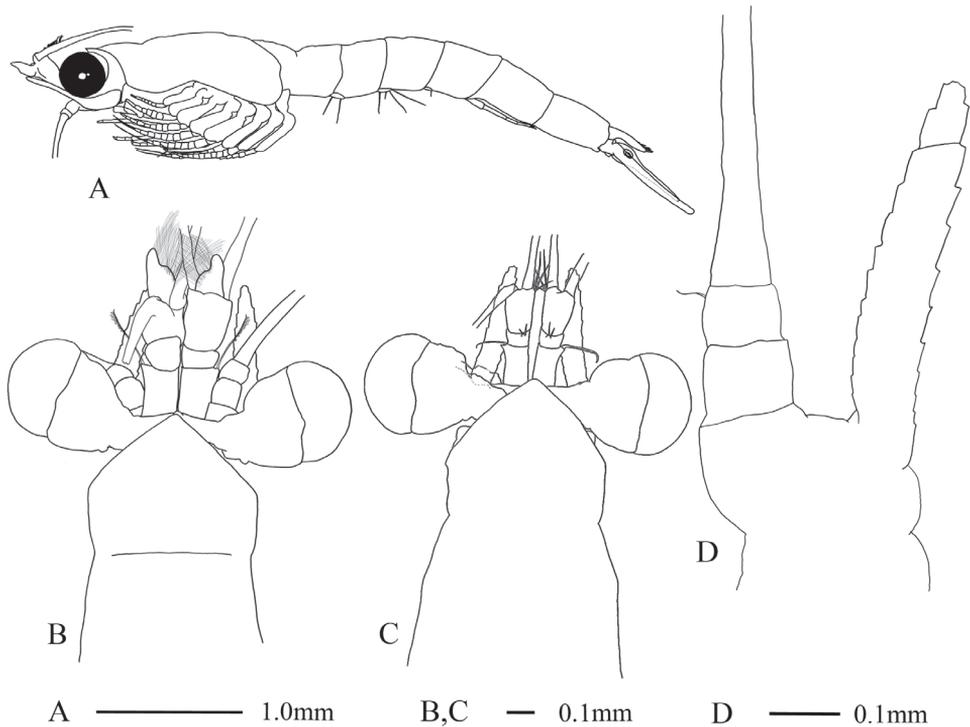
Figs 1–4

**Type series.** Holotype (NSMT-Cr 24246), adult male (BL, 4.1 mm); allotype (NSMT-Cr 24247), adult female with embryos (BL, 4.5 mm); paratypes (NSMT-Cr 24248), 3 adult males (BL, 5.0, 4.8 and 4.2 mm) and 2 adult females with embryos (BL, 4.2 and 4.7 mm); Chaolao Beach, Chantaburi Province, Thailand, 12°31.58'N, 101°55.21'E; collected with a hand net (mesh size, 0.33 mm; mouth diameter, 30 cm) by skin diving on a coral reef 3–5 m deep on November 28, 2010 by M. Moriya.

**Description.** Body slender (Fig. 1A). Carapace produced anteriorly as a low triangular rostrum with a moderately pointed apex, uncovering eyestalks almost completely (Fig. 1B).

Eyes large, cornea globular, extending laterally beyond the lateral margin of carapace (Fig. 1B, C).

Antennular peduncle more robust in male (Fig. 1B) than that of female (Fig. 1C), first segment as long as third, armed with single seta at anterolateral corner, second segment shortest. In female (Fig. 1C), first segment armed with single seta at anterolateral corner, third segment as long as combined length of first and second segments.



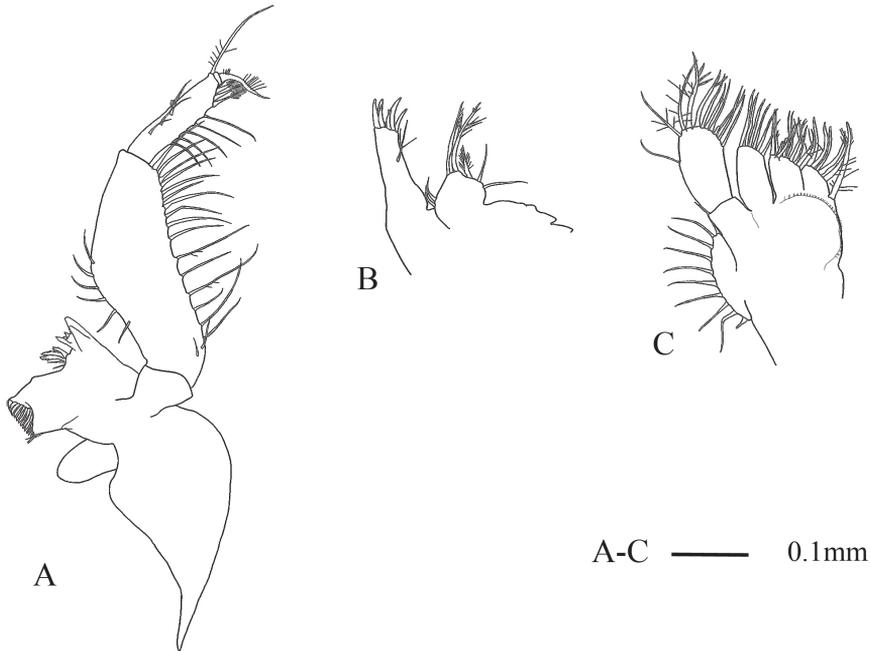
**Figure 1.** *Anisomysis (Anisomysis) spinaintus* sp. n., **A, B, D** holotype **C** allotype **A** lateral view **B** anterior part of body **C** anterior part of body **D** antenna.

Antennal scale slightly beyond anterior margin of antennular peduncle in male (Fig. 1B), and beyond anterior margin in female (Fig. 1C); 5.9 times as long as broad, slightly curved outward in male (Fig. 1D), 6.7 times as long as broad in female. Antennal peduncle (Fig. 1B, C) short, not reaching the middle of antennal scale in both sexes.

Mandibular palp (Fig. 2A) 3-segmented; second segment widened mesially at around mid-length, armed with setae on both margins, without prominent denticles; third segment 0.6 times as long as second, rectangular, armed with five setae on margin increasing in length distally, with four barbed setae on distal margin and 1 recurved and barbed seta and one long seta at distomedial corner. Maxillule and maxilla as shown in Fig. 2B and C, respectively.

First thoracopodal endopod (Fig. 3A) short and robust, armed with straight, strong terminal claw. Second thoracopodal endopod (Fig. 3B) short; merus as long as carpopropodus, dactylus slightly longer than broad. Third to sixth thoracopodal endopods (Fig. 3C–F) with carpopropodus divided distally into two segments, seventh and eighth thoracopodal endopods (Fig. 3G, H) with undivided carpopropodus in both sexes. Flagelliform part of first and eighth thoracopodal exopods 7-segmented (Fig. 3A, H) and second to seventh 8-segmented (Fig. 3B–G). Basal plates of eight thoracopodal exopods with rounded outer distal corner.

Abdomen (Fig. 1A) long and slender, sixth somite almost as long as fifth.



**Figure 2.** *Anisomysis* (*Anisomysis*) *spinaintus* sp. n., **A-C** holotype; **A** mandible and mandibular palp (right side) **B** maxillule **C** maxilla.

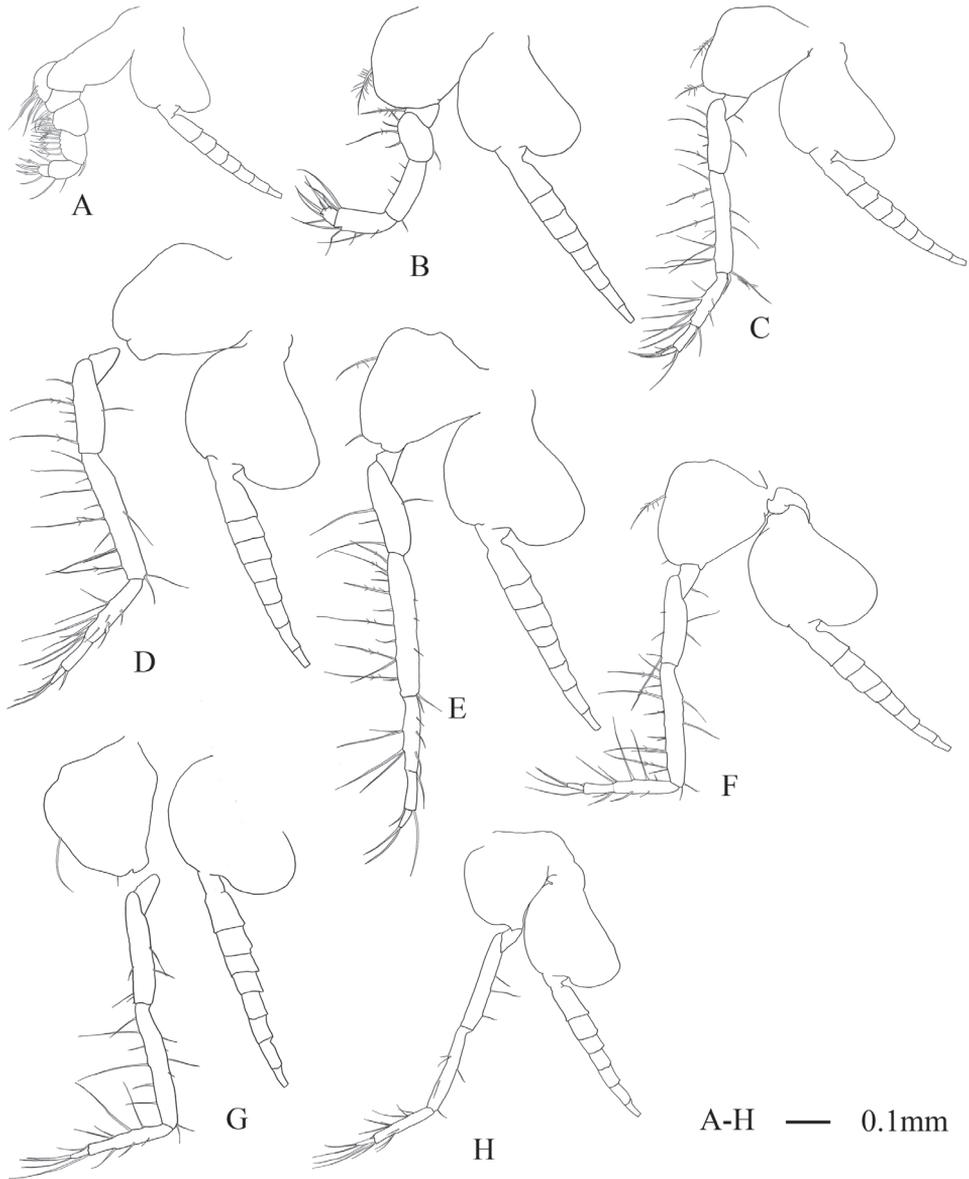
First, second, third, and fifth pleopods of males and all pleopods of females rudimentary. Fourth male pleopod (Fig. 4A) biramous; endopod thin lobed with 1 seta, exopod long, 3-segmented, extended to anterior margin of sixth abdominal somite including terminal setae (Fig. 1A); First segment as long as second and third segments combined; second segment shortest; segment length ratios 2.6:1:1.6; third segment with two terminal setae, inner seta slightly shorter than outer, and stout and swollen in proximal part and barbed in distal part, outer seta slender and naked.

Uropod (Fig. 4B) slender, setose; endopod straight, 1.8 times longer than telson excluding apical spines, no spine in statocyst region; exopod slightly curved outward, 1.2 times as long as endopod.

Telson (Fig. 4B) nearly  $3/4$  length of sixth abdominal somite, 1.7 times as long as broad at base, narrower distally, with apical cleft; lateral margin armed on distal half with 4-7 spines increasing in length distally; distal margin of each apical lobe armed with 2-4 large subequal spines. Apical cleft  $2/5$  length of telson, slightly more than  $1/2$  as broad as base at level of cleft, with rounded bottom and 6-9 spines.

**Etymology.** The specific name is derived from Latin *spina intus*, meaning spine on the inside, referring to the apical cleft of telson armed with spines.

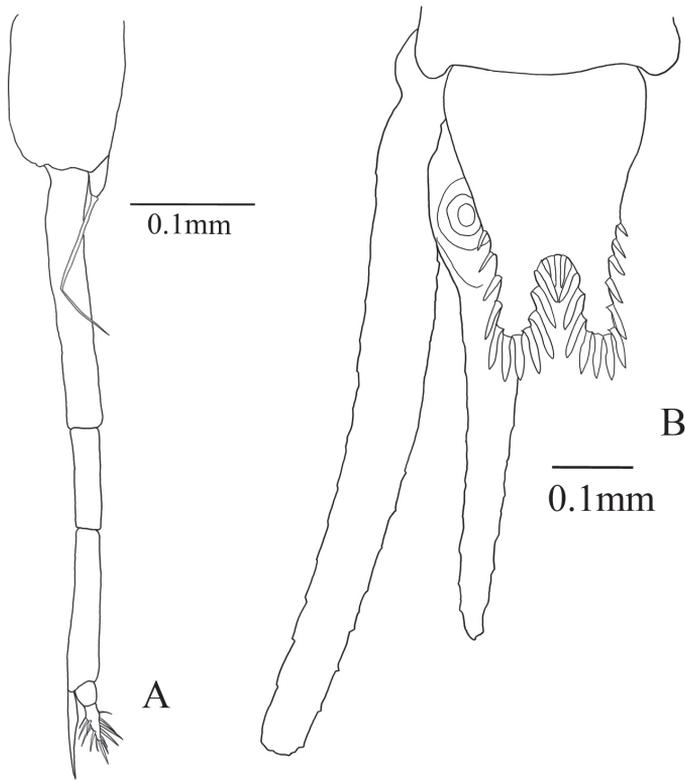
**Remarks.** The most noticeable characteristic of *A. (A.) spinaintus* is the presence of 6-9 spines on the apical cleft of telson. This new species resembles *A. (A.) incisa* Tattersall, 1936; *A. (A.) hawaiiensis* Murano, 1995; and *A. (A.) aikawai* Ii, 1964, which was re-described by Murano and Fukuoka (2003) on the basis of the specimens from Nomo,



**Figure 3.** *Anisomysis (Anisomysis) spinaintus* sp. n., **A–H** holotype **A** 1<sup>st</sup> thoracopod **B** 2<sup>nd</sup> thoracopod **C** 3<sup>rd</sup> thoracopod **D** 4<sup>th</sup> thoracopod **E** 5<sup>th</sup> thoracopod **F** 6<sup>th</sup> thoracopod **G** 7<sup>th</sup> thoracopod **H** 8<sup>th</sup> thoracopod.

Nagasaki, Japan, by the form of the apical cleft of telson. The present species is distinguished from *A. (A.) incisa* and *A. (A.) hawaiiensis* by the presence of spines on the apical cleft of the telson, and from *A. (A.) aikawai* by the deeper apical cleft and larger number of spines on the telson. Differences among these four species are summarized in Table 1.

**Distribution.** Only known from the type locality.



**Figure 4.** *Anisomysis* (*Anisomysis*) *spinaintus* sp. n., **A, B** holotype **A** 4<sup>th</sup> pleopod **B** uropods and telson.

**Table I.** Morphological differences among *A. (A.) spinaintus* n. sp; *A. (A.) incisa* Tattersall, 1936; *A. (A.) hawaiiensis* Murano, 1995, *A. (A.) aikawai* Ii, 1964; and *A. (A.) aikawai* Ii, 1964, re-described by Murano and Fukuoka (2003).

	<i>A. (A.) spinaintus</i> sp. n.	<i>A. (A.) incisa</i>	<i>A. (A.) hawaiiensis</i>	<i>A. (A.) aikawai</i>
Carpopropodus of 3 <sup>rd</sup> to 8 <sup>th</sup> thoracopodal endopod	3 <sup>rd</sup> to 6 <sup>th</sup> divided distally into 2 segments	Unsegmented	Unsegmented	8 <sup>th</sup> divided distally into 2 segments (at least)
Exopod of 4 <sup>th</sup> male pleopod: length	Anterior margin of 6 <sup>th</sup> abdominal somite	Backwards to level of the apical lobes of the telson	Middle of telson	Backwards to the posterior end of the 5 <sup>th</sup> abdominal somite
Telson: apical cleft	Deep	Deep	Deep	Deep
Spines on each lateral margin of telson	12 or 13	9 or 10	10	11 or 12 (9 or 10) #
Spines on each lateral margin of telson cleft	8	0 (un-armed)	0 (un-armed)	6 (4) #

# Re-described by Murano and Fukuoka (2003).

***Anisomysis (Anisomysis) phuketensis* sp. n.**

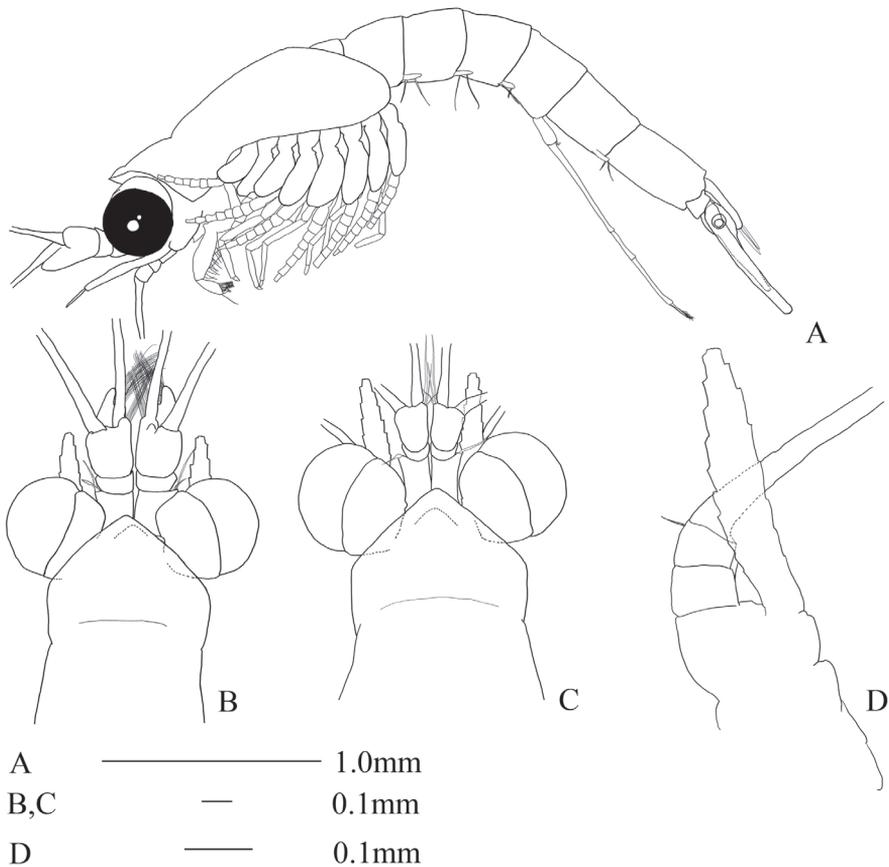
<http://zoobank.org/1C987A5B-8D8F-436B-A3C3-5B1739089E97>

Figs 5–8

**Type series.** Holotype (NSMT-Cr 24249), adult male (BL, 3.6 mm); allotype (NSMT-Cr 24250), adult female with embryos (BL, 3.9 mm); paratypes (NSMT-Cr 24251), 2 adult males (BL, 3.8, 4.0 mm) and 2 adult females with embryos (BL, 3.2, 3.6, 3.4 mm); Ko Lon, Phuket Is., Thailand, 7°47.01'N, 98°21.30'E; collected with a hand net (mesh size, 0.33 mm; mouth diameter, 30 cm) by skin diving in a coral reef of 2–3 m deep, December 3, 2010 by M. Moriya.

**Description.** Body slender (Fig. 5A). Carapace extending anteriorly into obtusely triangular rostrum with bluntly pointed apex, covering bases of antennules (Fig. 5B, C).

Eyes large, cornea occupying half of eye in dorsal view (Fig. 5A–C). Eyestalk very short, without papilliform process on dorsal surface.

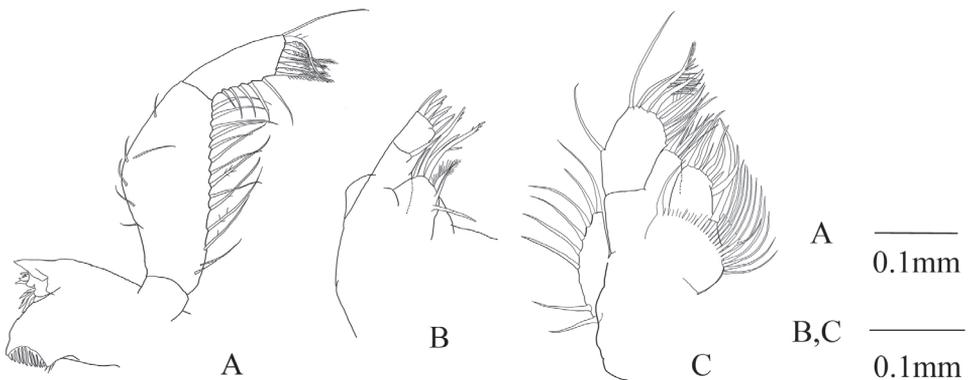


**Figure 5.** *Anisomysis (Anisomysis) phuketensis* sp. n., **A, B, D** holotype **C** allotype **A** lateral view **B** anterior part of body **C** anterior part of body **D** antenna.

Antennular peduncle more robust in male (Fig. 5B) than that in female (Fig. 5C); first segment shorter than third, armed with two setae at anterolateral corner; second segment shortest. In female (Fig. 5C), first segment armed with single seta at anterolateral corner.

Antennal scale (Fig. 5D) closely near the anterior margin of antennular peduncle in male (Fig. 5B), well beyond anterior margin in female (Fig. 5C); 5.5 times as long as broad in male, 6.1 times as long as broad in female. Antennal peduncle short, not reaching middle of antennal scale in both sexes (Fig. 5D).

Mandibular palp (Fig. 6A) 3-segmented; second segment widened mesially at around mid-length, armed with setae on both margins, without triangular processes; third segment rectangular, 0.5 times as long as second segment, outer margin armed with 5 marginal setae increasing in length distally, distal margin with 5 barbed setae on margin, 1 recurved and barbed seta and 1 long seta at distomedial corner. Maxillule and maxilla as shown in Fig. 6B and C, respectively.

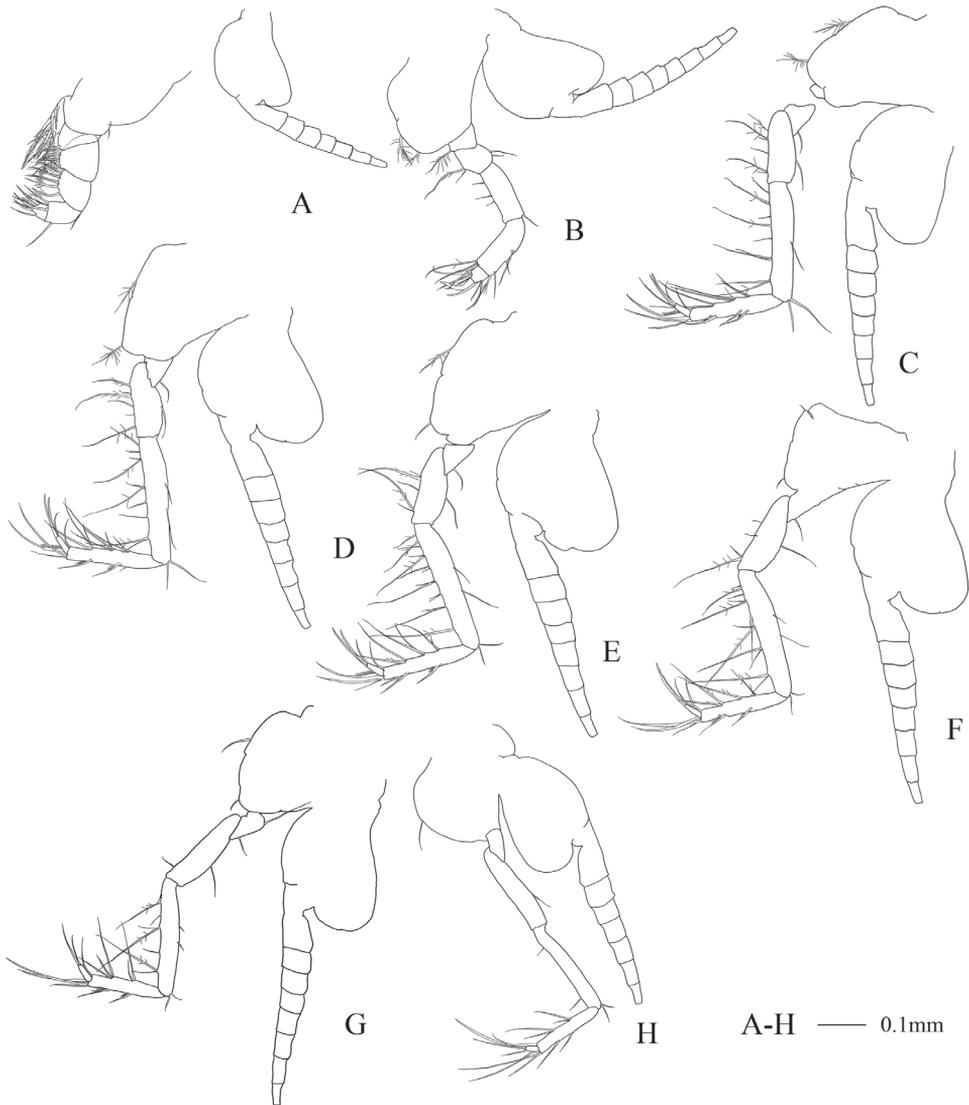


**Figure 6.** *Anisomysis* (*Anisomysis*) *phuketensis* sp. n., **A–C** holotype **A** mandible and mandibular palp (right side) **B** maxillule **C** maxilla.

First thoracopodal endopod (Fig. 7A) short and stout, armed with straight, strong terminal claw. Second thoracopodal endopod (Fig. 7B) short; merus as long as carpopodus, dactylus with strong, curved terminal claw. Third to eighth thoracopodal endopods (Fig. 7C–H) with undivided carpopodus in both sexes. Flagelliform part of first and eighth thoracopodal exopods 7-segmented (Fig. 7A, H) and second to seventh 8-segmented (Fig. 7B–G). Basal plate of eight thoracopodal exopods with rounded outer distal corners.

Abdomen (Fig. 5A) long and slender, sixth somite 1.3 times longer than fifth.

First, second, third, and fifth pleopods of male and all pleopods of female rudimentary. Fourth male pleopod (Fig. 8A) biramous; endopod minute and thin lobe with 4 setae; exopod long, three-segmented, overreaching distal end of telson (Fig. 5A). First

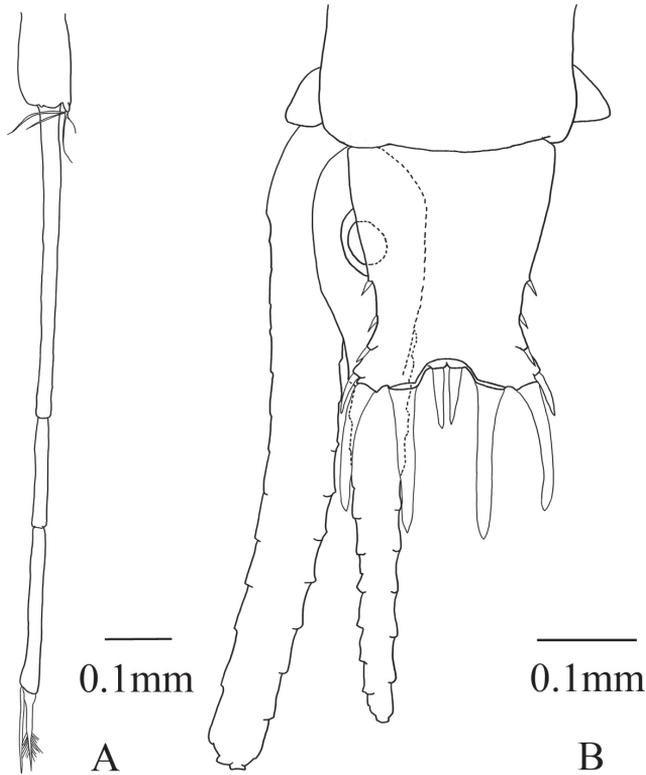


**Figure 7.** *Anisomysis (Anisomysis) phuketensis* sp. n., **A–H** holotype **A** 1<sup>st</sup> thoracopod **B** 2<sup>nd</sup> thoracopod **C** 3<sup>rd</sup> thoracopod **D** 4<sup>th</sup> thoracopod **E** 5<sup>th</sup> thoracopod **F** 6<sup>th</sup> thoracopod **G** 7<sup>th</sup> thoracopod **H** 8<sup>th</sup> thoracopod.

segment longer than second and third segments combined; second segment shortest; segment length ratios 3:1:1.5; third segment with two terminal setae, almost equal in length, outer setae slender and naked, inner setae swollen at base and barbed on distal half.

Uropod slender, setose around (Fig. 8B); endopod straight, 1.5 times longer than telson excluding apical spines, no spine in statocyst region; exopod slightly curved outward, 1.1 times as long as endopod.

Telson (Fig. 8B) short, nearly 3/5 length of sixth abdominal somite, 1.2 times longer than broad at base, compressed around distal 1/4, then expanding distally, dis-



**Figure 8.** *Anisomysis* (*Anisomysis*) *phuketensis* sp. n., **A, B** holotype **A** 4<sup>th</sup> pleopod **B** uropods and telson

tal margin slightly narrower than base; lateral margin armed with 4-5 short spines; apex of telson concave at the middle with paired spines almost equal in length, apical margin truncate with two long stout spines, outer spine curved inward, slightly shorter than inner straight spine.

**Etymology.** The species is named after the type locality.

**Remarks.** The most noticeable characteristic of *A. (A.) phuketensis* is the form of the telson. This species resembles *A. (A.) robustispina* Panampunnayil, 1984 and *A. (A.) truncata* Panampunnayil, 1993 in the presence of the peculiar long stout spines on the apical margin of telson. However, *A. (A.) phuketensis* is distinguished from *A. (A.) robustispina* by the following characters: only two long stout spines on telson (three in the latter), the length/width ratio of telson being 1.2 (1.6 in the latter), the length ratio of uropodal endopod to telson being 1.5 (2.3 in the latter). Although the telson of *A. (A.) truncata* is also armed with two pairs of stout apical spines, the outer spines are twice as long as the inner (subequal in *A. (A.) phuketensis*) and lacks the medial depression with two small spines, which is present in *A. (A.) phuketensis* and *A. (A.) robustispina*.

**Distribution.** The type locality and Ko Chueak, Hat Chao Mai National Park, Trang Province, Thailand.

**Key to the subgenera of the genus *Anisomysis* (cited from Murano and Fukuoka 2003)**

- 1 Body rather strongly built, gibbous; abdomen flexed ventrally; eye large, with cornea divided into two parts by groove.....***Pseudanisomysis* Murano & Fukuoka, 2003**
- Body slender, straight; eye globular, expanded, not divided into two portions .....**2**
- 2 Mandibular palp with second segment armed with triangular processes on mesial margin .....***Paranisomysis* Băcescu, 1973**
- Mandibular palp with second segment armed with normal setae on both margins.....**3**
- 3 Carapace with spinules on antero-lateral margin; telson with un-articulated denticles on lateral margin .....***Javanisomysis* Băcescu, 1992**
- Carapace without spinules on antero-lateral margin; telson with articulated denticles on lateral margin .....***Anisomysis* Băcescu, 1973**

**Key to the species of the subgenus *Anisomysis*****Subgenus *Anisomysis* Băcescu, 1973**

**Type species.** *Anisomysis laticauda* Hansen, 1910.

**Description.** Body straight, slender, not hispid. Cornea of eye large, globular, not divided into two portions. Antennular peduncle having neither expanded lobe nor finger-like process on second segment. Second segment of mandibular palp foliate, without triangular denticles on mesial margin. Telson variable with basally articulated denticles on lateral margin.

- 1 Telson longer than last abdominal somite.....***A. sirielloides* Băcescu, 1975**
- Telson shorter than last abdominal somite.....**2**
- 2 Telson without distal cleft.....**3**
- Telson with distal cleft.....**11**
- 3 Telson triangular with narrow apex .....**4**
- Telson with rounded or truncate distal margin .....**5**
- 4 Rostrum triangular with narrowly rounded apex; exopod of fourth male pleopod with second segment 1/3 as long as third segment; marginal spines of telson increasing distally in length, apical spine 3 times as long as broad at base.....***A. mixta* Nakazawa, 1910**
- Rostrum broadly rounded or triangular with broadly rounded apex; exopod of fourth male pleopod with second segment about 4/5 as long as third segment; marginal spines of telson subequal in length, apical spine 1.5 times as long as broad at bas.....***A. australis* Zimmer, 1918**
- 5 Distal margin of telson rounded .....**6**

–	Distal margin of telson truncate or weakly truncate .....	7
6	Telson 1.5 times as long as broad, with 10–12 spines on posterior half of each lateral margin.....	<b><i>A. chessi</i> Murano, 1983</b>
–	Telson nearly twice as long as broad, with 7–8 spines on posterior 2/3 of each lateral margin.....	<b><i>A. quadrispinosa</i> Wang, 1989</b>
7	Telson with constriction, more than 10 spines on each lateral margin .....	<b><i>A. enwetakensis</i> Murano, 1983</b>
–	Telson without constriction .....	<b>8</b>
8	Telson armed with 4–5 spines on each lateral margin .....	<b>9</b>
–	Telson armed with 9–13 spines on each lateral margin .....	<b>10</b>
9	Telson rounded triangular with weakly truncate distal margin; distal spines of telson subequal in size.....	<b><i>A. levi</i> Bănescu, 1973</b>
–	Telson trapezoid with truncate distal margin; distal spines of telson longer and stouter than lateral spines.....	<b><i>A. truncata</i> Panampunnayil, 1993</b>
10	Each lateral margin of telson with 9 spines. Exopod of fourth male pleopod reaching tip of telson .....	<b><i>A. bacescui</i> Pillai, 1976</b>
–	Each lateral margin of telson with 10–13 spines. Exopod of fourth male pleopod reaching beyond base of telson.....	<b><i>A. comorensis</i> Wooldridge &amp; Mees, 2004</b>
11	Inner margin of telson cleft unarmed with spines.....	<b>12</b>
–	Inner margin of telson cleft armed with spines.....	<b>22</b>
12	Uropodal endopod with process on mesial margin of statocyst region.....	<b>13</b>
–	Uropodal endopod without process on mesial margin of statocyst region....	<b>14</b>
13	Process on uropodal endopod blunt, without articulation at base .....	<b><i>A. bifurcata</i> Tattersall, 1912</b>
–	Process on uropodal endopod acutely pointed, with articulation at base .....	<b><i>A. spinata</i> Panampunnayil, 1993</b>
14	Each apical lobe of telson with single spine.....	<b>15</b>
–	Each apical lobe of telson with 2 or 3 spines .....	<b>19</b>
15	Telson cleft about half of telson length .....	<b>16</b>
–	Telson cleft less than 1/3 of telson length .....	<b>17</b>
16	Rostrum pointed; eyestalk with papilliform process; telson with 2 or 3 spines on lateral margin of each posterior lobe .....	<b><i>A. megalops</i> (Illig, 1913)</b>
–	Rostrum rounded; eyestalk without papilliform process; telson with 5 or 6 spines on lateral margin of each posterior lobe.....	<b><i>A. nana</i> Murano, 1995</b>
17	Each lateral margin of telson with 11–20 spines... <b><i>A. minuta</i> Liu &amp; Wang, 1983</b>	
–	Each lateral margin of telson with less than 10 spines .....	<b>18</b>
18	Each lateral margin of telson with 5–9 short slender spines. Cleft of telson 1/3 length of telson.....	<b><i>A. pelewensis</i> Ii, 1964</b>
–	Each lateral margin of telson with 3 small spines. Cleft of telson 1/5 length of telson.....	<b><i>A. unispinosa</i> Wooldridge &amp; Mees, 2004</b>
19	Telson narrowing abruptly at distal 1/3, each lateral margin with 2 spines at narrow part; each apical lobe of telson with 2 short spines .....	<b><i>A. kunduchiana</i> Bănescu, 1975</b>

- Telson gradually narrowing, each lateral margin with more than 4 spines; each apical lobe of telson with 2 or 3 spines.....**20**
- 20 Telson with V-shaped cleft, each lateral margin with 7–11 spines; each apical lobe of telson with 2 spines.....***A. hawaiiensis* Murano, 1995**
- Telson with U-shaped cleft, each lateral margin with 4–7 spines .....**21**
- 21 Each apical lobe of telson with 3 spines, each lateral margin armed with 6 or 7 spines..... ***A. incisa* Tattersall, 1936**
- Each apical lobe of telson with 2 spines, each lateral margin armed with 4 to 6 spines..... ***A. pescaprae* Connell, 2009**
- 22 Posterior margin of telson narrow; each apical lobe of telson with 1 spine ....  
.....***A. extranea* Murano, 1995**
- Posterior margin of telson broad; each apical lobe of telson with more than 3 spines.....**23**
- 23 Distal margin of telson with median depression, armed with more than 4 spines.....**24**
- Distal margin of telson with slight median sinus, armed with 2 spines.....**30**
- 24 Bottom of telson cleft convexed.....**25**
- Bottom of telson cleft rounded .....**26**
- 25 Telson 1.3 times as long as broad. Exopod of fourth male pleopod not extending beyond anterior margin of telson .....***A. hanseni* Nouvel, 1967**
- Telson 1.5 times as long as broad. Exopod of fourth male pleopod extending to distal end of telson.....***A. mullini* Murano, 1987**
- 26 Telson cleft with bottom spines only ..... ***A. neptuni* Connell, 2009**
- Telson cleft with spines entirely covered .....**27**
- 27 Exopod of fourth male pleopod extending to anterior margin of last abdominal somite .....**28**
- Exopod of fourth male pleopod extending to or beyond posterior margin of last abdominal somite .....**29**
- 28 Apical cleft as long as 1/9 of telson, each lateral margin of telson with 3 or 4 spines..... ***A. aikawai* Ii, 1964**
- Apical cleft as long as 2/5 of telson, each lateral margin of telson with 5 or 6 spines.....***A. spinaintus* sp. n.**
- 29 Exopod of fourth male pleopod extending to middle of telson, second segment 1.6 times longer than third .....***A. hashizumei* Fukuoka & Murano, 1997**
- Exopod of fourth male pleopod extending slightly beyond anterior margin of telson, second segment slightly longer than third ....***A. laticauda* Hansen, 1910**
- 30 Distal margin of telson armed with 2 or 3 pairs of long and robust spines **31**
- Distal margin of telson without long and robust spines.....**32**
- 31 Posterior margin of telson broader than basal width, with 3 pairs of long and robust spines, about 1/3 of telson length.....  
.....***A. robustispina* Panampunnayil, 1984**
- Posterior margin of telson equal to or narrower than basal width, with 2 pairs of long and robust spines, about 2/5 of telson length ...***A. phuketensis* sp. n.**

32	Telson 1.3–1.4 times as long as broad.....	33
–	Telson 1.5–1.8 times as long as broad.....	35
33	Posterior 2/3 of telson gradually narrowing distally; lateral spines of telson considerably reduced in size.....	<i>A. vasseuri</i> Ledoyer, 1974
–	Posterior 1/4 to 1/3 of telson almost parallel-sided; lateral spines of telson normally developed.....	34
34	Rostrum broadly rounded; uropodal endopod subequal to exopod in length; length ratios of 3 exopod segments of fourth male pleopod 3.1 : 1 : 1.5.....	<i>A. rotunda</i> Murano & Fukuoka, 2003
–	Rostrum triangular with rounded apex; uropodal endopod clearly shorter than exopod; length ratios of 3 exopod segments of fourth male pleopod 5.5 : 1 : 2.6 .....	<i>A. maldivensis</i> Murano & Fukuoka, 2003
35	Lateral spines of telson considerably reduced in size.....	36
–	Lateral spines of telson normally developed .....	37
36	Antennal scale not extending to distal end of antennular peduncle in male, slightly beyond in female, 6 times as long as broad; telson 1.5 times as long as broad, with 7 spines on each lateral margin.. <i>A. boraboraensis</i> Murano, 1995	
–	Antennal scale extending beyond distal end of antennular peduncle in both sexes, 7 times as long as broad; telson 1.7 times as long as broad, with 8 or 9 spines on each lateral margin .....	<i>A. parvispina</i> Murano & Fukuoka, 2003
37	Lateral depression of telson clear at distal quarter; distal margin of telson broad, with 4 or 5 pairs of long spines .....	<i>A. brevicauda</i> Wang, 1989
–	Lateral depression of telson very slight if present; distal margin of telson narrow, with 3 pairs of long spines.....	<i>A. akajimaensis</i> Murano, 1990

**Note about the subgenus *Pseudanisomysis***

In the middle of September 2015, the subgenus *Pseudanisomysis* is treated as a junior synonym of the subgenus *Carnegieomysis* in the World Register of Marine Species (WoRMS: Mees, 2015). The reference is Mees J (2015) *Anisomysis* (*Carnegieomysis*) W. Tattersall, 1943. In: Mees J, Meland K (Eds.) World List of Lophogastrida, Stygiomysida and Mysida. Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=456543> on 2015–09–29.

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## References

- Băcescu M (1973) *Anisomysis levi* sp. n. from the Red Sea and the dichotomic key of the species belonging to the genus, with description of a new taxon, *Paranisomysis* n. sg. Revue Roumaine de Biologie: Série de Zoologie 18: 173–180.
- Băcescu M (1975) Contributions to the knowledge of the mysid (Crustacea) from the Tanzanian waters. University Science Journal (University of Dar es Salaam) 1: 39–61.
- Băcescu M (1992) *Javanisomysis gutzui*, n. sg., sp. n., mysidacé grégaire des eaux Indonésiennes. Revue Roumaine de Biologie: Série de Biologie Animale 37: 79–86.
- Connell AD (2009) The genus *Anisomysis* (Crustacea: Mysidae) from the east coast of South Africa – descriptions of three new species, and range extensions of two known species. African Natural History 5: 17–30.
- Fukuoka K, Murano M (1997) Mysidacea from coastal waters of Iriomote Island, Ryukyu Islands, southwestern Japan, with descriptions of three new species. Journal of Crustacean Biology 17: 520–537. doi: 10.2307/1549445
- Hansen HJ (1910) The Schizopoda of the Siboga Expedition. Siboga Expedition 37: 1–123.
- Hansen HJ (1912) 27. The Schizopoda. Report on the scientific results of the expedition to the tropical Pacific by the U.S. Fish Commission Steamer Albatross, the Schizopoda, Memoirs of the Museum of Comparative Zoology at Harvard College 35: 173–296.
- Ii N (1964) Fauna Japonica, Mysidae (Crustacea). Biogeographical Society of Japan, 610 pp.
- Illig G (1913) Ein weiterer Bericht über die Schizopoden der Deutschen Tiefsee-Expedition 1898–1899. Zoologischer Anzeiger 43: 271–273.
- Ledoyer M (1974) *Anisomysis vasseuri* sp. n. Mysidacé nouveau vivant a l'entrée des grottes sous-marines récifales. Tethys 5: 361–366.
- Liu R, Wang S (1983) On three new species of Mysidacea (Crustacea) from the coastal waters of Guangdong, China. Oceanologia et Limnologia Sinica 14: 522–530.
- Mees J (2015) Mysida. World Register of Marine Species. <http://www.marinespecies.org/aphia.php?p=taxdetails&id=149668> [accessed on 2015–08–20]
- Mees J, Meland K (2015) World List of Lophogastrida, Stygiomysida and Mysida. World Register of Marine Species. <http://www.marinespecies.org/aphia.php?p=taxdetails&id=456543> [accessed on 2015–09–29]
- Murano M (1983) Mysidacea fauna from Enewetak Lagoon, Micronesia. Bulletin of Plankton Society of Japan 30: 81–90.
- Murano M (1987) A new species of the genus *Anisomysis* from the Great Barrier Reef (Mysidacea). Crustaceana 52: 47–52. doi: 10.1163/156854087X00051
- Murano M (1990) Mysidacea from coastal water of Akajima Island, Ryukyu Islands. Journal of Tokyo University of Fisheries 77: 189–212.
- Murano M (1995) New and already known species of the genus *Anisomysis* (Mysidacea) from Hawaii and the Society Islands. Journal of Crustacean Biology 15: 355–364. doi: 10.2307/1548962

- Murano M, Fukuoka K (2003) A systematic study of the genus *Anisomysis* (Crustacea: Mysida: Mysidae), with description of six new species. Bulletin of the National Science Museum, Tokyo, Ser. A 29: 65–102.
- Nakazawa K (1910) Notes on Japanese Schizopoda. Annotationes Zoologicae Japonenses 7: 247–261.
- Nouvel H (1967) Mysidacés récoltés par S. Frontier a Nosy-Bé. IV. *Mesacanthomysis pygmaea* n. gen., sp. n. et *Anisomysis hanseni* sp. n. Bulletin de la Société d'Histoire Naturelle de Toulouse 106: 105–121.
- Nouvel H (1973) Un Mysidacé nouveau de la Nouvelle-Calédonie: *Anisomysis thurneysse* sp. n. Bulletin du Muséum National d'Histoire Naturelle 3e série, Zoologie 124: 1453–1459.
- Panampunnayil SU (1984) Two new species belonging to the genus *Anisomysis* (Crustacea, Mysidacea) and a new record of *Anisomysis bipartoculata* from the Indian Ocean. Journal of Plankton Research 6: 943–952. doi: 10.1093/plankt/6.6.943
- Panampunnayil SU (1993) Two new species of *Anisomysis* (Crustacea, Mysidacea) from the Lakshadweep Archipelago. Journal of Plankton Research 15: 1141–1148. doi: 10.1093/plankt/15.10.1141
- Pillai NK (1976) Observations on two Indo-West Pacific mysids. Aquatic Biology 1: 65–76.
- Sawamoto S (2014) Current status of mysid taxonomy in Southeast Asia. Marine Research in Indonesia (Proceedings of LIPI-JSPS Joint Seminar on Coastal Ecosystems in Southeast Asia, 2012). Marine Research in Indonesia 39: 1–14.
- Sawamoto S, Srinui K, Moriya M (2015) Re-definition of the genus *Javanisomysis* Băcescu, 1992 as a subgenus in the genus *Anisomysis* Hansen, 1910 (Mysida, Mysidae) and a new species of the subgenus from a coastal water in Phuket, Thailand. Crustaceana 88: 809–838. doi: 10.1163/15685403-00003455
- Tattersall WM (1912) On the Mysidacea and Euphausiacea collected in the Indian Ocean during 1905. Transactions of the Linnean Society of London, Zoology, ser. 2 15: 119–136, 2 pls.
- Tattersall WM (1936) Mysidacea and Euphausiacea. British Museum (Natural History), Great Barrier Reef Expedition 1928–29. Scientific Reports 5: 143–176.
- Tattersall WM (1943) Biological results of last cruise of Carnegie IV. The mysids. In: Ault JP (commander), Scientific results of cruise VII of the Carnegie during 1928–1929, Biology IV. Publ. Carnegie Inst. Washington, No. 555: 61–72.
- Wang S (1989) On two new species of genus *Anisomysis* (Crustacea Mysidacea) from the South China Sea. Studia Marina Sinica 30: 229–237.
- Wittmann KJ, Ariani AP, Lagardère JP (2014) Orders Lophogastrida Boas, 1883, Stygiomysida Tchindonova, 1981, and Mysida Boas, 1883 (also known collectively as Mysidacea). In: von Vaupel Klein JC, Charmantier-Daures M, Schram FR (Eds) Treatise on Zoology—Anatomy, Taxonomy, Biology. The Crustacea. Revised and updated, as well as extended from the *Traité de Zoologie*, Vol. 4 Part B (54). Koninklijke Brill NV, Leiden, 189–396, 404–406. doi: 10.1163/9789004264939\_006
- Wooldridge T, Mees J (2004) Mysidacea from the Comoros Archipelago with descriptions of two new species. Annals of the South African Museum 112: 98–102.
- Zimmer C (1918) Neue und wenig bekannte Mysidaceen des Berliner Zoologischen Museums. Mitteilungen aus dem Zoologischen Museum in Berlin 9: 13–26.

