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Lithobius (*Monotarsobius*) *zhangi* sp. n., a new species from Eastern China (Chilopoda, Lithobiomorpha, Lithobiidae)

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Abstract

Lithobius (*Monotarsobius*) *zhangi* **sp. n.** (Lithobiomorpha: Lithobiidae), recently discovered from Nanshan Park, Yantai City, Shandong Province, and Wuyishan County, Nanping City, Fujian Province, from China, is described. Morphologically it resembles *L.* (*M.*) *songi* Pei, Ma, Shi, Wu, Zhou, 2011 from Province Hebei, China, but can be readily distinguished from the latter by antennae composed of 15+15–19+19 articles versus 19+19–21+21 articles, terminal claw of female gonopods inner tooth broader than the outer vs dorsal and ventral tooth about same in size, ventral plectrotaxy 01320, dorsal plectrotaxy 10210 in the 14th legs, 01210 and 10200 respectively in *L.* (*M.*) *songi*. A key to the *Lithobius* (*Monotarsobius*) species of China and Korea is presented.

Keywords

Lithobiidae, *Lithobius* (*Monotarsobius*) *zhangi*, Shandong Province, China, identification key

Introduction

The centipede subgenus *Lithobius* (*Monotarsobius*) Verhoeff, 1905 (Lithobiomorpha: Lithobiidae) is characterized by the presence of fused tarsi of legs 1–13 and antennal articles fixed at 20 or thereabouts (Eason 1992), this subgenus comprises 114 species known

from Asia, Europe, and North Africa (Pocock 1895; Trotzina 1895; Attems 1901, 1904; Dobroruka 1960, 1979; Zalesskaja 1978; Farzalieva and Zalesskaja 2002; Farzalieva 2006; Zapparoli 2006; Zapparoli and Edgecombe 2011; Dányi and Tuf 2012).

Lithobiomorph centipedes of China are poorly known as only sixty-nine species and subspecies are hitherto known from the country (Attems 1938, 1953; Takakuwa 1939, 1940; Takakuwa and Takashima 1949; Chamberlin and Wang 1952; Wang 1959, 1963; Zalesskaja 1978; Wang and Mauriès 1996; Zhang, 1996; Eason 1997, 1997; Chao 2005; Zapparoli 2006; Ma et al. 2007a, b, c; 2008a, b, c, d; Ma et al. 2009a, b; 2012a, b; 2013; 2014; Pei et al. 2010; 2011a, b). The subgenus *Lithobius* (*Monotarsobius*) is among the poorly studied taxa of China, with only ten species being up to now registered from its territory. None of them has hitherto been documented from Shandong Province. Herewith we describe a new species recently found in Shandong and Fujian Provinces.

Methods

All specimens were hand-collected under leaf litter or stones. The material was examined with the aid of a Motic-C microscope, made in China. The colour description is based on specimens in 75% ethanol, and body length is measured from anterior margin of the cephalic plate to posterior end of postpedal tergite. Type specimens are preserved in 75% ethanol and deposited in the department of Life Sciences, Hengshui University, Hengshui, China. The terminology of the external anatomy follows Bonato et al. (2010).

The following abbreviations are used in the text and the tables: T, TT = tergite, tergites; S, SS = sternite, sternites; C = coxa, Tr = trochanter, P = prefemur, F = femur, Ti = tibia, a = anterior, m = median, p = posterior.

Taxonomic part

Lithobiidae Newport, 1844

***Lithobius* (*Monotarsobius*) *zhangi* sp. n.**
http://zoobank.org/32726748-44E8-452C-A4FC-C461A084B73B
Figs 1–6

Material examined. Holotype. ♀ (Figure 1), body length 8.0 mm, cephalic plate 0.5 mm long, 0.5 mm broad, Nanshan Park, Yantai City, Shandong Province, 37°05'N, 121°04'E, 27 m, 5 July 2005, leg. Huiqin Ma. **Paratypes.** 2 ♀♀, same data as holotype.

Other material. 15 ♀♀, 2 ♂♂, Wuyishan County, Nanping City, Fujian Province, 27°43'N 118°01'E, 238 m, 10 August 2010, leg. Feng Zhang and Huiqin Ma.

Etymology. The specific name is a patronym in honor of the myriapodologist Professor Chongzhou Zhang, Academician at the Chinese Academy of Sciences.

Diagnosis. A *Lithobius* (*Monotarsobius*) species with body length 7.0–8.0 mm, antennae composed of 15–19 articles; 5–6 ocelli on each side, arranged in 2 irregular rows, the terminal ocellus comparatively large; Tömösváry's organ moderately small, slightly smaller than adjoining ocelli; 2+2 coxosternal teeth; porodonts moderately slender, posterolateral to the most lateral teeth; posterior angles of all tergites without triangular projections; coxal pores 1222, oval to round; female gonopods with 2+2 small, coniform spurs; terminal claw of the third article tridentate; male gonopods short and small, with 1 long seta on the terminal segment.

Description. Body length: 7.0–8.0 mm, cephalic plate 0.5–0.6 mm long, 0.5–0.6 mm wide.

Colour: basal antennal articles lavender, the 7–8 article gradually turning to yellow-brown, distalmost article yellow-brown; tergites pale brown to chestnut-brown; cephalic plate, TT1, 14 and 15 yellow-brown; pleural region pale grey to lavender; sternites pale grey to gray; distal part of forcipules brown, basal and proximal parts of forcipules, forcipular coxosternite and SS 14 and 15 pale yellow-brown with greyish hue; all legs lavender, the distal of every article of all legs slightly dark, the tarsus of all legs yellow-brown.

Antennae: 15–19 articles (Figure 1); basal article slightly longer than wide, second one markedly longer than wide, following articles gradually shortening, distal article up to 2.0–2.5 times as long as wide. Abundant setae on the antennal surface, less so on the basal articles, gradually increasing in density to about sixth article, then more or less constant.

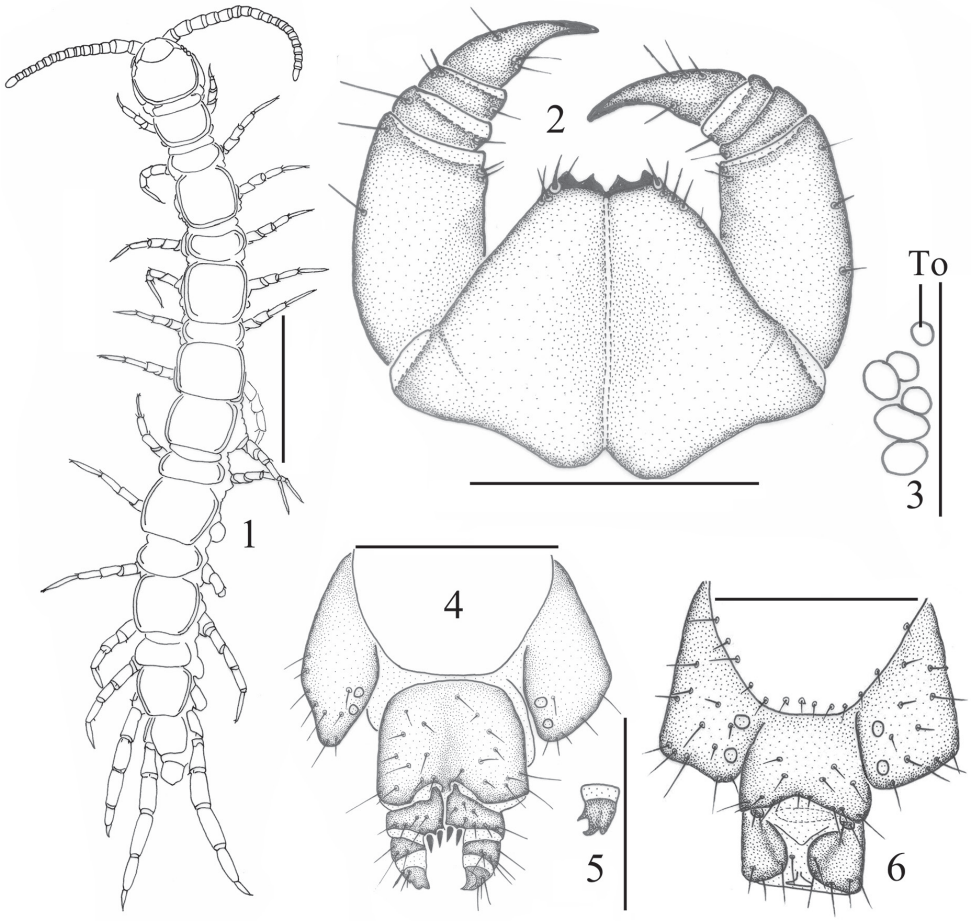
Cephalic plate smooth, convex, width approximately equal to length; tiny setae emerging from pores scattered very sparsely over the whole surface; frontal marginal ridge with shallow anterior median furrow; short to long setae scattered along the marginal ridge of the cephalic plate; lateral marginal ridge discontinuous, posterior marginal ridge moderately broader, straight or slightly bulging.

Five–six oval to rounded ocelli on each side (Figure 3) in two irregular rows; the terminal ocellus comparatively large; other ocelli about equal in size apart the ocelli adjoining to the ventral; all ocelli domed, translucent, usually darkly pigmented.

Tömösváry's organ situated at the anterolateral margin of the cephalic plate, slightly smaller than the adjoining ocelli and lying well apart from them (Figure 3-To).

Coxosternite subtrapezoidal (Figure 2), anterior margin narrow; median diastema moderately deep, V-shaped; anterior margin with 2+2 teeth; porodonts slender, lying posterolateral to the most lateral teeth (Figure 4); some long setae scattered on the ventral side of coxosternite.

All tergites smooth, without wrinkles, backside slightly hunched; T 1 posterolaterally narrower than anterolaterally, generally trapeziform, narrower than T 3 and the cephalic plate, the cephalic plate slightly wider than T 3 or equal to T 3; posterior margin of T 1 straight or slightly convex, its posterior marginal ridge continuous; posterior margin of TT 3, 5, 8, 10, 12 and 14 shallow concave, posterior marginal ridge of TT 3, 5, 8, 10 and 12 discontinuous; all posterior angles generally rounded, without



Figures 1–6. *Lithobius* (*Monotarsobius*) *zhangi* sp. n., **1–5** holotype, female: **1** habitus, dorsal view, scale bar 1 mm; **2** forcipular segment, ventral view, scale bar 500 μm; **3** ocelli and Tömösváry's organ (To), lateral view, scale bar 250 μm; **4** posterior segments and gonopods, ventral view, scale bar 500 μm; **5** terminal claw of right gonopod, dorsal view, scale bar 250 μm; **6** paratype, male: posterior segments and gonopods, ventral view, scale bar 500 μm.

triangular projections; lateral marginal ridge of all tergites continuous (Figure 1); tiny setae scattered very sparsely over the surface.

Posterior side of sternites narrower than the anterior one, generally trapeziform, comparatively smooth, setae emerging from pores scattered very sparsely on the surface, slightly thicker setae on the surface of the anterior part of each sternite; A pair of longer setae approximately symmetrical on the surface of both the anterior and the posterior part of each sternite; 2–3 longer setae on both anterior lateral borders, 1–2 comparatively long setae scattered sparsely on posterior margin of sternites.

Legs strong, tarsal articulation not defined on legs 1–13, tarsal articulation well defined on legs 14–15; all legs with fairly long curved claws; anterior and posterior ac-

Table 1. Leg plectrotaxy of *L.* (*M.*) *zhangi* sp. n.

Legs	Ventral					Dorsal				
	C	Tr	P	F	Ti	C	Tr	P	F	Ti
1	-	-	p	am	m	-	-	p	a	a
2	-	-	p	am	m	-	-	p	ap	a
3–9	-	-	-	am	m	-	-	p	ap	ap
10	-	-	-	am	m	-	-	p	p	ap
11	-	-	p	am	m	-	-	p	p	ap
12	-	-	p	am	m	-	-	mp	p	a
13	-	-	p	am	m	-	-	mp	p	p
14	-	m	amp	am	-	a	-	mp	p	-
15	-	m	amp	am	-	a	-	mp	-	-

cessory spines on legs 1–14; anterior accessory spine moderately long and slender, the posterior one slightly strong; the anterior accessory spines form relatively large angles with the pretarsus, the posterior accessory spines form relatively small angles with the pretarsus; no anterior accessory spines on legs 15. Short to comparatively long setae scattered very sparsely over the surface of all segments of all legs, more setae scattered on the surface of tarsus, slightly thick setae arranged in a row on the ventral side of tarsus; legs 14 and 15 absence of secondary sexual characters on femur or tibia, obvious thicker and stronger than other legs, tarsus 1 about 3.3–4.5 times as long as wide, tarsus 2 about 65%–82% the length of tarsus on legs 15. Leg plectrotaxy as in Table 1.

Coxal pores 1222, round or slightly ovate, coxal pore field in a relatively flat surface.

Female S 15 anterolaterally broader than posterolaterally, generally trapeziform, posteromedially straight, generally yellow-brown; short to long setae scattered sparsely on the surface and the lateral margin, 2 longer setae on posterior lateral borders; sternite of genital segment usually well chitinised, wider than long; relatively long setae scattered over the ventral surface of the genital segment, few setae near S 15, regularly fringed with longer setae along the posterior margin; posterior margin of genital sternite deeply concave between the condyles of gonopods, except for a small, median approximately triangular bulge. Gonopods: first article fairly broad, bearing 7–8 long setae arranged in three irregular rows; 2+2 moderately small, blunt, coniform spurs, inner spur slightly smaller than the outer (Figure 4); second article with 3–4 rather long setae, arranged in two irregular rows on the ventral side, third article with 2 comparatively long setae lying on the ventral side, terminal claw tridentate, the inner broader than the outer (Figure 5).

Male S 15 posterolaterally narrower than anterolaterally, generally trapeziform, posteromedially straight, sparsely covered with short to long setae; the sternite of the genital segment wider than long, usually well sclerotised. Posterior margin quite deeply concave between the gonopods, without a medial bulge; comparatively long setae evenly scattered on the ventral surface of the genital segment, few setae near S 15, gonopod short, consisting of a small bulge, with two long setae, apically slightly sclerotised (Figure 6).

Habitat. The specimens were collected in a *Larix* forest. The species inhabits moderately moist habitats under roadside stones and forest floor.

Remarks. The new species is morphologically close to *L.* (*M.*) *songi* Pei, Ma, Shi, Wu, Zhou, 2011 from Province Hebei, China, with which it shares the following traits: 2+2 coxosternal teeth, 2+2 spurs of female gonopods and 1222 coxal pores, the terminal claw of the female gonopods tridentate. It can however be distinguished from the latter by antennae composed of 15+15–19+19 articles versus 19+19–21+21 articles, terminal claw of female gonopods inner tooth broader than the outer vs dorsal and ventral tooth about same in size, ventral plectrotaxy 01320, dorsal plectrotaxy 10210 in the 14th legs, 01210 respectively 10200 in *L.* (*M.*) *songi*. The new species is morphologically close to *L.* (*M.*) *dziadoszi* Matic, 1970, from Korea, with which it shares the following traits: antennae composed of 15–20 articles, 2+2 coxosternal teeth, 2+2 spurs of female gonopods, the terminal claw of the female gonopods tridentate. It can however be distinguished from the latter by 5–6 ocelli versus 7 ocelli, Tömösváry's organ smaller than adjoining ocellus versus larger than adjoining ocellus, 1222 coxal pores other than 3333 coxal pores, male legs 15 absence of secondary sexual characters on femur versus presence secondary sexual characters, ventral plectrotaxy 01320, dorsal plectrotaxy 10210 in the 14th legs, 01321 respectively 10310 in *L.* (*M.*) *dziadoszi*. The new species is morphologically close to *L.* (*M.*) *riedeli* Matic, 1970, from Korea, with which it shares the following traits: antennae composed of 15–19 articles, 2+2 coxosternal teeth, 2+2 spurs of female gonopods, the terminal claw of the female gonopods tridentate. It can however be distinguished from the latter by Tömösváry's organ smaller than adjoining ocellus versus larger than adjoining ocellus, 1222 coxal pores other than 2222 or 3333 coxal pores, male legs 15 absence of secondary sexual characters on femur versus presence secondary sexual characters, ventral plectrotaxy 01320, dorsal plectrotaxy 10210 in the 14th legs, 01210 respectively 10200 in *L.* (*M.*) *riedeli*. The new species is morphologically close to *L.* (*M.*) *mroczkowskii* Matic, 1970, from Korea, with which it shares the following traits: 5–6 ocelli, 2+2 coxosternal teeth, 2+2 spurs of female gonopods, male legs 15 absence of secondary sexual characters on femur. It can however be distinguished from the latter by antennae composed of 15–19 articles versus 20–21 articles, 1222 coxal pores other than 3343 or 4564 coxal pores, the terminal claw of the female gonopods tridentate other than simple, ventral plectrotaxy 01320, dorsal plectrotaxy 10210 in the 14th legs, 01332 respectively 10311 in *L.* (*M.*) *mroczkowskii*.

Key to the Chinese and Korean species of *Lithobius* (*Monotarsobius*)

To assist in the identification of the Chinese and Korean of *Lithobius* (*Monotarsobius*), the following key is offered. This key emphasizes characters that can be examined without high-magnification microscopy; moreover, these characters are specific to the taxa occurring in China and Korea.

1 1111 coxal pores ..... ***L.* (*M.*) *monoforaminis* Ma, Pei, Wu, Lin, Gai, 2012**
– At least 1222 coxal pores **2**
2 4–6 coxal pores **3**
– At most 3 coxal pores. **5**
3 8–11 ocelli on each side of cephalic plate ... ***L.* (*M.*) *crassipes* L. Koch, 1862**
– 5–6 ocelli on each side of cephalic plate **4**
4 5555 coxal pores, 3+3, 4+4, 3+4 spurs of female gonopods ..... ***L.* (*M.*) *ramulosus* (Takakuwa, 1941)**
– 3343 or 4564 coxal pores, 2+2 spurs of female gonopods ..... ***L.* (*M.*) *mroczkowskii* Matic, 1970**
5 Four ocelli on each side of cephalic plate, 17+17 antennal articles ..... ***L.* (*M.*) *crassus* (Loksa, 1965)**
– Five or more ocelli on each side of cephalic plate, antennal not less than 18+18 articles **6**
6 Tömösváry's organ smaller than adjoining ocellus **7**
– Tömösváry's organ larger than adjoining ocellus or about same in size **8**
7 With anterior spine on prefemur on legs 14–15 ..... ***L.* (*M.*) *zhangi* sp. n.**
– Without anterior spine on prefemur on legs 14–15 ..... ***L.* (*M.*) *songi* Pei, Ma, Shi, Wu, Zhou, 2011**
8 male legs 15 presence secondary sexual characters, the terminal claw of the female gonopods tridentate **9**
– male legs 15 absence secondary sexual characters, the terminal claw of the female gonopods not tridentate **10**
9 Ventral plectrotaxy 01210, dorsal plectrotaxy 10200 in the 14th legs ..... ***L.* (*M.*) *riedeli* Matic, 1970**
– Ventral plectrotaxy 01321, dorsal plectrotaxy 10310 in the 14th legs ..... ***L.* (*M.*) *dziadoszi* Matic, 1970**
10 Tömösváry's organ larger than the biggest ocellus ..... ***L.* (*M.*) *holstii* (Pocock, 1895)**
– Tömösváry's organ smaller than the biggest ocellus **11**
11 With one protuberance at the end of the dorsal of tibia of 15 legs in male .... ***L.* (*M.*) *ferganensis* (Trotzina, 1894)**
– Without protuberance at the end of the dorsal of tibia of 15 legs in male . 12
12 With posterior spine on prefemur on legs 11–13 ..... ***L.* (*M.*) *obtusus* (Takakuwa, 1941)**
– Without posterior spine on prefemur on legs 11–13 ..... ***L.* (*M.*) *subspinipes* Ma, Pei, Zhu, Zhang, Liu, 2009**

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The millipede genus *Eviulisoma* Silvestri, 1910 in Kenya, with descriptions of new species (Diplopoda, Polydesmida, Paradoxosomatidae)

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Abstract

The genus *Eviulisoma*, the largest among Afrotropical Paradoxosomatidae, currently encompasses 36 species or subspecies, including six new from Kenya: *E. ngaia* **sp. n.**, *E. ngaiaorum* **sp. n.**, *E. taitaorum* **sp. n.**, *E. taita* **sp. n.**, *E. kirimeri* **sp. n.** and *E. kakamega* **sp. n.** In addition, *E. alluaudi* Brolemann, 1920 and *E. silvestre* (Carl, 1909) are recorded for the first time beyond their type localities in Kenya and Tanzania, respectively, based on new material from Kenya. A key is given to all ten species of the genus presently reported from Kenya.

Keywords

Diplopoda, *Eviulisoma*, taxonomy, new species, key

Introduction

The genus *Eviulisoma* Silvestri, 1910 is the largest among Afrotropical Paradoxosomatidae, currently known to encompass 30 species or subspecies in central and eastern Africa (Nguyen and Sierwald 2013). The reader is referred to Jeekel (2003) for a most useful review of taxonomic research into *Eviulisoma*, a detailed new diagnosis, an outline of informal species groups and a key to most of the constituent species.

The following checklist of *Eviulisoma* species or subspecies has been extracted from Jeekel (2003) and Nguyen and Sierwald (2013):

1. *E. cavallii* (Silvestri, 1907), the type species, from Uganda and Rwanda;
2. *E. alluaudi* Brolemann, 1920, from Kenya;
3. *E. boranicum* Manfredi, 1939, from Ethiopia;
4. *E. castaneum* Attems, 1953, from the Democratic Republic of the Congo;
5. *E. cervicorne* (Attems, 1927), from an unknown locality in Africa;
6. *E. congicolens* (Chamberlin, 1927), from the Democratic Republic of the Congo;
7. *E. cylindricum* Attems, 1953, from the Democratic Republic of the Congo;
8. *E. cylindricum simile* Attems, 1953, from the Democratic Republic of the Congo;
9. *E. dabagaense* Kraus, 1958, from Tanzania;
10. *E. debile* Attems, 1938, from the Democratic Republic of the Congo;
11. *E. egregium* Attems, 1938, from the Democratic Republic of the Congo;
12. *E. fossiger* (Carl, 1909), from Tanzania;
13. *E. graueri* Attems, 1944, from the Democratic Republic of the Congo;
14. *E. insulare* Brolemann, 1920, from Zanzibar Island, Tanzania;
15. *E. iugans* (Chamberlin, 1927), from the Democratic Republic of the Congo;
16. *E. iuloideum* (Verhoeff, 1941), from Tanzania;
17. *E. jeanneli* Brolemann, 1920, from Kenya;
18. *E. kwabuniense* Kraus, 1958, from Tanzania;
19. *E. lanceolatum* Attems, 1953, from the Democratic Republic of the Congo;
20. *E. muturanum* Attems, 1937, from both the Democratic Republic of the Congo and the Republic of the Congo (Brazzaville);
21. *E. obesum* Attems, 1953, from the Democratic Republic of the Congo;
22. *E. obscurum* Attems, 1937, from the Democratic Republic of the Congo;
23. *E. pallidum* Attems, 1939, from Kenya;
24. *E. schoutedeni* (Attems, 1929), from the Democratic Republic of the Congo;
25. *E. silvaticum* Attems, 1953, from Rwanda;
26. *E. silvestre* (Carl, 1909), from Tanzania;
27. *E. somaliense* Ceuca, 1971, from Somalia;
28. *E. tertalinus* Manfredi, 1941, from Ethiopia;
29. *E. tritonium* Attems, 1937, from the Democratic Republic of the Congo;
30. *E. ussuwiense* (Carl, 1909), from Tanzania.

Prompted by the discovery of several new or poorly-known congeners in Kenya, eastern Africa, this paper focuses on their descriptions or records, as well as presenting a key to all *Eviulisoma* species currently known to occur in Kenya.

Material and methods

The material underlying the present contribution was taken in Kenya in 1999–2004. Most of the types are housed in the collection of the Royal Museum for Central Africa,

Tervuren, Belgium (MRAC), a few paratypes have been donated to the Zoological Museum, Moscow State University, Moscow, Russia (ZMUM + entry number).

SEM micrographs were taken using a JEOL JSM-6480LV scanning electron microscope. After examination, SEM material was removed from stubs and returned to alcohol, all such samples being kept in MRAC.

Line drawings were very skillfully executed by Mrs Nadine Van Noppen (MRAC).

Results

***Eviulisoma ngaia* sp. n.**
http://zoobank.org/F10DDEC4-2738-42E8-833A-A08B839D5DFB
Fig. 1, Map 1

Type material. Holotype ♂ (MRAC 20799), Kenya, Ngaia Forest, N00°19', E38°02', ca 1070 m a.s.l., 2.XII.2002, leg. D. VandenSpiegel.

Paratypes: 3 ♂, 1 ♀, 1 juv. (MRAC 22634), 1 ♂ (ZMUM ϱ2442), same data, together with holotype; 1 ♂ (MRAC 20703), same data, 3.XII.2002, leg. D. VandenSpiegel.

Name. To emphasize the type locality, a noun in apposition.

Diagnosis. Differs from all congeners but *E. ngaiaorum* sp. n. in the absence of a sternal excavation in ♂ segment 6, from *E. ngaiaorum* sp. n. in the absence of sternal cones in the ♂ and by the presence of a well-developed, phylloid, postfemoral process of the gonopod (Fig. 1C-E). See also Key below.

Description. Length of holotype ca 16 (♂), of adult paratype ca 18 mm (♀), width of midbody metazonae 1.5–1.6 (♂) or 2.0 mm (♀). Coloration uniformly yellowish, often with an annulated pattern of slightly more intense yellowish to marbled reddish yellow metazonae. Legs usually slightly lighter to nearly pallid.

Body subcylindrical, metazonae only faintly vaulted laterally compared to prozonae (Fig. 1A, B). In width, collum > segment 2 > head = segments 5-16 > 3 = 4 (♂) or head = segments 6-16 > 2 = 4 (♀); body behind segment 17 gradually tapering towards telson. Clypeolabral region rather densely setose, vertigial region bare (Fig. 1A). Antennae medium-sized, only slightly clavate, reaching behind body segment 2 (♂) or its midpoint (♀) when stretched dorsally; in length, antennomere 2 = 3 = 6 > 4 = 5 > 1 = 7; antennomeres 5 and 6 each with a distodorsal compact group of tiny bacilliform sensilla (as in Fig. 4G). Paraterga nearly missing, on each side a large, broadly rounded, ventrolateral lobe only in collum; a modest, caudally invariably rounded ridge demarcated by a premarginal lateral sulcus only dorsally in segment 2, thereafter totally wanting (Fig. 1A, B). Ozopores lateral, rather inconspicuous (as in Fig. 4B, C), lying at ca 1/3 of metazonite length in front of caudal margin (Fig. 1B). Body surface dull to poorly shining, smooth, microalveolate to faintly shagreened. Axial line missing. A transverse metatergal pigmented line traceable only dorsally in caudal 1/3 on segments 5–18, absent from 19th. Tergal setae short, mostly ca 1/4–1/5 as long as metazonite, largely abraded, pattern traceable only as 2+2 or 3+3 setae, but not their insertion points, placed in anterior 1/3 of metaterga. Stricture dividing pro- and metazonae rather thin, shallow, smooth. Pleurosternal carinae rather evident, arcuate ridges devoid of

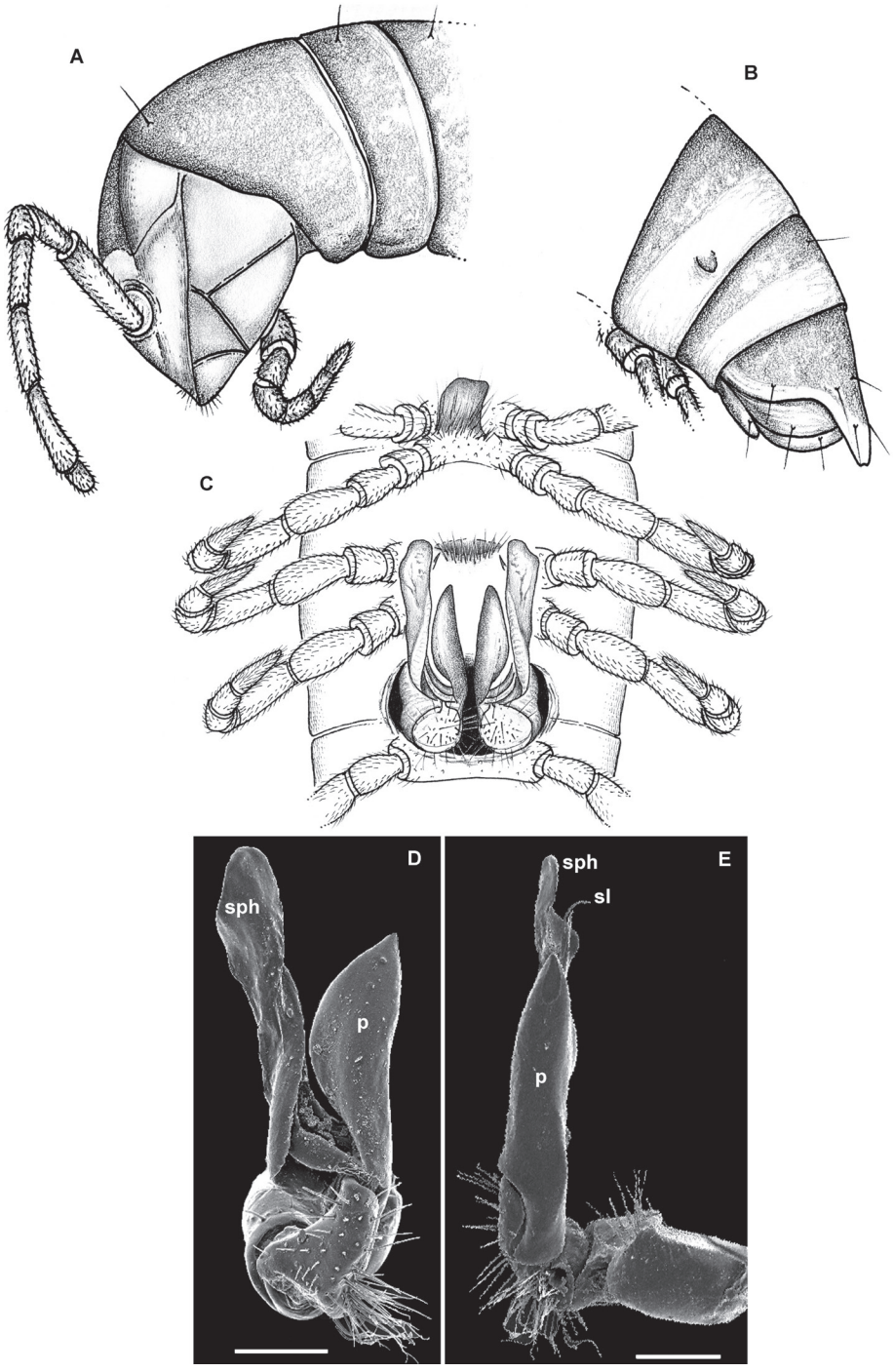
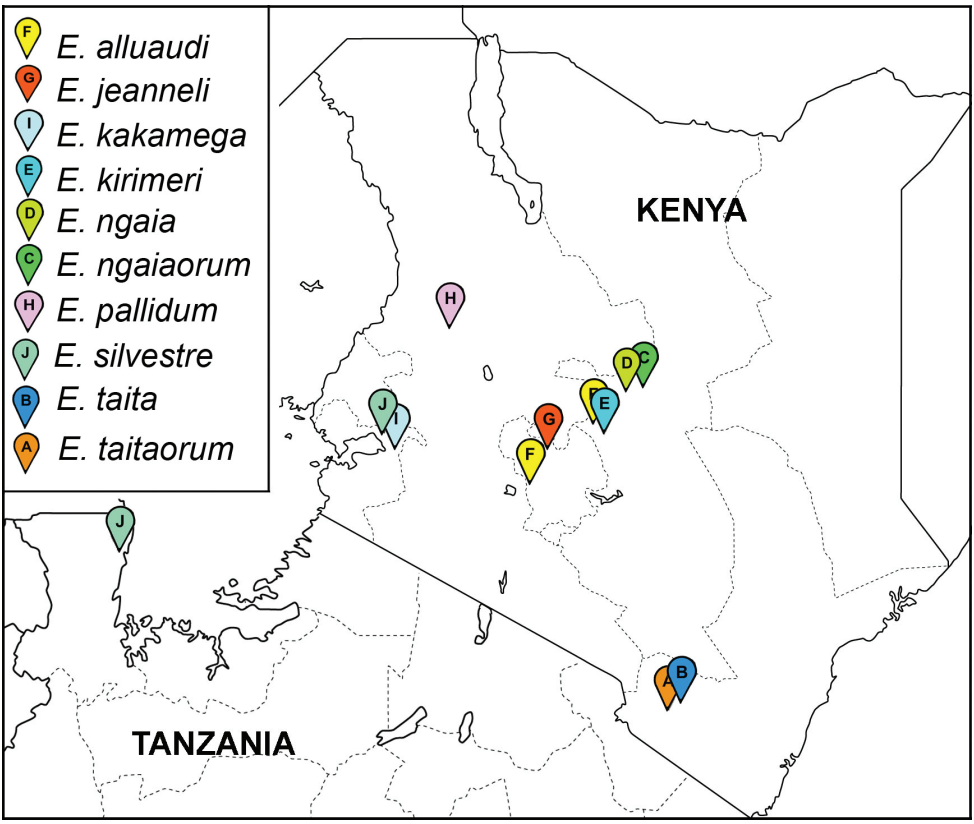


Figure 1. *Eviulisoma ngaia* sp. n., ♂ paratype. **A** anterior part of body, lateral view **B** posterior part of body, lateral view **C** body segments 5–7, ventral view **D, E** right gonopod, ventral and mesal views, respectively. Scale bars: 0.2 mm (**D, E**); **A–C**, drawn not to scale. Designations in text.



Map 1. Distribution of *Eviulisoma* species in Kenya*: E. taitaorum* sp. n. (**A**), *E. taita* sp. n. (**B**), *E. ngaiaorum* sp. n. (**C**), *E. ngaia* sp. n. (**D**), *E. kirimeri* sp. n. (**E**), *E. alluaudi* Brolemann, 1920 (**F**), *E. jeanneli* Brolemann, 1920 (**G**), *E. pallidum* Attems, 1939 (**H**), *E. kakamega* sp. n. (**I**), *E. silvestre* (Carl, 1909) (**J**).

a caudal tooth, visible until segment 10 (♂) or 7 (♀). Epiproct (Fig. 1B) long, flattened dorsoventrally, very faintly concave apically, subapical lateral papillae small, but evident, removed unusually far forward from tip. Hypoproct nearly semi-circular, caudal 1+1 setae clearly separated, borne on minute knobs and clearly removed from caudal margin.

Sternites generally without modifications, densely setose, cross-impressions evident, but axial impressions especially weak; a subquadrate, densely setose lobe between ♂ coxae 4 (Fig. 1C), sternite between ♂ coxae 5 caudally, sterna between ♂ coxae 6 and 7 entirely and clearly flattened. Legs densely setose, rather short, with neither adenostyles nor dorsally bulged prefemora, 1.1–1.2 (♂) or 0.9–1.0 (♀) times as long as body height; ♂ tibial and tarsal brushes consisting of modified setae (as in Fig. 4D–F), present until a few last leg-pairs, tibiae thereby being a little, but clearly shorter than tarsi; ♀ tarsi ca 1.5 times as long as tibiae (as in Fig. 4B).

Gonopods (Fig. 1C-E) compact, with a lamellar solenophore (**sph**) (= tibiotarsus in Jeekel's (2003) terminology) about as long as a flagelliform solenomere (**sl**), both being considerably higher than a simple, phylloid, postfemoral process (**p**).

Vulvae densely setose, without peculiarities, as in Fig. 4M, N.

Remarks. Due to flattened, not deeply excavate, sterna between ♂ coxae 6 and 7, this species resembles *Eoseviulisoma* Brolemann, 1920, but the presence of a central lobe between ♂ coxae 4 warrants the assignment of this species to *Eviulisoma*. Brolemann (1920: 163) diagnosed *Eoseviulisoma* as follows.

«Sous-genre *Eviulisoma*, s. str. — Un prolongement entre les pattes de la 4e paire. Une excavation sternale accentuée au 6e segment. — Tronc du télopodite des gonopodes plus court que les rameaux. Suture transverse des métazonites lisse. — Type: *E. Cavalli* Silv.

Sous-genre *Eoseviulisoma*, nov. — Pas de prolongement entre les pattes de la 4e paire. — Excavation sternale du 6e segment très faible. — Tronc du télopodite des gonopodes plus long que les rameaux. — Suture transverse des métazonites perlée. — Type: *E. julinum* Att.»

***Eviulisoma ngaiaorum* sp. n.**

http://zoobank.org/6119F18D-FF35-4C1C-A46E-35E1F8FD51B0

Fig. 2, Map 1

Type material. Holotype ♂ (MRAC 20806), Kenya, Ngaia Forest, N00°19', E38°02', ca 1070 m a.s.l., 3.XII.2002, leg. D. VandenSpiegel.

Paratypes: 1 ♂ fragment, 1 ♂ subadult, 8 juv. (MRAC 20806), same data, together with holotype.

Name. To emphasize the type locality, in Latin meaning "a dweller of Ngaia".

Diagnosis. Differs from all congeners but *E. ngaia* sp. n. in the absence of a sternal excavation in ♂ body segment 6, from *E. ngaia* sp. n. in the presence of sternal cones in the ♂ and only a vestigial gonopod postfemoral process (Fig. 2C–E). See also Key below.

Description. Length of adults ca 20 mm (♂ holotype), width of midbody metazonae 2.2 mm (both ♂ holotype and ♂ fragment paratype). Juveniles entirely pallid.

Coloration and other adult characters as in *E. ngaia* sp. n., except as follows.

Transverse metatergal sulcus/line wanting. Tergal setae mostly retained, pattern 3+3 (Fig. 2B). Pleurosternal carinae rather evident, arcuate ridges devoid of a caudal tooth, visible until segment 15 (♂). Epiproct (Fig. 2B) subtruncate apically, subapical lateral papillae rather large and only poorly removed from tip.

Sternites behind gonopods with a distinct sharp cone near each ♂ coxa, each caudal pair per diplosegment being a little stronger than anterior one. Setose lobe between ♂ coxae 4 (Fig. 2C) faintly concave at tip. Sterna between ♂ coxae 6 and 7 (Fig. 2C) clearly flattened, their coxae being a little enlarged and conical distoventrally. Legs densely setose, rather short, 1.2–1.3 times as long as body height (♂), tibiae behind gonopods thereby being mostly subequal in length to tarsi; ♂ tibiae and tarsi with ventral brushes until last two leg-pairs.

Gonopods (Fig. 2C–E) with a lamellar, lateroparabasally strongly expanded solenophore (**sph**) carrying a large apical claw and two pre-apical teeth, one mesal (**m**), the other lateral (**l**); a flagelliform solenomere (**sl**) about as long as to reach bases of both **l** and **m**; postfemoral process (**p**) very short, fold-shaped, vestigial.

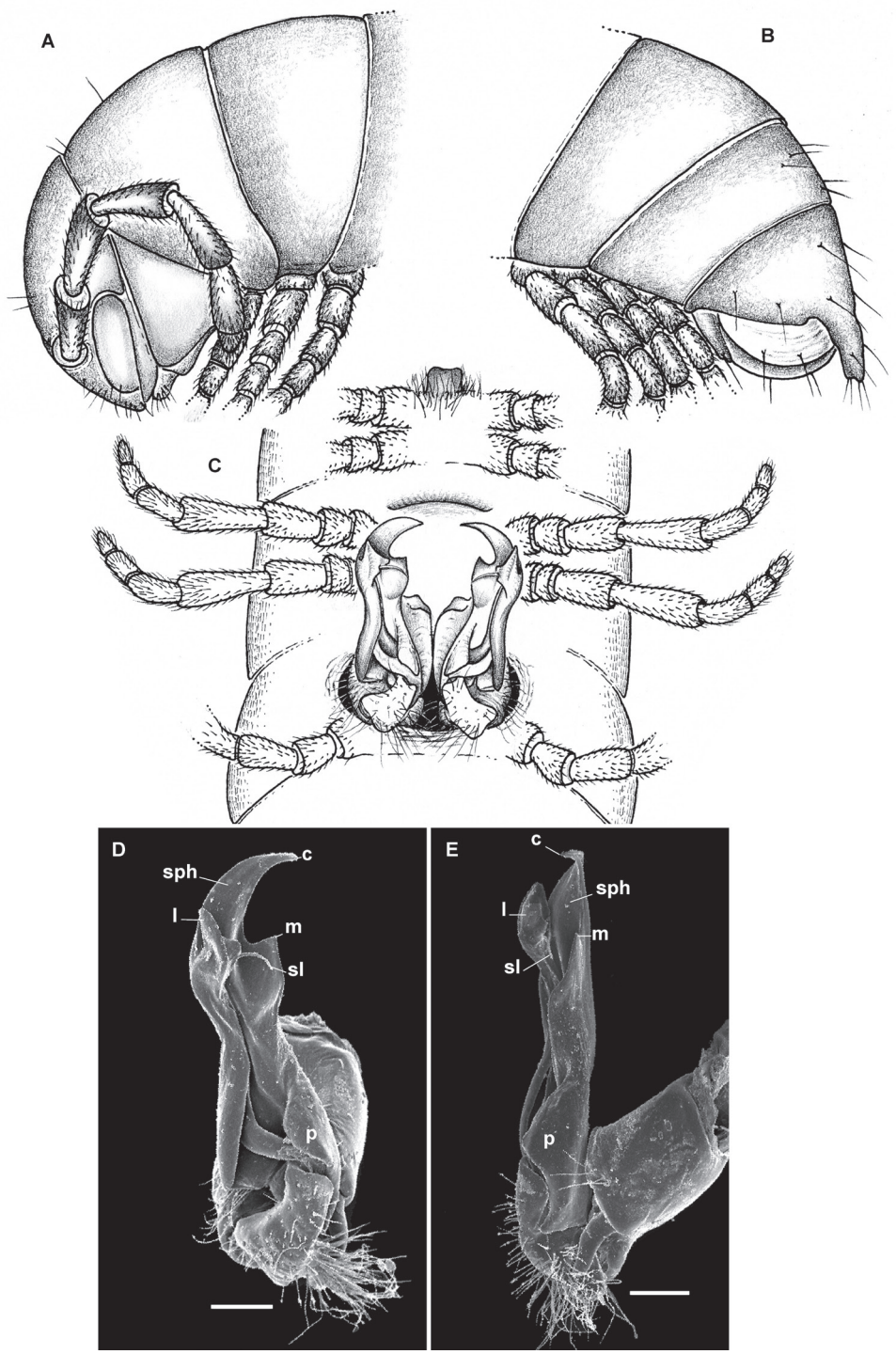


Figure 2. *Eviulisoma ngaiaorum* sp. n., ♂ holotype (**A–C**) & ♂ paratype (**D, E**). **A** anterior part of body, lateral view **B** posterior part of body, lateral view **C** body segments 5–7, ventral view **D, E** right gonopod, ventral and mesal views, respectively. Scale bars: 0.1 mm (**D, E**); **A–C**, drawn not to scale. Designations in text.

***Eviulisoma taitaorum* sp. n.**
http://zoobank.org/83C83C70-46C1-4AC7-9D13-EEA08A149E11
Figs 3, 4, Maps 1, 2

Type material. Holotype ♂ (MRAC 22630), Kenya, Taita Hills, Chawia Forest, 1500 m a.s.l., S03°29', E38°20', pitfall trapping, 1–20.VI.1999, leg. R. Mwakos.

Paratypes: 3 ♂, 1 ♀, 8 juv. (MRAC 18071), same data, together with holotype; 3 ♂ (MRAC 18016), same locality, 1500 m a.s.l., pitfall traps, 10–26.VI.1999, leg. R. Mwakos; 3 ♀ (MRAC 18096), same locality, 1500 m a.s.l., pitfall traps, III–IV.1999, leg. L. Rogo; 1 ♀, 3 juv. (MRAC 17993), same locality, 1500 m a.s.l., III–IV.1999, leg. D. VandenSpiegel; 3 juv. (MRAC 18505), same locality, Winkler extraction, 7.XII.1999, leg. D. VandenSpiegel & J. P. Michiels; 1 ♂, 2 ♀, 5 juv. (MRAC 18424), same locality, 7.XII.1999, leg. D. VandenSpiegel & J. P. Michiels; 1 ♂ fragment, 1 ♀, 2 juv. (MRAC 18043), 1 ♂, 1 ♀ (ZMUM ϱ2443), Taita Hills, Ngangao Forest, S03°22', E38°21', 1820 m a.s.l., 17.VIII.1999, leg. R. Mwakos; 1 ♂ fragment, 20 ♀ (MRAC 18476), same locality, 4.XII.1999, leg. D. VandenSpiegel & J. P. Michiels; 3 ♀, 3 juv. (MRAC 18008), same locality, 1820 m a.s.l., 19.VI.1999, leg. D. VandenSpiegel; 1 ♀ (MRAC 18090), same locality, 1820 m a.s.l., pitfall traps, III–IV.1999, leg. D. VandenSpiegel, 1 ♂ (MRAC 18036), same locality, 1820 m a.s.l., pitfall traps, 15–17.III.1999, leg. L. Rogo; 1 ♂ (MRAC 22622), Taita Hills, Fururu Forest, S3°26', E38°20', 9.XII.1999, leg. D. VandenSpiegel & J. P. Michiels; 1 ♂, 1 ♀, 7 juv. (MRAC 22623), same locality, pitfall traps, 14–17.XII.2004, leg. A. Bwong, J. Mwandoe & J. Measey; 1 ♂, 1 ♀, 3 juv. (MRAC 18083), Taita Hills, Vuria Forest, S03°24', E38°17', 2200 m a.s.l., 26.VI.1999, leg. D. VandenSpiegel; 1 ♀ (MRAC 18459), Taita Hills, Sagala Forest, S03°50', E38°58', 5.XII. 1999, leg. D. VandenSpiegel & J. P. Michiels.

Non-types: ca 30 juv. (MRAC 18.543), Taita Hills, Fururu Forest, S03°26', E38°20', Winkler extraction, 9.XII.1999; 1 ♀ (MRAC 18441), Taita Hills, Wundanyi, near house, S03°24'07", E38°21'49", 6.XII. 1999, all leg. D. VandenSpiegel & J. P. Michiels.

Name. To emphasize the type locality, in Latin meaning "a dweller of Taita".

Diagnosis. Differs from all congeners in the remarkable size dimorphism, coupled with absence of a sternal lobe between ♂ coxae 4, as well as the subequally long and slender solenophore (**sph**) and postfemoral process (**p**) (Figs 3C, 4H–L). See also Key below.

Description. Length of adults ca 17–20 (♂ holotype and some ♂ & ♀ paratypes from Chawia, Fururu and from Ngangao) or 28–38 mm (most of ♂ & ♀ paratypes from Fururu and Ngangao, all few paratypes from Sagala and Vuria), width of midbody metazonae 1.7–1.8 (♂ holotype and some ♂ paratypes) up to 2.0 mm (♀ paratypes from Chawia) or 2.5–2.6 (most of ♂ paratypes from Fururu and Ngangao) up to 3.0–3.8 mm (most of ♀ paratypes from Fururu and Ngangao).

Coloration from pallid, via light pinkish or marbled pinkish brown to nearly chocolate brown, pattern often annulated due to darker metazonae, including later instars of larger morph. Legs pallid to yellowish, earlier instars always entirely pallid. Sometimes a narrow, darker, pigmented axial line and a similar transverse line in caudal 1/3 of metaterga.

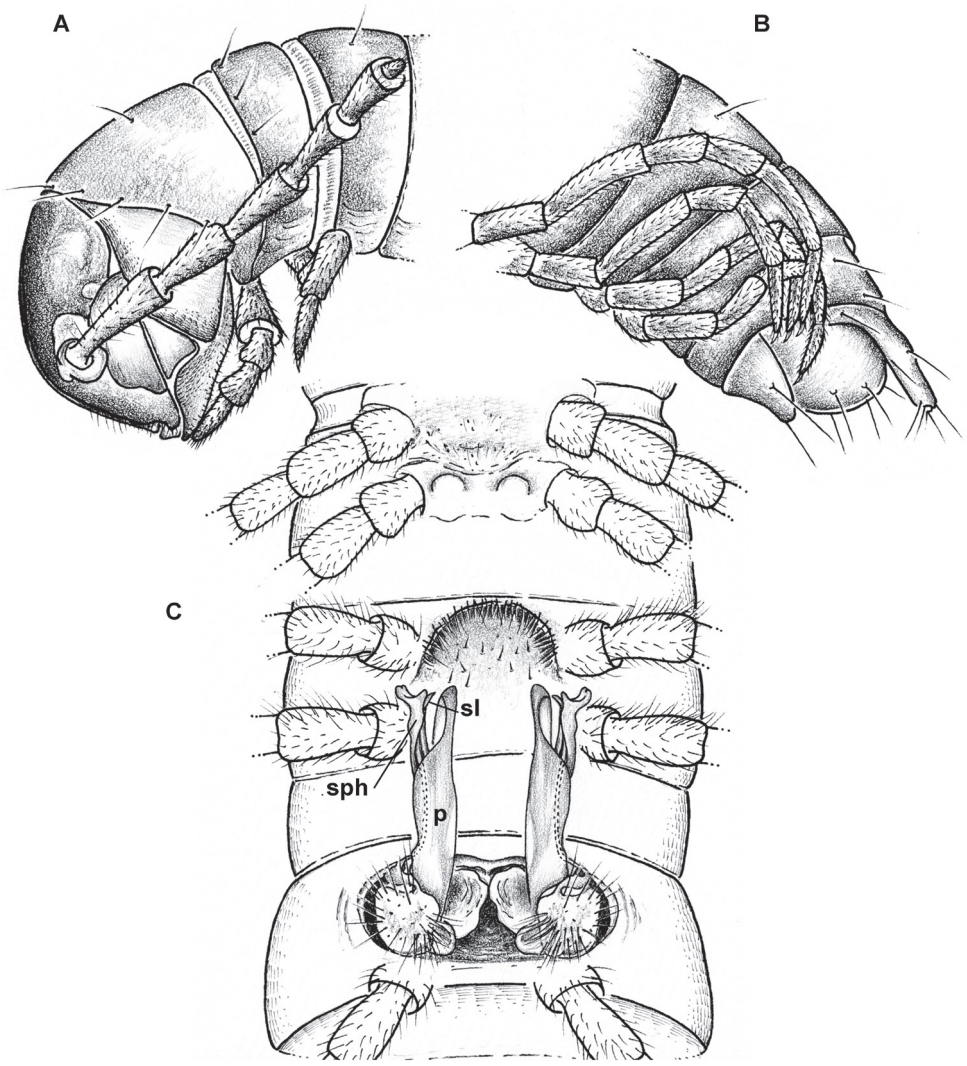


Figure 3. *Eviulisoma taitaorum* sp. n., ♂ paratype. **A** anterior part of body, lateral view **B** posterior part of body, lateral view **C** body segments 5–7, ventral view. Drawn not to scale. Designations in text.

All characters as in *E. ngaia* sp. n. (Fig. 4A–G, M, N), except as follows.

Surface rather smooth and shining (Figs 3, 4A–C, H), near ozopores faintly rugulose longitudinally, slightly microgranulate below in ♂. Hypoproct more narrowly rounded up to nearly pointed between 1+1 caudal setae (♂). Pleurosternal carinae faint (Fig. 4H), mostly line-shaped, visible until segment 17 (♂) or 15 (♀). Sterna between ♂ coxae 4 and 5 each with a pair of paramedian cones caudally, devoid of any central lobes (Figs 3C, 4H); sterna between ♂ coxae 6 and 7 unusually deeply excavate and ledge-shaped for accommodation of gonopod tips (Fig. 4H), the excavation's

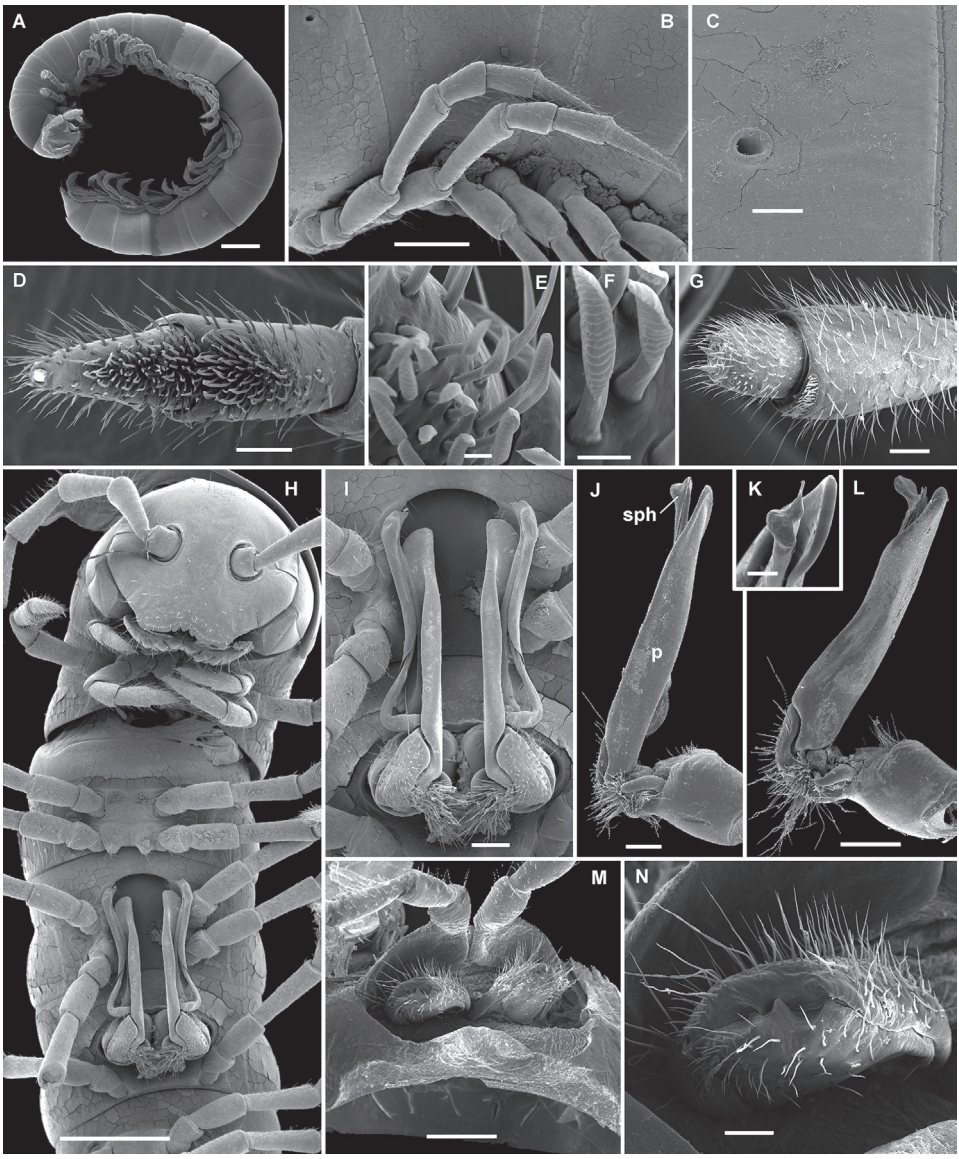
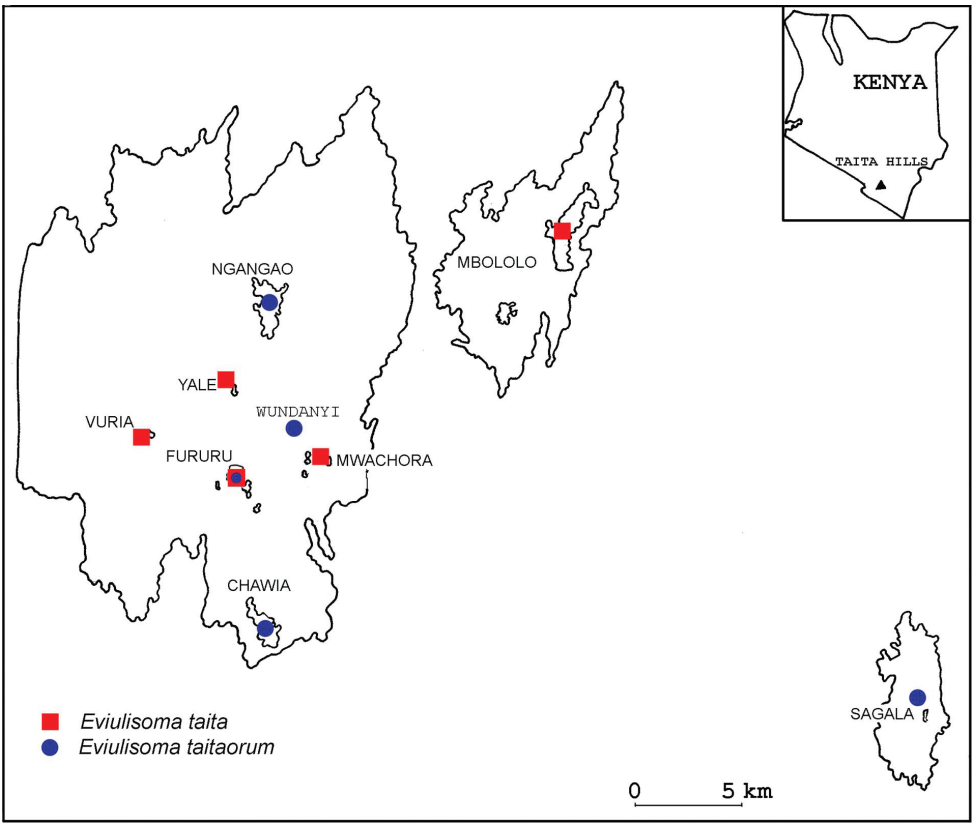


Figure 4. *Eviulisoma taitaorum* sp. n., ♂ (**A, D–L**) & ♀ paratypes (**B, C, M, N**). **A** habitus, lateral view **B** midbody legs, lateral view **C** ozopore, lateral view **D** ventral brushes on tibia and tarsus, ventral view **E, F** modified setae of ventral brushes, ventral view **G** antennomeres 6–8, sublateral view **H** anterior part of body, ventral view **I** both gonopods in situ, ventral view **J, L** right gonopod, mesal and submesal views, respectively **K** gonopod tip, sublateral view **M** both vulvae in situ, ventrocaudal view **N** right vulva, ventrocaudal view. Scale bars: 1.0 (**A**), 0.5 (**B, H, M**), 0.2 (**I, J, L**), 0.1 (**C, D, G, K, N**) & 0.01 mm (**E, F**). Designations in text.

frontal edge being densely setose (Fig. 3C). Postgonopodial sterna with small, but evident, sometimes pointed cones near each coxa, anterior pair being always smaller than caudal one on each diplosegment. ♂ tarsi either a little longer than tibiae (usually



Map 2. Distribution of *Eviulisoma taitaorum* sp. n. (blue dot) and *E. taita* sp. n. (red square) in the Taita Hills, Kenya.

smaller morph) or both subequal in length (usually larger morph). Legs 1.2–1.4 (♂) or 0.8–0.9 (♀) times as long as body height. ♂ tibiae and tarsi with ventral brushes until last two leg-pairs, their setae being flattened, same as in other new species (Fig. 4D–F).

Gonopods (Figs 3C, 4H–L) very slender, with solenophore (**sph**), postfemoral process (**p**) and solenomere (**sl**) subequal in length.

Vulvae without peculiarities, as in Fig. 4M, N.

Remarks. This new species seems remarkable in being represented by two different size morphs which invariably co-occur at least in sufficiently rich samples and show no intermediates. Thus, in one sample from Chawia the adult ♂♂ can vary in size by 1.5–2.0 times. Larger animals tend to be darker than smaller ones.

Such a strong size morphism could be advantageous for the local populations in variably adverse ecological conditions, possibly allowing selection for different life strategies.

The above two species from Taita Hills show parapatry (Map 1), co-occurring only in Fururu Forest.

In addition, the absence of a central lobe between ♂ coxae 4 is rather characteristic of *Eoseviulisoma* Brolemann, 1920, but the smooth metazonital suture, the structure of the gonopods and the deeply excavate sterna between ♂ coxae 6 and 7

warrant the assignment of this species to *Eviulisoma* (cf. Brolemann 1920). This is just another example that these two genera may well prove to be synonymous. Both Brolemann (1920) and Attems (1937) had treated *Eoseviulisoma* as only a subgenus of *Eviulisoma*, but Hoffman (1953) elevated the former to the rank of a full genus which currently includes only 2–3 species from Tanzania and the Democratic Republic of the Congo.

***Eviulisoma taita* sp. n.**
http://zoobank.org/2D851CA6-F810-4A2D-BE59-EC7EC6FB294E
Figs 5, 6, Maps 1, 2

Type material. Holotype ♂ (MRAC 22631), Kenya, Taita Hills, Mbololo Forest, S03°22.56', E38°20.70', 1800–1900 m a.s.l., pitfall traps, III–IV.1999, leg. L. Rogo.

Paratypes: 17 ♂, 13 ♀, 4 juv. (MRAC 18084), 1 ♂, 1 ♀ (ZMUM ϱ2444), same data, together with holotype; 2 ♂, 2 ♀ (MRAC 18029), same locality, pitfall traps, 3.VII-2.VIII.1999, leg. R. Mwakos; 1 ♂, 1 ♀ (MRAC 18412), same locality, 8.XII.1999, leg. D. VandenSpiegel & J. P. Michiels; 1 ♀, 1 juv. (MRAC 17990), same locality, 22.VI.1999, leg. D. VandenSpiegel; 9 ♂, 8 ♀, 33 juv. (MRAC 18039), same locality, 1800–1900 m a.s.l., sieving, 2–10.VII.1999, leg. R. Mwakos; 1 ♀ (MRAC 17976), same locality, 21.VI.1999, leg. D. VandenSpiegel; 1 ♂, 1 ♂ fragment, 1 ♀, 1 ♀ fragment (MRAC 18414), same locality, 8.XII.1999, leg. D. VandenSpiegel & J. P. Michiels; 3 ♂, 1 ♀ (MRAC 18100), Taita Hills, Yale Forest, 1840 m, S03°39', E38°33', pitfall traps, III–IV.1999, leg. L. Rogo; 4 ♂, 4 ♀, 22 juv. (MRAC 18451), Taita Hills, Fururu Forest, S03°26', E38°20', 9.XII.1999; 1 ♂, 3 ♀, 20 juv. (MRAC 18495), same locality, Winkler extraction, 9.12.1999; 5 ♂, 4 ♀, 1 juv. (MRAC 18576), Taita Hills, Mwachora Forest, Winkler extraction, 10.XII.1999, all leg. D. VandenSpiegel & J. P. Michiels; 2 ♂ (MRAC 22632), same data; 1 ♂, 1 ♀, 1 ♀ fragment, 1 juv. (MRAC 22633), same locality, 15.II.2004, leg. T. Spanhove & M. Chovu.

Name. To emphasize the type locality, a noun in apposition.

Diagnosis. Differs from congeners by a broadly and regularly rounded hypoproct, coupled with the presence of sternal cones behind ♂ body segment 7, and the lamellar, slender, apically unciform and bidentate solenophore (**sph**) carrying a lateral tooth midway (**t**) and reaching about as long as a flagelliform solenomere (**sl**), both **sph** and **sl** being considerably higher than a rather simple, similarly slender, postfemoral process (**p**). See also Key below.

Description. Length of adults ca 16–23 (♂) or 18–28 mm (♀), width of midbody metazonae 1.5–2.7 (♂) or 2.0–3.7 mm (♀). Holotype ca 16 mm long and 1.6 mm wide on midbody metazonae.

Coloration from pallid to annulated chocolate brown due to darker metazonae, often with a thin axial pigment line and a similar transverse pigment line in posterior 1/3 of metaterga.

Other adult characters as in *E. ngaia* sp. n., except as follows.

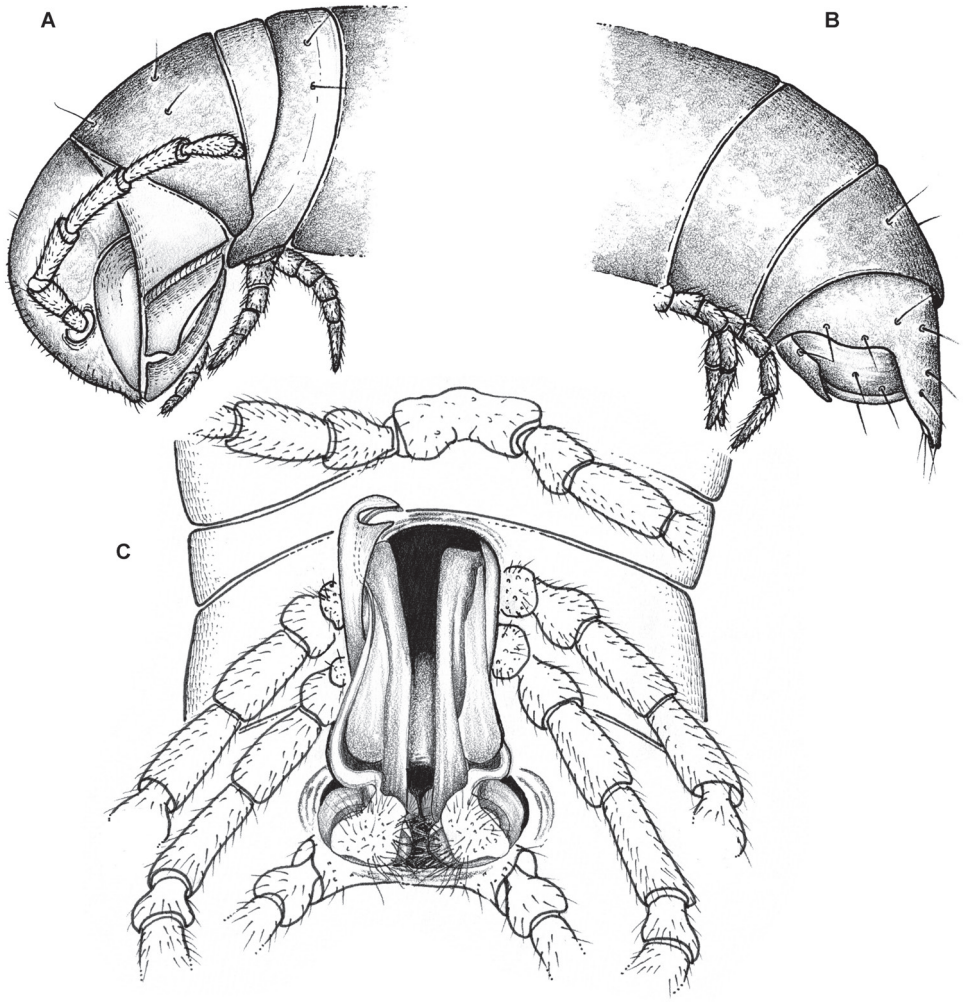


Figure 5. *Eviulisoma taita* sp. n., ♂ paratype. **A** anterior part of body, lateral view **B** posterior part of body, lateral view **C** body segments 5–7, ventral view. Drawn not to scale.

Vertigial region with a few setae (Figs 5A, 6D). Stricture between pro- and metazonae very delicately striolate. Tegument generally smooth, often with only a few arcuate striae near and below ozopores. Pleurosternal carinae rather evident, arcuate ridges devoid of a caudal tooth, visible until segment 16 (♂, ♀). Epiproct long (Fig. 5B), faintly concave apically, subapical lateral papillae evident, well removed from tip. Hypoproct broadly rounded.

Setose lobe between ♂ coxae 4 (Fig. 6E) low, subtrapeziform, slightly rounded apically. Sternite between ♂ coxae 5 densely setose, with paramedian cones caudally (Fig. 6E); sterna between ♂ coxae 6 and 7 unusually deeply excavate and ledge-shaped for accommodation of gonopod tips (Figs 5C, 6D–F), the excavation's frontal edge being sparsely setose

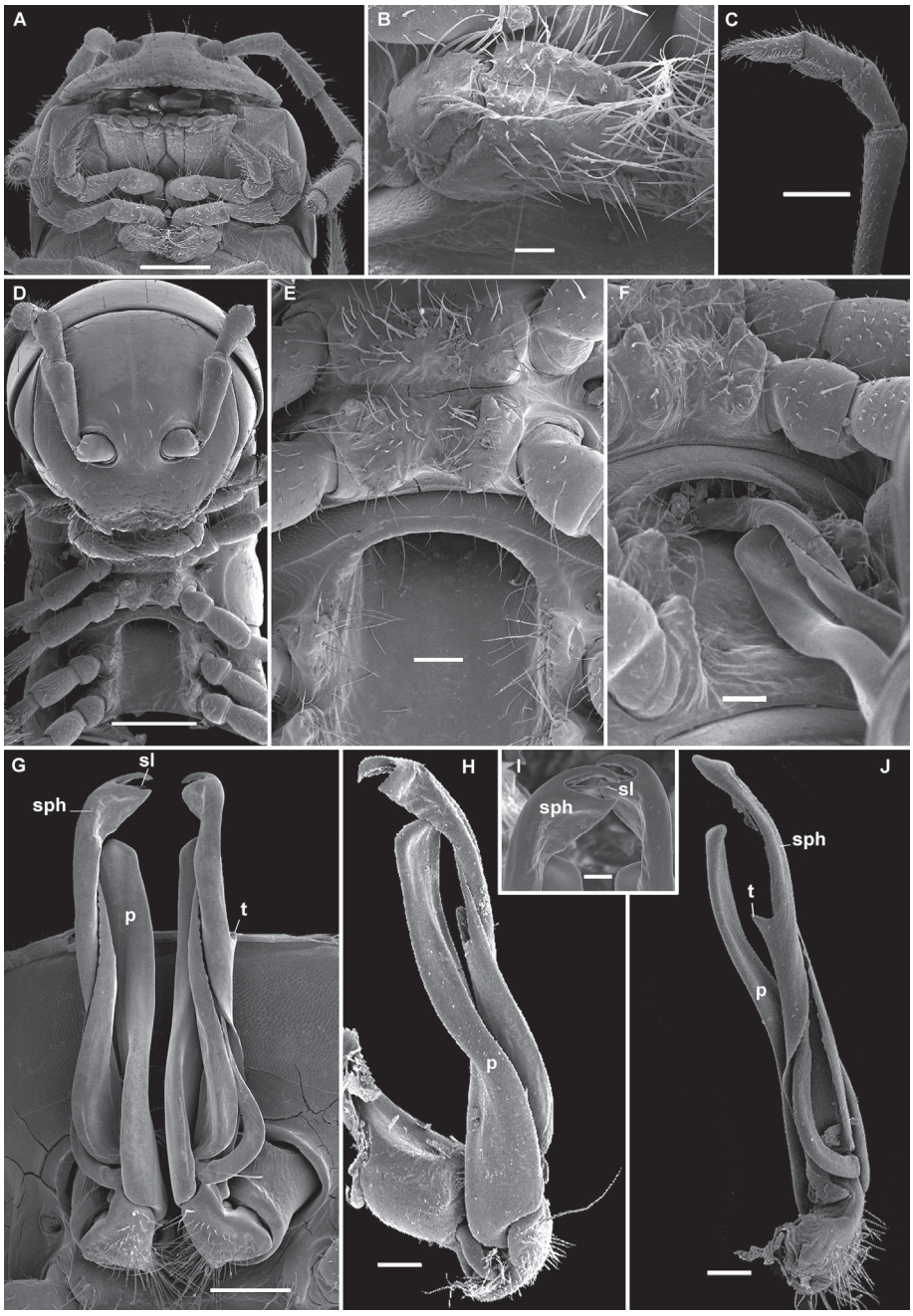


Figure 6. *Eviulisoma taita* sp. n., ♀ (**A, B**) & ♂ (**C–J**) paratypes. **A, D** anterior part of body, ventrocaudal and ventral views, respectively **B** right vulva, ventrocaudal view **C** distal part of a midbody leg, lateral view **E** sterna between coxae 4–7, ventral view **F** same, but with left gonopod placed into sternal pocket-shaped excavation **G** both gonopods in situ, ventral view **H, J** left gonopod, mesal and lateral views, respectively **I** tips of both gonopods in situ, ventral view. Scale bars: 0.5 (**A, C, D**), 0.2 (**G**), 0.1 (**E, F, H, J**) & 0.05 mm (**B, I**). Designations in text.

(Fig. 6E). Postgonopodial sterna mostly with small, low, blunt cones near each coxa, anterior pair being even smaller than caudal one on each diplosegment. ♂ tarsi considerably to only slightly longer than tibiae (Fig. 6C). Legs 1.5–1.6 (♂) or 0.9–1.1 (♀) times as long as body height. ♂ tibiae and tarsi with ventral brushes until last two leg-pairs (Fig. 6C).

Gonopods (Figs 5C, 6F–J) with a lamellar, slender, apically unciform and bidentate solenophore (**sph**) carrying a lateral tooth midway (**t**) and being about as long as a flagelliform solenomere (**sl**), both **sph** and **sl** considerably higher than a rather simple, similarly slender, postfemoral process (**p**).

Vulvae without peculiarities, as in Fig. 6A, B.

***Eviulisoma kirimeri* sp. n.**
http://zoobank.org/D7ED4341-A7DE-494E-9041-21EF9E026D42
Fig. 7, Map 1

Type material. Holotype ♂ (MRAC 22624), Kenya, Kirimeri Forest near Runyenyere, S00°25', E37°33', 1700 m a.s.l., sieved litter, 27.IV.2004, leg. D. Vanden-Spiegel, R. Jocqué & C. Warui.

Paratype: 1 ♂ (MRAC 22625), same data, together with holotype.

Name. To emphasize the type locality, a noun in apposition.

Diagnosis. Differs from congeners in the epiproct showing two distinct apical claws directed ventrad (Fig. 7b), as well as the gonopods being divergent, rather loose, with a complex, lamellar, apically unciform (**u**) solenophore (**sph**) partly sheathing a longer flagelliform solenomere (**sl**); postfemoral process (**p**) very simple, sickle-shaped (Fig. 7C-F). See also Key below.

Description. Length of ca 15–16 mm, width of midbody metazonae 1.5 (♂ holotype) or 1.7 mm (♂ paratype). Coloration entirely pallid.

Other adult characters as in *E. ngaia* sp. n., except as follows.

Clypeolabral region rather sparsely setose (Fig. 7A). Stricture between pro- and metazonae very delicately striolate. Tegument generally smooth, often with only a few arcuate striae near and below ozopores. Pleurosternal carinae rather evident, arcuate ridges devoid of a caudal tooth, visible until segment 15 (♂). Epiproct (Fig. 7B) faintly concave between two evident, claw-shaped, apical papillae directed ventrad; subapical lateral papillae evident, rather well removed from tip. Hypoproct subtriangular, pointed between 1+1 submarginal setae borne on minute knobs.

Setose lobe between ♂ coxae 4 (Fig. 7C) roundly subtriangular. Sternite between ♂ coxae 5 flattened; sterna between ♂ coxae 6 and 7 unusually deeply excavate and ledge-shaped for accommodation of gonopod tips, the excavation's frontal edge being densely setose (Fig. 7C). Postgonopodial sterna with small, but evident, almost sharp cones near each coxa, anterior pair being smaller than caudal one on each diplosegment. ♂ tarsi largely considerably longer than tibiae (Fig. 7C). Legs 1.2–1.3 times as long as body height (♂). All ♂ telopodite segments distal to coxa or prefemur with dense ventral brushes, but last leg-pair with ventral brushes retained only on tibiae and tarsi.

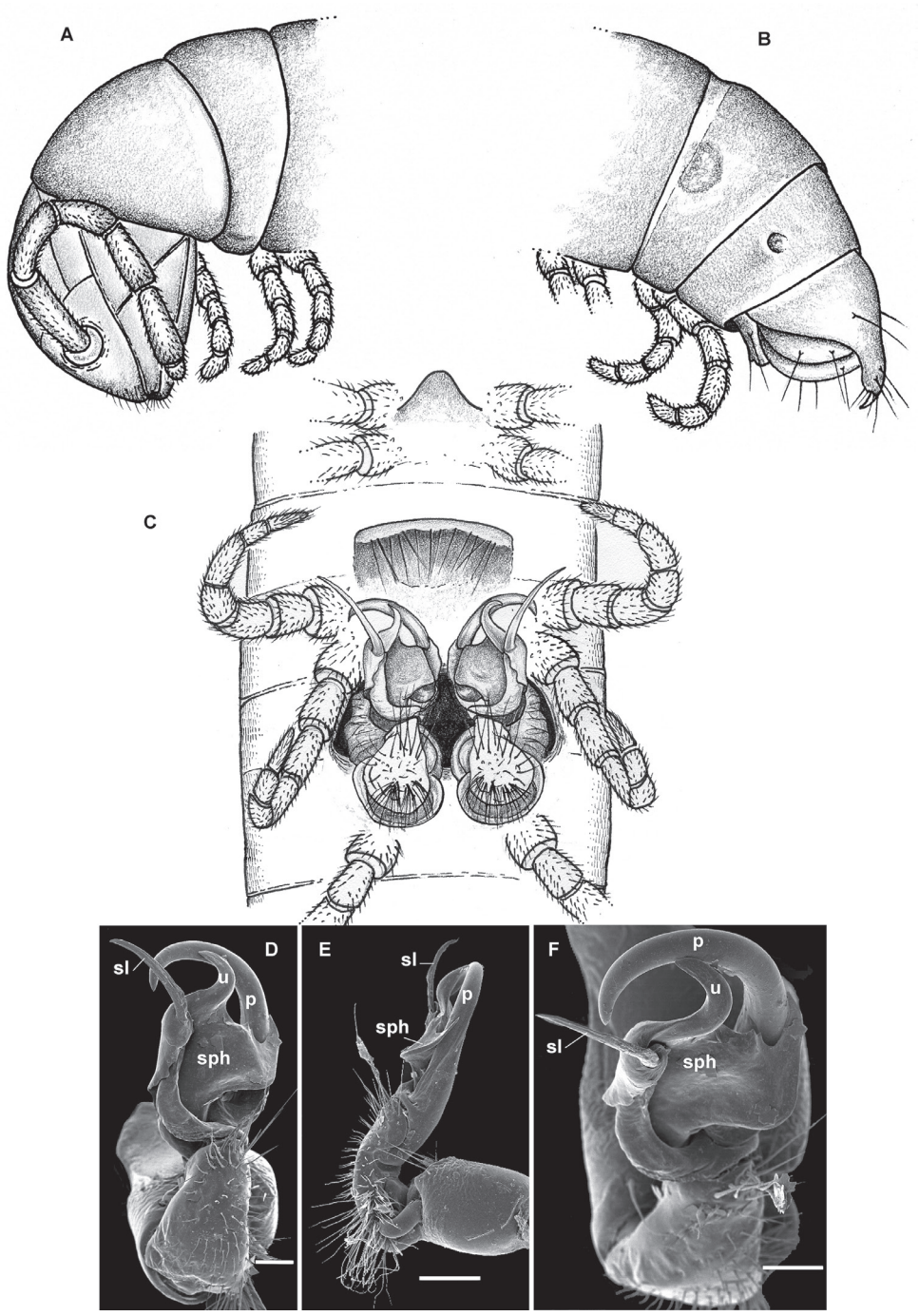


Figure 7. *Eviulisoma kirimeri* sp. n., ♂ paratype. **A** anterior part of body, lateral view **B** posterior part of body, lateral view **C** body segments 5–7, ventral view **D–F** left (**D, F**) and right (**E**) gonopod, ventral, mesal and anteroventral views, respectively. Scale bars: 0.2 (**E**) & 0.1 mm (**D, F**); **A–C**, drawn not to scale. Designations in text.

Gonopods (Fig. 7C–F) rather loose, divergent, with a complex, lamellar, apically unciform (**u**) solenophore (**sph**) partly sheathing a longer and flagelliform solenomere (**sl**); postfemoral process (**p**) very simple, strong and sickle-shaped.

***Eviulisoma kakamega* sp. n.**
http://zoobank.org/C175D502-7342-4456-9B79-C73CD155A752
Figs 8, 9, Map 1

Type material. Holotype ♂ (incomplete, only head and first 13 segments present) (MRAC 20771), Kenya, Likhanda Hills, Kakamega Forest, S00°13', E34°54', pitfall traps, 5.II.2002, leg. D. S. Smith.

Paratypes: 1 ♂ (incomplete, lacking gonopods and five posteriormost segments), 4 ♀, 5 juv., 1 fragment (MRAC 20772), same data, together with holotype.

Name. To emphasize the type locality, a noun in apposition.

Diagnosis. Differs from congeners by the gonopod solenophore (**sph**) being complex, cup-shaped, lamellar, about as long as a flagelliform solenomere (**sl**), flanked medially by a long, subspiniform, postfemoral process (**p**) (Figs 8C, 9C–E). See also Key below.

Description. Length of ♀ ca 22–23 mm, width of midbody metazonae 2.1 (♂ holotype), 2.7 (♂ paratype) or 3.1–3.3 mm. Coloration uniformly light pinkish yellow, legs lighter yellow.

Other adult characters as in *E. ngaia* sp. n., except as follows.

Vertigial region with a few setae (Figs 8A, 9A). Stricture between pro- and metazonae very delicately striolate. Tegument generally smooth, often with only a few arcuate striae near and below ozopores. Pleurosternal carinae rather evident, arcuate ridges devoid of a caudal tooth, visible at least until segment 15 (♂, ♀). Epiproct long (Fig. 8B), faintly concave between two small apical papillae, subapical lateral papillae evident, only slightly removed from tip (♀). Hypoproct semi-circular, regularly and broadly rounded, 1+1 submarginal setae borne on minute knobs and a little removed from margin.

Setose lobe between ♂ coxae 4 (Figs 8C, 9C) low, broad, clearly concave apically. Sternite between ♂ coxae 5 slightly elevated due to small caudolateral cones (Fig. 9C); sterna between ♂ coxae 6 and 7 unusually deeply excavate and ledge-shaped for accommodation of gonopod tips, the excavation's frontal edge being densely setose (Figs 8C, 9C). Postgonopodial sterna with small, but evident, often sharp cones near each coxa, anterior pair being smaller than caudal one on each diplosegment. ♂ tarsi considerably longer than tibiae. Legs 1.4–1.5 (♂) or 1.1–1.2 (♀) times as long as body height. Dense ventral brushes on ♂ tibiae and tarsi present (Fig. 9B).

Gonopods (Figs 8C, 9C–E) rather compact, highly complex due to an apically cup-shaped, lamellar solenophore (**sph**) about as long as a flagelliform solenomere (**sl**), flanked medially by a long, subspiniform postfemoral process (**p**).

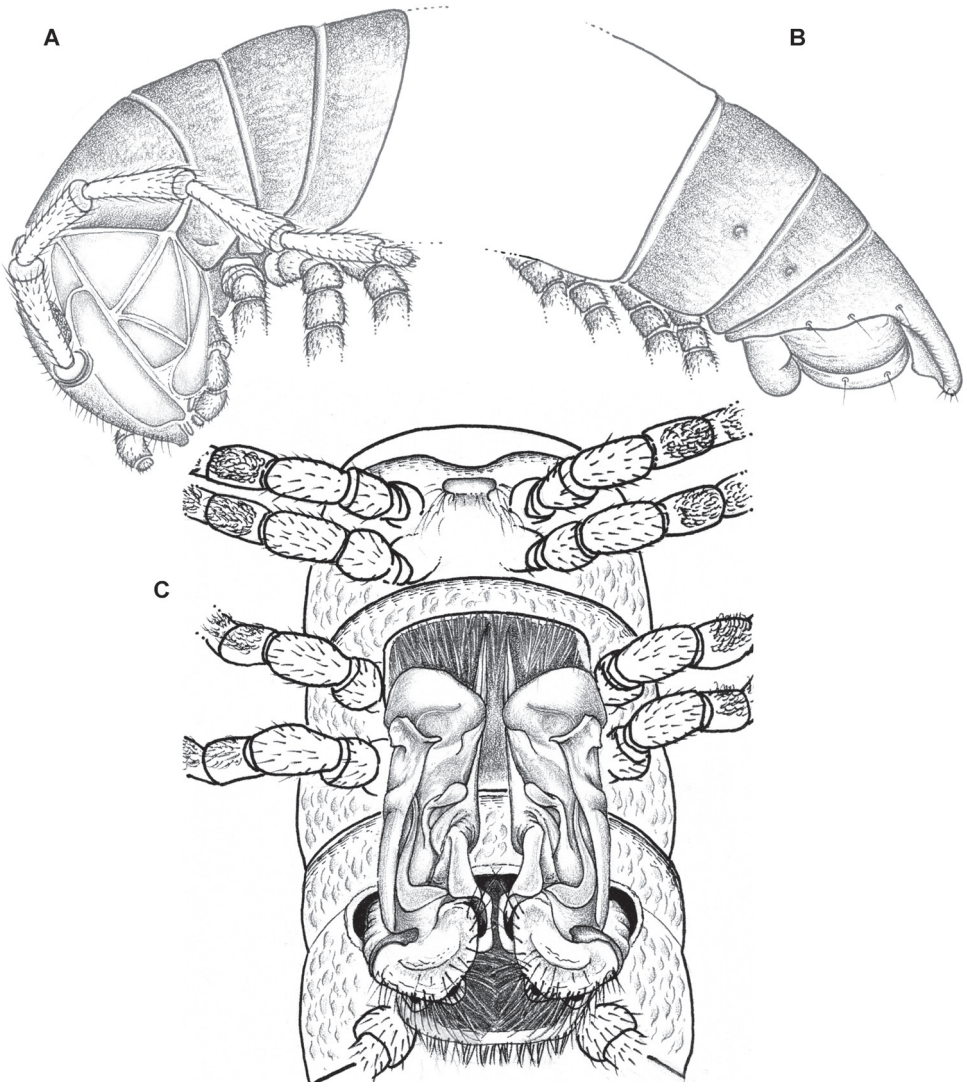


Figure 8. *Eviulisoma kakamega* sp. n., ♂ holotype (**A, C**) & ♀ paratype (**B**). **A** anterior part of body, lateral view **B** posterior part of body, lateral view **C** body segments 5–7, ventral view. Drawn not to scale.

Eviulisoma alluaudi Brolemann, 1920

Fig. 10, Map 1

Material. 3 ♂, 16 ♀, 4 juv. (MRAC 22626), 1 ♂, 1 ♀ (ZMUM ϱ2445), Kenya, Chogoria Forest, S0°11'13", E37°28'07", 2658 m a.s.l., bamboo forest, sieved litter and beaten from bamboos, 24.IV.2004, leg. D. VandenSpiegel, R. Jocqué & C. Warui.

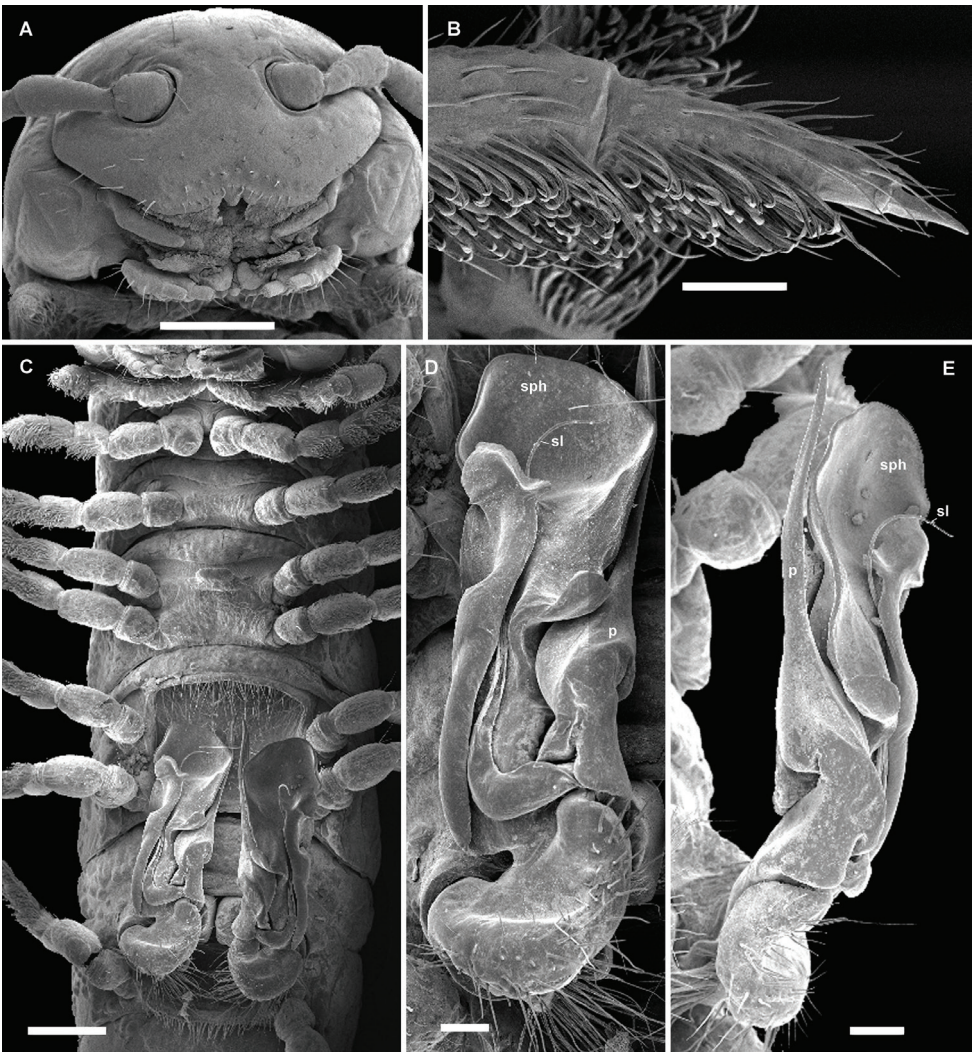


Figure 9. *Eviulisoma kakamega* sp. n., ♂ paratype. **A** anterior part of body, ventral view **B** ventral brushes on tibia and tarsus, lateral view **C** body segments 2–7, ventral view **D, E** right gonopod, ventral and lateral views, respectively. Scale bars: 0.5 (**A, C**), 0.2 (**D, E**) & 0.1 mm (**B**). Designations in text.

Remarks. The above is only the second record of this species beyond the type locality: alpine meadows and a forest at 3100 m and 2600 m a.s.l., respectively, on Mt. Kinangop, S00°11', E37°28', Aberdare Ridge, Kenya (Brolemann 1920, Attems 1939). Even though the new samples, which are in rather poor condition, fully match Brolemann's (1920) excellent original description, we provide additional illustrations (Fig. 10) to document the identity of this obviously high-montane species which appears to be more widely distributed at least in Kenya (Map 1). The shapes and proportions of the solenophore (**sph**), solenomere (**sl**) and postfemoral process (**p**) are quite characteristic.

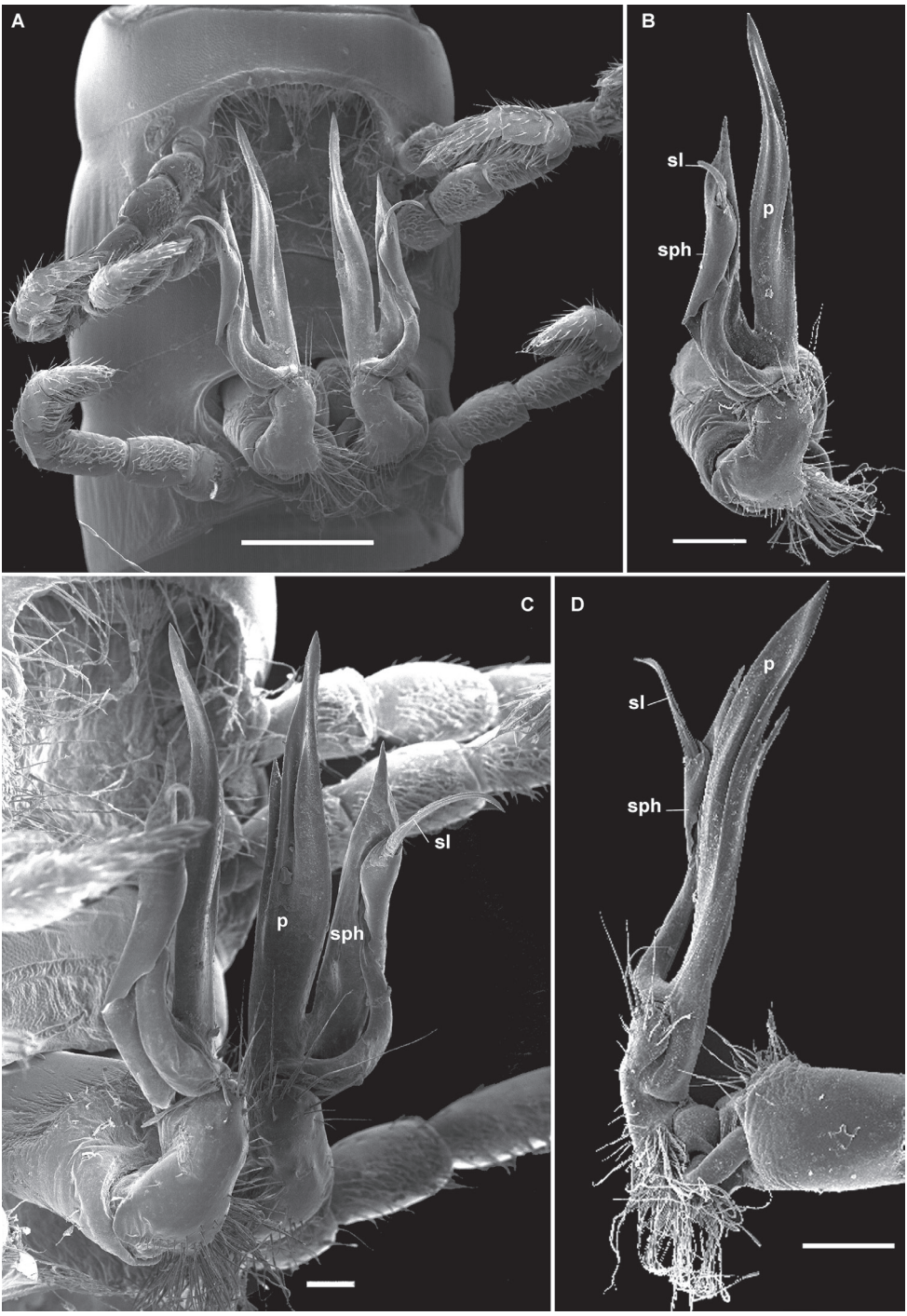


Figure 10. *Eviulisoma alluaudi* Brolemann, 1920, ♂ from Chogoria Forest. **A, C** body segments 6 & 7, ventral and ventrolateral views, respectively **B, D** right gonopod, ventral and mesal views, respectively. Scale bars: 0.5 (**A, C**), 0.2 (**D, E**) & 0.1 mm (**B**). Designations in text.

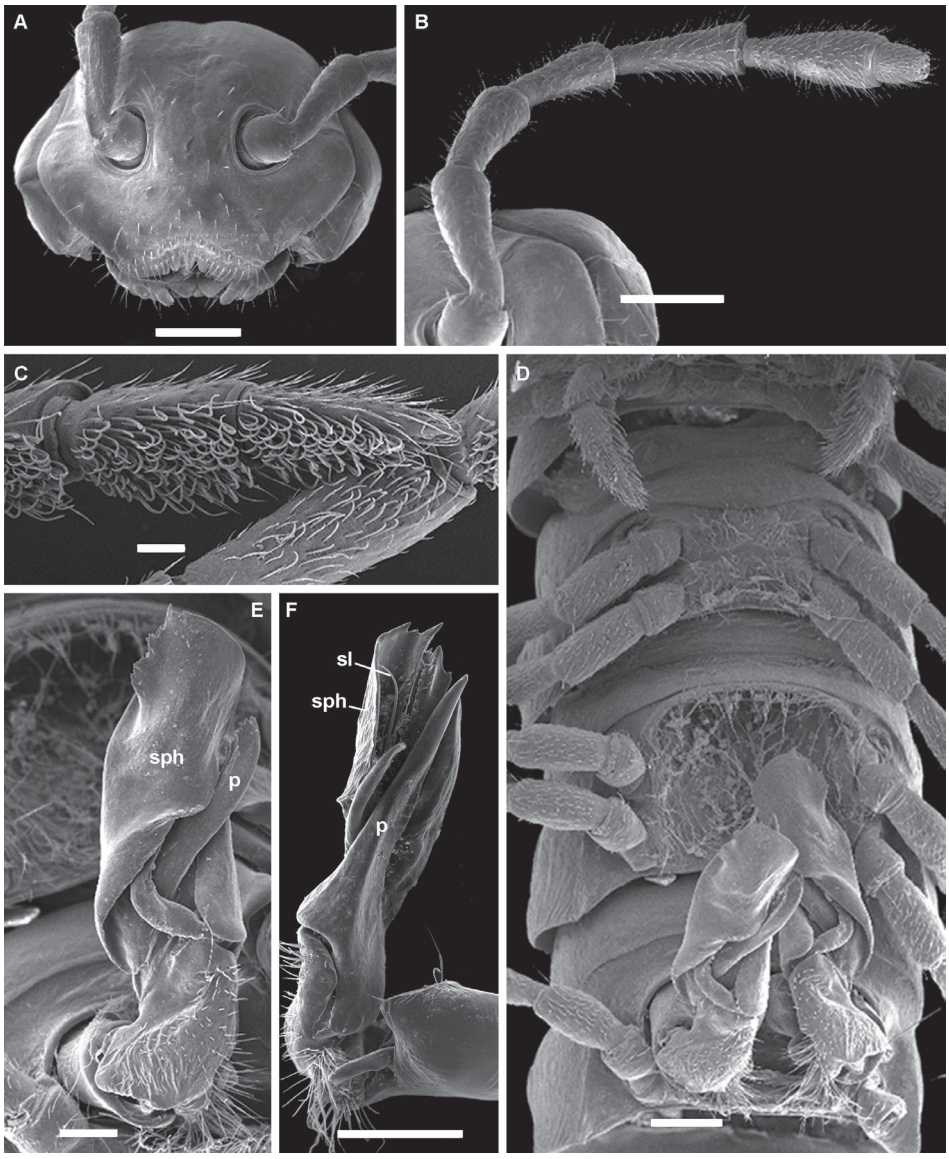


Figure 11. *Eviulisoma silvestre* (Carl, 1909), ♂ from Kakamega Forest, Kenya. **A** head, frontal view **B** antenna, frontal view **C** ventral brushes on tibia and tarsus, ventral view **D** body segments 4–7, ventral view **E, F** right gonopod, ventral and mesal views, respectively. Scale bars: 0.5 (**A, B, D, F**), 0.2 (**E**) & 0.1 mm (**C**). Designations in text.

Eviulisoma silvestre (Carl, 1909)

Fig. 11, Map 1

Material. 1 ♂, 1 ♂ (incomplete, only last 8 segments present) (MRAC 22627), Kenya, Likhanda Hills, Kakamega Forest, S00°13', E34°54', pitfall traps, 28.IX.2002; 1 ♂ (incom-

plete, only segments 8–20 present) (MRAC 22628), same locality, pitfall traps, 6.IV.2002; 1 ♂ (MRAC 22629), same locality, pitfall traps, 6.VII.2002, all leg. D. S. Smith.

Remarks. This is only the second record of this species which has hitherto been known solely from Bakoba, S00°11', E37°28', Tanzania (Carl 1909). First described as a variety of *E. fossiger* (Carl, 1909), it has since been treated (Hoffman 1953) as a species of full rank, recently very nicely revised and illustrated by Jeekel (2003) from type material. Even though the new samples, which are in rather poor condition, fully match Carl's (1909) original description and Jeekel's (2003) redescription, we provide additional illustrations (Fig. 11) to document the identity of this species. The shapes and proportions of the solenophore (**sph**), solenomere (**sl**) and postfemoral process (**p**) which has a conspicuous, parabasal, unciform branch (**h**) are quite characteristic. *E. silvestre* appears to be very widely distributed, occurring not only in Tanzania, but also in Kenya (Map 1).

Key to *Eviulisoma* species known from Kenya, based mainly on ♂ characters

1 Sterna between ♂ legs 6 and 7 flattened, not excavate (Figs 1C, 2C). Paraterga 2 present, however small. Ngaia Forest (N 00°19', E 38°02') **2**

– Sterna between ♂ legs 6 and 7 deeply excavate and ledge-shaped for accommodation of gonopod tips (Figs 3C, 4H, I, 5C, 6E, F, 7C, 8C, 9C, 10C, 11D). Paraterga 2 sometimes totally absent **4**

2 Sternal cones absent. Sternal lobe between ♂ coxae 4 large (Fig. 2C). Gonopod postfemoral process (**p**) large, phylloid, acuminate, but much shorter than a digitiform, suberect, apically rounded, lamellar solenophore (**sph**) (Fig. 2C–E) ..... ***E. ngaia* sp. n.**

– Sternal cones present, starting from ♂ body segment 8. Sternal lobe between ♂ coxae 4 rather small, slightly concave (Fig. 1C). Gonopod postfemoral process (**p**) vestigial, solenophore (**sph**) longest and claw-shaped apically (**c**), with two characteristic teeth (**m** and **l**) in distal 1/3 (Fig. 1C–E) .... ***E. ngaiaorum* sp. n.**

3 All ♂ telopodite segments distal to coxa or prefemur with ventral brushes. Epiproct with two distinct apical claws directed ventrad (Fig. 7b). Gonopods divergent, rather loose, with a complex, lamellar, apically unciform (**u**) solenophore (**sph**) partly sheathing a longer and flagelliform solenomere (**sl**); postfemoral process (**p**) very simple, strong and sickle-shaped (Fig. 7C–F) ... . ***E. kirimeri* sp. n.**

– Only 2–3 last telopodite segments distal to coxa or prefemur in ♂ with ventral brushes. Epiproct with only inconspicuous apical papillae. Gonopods either held parallel to each other or somewhat convergent, always compact **4**

4 Paraterga 2 wanting **5**

– Paraterga 2 at least traceable **7**

5 Sternal cones totally absent. Hypoproct acute caudally. Gonopod postfemoral process longest, erect, digitiform, fringed at base on mesal face; both sole-

nophore and solenomere only a little shorter, subequal in length, distal 1/3 of solenophore a subflagelliform branch..... ***E. pallidum***

– Sternal cones present at least between each caudal leg-pair per ♂ diplosegment following 7th. Hypoproct rounded caudally. Gonopod structure different, postfemoral process much longer than a similarly spiniform solenophore showing a fold for sheathing a likewise long solenomere in distal 1/3 extent..... **6**

6 Sternal cones present only between each caudal leg-pair per ♂ diplosegment following 7th. Gonopod postfemoral process simple, not grooved longitudinally..... ***E. jeanneli***

– Sternal cones small, but present between both leg-pairs per ♂ diplosegment following 7th. Gonopod postfemoral process (**p**) more complex, grooved longitudinally, with a dorsal spinule in distal half (Fig. 10)..... ***E. alluaudi***

7 Sternal lobe between ♂ coxae 4 missing (Fig. 3C). Gonopods (Figs 3C, 4H–L) very slender, with solenophore (**sph**), postfemoral process (**p**) and solenomere (**sl**) subequal in length. Sufficiently abundant samples revealing two distinct size morphs, with midbody widths being 1.5–2.7 or 2.0–3.7 mm..... ***E. taitaorum* sp. n.**

– Sternal lobe between ♂ coxae 4 usually present. Gonopods different. No distinct size morphs noted even in the syntopically occurring congener, *E. taita* sp. n..... **8**

8 Hypoproct trapeziform, with a sharp tooth caudally. Sternal lobe between ♂ coxae 4 very small to missing. Sternal cones behind body segment 7 absent. Gonopod postfemoral process long and subspiniform, nearly as long as solenomere and a lamellar, fold-shaped solenophore, the latter showing a parabasal, unciform process about half as long as postfemoral process..... ***E. silvestre***

– Hypoproct broadly and regularly rounded caudally. Sternal lobe between ♂ coxae 4 always quite conspicuous. Sternal cones behind ♂ body segment 7 present. Gonopods different **9**

9 Gonopods (Figs 5C, 6F–J) with a lamellar, slender, apically unciform and bidentate solenophore (**sph**) carrying a lateral tooth midway (**t**) and reaching about as long as a flagelliform solenomere (**sl**), both **sph** and **sl** being considerably higher than a rather simple, similarly slender, postfemoral process (**p**)..... ***E. taita* sp. n.**

– Gonopod solenophore (**sph**) complex, cup-shaped, lamellar, about as long as a flagelliform solenomere (**sl**), flanked medially by a long, subspiniform, postfemoral process (**p**) (Figs 8C, 9C–E) ..... ***E. kakamega* sp. n.**

Conclusion

At least in Kenya, several places appear to support two *Eviulisoma* species, e.g. Ngaia Forest, Taita Hills and Kakamega Forest. Furthermore, one of the species from Taita Hills demonstrates remarkable size dimorphism, when adult males can vary in size by

1.5–2.0 times, and is parapatric with a second *Eviulisoma* species. We are not aware of anything similar among other Paradoxosomatidae, but some Odontopygidae, a purely Afrotropical family of Spirostreptida, also show surprisingly distinct size dimorphism (Didier VandenSpiegel, unpublished results). As noted above, this variability may be advantageous for the local populations in adverse ecological conditions, possibly allowing for selection of different life strategies.

Last but not least, even though *Eviulisoma* is already the largest paradoxosomatid genus in tropical Africa, at the moment counting 36 species or subspecies, there is little doubt that numerous further species will be discovered in the region.

Acknowledgements

We are most grateful to all collectors who entrusted us their valuable material for treatment. Didier VandenSpiegel's research visit to the Natural History Museum Vienna in 2005 to check the type material of *Eviulisoma* available there was partly financed by that museum. Sergei I. Golovatch is most obliged to the Musée Royal de l'Afrique Centrale, Tervuren for the invitation to work on this project.

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RESEARCH ARTICLE

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A new species of *Halicyclops* (Copepoda, Cyclopoida, Cyclopidae) from a lagoon system of the Caribbean coast of Colombia

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Abstract

Plankton samples obtained from the lagoon system Laguna Navío Quebrado, in northern Colombia, yielded male and female specimens of an undescribed cyclopoid copepod of the genus *Halicyclops*. The new species belongs to the highly diverse and widely distributed *thermophilus*-complex. It closely resembles *H. clarkei* Herbst, 1982 from Louisiana and *H. bowmani* Rocha & Iliffe, 1993 from Bermuda. These species share the same armature of P1-P4EXP3, with a 3443 spine formula and the terminal antennary segment with 5 setae. However, *H. gaviriai* **sp. n.** can be separated from both *H. clarkei* and *H. bowmani* by the morphology of the anal pseudoperculum, the proportions of the fourth antennulary segment, the length of the inner basipodal spine of P1, the P1EXP/inner basipodal spine inner length ratio and the length/width ratio of the caudal rami. This is the third species of *Halicyclops* recorded from Colombia and the first one described from this country. With the addition of *H. gaviriai* **sp. n.**, the number of species of *Halicyclops* known from the Neotropics increases to 19. The regional diversity of the genus is probably underestimated.

Keywords

Brackish waters, taxonomy, crustaceans, halicyclopines, lagoon systems biota

Introduction

The cyclopoid copepod genus *Halicyclops* is the most speciose in the subfamily Halicyclopinae; currently, it is known to contain 111 species and subspecies (Boxshall 2014) and is in need of revision. Members of this genus are cosmopolitan and planktonic forms (Chang 2012; Ueda and Nagai 2012), inhabiting chiefly coastal brackish water habitats, but some species can be found in freshwater habitats (Rocha 1995; Bazilevich and Kaftannikova 1970; Defaye and Dussart 1988; Fuentes-Reinés and Zoppi 2013).

In the Americas, Brazil and the United States are the countries with most records of *Halicyclops* (Wilson 1958; Herbst 1977, 1982; Rocha 1983, 1984, 1991, 1995, Rocha and Hakenkamp 1993). According to Rocha et al. (1998) there are about 17 species of *Halicyclops* recorded in the Caribbean region and this figure remained stable until the recent description of a new species from Argentina (Menu-Marque and Sorarrain 2007). In Colombia, the knowledge about this genus is still very limited; up to now, only two species, *H. venezuelaensis* Lindberg, 1954 and *H. exiguus* Kiefer, 1934 have been reported from two Caribbean localities of Colombia: Ciénaga Grande de Santa Marta, Magdalena and Laguna Navío Quebrado, La Guajira, respectively (Fuentes-Reinés et al. 2013, Fuentes-Reinés and Suárez-Morales unpubl. data). The few reports of *Halicyclops* in Colombia together with the high potential diversity of the genus in the area emphasizes the importance and necessity of intensifying the biological research in fresh and brackish water body in the country to improve our knowledge about the copepod fauna living in these environments. During a survey of the plankton community of the lagoonal system of Laguna Navío Quebrado, in the Colombian coast of the Caribbean, male and female specimens of an undescribed species of *Halicyclops* were collected. The aim of this paper is to describe this new species and compare it with its closest congeners.

Methods

Plankton samples were taken monthly from the Laguna Navío Quebrado, Colombia (11°25'N, 73°5'W) between April and December 2012, mainly in the littoral areas with vegetation (macrophytes and mangrove) but also from open water in areas close to oyster banks. Water salinity was measured with a WTW 3111 conductivity meter. Water samples were collected using a bucket of 25 L at both vegetation areas and shallow open water. Samples were filtered with a zooplankton net (45 μm) and preserved in 70% ethanol. Copepods were sorted from the original samples and then processed for taxonomical identification. Dissected specimens and appendages were mounted in glycerine and sealed with Canada balsam. Drawings were made with the aid of a camera lucida mounted on an Olympus BX51 compound microscope equipped with Nomarski DIC. The specimens were measured in lateral position, from the anterior end of the rostral area to the posterior margin of the caudal ramus. The specimens examined were deposited at the Museo de Colecciones Biológicas at the Universidad

del Atlántico (UARC), Colombia and in the Collection of Zooplankton (ECO-CHZ) held at El Colegio de la Frontera Sur (ECOSUR), Chetumal, Mexico. Morphological terminology follows Huys and Boxshall (1991). The following abbreviations are used in the description: P1–P6= first to sixth swimming legs, EXP= exopod, ENP= endopod.

Results

Taxonomy

Order Cyclopoida Burmeister, 1834
Family Cyclopidae Dana, 1846
Subfamily Halicyclopinae Kiefer, 1927
Genus *Halicyclops* Norman, 1903

***Halicyclops gaviriai* sp. n.**
http://zoobank.org/6F89C13E-501E-4CAE-82FA-89D435118FCD

Material examined. Adult female holotype (UARC393Z), Laguna Navío Quebrado, Colombia, limnetic plankton sample, 7 N., 2007, coll. Juan M. Fuentes-Reinés. Male allotype (UARC394Z), both partially dissected. Paratypes: ten females and four males, undissected, ethanol-preserved, vial (UARC395Z), plus one dissected female, slides (UARC399Z-403Z) and one dissected male (UARC397Z). Three adult females from same locality and date, two of them undissected, ethanol-preserved, in vial, one mounted on slide (ECO-CHZ-09267).

Type locality. Laguna Navío Quebrado, La Guajira, northern Colombia (11°25'N; 73°5'W).

Description of female. Habitus in dorsal position as in Figure 1A, body wide, robust in the anterior part, rostrum subtriangular. Body length, excluding caudal setae, 560–602 μm (average = 581 μm; *n*= 10; holotype: 574 μm). Rostrum strong, subtriangular (Fig. 1D). Labrum represented by widely rounded plate ornamented with marginal rows of spinules at both sides of teeth. Labrum armed with 10–12 teeth of different sizes, outermost being largest (Fig. 1C).

Cephalosome with large rounded dorsal integumental window. Urosome with four segments, genital double somite as long as wide with slight lateral protrusion at halflength and rounded integumental window on each side of posterior half (Fig. 3A). Seminal receptacle as shown in Fig. 3C, anal pseudoperculum, formed by slightly curved expansion of hyaline frill, posterior margin with irregularly serrate pattern (Fig. 3B) with adjacent rows of minute spinules. Caudal ramus about 1.2 as long as wide, outer seta III 1.4 times as long as ramus, apical seta V about twice time as long as seta IV (Fig. 1A), latter caudal seta with heteronomous ornamentation, with inner

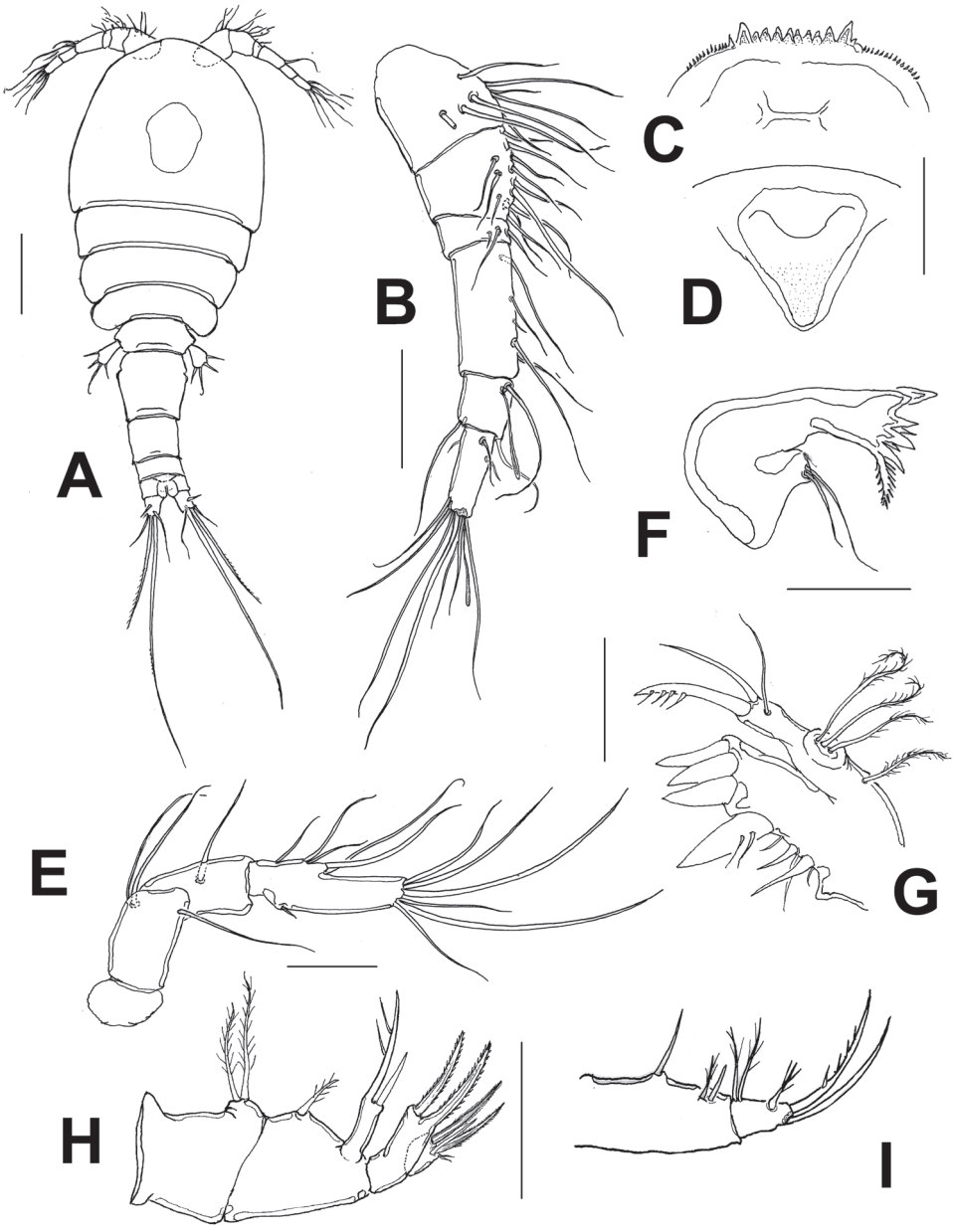

Figure 1. *Halicyclops gaviriai* sp. n., adult paratype female from northern Colombia. **A** habitus, dorsal view **B** antennule **C** labrum, ventral **D** rostrum **E** antenna **F** mandible **G** maxillule **H** maxilla **I** maxilliped. Scale bars: **A**=100 μm, **B**–**F**= 50 μm.

margin spinulated, outer margin with setules (Fig. 3D). Dorsal caudal seta (VII) 2.4 times as long as ramus.

Antennules 6-segmented, setal formula as follows, s=setae, ae=aesthetasc: 1(8s), 2(12s), 3(3s), 4(5s), 5(3+ae), 6(10+ae); fourth segment about 1.7 times as long as wide (Fig. 1B).

Antenna consisting of 4 segments, coxa reduced and unarmed, basis with 2 setae at inner corner; seta representing EXP present. ENP two-segmented. Proximal endopodal segment with a seta on middle inner margin. Terminal endopodal segment about 1.4 times as long as preceding segment armed with 5 inner setae and 7 apical setae plus short spinule on proximal outer margin. Length/wide ratio of second segment about 2.3 (Fig. 1E).

Mandible with well-developed coxal gnathobase, armed with 7 teeth plus outermost dorsal pinnate seta. Palp reduced, represented by 2 naked setae inserted on small protuberance, one seta about 1/3 times as long as the other one (Fig. 1F)

Maxillule with praecoxal arthrite bearing four strong tooth-like spines distally, inner spine strongest, with two proximal subequal setae, inner surface with two robust setal elements and one regular seta. Palp two-segmented, basis with 4 setae, endopodite represented by single oval-shaped segment, armed with three subequal, lightly setulated setae (Fig. 1G).

Maxilla 4-segmented, comprising praecoxa, coxa, basis and 1-segmented endopod. Praecoxal endite robust, armed with 3 setae and 2 spiniform elements on inner margin, with distal set of four robust claw-like spines. Basis with three elements including a claw-like spine, one naked stout seta and a short slender seta, exopod represented by single proximal seta. Endopod with 3 setae (Fig. 1H).

Maxilliped 2-segmented, armed with 3 setal elements on basal segment and 5 setae on distal segment, one of them subdistal, two distal (Fig. 1I).

P1-P4 exopod and endopod 3-segmented (Fig. 2A–D), armed as in Table 1. Spine inserted at inner corner of P1 basis reaching distal margin of second endopodal segment of P1 (Fig. 2A). EXP/inner spines of P1 basis ratio = 1.63. P2–P3 similar each other (Fig. 2B, C). Outer basipodal seta present in P1, P3 and P4, absent in P2. P4ENP3 about 1.7 times as long as wide, with four pinnate spines (I-IV) and inner lateral seta (arrowed in Fig. 2E), inner apical spine (III in Fig. 2E) as long as segment and 1.4 times as long as outer apical spine (II). Inner lateral spine (IV) 1.5 times as long as segment. Inner lateral seta spiniform, ornamented with short stiff setules.

P5 exopod subrectangular (Fig. 3E), about 1.56 times as long as wide, armed with 3 spines, all of them shorter than segment, plus one flexible seta 1.2 times as long as segment; relative length of elements from inner to outer margin as follows 0.66, 1.0; 0.46; 0.6.

Description of male. Habitus resembling that of female, body length, excluding caudal setae= 420μm; (average = 410 μm; n = 10; holotype: 420 μm). Cephalosome with middle integumental window dorsally and lateral window on posterior margin. Second and third somites of prosome with integumental windows laterally, the latter being smallest (Fig. 3G). Rostrum as in female, antennules geniculate, 14-segmented (Fig. 3H), antennular segments 10-12 with modified brush-like setae (detail in Fig. 3H). Antenna, maxilla, maxillule, mandible and maxilliped as in female. Urosome

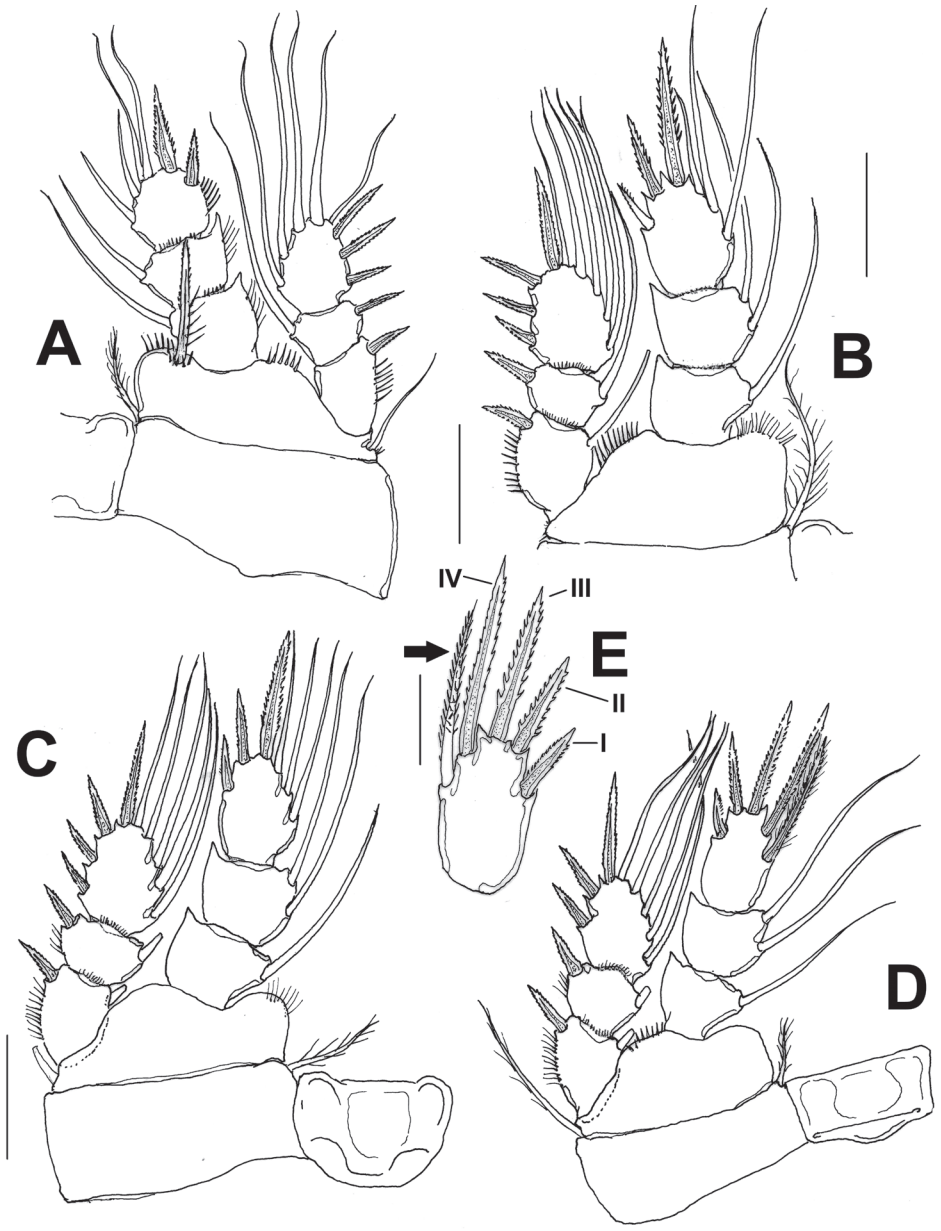

Figure 2. *Halicyclops gaviriai* sp. n., adult holotype female from northern Colombia. **A** leg 1 **B** leg 2 **C** leg 3 **D** leg 4 **E.** leg 4 terminal endopodal segment showing details of armature. Scale bars: **A–D**= 50 µm, **E**=25 µm.

with six somites, third somite with integumental window dorsally (Fig. 3F), caudal rami as in female.

P1-P4 as in female (Fig. 4A–C), P5 exopod subrectangular, about 1.27 as long as wide, and bearing 3 spines and 2 setae (Fig. 4D), relative length of elements from inner

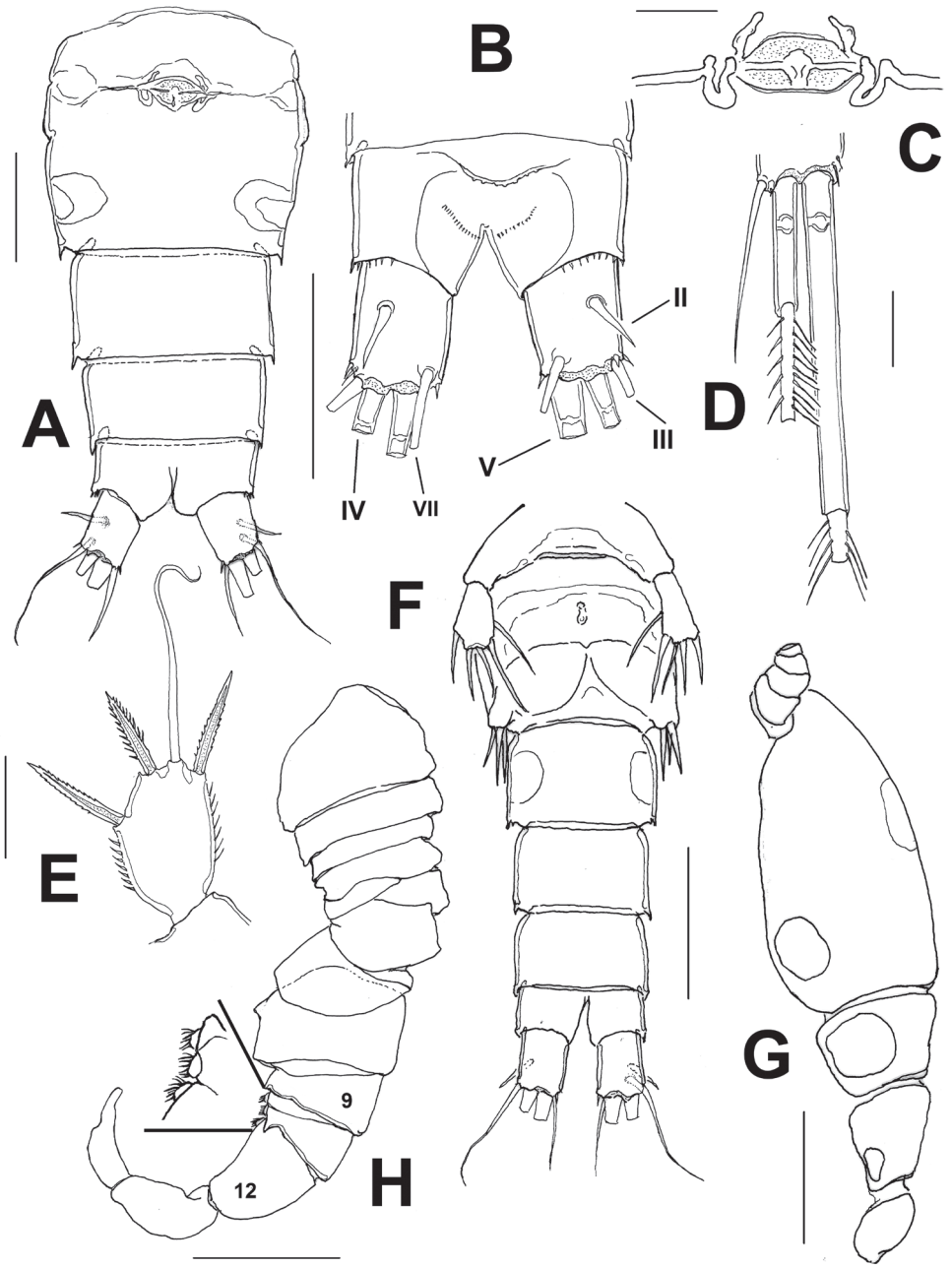

Figure 3. *Halicyclops gaviriai* sp. n., adult holotype female from northern Colombia. **A** urosome showing genital somite, ventral view **B** anal somite showing anal pseudoperculum, dorsal view **C** internal structures of genitalia, ventral view, **D** proximal section of middle apical setae of caudal ramus **E** leg 5; adult male from same locality **F** urosome, ventral view **G** lateral view of cephalothorax showing position of integumental windows **H** geniculate antennule, showing brush-like modified setae on segments 10–12. Scale bars: **A,B, F, H** =50 μm, **C**–**E**= 25 μm, **G**=100 μm.

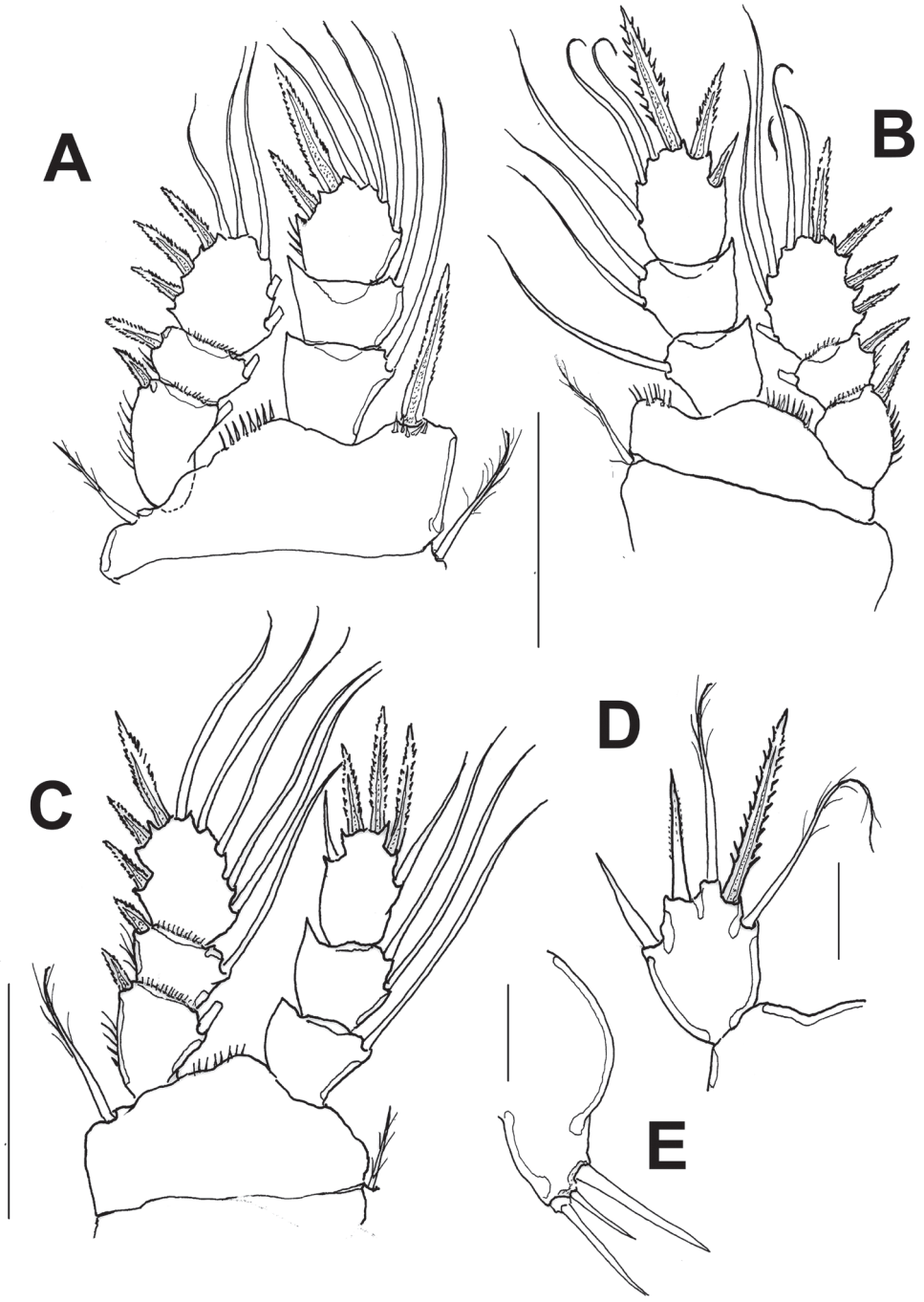

Figure 4. *Halicyclops gaviriai* sp. n., adult male allotype from northern Colombia. **A** leg 1 **B** leg 2 **C** leg 4 **D** leg 5 **E** leg 6. Scale bars: **A**–**C**= 50 μm, **D**=25 μm, **E**= 10 μm.

Table 1. Armature formula of legs 1–4.

	coxa	basis	exopod	endopod
Leg 1	0-1	1-I	I-0, I-1,III-1,4	0-1,0-1,II-2,2
Leg 2	0-1	0-0	I-1,I-1,III-1-1,4	0-1,0-2, III-3
Leg 3	0-1	1-0	I-1,I-1,III-I-1,4	0-1,0-2, III-3
Leg 4	0-1	1-0	I-1, I-1, II-I1-4	0-1,0-2, I-II-II

to outer margin as follows 1.0, 0.8, 1.0; 0.6, 0.5. Sixth leg represented by plate with three elements, two stout setae, middle seta shortest (Fig. 4E).

Etymology. The species is named after Dr. Santiago Gaviria for his work on Colombian copepods and his leadership in the formation of new generations of planktologists.

Remarks. *Halicyclops gaviriai* sp. n. is assigned to the group of species "B" of *Halicyclops* with a 3443 spine formula; this is the most diverse group containing 74 species (see Pesce 2014). One of its subgroups, including approximately 15 species (Pesce 2014) is the *thermophilus*-complex, proposed by Herbst (1983). Species in this group share the presence of a chitinous blunt hook-like process on each side of the genital double-somite, but in *H. gaviriai* this process is reduced or absent. Other characters related to this group include: inner distal margin of the basis of leg 1 devoid of setae, thus diverging from *H. gaviriai* sp. n. with a well-developed inner basipodal spine. Two characters of the *thermophilus* group present in our specimens are: intercoxal sclerite of P1-P4 naked, and regular, unmodified setae on P4 EXP2-3. Because of the absence of the main group characters, the new species is not assigned to the *thermophilus*-complex. In Colombia, only one species of the *thermophilus* group has been hitherto recorded: *H. venezuelaensis* Lindberg, 1954.

Among the species of *Halicyclops* reported from the Caribbean region and adjacent areas (Rocha et al. 1998), *H. gaviriai* sp. n. closely resembles *H. clarkei* Herbst, 1982 described from Louisiana and *H. bowmani* Rocha & Iliffe, 1993 from Bermuda. Both of them lack strong processes on the genital double-somite and have a P1 with a strong inner basipodal spine (Herbst 1982; Rocha and Iliffe 1993; Pesce 2014). When the most recent key to the Neotropical species of *Halicyclops* (Rocha et al. 1998) is followed, our specimens from Colombia key down to a couplet leading to these two species (*H. clarkei*, *H. bowmani*). They share the same spine formula of P1-P4EXP3 (3443), the P4EXP3 with 3 spines on the outer margin, and the terminal antennulary segment with 5 lateral setae. The female fifth legs of these species are also very similar (Herbst 1982; Rocha 1991). However, *H. gaviriai* sp. n. can be separated from both *H. clarkei* and *H. bowmani* by differences in several characters. In *H. clarkei* the integumental windows of the genital double-somite are rounded and relatively small (Herbst 1982, fig. 15) whereas they are oblong and larger in the new species (Fig. 3A). The morphology and ornamentation of the anal pseudoperculum has been regarded of taxonomical value to distinguish species in this group (Rocha and Iliffe 1993; Pesce

2014). This structure is slightly curved and bears tiny denticles along the free margin in *H. clarkei* (Herbst 1982, fig. 16), it is strongly developed and coarsely serrate in *H. bowmani* (Rocha and Illife 1993, fig. 27), and it has shallow, irregular indentations, and is slightly curved in the new species (Fig. 3B).

The length/width ratio of the fourth antennulary segment differs in these species, it is much shorter in *H. gaviriai* (ratio = 1.7) *vs.* 2.5 in *H. bowmani* (Rocha and Iliffe 1993) and 2.7 in *H. clarkei* (Herbst 1982, fig. 18). Also, in *H. clarkei* the inner basipodal spine of P1 is long, slender, it reaches half of P1ENP3 (Herbst 1982, fig. 19), in *H. bowmani* this spine is more robust and shorter, it doesn't reach the distal margin of P1ENP2 (Rocha and Illife 1993, fig. 29), whereas in *H. gaviriai* this spine reaches the distal margin of P1ENP2 (Fig. 2A). The length ratio P1EXP/basipodal spine is about 2.0 in *H. bowmani* (Rocha and Iliffe 1993, fig. 29), 1.42 in *H. clarkei* (Herbst 1982, fig. 19), and 1.63 in *H. gaviriai*.

The armature details of P4ENP3 shows some additional differences among these species; this segment is armed with 4 spines and one spiniform, distally serrate seta in both *H. clarkei* (Herbst 1982, fig. 22; Rocha 1991, fig. 10) and *H. gaviriai* sp. n., while in *H. bowmani* the armature consists of 3 spines and 2 stout, plumose setae (Rocha and Iliffe 1993, fig. 31). Also, in *H. clarkei* the inner apical spine of P4ENP3 is as long as the segment (Herbst 1982, fig. 22; Rocha and Hakenkamp 1993), whereas in both our specimens from Colombia and in *H. bowmani* (Rocha and Iliffe, 1993, fig. 31) this spine is 1.25 times as long as the segment (Fig. 2D, E). The proportions of the caudal ramus have some variation among these species, the length/width ratio is about1.5 in *H. clarkei*, 1.3 in *H. bowmani*, and 1.2 in *H. gaviriai* sp. n. The inner/outer apical caudal setae length ratio is 1.8 in both the new species and in *H. bowmani* (Rocha and Iliffe 1993, fig. 28) *vs.* 2.3 in *H. clarkei* (Herbst 1982, fig. 14). The body size of these species show some additional differences: measuring 560-602 μm, the female of the new species *H. gaviriai* is larger than those of *H. bowmani* (500-530 μm) (Rocha and Iliffe 1993), but smaller than the females of *H. clarkei* (698 μm) (Herbst 1982).

The new species has also affinities with *Halicyclops* cf. *clarkei* from Panama (Rocha, 1991), but can be easily distinguished from the new species from Colombia by the armature of the female P5, in *Halicyclops* cf. *clarkei* the outermost spine is slightly shorter than the innermost and both are longer than the terminal segment (Rocha 1991, fig. 13), but in the new species the innermost spine is as long as the segment and the outermost spine is shorter than the segment (Fig. 3E). According to Rocha (1991), in both *Halicyclops* cf. *clarkei* from Panama and *H. clarkei* from the type locality in Louisiana the length/width ratio of the fourth antennulary segment are identical, about 2.7; this value diverges from that found in *H. gaviriai* (1.7). Also, in *Halicyclops* cf. *clarkei* the ENP3 of P2-P3 have the proximalmost inner seta modified as a stiff ornamented seta as the proximal seta of ENP3 of P4 (Rocha 1991, fig. 10), but in the new species these seta are unmodified, flexible elements (Fig. 2B, C). Rocha (1991) stated that the differences between the Panama specimens of *H.* cf. *clarkei* and those from the type locality in Louisiana are probably related to different species.

The male of the new species *H. gaviriai* differs from the male of *H. clarkei* in the presence of modified setae on the antennular segments 10-11, lacking in *H. clarkei* (Herbst 1982, fig. 25). Also, the length/width ratio of P4ENP4 is about 1.63 times as long as wide in *H. gaviriai* sp. n., while in *H. clarkei* is 1.53. The length/width ratio of P5EXP is about 1.27 in *H. gaviriai* sp. n., vs. 1.64 in *H. clarkei* (Herbst 1982, fig. 26). In *H. clarkei* the outer seta of P6 is clearly longer than the inner spine (Herbst 1982, fig. 27), whereas in *H. gaviriai* sp. n., the opposite condition occurs, the outer seta is shorter. Unfortunately, the male of *H. bowmani* remains unknown (Rocha and Iliffe 1993) and could not be compared with the male of the new species.

Halicyclops gaviriai sp. n. is characterized by a unique combination of characters including: 1) last antennary segment with five lateral setae, 2) length/wide ratio of same segment over than twice as long as wide, 3) fourth segment of female antennule over than twice as long as wide, 4) inner basipodal spine of P1 reaching the posterior border of the ENP2 of P1, 5) ENP3 of P4 with four serrate spines and one seta, and 6) P5 about 1.45 times as long as wide, apical seta longer than the segment.

Distribution and ecology. Halicyclops gaviriai sp. n. is currently known from a single locality only, the protected coastal system Laguna Navío Quebrado, on the Caribbean coast of Colombia. This species was recorded in both the limnetic region and the vegetation zones, being more frequent in the former habitat. This large (surface area of 10.7 km^2) lagoon system is a shallow water body (depth 0.3–1.1 m), whose temperature varies over the seasons in the range of 28–31 °C; pH values during sampling ranged between 7.8 and 8.3 and salinity was 28 PSU. This habitat diverges from that of one of its closest congeners, *H. bowmani*, a stygobitic form recorded only from an anchialine system of Bermuda (Rocha and Iliffe 1993). The known habitat of its other close congener, *H. clarkei*, is Lake Peigneur, a former freshwater system whose salinity drastically increased since 1980 after a failed oil drill deeply modified the system (Zio and Aven 2013). The samples examined by Herbst (1982) were obtained before this event, in 1977; he reported a low salinity range (0–5 psu) for this species. Hence, it is an intriguing question if this presumably endemic species was able to adapt to the new conditions and is still extant in the locality or adjacent areas.

The number of Neotropical species recognized by Rocha et al. (1998) was 17 and it remained stable in the region until the recent description of *H. ramirezi* from Argentina (Menu-Marque and Sorarrain, 2007) and the addition of this new species from Colombia, thus raising the number of known Neotropical species to 19. Furthermore, some nominal species in the literature such as *H.* cf. *clarkei* from Panama (Rocha 1991) probably represent undescribed species. The diversity of the genus in the region could be underestimated and certainly deserves further investigations.

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Molecular and morphological differentiation between *Aphis gossypii* Glover (Hemiptera, Aphididae) and related species, with particular reference to the North American Midwest

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Abstract

The cotton aphid, *Aphis gossypii*, is one of the most biologically diverse species of aphids; a polyphagous species in a family where most are host specialists. It is economically important and belongs to a group of closely related species that has challenged aphid taxonomy. The research presented here seeks to clarify the taxonomic relationships and status of species within the *A. gossypii* group in the North American Midwest. Sequences of the mitochondrial cytochrome oxidase 1 (COI), nuclear elongation factor 1-α (EF1-α), and nuclear sodium channel para-type (SCP) genes were used to differentiate between *A. gossypii* and related species. *Aphis monardae*, previously synonymised with *A. gossypii*, is re-established as a valid species. Phylogenetic analyses support the close relationship of members of the *A. gossypii* group native to North America (*A. forbesi*, *A. monardae*, *A. oestlundi*, *A. rubifolii*, and *A. rubicola*), Europe (*A. nasturtii*, *A. urticata* and *A. sedi*), and Asia (*A. agrimoniae, A. clerodendri, A. glycines, A. gossypii, A. hypericiphaga, A. ichigicola, A. ichigo, A. sanguisorbicola, A. sumire* and *A. taraxicicola*). The North American species most closely related to *A. gossypii* are *A. monardae* and *A. oestlundi*. The cosmopolitan *A. gossypii* and *A. sedi* identified in the USA are genetically very similar using COI and EF1-α sequences, but the SCP gene shows greater genetic distance between them. We present a discussion of the biological and morphological differentiation of these species.

Keywords
Aphid, host plant, morphology, phylogeny, sequence divergence, status novus

Introduction

Host plant association is often one of the main characters used to distinguish between closely related aphid species. However, host association can also be one of the main sources of misidentification of host-alternating aphids. These aphids migrate between taxonomically distant hosts, usually between woody and herbaceous plants. Taxonomic problems have been created when aphid morphs from primary (woody) host plants have been treated as separate species from those found living on secondary (herbaceous) or summer host plants. Host alternation provides an opportunity for aphids to acquire new hosts and may be a key to the rapid diversification of some groups of aphids (Eastop 1971, Dixon 1973, von Dohlen and Moran 2000), thereby leading to hard-to-distinguish species complexes. The evolution of *Aphis*, the largest aphid genus by a margin, is associated with the rapid diversification of herbaceous angiosperms (Heie 1996).

The *Aphis gossypii* group contains economically important and taxonomically problematic species, with *A. gossypii* Glover itself being the most biologically diverse and hence taxonomically challenging (Blackman and Eastop 2007). It has many different primary and secondary host plants and exhibits both holocyclic and anholocyclic life cycles (Kring 1955, Blackman and Eastop 2006, Margaritopoulus et al. 2006). Its taxonomic complexity is attested to by its 42 available synonyms, including the native North American species, *Aphis monardae* Oestlund (Favret 2014). Eastop and Hille Ris Lambers (1976) established this synonymy without comment. Lagos (2007) found that *A. monardae* is distinct morphologically from *A. gossypii* and treated it as a valid species. No type specimen of *A. monardae* could be found at the time, and no molecular or biological evidence was available to support this decision. The research presented here contains both molecular and biological evidence as well as an examination of material collected by Oestlund from *Monarda* spp.

In Europe, there are approximately 20 aphid species morphologically similar to *A. gossypii* (Stroyan 1984, Heie 1986). Several studies using mitochondrial, nuclear, and intron length polymorphism in the sodium channel para-type (SCP) genes have achieved some resolution discriminating *A. gossypii* and other *Aphis* species (Coeur d'acier et al. 2007, Foottit et al. 2008, Coccuzza et al. 2009, Carletto et al. 2009b, Kim et al. 2010a, Komazaki et al. 2010, Favret and Miller 2011). In North America, morphological studies show that species of the *A. gossypii* group can be misidentified easily (Voegtlin et al. 2004, Lagos 2007). The discrimination of species closely related to *A. gossypii* is of particular importance due to the recent introduction into North America of the soybean aphid, *A. glycines* Matsumura (Voegtlin et al. 2004). This species is obligately holocyclic and heteroecious, feeding on soybean, *Glycine max* (L.) Merr., as secondary host, and on *Rhamnus* spp. as primary host. *Aphis gossypii* has also

been reported to colonize soybean in North America (Blackman and Eastop 2007), and while its colonization on soybeans is uncommon in the north central United States, some soybean-collected insect samples from Alabama, Georgia, Kansas, Louisiana, and Mississippi contained only *A. gossypii* or a mixture of both species (personal observation, Illinois Natural History Survey (INHS) insect collection records). These collections suggest that *A. gossypii* may be more common on soybeans in southern regions. There are no records of the exotic *A. nasturtii* Kaltenbach feeding on soybeans, and attempts to culture *A. nasturtii* on soybeans were not successful (David Ragsdale, personal communication); however, this species shares the primary host, *Rhamnus* spp., with both *A. glycines* and *A. gossypii*.

We here elucidate the phylogenetic relationship of species morphologically close to *A. gossypii* and the taxonomic status of *A. monardae* in the North American Midwest.

Materials and methods

Aphid collections: Aphids were collected from their primary and/or secondary host plants from different sites in China, France, Italy, Japan, Spain and the USA, with the majority of the material originating from the Midwest of the USA. When possible, aphids were collected alive and reared on the host plant for the maturation of late instar nymphs. Adults were preserved in 95% ethanol and stored at -20°C until DNA extraction and microscope slide preparation. Collection data with INHS Insect Collection specimen voucher numbers are presented in Suppl. material 1.

Morphology: Archival microscope slides were prepared using the technique described by Pike et al. (1991). Individuals were selected from the same colonies as those selected for DNA extraction. Photographs of mounted specimens and measurements were taken using a Leica DM 2000 digital camera and SPOT Software 4.6 (Diagnostic Instruments, Inc). Analyses of variance of diagnostic characters, such as the distance from the base of the third antennal segment to the first secondary sensorium, the ratio of the lengths of the processus terminalis and the base of the sixth antennal segment, and the ratio of the lengths of the siphunculus and the cauda, were tested using JMP, Version 7 (SAS Institute Inc., Cary, NC, 1989–2007). Species identification of slide-mounted material was done by the first author, using published keys (Oestlund 1887, Gillette 1927, Hottes and Frison 1931, Palmer 1952, Kring 1955, Cook 1984, Voegtlin et al. 2004) and authoritatively identified specimens in the insect collections of the INHS and the University of Minnesota. Identifications of slide-mounted specimens were referenced to the aphid colony-mates used in the molecular analyses.

DNA extraction, PCR amplification, and sequencing: Two or three specimens per colony were sequenced individually. Individual specimens were crushed in a 1.5 ml microcentrifuge tube and DNA was extracted and purified using the QIAamp DNAmicrokit (QIAGEN Inc., Valencia, CA). The mitochondrial gene Cytochrome Oxidase I (COI) was amplified in two overlapping fragments: 5' fragment with forward primer C1-J-1718 (Simon et al. 1994) and internal reverse primer C1-J-2411 (Lagos et al.

2012); 3' fragment with internal forward primer C1-N-2509 (Lagos et al. 2012) and reverse primer TL2-N-3014 (Simon et al. 1994). The nuclear gene Elongation Factor-1-α (EF1-α) the following primers were used: EF3F (Lagos et al. 2012) and EF2 (Palumbi 1996). The length polymorphism of an intron in SCP was sequenced using the primers Aph13 and Aph15 (Carletto et al. 2009a). All primers were synthesized by Invitrogen™ Corporation (Carlsbad, CA). PCR used PuReTaq™ Ready-To-Go™ PCR 0.2 ml beads (GE Healthcare UK) mixed with 20 µl of PCR-grade water, 1 µl of F and R primers at 10 µM, and 3 µl of genomic DNA solution. The thermal cycler protocol used to amplify COI and EF1-α was: 95°C 2 min (95°C 30s; 53°C 30s; 72°C 120s) 40x. For SCP, it was: 95°C 3 min (94°C 60s, 55°C 45s, 72°C 60s) 40x. PCR products were run on a 1% agarose gel for 40 min at 90 v, and visualized with GelGreen nucleic acid stain (Biotium Inc, California, USA). Most PCR products were purified using QIAquick® (QIAGEN Inc.) kit. PCR products that included the co-amplification of non-specific bands were gel purified using Zymoclean ™ gel DNA recovery kit (Zymo Research, USA). The concentration of PCR products was measured using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). PCR products were sequenced in both directions using 3 µl of a mixture of BigDye® Terminator v3.1, dGTP BigDye Terminator v3.0, and buffer in a ratio of 2:1:1 respectively, 1.6 µl of 2 µM primer primers, differing amounts of DNA, and 1 µl of dimethyl sulfoxide (DMSO) (SIGMA-ALDRICH®, St Louis, MO). Sequencing reactions were run using the following protocol: 96°C 2 min (95°C 20s; 50°C 5s; 60°C 240s) 25x. Sequencing reactions were cleaned using Performa® DTR Ultra 96-Well Plates (EdgeBioSystems, Gaithersburg, MD) and run on ABI 3730 at the Keck Center (University of Illinois at Urbana-Champaign). Raw sequence data were examined and assembled using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI). Sequences were then aligned with Clustal X (version 2.0, 2007; Larkin et al. 2007). Three introns in EF1-α were identified and used in this study. Nucleotide sequences were deposited in GenBank (Suppl. material 1). Pairwise distances were obtained using PAUP 4.0b10 based on the Kimura two-parameter model (Swofford 2001).

The COI sequence of the *A. gossypii* neotype specimen (GU591547) and 25 EF1-α sequences of *Aphis* spp. (especially those of species closely related to *A. gossypii*) were retrieved from GenBank: EU019867, EU019869, EU019871, EU019872, EU019873, EU019874, EU019875, EU019876, EU019878, EU019879, EU358904, EU358907, EU358911, EU358915, EU358916, EU358917, EU358924, EU358926, EU358927, GU205375 and GU205376.

Phylogenetic analysis: Modeltest 3.7 (Posada and Crandall 1998) was used to select the best-fit nucleotide substitution model. Single sets of gene sequences were analyzed using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2003) to execute Bayesian analyses. For single analysis, four chains were run. The number of generations was 5,000,000 with a burn-in of 250 trees and frequency sampling of 100 generations with rates equal to variable gamma as a model of substitution of nucleotides. *Rhopalosiphum maidis* (Fitch) (Aphidinae: Aphidini), and *Hyadaphis tataricae* Aizenberg and *Uroleucon helianthicola* (Olive) (Aphidinae: Macrosiphini) were selected as outgroups.

Aphid biology: Two growth chambers were used to examine various aspects of the biology of *A. monardae*, *A. gossypii*, and *A. sedi* Kaltenbach, in order to discern differences in their life cycle. Experimental plants were grown in a greenhouse in 12.7 cm diameter pots and isolated in 13.5 by 13.5 by 22.5 inches cages. Chamber A was set at 12°C and short photoperiod (8L:16D), conditions that will trigger the development of sexual morphs. Colonies of *A. monardae* on *Monarda fistulosa* L., *A. sedi* on *Hylotelephium telephium* (L.) H.Ohba, and *A. gossypii* on *Cucurbita pepo* L. and *Rhamnus cathartica* L. were exposed to these conditions for extended lengths of time. Samples of *A. monardae* and *A. sedi* were collected on a weekly basis from the host plants listed above and examined for the presence of sexual morphs. In the cages of *A. gossypii* weekly samples were taken from *R. cathartica*.

The B chamber was set at 24°C with constant illumination (24 hours) to keep colonies and test host plant specificity of the three species mentioned above. The following experiments were done in chamber B: a *Monarda fistulosa* plant infested with *A. monardae* was placed into a cage with an aphid-free *C. pepo* plant and left for a several weeks. Biweekly examination of the *C. pepo* plants was made to determine if *A. monardae* had colonized them. A *Cucurbita pepo* plant infested with *A. gossypii* was placed into a cage with aphid-free *M. fistulosa* and *H. telephium* and left for several weeks. Biweekly examination of *M. fistulosa* and *H. telephium* was made to see if *A. gossypii* had colonized them. A *Hylotelephium telephium* plant infested with *A. sedi* was placed into a cage with aphid-free *C. pepo* and left for several weeks. Biweekly examination of *C. pepo* was made to see if *A. sedi* had transferred to them. An entire tree of *R. cathartica* infested with *A. gossypii* was isolated in a 2 by 2 by 2-m walk-in cage in May of 2011 on the grounds of the South Farms of the University of Illinois (Suppl. material 1). The temperature ranged between 10 and 22 °C, http://www.isws.illinois.edu/atmos/statecli/cuweather/. Aphid-free *C. pepo*, *H. telephium* and *Glycine max* were placed into the cage to document the potential infestation of these secondary hosts under natural environmental conditions.

Results

Phylogenetic analysis

A total of 160 COI sequences from 28 species, 133 EF1-α sequences from 36 species, and 13 SCP sequences from 6 species were used in this study. After alignment and excluding the primer sites, 1,290, 1,078 and 703 bp for COI, EF1-α (including gaps and introns) and SCP were used in the analysis, respectively. COI sequence divergence between species of the *A. gossypii* species group ranged from 0.08% (between *A. gossypii* and *A. sedi*) to 3.04% (between *A. gossypii* and *A. monardae*). The sequence divergence of *A. glycines* and *A. nasturtii* (sharing a winter host plant with *A. gossypii*), as compared with the species of the *gossypii* group, ranged from 5.25% (between *A. gossypii* and *A. glycines*) to 6.97% (between *A. nasturtii* and *A. sedi*) (Table 1). The

Table 1. Range of Kimura 2 Parameter pair-wise inter- and intraspecific sequence divergence (%) for COI sequences.

	A. forbesi	***A. glycines***	***A. gossypii***	***A. monardae***	***A. nasturtii***	***A. oestlundi***	***A. sedi***
A. forbesi	0.00						
A. glycines	5.49–5.73	0.00					
A. gosyypii	6.27- 6.35	5.25–5.92	0.00–0.54				
A. monardae	6.27	5.75–5.85	2.70–3.04	0.00–0.08			
A. nasturtii	5.73–5.77	7.03–7.15	6.66–6.89	6.50–6.73	0.00–0.08		
A. oestlundi	6.35	5.67–5.85	2.37–2.57	1.57–1.81	6.57–6.68	0–0.16	
A. sedi	6.2–6.35	5.51–5.76	0.08–0.70	2.62–3.02	6.54–6.97	2.37–2.77	0.00–0.54

Table 2. Range of Kimura 2 Parameter pair-wise inter- and intraspecific sequence divergence (%) for EF1-α and SCP sequences.

	A. gossypii		***A. monardae***		***A. oestlundi***		***A. sedi***	
	EF1-α	**SCP**	**EF1-α**	**SCP**	**EF1-α**	**SCP**	**EF1-α**	**SCP**
A. gossypii	0.40–0.87	0.14–0.84						
A. monardae	0.54–0.97	1.12–1.98	0.00–0.11	0.14–0.28				
A. oestlundi	0.76–1.20	1.12–1.83	0.87–0.98	0.42–0.64	0.00	0.00		
A. sedi	0.11–0.76	0.84–1.84	0.65–0.76	1.26	0.87	1.26	0.00–0.22	0.00

sequence divergences of EF1-α and SCP are presented in Table 2. Generally, COI sequences were more conserved than EF1-α, which in turn were more conserved than SCP.

The cladograms using COI (Figure 1) and EF1-α (Figure 2) showed a high level of agreement. The COI analysis supported the monophyly of a group of species (Clade A) related to *A. gossypii*, including *A. glycines, A. nasturtii*, and a still more closely related group that are regarded as members of the *A. gossypii* complex (Clade D). Within Clade A are several supported groups: Clade H of *A. rubifolii* (Thomas) and *A. rubicola* Oestlund (PP:1.00); Clade I of *A. nasturtii* and *A. urticata* Gmelin (PP:1.00); Clade B of *A. glycines* with the *A. gossypii* complex (PP:0.99). The *A. gossypii* complex is itself well supported (Clade D, PP:0.99), and includese two groups: Clade E of *A gossypii* and *A. sedi* (PP:0.99) and Clade F of *A. oestlundi* Gillette*, A. monardae*, and several possible new species.

The dendrogram inferred by MrBayes using EF1-α (Figure 2) is congruent with that of COI for some taxa mentioned above (clade A, PP:0.99), although lack of resolution prevented recovery of a monophyletic Midwest *A. gossypii* complex. The close relationship of *A. nasturtii* and *A. urticata* is robustly supported (Clade G, PP:1.00) as is clade B (PP:0.99). Clade A in the COI analysis is polyphyletic in the EF1-α analysis and includes Asian species *A. ichigicola* Shinji and *A. ichigo* Shinji (Clade F, PP:0.97); *A. glycines* and *A. sanguisorbicola* Takahashi (Clade E, PP:0.98). Clade C is poorly supported and presents polytomies of species closely related to *A. gossypii*:

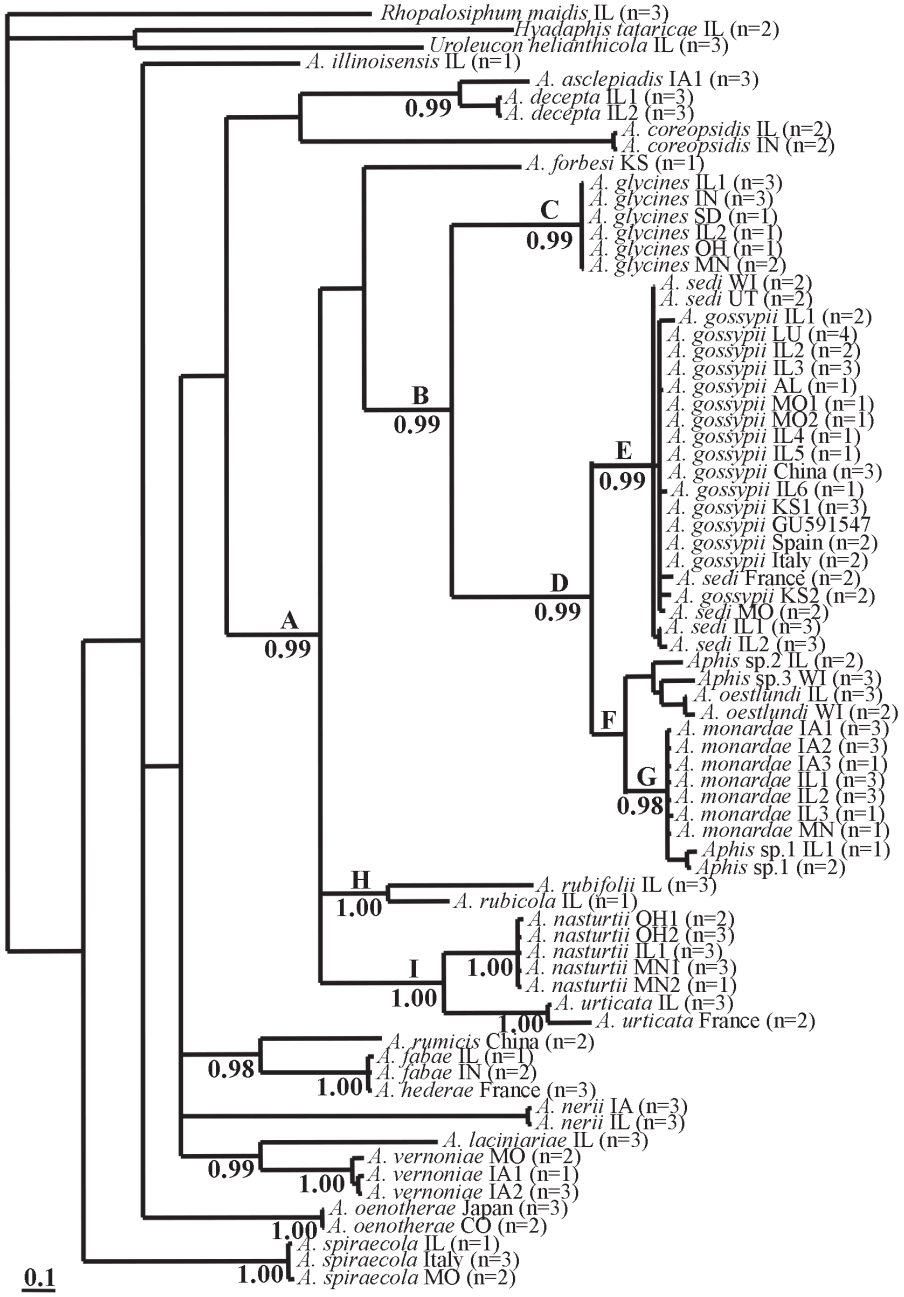

Figure 1. Cladogram inferred based on analysis of COI with MrBayes. Support values (Posterior Probabilities) are below branches. Values under 0.95 are not presented. Species names are followed by collection locality (USA: AL (Alabama), CO (Colorado), IA (Iowa), IL (Illinois), IN (Indiana), KS (Kansas), LA (Louisiana), MO (Missouri), MN (Minnesota), OH (Ohio), SD (South Dakota), WI (Wisconsin)), and number of haplotypes.

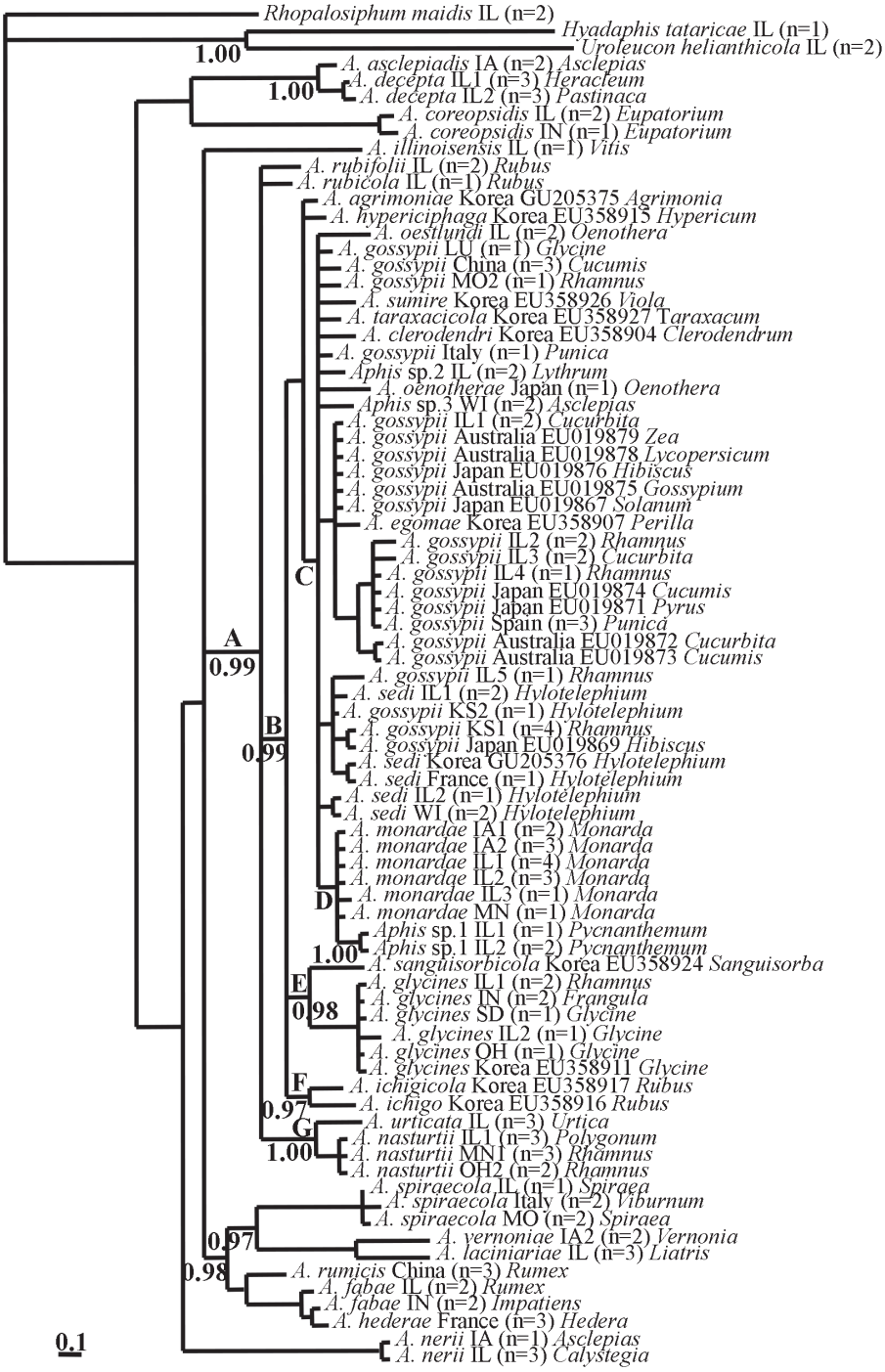

Figure 2. Cladogram inferred based on analysis of EF1-α with MrBayes. Support values (Posterior Probabilities) are below branches. Values under 0.95 are not presented. Species names are followed by collection locality, number of haplotypes and genus of host plant.

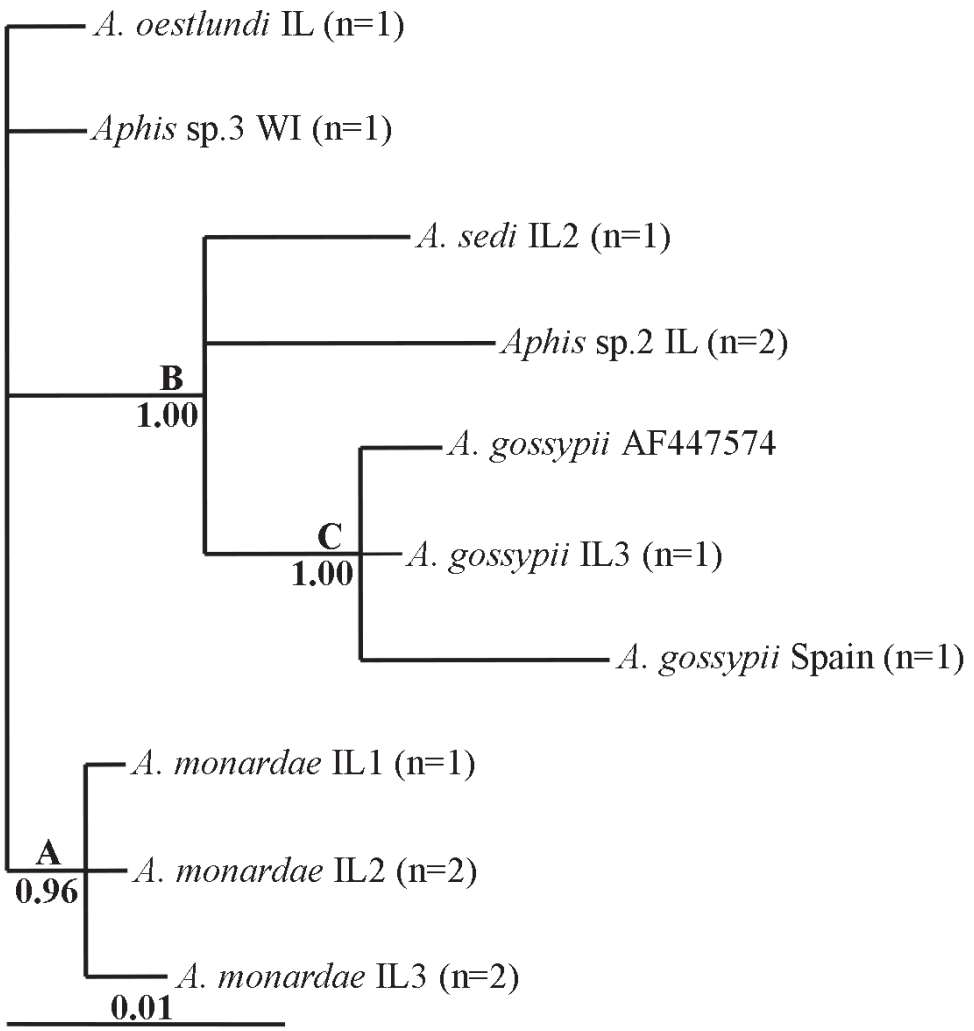

Figure 3. Inferred relationships using the SCP gene based on analysis with MrBayes. Support values (Posterior Probabilities) are below branches. Species names are followed by the collection locality (USA) and number of haplotypes.

A. sedi, *A. oenotherae* Oestlund; the Asian taxa *A. egomae* Shinji, *A. sumire* Moritsu, *A.* taraxacicola (Börner), and *A. clerodendri* Matsumura; and the North American species *A. monardae* and *A. oestlundi*.

The SCP gene was difficult to amplify and thus we only acquired sequences for six taxa. The Bayesian cladogram using SCP (Figure 3) shows two groups strongly supported: *A. monardae* (Clade A, PP: 0.96) and the group comprised of *A. sedi*, *Aphis* sp.2 and *A. gossypii* (Clade B, PP:1.00).

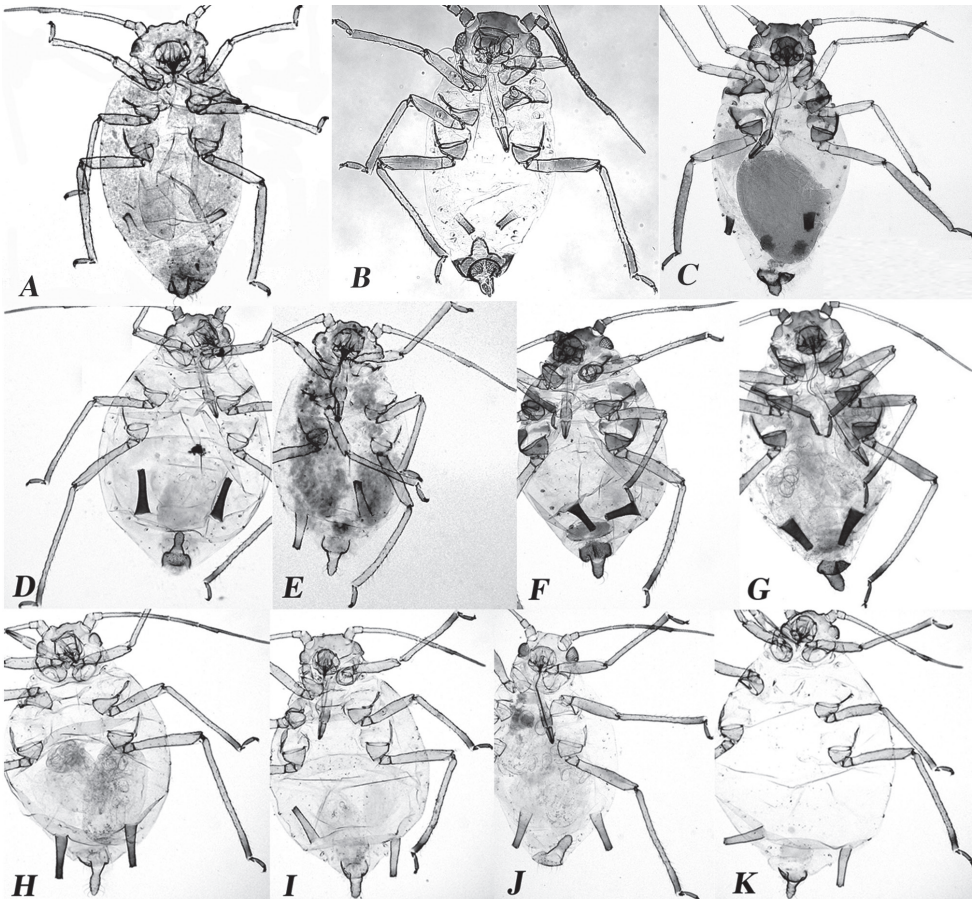

Figure 4. Habitus images of slide-mounted sexual morphs of **A** ovipara of *A. monardae* **B** male of *A. monardae* **C** ovipara of *A. sedi*, and apterous viviparae of **D** *A. gossypii* **E** *A. gossypii* **F** *A. gossypii* **G** *A. sedi* **H** *A. glycines* **I** *A. monardae* **J** *A. nasturtii* **K** *A. oestlundi*.

Biological evidence

After four weeks under conditions of reduced temperature and photoperiod, colonies of *A. monardae* reared on *M. fistulosa* produced oviparae and apterous males (Figure 4A–B). Also, sexual morphs were collected in the field (Middlefork Savanna, Lake County) at the beginning of October (Suppl. material 1). *Aphis sedi* on *H. telephium* produced oviparae (Figure 4C) but no males were found. Voucher slides of both species are deposited in the INHS insect collection with the following catalog numbers: *A. monardae*, 512858-512865; *A. sedi*, 511202-511208 and 511559-511573. In chamber A, no sexual morphs of *A. gossypii* were found after two months exposure to the low temperatures and reduced photoperiod and weekly collections on *R. cathartica*.

The outdoor experiments located at the South farms of the University of Illinois were evaluated after 25 days. Alate viviparae of *A. gossypii* were seen on *H. telephium*

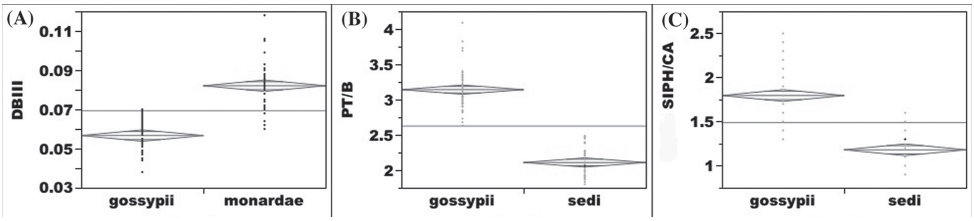

Figure 5. Analysis of variance of morphological characters useful to discriminate *Aphis gossypii, A. monardae*, and *A. sedi*. The gray line represents the median. The gray diamond represents the means and standard deviation. A 95% level indicates a significant difference. **A** distance from the base of antennal segment III to the first secondary sensorium (DBIII) between *A. gossypii* and *A. monardae* **B** ratio of length of processus terminalis (PT) to the base of last antennal segment **B** between *A. gossypii* and *A. sedi* **C** ratio of length of siphunculi (SIPH) to the length of cauda (CA) between *A. gossypii* and *A. sedi.*

and *G. max* but they did not produce offspring, however, alates that moved to *C. pepo* did produce apterous and alate viviparae. Voucher slides are deposited in the INHS insect collection numbers: 512851-512857. The colonies of *A. gossypii* reared on *C. pepo* were set in a growth chamber B where they grew rapidly. Potted *M. fistulosa* were placed in this chamber and were colonized by *A. gossypii*. Clean plants of *C. pepo* that were later exposed in the same chamber to a colony of *A. monardae* were not colonized. A colony of *A. sedi* begun with fundatrices from *H. telephium* was exposed to *C. pepo* in growth chamber B for several weeks, but the aphids did not transfer to and establish on this plant.

Comparison of *Aphis monardae* and *Aphis gossypii*

In both the COI and EF1-α analyses, *A. monardae* was readily distinguished from *A. gossypii* (Figure 1 Clade G, Figure 2 Clade D). *Aphis monardae* and *A. gossypii* are also differentiable morphologically: 1) the siphunculi of apterous morph are darker in *A. gossypii* than in *A. monardae*, and 2) and secondary sensoria on antennal segment IV are always absent in alate viviparae of *A. gossypii*, but present in *A. monardae* (Suppl. material 2). A third, novel morphological character, the distance from the base of antennal segment III to the first secondary sensorium (DBIII) in alate viviparae also separates these species consistently. In *A. gossypii,* the secondary sensoria are uniformly distributed along the segment (Figure 6B) but not in *A. monardae* (Figure 6C). The means of the distance from the base of antennal segment III to the basal margin of the first secondary sensorium of *A. gossypii* and *A. monardae* are 0.06 and 0.08 mm, respectively (Figure 5A, F ratio=152.3, df=1, P<0.0001). Evidence in support of the reproductive isolation of this species is the presence of oviparae (Figure 4A) and apterous males of *A. monardae* (Figure 4B) on *M. fistulosa* (INHS insect collection numbers: 511335-511344 and 512858-512865, respectively), as well as a COI sequence divergence of 2.7-3.04% between *A. gossypii* and *A. monardae* (Table 1).

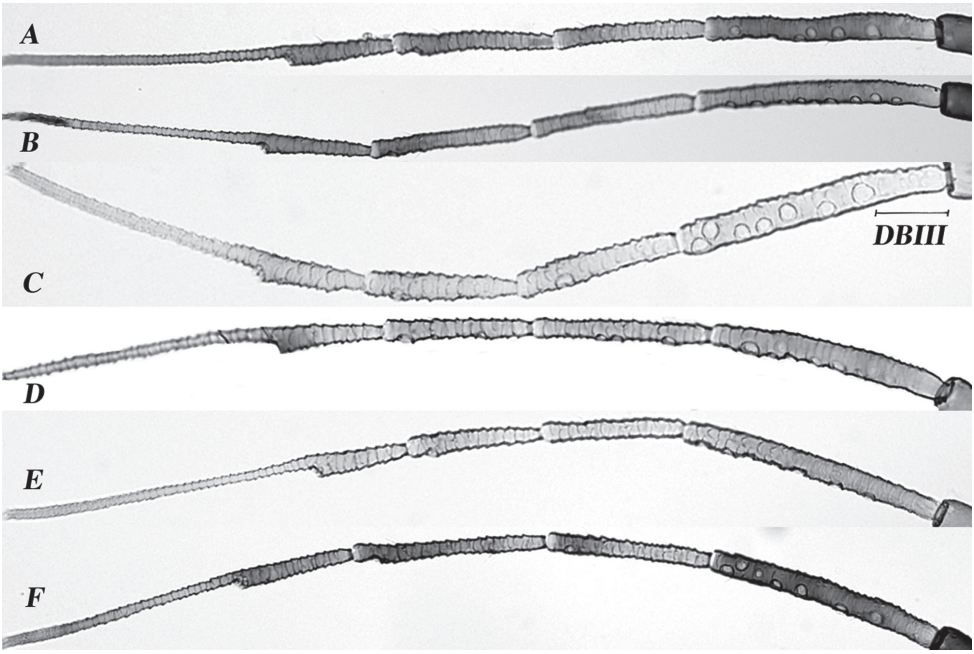

Figure 6. Antennal segments (II-VI) of alate viviparae **A** *A. glycines* **B** *A. gossypii* **C** *A. monardae*, showing distance from the base of antennal segment III to the first secondary sensorium, DBIII **D** *A. nasturtii* **E** *A. oestlundi* **F** *A. sedi*.

Redescription of *Aphis monardae* Oestlund, 1887

Diagnosis: Siphunculi of apterous morph pale, dark distally. When alive, light yellow to light green, body covered with white wax (Figure 8B). In alate viviparae: secondary sensoria on antennal segment IV present (Figure 6C). The distance from the base of antennal segment III to the first secondary sensorium (DBIII) 0.06-0.12 (0.08).

Neotype: Apterous viviparous female. USA: Minnesota; Douglas County; on *Monarda fistulosa* L.; 45.8160°N, 95.7472°W; 19.viii.2010; D. Lagos. Neotype apterous viviparous female (INHS Insect Collection 513070)**.** Body1.4, URS 0.09, accessory setae 2, antennal segments: III 0.16, IV 0.08, V 0.09, B 0.08, Pt 0.18, LHIII 0.010, hind tibiae 0.50, HT2 0.08, width of tubercle on abdominal tergite I 0.020, width of tubercle on abdominal tergite VII 0.018, siphunculus 0.19, cauda 0.12, with 5 setae, abdominal tergite VIII with 2 setae, sub-genital plate with 3 setae on anterior part.

See Suppl. material 2 for morphological measurements of the four morphs of *A. monardae*. Additional images of *A. monardae* can be found in Lagos et al. (2014a).

Apterous viviparae (n= 40). *Color in life* (Figure 8B): Head, thorax and abdomen vary from light yellow to light green. *Color of cleared specimens* (Figure 4I): *Head:* dusky. All antennal segments pale, except the sixth throughout, which is dusky. Secondary sensoria absent. URS does not reach the hind coxae. *Thorax:* Coxae, trochanters and all femora dusky. All hind tibiae dusky and dark distally. *Abdomen:* Cauda

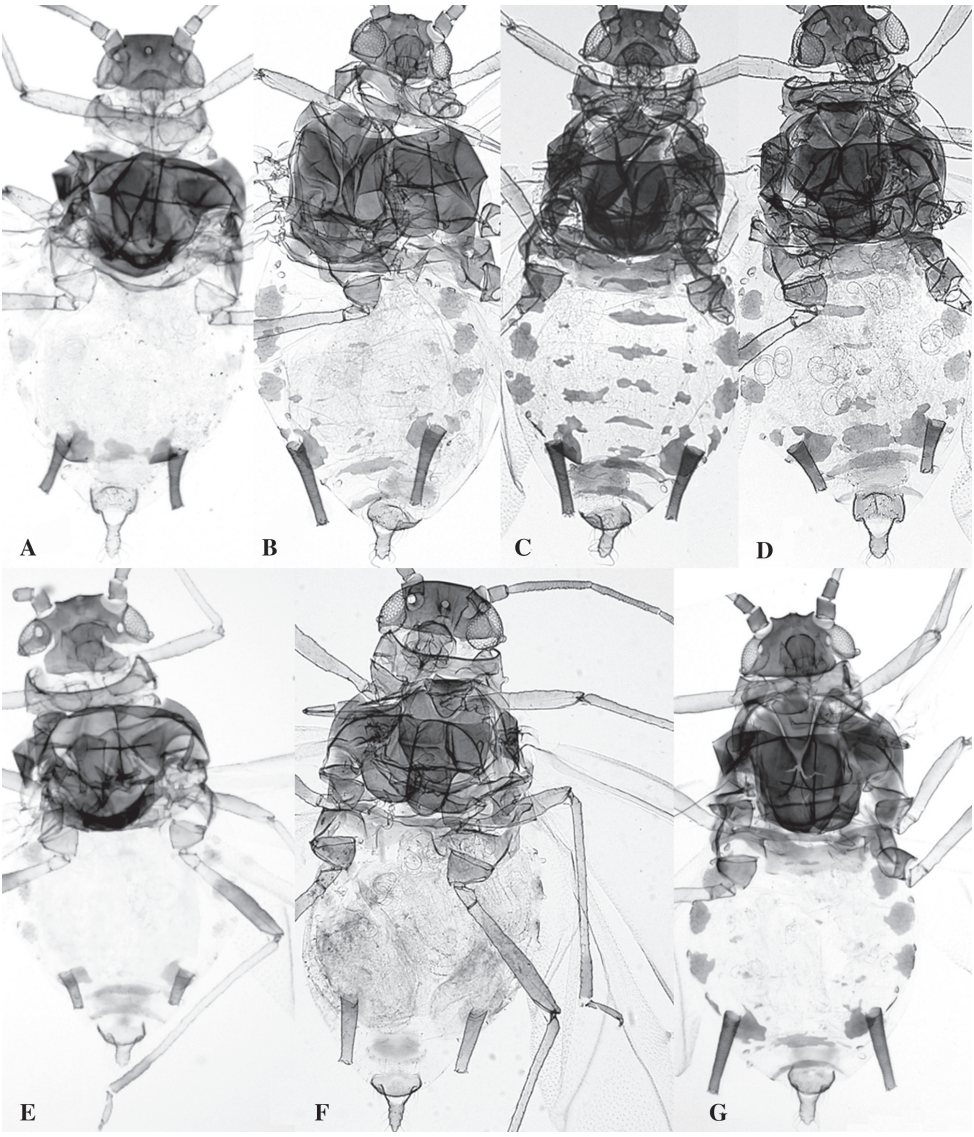

Figure 7. Body of alate viviparae. **A** *A. glycines* **B** *A. gossypii* **C** *A. gossypii* **D** *A. sedi* **E** *A. monardae* **F** *A. nasturtii* **G** *A. oestlundi*.

slightly dusky, tongue-shaped. Siphunculi dusky and dark distally, imbricated with flange. Marginal sclerites pale. Marginal tubercles only present on abdominal segments I and VII. Dorsal abdomen without sclerites. Pre and post-siphuncular sclerites. Abdominal tergite VIII with 2 setae. Subgenital plate complete, slightly dusky with 2-7 setae on anterior part. Cuticle with reticulation.

Alate viviparae (n= 59). *Color in life* (Figure 8B): Head and thorax brown. Abdomen green. *Color of cleared specimens* (Figure 7E): *Head*: dark. Antennal segments:

Figure 8. *Aphis* species of the *A. gossypii* complex. **A** Apterous vivipara of *A. gossypii* on *Rhamnus cathartica* **B** Nymphs, apterous and alate viviparae of *A. monardae* on *Monarda fistulosa* **C** Apterous ovipara of *A. monardae* **D** Nymphs and apterous male (brownish in the center of the image) of *A. monardae* **E** Nymphs and alate vivipara of *A. gossypii* on *Cucurbita pepo* **F** Nymphs and apterous vivipara of and *A. oestlundi* on *Oenothera biennis* **G** Apterous vivipara (top) and apterous ovipara (bottom) of *A. sedi* on *Hylotelephium telephium*.

first and second dark, the rest dusky. Secondary sensoria present on and III and IV. Arrangement of secondary sensoria in a single row on the distal half (Figure 6C). *Thorax:* All femora dusky except in the base. Hind coxa dark. Hind trochanters paler than coxa. Hind tibiae dark distally. *Abdomen:* Cauda pale or slightly dusky. The cauda parallel-sided with constriction near the base. Siphunculi dark throughout,imbricated with flange. Pre-siphuncular sclerites absent. Post-siphuncular sclerites dusky. Marginal sclerites pale. Marginal tubercles only present on abdominal segments I and VII. Dorsal abdomen with small transverse sclerites on VI, VII and VIII. Abdominal tergite VIII with 2 setae. Subgenital plate complete, slightly dusky, with 2-7 setae on anterior part. Cuticle without reticulation.

Oviparae (n= 26). *Color in life* (Figure 8C): *Head*: varies from light brownish to dark green. Antennal segments: first, second and ¾ of third pale yellowish, the rest dusky. *Thorax:* Coxae and trochanters pale or dusky. Fore femora dusky throughout, mid-femora dusky except at base, hind femora dark except at base. Tibiae dusky distally and tarsi dusky. *Abdomen*: Cauda dark green. Siphunculi lighter than dark green abdomen. *Color on slide and morphological characters* (Figure 4A): *Head*: Dusky without frontal setae. Antennal tubercle undeveloped. Antennae five-six segmented, shorter than body. Antennal segments: first, second, third and four pale, the rest dusky. Rostrum reaches mesocoxae. *Thorax:* Coxae and trochanters dusky. All femora dusky throughout. Tibiae and tarsi dusky throughout. *Abdomen*: Cauda dusky, parallel-sided with blunt tip and bearing 6-8 setae. Siphunculi pale, smooth with flange. Pre and post-siphuncular sclerites absent. Marginal tubercles only present on abdominal segments I and VII. Dorsum

of abdomen without sclerites. Abdominal tergite VIII with 4-8 setae. Subgenital plate dark, with 4-17 setae on anterior part. Cuticle without reticulation.

Alate male (n=17). *Color in life* (Figure 8D): *Head*: brownish. Antennae: blackish. *Thorax:* greenish. Legs light brown and tibiae distally dark as well as tarsi. *Abdomen*: Cauda dark green. Siphunculi lighter than dark green abdomen. *Color on slide and morphological characters* (Figure 4B): *Head*: dark. Antennae dark with secondary sensoria scattered on segments III, IV, and V. *Abdomen*: Cauda pale or dusky, parallel-sided with blunt tip and bearing 3-6 setae. Marginal tubercles present on abdominal segments I and VII. Dorsum of abdomen without large transverse sclerites. Male genitalia with 2 short claspers anteriorly and aedeagus centrally.

Comparison of *Aphis sedi* and *Aphis gossypii*

The distinction of *A. sedi* from *A. gossypii* is supported by phenotypic characters of specimens in collections included in Tables S1 and S2. In addition, morphological characters such as the ratio of the lengths of the processus terminalis and the base of the sixth antennal segment (Suppl. material 2, Figure 5B: F ratio=498.1, df=1, P<0.001) and the ratio of the lengths of the siphunculus and the cauda (Suppl. material 2, Figure 5C: F ratio=168.5, df=1, P<0.001) of apterous viviparae can be useful to discriminate these species. Interestingly, only oviparae of *A. sedi* reared on *Hylotelephium telephium* were collected under laboratory conditions (Figures 4C and 8G). In contrast with the morphological differences, the interspecific genetic divergences using COI and EF1-α sequences of *A. gossypii* and *A. sedi* are less than 1% (Tables 1 and 2). SCP showed greater genetic divergence between these two species, namely 0.84–1.84% (Table 2).

Comparison of *Aphis gossypii* with *Aphis forbesi*, *Aphis glycines* and *Aphis nasturtii*

These species are sometimes misidentified because they share some morphological characters on either apterous or alate morphs. Moreover, the pair-wise sequence divergences using COI sequences between *A. gossypii* and *A. forbesi* Weed, *A. glycines* and *A. nasturtii* are up to 5% (Table 1). Here we present some characters that can be useful for their discrimination. Apterous viviparae of *A. gossypii* can be differentiated from those of *A. forbesi* by the width of the marginal tubercles on abdominal segments I and VII (maximally 0.011 in *A. gossypii* and minimally 0.025 in *A. forbesi*; range for *A. gossypii* is given in Suppl. material 2), number of antennal segments and color pattern of siphunculi. Apterae of *A. gossypii* are differentiated from those of *A. glycines* by the shape of the cauda (Figures D-F, H) and the number of caudal setae, and from those of *A. nasturtii* by the absence of marginal tubercles on abdominal segments II and VI (Figure 4J). Alate viviparae of *Aphis gossypii* can be differentiated from those of *A. forbesi* by the number of secondary sensoria on III and the DBIII (Suppl. material 2), from those of *A. glycines* by the color of the hind coxae and marginal sclerites (Figure 7A) and from

those of *A. nasturtii* by the number of secondary sensoria on antennal segments III, IV and V, absence of marginal tubercles on abdominal segments II and VI, and shape of cauda (Figure 7F). More figures and morphological characters have been uploaded in Lagos et al. 2014a.

Dichotomous keys to apterous and alate viviparous females of the *Aphis gossypii* complex in the Midwest

Many dichotomous keys to subsets of *Aphis* have been written (Hottes and Frison 1931, Palmer 1952, Rojanavongse and Robinson 1977, Cook 1984, Stroyan 1984, Heie 1986, Brown 1989, García Prieto et al. 2005, Blackman and Eastop 2006) when morphological characters were not useful to discriminate between species, host plant associations have been used. Unfortunately, in the Midwest *A. gossypii* has been found on most of the host plants of other *Aphis* species included in this complex (*A. gossypii*, *A. monardae* stat. nov., *A. oestlundi* and *A. sedi*). The alternative key that we present below is based on specimens from collections made in the Midwest, and molecular data for specimens from these collections (Tables 1 and 2) supports our morphologically based identifications. Morphological data for these species is shown in Suppl. material 2. For some comparative morphometric data of European specimens of *A. gossypii* and *A. sedi* see Stroyan (1984), Heie (1986), Brown (1989) and García Prieto et al. (2005). The key is specific to Midwest collected specimens and may not be reliable in other geographic regions. It also demonstrates the difficulty of separating these closely related species using only morphological characters.

Key to apterous viviparae

1 Cauda pale, most often with constriction at midpoint, with 4–7 setae. Antennae five or six segmented. Siphunculi pale, distally dusky. Summer morphs. Polyphagous (Figure 4E) ..... ***A. gossypii***

– Cauda dusky or dark **3**

3 Siphunculi dark all throughout **4**

– Siphunculi dusky or lighter at the base **7**

4 Cauda constricted **5**

– Cauda not constricted **7**

5 Cauda spoon-shaped, distinctly constricted, with 4–7 setae. Ratios PT/B 2.6–4.1, SIPH/CA 1.3–2.5. Polyphagous (Figure 4D) ..... ***A. gossypii***

– Cauda slightly constricted **6**

6 Cauda slightly constricted at midpoint, with 4–5 setae. Ratios PT/B 2.0–2.7, SIPH/CA 1.5–2.2. On *Oenothera* spp. (Figure 4K) ..... ***A. oestlundi***

– Cauda elongate, parallel-sided, with acute tip and slight constriction at the base, and with 4–8 setae. Ratios PT/B 1.8–2.5, SIPH/CA 0.9–1.6. On

Hylotelephium spp. (and elsewhere recorded from *Sedum* spp. and some other Crassulaceae) (Figure 4G) ..... ***A. sedi***

7 Siphunculi lighter at the base, dusky distally. Cauda tongue-shaped, with 6–9 setae. Ratios PT/B 1.7–2.9, SIPH/CA 1.3–1.7. On *Monarda* spp. (Figure 4I) ..... ***A. monardae***

– Siphunculi dusky. Cauda tongue-shaped, with 4–7 setae. Ratios PT/B 2.6–4.1, SIPH/CA 1.3–2.5. Polyphagous (Figure 4F) ..... ***A. gossypii***

Key to alate viviparae

1 Cauda tongue-shaped, with 3–9 setae, without sclerites on dorsum abdominal segments I, II, and III. Secondary sensoria on antennal segment III (4–9), IV (0–3). DBIII 0.07–0.12 (Figure 6C). Ratios PT/B 1.9–3, SIPH/CA 1.1–1.8. (Figure 7E) ..... ***A. monardae***

– Cauda constricted, sometimes with sclerites on dorsum of abdominal segments I, II, and III **2**

2 Antenna VI PT/B 2.1–3.6. Secondary sensoria on antennal segment III (4–10) DBIII 0.04–0.07 (Figure 6B). Sometimes with transverse sclerites on dorsum of all abdominal segments (Figures 7B-C). Ratio SIPH/CA 1.1–2.3. Polyphagous ..... ***A. gossypii***

– Antenna VI PT/B 1.9–2.3. Secondary sensoria on antennal segment III (7–10) and IV (0–2) (Figure 6F). Sometimes with transverse sclerites on dorsum of all abdominal segments (Figure 7D). Ratio SIPH/CA 0.9–1.5. On *Hylotelephium* spp. ..... ***A. sedi***

– Antenna VI PT/B 2.2–2.9. Secondary sensoria on antennal segment III (2–8) (Figure 6E). Never with sclerites on dorsum of abdomen (Figure 7G). Ratio SIPH/CA 1.8–2.1. On *Oenothera* spp. ..... ***A. oestlundi***

Discussion

The analysis of different species included in this study largely corroborates the results obtained by Coeur d'acier et al. (2007), Kim and Lee (2008), Kim et al. (2010a), Kim et al. (2010b), Kim et al. (2011) and Lagos et al. (2014b). The *gossypii* complex in the North American Midwest contains the following native species, *A. oestlundi* and *A. monardae*, and the invasive species *A. gossypii* and *A. sedi*. Collection host records for *A. gossypii* show that it has been collected on *Oenothera* and *Monarda*, the host plants of the native *Aphis* species listed above (Blackman and Eastop 2006). Collection records for the native species suggest a very limited host range, in contrast with the highly polyphagous *A. gossypii*. Our results indicate that these species can be differentiated by morphological characters as well as host association. Data from this study confirms the finding of Lagos (2007) that *A. monardae* is a valid species and not a synonym of *A. gossypii* (Eastop and Hille Ris

Lambers 1976). The novel character (distance from the base of antennal segment III to its first secondary sensorium, DBIII) is useful to differentiate alate viviparae of *A. monardae* and *A. gossypii* when they are collected together in traps. The sexual morphs collected on *Monarda* under laboratory and field conditions indicate that *A. monardae* has a monoecious holocyclic life cycle. A neotype of *A. monardae* has to be designated according to the Article 75.3 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). Concomitant with the redescription of the species, we here designate a neotype of *A. monardae* from the state of Minnesota on *Monarda fistulosa*. Slides deposited by O.W. Oestlund in the Insect Collection of the University of Minnesota show collection data no earlier than 1896. However, the first description of *Aphis monardae* was published in 1887 and the original description specified neither a type nor the type locality (Oestlund 1887). The comparison of apterae, alatae and oviparae of Oestlund's collections match the morphological characters of those collected recently (Suppl. material 1). Some slides made by Oestlund were remounted in 1968 so it was possible to better see the characters. For a neotype we chose a more recently collected specimen taken in Minnesota as it more clearly shows color pattern and other characters used in the redescription.

The discrimination of *A. gossypii* and *A. sedi* is clear when the aphids are alive (Figure 8). The identification problem arises when we examine samples that have lost their color by being stored in ethanol. Molecular data also are helpful. The pair-wise sequences divergences between these species using SCP are higher than for COI and EF1-α sequences (Tables 1 and 2). This marker also successfully differentiated the cryptic species *A. gossypii* and *A. frangulae* (Carletto et al. 2009a). Results obtained in this study corroborate the biological and morphological findings of Kring (1955), who found that *A. sedi* is holocyclic monoecious on *Hylotelephium*. In this study, only apterous oviparae were collected under laboratory conditions conducive to the production of sexuales (Figure 4C). Kring's morphological observations showed that the ratio of the processus terminalis to the base of the last antennal segment (PT/B), and the ratio of the length of the siphunculus to the length of the cauda (SIPH/CA), are both greater in *A. gossypii* than *A. sedi* for all morphs (Suppl. material 2). Although the above characters are useful to differentiate these species, their identification (especially the alate viviparae) is still problematic because of their similar morphology and because these ratios overlap (Figures 5B–C).

The inclusion of *A. glycines*, *A. gossypii* and *A. nasturtii* in strongly supported clades (Clade A, Figures 1 and 2) is consistent with the findings of Foottit et al. (2008) but in disagreement with those of Kim et al. (2010a). Interestingly, these three invasive species share a winter host plant, *Rhamnus* spp., but this is not the only known overwintering host for *A. gossypii*. This indiscriminate behavior, in addition to multiple species sharing winter hosts, raises the possibility of interspecies hybridization (Müller 1986, Rakauskas 2003). Hybridization may or may not be successful but should be detectable in studies of gene flow and phenotypic characterization of putative hybrids.

The species regarded here as members of the *A. gossypii* complex, *A. gossypii*, *A. sedi*, *A. oestlundi* and *A. monardae* (Clade D), exhibit interesting biological, morphological

and molecular patterns. *Aphis gossypii* has been shown to colonize numerous secondary host plants including those of closely related taxa (Stroyan 1984, Heie 1986, Blackman and Eastop 2006). Moreover, it is one of the few *Aphis* species with multiple primary host plants (Blackman and Eastop 2006). By contrast, the native taxa related to it and found in the Midwest have or are presumed to have monoecious holocyclic life cycles (see Suppl. material 1 for host plant information). *Aphis oestlundi*, *A. monardae* and *A. sedi* have wingless males, a characteristic that would contribute to the genetic isolation of these species. These sibling species possess morphological characters useful for diagnostic purposes (Suppl. material 2) and the values that support interspecific sequences divergences (Table 2) are similar to those found by Foottit et al. (2008) and Favret and Miller (2011). The identification of species related to *A. gossypii* is made more difficult because they feed on host plants that can also serve as host to *A. gossypii*. Interestingly, however, their colors in life differ and can be useful for identification. For example, *A. gossypii* is dark green or light brownish and its siphunculi are dark throughout (Figures 8A, E), although this can vary in summer dwarf specimens. *Aphis gossypii* is mostly darker than *A. monardae*, which is light yellow or green (Figures 8B, C), and *A. oestlundi* is light yellow (Figure 8F). The color of *A. sedi* is dark green (Figure 8G), like *A. gossypii*, although it has more white wax on its body (García Prieto et al. 2005).

The COI sequence divergence values obtained in this study are similar to those obtained in other studies (Cocuzza et al. 2009, Cognato 2006, Coeur d'acier et al. 2007, Foottit et al. 2008, Favret and Miller 2011, Wang and Qiao 2009). Moreover, the low pair-wise sequence divergences found between some species such as *A. gossypii* and *A. sedi* (Table 1) are consistent with those obtained by other workers such as Piffaretti et al. (2012). While COI data have been found useful to discern the phylogenetic relationships of many taxa, the use of COI sequence divergences to set cut-off points that can differentiate *Aphis* species should be used with caution, since it may lead to the misidentification of new species, a conclusion drawn by other studies for several orders of insects (Blaxter 2004, Hebert et al. 2004, Nadler 2002, Will and Rubinoff 2004, Smith et al. 2008).

Our work suggests the possible existence of three undescribed Midwestern species (*Aphis* spp. 1, 2, and 3) within the *gossypii* complex. Further studies need to be done to validate their status. It is likely that additional new species will be found within this group as material is gathered from a larger geographical area and combined molecular, morphological and biological data are used to analyze the new taxa. The use of multiple primary hosts is unusual for any species, thus lineages within the *gossypii* complex that select and limit themselves to specific hosts may be driving the speciation process within this group (Peccoud et al. 2010, Kim et al. 2011).

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Supplementary material 1

Table S1. Collection information.

Authors: Doris M. Lagos, Colin Favret, Rosanna Giordano, David J. Voegtlin

Data type: species data

Explanation note: Collection information for specimens included in this study. INHS voucher and GenBank accession numbers are for specimens originating from a specific collection.

Supplementary material 2

Table S2. Morphological characters useful to discriminate *A. gossypii*, *A. monardae*, *A. oestlundi* and *A. sedi*.

Authors: Doris M. Lagos, Colin Favret, Rosanna Giordano, David J. Voegtlin

Data type: measurement data

Explanation note: Morphological characters useful to discriminate *A. gossypii*, *A. monardae, A. oestlundi* and *A. sedi*. For all measurements and counts the range is given and the mean is in parentheses. All measurements in mm. Abbreviations: B base of last antennal segment, CA cauda, DBIII: Distance from the base of antennal segment III to the first secondary sensorium, HT2 second hind tarsus, LHIII longest Hair on ant. segm. III, PT: Processus terminalis, SIPH siphunculi, URS ultimate rostral segment. Data of oviparae of *A. gossypii* from Kim et al. (2010a).

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Revision of the plant bug genus *Xenocylapidius* (Hemiptera, Heteroptera, Miridae, Cylapinae), with descriptions of five new species from Australia and New Caledonia

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Abstract

The genus *Xenocylapidius* Gorczyca, 1997 is revised. Five new species: *Xenocylapidius acutipennis* **sp. n.**, *X. ater* **sp. n.**, *X. bimaculatus* **sp. n.**, *X. gemellus* **sp. n.**, and *X. rolandi* **sp. n.** are described from Australia and New Caledonia. Illustrations of the male genitalia, color photographs of dorsal and lateral views of the adults of all species, and key to species of the genus *Xenocylapidius* are provided.

Keywords

Heteroptera, Miridae, Cylapinae, *Xenocylapidius*, new species, keys, Australian Region, Australia, New Caledonia

Introduction

With 75 species included in 28 genera (Schuh 2002–2013; Gorczyca 2006; Wolski 2012; Wolski and Gorczyca 2014) the Cylapinae in the Australian Region remain one of the most poorly known mirid subfamilies. Most of our knowledge about the Australian representatives of the Cylapinae is based on Carvalho and Lorenzato (1978), who reviewed the Papuan cylapines, Cassis et al. (2003), and Moulds and Cassis (2006), who provided revisionary treatments of the Australian species of Vaniini and the genus *Peritropis* Uhler, 1981, respectively.

The genus *Xenocylapidius* was described by Gorczyca (1997) to accommodate a new species *X. tamasi.* Subsequently, Gorczyca (1999) added two species – *X. australis* and *X. gressitti,* provided a redescription of the genus and type species and a key to species. Gorczyca (2006) transferred *Rhinomiridius bioculatus* Girault to *Xenocylapidius* and synonymized *X. australis* with this species.

In this contribution, we revise the genus *Xenocylapidius* and describe five new species. All previously known species are diagnosed, and identification key to species is provided.

Materials and methods

Observations were made using an Olympus SZX12 stereomicroscope and an Olympus BX50 optical microscope. Color pictures of the adults (Figs 1–15) were taken with an ALTRA 20 digital camera. Additional information on the pictured specimens is given in the species treatments.

Measurements were taken using an eyepiece (ocular) micrometer; all measurements are given in millimeters. The total body length is defined by the length from the apex of the clypeus to the posterior margin of the membrane, and the body width by the length between the lateral margins of the hemelytra at their widest point. Lengths and widths of the head are defined as follows: length, from the apex of the clypeus to the occipital carina; width, between the outer margins of each eye; diameter of eye, between the outer and inner margin of eye; length of the antennal and labial segments, between the base and apex. Lengths and widths of the pronotum are defined as follows: length, measured between the anterior and posterior margins; width of the anterior margin, between anterior angles; length of lateral margin, between the anterior and humeral angles; width of the posterior margin, between the humeral angles.

Dissections of male genitalia were done according to Kerzhner and Konstantinov (1999). The terminology of the male genitalic structures follows Konstantinov (2003) and Cassis (2008). The following additional terms for the elements of the endosoma are used in this paper:

AR – apical ring – apical portion of basal sac, composed of tiny spiculi and denticles, not forming a fully closed ring;

BP – basal plate – irregularly shaped, sclerotized plated situated at base of the endosoma;

BSC – basal sac – sclerotized sac situated at base of the endosoma, almost entirely embracing sclerotized portion of ductus seminis inside the endosoma (DSS);
DLS – dextrolateral sclerite – situated on the dextrolateral portion of the apical part of the endosoma;
DSS – sclerotized portion of ductus seminis inside endosoma;
MS – medial sclerite – situated at middle of the endosoma, with base localized near apex of DSS;
SLS – sinistrolateral sclerite – situated on the sinistrolateral portion of the apical part of the endosoma;
SP1, SP2, and SP3 – endosomal spiculi – bundles of spiculi situated basally, medially, and apically on the endosoma.

The material examined includes specimens borrowed from the institutions listed below:

BPBM Department of Entomology Collection, Bernice P. Bishop Museum, Honolulu, Hawaii, USA;
HNHM Hungarian Natural History Museum, Budapest, Hungary;
MNHN Museum National d'Histoire Naturelle, Paris, France;
NHRS Naturhistoriska Riksmuseet, Stockholm, Sweden;
US Department of Zoology, University of Silesia, Katowice, Poland;
USNM National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA;
ZSM Zoologische Staatssammlung München, Munich, Germany.

Taxonomy

Xenocylapidius Gorczyca

Xenocylapidius: Gorczyca 1997: 179, 183 (sp. n.), 1999: 16 (key to species), 2000: 49 (list), 2006: 70 (catalog); Chérot and Gorczyca 1999: 217 (note); Carpintero and Chérot 2014: 62 (note).

Diagnosis. Recognized by the following combination of characters: labial segment II subdivided medially or subapically; lateral margin of pronotum somewhat elevated; scent gland efferent system broad, occupying entire ventral margin of metepisternum; endosoma with a characteristic sclerotized basal sac with a relatively broad, ringlike structure apically (AR = apical ring) that is composed of numerous denticles and spiculi (Figs 16–17, 21–22, 26–27, 32–33, 37–38, 42–43); left paramere with a long, protruding sensory lobe (SL) (Figs 18–19, 23–24, 28–29, 34–35, 39–40, 44–45).

Redescription. STRUCTURE, TEXTURE, AND VESTITURE (Figs 1–15). Macropterous, elongate oval. ***Head***. Elongate horizontally, conical; antennal segment I gradually thickened toward apex, covered with sparse, short, adpressed setae and some-

times covered with a few bristlelike, protruding setae apically; segment II weakly broadened toward apex, covered with moderately dense, semirecumbent setae and sometimes with sparse, bristlelike, protruding setae on apical half; segments III and IV thin, with diameter about twice as thin as diameter of segment II, mixed with long, moderately dense, semirecumbent setae and with a few, bristlelike, protruding setae; labium thin, reaching medial part of abdomen or beyond; segment I subdivided near medial part, extending beyond base of head to anterior edge of xyphus; segment II subdivided subapically. ***Thorax***. *Pronotum*. Trapezoidal; collar present, thin; humeral angle usually furnished with single, bristlelike, rather long, protruding seta; calli moderately convex, broad, occupying anterior two thirds of pronotum; lateral margin usually strongly carinate and somewhat elevated, rarely weakly carinate and not elevated; posterior margin arcuate. *Mesoscutum and scutellum*. Mesoscutum well exposed; scutellum flattened or weakly convex. *Thoracic pleura*. Proepisternum and proepimeron shiny; remaining pleura matte; scent gland efferent system broad, occupying entire ventral margin of metepisternum. *Hemelytron*. Usually covered with very short, relatively dense, adpressed, black setae, rarely with sparse, relatively long, protruding setae; membrane with major cell nearly rectangular, minor cell clearly present. *Legs*. Relatively long; profemur usually with several protruding, thick, relatively long setae on inner surface; tarsus bisegmented; tarsomere II subdivided medially; pretarsal claw toothed subapically.

Male genitalia. *Aedeagus* (Figs 16–17, 21–22, 26–27, 32–33, 37–38, 42–43). Ductus seminis thin, with an outer wall fine and membranous; base of endosoma with a sclerotized sac (BSC), occupying one third to almost half of endosoma, enveloping sclerotized part of ductus seminis inside endosoma (DSS), with a large, not fully closed apical ring (AR) composed of tiny spiculi or/and denticles; secondary gonopore distinct; endosoma usually with 1–3 bundles of distinct spicules (SP1, SP2, and SP3); base of endosoma sometimes with an irregular, sclerotized plate (BP = basal plate); medial portion of endosoma often with a large sclerite (MS = medial sclerite); apical portion of endosoma with 1-2 large sclerites (dextrolateral sclerite = DLS and a sinistrolateral sclerite SLS). *Left paramere* (Figs 18–19, 23–24, 28–29, 34–35, 39–40, 44–45). Apical process: dorsal view: extreme apex strongly narrowed, usually rounded and weakly curved; paramere body: dorsal surface with bundle of thick, protruding setae; sensory lobe: convex and stout.

Remarks. *Xenocylapidius* is differentiated from other genera of Cylapinae primarily by the presence of the characteristic sclerotized sac at the base of endosoma (BS = basal sac) with the apical portion composed of numerous denticles and spiculi (AR = apical ring) surrounding well developed sclerotized part of ductus seminis inside endosoma (DSS) (Figs 16–17, 21–22, 26–27, 32–33, 37–38, 42–43) and by the large, stout sensory lobe (SL) of the left paramere (Figs 18–19, 23–24, 28–29, 34–35, 39–40, 44–45). In other Cylapinae the endosoma is usually furnished with more or less developed sclerotized part of the ductus seminis (DSS) (e.g. Carvalho and Fontes 1968; Carvalho and Lorenzato 1978; Cassis et al. 2003; Wolski 2010, 2013; Wolski and Henry 2012, Wolski and Gorczyca 2012, 2014) but it never is embraced by the basal sac (BS) as in *Xenocylapidius*.

Xenocylapidius is superficially similar to *Peritropis* Uhler, primarily in sharing elevated lateral margins of pronotum but can be easily distinguished by the shape of the male genitalia.

Key to species of *Xenocylapidius*

1 Hemelytron with mottled, brown to blackish and yellow to dirty yellow coloration (Figs 1, 4, 5, 6, 8) **2**

– Hemelytron uniformly blackish (Fig. 2) or blackish or chocolate with a white patch near base of corium and embolium (Figs 3, 7), color never mottled ... **6**

2 Metafemur brown to dark brown with large, yellow patches (Fig. 8); endosoma with basal sac entirely covered with small denticles (Fig. 43) ..... ***X. tamasi* Gorczyca**

– Metafemur uniformly dirty yellow to black (Figs 1, 4, 6); endosoma with basal sac without small denticles posteriorly (*X. acutipennis* and *X. gemellus*) (Figs 17, 33) **3**

3 Apical half of antennal segment II mixed with dense, fine, semirecumbent setae and sparse, protruding, bristlelike setae..... ***X. bioculatus* (Girault)**

– Apical half of antennal segment II with only fine, semirecumbent setae, without sparse, protruding, bristlelike setae **4**

4 Pronotal collar indistinct; yellow mottling on hemelytron composed of relatively small patches and spots (Fig. 6) ..... ***X. gressitti* Gorczyca**

– Pronotal collar well developed; yellow mottling on hemelytron composed of large patches (Figs 1, 5)..... **5**

5 Antennal segment II dark brown (Fig. 9); endosoma with two apical sclerites (DLS and SLS); medial sclerite (MS) long, weakly curved, tapering toward apex, sharply pointed (Fig. 16) .. ***X. acutipennis* Wolski & Gorczyca, sp. n.**

– Antennal segment II brownish yellow; endosoma with only one apical sclerite (SLS); medial sclerite (MS) with basal one third nearly rounded, apical two thirds tapering toward apex, sharply pointed apically (Fig. 32) ..... ***X. gemellus* Wolski & Gorczyca, sp. n.**

6 Hemelytron entirely black (Fig. 2)..... ***X. ater* Wolski & Gorczyca, sp. n.**

– Hemelytron chocolate brown or black, with a large, white patch near base of corium (Figs 3, 7) **7**

7 Hemelytron chocolate brown with a large, white patch near base of corium and with a small white patch on embolium apically (Fig. 3) ..... ***X. bimaculatus* Wolski & Gorczyca, sp. n.**

– Hemelytron black with a large, white patch near base of corium and with a large, white patch on apex of embolium, apicolateral surface of corium, and medial portion of inner margin of cuneus (Fig. 7) ..... ***X. rolandi* Wolski & Gorczyca, sp. n.**

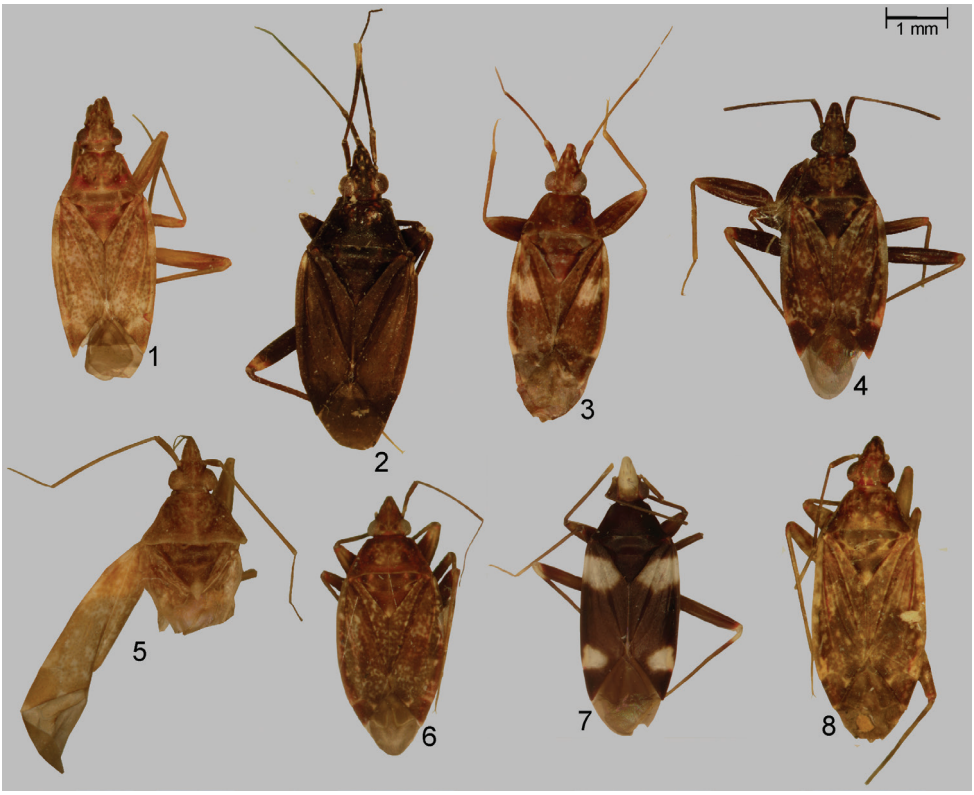
***Xenocylapidius acutipennis* Wolski & Gorczyca, sp. n.**
http://zoobank.org/C6849117-A190-43E2-8017-34C5A33D03F4
Figures 1, 9, 16–20, 31

Diagnosis. Recognized by the dorsum mottled with brownish yellow (Fig. 1); the dark brown antennal segment II; the endosoma with two bundles of spiculi (SP1 and SP2); the medial sclerite (MS) long, weakly curved, tapering toward apex, sharply pointed; the sinistrolateral sclerite (SLS) large, occupying almost half of endosoma, strongly broadened basally, constricted medially; the clublike dextrolateral sclerite (DLS) (Fig. 16); and the right paramere sickle-shaped (Fig. 20).

Most similar to *X. gemellus* in sharing the brownish yellow mottling on dorsum (Figs 1, 5), the rounded extreme apex of apical process of left paramere when viewed dorsally (Figs 19, 35), and the sickle-shaped right paramere. This new species can, however, be distinguished by the dark brownish antennal segment and shape of the endosoma (Figs 16).

Description. *Male*. **COLORATION** (Figs 1, 9). Dorsum mostly with mottled, brownish yellow coloration. ***Head***. Vertex and frons mottled with dark brown and yellow; remainder of head dark red with yellow mottling; antennal segment I dirty yellow, with an indistinct, dark yellow tinge basally and with a reddish tinge occupying apical one third of inner surface; segment II dark brown; labium dark brown with indistinct, dirty yellow areas. ***Thorax***. *Pronotum*. Collar yellow; calli dark brown, with broad, yellowish mottling; anterior margin weakly tinged with red medially; lateral margin and posterior lobe dark brown, tinged with red and dirty yellow; humeral angle and medial portion of posterior margin yellow. *Mesoscutum and scutellum*. Mostly reddish; mesoscutum weakly tinged with dark brown medially and with dirty yellow area bordering portion being depressed onto lateral margin; scutellum reddish with dirty yellow patch apically. *Thoracic pleura*. Proepimeron and proepisternum mostly dark brown with reddish areas; remaining pleura reddish, with indistinct yellowish areas. *Hemelytron*. Corium and clavus dark brown, mottled with yellow; cuneus dark brown, weakly tinged with red, inner angle yellow, apex with a small, dirty yellow patch; membrane fuscous with indistinct, dirty yellow areas. *Legs*. Procoxa dark brown, dirty yellow apically; meso- and metacoxae yellow; femora dirty yellow brown with reddish areas; tibiae dark brown; tarsi dirty yellow brown. ***Abdomen***. Dark brown with large dirty yellow areas. **STRUCTURE, TEXTURE, AND VESTITURE** (Figs 1, 9). ***Head***. Antennal segment II weakly broadened toward apex, covered with moderately dense, adpressed and semirecumbent setae, sparse on basal one-fifth of segment II and dense on remainder of segment. ***Thorax***. *Pronotum*. Lateral margins sharply carinate, somewhat elevated. *Mesoscutum and scutellum*. Scutellum weakly convex. *Hemelytron*. Covered with short, relatively dense, adpressed, black setae.

Male genitalia. *Aedeagus* (Figs 16–17). Basal sac (BSC) occupying one third of endosoma; sclerotized portion of ductus seminis inside endosoma (DSS) ovoid; secondary gonopore nearly circular, not fully closed; basal plate (BP) irregular in shape; apex of endosoma with two bundles of spiculi (SP1 and SP2); medial sclerite (MS)



Figures 1–8. Dorsal habitus color photographs of *Xenocylapidius* spp.: **1** *X. acutipennis* (holotype) **2** *X. ater* (holotype) **3** *X. bimaculatus* (holotype) **4** *X. bioculatus* (♀: Australia N. S. W., Manly nr Sydney, North Head 16–21.2., D. Shcherbakov 1997) **5** *X. gemellus* (holotype) **6** *X. gressitti* (holotype) **7** *X. rolandi* (holotype) **8** *X. tamasi* (♀: New Caledonia, Foret di Thi, 29.X.–1.XI.1967).

long, weakly curved, tapering toward apex, sharply pointed; sinistrolateral sclerite (SLS) large, occupying almost half of endosoma, strongly broadened basally, constricted medially, and broadened, nearly cylindrical on apical half; dextrolateral sclerite (DLS) somewhat smaller than SLS, clublike. *Left paramere* (Figs 18–19). Apical process: lateral view: weakly tapering toward apex, obtuse apically; dorsal view: lateral margins weakly curved, extreme apex rounded; paramere body: dorsal view: weakly broadened toward apex; sensory lobe: massive, just slightly tapering toward apex, obtuse. *Right paramere* (Fig. 20). Sickle-shaped; apical process: long, thin, arcuate, just slightly narrowed toward apex; paramere body: thin, dorsal margin straight, ventral margin weakly arcuate.

Measurements. ♀/♂ (n=2, holotype measurements second). *Body*. Length 6.00/4.70, width 2.15/1.76. *Head*. Length 1.00/0.98, width 0.85/0.79, interocular distance 0.35/0.35. *Antenna*. Length of segment I 0.74/0.64, II 1.83/1.83 (III and IV missing in both specimens). *Labium*. Length of segment I 0.98/0.95 (II, III, and IV immeasurable in both specimens). *Pronotum*. Length 0.85/0.73, width of ante-

rior margin 0.68/0.65, length of lateral margin 0.98/0.80, width of posterior margin 1.60/1.38.

Female. Similar to male in coloration, structure, texture, and vestiture.

Biology. Unknown.

Distribution. Australia (Queensland) (Fig. 31).

Etymology. The specific name is derived from the Latin "acutus", meaning sharpened, and is used to denote the sharply pointed mesial process (MS) of the endosoma.

Type material. Holotype ♂: Malanda; Queensl[and] Mjöberg; Swedish Museum of Natural History Stockholm NHRS (NHRS); paratype 1 ♀: Glen Lamington Queensl[and] Mjöberg; Swedish Museum of Natural History Stockholm NHRS (NHRS).

Xenocylapidius ater Wolski & Gorczyca, sp. n.

http://zoobank.org/DF204786-7FCE-4940-B323-99FA280CCF46

Figures 2, 10, 21–25, 31

Diagnosis. Recognized by the black dorsal coloration (Fig. 2); the antennal segment II with a yellow annulation apically (Figs 2, 10); the endosomal dextrolateral sclerite (DLS) nearly square on basal one third and triangular on apical two thirds (Fig. 21); the extreme apex of apical process of left paramere when viewed dorsally nearly conelike (Fig. 24); the sensory lobe (SL) of left paramere long, weakly arcuate in dorsal view (Fig. 24); and the right paramere with an apical process broadened with a narrow, nearly conelike process apically (Fig. 25).

Most similar to *X. rolandi* in sharing blackish dorsal coloration (Figs 2, 7). *Xenocylapidius ater* can, however, be easily distinguished by the lack of large white patches on hemelytron (Fig. 2), the coloration of antennal segment II (Figs 2, 10), and the shape of the male genitalia (Figs 21–25).

Description. *Male*. **COLORATION** (Figs 2, 10). Dorsum mostly blackish with small yellow and dirty yellow areas. ***Head***. Black with yellowish patches; vertex with two yellow patches each situated behind each eye and with additional two longitudinal, yellow patches, each bordering inner margin of each eye, vertex also with a small yellow patch medioapically; frons with two groups of several small, yellowish patches, each situated laterally, near inner margin of eye, frons also with a small, yellow patch medioapically, nearly bordering base of clypeus; clypeus with a short, longitudinal, yellow patch basally; mandibular plate with two small, yellow patches basally, each bordering base of clypeus; mandibular plate also with a yellow line along entire length of ventral margin; ventral surface of maxillary plate and dorsal surface of gula, bordering maxillary plate with a relatively large, yellow patch; gula with a relatively large, yellow patch bordering ventral margin of eye; antenna black except for contrasting yellow annulation at apical one fifth of antennal segment II; labium black with an indistinct, dirty yellowish annulation medially. ***Thorax***. *Pronotum*. Black with a broad, dirty yellow mottling on pronotal calli. *Mesoscutum and scutellum*. Black. *Thoracic pleura*. Blackish. *Hemelytron*. Blackish; base of embolium with a small, yellow patch. *Legs*.

Figures 9–15. Color photographs of *Xenocylapidius* spp., lateral views: **9** *X. acutipennis* (holotype) **10** *X. ater* (holotype); 11. *X. bimaculatus* (holotype) **12** *X. bioculatus* (♀: Australia N. S. W., Manly nr Sydney, North Head 16–21.2., D. Shcherbakov 1997) **13** *X. gressitti* (holotype) **14** *X. rolandi* (holotype) **15** *X. tamasi* (♀: New Caledonia, Foret di Thi, 29.X.–1.XI.1967).

Pro- and mesocoxae black; metacoxa dirty yellow; femora black; mesofemur with a small, dirty yellow patch subapically; metafemur with relatively broad, yellow annulation subapically; tarsi dirty yellow. ***Abdomen***. Blackish with indistinct, dirty yellowish areas. **STRUCTURE, TEXTURE, AND VESTITURE** (Figs 2, 10). Antennal segment II weakly broadened toward apex, covered with moderately dense, adpressed and semirecumbent setae, sparse on basal one-fifth of segment II and dense on remainder of segment. ***Thorax***. *Pronotum*. Lateral margins sharply carinate, somewhat elevated. *Mesoscutum and scutellum*. Scutellum weakly convex. *Hemelytron*. Covered with very short, relatively dense, adpressed, black setae.

Male genitalia. *Aedeagus* (Figs 21–22). Basal sac (BSC) occupying one third of endosoma; sclerotized portion of ductus seminis inside endosoma (DSS) stout, with sinuate margins; basal plate (BSC) nearly cylindrical, thin, and sinuate at basal two thirds, nearly rectangular at apical one third; dextrolateral sclerite (DLS) nearly square on basal one third and triangular on apical two thirds. *Left paramere* (Figs 23–24). Apical process: lateral view: nearly cylindrical, weakly constricted medially; dorsal view: weakly tapering toward apex; extreme apex nearly conelike; paramere body: lateral view: dorsal surface covered with dense, long, protruding setae; dorsal view: sensory lobe: long, weakly arcuate. *Right paramere* (Fig. 25). Apical process: broadened with a narrow, nearly conelike process apically; paramere body weakly arcuate, covered with sparse, long, protruding setae.

Measurements. Holotype ♂: *Body*. Length 5.3, width 2.15. *Head*. Length 1.0, width 0.88, interocular distance 0.35. *Antenna*. Length of segment I 0.71, II 1.82, III 0.62, IV (missing). *Labium*. Length of segment I 0.87, II 1.43, III 0.85, IV 0.7. *Pronotum*. Length 0.82, width of anterior margin 0.75, length of lateral margin 1.00, width of posterior margin 1.75.

Female. Unknown.

Biology. Unknown.

Distribution. Australia (Western Australia) (Fig. 31).

Etymology. The specific name is derived from the Latin "ater", meaning black, and is used to denote the blackish dorsal coloration.

Type material. Holotype ♂: Australia, WA 06/85, 30 km nnw. Leonora 28.61799S, 121.19967E, 441 m, 30.1.2006, M. Baehr (ZSM).

Xenocylapidius bimaculatus Wolski & Gorczyca, sp. n.

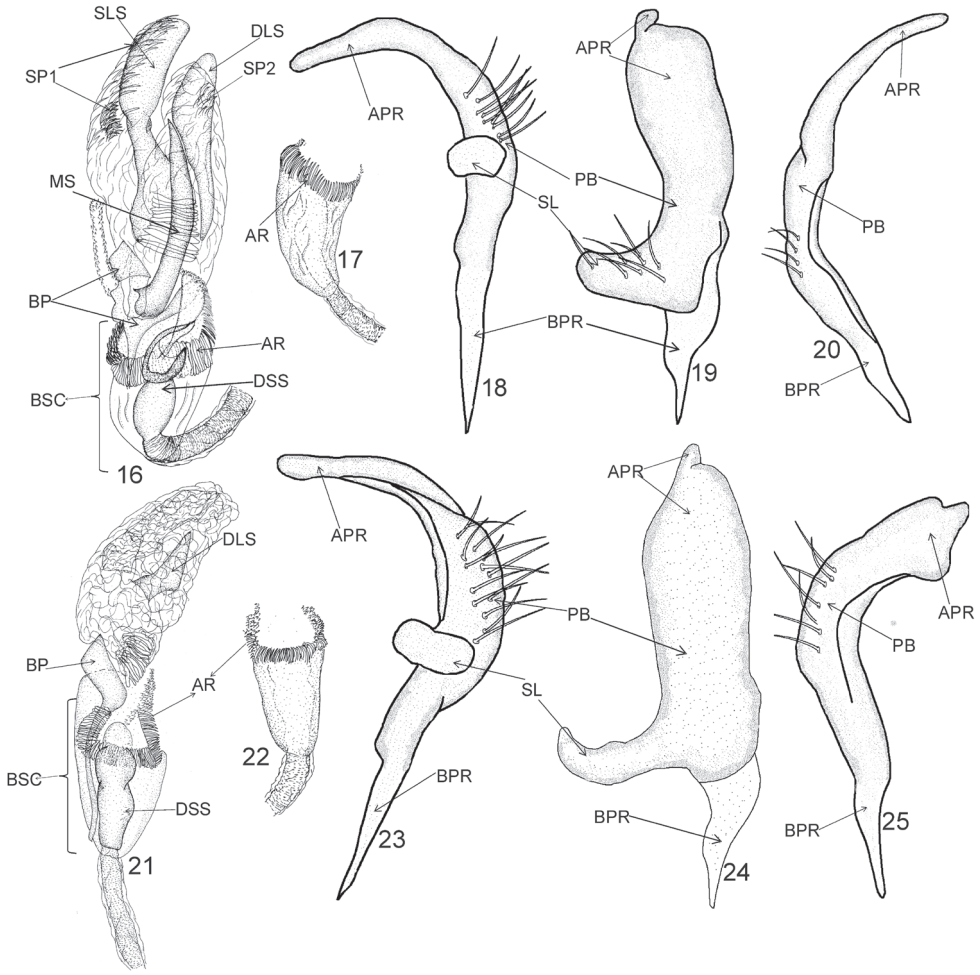
http://zoobank.org/D2ED9060-36D9-4C86-A273-33EB7D120100

Figures 3, 12, 26–31

Diagnosis. Recognized by the chocolate brown dorsum with two large whitish patches, each situated near base of the hemelytron (Fig. 3); the medial sclerite (MS) stout, large, occupying almost half of endosoma, tapering toward apex, sharply pointed (Fig. 26); the extreme apex of apical process of the left paramere weakly arcuate, nearly conelike (Fig. 24); the right paramere with an apical process ovoid, with a basal, small, obtuse process dextrolaterally and paramere body rather thin, nearly cylindrical, and very weakly arcuate at apical half, strongly broadened at basal half (Fig. 30).

Most similar to *X. rolandi* in sharing a large, pale patch near base of hemelytron (Figs 3, 7). The present new species can, however, be distinguished by the chocolate brown dorsum (Fig. 3) and the shape of the male genitalia (Figs 26-30).

Description. *Female*. **COLORATION** (Figs 3, 11). Dorsum chocolate brown, with yellow areas. ***Head***. Chocolate brown with whitish areas; posterior margin of vertex with two indistinct, dirty yellow patches, each situated mediolaterally, vertex also with two longitudinal, yellowish patches, each bordering inner margin of each eye and with a longitudinal, yellow stripe medially; frons with two yellow patches, each situated laterally and with yellow patch medioapically, bordering clypeus; clypeus with a short, longitudinal, yellow patch basally; mandibular plate with two small, yellow patches basally, each bordering base of clypeus, mandibular plate also with a yellow line along entire length of ventral margin; gula with relatively large, yellow patch bordering ventral margin of eye; antennal segment I chocolate brown with a yellowish annulation near base; segment II dirty yellow to brown, apical one third dark brown; segments III and IV dark brown; labium yellow, with fuscous areas. ***Thorax***. *Pronotum*. Chocolate brown, with indistinct yellow mottling on anterior half of calli and with indistinct yellow stripe medially, originating from middle of pronotal calli and ending at posterior margin. *Mesoscutum and scutellum*. Chocolate brown with a pale patch apically. *Thoracic pleura*. Chocolate brown. *Hemelytron*. Chocolate brown with indistinct yellowish shades and more or less developed whitish areas; embolium with a small whitish patch basally and apically; corium and embolium with a large, whitish patch near base; cuneus with a small yellow patch apically; membrane chocolate brown, membrane venation whitish. *Legs*. Procoxa chocolate; meso- and metacoxae



Figures 16–25. Male genitalia of *X. acutipennis* (**16–20**) and *X. ater* (**21–25**): **16, 21** Endosoma (dorsal view) **17, 22** Basal sac of endosoma (ventral view) **18, 23** Left paramere (left lateral view) **19, 24** Left paramere (dorsal view) **20, 25** Right paramere (right lateral view). APR = apical process of paramere; AR = apical ring of endosomal basal sac; BP = basal plate; BPR = basal process of paramere; BSC = basal sac; DLS = dextrolateral sclerite; DSS = sclerotized portion of ductus seminis inside endosoma; MS = medial sclerite; PB = paramere body; SL = sensory lobe; SLS = sinistrolateral sclerite; SP1 and SP2 = endosomal spiculi.

yellow; profemur chocolate brown; protibia brownish; protarsus dirty yellow. ***Abdomen***. Brown with yellow areas. **STRUCTURE, TEXTURE, AND VESTITURE** (Figs 3, 11). ***Head***. Antennal segment II weakly broadened toward apex, covered sparse, adpressed setae, sparse on basal one-fifth of segment II and dense on remainder of segment. ***Thorax***. *Pronotum*. Lateral margins sharply carinate, somewhat elevated. *Mesoscutum and scutellum*. Scutellum weakly convex. *Hemelytron*. Covered with short, relatively dense, adpressed, black setae.

Male. Similar to female in coloration, structure, texture, and vestiture.

Male genitalia. *Aedeagus* (Figs 26–27). Basal sac (BSC) occupying one third of endosoma; apex of endosoma with a single bundle of spiculi (SP1); medial sclerite (MS) stout, large, occupying almost half of endosoma, tapering toward apex, sharply pointed. *Left paramere* (Figs 28–29). Apical process: lateral view: broadened basally, cylindrical at apical two-thirds, obtuse; dorsal view: lateral margins weakly sinuate; extreme apex weakly arcuate, nearly conelike; sensory lobe: smassive, just slightly arcuate, obtuse. *Right paramere* (Fig. 30). Apical process: ovoid, with a basal, small, obtuse process dextrolaterally; paramere body: rather thin, nearly cylindrical, and very weakly arcuate at apical half, strongly broadened at basal half, covered with a few long, protruding setae sinistrolaterally.

Measurements. ♀/♂ (n=3, holotype measurements in parentheses). *Body*. Length 4.30–4.70/4.00 (4.70), width 1.65–1.75/1.65 (1.75). *Head*. Length 0.70–0.82/0.88 (0.82), width 0.70–0.73/0.70 (0.73), interocular distance 0.32–0.33/0.30 (0.32). *Antenna*. Length of segment I 0.44–0.50/0.45 (0.50), II 1.20–1.35/1.25 (1.35), III 0.60–0.65/0.63 (0.65) (IV missing in examined specimens). *Labium*. I (holotype) 0.80 (remaining segments immeasurable in examined specimens). *Pronotum*. Length 0.65–0.68/0.65 (0.68), width of anterior margin 0.63–0.65/0.58 (0.65), length of lateral margin 0.73–0.75/0.78 (0.75), width of posterior margin 1.30–1.38/1.33 (1.38).

Biology. Unknown.

Distribution. Australia (South Australia) (Fig. 31).

Etymology. The specific name is derived from the Latin "bi", meaning two, and "macula", meaning spot, and is used to denote the presence of two large dorsal patches, each situated near base of each hemelytron.

Type material. Holotype ♀: Australien 78, Wilpena Pound, Flinders Range, SA, 25.12.1972, M. Baehr (ZSM). Paratypes 1 ♀ and 1 ♂: same data as for holotype (ZSM).

Xenocylapidius bioculatus (Girault)

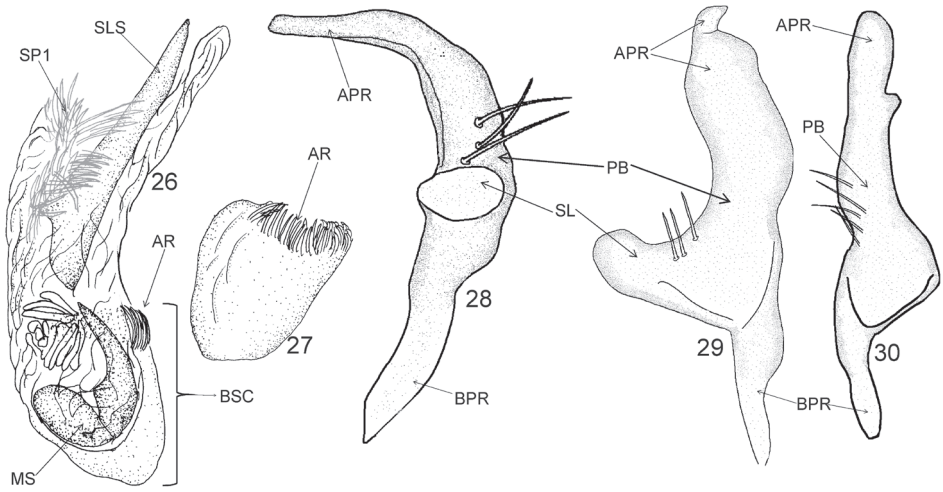
Figures 4, 11, 31

Rhinomiridius bioculatus Girault 1934: l (sp. n.); Carvalho 1957: 24 (catalog), 1974: 43 (list of types of species described by Girault); Cassis and Gross 1995: 150 (list); Schuh 1995: 36 (catalog); Gorczyca and Chérot 1998: 24 (note).

Xenocylapidius australis Gorczyca, 1999: 16, 17, Fig. 2 (sp. n.), (synonymized by Gorczyca 2006) (BPBM).

Diagnosis. Recognized by the following set of characters: dorsum with a mottled, blackish yellow coloration (Fig. 4); apical half of antennal segment II with dense, fine, semirecumbent setae and with sparse, protruding, bristlelike setae; femora entirely blackish (Fig. 4).

Most similar to *X. acutipennis*, *X. gemellus*, *X gressitti*, and *X. tamasi* in sharing mottled dorsal coloration (Figs 1, 4, 5–6, 8). *Xenocylapidius bioculatus* can, however, be distinguished by the presence of bristlelike setae on the antennal segment II and the uniformly black coloration of femora (Fig. 4)



Figures 26–30. Male genitalia of *X. bimaculatus*: **26** Endosoma (dorsal view) **27** Basal sac of endosoma (ventral view) **28** Left paramere (left lateral view) **29** Left paramere (dorsal view) **30** Right paramere (right lateral view). APR = apical process of paramere; AR = apical ring of endosomal basal sac; BPR = basal process of paramere; BSC = basal sac; DSS = sclerotized portion of ductus seminis inside endosoma; PB = paramere body; SL = sensory lobe; SLS = sinistrolateral sclerite; SP1 = endosomal spiculi.

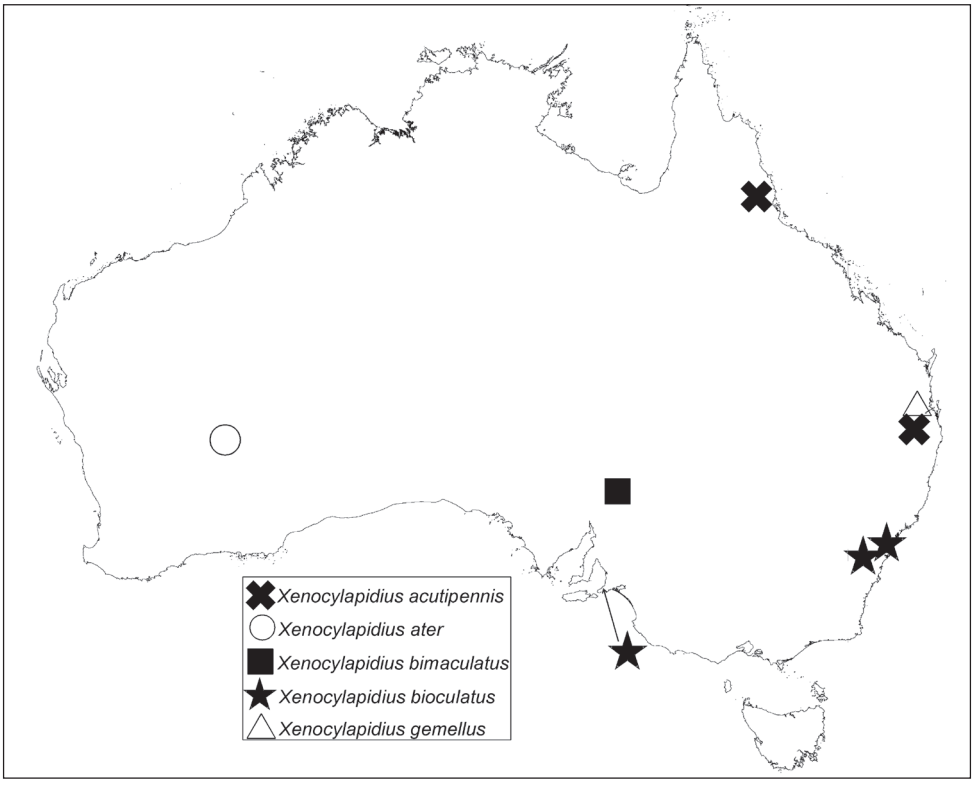


Figure 31. Distribution map of *Xenocylapidius* spp.

Biology. Unknown.

Distribution. Australia (New South Wales, South Australia) (Fig. 31).

Examined material. Holotype of *X. australis* ♀: Australia N. S. W., Manly nr Sydney, North Head 16-21.2., D. Shcherbakov 1997 (US); 1?: Mt. Gibraltar National Park, N.S.W., 24 Feb 1965, D.K. McAlpine; Eeastern scarp, c. 3000 ft.; Carvalho to Drake Coll 1993 (USNM).

Xenocylapidius gemellus Wolski & Gorczyca, sp. n.

http://zoobank.org/DFE7AE29-2127-47BD-A66F-ED2B6D9822AC

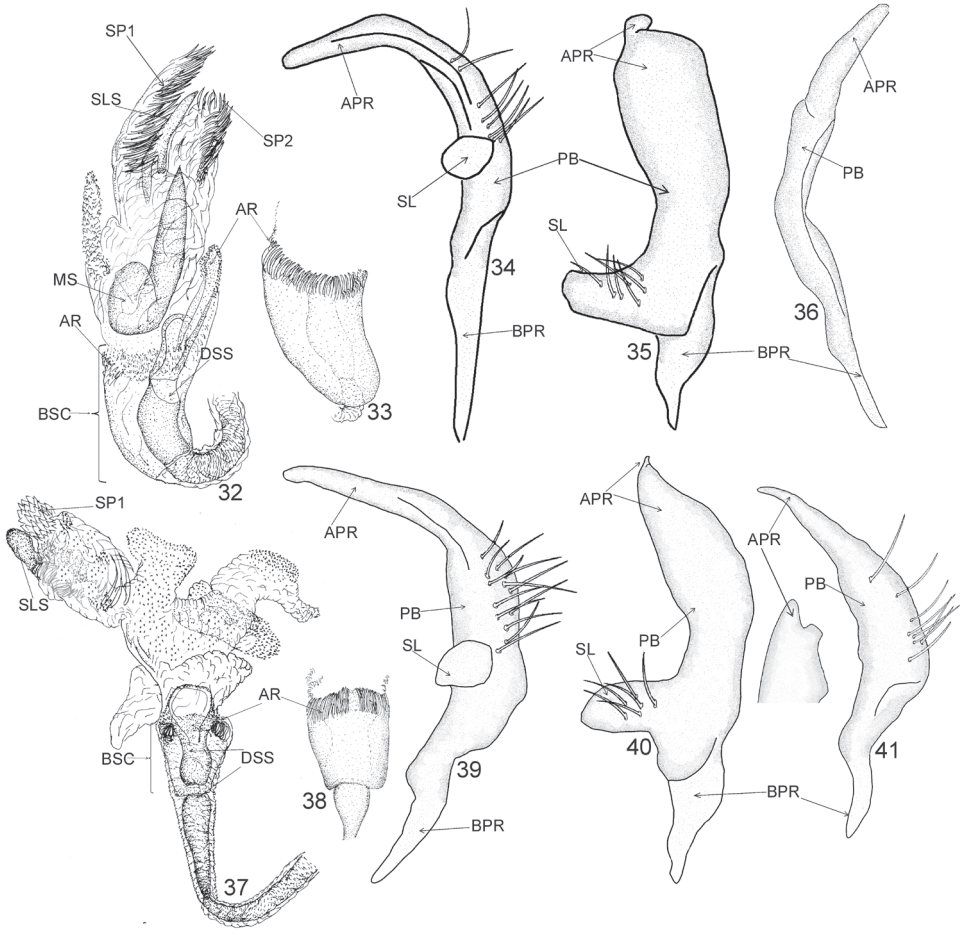
Figures 5, 31–36

Diagnosis. Recognized by the mottled, brownish yellow coloration (Fig. 5); the dirty yellow antennal segment II (Fig. 5); the medial sclerite (MS) stout, occupying more than one third of endosoma, basal one third nearly rounded, apical two thirds tapering toward apex, sharply pointed apically; the endosomal sinistrolateral sclerite (SLS) relatively small, occupying one fourth of endosoma, bifurcate at basal one third, remainder of sclerite cylindrical, somewhat narrowed apically (Fig. 32); the extreme apex of apical process of left paramere rounded in dorsal view (Fig. 35); and the right paramere sickle-shaped (Fig. 36).

Most similar to *X. acutipennis* in sharing a brownish yellow mottling on dorsum (Figs 1, 5), the rounded extreme apex of apical process of the left paramere when viewed dorsally (Figs 19, 35), and sickle-shaped right paramere. This new species can, however, be distinguished by the dark dirty yellow antennal segment (Fig. 5) and the shape of the endosoma (Figs 32).

Description. *Male*. **COLORATION** (Fig. 5). Dorsum dark brown with dirty yellow and whitish areas. ***Head***. Dark brown dirty yellow; antenna dirty yellow; labium yellowish. ***Thorax***. *Pronotum*. Dark brown dirty yellow. *Mesoscutum and scutellum*. Dark brown with a whitish patch apically. *Thoracic pleura*. Dark brown with brown and dirty yellow areas. *Hemelytron*. Brown, mottled with yellow; membrane grey, venation dirty yellowish white. *Legs*. Procoxa dark brown; meso- and metacoxa dirty yellowish; pro- and mesofemur dark brownish; remaining segments of pro- and mesoleg dirty yellow. ***Abdomen***. Dirty yellow. **STRUCTURE, TEXTURE, AND VESTITURE** (Fig. 5). ***Head***. Antennal segment II weakly broadened toward apex, covered with moderately dense, semirecumbent setae, sparse on basal one-fifth of segment II and dense on remainder of segment. ***Thorax***. *Pronotum*. Lateral margins sharply carinate, somewhat elevated. *Mesoscutum and scutellum*. Scutellum weakly convex. *Hemelytron*. Covered with short, relatively dense, adpressed, black setae.

Male genitalia. *Aedeagus* (Figs 32–33). Basal sac occupying one third of endosoma, apical ring (AR) extended into long, irregular, apically broadened and serrate sclerite dextrolaterally; sclerotized portion of ductus seminis inside endosoma (DSS) arcuate, nearly cylindrical at basal two-thirds, apically extended into an irregular, nearly ovoid plate; apical one third of endosoma with two bundles of spiculi (SP1 and



Figures 32–41. Male genitalia of *X. gemellus* (**32–36**) and *X. rolandi* (**37–41**): **32, 37** Endosoma (dorsal view) **33, 38** Basal sac of endosoma (ventral view) **34, 39** Left paramere (left lateral view) **35, 40** Left paramere (dorsal view) **36, 41** Right paramere (right lateral view). APR = apical process of paramere; AR = apical ring of endosomal basal sac; BPR = basal process of paramere; BSC = basal sac; DSS = sclerotized portion of ductus seminis inside endosoma; MS = medial sclerite; PB = paramere body; SL = sensory lobe; SLS = sinistrolateral sclerite; SP1 and SP2 = endosomal spiculi.

SP2); medial sclerite (MS) stout, occupying more than one third of endosoma, basal one third nearly rounded, apical two thirds tapering toward apex, sharply pointed apically; sinistrolateral sclerite (SLS) relatively small, occupying one fourth of endosoma, bifurcate at basal one third, remainder of sclerite cylindrical, somewhat narrowed apically. *Left paramere* (Figs 34-35). Apical process: lateral view: broadened and weakly arcuate basally, slightly tapering toward apex, obtuse apically; dorsal view: lateral margins weakly arcuate, extreme apex rounded; sensory lobe: stout, obtuse apically. *Right paramere* (Fig. 36). Sickle-shaped; apical process: relatively long, weakly curved and slightly tapering toward apex; paramere body: thin, arcuate.

Measurements. Holotype ♂: *Body*. Length 5.50, width 2.00. *Head*. Length 0.88, width 0.77, interocular distance 0.33. *Antenna*. Length of segment I 0.75, II 1.8, III 0.75, IV (partly broken). *Labium*. Immeasurable in specimen examined. *Pronotum*. Length 0.83, width of anterior margin 0.68, length of lateral margin 0.90, width of posterior margin 1.70.

*Female***.** Unknown.

Biology. Unknown.

Distribution. Australia (Queensland) (Fig. 31).

Etymology. The specific name is derived from the Latin "gemellus", meaning twin, and is used to denote the similarity of this species to *X. acutipennis*.

Type material. Holotype ♂: QUEENSLAND, Cedar Creek, Mars 1910, E. Mjöberg (NHRS).

Xenocylapidius gressitti Gorczyca

Figures 6, 13, 48

Xenocylapidius gressitti Gorczyca 1999: 16, 19, Fig. 3 (sp. n.), 2006: 70 (catalog).

Diagnosis. Recognized by the following set of characters: dorsum with a mottled, dark brownish yellow coloration (Fig. 6); pronotal collar indistinct; femora entirely blackish, except for pale yellow annulation at basal one third of mesofemur.

Most similar to *X. acutipennis*, *X. bioculatus*, *X. gemellus*, and *X. tamasi* in sharing mottled dorsal coloration (Figs 1, 4, 5–6, 8). *Xenocylapidius gressitti* can, however, be distinguished by the coloration of femora.

Biology. Unknown.

Distribution. New Caledonia (North Province) (Fig. 48).

Type material. Holotype ♀: New Caledonia, Col des Roussettes, 450-550 m, 4–6. II.63; J. L. Gressitt Collector (**BPBM**).

Xenocylapidius rolandi Wolski & Gorczyca, sp. n.

http://zoobank.org/87AF24A7-F7A9-481E-A200-2EEB45EDC679

Figures 7, 14, 37–41, 47–48

Diagnosis. Recognized by the white head, with a fuscous vertex (Figs 7, 47); the blackish hemelytron with two large, white patches at base and at apex of corium (Figs 7, 47); the sclerotized portion of ductus seminis (DSS) composed of two parts: basal one, relatively long, gradually broadened toward apex and apical one, weakly ovoid basally and rounded apically; the apical half of endosoma composed of five strongly membranous lobes covered with tiny denticles; the endosomal sinistrolateral sclerite (SLS) small, nearly ovoid, with serrate margins (Fig. 37); and the apical process of

right paramere tapering toward apex, with a subapical, short, obtuse process dextrolaterally (Fig. 41).

Most similar to *X. bimaculatus* in sharing large, pale patch near base of hemelytron (Figs 4, 7, 47). The present new species can, however, be distinguished by the blackish dorsum, with a large, white patch situated on hemelytron apically (Fig. 7, 47), and the shape of the male genitalia (Figs 37–41).

Description. *Male*. **COLORATION** (Figs 7, 14, 47). Dorsum blackish with large white areas. ***Head***. Mostly white; vertex fuscous; frons with two small, fuscous patches, each contiguous with inner margin of each eye and surrounding antennal insertion; gula blackish; antennal segments I and II fuscous; labial segment I blackish; remainder of labium dirty yellow. ***Thorax***. *Pronotum*. Black. *Mesoscutum and scutellum*. Black. *Thoracic pleura*. Black. *Hemelytron*. Mostly black; corium and clavus with large, white patch near base; apex of embolium, apicolateral surface of corium, and medial portion of inner margin of cuneus with a large white patch; membrane dark grey. *Legs*. Procoxa black; meso- and metacoxae yellow, with a fuscous patch basally; femora and tibiae black; metafemur with a narrow, reddish annulation subapically and yellow, narrow annulation apically; metatibia with a yellow annulation basally and dirty yellow tinge at apical one third; tarsi dirty yellow. ***Abdomen***. Black. **STRUCTURE, TEXTURE, AND VESTITURE** (Figs 7, 14, 47). ***Head***. Antennal segment II weakly broadened toward apex, covered with moderately dense, semirecumbent setae, sparse on basal one-fifth of segment II and dense on remainder of segment, apical one fourth also with sparse, bristlelike, protruding setae. ***Thorax***. *Pronotum*. Lateral margins incarinate, not elevated. *Mesoscutum and scutellum*. Scutellum flattened. *Hemelytron*. Covered with very short, relatively dense, adpressed, black setae.

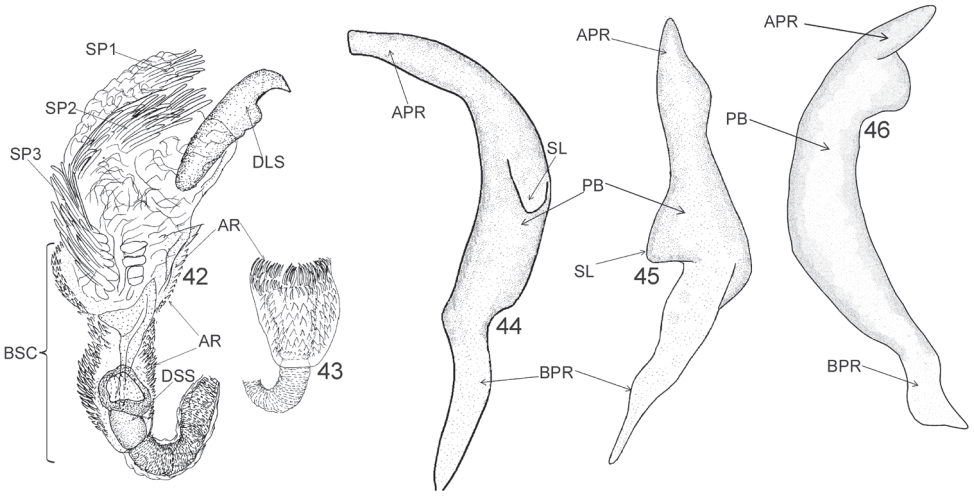
Male genitalia. *Aedeagus* (Figs 37–38). Basal sac (BSC) nearly square; sclerotized portion of ductus seminis of endosoma (DSS) composed of two parts: basal one, relatively long, gradually broadened toward apex and apical one, weakly ovoid basally and rounded apically; apical half of endosoma composed of five strongly membranous lobes covered with tiny denticles; apical portion of endosoma with a single bundle of short spiculi (SP1); sinistrolateral sclerite (SLS) small, nearly ovoid, with serrate margins. *Left paramere* (Figs 39–40). Apical process: lateral view: slightly tapering toward apex, very weakly curved subapically; dorsal view: strongly tapering toward apex; sensory lobe: stout, obtuse. *Right paramere* (Fig. 41). Apical process: tapering toward apex, thin; dorsal view: tapering toward apex, with subapical, short, obtuse process dextrolaterally; paramere body: dorsal surface with sparse, long, protruding setae.

Measurements. Holotype ♂: *Body*. Length 4.75, width 1.70. *Head*. Length 0.80, width 0.70, interocular distance 0.33. *Antenna*. Length of segment I 0.65, II 1.48 (III and IV missing). *Labium*. Length of segment I 0.87 (II, III, and IV immeasurable). *Pronotum*. Length 0.60, width of anterior margin 0.63, length of lateral margin 0.70, width of posterior margin 1.32.

Female. Unknown.

Biology. Unknown.

Distribution. New Caledonia (South Province) (Fig. 48).



Figures 42–46. Male genitalia of *X. tamasi*: **42** Endosoma (dorsal view) **43** Basal sac of endosoma (ventral view) **44** Left paramere (left lateral view) **45** Left paramere (dorsal view) **46** Right paramere (right lateral view). APR = apical process of paramere; AR = apical ring of endosomal basal sac; BPR = basal process of paramere; BSC = basal sac; DLS = dextrolateral sclerite; DSS = sclerotized portion of ductus seminis inside endosoma; PB = paramere body; SL = sensory lobe; SP1, SP2, and SP3 = endosomal spiculi.

Etymology. We are happy to name this species after our friend and colleague and the collector of the type specimen Roland Dobosz (Upper Silesian Museum, Bytom, Poland).

Type material. Holotype ♂: New Caledonia (S), 22°16.8'S, 166°53.5'E, Pic du Grand Kaori, 26. 12. 2006, 240 m, night coll. (lamp & beating), leg. R. Dobosz & M. Wanat; 5915/1788, coll. (MNHN).

Xenocylapidius tamasi Gorczyca

Figures 8, 15, 42–46, 48

Xenocylapidius tamasi Gorczyca 1997: 179, Figs l, 3, 6 (sp. n.), 1999: 16, figs 7–9 (redescription, male genitalia), 2006: 70, Fig. 23 (catalog)

Diagnosis. Recognized by the mottled, dark brown, dorsal coloration (Fig. 8), the femora mottled with dark brown and yellow (Figs 8, 15), the endosoma with three bundles of spicules: one situated medially, second subapically, and third apically (Fig. 42), the endosomal basal sac (BSC) occupying half of endosoma, entirely covered with small denticles (Figs 42–43), the endosomal dextrolateral sclerite (DLS) large, occupying nearly one third of endosoma, weakly broadened toward apex, hook-shaped apically (Fig. 42), the sensory lobe (SL) of left paramere short and obtuse in dorsal view (Fig. 45), the right paramere with apical process broadened, with long apical process, weakly tapering toward apex (Fig. 46).

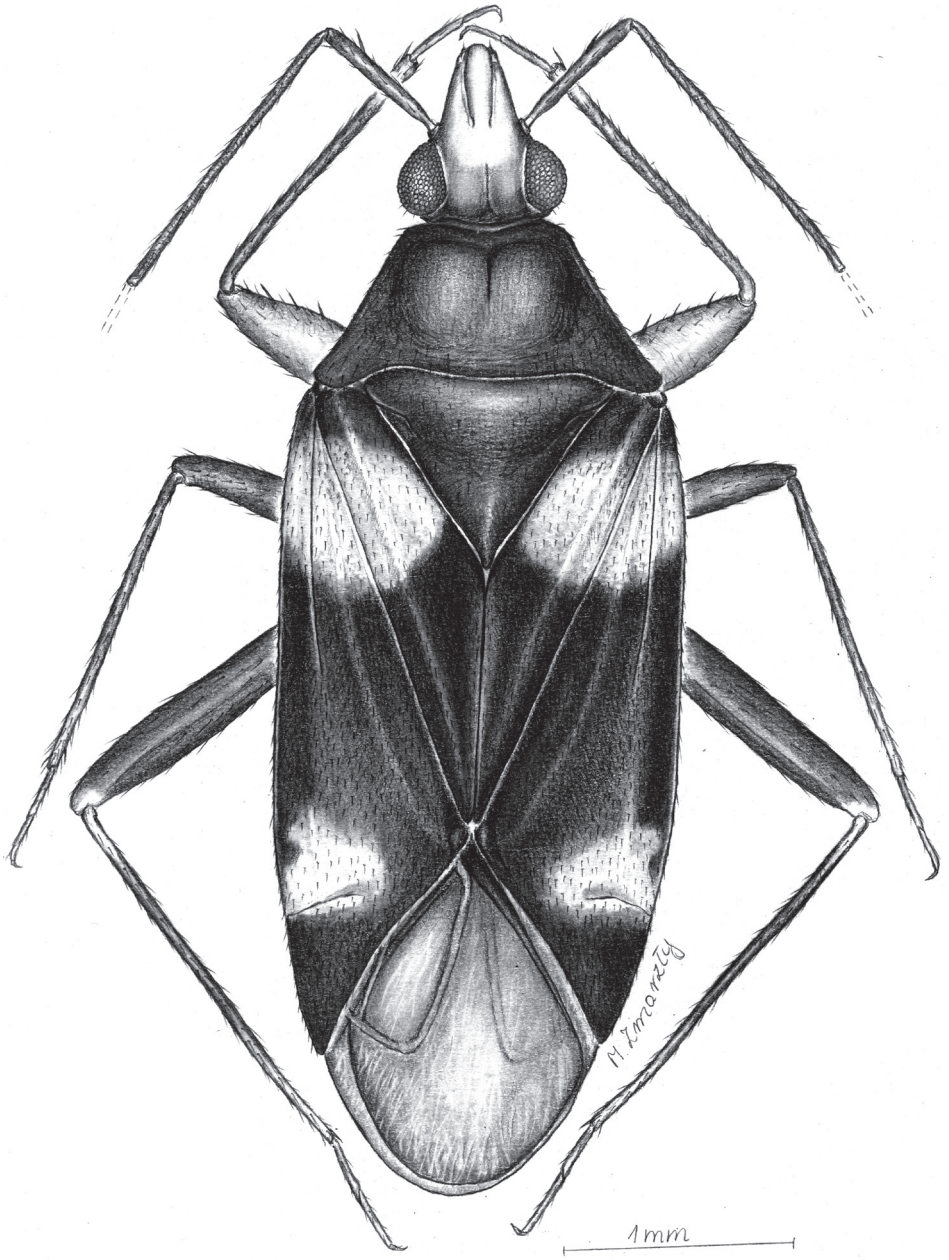


Figure 47. Dorsal habitus drawing of *X. rolandi* (holotype).

Most similar to *X. acutipennis*, *X. bioculatus*, *X. gemellus*, and *X. gressitti* in sharing mottled dorsal coloration (Figs 1, 4, 5–6, 8). *Xenocylapidius tamasi* can, however, be distinguished by the coloration of femora. From *X. acutipennis* and *X. gemellus* it can be distinguished by the shape of the male genitalia (Figs 42–46).

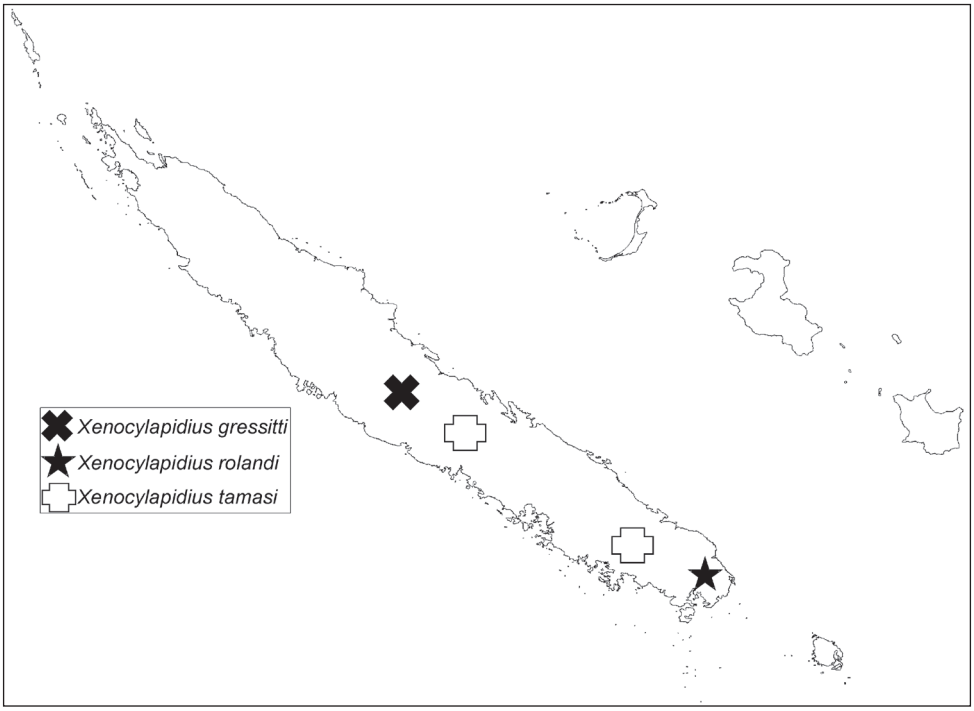


Figure 48. Distribution map of *Xenocylapidius* spp.

Male genitalia. *Aedeagus* (Figs 42–43). Basal sac (BSC) occupying half of endosoma, entirely covered with small denticles; endosoma with three bundles of spicules: one situated medially, second subapically, and third apically; dextrolateral sclerite (DLS) large, occupying nearly one third of endosoma, weakly broadened toward apex, hook-shaped apically. *Left paramere* (Figs 44–45). Apical process: lateral view: very weakly broadened at basal two thirds, cylindrical at apical one third, blunt; dorsal view: basal half with sinistrolateral margin weakly convex and dextrolateral margin strongly convex, apical half tapering toward apex; sensory lobe: short and obtuse. *Right paramere* (Fig. 46). Apical process: broadened, with long apical process, weakly tapering toward apex; paramere body: relatively broad, arcuate.

Biology. Unknown.

Distribution. New Caledonia (South Province) (Fig. 48).

Type material. Holotype ♀: New Caledonia, Col d' Amieu, Ht. Rembtai; 19. I. 1977, leg. J. Balogh; holotype [red label]; *Xenocylapidius tamasi* gen et sp. n., det. J. Gorczyca, 1997 (HNHM).

Additional examined material. 2 ♂♂ and 1 ♀: New Caledonia, Mt. des Koghis, 300–600 m, 19. III. 1968; J.L. Gressitt & T.C. Maa Collectors, Bishop Museum; 1 ♀: New Caledonia, Foret di Thi, 29.X. – 1.XI.1967; J. & M. Sedlacek Collectors, Bishop (US).

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Diversity, distribution and biology of Romanian flat-footed flies (Diptera, Opetiidae and Platypezidae) with taxonomic notes on *Callomyia saibhira* Chandler

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Abstract

Altogether 18 species of the families Opetiidae and Platypezidae are reported from Romania, based on newly studied material and previously published records. The following three species are recorded from Romania for the first time: *Agathomyia vernalis* Shatalkin, 1981, *Callomyia saibhira* Chandler, 1976, and *Lindneromyia hungarica* Chandler, 2001. The presented differential diagnosis and a detailed redescription of body and genitalia of the male of *Callomyia saibhira* are based on one specimen which is the first male found in Europe. Information about distribution and biology of all 18 Romanian species is provided as well as photographs of selected important species. Finally, a new checklist of all Romanian species is given.

Keywords

Diptera, Opetiidae, Platypezidae, *Callomyia saibhira* redescription, Palaearctic Region, Romania, distribution, biodiversity, new records, biology

Introduction

The Opetiidae and Platypezidae are basal cyclorrhaphous families of Diptera, belonging to the superfamily Platypezoidea. The European species are small brachycerous flies, ranging from 1.4 to 6.0 mm in wing length. Their coloration is often black

(males) or composed of black, orange and grey (females), and some species have silvery grey reflective patterns. The males have larger heads with holoptic eyes, while the female eyes are dichoptic. The larvae may be flat or cylindrical. All known larvae are mycophagous and feed by burrowing in the tissue of fungus fruiting bodies, at the surface of the gills of gill fungi, or on fungal mycelia under bark of dead trees; one species, *Agathomyia wankowiczii* (Schnabl, 1884), is gall-forming on sporocarps of a polypore. Adults of European species may be observed running rapidly on broad leaves in forested habitats; females may be observed ovipositing on host fungi.

These two families of flat-footed flies include 44 species in 13 genera in Europe (Chandler 2001, 2004). Current literature (Chandler 2001, 2004) lists only six species from Romania. However, during the preparation of this paper we have noticed that some localities of Platypezidae given by Thalhammer (1899) and Szilády (1941) are in fact in the present territory of Romania. Unfortunately, the material of Thalhammer (1899) and Szilády (1941) was destroyed by fire in 1956 (Papp 2001), but we consider their determinations to be correct and thus their records are included in the present paper. The monograph of the European species by Chandler (2001) summarizes all known (up to year 2000) data on adult and larval morphology, biology, distribution, systematics, including keys to the species. The nomenclature and classification used here therefore follow Chandler (2001).

Material and methods

Specimens were examined with an Olympus SZX10 binocular microscope. Photographs were taken by Canon 600D and/or 60D with MPE-65 macro lens and in some cases combined from multiple layers using Helicon Focus Pro 5.2. Drawings and photographs were edited in CorelDRAW 12 and Corel PHOTO-PAINT 12 graphic software. Morphological terminology follows Cumming and Wood (2009) and Chandler (2001), terminology of male genitalia follows Chandler (2001), Chandler and Shatalkin (1998), and is supplemented in parentheses by terminology adopted from Cumming and Wood (2009). The material examined is now deposited in the Silesian Museum, Opava, Czech Republic (SMOC, all specimens collected by J. Roháček) and the National Museum, Praha, Czech Republic (NMPC, remaining specimens).

Distributional data follows Chandler (2001, 2004) and are supplemented by data of Thalhammer (1899), Szilády (1941), Carles-Tolrá and Báez (2002), Ševčík (2004), Pakalniškis et al. (2006), Schacht (2006), Ståhls and Kahanpää (2006), Tkoč and Vaňhara (2006, 2008), Roháček and Ševčík (2007, 2011), Vaňhara (2009), Andrade and Almeida (2010), Ebejer and Andrade (2010), Tkoč (2011), Tkoč et al. (2012), Claussen (2013) and Ståhls (2014).

The following abbreviations are used in the text: I–XII – January to December, BMNH – The Natural History Museum, London, United Kingdom, JR – Jindřich Roháček, ER – European Russia, FE – Far East of Russia, MT – Michal Tkoč, NMPC – National Museum, Praha, Czech Republic, SMNS – Staatliches Museum

für Naturkunde, Stuttgart, Germany, SMOC – Silesian Museum, Opava, Czech Republic. The species with asterisk (*) in front of their names represent new records for Romania. The translations of original localities from Romanian are in square brackets [ ], together with names of the respective county and historical region.

Results

Family Opetiidae

***Opetia nigra* Meigen, 1830**
Figure 1

Published records. Orlát [Orlat, Sibiu, Transilvania] (Thalhammer 1899); Mehádia [Mehadia, Caraș-Severin, Banat] (Szilády 1941).

Material examined. 1 ♂, 1. vi. 2008, Banat, Sfânta Elena, 4km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegetation along brook, JR leg.

Distribution. Palaearctic species. Recorded in Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Luxembourg, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Sweden, Switzerland and Russia (ER).

Biology. The adults run on broad leaves in wooded biotopes, where they sometimes form swarms. Its larvae are unknown. The adults were reared from very rotten beech wood and leaf litter (Speight et al. 1990, Chandler 2001) but the exact development substrate of the larvae remains unknown and thus these records need confirmation. The males can sometimes be caught by light trap (Chandler 2001), while the females can be collected by pitfall traps (Vaňhara 1986). The adults (mostly males) can be collected by sweeping undergrowth of various forests, sweeping on *Atropa belladonna* leaves proved to be particularly productive (pers. obs.). The species is bivoltine, adult flight period in central Europe is V–VI and VIII–X.

Family Platypezidae
Subfamily Callomyiinae

***Agathomyia antennata* (Zetterstedt, 1819)**
Figure 2

Published records. Mehádia [Mehadia, Caraș-Severin, Banat]; Szászka [Szászka, Caraș-Severin, Banat] (Szilády 1941).

Material examined. 1 ♀, 31. v. 2008, Banat, Sfânta Elena, 1 km E, Alibeg brook valley (Figure 18), 230 m a.s.l., 44°40'37"N, 21°43'32"E, sweeping undergrowth of

Figure 1. *Opetia nigra* Meigen, 1830, male habitus. Photo by J. Roháček.

Figure 2. *Agathomyia antennata* (Zetterstedt, 1819), female habitus. Photo by D. Gavryushin.

deciduous forest, JR leg.; 3 ♀♀, 1. vi. 2008, Banat, Sfânta Elena, 4 km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegetation along brook, JR leg.; 1 ♀, 4. vi. 2008, Banat, Berzasca, 2 km NE, Berzasca

river valley, 85 m a.s.l., 44°39'09"N, 21°57'47"E, sweeping riverside vegetation, JR leg.; 1 ♀, 19. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N, 23°32'02"E, sweeping on *Quercus* sp., MT leg.

Distribution. Palaearctic species reaching to Oriental region (Taiwan). Recorded in Austria, Belgium, Croatia, the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Italy, Lithuania, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Spain, Sweden, Switzerland and Russia (ER, FE).

Biology. Most common species of the genus in the Palaearctic Region. The adults of both sexes are often found running on *Petasites* sp. leaves (pers. obs.). Larvae develop in *Bjerkandera adusta* (Ševčík 2010). Adult flight period is in IV–IX.

Agathomyia collini Verrall, 1901

Published records. Mehadia, Karaš-Severin [Caraș-Severin, Banat], 3.vii.1912, Oldenberg coll. (SMNS) (Czerny 1930, Chandler 2001).

Distribution. Palaearctic species. Recorded in the Czech Republic, France, Great Britain, Hungary, Romania, Slovakia, Spain, Russia (ER, FE) and Georgia (North Ossetia) in the Caucasus (Shatalkin 1992).

Biology. Larvae are unknown; Chandler (2001) mentioned possible association with *Phellinus pomaceus* growing on apple or plum trees based on information provided by Morley (1918). However, this host association needs confirmation. The adults can be swept on forest undergrowth formed mainly by *Lunaria rediviva* (Roháček and Ševčík 2011; pers. obs.). Adults occur in IV–IX.

Agathomyia falleni (Zetterstedt, 1838)
Figure 3

Published records. Szászka [Szászka, Banat, Caraș-Severin] (Szilády 1941).

Distribution. Palaearctic species. Recorded in Austria, Croatia, the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Lithuania, the Netherlands, Romania, Poland, Slovakia, Sweden, Switzerland and Russia (ER).

Biology. Host fungus is *Bjerkandera adusta* (see Chandler 2001). The females can be observed during oviposition on sporocarps of the host fungi on tree stumps (Figure 3). It is a species with autumnal activity; adult flight period is in IX–XI.

Agathomyia setipes Oldenberg, 1916
Figure 4

Published records. 1 ♂, Czerna Ufers [=Cerna river banks], Herkulesbad [Băile Herculane, Caraș-Severin, Banat], 13.vii.1912 (Oldenberg 1916, Czerny 1930).

Figure 3. *Agathomyia falleni* (Zetterstedt, 1838), female ovipositing on undetermined yellow slime mold (Mycetozoa) on stump overgrown by *Bjerkandera adusta*. Photo by M. Tkoč.

Figure 4. *Agathomyia setipes* Oldenberg, 1916, female showing abdominal pattern. Photo by J. Roháček.

Distribution. Palaearctic species. Recorded in Croatia, the Czech Republic, Slovakia, Hungary, Romania and Russia (FE).

Biology. Unknown. Adults are usually swept from vegetation along brooks in forests (Roháček and Ševčík 2009). Very rare species known from only a few specimens from the whole of Europe; adult flight period is in VII–X.

**Agathomyia vernalis* Shatalkin, 1981

Figure 5

Material examined. 1 ♀, 19. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N, 23°32'02"E, sweeping on *Fagus sylvatica*, MT leg.

Distribution. Palaearctic species. Recorded in the Czech Republic, Finland, Germany, Slovakia, Switzerland and Russia (ER). **New record for Romania.**

Biology. Very rare species with early flight period. The individuals are collected only in IV and V and were almost exclusively females. Larval biology and host fungus are unknown. Tkoč and Barták (2013) recorded this species in numbers from a pyramidal (emergence) trap baited with dead wood.

Agathomyia viduella (Zetterstedt, 1838)

Figure 6

Published records. Mehádia [Mehadia, Caraș-Severin, Banat] (Szilády 1941).

Material examined. 1 ♀, 1. vi. 2008, Banat, Sfânta Elena, 4 km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegetation along brook, JR leg.

Distribution. Palaearctic species. Recorded in the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Lithuania, Montenegro, the Netherlands, Norway, Poland, Romania, Slovakia, Sweden, Switzerland and Russia (ER, FE).

Biology. Uncommon species with adults occurring in undergrowth and along brooks in humid deciduous and mixed forests (Roháček and Ševčík 2009) and are often observed running on *Petasites* sp. leaves together with *A. antennata* (pers. obs.). The main flight period of adults ranges from V to VII. Host fungus is unknown.

Callomyia amoena Meigen, 1824

Figure 7

Published records. Mehádia [Mehadia, Caraș-Severin, Banat]; Orsova [Orșova, Mehedinți, Banat] (Thalhammer 1899); 1 ♂, 1 ♀, Retezatului Mts, near Hobita,

Figure 5. *Agathomyia vernalis* Shatalkin, 1981, female habitus in lateral view. Photo by M. Tkoč.

Figure 6. *Agathomyia viduella* (Zetterstedt, 1838), female habitus. Note the glossy frons as the main diagnostic character of females of this species. Photo by M. Tkoč.

Figure 7. *Callomyia amoena* Meigen, 1824, male habitus. Photo by D. Gavryushin.

Calana [Hobița, Hunedoara], 29. vi. 1969, in mature pine forest, B.H. Cogan and R.I. Vane-Wright leg. (BMNH) (Chandler 2001, in litt.).

Material examined. 1 ♀, 1 vi. 2008, Banat, Sfânta Elena, 4 km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegetation along brook, JR leg.; 1 ♂ 1 ♀, 2. vi. 2008, Banat, Radimna near Pojejena, 140 m a.s.l., 44°49'17"N, 21°33'31"E, sweeping undergrowth of alder forest, JR leg.; 1 ♂, 19. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N, 23°32'02"E, sweeping on *Acer campestre*, MT leg.

Distribution. Palaearctic species. Recorded in Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Lithuania, the Netherlands, Norway, Poland, Slovakia, Spain, Sweden, Switzerland, Romania and Russia (ER).

Biology. Common species, the larvae live on mycelia under bark of fallen trunks of various trees. A record from mycelia on bark on the underside of aspen trunks lying on the ground was mentioned by Krivosheina (2008). Flight period of adults ranges from V to X.

Callomyia elegans Meigen, 1804

Figure 8

Published records. Mehádia [Mehadia, Caraș-Severin, Banat] (Thalhammer 1899).

Figure 8. *Callomyia elegans* Meigen, 1804, female habitus. Photo by D. Gavryushin.

Distribution. Palaearctic species. Recorded in Andorra, Austria, the Czech Republic, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Lithuania, the Netherlands, Norway, Poland, Slovakia, Sweden, Switzerland, Romania and Russia (ER).

Biology. Unknown, the larvae probably live on mycelia under bark of fallen trunks of various trees (similarly to other species of *Callomyia*, see Krivosheina 2008). Rare species with no recent records from Central and South Europe, population densities of this species are very low or undetectable. Adult flight period is in IV–VIII.

**Callomyia saibhira* Chandler, 1976

Figs 9–10

Material examined. 1 ♂, 1. vi. 2008, Banat, Sfânta Elena, 4 km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegetation along brook, JR leg.

Differential diagnosis. Male of *C. saibhira* differs from *C. amoena* and *C. elegans* by having darker halteres that are not orange (brown with knob black). *C. speciosa* have longer arista and shorter first flagellomere and upper part of pleura is not so silvery grey dusted as in *C. saibhira*. From its most similar species, *C. dives* Zetterstedt, 1838, it differs by a clear wing membrane, brown palpus and different genitalia, basal lobe of gonopod is shorter (Figure 10). Females have unique abdominal coloration: tergites 1–4 (T1–4) are orange yellow with narrow brown hind margins on T2–4, only T5 is black, T6 and terminal segments are silvery grey dusted.

Figure 9. *Callomyia saibhira* Chandler, 1976, male from Romania: body in lateral view. Photo by M. Tkoč.

Redescription. ***Male.*** Body length 4.1 mm. Wing length 3.8 mm.

Head black with silvery grey dusting. Antenna dark brown, scapus with dorsal seta reaching to tip of pedicel, pedicel with one strong dorsal seta reaching to the middle of first flagellomere, both sides of pedicel with 3 short setae, 1 short seta on ventral position. First flagellomere conical, twice as long as pedicel. Second and third flagellomere long. Arista half of antennal length. Two pairs of small frontal setae. Ocellar tubercle dull brown, with one pair of ocellar setae and one pair of small postocellar setae. Postocular setae long, their apices visible in anterior view. Face and parafacial bare, silvery grey dusted. Gena, occiput and postgena with long black setae. Occiput black, silvery grey dusted. Palpi brown with short black setae, proboscis brown with pale pubescence.

Thorax velvet black with silvery grey dusted areas. Two very inconspicuous median dorsal grey stripes between dorsocentral and acrostichal setae ending in anterior two thirds of scutum. Posterior sides of scutum silvery shining. Pleural sides of thorax without setae, silvery grey coloured. All thoracic setae black. Uniserial row of acrostichal setae, two rows of about 10 dorsocentral setae. Humeral callus with top brownish, with 2 humeral setae; 4 small posthumeral setae. One postalar seta. Notopleural group composed of 6 setae: 1st long, 2nd– 4th short, 5th–6th long. Notopleural area silvery grey dusted. Two long presutural and 4–5 small postsutural setae. Scutellum black, with 2 prominent scutellar setae on each side. Haltere brownish with knob black.

Wing hyaline with brown to dark brown veins. Subcostal cell (sc) yellow tinted and with microtrichia. Wing surface not uniformly covered with microtrichia, microtrichia present on anal lobe, posterior and distal part of wing. First longitudinal vein (R_1)

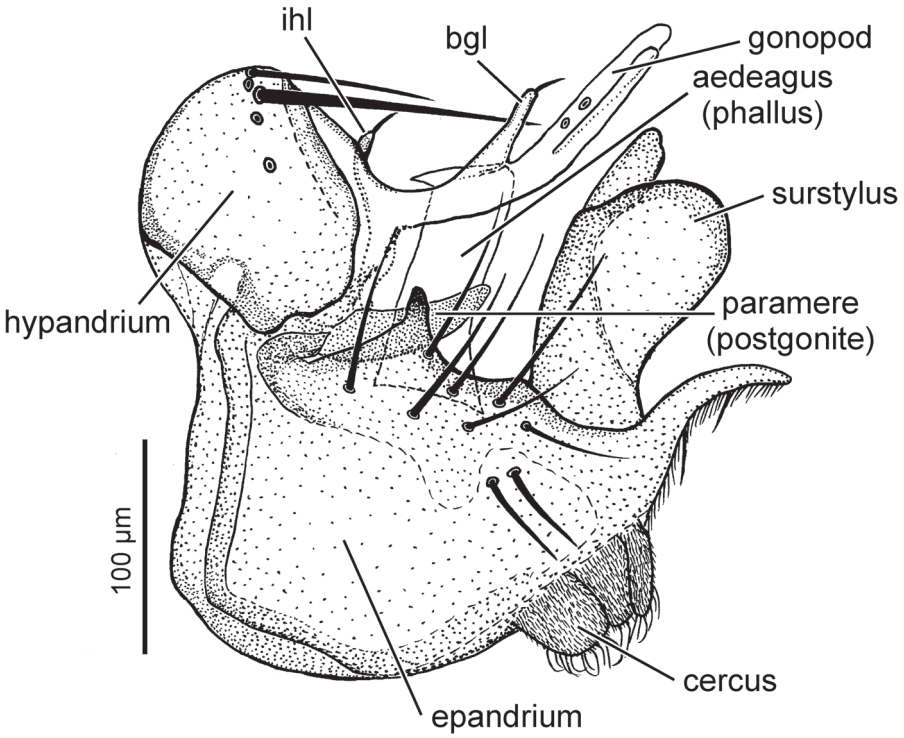

Figure 10. *Callomyia saibhira* Chandler, 1976, male genitalia of specimen from Romania: right lateral view. (bgl – basal gonopodal lobe, ihl – inner hypandrial lobe).

bearing 9–10 spines. Anterior (r-m) and posterior (dm-cu) crossveins present. Costal cell (c) equal to sc in length. Posterior crossvein (dm-cu) twice as long as distal part of the fifth longitudinal vein (CuA_1). Anal cell (cup) elongated, its length about three times portion of anal vein (A_1+CuA_2) beyond it.

Legs slender, brown, slightly silvery shiny. All coxae silvery dusted with black setae, yellow distally. Fore femur with longer fine ventral setae distally. Fore femur with 1 ox-horn seta. Apices of femora and basal parts of tibiae (="knees") yellow. Fore tibia with 1 anteroventral spur. Fore tarsomeres I–II yellow. Mid tibia bearing short dorsal seta above middle (anterodorsal seta absent) and two long ventral apical spurs. Hind femur of the same width as hind tibia. Hind tarsomere I with ventral seta above middle.

Abdomen black with silver-grey coloured markings. Setae on abdomen fine and black. Tergites 1 and 2 (T1+2) more setulose than the others, T3+T4 sparsely setulose. T1 black, its anterior half shiny silvery grey in lateral view. T2 black with silvery grey marking on posteroventral area. In lateral view this marking occupies posterior third of T2. T3 black, with similar (but smaller) marking, mainly on ventral part. T4 black, with silvery grey marking on posteroventral area occupying posterior two thirds in lateral view. T5 black. T6 black with posterior border grey. T7 small, entirely grey. Sternite 8 also grey, without setae.

Genitalia (Figure 10) with epandrium grey, cercus brownish, surstylus and hypandrium shiny amber-brown. Paramere (postgonite) slightly curved dorsally with base narrower, its broader apical part slightly tapered towards the rounded apex. Aedeagus (phallus) broad in lateral view, its dorsal apex with sharp tooth anteriorly, bluntly rounded posteriorly. Hypandrium with small inner hypandrial lobe (ihl) sub-basal to gonopod. Gonopod (hypandrial lobe) trifid, with shorter basal gonopodal lobe (bgl) and longer terminal part deeply bifid forming two slender apical lobes. Surstylus with basal part narrower than its apical rounded part, the latter with a digitiform dorsal process. Terminal lobe of epandrium gradually tapered and slightly curved, covered by setulae and with 2 longer setae. Ventral part of epandrium with 5 prominent setae two smaller setae and two additional smaller setae positioned more ventrally. Cercus covered by microtrichia and with short curly setulae on apex.

Female. Not studied, for description see Chandler (1976, 2001).

Distribution. Palaearctic species. Hitherto recorded only from Bulgaria (Chandler 1976) and the Far East of Russia (Shatalkin 1985). **New record for Romania.**

Biology. Unknown. The larvae of other European *Callomyia* species develop on mycelia under bark of various trees (see above under *C. amoena*). The adult male examined was swept from vegetation close to a cave along a small brook (Figure 17). The known flight period in Europe is in VI.

Comments. This is the second specimen and first male of the species from Europe. Other known material (♂♂ and ♀♀) was collected in the Far East of Russia, Amur region (Shatalkin 1985). It is similar to *Callomyia dives* in the morphology of males, but the silver coloration on the thorax and abdomen is less developed. Also, the basal lobe of the gonopod (bgl) is shorter (Figure 10). This species seems to have an inland distribution, whereas *C. dives* is found mostly on islands or close to coastal zones of Europe.

Chandler (2001) figured genitalia of an Amur specimen collected by Shatalkin. However, the genitalia of our specimen do not entirely fit into the description and figure of Chandler (2001). The main differences appear to be as follows (see Figure 10): presence of inner hypandrial lobe (ihl) positioned sub-basally to gonopod (this character is present in more *Callomyia* species, but usually omitted in the descriptions and figures in the literature); paramere (postgonite) is wider in lateral view, curved dorsally, with narrow basal part; morphology and position of basal gonopodal lobe (bgl) is very similar to that of Chandler (2001), but the bifurcation of terminal part of gonopod is more profound; the apical part of surstylus is less rounded and its basal part is only slightly narrower than apical part; cerci have shorter curly setulae.

Callomyia speciosa Meigen, 1824

Figure 11

Published records. 1 ♂, Retezatului Mts, near Hobita, Calana [Hobița, Hunedoara], 29. vi. 1969, in mature pine forest, B.H. Cogan and R.I. Vane-Wright leg. (BMNH) (Chandler 2001, in litt.).

Figure 11. *Callomyia speciosa* Meigen, 1824, female habitus. Photo by D. Gavryushin.

Material examined. 1 ♀, 31. v. 2011, Banat, Sfânta Elena, 1 km E, Alibeg brook valley (Figure 18), 230 m a.s.l., 44°40'37"N, 21°43'32"E, sweeping undergrowth of deciduous forest, JR leg.; 1 ♂, 1 vi. 2008, Banat, Sfânta Elena, 4 km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegetation along brook, JR leg.; 1 ♀, 3. vi. 2008, Banat, Sfânta Elena, 1 km E, Alibeg brook valley (Figure 18), 230 m a.s.l., 44°40'37"N, 21°43'32"E, sweeping vegetation along brook, JR leg.

Distribution. Palaearctic species. Recorded in Andorra, Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Italy, Israel, Lithuania, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Russia (ER) and Caucasus.

Biology. Less common than *C. amoena* but widely distributed throughout Europe. The larvae also live on mycelia under bark of various trees (reared from mycelium on surface of a fallen hazel trunk by Krivosheina (2008)). Adults fly in V–IX.

Subfamily Platypezinae

Seri obscuripennis (Oldenberg, 1916)

Figure 12

Published records. 1 ♂, Herkulesbad [Băile Herculane, Caraș-Severin, Banat], 6.vi.1904, Kertész lgt. (Oldenberg 1916, Czerny 1930); Mehádia [Mehadia, Caraș-Severin, Banat] (Szilády 1941).

Figure 12. *Seri obscuripennis* (Oldenberg, 1916), male habitus. Photo by D. Gavryushin.

Distribution. Palaearctic species. Recorded in Austria, the Czech Republic, Finland, Germany, Great Britain, Hungary, the Netherlands, Norway, Poland, Romania, Slovakia, Sweden, Switzerland and Russia (ER, FE).

Biology. Rather rare in Europe but more common in the Far East of Russia (Shatalkin 1985, Chandler 2001). Its larvae develop in several species of *Polyporus* (Ševčík 2010). Adults occur in VI–VII and IX.

Bolopus furcatus (Fallén, 1826)

Published records. Mehádia [Mehadia, Caraș-Severin] (Szilády 1941).

Distribution. Palaearctic species. Recorded in Austria, Belgium, the Czech Republic, Denmark, Finland, Germany, Great Britain, Hungary, Ireland, Poland, Romania, Slovakia, Sweden, Switzerland, the Netherlands, Russia (ER).

Biology. Immature stages develop in *Polyporus squamosus* (Chandler 2001, Ševčík 2010). Adult flight period is from IV to IX.

Polyporivora ornata (Meigen, 1838)

Figure 13

Published records. Szászka [Szászka, Caraș-Severin, Banat] (Szilády 1941).

Figure 13. *Polyporivora ornata* (Meigen, 1838), male habitus. Photo by D. Gavryushin.

Material examined. 1 ♂, 19. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N, 23°32'02"E, sweeping on *Fagus sylvatica*, MT leg.

Distribution. Palaearctic species. Recorded in Belgium, the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Lithuania, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Spain, Sweden, Switzerland and Russia (ER).

Biology. Immature stages develop in *Trametes versicolor* (Ševčík 2010). Adult flight period ranges from V to X, the species is bivoltine.

Paraplatypeza atra (Meigen, 1804)

Published records. Mehádia [Mehadia, Caraș-Severin, Banat] (Thalhammer 1899); Mehádia [Mehadia, Caraș-Severin, Banat], Radna-Borberek [Rodna, Bistrița-Năsăud, Transilvania] (Szilády 1941).

Material examined. 1 ♂, 1. vi. 2008, Banat, Sfânta Elena, 4 km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegetation along brook, JR leg.; 1 ♀, 18. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N (46.07168), 23°32'02"E (23.53429), sweeping on *Fagus sylvatica*, K. Blahová & MT leg.

Distribution. Palaearctic species. Recorded in Austria, Belgium, the Czech Republic, Denmark, Finland, France, Germany, Great Britain, Hungary, Ireland, Italy, Lithuania, the Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Sweden, Switzerland and Russia (ER).

Biology. Common species, larvae develop in various species of *Pluteus*, mainly in *Pluteus cervinus* (Ševčík 2010). Adult flight period ranges from IV to XI.

Figure 14. *Paraplatypeza bicincta* (Szilády, 1941), female habitus. Photo by D. Gavryushin.

***Paraplatypeza bicincta* (Szilády, 1941)**
Figure 14

Published records. Szászka [Szászka, Caraș-Severin, Banat] (Szilády 1941).

Distribution. Palaearctic species reaching to Oriental region. Distributed in the Czech Republic, Great Britain, Finland, Norway, Slovakia, Switzerland, Sweden, Russia and Myanmar [=Burma].

Biology. Rare species associated with *Pluteus* sp. The adults were reared from *Pluteus cervinus* several times by the first author (not published). Adult flight period ranges from VIII to X.

***Lindneromyia dorsalis* (Meigen, 1804)**
Figure 15

Published records. 1♂, „Bucarest" [București], A.L. Montandon leg., ex E. Brunetti coll. (BMNH) (Chandler 2001, in litt.).

Material examined. 1 ♂, 25. v. 2013, Mer occ., Muntii Locvei Mts., Sfanta Elena env., cca 44°40'N, 21°43'E, B. Mocek leg.; 1 ♀, 30. v. 2008, Banat, Latunas, 3 km W nr. Comoraste, 110 m a.s.l., 45°13'16"N, 21°28'10"E, sweeping over boggy meadow, JR leg.; 1 ♂, 1. vi. 2008, Banat, Sfânta Elena, 4 km NE, Kulhavá skála, Vranovec cave (Figure 17), 300 m a.s.l., 44°42'12"N, 21°43'52"E, sweeping vegeta-

Figure 15. *Lindneromyia dorsalis* (Meigen, 1804), female habitus. Photo by D. Gavryushin.

tion along brook, JR leg.; 1 ♀, 19. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N(46.07168), 23°32'02"E(23.53429), sweeping on *Fagus sylvatica*, MT leg.; 1 ♀, 19. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N (46.07168), 23°32'02"E (23.53429), sweeping on *Fagus sylvatica*, MT leg.; 2 ♀, 18. v. 2011, Alba, Alba Iulia, 1 km E, 380 m a.s.l., 46°04'18"N (46.07168), 23°32'02"E (23.53429), sweeping on *Fagus sylvatica*, K. Blahová & MT leg.

Distribution. Palaearctic species. Recorded in Andorra, Austria, Belgium, Cyprus, the Czech Republic, Denmark, France, Germany, Great Britain, Greece, Hungary, Italy, Israel, Morocco, the Netherlands, Poland, Portugal, Romania, Slovakia, Spain, Switzerland, Turkey and Russia (ER).

Biology. Larvae mostly develop in fruiting bodies of various *Agaricus* sp. There are several unconfirmed records from other soft fungi (Chandler 2001). The adult females may be observed during oviposition directly on fruiting bodies. Adults occur in V–XI.

**Lindneromyia hungarica* Chandler, 2001

Figure 16

Material examined. 1 ♀, 1. vi. 2008, Banat, Sfânta Elena, 2.5 km NE, 420 m a.s.l., 44°41'44"N, 21°43'10"E, sweeping over meadow, JR leg.

Figure 16. *Lindneromyia hungarica* Chandler, 2001, female habitus. Photo by D. Gavryushin.

Distribution. Palaearctic species. Recorded in Austria, the Czech Republic, France, Germany, Great Britain, Hungary, Portugal, Slovakia, Spain and Switzerland. **New record for Romania.**

Biology. The species was only recently separated from *L. dorsalis* and their larvae can develop together with those of *L. dorsalis* in the same fruiting body of an *Agaricus* sp. (Chandler 2001, Tkoč and Vaňhara 2008). Occasionally, the species can be caught on sporocarps of other fungi, e.g. Roháček and Ševčík (2013) collected one female on *Meripilus giganteus* in a park of Opava city (Czech Republic). Adults can be found in V–X.

Checklist of the Romanian Opetiidae and Platypezidae

Family Opetiidae
- *Opetia nigra* Meigen, 1830

Family Platypezidae
- Subfamily Callomyiinae
  - *Agathomyia antennata* (Zetterstedt, 1819)
  - *Agathomyia collini* Verrall, 1901
  - *Agathomyia falleni* (Zetterstedt, 1838)

Agathomyia setipes Oldenberg, 1916
Agathomyia vernalis Shatalkin, 1981
Agathomyia viduella (Zetterstedt, 1838)
Callomyia amoena Meigen, 1824
Callomyia elegans Meigen, 1804
Callomyia saibhira Chandler, 1976
Callomyia speciosa Meigen, 1824

Subfamily Platypezinae
Seri obscuripennis (Oldenberg, 1916)
Bolopus furcatus (Fallén, 1826)
Polyporivora ornata (Meigen, 1838)
Paraplatypeza atra (Meigen, 1804)
Paraplatypeza bicincta (Szilády, 1941)
Lindneromyia dorsalis (Meigen, 1804)
Lindneromyia hungarica Chandler, 2001

Discussion

Altogether 18 species of the families Opetiidae and Platypezidae are reported from Romania, representing 40.9 % of all flat-footed flies known from Europe. This number is far from the total number of species in this country and more research on flat-footed flies is needed to understand their distribution in Romania and Europe as a whole. Comparing the species number of the family Opetiidae and Platypezidae from Romania with the countries related to the Carphathian-Pannonian region it is an average number of species: Austria has 17 species (Chandler 2004), the Czech Republic 34 (Vaňhara 2009), Hungary 27 (Papp 2001, Weele 2001, Chandler 2004), Poland 25 (Chandler 2004), Slovakia 34 (Vaňhara 2009); the flat-footed fly fauna of Croatia, Serbia, Slovenia and Ukraine has not been systematically studied, thus the numbers are very low and not useful for comparison.

The discovery of the first male of *Callomyia saibhira* in Romania is the most important result of this study. The genitalia figured herein do not entirely agree with the description and figure of Chandler (2001). The differences in male genitalia between the Romanian and Amur specimen highlighted above may be the result of simple variation, or could be caused by comparing our specimen (Figure 10) with the somewhat simplified illustration of Chandler (2001) or may point to a more complicated taxonomic problem. To resolve this question, additional fresh material from both Europe and the Far East of Russia is needed in order to study variability in the morphology of the male genitalia and to test for differences using molecular methods. It also needs to be determined, if the males assigned to this species are correctly associated with the females, which appears to be a problem in the Nearctic species of *Callomyia* (Chandler 2001; Tkoč 2012).

Figures 17–18. Habitats of Opetiidae and Platypezidae in Romania: **17** Kulhavá skála, Vranovec cave **18** Alibeg brook valley. Photos by J. Roháček.

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RESEARCH ARTICLE

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Coleophora nepetellae Baldizzone & Nel, a new species of the *C. lixella* group (Lepidoptera, Coleophoridae) from France and Italy

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Abstract

Coleophora nepetellae Baldizzone & Nel, **sp. n.** is described from the southern Alps (Italy and France). It belongs to the *Coleophora lixella* species group. Its host plants are *Nepeta nepetella* L. (Lamiaceae) and an unidentified Poaceae. The fifth instar larva, its case, the adult habitus, and genitalia are illustrated. The species is compared to *C. nevadella* Baldizzone, 1985, here newly confirmed from France and whose larvae feed on *Nepeta latifolia* DC. in the Eastern Pyrénées. DNA barcodes are shown to be distinct and congruent with morphological differences among species of the *lixella* group. Barcodes revealed that *C. tricolor* Walsingham, 1889, formerly known only from Great Britain, is also present in France and Greece.

Résumé

Coleophora nepetellae Baldizzone & Nel, **sp. n.** du groupe de *Coleophora lixella* Zeller, 1849., est décrite des Alpes méridionales (Italie et France). La plante-hôte de ponte est la Lamiacée *Nepeta nepetella* L. et celle de la larve à maturité est une espèce non-identifiée de Poaceae. La chenille L5 et son fourreau, l'habitus et les genitalia mâles et femelles sont figurés. L'espèce est comparée à *C. nevadella* Baldizzone, 1985, espèce ici confirmée pour la France où elle est inféodée à *Nepeta latifolia* DC. dans les Pyrénées-Orientales. Enfin, la validité de cette nouvelle espèce est confirmée par l'étude des codes-barres ADN du groupe *lixella*. Les codes-barres ont aussi révélé la présence de *C. tricolor* Walsingham, 1889 en France et en Grèce alors que l'espèce n'était auparavant connue que de la Grande-Bretagne.

* CXXXth contribution to the knowledge of Coleophoridae

Keywords
Coleophora caucasica, *Coleophora lixella*, *Coleophora malatiella*, *Coleophora nevadella*, *Coleophora ornatipennella*, *Coleophora samarensis*, *Coleophora tricolor*, DNA barcodes, genitalia, *Nepeta*

Introduction

The *Coleophora lixella* group is currently composed of the following seven species: *C. ornatipennella* (Hübner, 1796), *C. lixella* Zeller, 1849, *C. caucasica* Stainton, 1867, *C. tricolor* Walsingham, 1899, *C. malatiella* Toll, 1952, *C. nevadella* Baldizzone, 1985, and *C. samarensis* (Anikin, 2001). The group composition was initially circumscribed by Toll (1952), and subsequently expanded by Baldizzone (1985) and Anikin (2001) to include their newly described species.

The group is defined by the following combination of characters: apically falcate forewings; antennal scape with a long tuft, base of the flagellum thickened with spreading scales; very elongate, narrow tegumen with almost straight sides and abruptly widened and angled pedunculi; elongate and upcurved cucullus; very elongate vesica about twice the length of the phallus; very long phallic appendix with 10–20 coils; sterigma with two elongate, digitiform lateral bars; colliculum very slender and as long or longer than S8; spinulate section of ductus bursae with one very long coil; papillae anales thickened and melanized; larva feeding on two different host plants, the florets or seeds of a Lamiaceae in its early stages, switching to mining leaves of Poaceae in its late stages, making a different case on each host. It remains undetermined whether these features are apomorphic or have phylogenetic value, but members of the group share a distinctive external aspect and male and female genitalia, and the switch from dicot to monocot hosts during larval development is unique.

Emmet (1996, p. 128, 276) seems to be the sole author to have also included *C. ochrea* (Haworth, 1928) in the *lixella* group. However, his reason for doing so was not explicitely stated and was probably based on similarities that he mentioned in his diagnosis of the group, notably the tubular phallus, short sacculus, the antennal scape with a long tuft, the base of the flagellum thickened with spreading scales, and the falcate forewings. None of these characters is unique to the group. *Coleophora ochrea* differs in several male and female genitalia features from species of the *lixella* group, notably the differently shaped, shorter tegumen, short and spatulate cucullus, lack of lateral bars on the sterigma, colliculum shorter than S8, and the short and straight spinulate section of the ductus bursae. The case-making and feeding habits of its larva are different as well: unlike members of the *lixella* group, *C. ochrea* uses a single host plant during its development and enlarges its case in sections as it grows. Because of these differences, we exclude *C. ochrea* from the *lixella* group, although its affinities remain undetermined. The recent phylogenetic (molecular) framework for Coleophoridae (Bauer et al. 2012) did not analyze *C. ochrea*.

The most widespread species in the *lixella* group is *C. ornatipennella*, which is distributed over most of Europe and extends in Asia from Turkey to Siberia and China;

C. tricolor was known only from Great Britain but is here reported from continental Europe; *C. malatiella* is known from Romania, Turkey, Ukraine, and Iran; *C. nevadella* was formerly known only from Spain but its presence in the western Pyrénées region of France is established in the present work (see below); *C. samarensis* is known from southern Russia and from Georgia to the Ukraine; and *C. caucasica* is known from Georgia (where the type locality is), Armenia, and Turkey (Baldizzone et al. 2006). Details of the larval life history are provided in Emmet (1996) for the two British species, *C. lixella* and *C. tricolor*.

In recent years DNA barcoding of many specimens has led to a re-assessment of species occurrences (Landry et al. 2013) as well as revealed the presence of additional, undescribed species. Among the recently detected undescribed species is the one we describe as new in the present work. In 2001, JN had already recorded it in his Atlas under entry no 140b as "*Coleophora lixella (Eupista)* cf. *malatiella* Toll, 1952", in addition to noting its close relationship to specimens of *C. nevadella*. In 2012, GB found in the Varaita Valley (Cottian Alps, Piedmont) of northern Italy a large population of a *Coleophora* associated with *Nepeta nepetella*: the adults were bigger and looked different from typical *C. lixella*. Examination of the genitalia confirmed that it was an undescribed species closely related to *C. nevadella*, and that it matched species 140b reported in Nel (2001).

Taxonomy

Coleophora nepetellae **Baldizzone & Nel, sp. n.**
http://zoobank.org/8F1DE90F-4535-49E5-945E-1BBCFA933E3A
Barcode Index Number: BOLD:AAI9227

Type material. Holotype ♂ (genitalia slide Bldz 15711): [**Italy**] "PIEMONTE |V.[alle] Varaita | Pontechianale (CN) | Grangia del Rio 2000 m | 30-VII-2012 | G. Baldizzone *leg.*"; "Database # | CNCLEP | 00110051"; "Barcode of Life Project | Leg(s) removed | DNA extracted" [blue]. In coll. Baldizzone, Asti.

Paratypes: **Italy**: 14 ♂, 12 ♀, Piemonte, same locality and date as holotype, coll. Baldizzone, barcoded specimens # CNCLEP00110052–CNCLEP00110060; 14 ♂♂ (genitalia slide Bldz 15535), 12 ♀ (genitalia slide Bldz 15536, 15712), *ibidem*, 2-VIII-2012, coll. Baldizzone; 4 ♂, 8 ♀, *ibidem*, 22.VII.2013, coll. Baldizzone; 1 ♂, *ibidem*, 2.VIII.1986, G. Bassi leg., coll. Bassi.

France: 1 ♀, Alpes-Maritimes, Le Pra sur Tinée, 1700 m, 44.3227°N, 6.8849°E, ex *Nepeta nepetella*, 23.VII.2000, J. Nel *leg.*, coll. J. Nel, La Ciotat; 1 ♀, Alpes Maritimes, Roubion, 44.093°N, 7.0511°E, 1630 m, 9.VII.2011, Th. Varenne *leg.*, coll. Th. Varenne, Nice; 1 ♂, Alpes-Maritimes, Tende, col de Tende, 44.15°N, 7.5667°E, 1830 m, 21.VII.1995, Th. Varenne *leg.*, coll. Th. Varenne; 1 ♀, Alpes-Maritimes, Casterino, 44.0986°N, 7.5059°E, 2000 m, 13.VIII.2013, ex *Nepeta nepetella*, J. Nel *leg.*, coll. J. Nel; 1 ♂, Alpes-de-Haute-Provence, St-Ours near Meyronnes, 44.4805°N, 6.8086°E,

15.VII.2005, 1800 m, *leg*. Jacques Nel, specimen # CNCLEP00033958, genitalia slide MIC 6834, barcoded, Canadian National Collection, Ottawa; 1 ♀, Alpes-de-Haute-Provence, 2 km E Meyronnes, 44.4711°N, 6.8086°E, 1575 m, 2.VII.2005, *leg*. C & FK Gielis, specimen # CNCLEP00029208, genitalia slide MIC 6835, barcoded, coll. H. van der Wolf, Netherlands.

Diagnosis. *Coleophora nepetellae* is a relatively large *Coleophora*, whose forewings are predominantly yellow with fine silvery striae. It belongs to the *C. lixella* species group and is most similar to *C. nevadella*, both externally and in genitalia. The latter species was formerly known only from Spain (Vives Moreno 1991) but is here reported from France for the first time based on material collected by JN (see below).

Externally the adult of *C. nevadella* from the Sierra Nevada (the type locality in Spain) (Fig. 2) is on average smaller than that of *C. nepetellae*, its forewings are devoid of brown scales, and the silvery striae are inconspicuous or nearly absent. In male genitalia (Figs 6, 11), *C. nevadella* has the valvula smaller with its outer margin more oblique and its ventral margin barely reaches the dorsal edge of the sacculus, whereas in *C. nepetellae* the ventral margin of the valvula is extended nearly to the ventral edge of the sacculus. The apex of sacculus is more prominently bulged in *C. nepetellae* than in *C. nevadella*.

In female genitalia, *C. nepetellae* is easily distinguished from *C. nevadella* by the following differences: in *C. nevadella* the colliculum is more elongate and has two long and thin lateral bars that are extended nearly to the distal margin of the sterigma (Fig. 12); these bars are very short in *C. nepetellae* (Fig. 13); in *C. nevadella* the median lamina in the ostium bursae is long, thin, and extended posterad of the ostium whereas in *C. nepetellae* it is more robust, irregularly delineated and with sclerotized lateral extensions; finally the paired longitudinal bars of the sterigma are narrow with the inner margins smooth in *C. nevadella*, whereas they are rough-edged with small, spinulose protuberances in *C. nepetellae*.

Description. *Adult*. Wingspan 22–26 mm. Head white shaded with yellow on vertex. Antenna with scape cream yellow, with thick tuft of erect, concolorous scales; flagellum white annulated with pale ochreous yellow, nearly indistinctly so on upper surface, dark brown on lower surface; basal third with elongate cream-coloured scales. Labial palp white, third article about as long as second; second article with long tuft of erect scales on ventral surface. Thorax white with a median cream yellow line; tegula cream with thin white border.

Forewing with apex falcate, curvature variable; ground colour cream yellow, paler in dorsal half, costal half slightly darker from scattering of brown scales, mainly in area between median line and costa; apical portion with 4–5 short, oblique silvery striae; one fine silvery stria in subcostal area near base, widening to quarter of wing and lined with brown on costal side; second silvery stria in median area from basal third to margin; third silvery stria along anal fold and interrupted before margin; fourth silvery stria along dorsal margin, very short and inconspicuous. Fringe dark cream-coloured along costal margin and around falcate apex; on dorsal margin, fringe pale grey with a line of pale cream basally. Hindwing grey, sometimes with brownish hue, fringe coloured as in forewing. Abdomen pale dirty white.

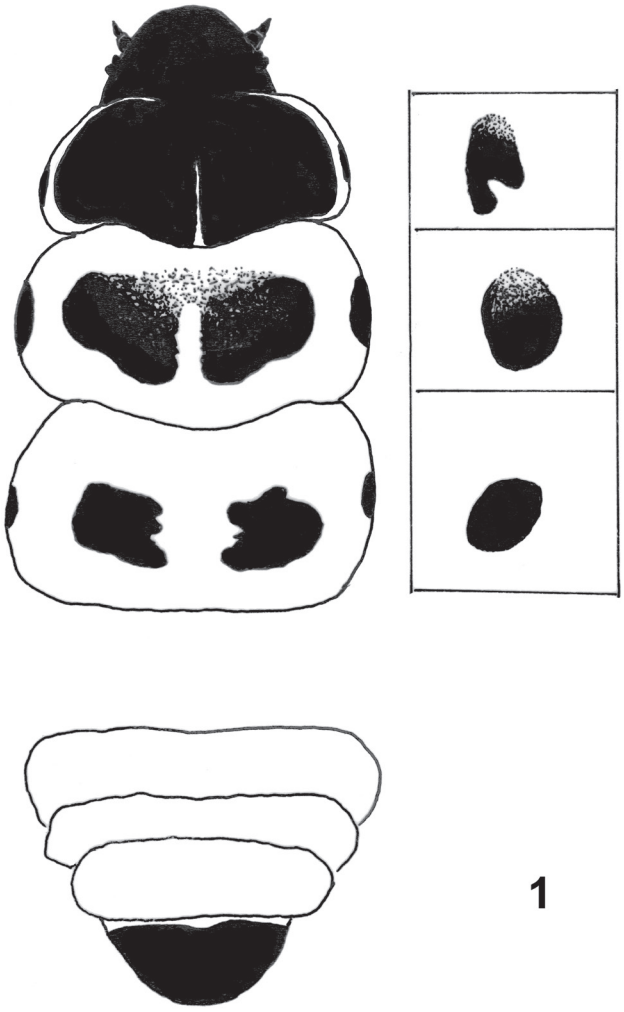
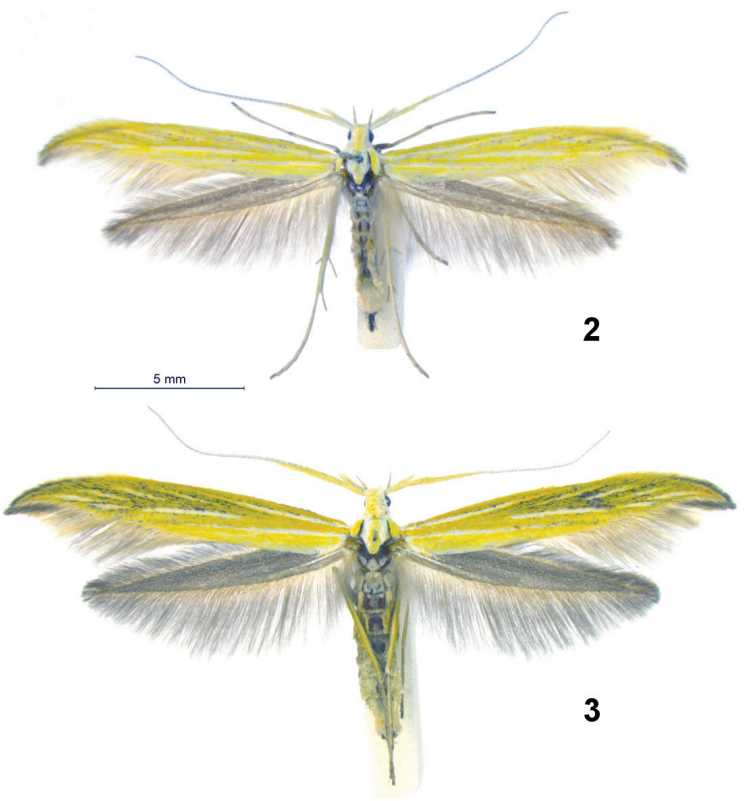


Figure 1. *C. nepetellae*, schematic illustration of 5th larval instar.

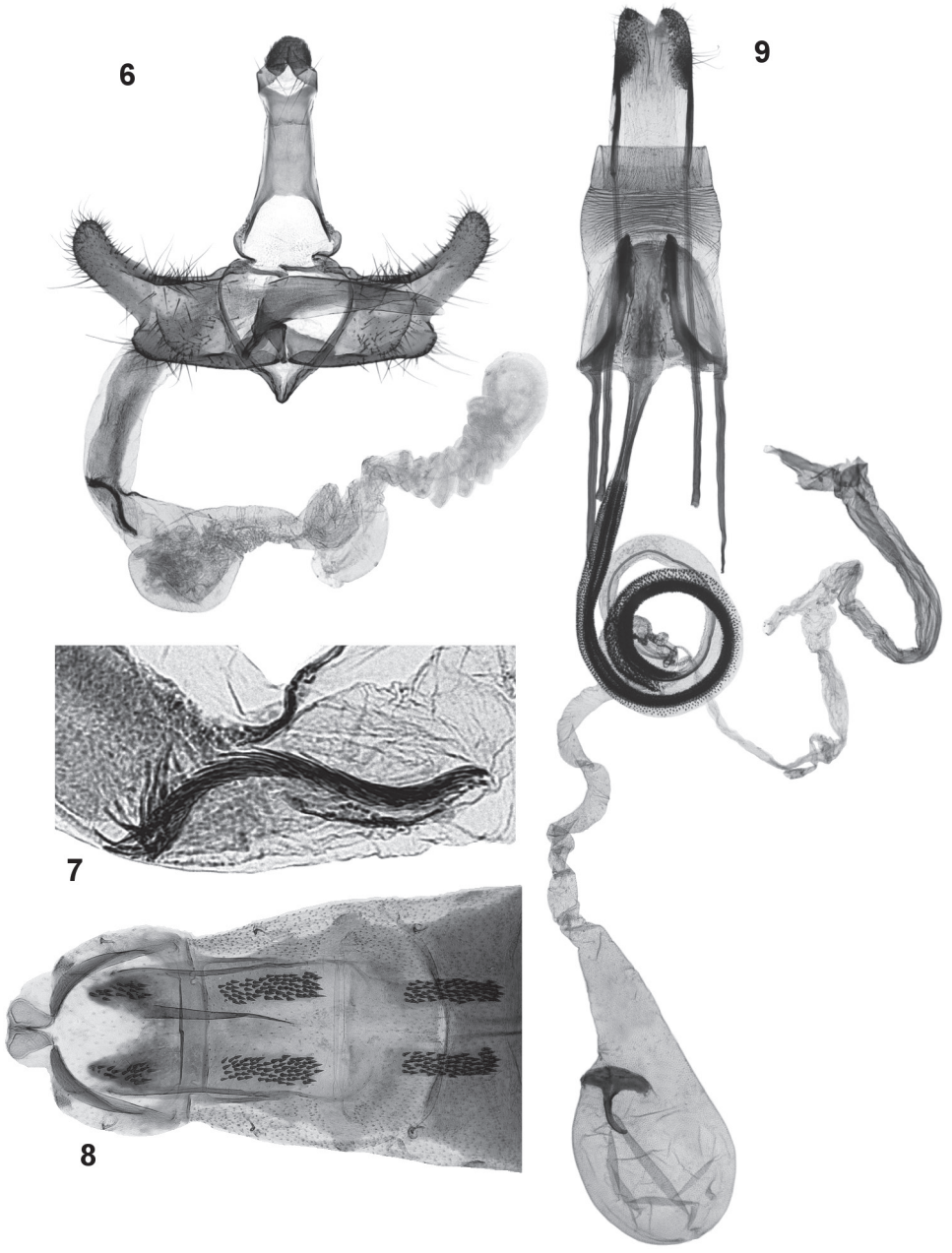
Abdominal apodemes (Fig. 8): Latero-anterior bars about twice as long as latero-posterior ones. Transverse bar long, proximal edge straight and thin, distal edge slightly convex around tergal sclerites. Tergal sclerites covered with conical spines, about 5–6× longer than wide (on T3).

Male genitalia (Figs 6, 7, 11): Gnathos knob large, globose. Tegumen narrow, elongate, pedunculi slightly outwardly flared. Transtilla thin, linear. Valvula wide with rounded ventral margin. Cucullus large, markedly sclerotized, wider basally, obliquely oriented, dorsal side slightly convex and apex rounded. Phallotheca elongate-conical, ventral portion less sclerotized. Cornuti (Fig. 7) thin, tightly arranged in elongate bundle.

Female genitalia (Figs 9, 13): Papillae anales markedly sclerotized, elongate. Posterior apophysis twice as long as anterior one. Sterigma conical laterally with pair of



Figures 2–5. Adults and larval case of *C. nepetellae* and *C. nevadella*. **2** *C. nevadella*, female: Spain, Sierra Nevada, Camino de la Veleta, 1600 m, 19.VII.1985, G. Baldizzone and E. Traugott-Olsen leg. (coll. Baldizzone) **3** *C. nepetellae*, female paratype: Italy, Piemonte, Valle Varaita, Pontechianale, Grangia del Rio, 2000 m, 22.VII.2013, G. Baldizzone leg., (coll. Baldizzone) **4** *C. nepetellae*, female paratype: France, Alpes Maritimes, Roubion, 1630 m, 09.VII.2011, Th. Varenne leg. (coll. Th. Varenne) **5** *C. nepetellae*, larva in it case: France, Alpes-de-Haute-Provence, Montagne de Lure, 1519 m, 31.V.2013, on grass near *N. nepetella*, J. Nel leg. (photo © Th. Varenne).



Figures 6–9. Genitalia of *C. nepetellae*. **6** male genitalia (genitalia prep. Bldz 15535) **7** male genitalia, closeup of cornuti (genitalia prep. Bldz 15711, holotype) **8** Male abdominal segments 1–3 (genitalia prep. Bldz 15535) **9** Female genitalia (genitalia prep. Bldz 15536).

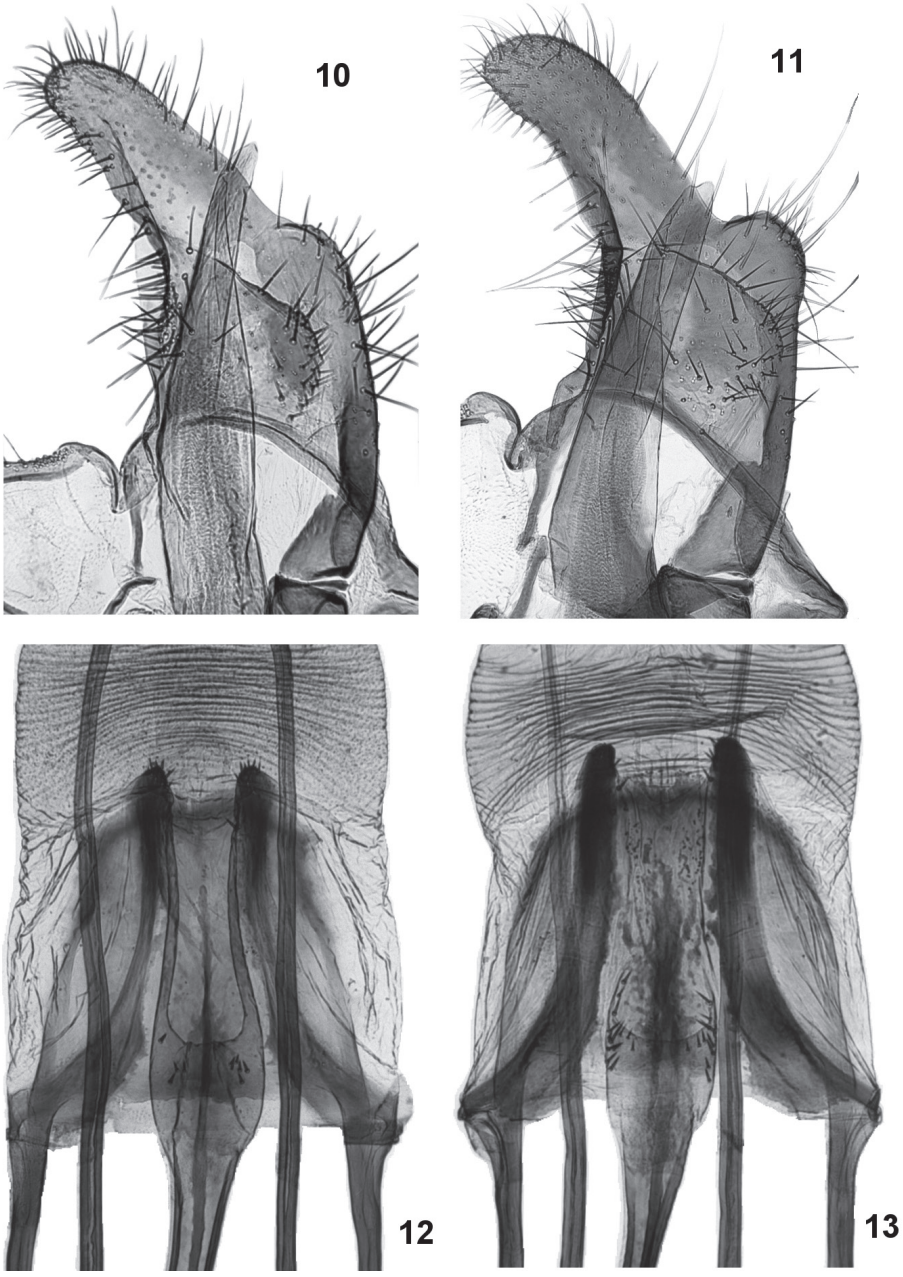
longitudinal sclerotized, outcurved bars, curvature more pronounced on outer side, distal portion of bars parallel to each other. Ostium bursae calix-shaped. Widest distal section of colliculum with small conical spines, proximal section narrowed with lateral edges more sclerotized, median lamina extended from smooth, looped section of ductus bursae to widened portion of colliculum. Ductus bursae lined with short conical spinules from anterior end of colliculum to first loop (about 4× length of sterigma); second loop without spinules (about 2× length of sterigma); anteriormost section of ductus transparent, without ornamentation. Corpus bursae ovoid with distal half tapered, signum leaf-like.

L5 larva (Fig. 1): Length 9 mm. Body brown with faint brown dorsal line interrupted at segment junctions. Head shiny black. Thoracic shields shiny black; prothoracic shield wide, very finely cracked along median axis from middle to posterior edge; mesothoracic shield made up of two wide plates irregular in shape, separated by a median gap except thinly and narrowly joined anteriorly; metathoracic shield made up of two smaller plates separated by a gap, irregular in shape with denticulate inner edges. Spiracular sclerites shiny black, present on all thoracic segments: oblong on prothorax, oval on mesothorax and metathorax, largest in size on mesothorax. Thoracic legs entirely shiny black. Prolegs on A3–A6 with 5–7 crochets in two uniordinal rows. Anal plate shiny black. Anal proleg half-moon-shaped, each with 15–18 crochets. (Description based on larva from France, Alpes-de-Haute-Provence, Montagne de Lure, 1519 m, 31.V.2013, found on grass beside *Nepeta nepetella*, J. Nel *leg*.).

Derivation of specific epithet. The species epithet is derived from the species name of its larval host, *Nepeta nepetella*.

Biology. The host plant of *Coleophora nepetellae* is *Nepeta nepetella* L. (Lamiaceae). This plant has narrow, dentate, whitish green leaves, and large white, hairy corollas with the pistils extended beyond the chalices. This is a mountain plant that occurs throughout the southern Alps, in France mainly in the most sun-exposed parts of the foothills, between 1000 and 2000 m in elevation, along roads, at the base of screes, or near sheepfolds. It blooms between mid-June and late August, depending on elevation and exposure. *Nepeta nepetella* is the initial food plant from which the larva makes its first case. The second host plant which serves to construct the final case is a unidentified Poaceae.

Oviposition and larval development between late summer and overwintering was not observed directly but it can be inferred with much certainty that the eggs are deposited on or in the chalices, and that the young larvae build a case from a seed, as do all the other species of the *lixella* group for which the biology is known. Overwintering probably takes place on the ground or in the litter. In the spring, it was observed that the young larva lives in a case made from a hollowed-out piece of grass. Grasses used for case-making are species with broad leaves that grow in close proximity to *Nepeta* plants which become the larval host. This habit of using different host plants before and after overwintering is exceptional among leaf-mining Lepidoptera and occurs among all the species of the *lixella* group for which the biology is known. The case is not enlarged during larval development (as in *C. ornatipennella*, for example) but is



Figures 10–13. Genitalia of *C. nepetellae* and *C. nevadella*. **10** *C. nevadella*, male genitalia, closeup of valva and distal portion of phallotheca (genitalia prep. Bldz 6162 – paratype): Spain, Granada, Sierra Nevada, 1000 m, Puerta de la Ragua, 20.VII.1969, K. Sattler & D.J. Carter (coll. BMNH) **11** *C. nepetellae* male genitalia, closeup of valva and distal portion of phallotheca (genitalia prep. Bldz 15535) **12** *C. nevadella*: female genitalia, detail of sterigma and colliculum (genitalia prep. Bldz 15713): Spain, Sierra Nevada, Camino de la Veleta, 2050 m, 22.VII.1985, G. Baldizzone and E. Traugott-Olsen (coll. Baldizzone) **13** *C. nepetellae* female genitalia, detail of sterigma and colliculum (genitalia prep. Bldz 15712).

abandoned for a new, larger one at each instar. The final case (Fig. 5) is constructed from a piece of mined grass leaf which is hollowed out. It is 13–15 mm long, about 3 mm at its widest girth, straw-coloured, slightly darkened, with longitudinal ridges made by the veins from the leaf used in its construction; the oral opening is rounded, at 30°; the anal end has the terminal 3 mm dorso-ventrally flattened; the median portion is slightly broader, fusiform.

Phenology. The species has one generation per year, with the adults emerging between July 20 and the first week of August in the Valle Varaita in Italy. In France at higher elevations adult emergence extends into the middle of August. In all locations adult flight coincides with the flowering of the host plant, *Nepeta nepetella*. The adults fly in bright sunshine, especially during the afternoon and take short flights among the flowering stems. The new species coexists with *C. lixella* which flies among *Thymus* cf. *serpyllum* L. (Lamiaceae), its host plant.

Type locality. Italy, Piemonte, Valle Varaita, Pontechianale, Grangia del Rio, 2000 m, 44.6625°N, 6.9939°E. The Grangia del Rio is a side valley of the Valle Varaita through which runs a tributary of the Varaita River. It is situated in the Cottian group of the Western Alps in northwestern Italy. The host plant, *Nepeta nepetella*, from which type material was obtained grows there within 30–40 feet of a pastoral trail and at the base of a rock slide.

Geographical distribution. In Italy the species is known only from the type locality in the Piemont Region. In France, it is recorded from the Alpes-Maritimes, Upper Var, Alpes-de-Haute-Provence, Hautes-Alpes, Isère, the Drôme, and further to the west from the Vaucluse where it is common on the slopes of Mont Ventoux wherever *Nepeta nepetella* grows.

Records of *C. nevadella* from France

In his Atlas of Coleophoridae of France, Nel (2001) recorded a species of the *lixella* group from the Eastern Pyrénées (Cerdagne area). He labelled it "*Coleophora lixella* (*Eupista*) cf. *nevadella* Baldizzone, 1985" (under his entry no 140c) and indicated that the identification was tentative. *Coleophora nevadella* was formerly known only from Spain (Vives Moreno 1991). Nel reported finding adults among *Nepeta latifolia* DC., a Lamiaceae distributed in the Iberian Peninsula which reaches its northern limit in southwestern France. We confirm here that *C. nevadella* is indeed the species tentatively reported from France by Nel (2001). The adults occur in July on blooming *Nepeta latifolia* plants, which is the likely oviposition host plant.

Record details. 3 ♂, 2 ♀,: France, Pyrénées-Orientales, Mont-Louis, route D10 to Sauto 11.VII.1990, imagos on *Nepeta latifolia*, J. Nel leg.; 2 ♂, ditto, 28.VII.1993. 6 ♂, Pyrénées-Orientales, Védrignans, 1400 m, 16.VII.1992, imagos on *Nepeta latifolia*, J. Nel leg.; 1 ♀, ditto, 30.VII.1993 (Jacques Nel Collection and Tyroler Landemuseen, Innsbruck). 1 ♂, Porté-Puymorens, vallon de Passet, 1650 m, 17.VII.2004, T Varenne leg. (Thierry Varenne Collection, Nice).

DNA barcode analysis

Tissue samples (dried legs) were shipped to the Canadian Centre for DNA Barcoding in Guelph for DNA extraction, amplification, and sequence analysis. Laboratory protocols at this facility have been optimized, and the current iteration can be accessed at http://www.ccdb.ca. In short, a small tissue sample is lysed and genomic DNA extracted using an automated, silica-based method; the COI barcode region is amplified via PCR using one or more primer sets (Hebert et al. 2013) and successful amplicons are then bi-directionally sequenced (deWaard et al. 2008). The resultant sequences, along with the voucher data, images, and trace files, are deposited in the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007; www.barcodinglife.org), with sequences > 600bp subsequently deposited in GenBank. Sequences longer than 100 bp were included in the analysis.

Barcoding efforts included the holotype and 11 paratypes of the new species as well as representatives of several species of the *lixella* group. Several specimens of this species group that had already been barcoded independently of the present work as part of the Lepidoptera barcoding campaigns were also selected for the comparative analysis.

The Barcode Identification Numbers (BINs) (Ratnasingham and Hebert 2013) in BOLD are used as registry designations for barcode clusters. Neighbor-joining trees and genetic distances were calculated with MEGA 5.05 (Tamura et al. 2011) using the Kimura two-parameter (K2P) model of base substitution (Kimura 1980). Details of the barcoded specimens and their photographs are available through the following dataset (http://dx.doi.org/10.5883/DS-CNEPETA). The same DOI provides access to the sequence records, trace files, and primer sequences used for PCR amplification, together with GenBank accession numbers.

DNA barcode results (Table 1, Fig. 14).

Eighty-seven specimens were successfully sequenced, resulting in a 658 bp, full-length barcode fragment for 63 specimens, and fragments of more than 600 bp for a further 17 specimens; two sequences longer than 500 bp, and five sequences shorter than 400 bp were also included in the analysis, whereas sequencing of 11 specimens failed. Only one of the analyzed species, *C. nevadella*, failed to yield any sequence, as did two of the *C. nepetellae* paratypes. Failure to obtain *C. nevadella* sequences (four specimens were processed) was disappointing because this is the species deemed morphologically most similar to *C. nepetellae*, with which it is compared in the diagnosis above.

Barcodes from the holotype and 11 paratypes of *C. nepetellae* were obtained. Six paratypes yielded full barcodes whereas five yielded sequences between 605–639 bp; the holotype barcode was 622 bp with two ambiguous positions. Barcodes of *C. nepetellae* were compared to barcodes from 12 other BINs representing at least nine distinct or putative species in the *lixella* group. Inter-group distances ranged from 1.4 to 10.2%, with an average of 7.4%. *Coleophora nepetellae* is markedly divergent from

Table 1. Percent sequence divergence in cytochrome *c* oxidase I gene among 87 specimens representing 13 species clusters (BINs) of the *Coleophora lixella* group. Cells below diagonal = mean inter-cluster distances in %; diagonal cells = mean intra-cluster distances. "BOLD:ABC1234" = Barcode Index Numbers.

	lixella-group I BOLD:AAB2197	tricolor BOLD:AAE8791	lixella BOLD:ACE7459	lixella BOLD:ACE7458	lixella BOLD:AAB2196	lixella-group II BOLD:AAC8630	lixella-group III BOLD:ACM4218	ornatipennella BOLD:AAB2195	caucasica BOLD:ABZ6687	lixella-group IV BOLD:ACM4689	malatiella BOLD:AAJ6597	nepetellae BOLD:AAB2198	samarensis BOLD:AAI9227
lixella-group I BOLD:AAB2197 (n=1)	n/c												
tricolor BOLD:AAE8791 (n=6)	7.0	0.8											
lixella BOLD:ACE7459 (n=6)	4.7	6.3	0.3										
lixella BOLD:ACE7458 (n=2)	5.5	6.4	1.6	0.2									
lixella BOLD:AAB2196 (n=8)	5.1	6.7	1.4	1.4	0.5								
lixella-group II BOLD:AAC8630 (n=4)	5.8	9.5	7.4	8.3	7.9	0.6							
lixella-group III BOLD:ACM4218 (n=1)	6.8	8.3	6.8	7.5	7.6	5.4	n/c						
ornatipennella BOLD:AAB2195 (n=27)	7.1	9.4	7.6	8.3	7.9	7.3	7.4	0.2					
caucasica BOLD:ABZ6687 (n=14)	9.1	10.2	9.7	10.1	10.0	8.9	8.9	3.5	0.7				
lixella-group IV BOLD:ACM4689 (n=1)	6.9	8.9	8.1	8.5	8.3	6.8	7.1	2.7	3.9	n/c			
malatiella BOLD:AAJ6597 (n=1)	7.5	8.9	7.6	7.9	7.7	8.4	8.6	8.2	9.6	7.4	n/c		
nepetellae BOLD:AAB2198 (n=12)	9.2	9.6	8.8	9.1	9.3	8.0	7.7	7.4	8.9	7.6	9.2	0.2	
samarensis BOLD:AAI9227 (n=4)	8.4	9.3	8.2	8.5	8.2	5.9	6.4	7.4	8.7	7.3	8.5	3.9	0.1

all other *lixella*-group clusters, with distances ranging from 3.9 to 9.6%, with *C. samarensis* the closest species. The intraspecific distance was low and varied from 0.1–0.8%, with an average of 0.3%. Despite the lack of *C. nevadella* sequences, the wide barcode

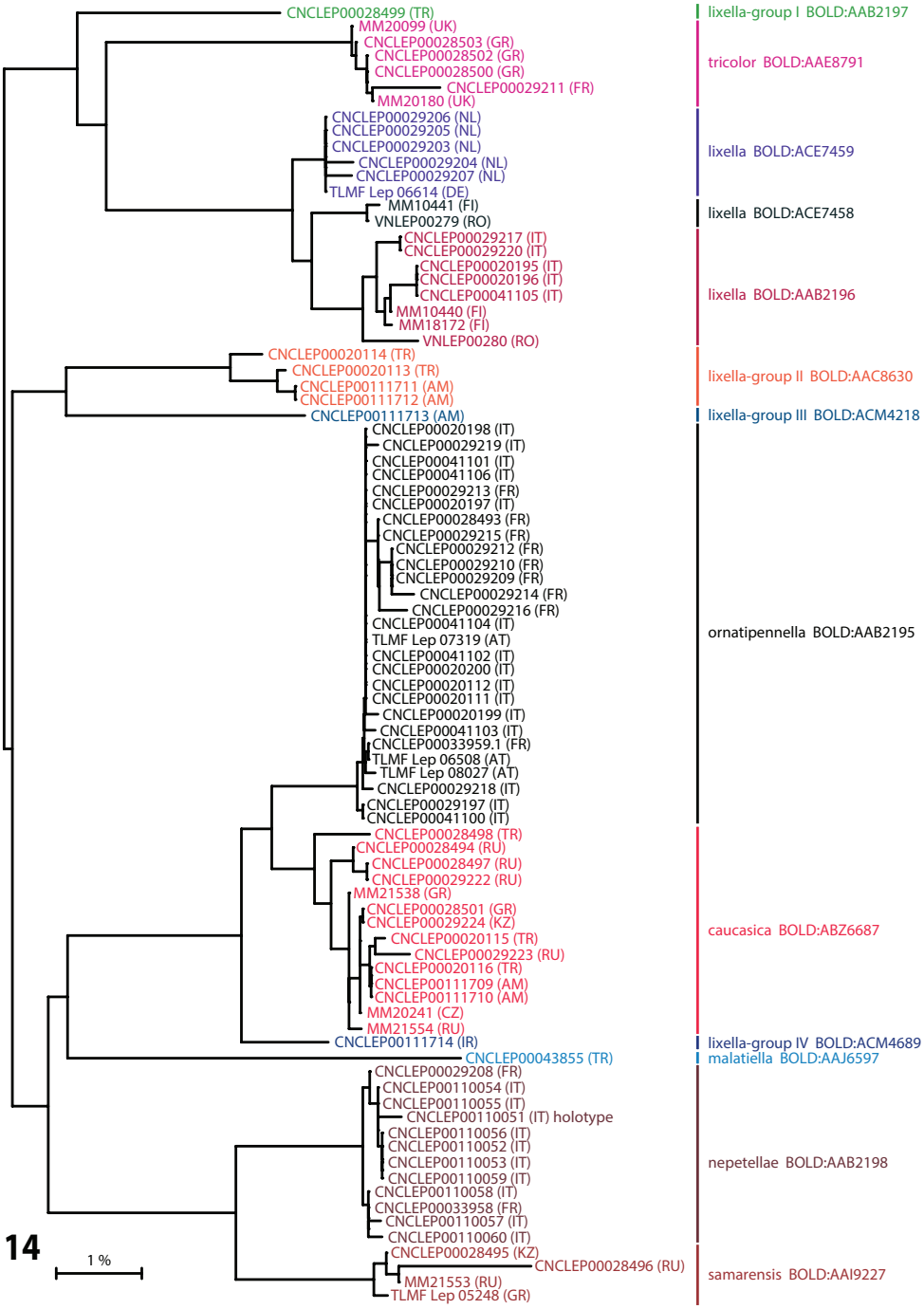


Figure 14. Neighbor-joining tree of K2P distances for the barcode region of the cytochrome *c* oxidase I gene among 87 specimens representing 13 species clusters of the *Coleophora lixella* group. End-branch labels are specimen ids followed by the geographic area in parentheses and the Barcode Index Number (BIN). Scale bar = 1%.

gaps observed among the established, morphologically distinct species of the *lixella* group strongly suggest that that species would also show marked barcode divergence from *C. nepetellae* and others in the group.

The majority of BINs had low intra-group divergence and thus seemed taxonomically well defined, including the new species, *C. nepetellae*. This is congruent with the relatively subtle but consistent differences observed in genitalia which have been used by authors to distinguish those species. However, some specimens in four BINs (BOLD:AAB2197, BOLD:AAC8630, BOLD:ACM4218, BOLD:ACM4689, also labelled as '*lixella*-group I–IV in Fig. 14) were characterized by pronounced barcode divergence similar or exceeding those of described species, suggesting that they constituted putative undescribed diversity, as has been documented elsewhere in Lepidoptera (for examples, see Huemer and Hebert 2011, Huemer et al. 2012, Huemer et al. 2013, Kaila and Mutanen 2012, Landry and Hebert 2013, Mutanen et al. 2012a, b, Segerer et al. 2011, Wilson et al. 2010). Additionally, specimens identified as *C. lixella* on the basis of genitalia separated into three different BINs which showed the shortest inter-group distances (1.4–1.6%) among all species analyzed. These *C. lixella* specimens were all from continental Europe. Retrospectively we observed that the three *C. lixella* BINs correlated with minor differences in genitalia among them but our sampling was too limited to clarify the situation. This suggests either marked haplotype variation within this species, or cryptic diversity. Further study is needed using more extensive material, but resolution of this problem is beyond the scope of the present paper.

New continental records of *C. tricolor*

Barcode Index Number: BOLD:AAE8791

Barcoding results revealed that *C. tricolor* occurs in southern France and in Greece (Fig. 14 and online dataset given above). This indicates that this species, previously thought to be restricted to Great Britain, has a much wider distributional range than previously considered. This also suggests that many existing continental records of *C. lixella* or *C. ornatipennella* that were not checked by genitalia dissections should be verified for their accuracy and for the possible misidentification of additional *C. tricolor* specimens.

Our results also highlight the difficulty in recognizing the species of the *lixella* group from morphology alone. Differences in external aspect, if present, are subtle and may be blurred by slight variations and compounded by specimen wear. Illustrations of the genitalia of species of the *lixella* group in the literature are: *Coleophora caucasica* (Anikin 2001); *lixella*, *malatiella*, *nepetellae* (as "*lixella* cf *nevadella*"), *lixella*, *ornatipennella* (Nel 2001); *nevadella* (Baldizzone 1985); *malatiella* (Toll 1952). These illustrations differ greatly in quality and comparisons are problematic. Genitalia differences are also small and careful preparations are required to examine and compare them. These will be dealt with more comprehensively in a work in preparation on the *lixella* group by the first author. However, we give here the main differences between *C. tricolor* and the pair *C. lixella* – *C. ornatipennella*, which most closely resemble each other.

Externally *Coleophora tricolor* can be tentatively distinguished from both *C. lixella* and *C. ornatipennella* by the fuscous annulations on the upperside of the distal half of the antenna, whereas the latter two species have that part of the antenna white, as do the other species of the *lixella* group. This character was pointed out by Emmet (1996: 276) but given its relative subtlety, it could be subject to geographic variation that has not been evaluated. Moreover, one must be careful to check the upper side of the antenna only: it is not uncommon for collection specimens to have the distal part of the antennae twisted around with the annulate lower sides turned upward.

In genitalia, males of *C. tricolor* have the ventral edge of the valvula conical, and the apex of the sacculus angular and broad; in *C. lixella* and *C. ornatipennella*, the ventral edge of the valvula is broadly rounded, as is the apex of the sacculus; the cucullus is more pronouncedly upturned in *C. lixella* than in the other two species. The appendix of the phallotheca has 13–14 coils in both *C. tricolor* and *C. ornatipennella*, versus only 10–11 in *C. lixella*; in *C. tricolor* the coils are wider in the middle of the appendix whereas in *C. ornatipennella* they are gradually widened towards the apex.

Females of *C. tricolor* have the colliculum about as long as the sterigma and the lateral bars of the sterigma with sharply delineated, concave outer edges and with apices outwardly curved; the spinules of the ductus bursae are small and restricted to the straight distal section except for a short patch at the anterior end of the first loop. Females of *C. lixella* have a similarly proportioned colliculum/sterigma but the apex of the lateral bars are straight and the outer edges sinuate, and the spinules of the ductus bursae larger and extended around three-quarters of the loop. In *C. ornatipennella* the colliculum is extremely narrow and more than 1.5x longer than the sterigma, the lateral bars of the sterigma are broader with diffusely delineated edges, and the spinules of the ductus bursae are much finer and extended only to about half of the loop.

Record details. 1 ♂: France, Provence-Alpes-Côte d'Azur, Hautes-Alpes, Les Laus, 6 km N Col d'Izoard, 1800 m, 30.VI.2003, C & FK Gielis leg., specimen CNCLEP00029211,genitalia slide MIC 6830, barcoded (coll. van der Wolf); 3 ♂: Greece, Macedonia, Kozani, near Xirolimni Village, 3.VI.2005, T. Nupponen leg., specimens CNCLEP00028500, 28502, 28503, genitalia slides MIC 5297, MIC 5299, barcoded (CNC).

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Acariform mites (Acariformes) - permanent symbionts of *Hapalomys delacouri* Thomas (Rodentia, Muridae) in Vietnam

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Abstract

Two new species of parasitic acariform mites (Acariformes) are described from the Delacour's marmoset rat *Hapalomys delacouri* Thomas (Rodentia: Muridae) in Vietnam: *Afrolistrophorus (Afrolistrophorus) hapalomys* **sp. n**. (Listrophoridae) and *Radfordia (Radfordia) mirabilis* **sp. n**. (Myobiidae). Based on morphological evidences, we show that species of both mite genera associated with *Hapalomys* Blyth do not demonstrate clear phylogenetic links with respective congeners from rodents of the closest genus *Chiropodomys* Peters (Rodentia: Muridae).

Keywords

Acariformes, Listrophoridae, Myobiidae, *Afrolistrophorus*, *Radfordia*, systematics, rodents, ectoparasites

Introduction

Marmoset rats of the genus *Hapalomys* Blyth (Rodentia: Muridae: Murinae) are medium-sized arboreal from Southeast Asia, with highly patchy distributions throughout their range from southern China to the Malay Peninsula. The genus consists of two

species, *Hapalomys delacouri* Thomas and *H. longicaudatus* Blyth (Musser 1972, Musser and Carleton 2005). Little is known about the life history of marmoset rats because of paucity of museum materials available for study. Parasitic mites of marmoset rats have never been reported.

Several specimens of the Delacour's marmoset rat *Hapalomys delacouri* were collected in southern Vietnam during the mammalogical surveys carried out by the Joint Vietnamese-Russian Tropical Research and Technological Centre (Abramov et al. 2012). In this paper, we describe two new mite species belonging to the families Listrophoridae and Myobiidae (Acariformes) collected from this host. Mites of both families are represented by permanent and highly specialized mono- or stenoxenous ectoparasites inhabiting the fur (Listrophoridae) and skin (Myobiidae) of mammals (Bochkov 2009, 2010).

Material and methods

In the field, the trapped hosts were individually wrapped in cheesecloth to prevent falling-out of ectoparasites and preserved in 70% ethanol. In the laboratory conditions, mites were collected from ethanol preserved hosts with fine forceps under dissection microscope and mounted in Hoyer's medium. Specimens were studied using a Leica microscope under phase contrast and Nomarsky (DIC) optics. Drawings were made with a camera lucida, and measurements were taken using a calibrated ocular micrometer. In the descriptions below, the idiosomal setation of listrophorid mites follows Griffiths et al. (1990) with modifications by Norton (1998) concerning coxal setae; the leg setation follows Grandjean (1939a). The idiosomal setation of myobiid mites follows Grandjean (1939b) as interpreted by Bochkov et al. (2008). All measurements are in micrometres (μm), provided for paratypes in parentheses, and were taken as follow: body length = the total length from the anterior extremity of the prescapular shield in listrophorids or the palpal extremites in myobiids to the posterior border of the body; body width = width at the level of setae *se* in listrophorids and setae *c2* in myobiids; length of dorsal shields(listrophorids) = maximum length, measured along the median line of the shields; length of opisthosoma (listrophorids) = length from the posterior margins of trochanter IV insertions to the posterior border of the opisthosoma; length of the posterior legs (listrophorids) = length from the most basal point of the trochanter to the apex of the tarsus, excluding pretarsus; tarsal length was measured without pretarsus. Host systematics follows Musser and Carleton (2005).

Abbreviations of institutions:

UMMZ University of Michigan Museum of Zoology, Ann Arbor, USA;
ZISP Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

Systematics

Family Listrophoridae Megnin & Trouessart
Genus *Afrolistrophorus* Fain
Subgenus *Afrolistrophorus* Fain

***Afrolistrophorus hapalomys* Bochkov & Abramov, sp. n.**
http://zoobank.org/ 8AADCBF1-40F9-4A00-9DB6-6ACDEFC2F49B
Figs 1, 2

Type material. Male holotype (ZISP L-T-9, AVB 10-0803-012), 7 male and 12 female paratypes (ZISP AVB 10-0803-012, 1-19) from *Hapalomys delacouri* Thomas (Rodentia: Muridae) [fur], VIETNAM: Binh Phuoc Province, Bu Gia Map National Park, 13 km NE Bu Gia Map Village, 540 m a.l.s., 12°11'37"N, 107°12'21"E, 13 January 2010, coll. A.V. Abramov (ZISP 99485). Mites removed by A.V. Bochkov.

Type deposition. Holotype and 17 paratypes deposited in ZISP, one female and one male paratypes in UMMZ.

Description. Male (holotype; paratypes = 7; Fig. 1). Body 360 long (350–385), 105 wide (100–110). Prescapular shield 110 long (105–110) with distinct median process. Postscapular shield 62 long (62-65), covered by 8-10 transverse markings; 2 anterior markings interrupted in median part. Median apodeme present. Hysteronotal shield 155 long (150–160), completely covered by few distinct striae from anterior margin to level of setal bases *e2*; these striae transverse in anterior one third of shield, oblique in middle, and almost longitudinal in posterior one third. Supranal concavity completely sclerotized. Shortest distance between postscapular and hysteronotal shields 10 (10–25). Setae *h2* 155 long (140–160); membranous setae *h3* well developed, about 35 wide, slightly overlapping, without ribs. Terminal cleft 30 long (30–37). Opisthosomal lobes about 20 maximum wide. Cuticle between coxal fields II not striated. Coxal apodemes III fused to each other. Aedeagus about 45 long. Diameter of adanal suckers about 8. Legs III and IV about 75 and 90 long, respectively. Tarsus III without dorso-subapical projection. Tarsi III 20 long (20–23) and tarsi IV 25 long (25–30). All setae of tarsi III and IV shorter than respective segments, excluding pretarsi; setae *d*III and *d*IV spur-like, setae *e*IV microspines. Solenidia *ω1*I, II 12–15 long, *ω3*I about 25 long, *φ*I, II 40–45 long.

Female (ranges for 10 paratypes, Fig. 2). Body 425–440 long, 100–115 wide. Prescapular shield 110–120 long. Anterior margin of prescapular shield with distinct median process. Postscapular shield about 75 long, covered by 7-9 transverse markings. Median apodeme present. Idiosomal surface between postscapular and hysteronotal shields with 3–4 transverse lines. Hysteronotal shield 70–80 long, crossed by 8–11 oblique striae, 3 posterior striae very short, situated medially. Hysteronotum posterior to hysteronotal shield with 18–20 transverse striae, sclerotized, but less than this shield properly. Opisthosoma about 180 long. Posterior end of opisthonotum without lateral

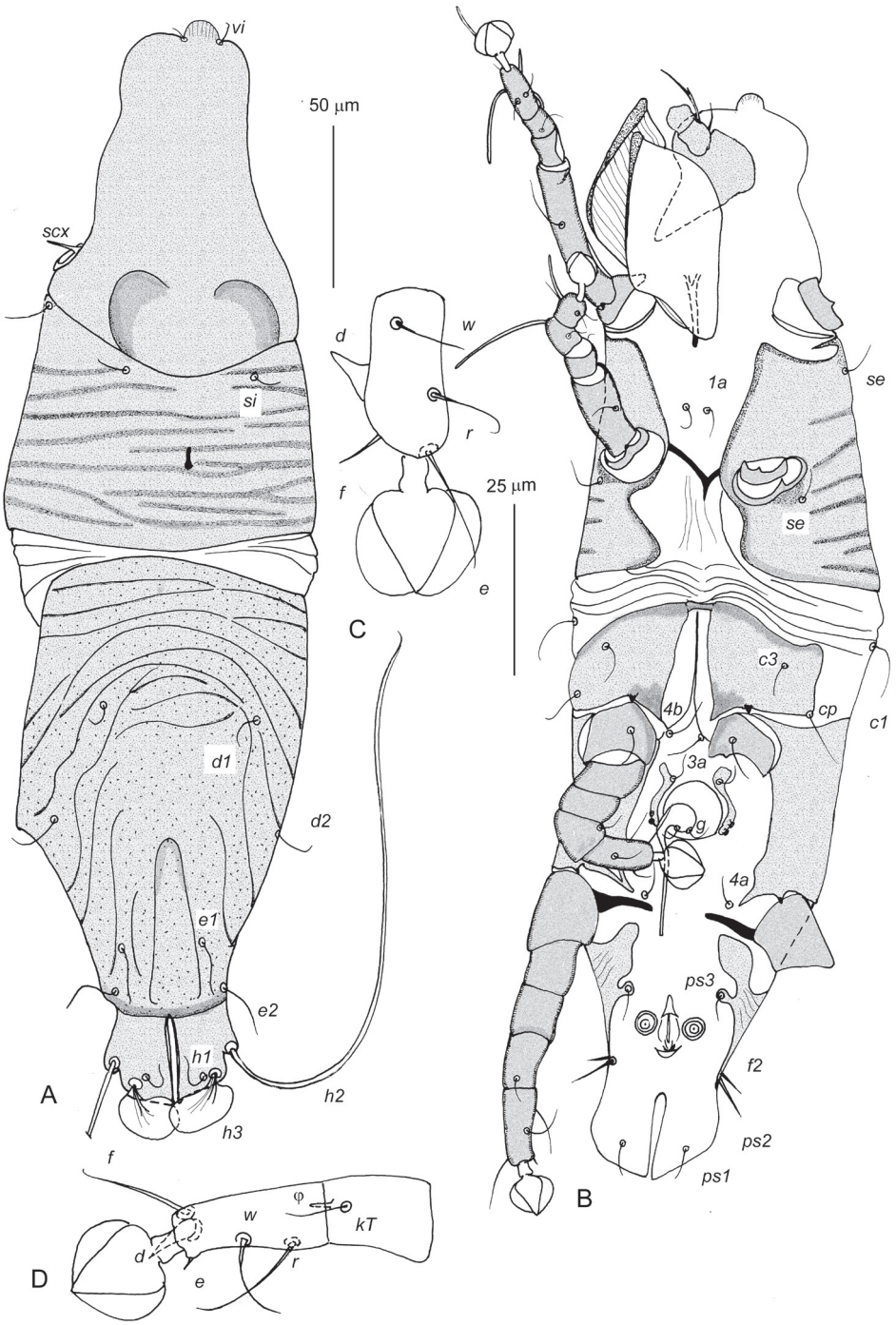

Figure 1. *Afrolistrophorus hapalomys* sp. n., male holotype, **A** dorsal view **B** ventral view **C** tarsus III in ventral view **D** tarsus IV ventral view. Scale bars: **A** and **B** = 50 μm; **C** and **D** = 25 μm.

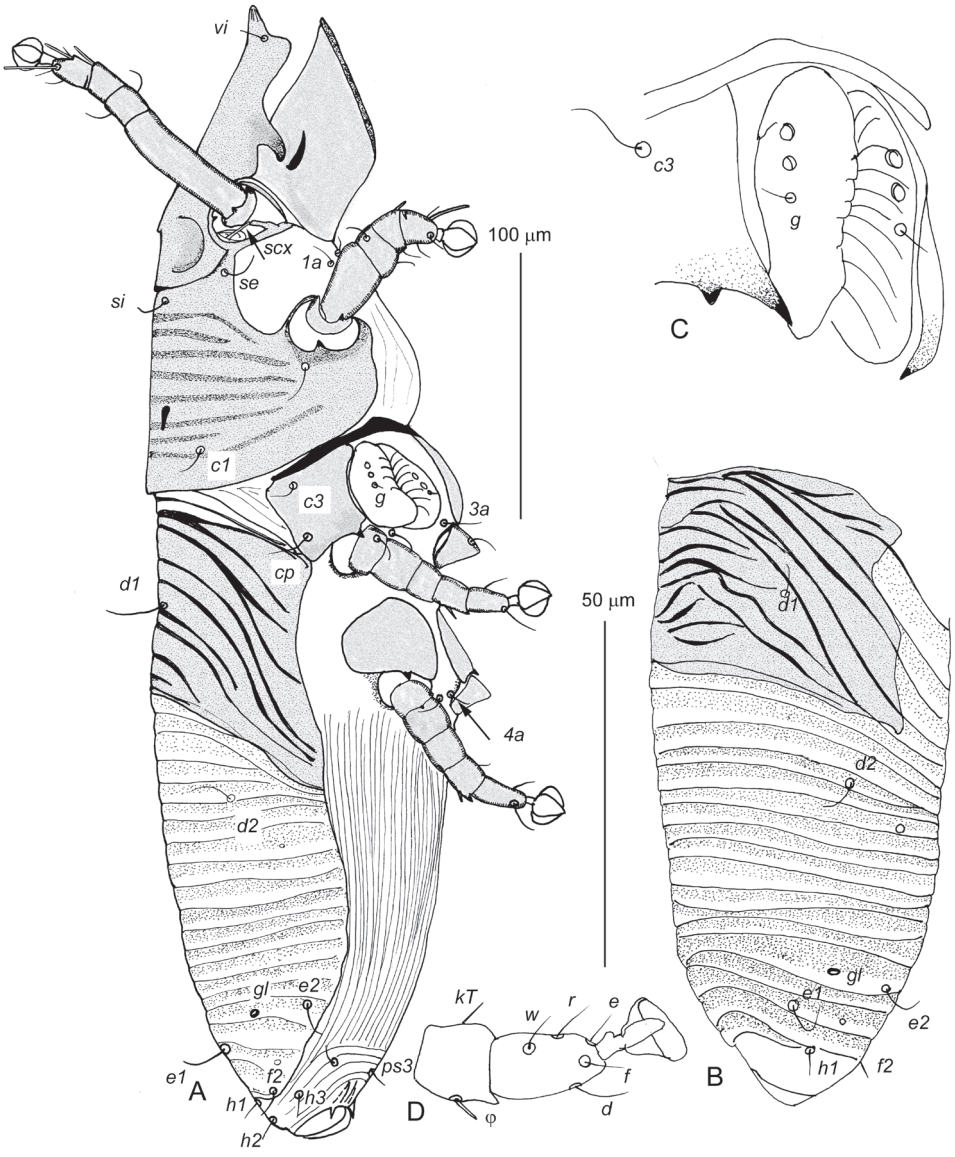

Figure 2. *Afrolistrophorus hapalomys* sp. n., female, **A** lateral view **B** opisthosoma in dorsal view **C** ovipore **D** tibia and tarsus III in ventral view. Scale bars: **A** and **B** = 100 µm; **C** and **D** = 50 µm.

sclerotized patches. Cuticle between coxal fields II not striated. Opisthogaster without scales or verrucae. Setae *h2* short, about 8 long, subequal in length to other opisthosomal setae. Setae *ps1, ps2,* and *4b* absent. Legs III and IV subequal, 65–70 long. Setae *d*III and *d*IV about 2 times shorter than respective tarsi, excluding pretarsus. Solenidia *ω1*I, II about 12 long, *ω3*I about 24 long, *φ*I, II about 8 long.

Etymology. The species name is derived from the generic name of the host and is a noun in apposition.

Differential diagnosis. This new species belongs to the "*apodemi"* species group, which includes twelve species parasitizing mostly Eurasian murines (Murinae). All species in this group have a median process on the anterior margin of the prescapular shield. In males, apodemes III are fused to each other; in females, the cuticle between coxal fields II is without distinct striations, setae *ps1* and *ps2* are either present or absent, setae *h2* are not longer than other opisthosomal setae (Bochkov and OConnor 2006; Bochkov et al. 2011). Among species of the "*apodemi*" group, the new species is close to *Afrolistrophoroides laonastes* Bochkov et al., 2011 from *Laonastes aenigmamus* Jenkins et al. (Rodentia: Diatomyidae) (Bochkov et al. 2011). In both species, the postscapular shield is distinctly developed and possesses several transverse markings and lacks a median sclerotized band; seta *d*III is much shorter than the respective tarsus (excluding the pretarsus); in males, the hysteronotal shield is ornamented in posterior part, setae *h3* are strongly widened and slightly overlap each other; in females, setae *4b*, *ps1* and *ps2* are absent, the ventral side of opisthosoma has no verrucae or scales. *Afrolistrophorus hapalomys* sp. n. differs from *A. laonastes* by the following characters. In both sexes of *A. hapalomys*, the postscapular and hysteronotal shields are covered by a few markings or striae (less than 15), setae *d*IV of tarsi IV are at least twice as short as the respective segment; in males, the supranal concavity is completely sclerotized, tarsi IV are without projections; in females, most striae of the hysteronotal shield are oblique, the posterior end of the opisthonotum is devoid of the lateral sclerotized patches. In both sexes of *A. laonastes*, the postscapular and hysteronotal shields are covered by numerous markings or striae (more than 20), setae *d*IV of tarsi IV are subequal or longer than the respective segment; in males, the supranal concavity is not sclerotized, tarsi IV have a distinct subapical projection; in females, striae of the hysteronotal shield are relatively straight, the posterior end of the opisthonotum has a pair of the lateral sclerotized patches.

Family Myobiidae Megnin
Genus *Radfordia* Ewing
Subgenus *Radfordia* Ewing

***Radfordia mirabilis* Bochkov & Abramov, sp. n.**
http://zoobank.org/0B4528B8-9C9B-4C20-8FF3-558911D7363F
Fig. 3

Type material. Female holotype (ZISP My-T-37, AVB 10-0803-012) and 1 female paratype (ZISP AVB 10-0803-012) from *Hapalomys delacouri* Thomas (Rodentia: Muridae) [skin], VIETNAM: Binh Phuoc Province, Bu Gia Map National Park, 13 km NE Bu Gia Map Village, 540 m a.l.s., 12°11'37"N, 107°12'21"E, 13 January 2010, coll. A.V. Abramov (ZISP 99485). Mites removed by A.V. Bochkov.

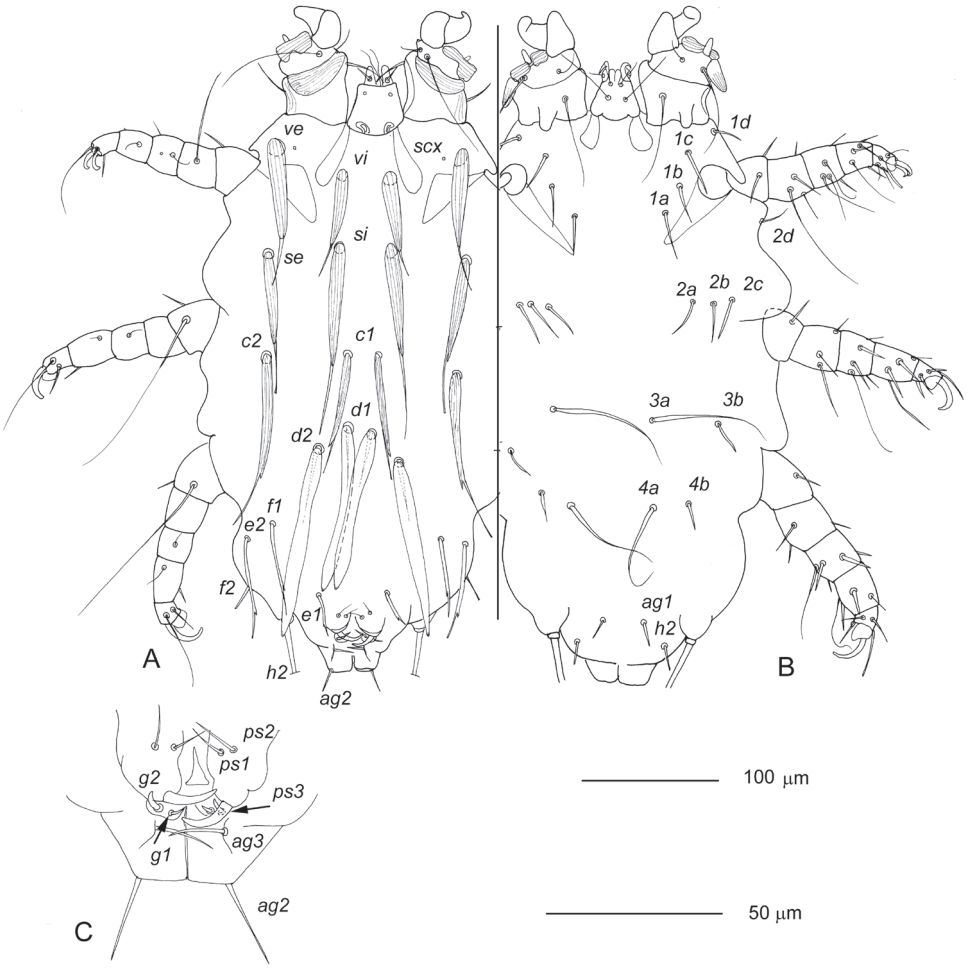

Figure 3. *Radfordia mirabilis* sp. n., female holotype, **A** dorsal view **B** ventral view **C** vulva. Scale bars: **A** and **B** = 100 μm; **C** = 50 μm.

Type deposition. Holotype and single paratype deposited in ZISP.

Description. Female (holotype, 1 paratype). Body 435 long (410), 245 wide (230). Body 1.8 times longer than wide. Setae *vi*, *ve*, *d1*, and *d2* 10–12 wide at base; *si* and *se2* about 7 wide at base; *c1* and *c2* about 5 wide at base, *e1*, *e2*, and *f2* about 3 wide at base. Apices of setae *si* reaching level of setal bases *d1*, apices of setae *se* reaching level of setal bases *c2*. Approximate distances between bases of setae: *vi–vi* 25, *si–si* 22, *c1–c1* 17, *c1–c2* 50, *d1–d1* 15, *d2–d2* 50, *c1–d1* 52, *d1–d2* 17. Setal bases *f1* situated close to *e2* than to *e1*, distance *e1–f1* about 50, *f1–e2* about 13. Setae *f2* situated at lateral margins of idiosoma. Lengths of setae: *vi* 75 (70), *ve* 90 (93), *si* 115 (110), *se* 87 (85), *c1* 63 (75), *c2* 125 (115) – all distinctly longitudinally striated; *d1* 115 (117) and *d2* about 125, membranous, without striae, *e1* about 27, *e2* about 75, *f1* about 60, *f2*

about 15, *h1* about 12, *h2* 310 (315), *ps1* and *ps2* about 11, *ps3* (hook-like) about 15, *g1* about 6, *g2* about 8, *1a, 2a* 25–28, *3a* about 80, and *4a* about 75, *ag1* and *ag3* 8–11, *ag2* about 12. Setae *3a* and *4a* slightly thickened. Apical segment of leg I without ventral projection. Setation for legs II-IV (solenidia in parentheses): tarsi 7(1)-6-6, tibiae 6-6-6, genua 7 (1)-6-5, femora 5-3-3, trochanters 3-3-3, coxae 4-2-2.

Etymology. This epithet refers the unusual external morphology of this species – *mirabilis* (Latin, wonderful).

Differential diagnosis. The subgenus *Radfordia* is separated onto two species groups, "*ensifera*" (setation of coxae II-IV 3-2-2) and "*lancearia*" (3-1-2) (Bochkov and Fain 2003; Bochkov 2009). The new species distinctly differs from all known species of both groups by the setation of coxae II-IV(4-2-2), position of setae *f2* on the lateral margins of the opisthosoma (vs. distant from the lateral margins in all other species) and the bases of *f1* and *e2* which are situated close to each other (vs. distant in all other species). Therefore, we establish for this new species a new species group *mirabilis*.

Discussion

The phylogenetic position of *Hapalomys* is still unclear because of the scarcity of museum specimens. Usually the genus is placed within *Micromys* division of the large muroid subfamily Murinae (Musser and Carleton 2005). Other authors suggested a close link between *Hapalomys* and *Chiropodomys* (Misonne 1969; Musser and Newcomb 1983; Chaimanee 1998; Musser and Carleton 2005). The both mite species from *Hapalomys* described herein strongly differ from the respective congeners described from rodents of the genus *Chiropodomys* Peters (Fain 1970, 1976; Bochkov and Fain 2003). *Afrolistrophorus chiropodomys* Fain, 1970 described from *Chiropodomys major* Thomas and *A. hapalomys* sp. n. belongs to the same species group "*apodemi*" but in the limits of this group they strongly differ from each other by the ornamentation of the dorsal shields in both sexes. *Radfordia chiropodomys* Fain, 1976 described from *Chiropodomys gliroides* (Blyth) is the typical representative of the species group "*ensifera*" (Bochkov and Fain 2003), whereas *R. mirabilis* sp. n. is a sole representative of a separate species group and morphology strongly different from all other representatives of this subgenus (see description). Based on morphological evidences, we conclude that species of both mite genera associated with *Hapalomys* Blyth do not demonstrate clear phylogenetic links with respective congeners from rodents of the closest genus *Chiropodomys*.

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