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The millipede genus *Orthomorpha* Bollman, 1893 in Laos (Diplopoda, Polydesmida, Paradoxosomatidae), with descriptions of new species

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Abstract

The genus *Orthomorpha* is currently represented in Laos by nine species, including three, *O. paviei* Brölemann, 1896, *O. communis* Likhitrakarn, Golovatch & Panha, 2011 and *O. cambodjana* (Attems, 1953), which are new to the fauna of the country, and further three new to science: *O. suberectoides* **sp. n.**, *O. gladiata* **sp. n.** and *O. sutchariti* **sp. n.**

Keywords

Millipede, *Orthomorpha*, taxonomy, new species, Laos

Introduction

The large Southeast Asian millipede genus *Orthomorpha* Bollman, 1893 has recently become the subject of a thorough review (Likhitrakarn et al. 2011), with the result that currently it comprises 51 species ranging from northern Myanmar and Thailand

southeastwards to Lombok Island, Indonesia. Among them, only three have hitherto been recorded in Laos: the pantropical anthropochore *O. coarctata* (De Saussure, 1860), as well as *O. rotundicollis* (Attems, 1937) and *O. scabra* Jeekel, 1964, both latter species also shared with Vietnam (Likhitrakarn et al. 2011, 2014).

The present paper puts on record a few more *Orthomorpha* from Laos, including three species heretofore known from the adjacent parts of Indochina (one also from as far south as Indonesia), and further three new to science.

Material and methods

The material was collected during a field trip to southern Laos undertaken in 2013 by SP and members of the Animal Systematics Research Unit, Chulalongkorn University. Live animals were photographed on the spot. Specimens were preserved in 75% ethanol, and morphological investigations were carried out in the laboratory using an Olympus stereomicroscope. Scanning electron micrographs (SEM) of gonopods coated with gold were taken using a JEOL, JSM–5410 LV microscope, returned to alcohol upon examination. Digital images of the specimens were taken in the laboratory and assembled using the "Cell^{D}" automontage software of the Olympus Soft Imaging Solution GmbH package. In addition, line drawings of gonopods were also prepared. All material is housed in the Museum of Zoology, Chulalongkorn University (CUMZ), Bangkok, Thailand.

Collecting sites were located by GPS using the WGS84 datum.

In the catalogue sections, D stands for the original description, subsequent descriptive notes or appearance in a key while M for a mere mention.

Taxonomic part

Order Polydesmida
Family Paradoxosomatidae Daday, 1889
Genus *Orthomorpha* Bollman, 1893

***Orthomorpha paviei* Brölemann, 1896**
http://species-id.net/wiki/Orthomorpha_paviei
Figs 1–3

Orthomorpha Paviei Brölemann, 1896: 1 (D).
Orthomorpha Paviei – Brölemann 1904: 8 (D).
?*Prionopeltis Paviei* – Attems 1914: 204 (M).
Pratinus paviei – Attems 1937: 122 (M).
Orthomorpha paviei – Jeekel 1963: 265 (M); 1964: 359 (M); 1968: 56 (M); Golovatch 1998: 42 (D); Enghoff et al. 2004: 34 (M); Enghoff 2005: 97 (M); Likhitrakarn et al. 2011: 52 (D).

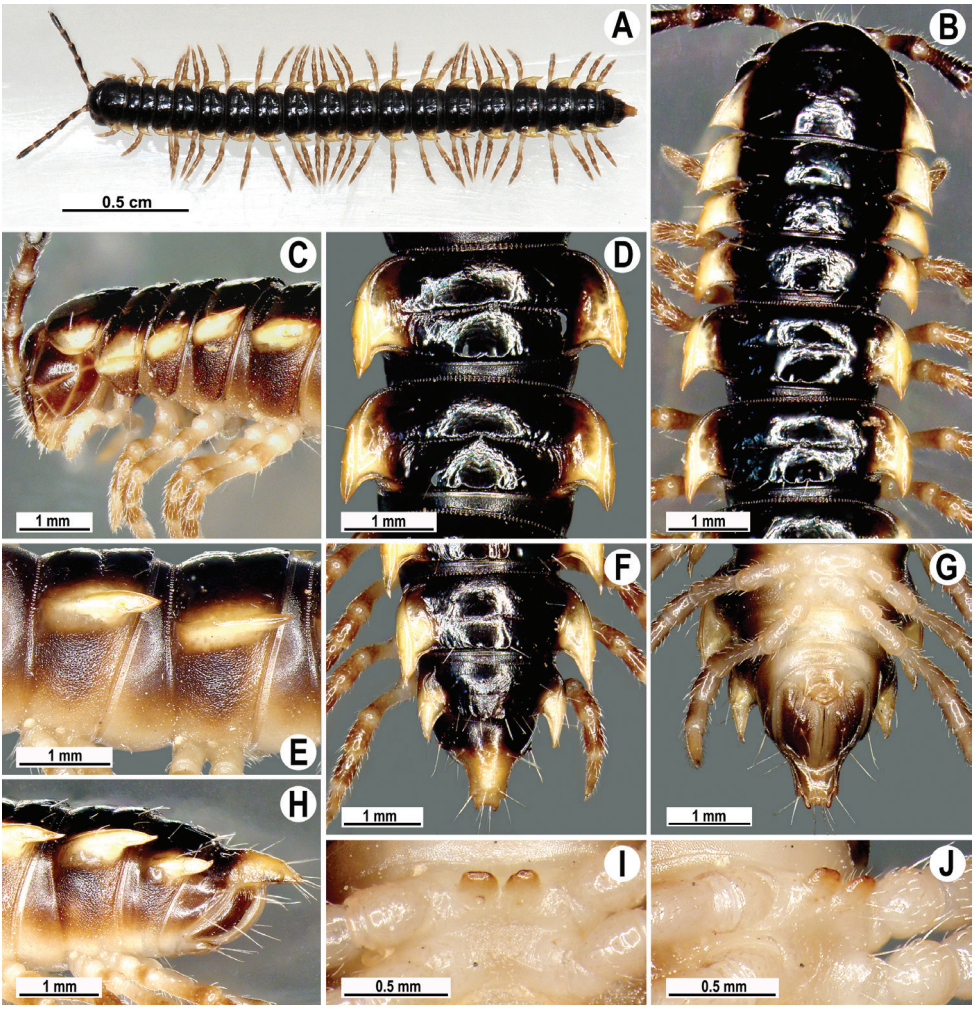

Figure 1. *Orthomorpha paviei* Brölemann, 1896, ♂ from Laos. **A** habitus, live coloration; **B, C** anterior part of body, dorsal and lateral views, respectively **D, E** segments 10 and 11, dorsal and lateral views, respectively **F–H** posterior part of body, dorsal, ventral and lateral views, respectively **I, J** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

Material examined: 1 ♂ (CUMZ), Laos, Champasak Province, Khong District, Khone Phapheng Waterfall, 82 m a.s.l., 13°57'47"N, 105°59'17"E, 23.07.2013, leg. W. Siriwut.

Diagnosis. According to the key in Likhitrakarn et al. (2011), this species is especially similar to *O. coarctata* (De Saussure, 1860), but differs in the paraterga more strongly developed, in having two, fully separated, sternal cones between ♂ coxae 4, and a nearly trifid gonopod tip with two clearly larger prongs being deeply split with a third, very small denticle lying ventrally at about midway of the second, slightly stronger prong.

Redescription. Length 25 mm (♂), width of midbody pro- and metazona 2.06 and 3.23 mm, respectively.

Coloration of live animals blackish (Fig. 1A), paraterga and epiproct contrasting dark yellow, head and antennae brownish, legs pale brownish; coloration in alcohol, after three months of preservation, faded to black-brown (Fig. 1B–H), paraterga and epiproct pale whitish yellow or pale brown, legs whitish to pale brown distally.

Clypeolabral region densely setose, vertigial region with a few setae only; epicranial suture distinct. Antennae long (Fig. 1A), reaching posterior end of body segment 4 when stretched dorsally. In width, head < segment 3 = 4 < collum < segment 2 < 5–17, gently and gradually tapering thereafter. Collum with three transverse rows of setae: 4+4 in anterior, 3+3 in intermediate, and 1+1 in posterior row; a very faint incision laterally near midway; caudal corner of paraterga pointed, dentiform, slightly upturned, but not drawn behind rear margin (Fig. 1B, C).

Tegument smooth and shining, prozona finely shagreened, metaterga smooth and delicately rugulose, leathery; surface below paraterga finely microgranulate. Postcollum metaterga with two transverse rows of setae traceable at least as insertion points when setae broken off: 2+2 in anterior (pre-sulcus), 3–4+3–4 in posterior (post-sulcus) row. Tergal setae long, strong, slender, about 1/3 of metatergal length. Axial line visible only on metaterga, slightly better developed on their posterior halves. Paraterga very strongly developed (Fig. 1B–H), mostly slightly upturned, all lying faintly below dorsum, set at about upper 1/3 of midbody height, subhorizontal, caudal corner almost or fully pointed, increasingly well spiniform and produced behind rear tergal margin until segment 18; paraterga very thin in lateral view, blunt blades, modestly enlarged in pore-bearing segments, thinner in poreless ones. Calluses on paraterga 2–4 delimited by a sulcus only dorsally, on following paraterga both dorsally and ventrally. Paraterga 2 broad, anterior edge angular, lateral edge with one larger and two smaller, but evident incisions in anterior 1/3; posterior edge well concave (Fig. 1B, D, F). Anterior edges of paraterga 3–9 clearly convex, of paraterga 10–18 nearly straight and slightly bordered. Lateral edge of paraterga with two slight, but evident incisions, one in anterior 1/3, the other in posterior 1/3. Posterior edge of paraterga clearly concave, especially well so in segments 16–19 (Fig. 1F–H). Ozopores evident, lateral, lying in an ovoid groove at about 1/4 of metatergite's length in front of caudal corner. Transverse sulcus usually distinct (Fig. 1C, E, H), complete on metaterga 5–19, incomplete and nearly wanting on 19th, shallow, nearly reaching bases of paraterga, rather faintly beaded at bottom. Stricture between pro- and metazona wide, clearly beaded at bottom down to base of paraterga (Fig. 1B, D, F). Pleurosternal carinae complete crests with a sharp caudal tooth in segment 2, a very sharp, caudal tooth in segments 3 and 4, a small, mostly sharp tooth in segments 5–9 (Fig. 1C, E). Epiproct (Fig. 1F, G) conical, flattened dorsoventrally, with two evident apical papillae; tip subtruncate; pre-apical papillae small, but visible, lying rather close to tip. Hypoproct roundly subtriangular, setiferous knobs at caudal edge evident and well-separated.

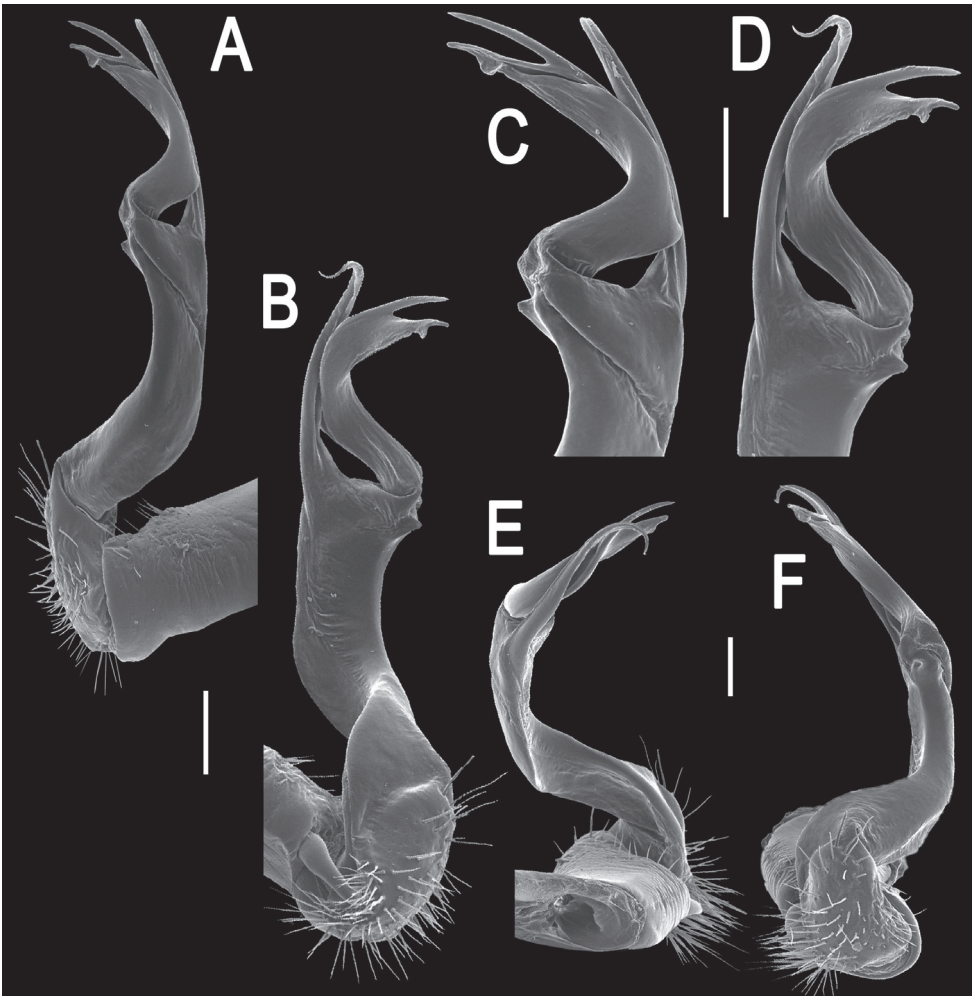

Figure 2. *Orthomorpha paviei* Brölemann, 1896, ♂ from Laos, left gonopod. **A, B** lateral and mesal views, respectively **C, D** telopodite, lateral and mesal views, respectively **E, F** distal part, subcaudal and suboral views, respectively. Scale bar: 0.2 mm.

Sterna sparsely setose, without modifications; two rather large, fully separated, sternal cones between ♂ coxae 4 (Fig. 1I, J). A paramedian pair of evident tubercles in front of gonopod aperture. Legs moderately long and slender, midbody ones ca 1.1–1.2 times as long as body height, prefemora without modifications, ♂ tarsal brushes present until legs of segment 8.

Gonopods (Figs 2, 3) with slender and long coxae, the latter with several setae distoventrally. Femorite about 3 times as long as prefemoral (= strongly setose) part. Femorite slender, slightly curved, postfemoral portion demarcated by an oblique lateral sulcus; tip of solenophore indistinctly trifid, both dorsal (**d**) and middle (**m**) prongs being sharp

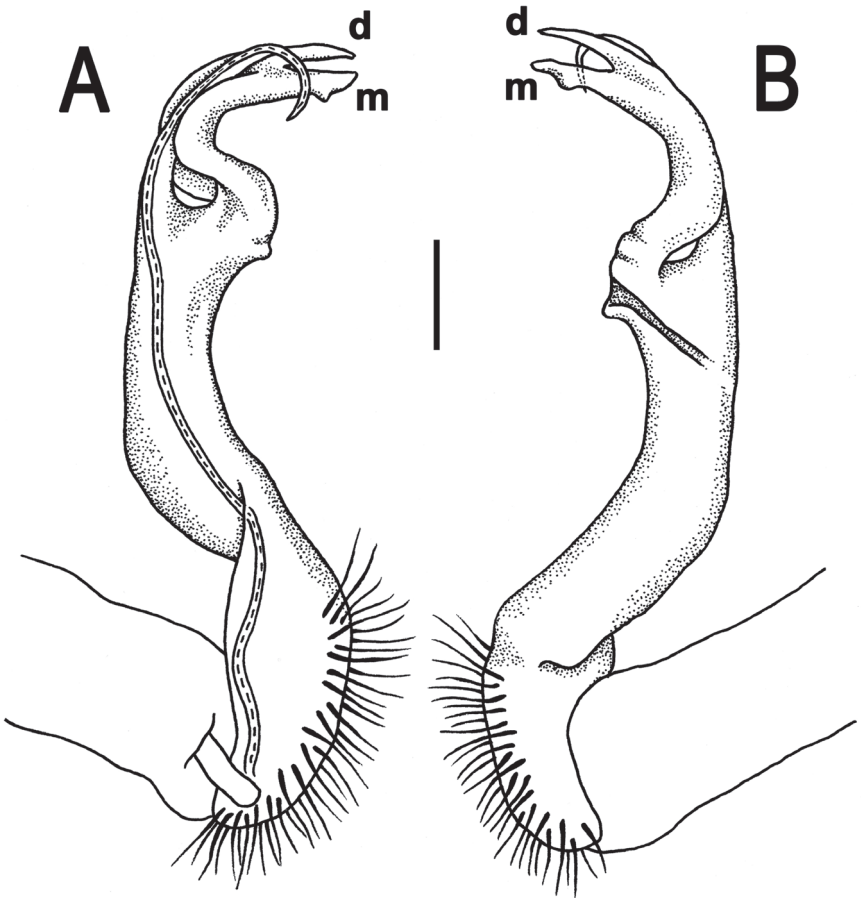

Figure 3. *Orthomorpha paviei* Brölemann, 1896, ♂. **A, B** left gonopod, mesal and lateral views, respectively. Scale bar: 0.5 mm.

and deeply split, middle prong slightly stronger, supplied with a minute, but evident subterminal knob/lobule ventrally at about midway; solenomere long and flagelliform.

Remarks. The new specimen agrees nearly fully with the most detailed and beautifully illustrated descriptions of the species given by Brölemann (1896, 1904), but the holotype is larger: body length 40 mm versus 25 mm, width of midbody metazona 4.25 mm versus 3.23 mm. In addition, a transverse sulcus is visible in segments 4–18 in the holotype versus segments 5–19 in the fresh ♂. Originally described from an unspecified locality in Thailand (Brölemann 1896, 1904), *O. paviei* is thus new to the fauna of Laos.

In having both apical prongs of the gonopods rather deeply split, this species is somewhat intermediate between the genera *Antheromorpha* Jeekel, 1968 and *Orthomorpha* Bollman, 1893 (Likhitrakarn et al. 2011).

The above detailed redescription is given not only to better outline the quite modest variations found in *O. paviei*, but also to serve as a pattern for further, more condensed descriptions or descriptive notes below.

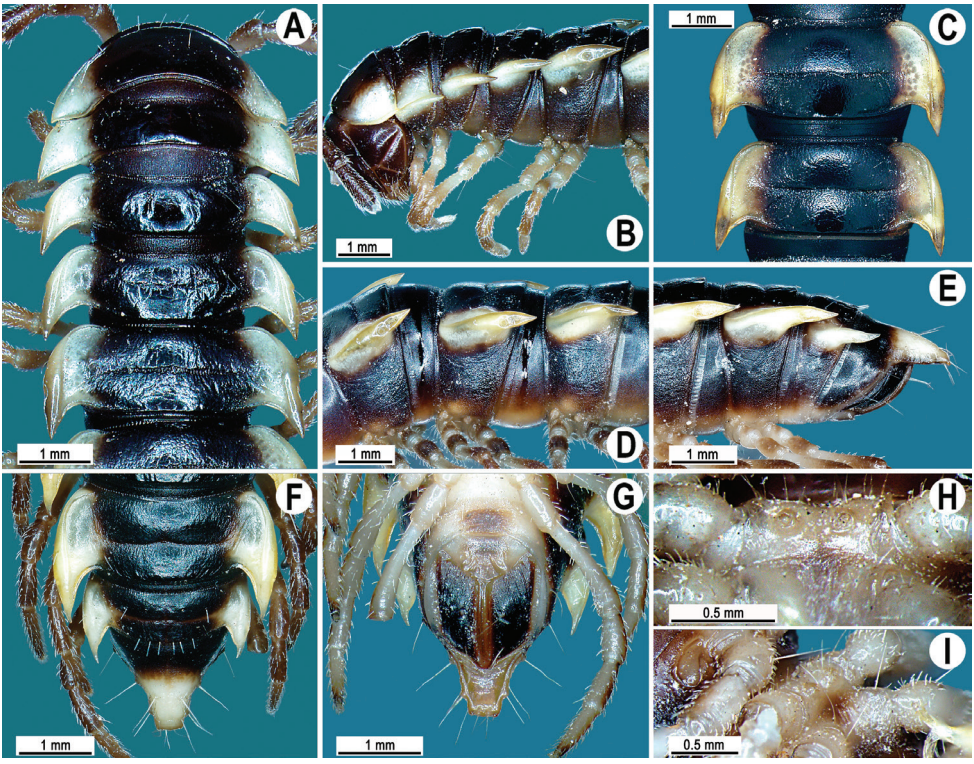

Figure 4. *Orthomorpha cambodjana* (Attems, 1953), ♂. **A, B** anterior part of body, dorsal and lateral views, respectively; **C** segments 10–11, dorsal view **D** segments 9–11, lateral view **E–G** posterior part of body, lateral, dorsal and ventral views, respectively; **H, I** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

***Orthomorpha cambodjana* (Attems, 1953)**
http://species-id.net/wiki/Orthomorpha_cambodjana
Figs 4–6

Pratinus cambodjanus Attems 1953: 168 (D).
Orthomorpha cambodjana – Jeekel 1963: 265 (M); 1964: 361 (M, D); 1968: 56 (M); Hoffman 1977: 700 (M); Golovatch 1998: 42 (M, D); Likhitrakarn et al. 2011: 66 (D).

Material examined. 2 ♂ (CUMZ), Laos, Attapu Province, Xaysetha District, Ban Kasom, 116 m a.s.l., 15°06'27"N, 106°51'14"E, 16.10.2013, leg. C. Sutcharit and W. Siriwut.

Descriptive notes. Length 32–35 mm (♂), width of midbody pro- and metazona 2.6–2.7 and 3.8–3.9 mm (♂), respectively.

Coloration in alcohol, after one month of preservation, blackish (Fig. 4A–F), paraterga and epiproct light yellow to whitish yellow; venter, legs and antennae whitish to pale brown distally (Fig. 4A–I).

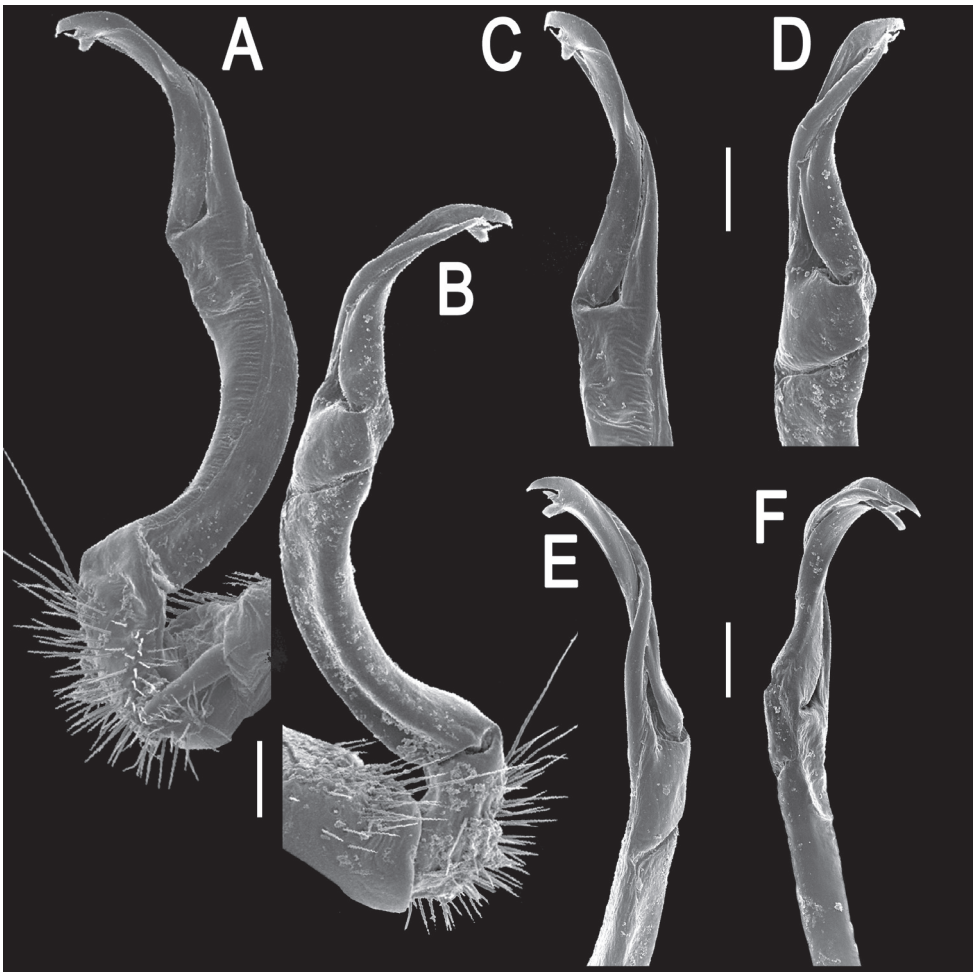

Figure 5. *Orthomorpha cambodjana* (Attems, 1953), ♂, right gonopod. **A, B** mesal and lateral views, respectively **C–F** distal part of left gonopod, sublateral, submesal, subcaudal and suboral views, respectively. Scale bar: 0.2 mm.

Antennae reaching segment 3 when stretched dorsally. Paraterga very strongly developed (Fig. 4A–G), anterolateral edge clearly convex. Calluses on paraterga 2 and 3 delimited by a sulcus only dorsally, following paraterga by a sulcus both dorsally and ventrally, strongly bordered. Pleurosternal carinae complete crests only in segment 2 (Fig. 4B, D, E), a small, sharp, caudal tooth in segments 3–7, a very faint tubercle until segment 15. Legs long and slender, midbody ones ca 1.1–1.3 times as long as body height, prefemora without modifications, tarsal brushes present until ♂ leg 7.

Gonopod solenophore with a tridentate tip (Figs 5, 6), dorsalmost prong being subacuminate, longer than both others, whereas middle denticle shortest and pointed.

Remarks. This species has hitherto been known only from a ♂ lectotype and a ♀ paralectotype, currently both rather strongly faded, taken at Sre-Umbell, Kampot

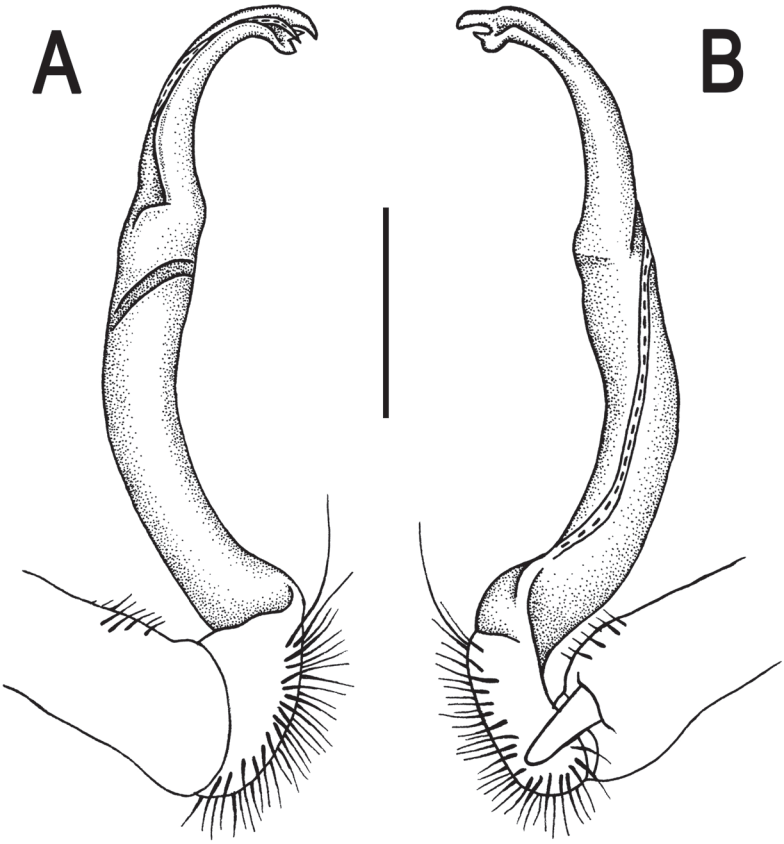

Figure 6. *Orthomorpha cambodjana* (Attems, 1953), ♂. **A, B** right gonopod, lateral and mesal views, respectively. Scale bar: 0.5 mm.

Province, southern Cambodia (Attems 1953, Likhitrakarn et al. 2011). The above fresh samples show only slight variations, mainly a darker coloration, as opposed to the types which have recently been redescribed in due detail (Likhitrakarn et al. 2011). This species is thus new to the fauna of Laos, suggesting a far wider distribution in Indochina.

***Orthomorpha communis* Likhitrakarn, Golovatch & Panha, 2011**
http://species-id.net/wiki/Orthomorpha_communis

Orthomorpha communis Likhitrakarn, Golovatch & Panha 2011: 37 (D).

Material examined. 1 ♂, 2 ♀ (CUMZ), Laos, Savannakhet Province, Atsaphangthong District, Ban Than Bang, 170 m, 16°42'12"N, 105°12'06"E, 19.10.2013, leg. C. Sutcharit and W. Siriwut.

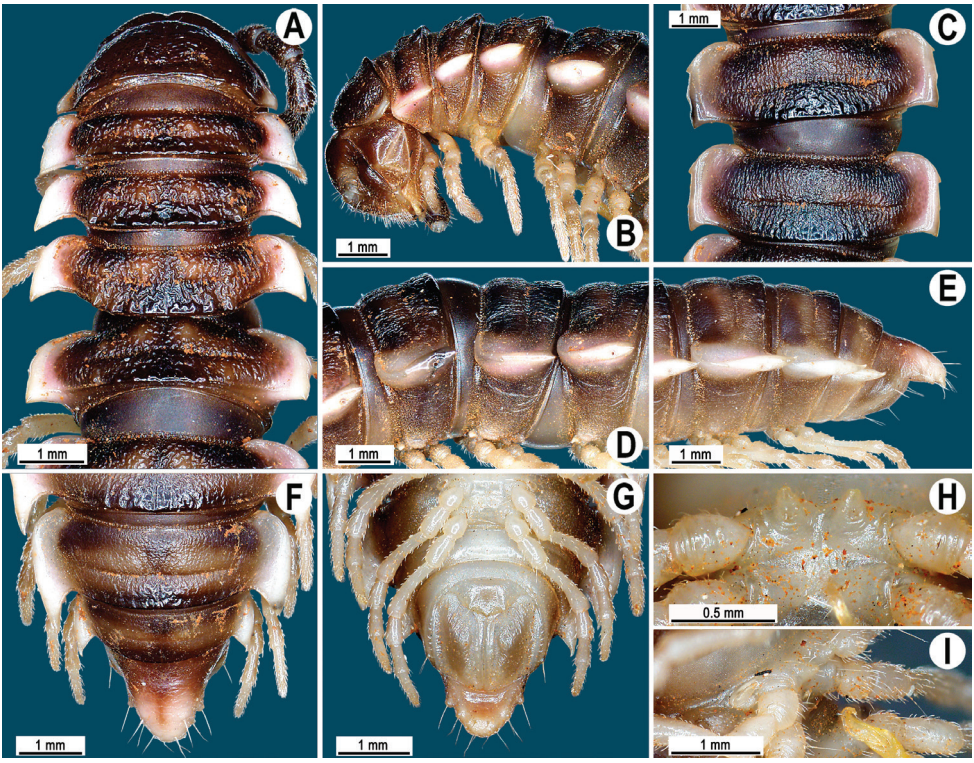

Figure 7. *Orthomorpha suberectoides* sp. n., ♂ holotype. **A, B** anterior part of body, dorsal and lateral views, respectively **C** segments 10–11, dorsal view **D** segments 9–11, lateral view **E–G** posterior part of body, lateral, dorsal and ventral views, respectively **H, I** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

Descriptive notes. Length 34 mm (♂) or 36–37 mm (♀), width of midbody pro- and metazona 2.9 and 4.5 mm (♂), 3.0–3.8 and 4.5–5.3 mm (♀), respectively.

Remarks. The above material fully agrees with the original, quite detailed description of this species (Likhitrakarn et al. 2011). It appears to be rather widespread not only in the eastern part of Thailand close to the frontier to Cambodia, but also in southern Laos. This species is new to the fauna of Laos, again suggesting a much wider distribution in Indochina.

***Orthomorpha suberectoides* sp. n.**
http://zoobank.org/695749B1-EFAF-4D08-BF0E-2C817DC63C95
http://species-id.net/wiki/Orthomorpha_suberectoides
Figs 7–9

Holotype ♂ (CUMZ), Laos, Attapu Province, Xaysetha District, Ban Lak No. 52, 224 m a.s.l., 15°09'24"N, 106°44'01"E, 16.10.2013, leg. C. Sutcharit.

Name. To emphasize the apparent similarity to *O. suberecta* Likhitrakarn, Golovatch & Panha, 2011; adjective.

Figure 8. *Orthomorpha suberectoides* sp. n., ♂ holotype, right gonopod. **A, B** mesal and lateral views, respectively **C–F** distal part of left gonopod, mesal, lateral, suboral and subcaudal views, respectively. Scale bar: 0.2 mm.

Diagnosis. This new species strongly resembles *O. suberecta*, especially as regards the shape of the paraterga, but differs by a larger body, in the paraterga being broader, the metatergal tegument clearly rugose, and the pleurosternal carinae more strongly developed.

Description. Length 35 mm (♂), width of midbody pro- and metazona 3.5 and 5.0 mm, respectively.

Coloration in alcohol, after one month of preservation, dark brown (Fig. 7A–F), paraterga and epiproct pale pinkish or pallid, head and antennae brownish, venter and legs pale whitish to pale brown (Fig. 7B–I).

All other characters as in *O. paviei*, except as follows.

Antennae (Fig. 7A) reaching posterior end of body segment 3 when stretched dorsally. In width, head < collum < segment 2 < 3 < 4 < 5–16, gently and gradually tapering thereafter. Collum with three transverse rows of setae: 4+4 in anterior, 2+2 in intermediate, and

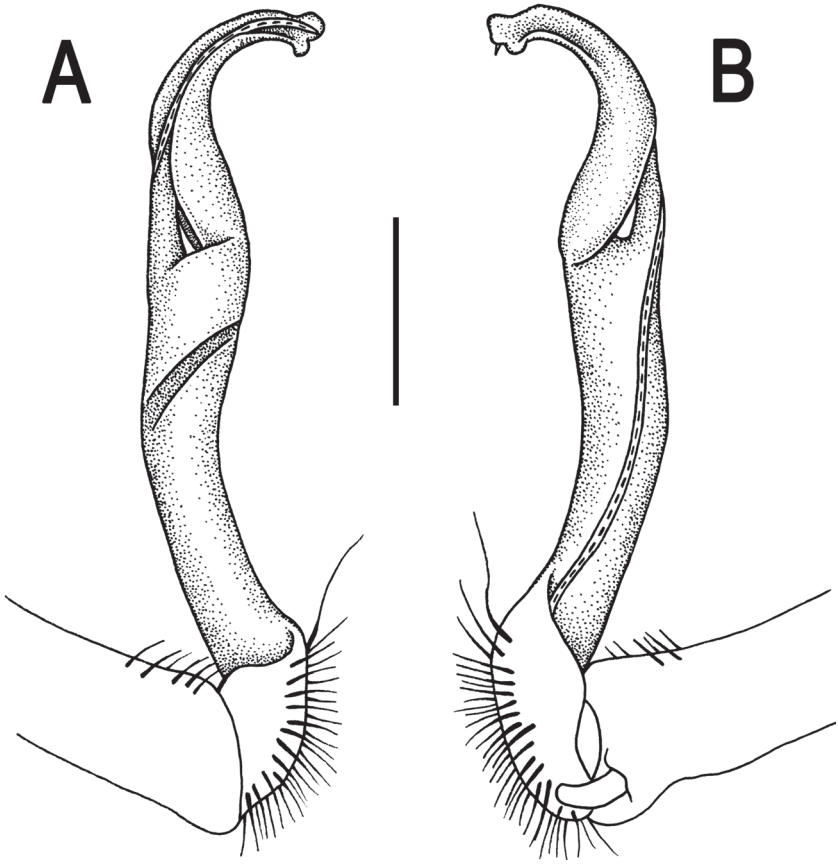

Figure 9. *Orthomorpha suberectoides* sp. n., ♂ holotype. **A, B** right gonopod, lateral and mesal views, respectively. Scale bar: 0.5 mm.

4+4 in caudal row; paraterga slightly declivous, broadly rounded and narrowly bordered, caudal corner pointed, dentiform, but not drawn behind rear margin (Fig. 7A, B).

Tegument shining and rugulose (Fig. 7A–F), metaterga rather obviously rugose, leathery, with traces of tubercles/wrinkles; surface below paraterga microgranulate and rugulose. Postcollum metaterga with two transverse rows of setae: 2+2, mostly abraded setae in anterior (pre-sulcus) row, 3+3 in posterior (post-sulcus) one, these setae or their traces being borne on evident cones growing stronger laterally, until segment 6 visible only as insertion points, tuberculations gradually growing smaller thereafter. Tergal setae simple, rather long, about 1/3 of metatergal length, mostly abraded. Axial line traceable, especially clear on collum and following metaterga. Paraterga very strongly developed (Fig. 7A–G), mostly slightly upturned, all lying faintly below dorsum, set at about half of midbody height, subhorizontal, in lateral view modestly enlarged in pore-bearing segments, thinner in poreless ones; shoulders broadly rounded, narrowly bordered, fused to callus; caudal corner almost or fully pointed, lying within rear tergal margin, after segment 16 drawn increasingly well beyond it, slightly curved mesad on

segments 17–19 (Fig. 7E–G). Calluses delimited by a sulcus only dorsally. Paraterga 2 broad, anterior edge convex, lateral edge with three small and acute denticles, the one near caudal corner being particularly small (Fig. 7A, B). Each following poreless segment with two incisions, each pore-bearing one with one, often evident incision in front of ozopore. Posterior edge of paraterga slightly concave, especially clearly so in segments 16–19. Ozopores evident, lateral, lying in an ovoid groove at about 1/4 of metatergite's length in front of caudal corner. Transverse sulcus usually distinct (Fig. 7A–F), complete on metaterga 5–19, incomplete on segment 4, narrow, not reaching bases of paraterga, ribbed at bottom. Stricture between pro- and metazona narrow, clearly ribbed at bottom down to base of paraterga (Fig. 7A–F). Pleurosternal carinae complete crests with a sharp caudal tooth in segments 2–7, a very sharp, caudal tooth in segments 8–17, a small, rather sharp tooth in segment 18 (Fig. 7B, D, E). Epiproct (Fig. 7F, G) conical, flattened dorsoventrally, with two evident apical papillae, both latter directed ventrocaudally and acute at tip; pre-apical papillae small denticles lying close to tip. Hypoproct (Fig. 7G) roundly subtriangular, setiferous knobs at caudal edge evident and well-separated.

Sterna sparsely setose, without modifications; cross-impressions shallow; a paramedian pair of evident, fully separated, small, sternal cones between ♂ coxae 4 (Fig. 7H, I). A paramedian pair of evident tubercles in front of gonopod aperture. Legs moderately long and slender, midbody ones ca 1.0–1.1 times as long as body height, prefemora without modifications, ♂ tarsal brushes absent.

Gonopods (Figs 8, 9) with slender and long coxae, the latter with several setae distoventrally. Femorite about 3 times as long as prefemoral portion. Femorite slender, slightly curved, postfemoral portion demarcated by an oblique lateral sulcus. Solenophore with *lamina lateralis* clearly smaller than *lamina medialis*, tip distinctly bilobed, dorsal lobule larger; solenomere long and flagelliform.

Remarks. According to the key in Likhitrakarn et al. (2011), this new species seems to be especially similar to *O. suberecta*. The latter taxon has originally been found near Cave Suwannnakhuha, Suwannnakhuha District, Nong Bua Lamphu Province, northeastern Thailand (Likhitrakarn et al. 2011), thus very far away (550 km) from the type locality of *O. suberectoides* sp. n. Both these species are thus distinct not only morphologically (see Diagnosis), but also geographically.

Orthomorpha gladiata **sp. n.**

http://zoobank.org/B785592D-54B8-4C4D-918A-F43D9ED88C4A
http://species-id.net/wiki/Orthomorpha_gladiata
Figs 10–12

Holotype ♂ (CUMZ), Laos, Champasak Province, Paksong District, Tadedu, 906 m a.s.l., 15°11'35"N, 106°06'07"E, 16.10.2013, leg. S. Panha and C. Sutcharit.

Paratype. 1 ♂ (CUMZ), same data, together with holotype.

Name. To emphasize the sword-shaped tip of the gonopod; adjective.

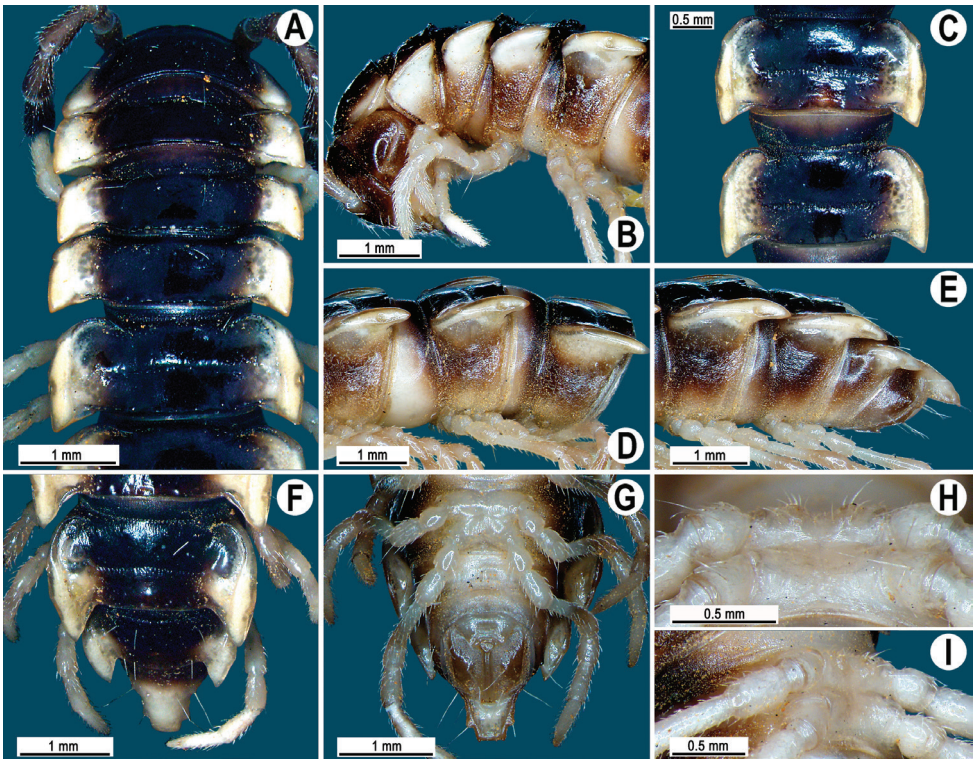

Figure 10. *Orthomorpha gladiata* sp. n., **A–B** ♂ paratype **C–I** ♂ holotype. **A, B** anterior part of body, dorsal and lateral views, respectively **C** segments 10–11, dorsal view **D** segments 9–11, lateral view **E–G** posterior part of body, lateral, dorsal and ventral views, respectively **H, I** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

Diagnosis. *O. gladiata* sp. n. seems to be especially similar to *O. hydrobiologica* Attems, 1930, a species known to range from eastern Java, Indonesia to Indochina (Likhitrakarn et al. 2011), but differs clearly in the tip of the solenophore being nearly monodentate and sword-shaped.

Description. Length 25–27 mm (♂), width of midbody pro- and metazona 1.7–1.9 and 2.8–2.9 mm, respectively.

Coloration in alcohol, after one month of preservation, dark brown (Fig. 10A–G), paraterga and epiproct light yellow or pale yellowish, head, surface below paraterga and antennae brownish, venter and legs whitish to pale brown (Fig. 10A–I).

All other characters as in *O. paviei*, except as follows.

Antennae (Fig. 10A) reaching body segment 3 when stretched dorsally. In width, head < collum < segments 3 and 4 < 2 = 5–16, gently and gradually tapering thereafter. Collum with three transverse rows of setae: 3+3 in anterior, 2+2 in intermediate, and 3+3 in posterior row; caudal corner of paraterga very narrowly rounded, slightly upturned, but not drawn behind rear margin (Fig. 10A, B).

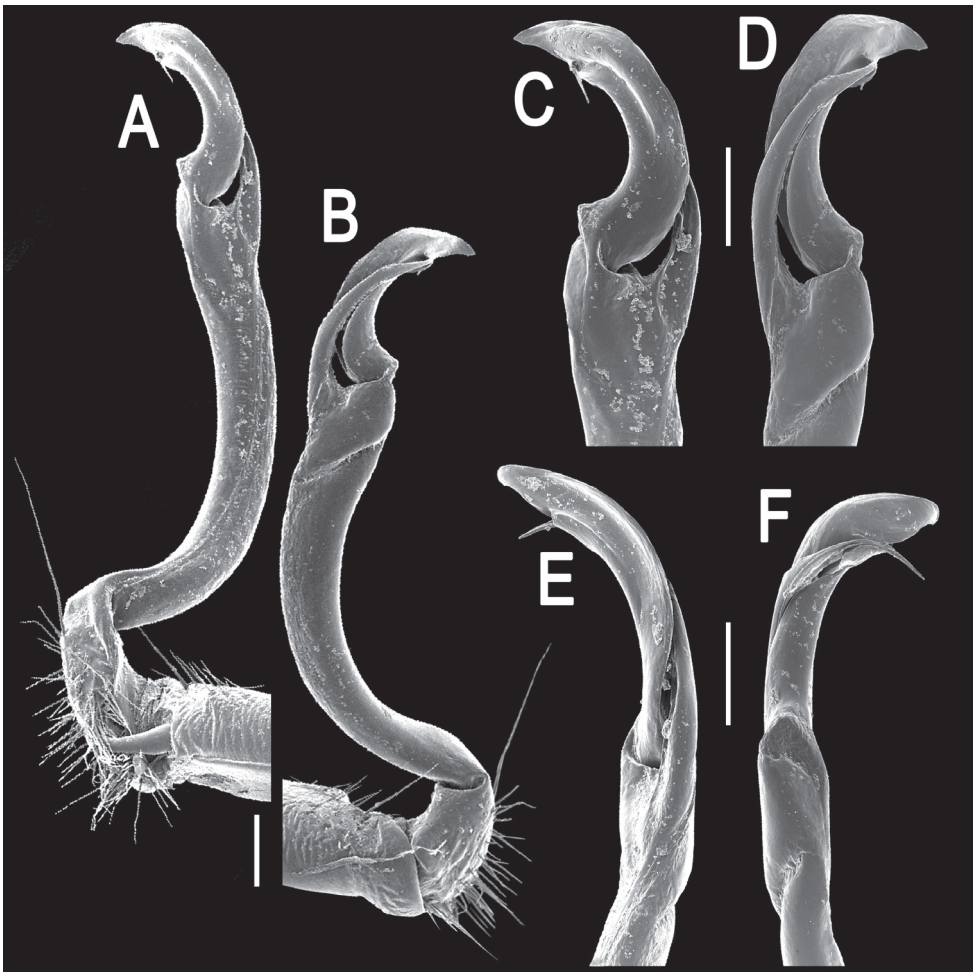

Figure 11. *Orthomorpha gladiata* sp. n., ♂ holotype, right gonopod. **A, B** mesal and lateral views, respectively **C–F** distal part of left gonopod, mesal, lateral, suboral and subcaudal views, respectively. Scale bar: 0.2 mm.

Postcollum metaterga with two transverse rows of setae traceable at least as insertion points when setae broken off: 2+2 in anterior (pre-sulcus) and 3+3 in posterior (post-sulcus) row, these setae being borne on small cones growing stronger laterally. Metaterga 17–19 with 4+4 setae in posterior row, likewise borne on evident cones growing stronger laterally. Axial line rather difficult to see, but traceable both on pro- and metaterga. Paraterga very strongly developed (Fig. 10A–G), mostly slightly upturned, all lying faintly below dorsum, set at about upper 1/4 of midbody height, mostly subhorizontal, caudal corner narrowly rounded to nearly pointed; paraterga very thin in lateral view, modestly enlarged in pore-bearing segments, thinner in poreless ones. Calluses on paraterga 2–4 delimited by a sulcus only dorsally, on following paraterga both dorsally and ventrally. Paraterga 2 broad, anterior edge rounded, lateral

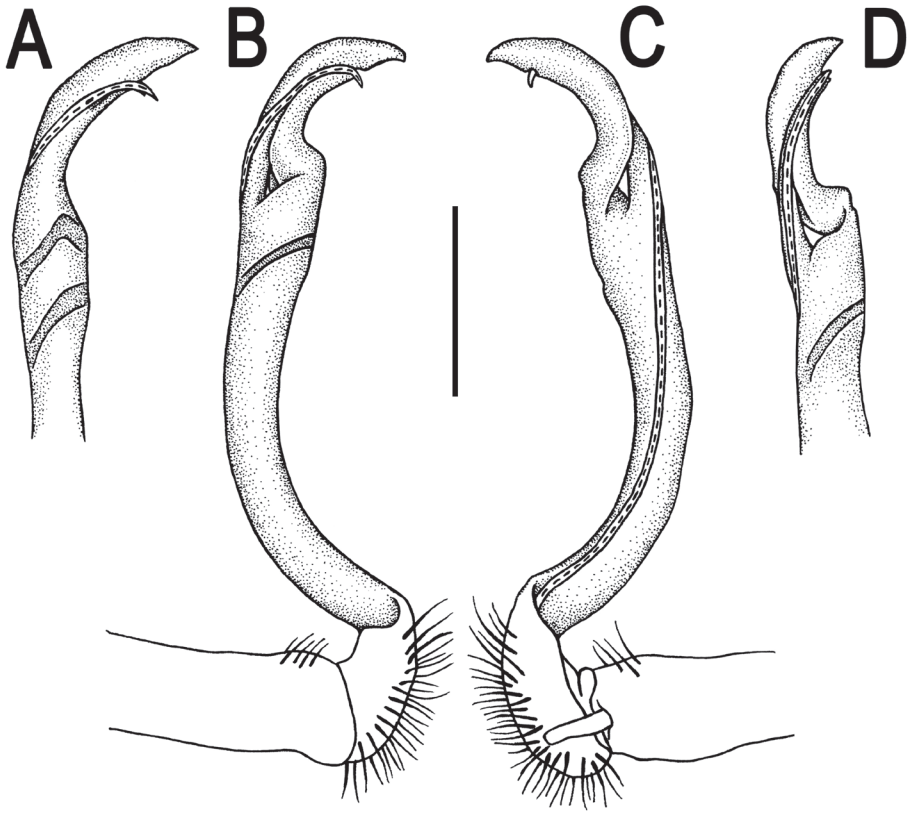

Figure 12. *Orthomorpha gladiata* sp. n., ♂ holotype. **A–D** right gonopod, suboral, lateral, mesal and subcaudal views, respectively. Scale bar: 0.5 mm.

edge with one larger and one smaller, but evident incision in anterior 1/3; posterior edge clearly concave (Fig. 10A, C, F). Anterior edges of paraterga broadly rounded and narrowly bordered, fused to callus. Lateral edge of paraterga with two slight, but evident incisions, one in anterior 1/3, the other in posterior 1/3. Posterior edge of paraterga clearly concave, lateral corner extending increasingly beyond rear tergal margin, especially strongly so in segments 17–19 (Fig. 10F, G). Transverse sulcus usually distinct (Fig. 10A, C, F), complete on metaterga 5–18, incomplete in segments 4 and 19, rather deep, reaching bases of paraterga, rather clearly ribbed at bottom. Stricture between pro- and metazona narrow, clearly ribbed at bottom down to base of paraterga (Fig. 10A–F). Pleurosternal carinae complete crests with a sharp caudal tooth in segments 2–4, a very sharp, caudal tooth in segments 5–7, a small, mostly sharp tooth until segment 16 (Fig. 10B, D, E). Epiproct (Fig. 10F, G) conical, flattened dorsoventrally, with two evident apical papillae; tip subtruncate; pre-apical papillae small, but visible, lying rather close to tip.

Two small, low, rounded, fully separated, sternal cones between ♂ coxae 4 (Fig. 10H, I). A paramedian pair of evident tubercles in front of gonopod aperture. Legs

rather long and slender, midbody ones ca 1.2–1.4 times as long as body height, prefemora without modifications, ♂ tarsal brushes present only on legs 1–3.

Gonopods (Figs 11, 12) with slender and long coxae, the latter with several setae distoventrally. Femorite about 3 times as long as prefemoral portion. Femorite very slender, slightly curved, postfemoral portion demarcated by an oblique lateral sulcus. Solenophore with a nearly monodentate and sword-shaped tip; solenomere long and flagelliform.

Remarks. The gonopod tip of *O. gladiata* sp. n. is much like in *O. parasericata* Likhitrakarn, Golovatch & Panha, 2010, from southern Thailand (Likhitrakarn et al. 2010, 2011), but the new species differs clearly not only in some somatic characters (e.g. the considerably broader paraterga), but also by the presence of an evident oblique sulcus demarcating a gonopod postfemoral portion.

***Orthomorpha sutchariti* sp. n.**
http://zoobank.org/D5E01463-1933-4A03-97C6-2D678FEAB8D2
http://species-id.net/wiki/Orthomorpha_sutchariti
Figs 13–15

Holotype ♂ (CUMZ), Laos, Champasak Province, Paksong District, Tadedu, 906 m a.s.l., 15°11'35"N, 106°06'07"E, 16.10.2013, leg. S. Panha and C. Sutcharit.

Paratype. 1 ♀ (CUMZ), same data, together with holotype.

Name. To honour Dr. Chirasak Sutcharit, Professor at the Department of Biology of Chulalongorn University, Bangkok, who participated in collecting the type specimens and taught the first author the basics of taxonomy.

Diagnosis. Differs in the colour pattern which has lighter caudal halves of the metaterga, the mostly strongly elevated and laterally bordered paraterga, and a trifid solenophore showing a subacuminate, longest, dorsal prong.

Description. Length ca 42 mm (♂) or 44.5 mm (♀), width of midbody pro- and metazona 3.4 and 4.8 mm (♂), 3.5 and 5.6 mm (♀), respectively.

Coloration in alcohol, after one month of preservation, rather uniformly dark brown (Fig. 13A–G) with lighter caudal halves of metaterga, paraterga and epiproct contrasting light yellow, mid-dorsal regions of prozona strongly infuscate, blackish like most of metaterga; antennae and legs brown to light brown (Fig. 13A–I).

All other characters as in *O. paviei*, except as follows.

Antennae (Fig. 13A, B) surpassing posterior end of body segment 3 (♂) or 2 (♀) when stretched dorsally. In width, head < collum < segment 3 = 4 < 2 < 5–15, gently and gradually tapering thereafter. Collum with three transverse rows of setae: 4+4 in anterior, 2+2 in intermediate, and 4+4 in posterior row; a very faint incision laterally near midway; caudal corner of paraterga very narrowly rounded, slightly upturned, but not drawn behind rear margin (Fig. 13A, B).

Tegument smooth and shining, metaterga smooth and delicately rugulose, leathery, posterior halves of metaterga rugose, with traces of tubercles/wrinkles; surface below paraterga finely microgranulate. Postcollum metaterga with two transverse rows of setae

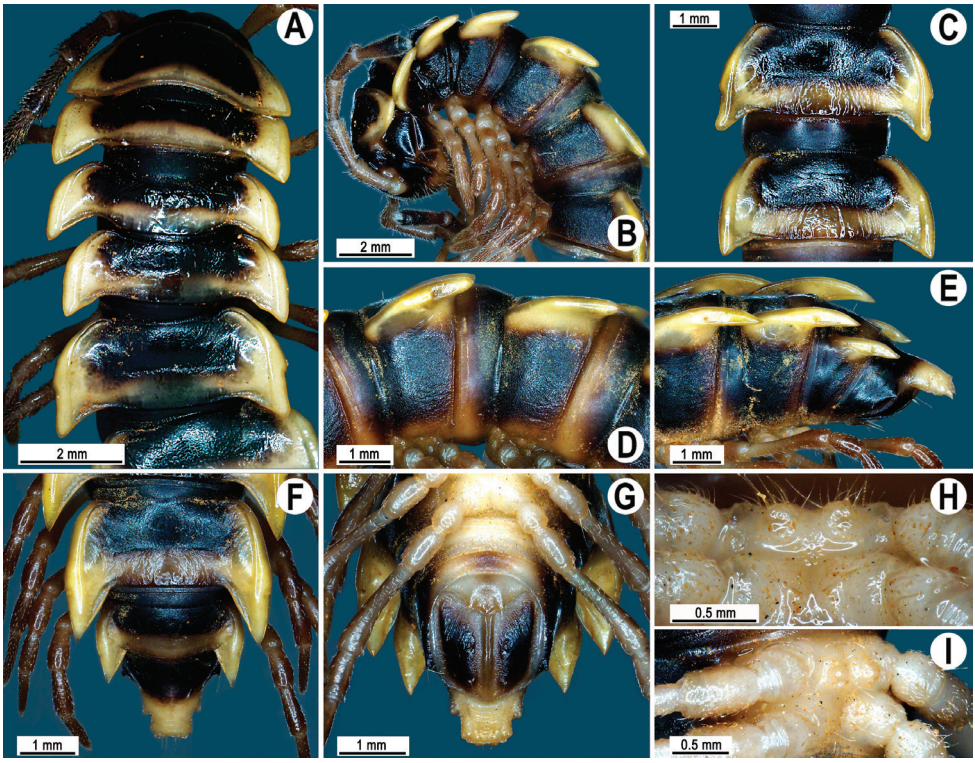

Figure 13. *Orthomorpha sutchariti* sp. n., ♂ holotype. **A, B** anterior part of body, dorsal and lateral views, respectively **C, D** segments 10–11, dorsal and lateral views, respectively **E–G** posterior part of body, lateral, dorsal and ventral views, respectively **H, I** sternal cones between coxae 4, subcaudal and sublateral views, respectively.

traceable at least as insertion points when setae broken off: 2+2 in anterior (pre-sulcus), 3+3 in posterior (post-sulcus) row. Axial line faint, but traceable both on pro- and metaterga. Paraterga very strongly developed (Fig. 13A–G), mostly slightly upturned, lying either faintly above (♂) or slightly below dorsum (♀), set at about upper 1/3 of midbody height, subhorizontal (♀), caudal corner very narrowly rounded, extended beyond rear tergal margin, increasingly well produced caudally in segments 16–19 (Fig. 13F, G); paraterga very thin in lateral view, blunt blades, modestly enlarged in pore-bearing segments, thinner in poreless ones. Calluses on paraterga 2 and 3 delimited by a sulcus only dorsally, on following paraterga both dorsally and ventrally. Paraterga 2 broad, anterior edge angular, lateral edge with one stronger and two smaller, but evident incisions in anterior 1/3; posterior edge clearly oblique (Fig. 13A, C, F). Anterior edges of paraterga broadly rounded and narrowly bordered, fused to callus. Transverse sulcus usually distinct (Fig. 13A, C, F), complete on metaterga 5–18, incomplete and nearly wanting on segments 4 and 19, shallow, not reaching bases of paraterga, rather faintly ribbed at bottom. Stricture between pro- and metazona narrow, clearly ribbed at bottom down to base of paraterga (Fig. 13A–F). Pleurosternal carinae complete crests

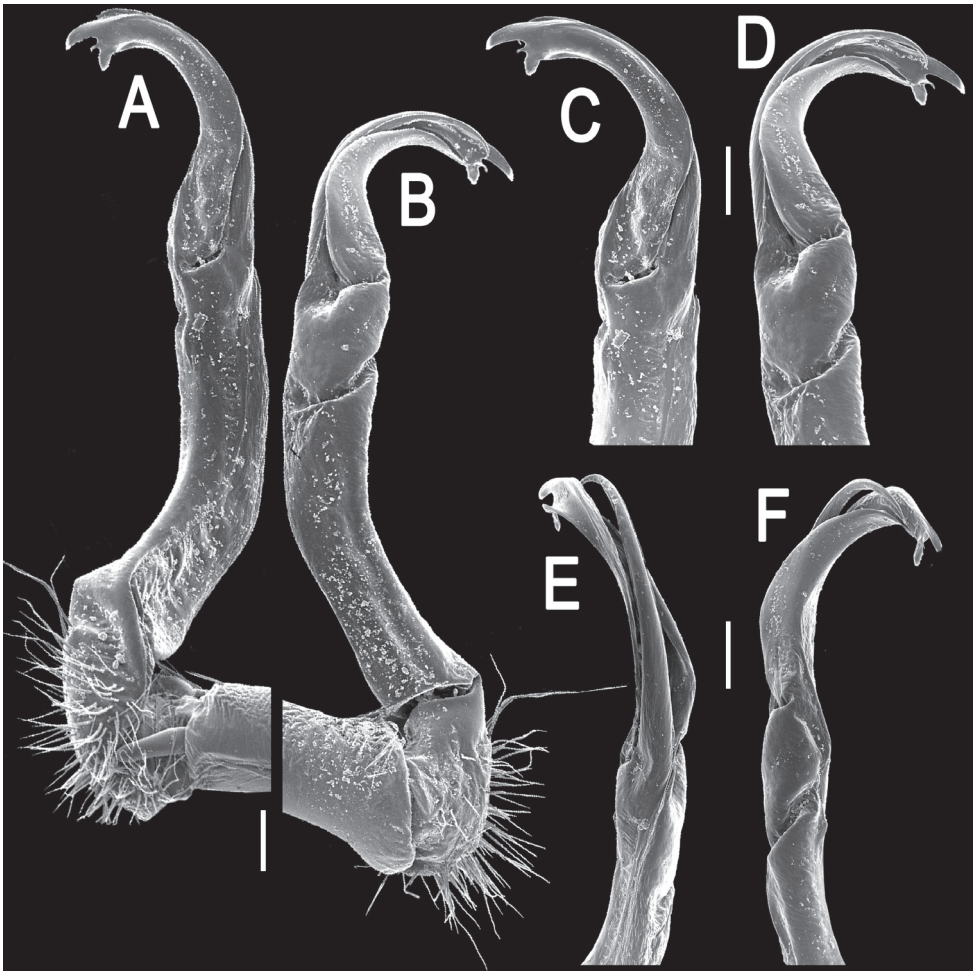

Figure 14. *Orthomorpha sutchariti* sp. n., ♂ holotype, right gonopod. **A, B** mesal and lateral views, respectively **C–F** distal part, mesal, lateral, suboral and subcaudal views, respectively. Scale bar: 0.2 mm.

with a sharp caudal tooth in segments 2–7 (♂) or 2–4 (♀), a very sharp, caudal tooth in segments 8–15 (♂) or 5–10 (♀), a small, mostly sharp tooth until segments 16–19 (♂) or 11–17 (♀) (Fig. 13B, D, E). Epiproct (Fig. 13F, G) conical, flattened dorsoventrally, with two evident apical papillae, both directed ventrocaudally and acute at tip; preapical papillae small, lying rather close to tip.

A pair of small, rounded, fully separated, sternal cones between ♂ coxae 4 (Fig. 13H, I). A paramedian pair of evident tubercles in front of gonopod aperture. Legs rather long and slender, faintly incrassate in ♂; midbody legs ca 1.2–1.4 (♂) or 0.9–1.1 (♀) times as long as body height, prefemora without modifications, ♂ tarsal brushes present until legs of segment 8.

Gonopods (Figs 14, 15) with slender and long coxae, the latter with several setae distoventrally. Femorite about 3 times as long as prefemoral portion. Femorite slender,

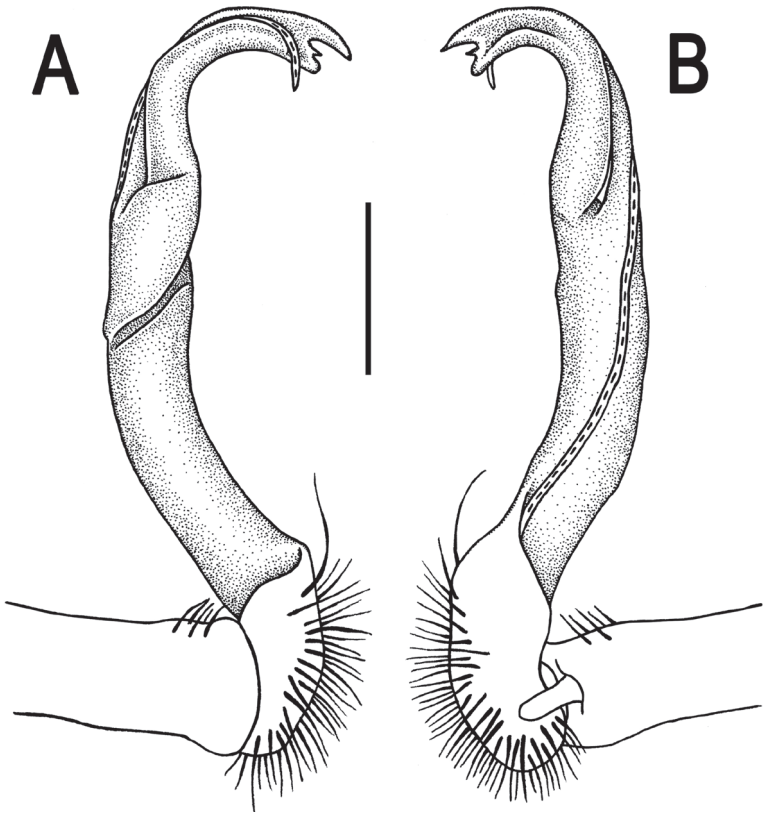

Figure 15. *Orthomorpha sutchariti* sp. n., ♂ holotype. **A, B** right gonopod, lateral and mesal views, respectively. Scale bar: 0.5 mm.

slightly curved, postfemoral portion demarcated by an oblique lateral sulcus; tip of solenophore trifid, with dorsal lobule being subacuminate and longest, middle denticle spiniform, shortest; solenomere long and flagelliform.

Remarks. The colour pattern of *O. sutchariti* sp. n. is similar to that observed in several species of the Oriental genus *Antheromorpha* Jeekel, 1968 (our personal observations), but the gonopod structure is typical of *Orthomorpha* spp. Due to the particularly short middle prong at the tip of the solenophore, this new species strongly resembles the numerous congeners within the former *weberi*-group (Likhitrakarn et al. 2011).

Conclusions

A total of 34 species of millipedes have hitherto been reported from Laos (Likhitrakarn et al. 2014). The present paper adds another three as new to the fauna of Laos, plus another three which are new to science. Since all these new records come from the southern parts of Laos alone (Fig. 16), the generic distribution which shows the north-

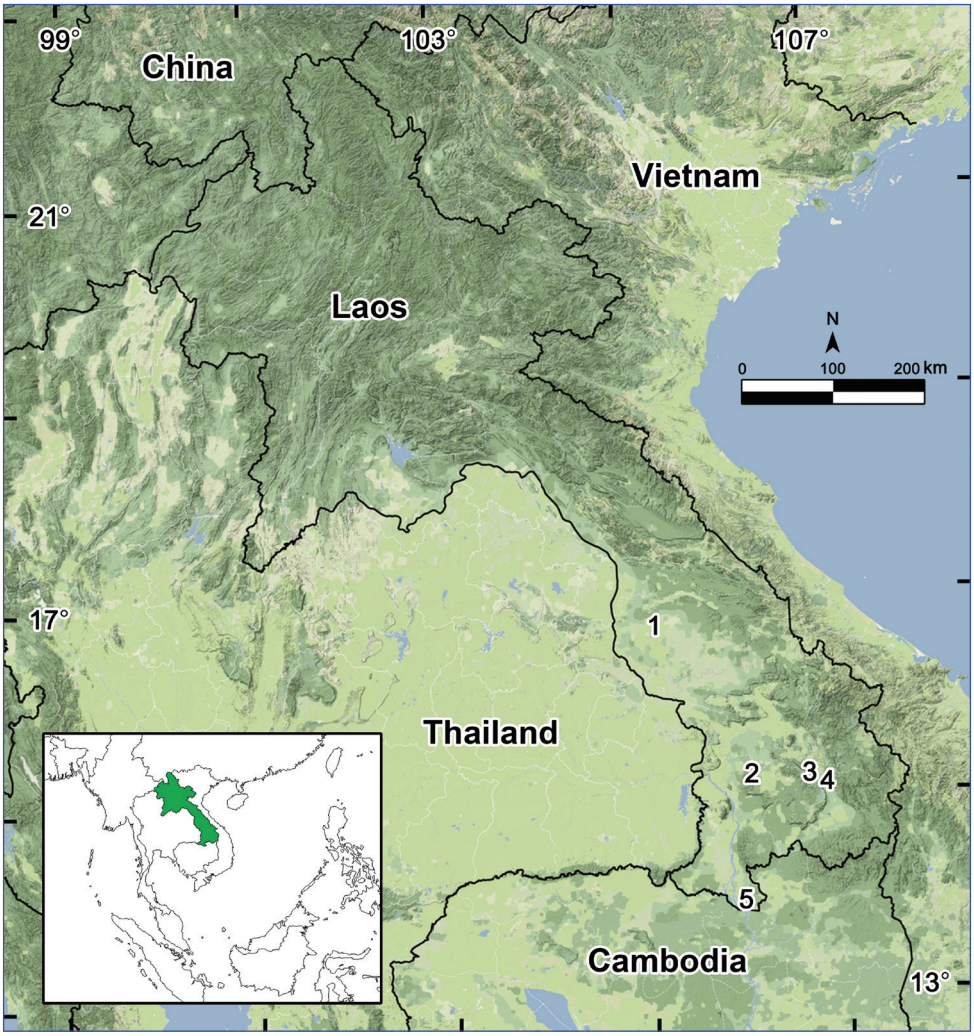

Figure 16. Distributions of the new records of *Orthomorpha* in Laos: **1** Ban Than Bang **2** Tadedu **3** Ban Lak No. 52 **4** Ban Kasom **5** Khone Phapheng Waterfall.

ern part of the country as being free from native *Orthomorpha* spp. remains unchanged (Likhitrakarn et al. 2011). There is little doubt that more *Orthomorpha* spp. will be found in countries, like Laos, that are home to this genus.

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Lectotype designations and nomenclatural changes in *Xylographus* Mellié (Coleoptera, Ciidae)

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Abstract

We designate lectotypes and propose nomenclatural changes in *Xylographus* Mellié (Coleoptera, Ciidae) based on type specimens deposited in the Museum of Comparative Zoology (USA), Museum für Naturkunde Berlin (Germany), the Natural History Museum (UK), Muséum d'Histoire Naturelle de la Ville de Genève (Switzerland), Muséum National d'Histoire Naturelle (France), Naturhistoriska Riksmuseet (Sweden) and Naturhistorisches Museum Wien (Austria). We designate lectotypes for the following species: *Cis fultoni* Broun, 1886, *Xylographus anthracinus* Mellié, 1849, *X. bicolor* Pic, 1916, *X. brasiliensis* Pic, 1916, *X. ceylonicus* Ancey, 1876, *X. contractus* Mellié, 1849, *X. corpulentus* Mellié, 1849, *X. dentatus* Pic, 1922, *X. gibbus* Mellié, 1849, *X. hypocritus* Mellié, 1849, *X. javanus* Pic, 1937, *X. lemoulti* Pic, 1916, *X. longicollis* Pic, 1922, *X. madagascariensis* Mellié, 1849, *X. nitidissimus* Pic, 1916, *X. perforatus* Gerstaecker, 1871, *X. porcus* Gorham, 1886, *X. punctatus* Mellié, 1849, *X. ritsemai* Pic, 1921, *X. rufescens* Pic, 1921, *X. rufipennis* Pic, 1934, *X. rufipes* Pic, 1930, *X. seychellensis* Scott, 1926, *X. subopacus* Pic, 1929, *X. subsinuatus* Pic, 1916, *X. suillus* Gorham, 1886, *X. testaceitarsis* Pic, 1916 and *X. tomicoides* Reitter, 1902. We propose the following **syn. n.** (senior synonym listed first): *X. anthracinus* = *X. testaceitarsis*, *X. brasiliensis* = *X. lucasi* Lopes-Andrade & Zacaro, *X. corpulentus* = *X. lemoulti* and *X. richardi* Mellié, *X. madagascariensis* = *X. eichelbaumi* Reitter, *X. rufipennis*, *X. seychellensis* Scott and *X. tarsalis* Fåhraeus, *X. nitidissimus* = *X. longicollis*, *X. subsinuatus* = *X. rufescens*. We exclude three species from *Xylographus*: *Cis renominatus,* **nom. n.** (for *X. dentatus* Pic, 1922, not *C. dentatus* Mellié, 1849), *Paratrichapus fultoni* (Broun, 1886), **comb. n.** and *P. javanus* (Pic, 1937), **comb. n.**

Keywords

Ciid, minute tree-fungus beetle, Orophiini, type material

Introduction

Xylographus Mellié (Coleoptera, Ciidae, Orophiini) is a genus of minute tree-fungus beetles with 36 described species, occurring in most continental and insular lands of tropical and subtropical regions (Lawrence and Lopes-Andrade 2010, Sandoval-Gómez et al. 2011). The name *Xylographus* was mentioned for the first time in the catalogue of Dejean (1835), but became available only after its description by Mellié (1847). Six species names were cited in the original description of the genus, but only one of them was available, *Cis bostrichoides* Dufour, 1843, being its type species by monotypy. Afterwards, Mellié (1849) described the other five species and proposed three more, respectively: *X. anthracinus* Mellié, 1849, *X. contractus* Mellié, 1849, *X. corpulentus* Mellié, 1849, *X. gibbus* Mellié, 1849, *X. hypocritus* Mellié, 1849, X. *madagascariensis* Mellié, 1849, *X. punctatus* Mellié, 1849 and *X. richardi* Mellié, 1849. Moreover, he synonymized *Cis cribatus* Lucas, 1849 with *X. bostrichoides*.

In the late XIX century, six species of *Xylographus* were described: *X. perforatus* Gerstaecker, 1871, *X. tarsalis* Fåhraeus, 1871, *X. ceylonicus* Ancey, 1876, *X. latirostris* Gorham, 1886, *X. porcus* Gorham, 1886 and *X. suillus* Gorham, 1886. *Xylographus latirostris* was later transferred to *Ceracis* Mellié, 1847 by Lawrence (1971) and *Cis fultoni* Broun, 1886 to *Xylographus* by Kuschel (1990).

The first half of the XX century was marked by a considerable increase in number of *Xylographus*, with the description of 19 species. Edmund Reitter described three species: *X. tomicoides* Reitter, 1902, *X. eichelbaumi* Reitter, 1908 and *X. globipennis* Reitter, 1911. Maurice Pic was the most prolific author, describing 14 species: *X. bicolor* Pic, 1916a, *X. brasiliensis* Pic, 1916a, *X. lemoulti* Pic, 1916b, *X. nitidissimus* Pic, 1916a, *X. subsinuatus* Pic, 1916b, *X. testaceitarsis* Pic, 1916a, *X. ritsemai* Pic, 1921, *X. rufescens* Pic, 1921, *X. dentatus* Pic, 1922, *X. longicollis* Pic, 1922, *X. subopacus* Pic, 1929, *X. rufipes* Pic, 1930, *X. rufipennis* Pic, 1934 and *X. javanus* Pic, 1937. However, these species are difficult to recognize, because their original descriptions are very brief, lacking adequate diagnostic characteristics and some of them may constitute synonyms of species previously proposed by other authors (Sandoval-Gómez et al. 2011). Scott (1926) described *X. seychellensis*, but indicated that it could be a synonym of one of the Afrotropical species described by Pic, which he could not examine. Blair (1940) described *X. bynoei*.

In the second half of the XX century only two species were described: *X. nakanei* Nobuchi, 1955 and *X. scheerpeltzi* Nobuchi & Wada, 1956. *Xylographus nakanei* was proposed as junior synonym of *Paraxestocis unicornis* Miyatake, 1954 by Kawanabe (1995). Finally, after almost a half century without new descriptions of *Xylographus*, *X. lucasi* was described by Lopes-Andrade and Zacaro (2003). Ferrer (1997) designated lectotypes of *Xylographus* species described by Fåhraeus (1871) and Reitter (1908). Later, in a paper on the Afrotropical *X. globipennis*, its lectotype was designated (Sandoval-Gómez et al. 2011).

Recently we had the opportunity to examine type material of the most important historical collections of *Xylographus*. During this work, we noted that some spe-

cies should be excluded from the genus and several synonyms were recognized. It is necessary to propose these nomenclatural acts now, before finishing the revision of *Xylographus*, because some names will soon be cited in ecological, cytotaxonomic and phylogenetic works on ciids. As most descriptions of *Xylographus* are based on syntypes, lectotype designations are necessary to fix clearly the concept of the names and to ensure the universal and consistent interpretation of them.

Material and methods

We examined 195 type specimens of *Xylographus* from the following institutions (preceded by acronyms used in this paper):

MCZ Museum of Comparative Zoology, Harvard University (Cambridge, Massachusetts, United States)
MFNB Museum für Naturkunde Berlin (Berlin, Germany)
MHNG Muséum d'histoire naturelle de la ville de Genève (Geneva, Switzerland)
MNHN Muséum National d'Histoire Naturelle (Paris, France)
NHM The Natural History Museum (London, United Kingdom)
NHMW Naturhistorisches Museum Wien (Wien, Austria)
NHRS Naturhistoriska Riksmuseet (Stockholm, Sweden)

We used the generic features of *Xylographus* cited by Sandoval-Gómez et al. (2011), the most important features proposed by Lawrence (1971) to recognize *Cis*, and the original description of *Paratrichapus* by Scott (1926), for making decisions on generic placement. *Paratrichapus* was described as having a 3-3-3 tarsal formula, but after studying its type material and images of microscope slide preparations by Hugh Scott, we observed that it was certainly 4-4-4 as in all other ciids. *Xylographus* and *Paratrichapus* are morphologically similar, so we propose the characteristics stated on Table 1 to differentiate them.

We have not located the types of *X. bostrichoides* and *X. richardi*. And we did not have access to type material of *X. scheerpeltzi*. In the case of *X. bostrichoides*, we had at hand several named historical specimens, including those used for its redescription by Mellié (1849). In the case of *X. richardi*, we had only a named specimen for examination. The description of *X. scheerpeltzi* is adequately detailed and includes information on the morphology of sclerites of male abdominal terminalia. In all other cases, we had access to the original type series and dissected male abdominal terminalia whenever necessary and possible. The morphology of sclerites of male abdominal terminalia of Ciidae is stable intraspecifically and distinctly varies interspecifically, even between closely related species (Antunes-Carvalho and Lopes-Andrade 2013, Oliveira et al. 2013).

We propose ten synonymies among the currently available names in *Xylographus*. For us, these are the most obvious cases that need solution. These names were proposed

Table 1. Main differences between *Xylographus* Mellié and *Paratrichapus* Scott.

Features	*Xylographus*	*Paratrichapus*
left mandible usually bearing an upward tooth in males	present in most species	absent
first labial palpomere	elongate, as long as or longer than the second one	shorter than the second one
pronotum punctation	dual, fine to coarse	single, always deep and coarse
prosternum	concave	biconcave
elytral length/elytral width	less than 1.15	more than 1.15
elytral length/pronotal length	less than 1.4	more than 1.4
protibial socketed spines	extending from the apex to almost its base	extending from the apex to at most its middle
first and second tarsomeres	subconical and well separated	subcylindrical and contiguous

based on slight color differences (for instance, those observed in teneral adults), subtle variations of male secondary sexual characteristics or based only on females. A single author, Maurice Pic, was responsible for half of the names here recognized as junior synonyms. He is known for having proposed thousands of new names of beetles based mostly on anecdotal descriptions and small type-series. Lack of access to type material was also a great problem. Scott (1926) described *X. seychellensis* stating that he did it with some hesitation, because he has not examined possible conspecifics, as *X. madagascariensis* and *X. eichelbaumi*, the senior and a junior synonym proposed here, respectively. The same was true to *X. lucasi*, whose authors (Lopes-Andrade and Zacaro 2003) described it without examining the type of *X. brasiliensis*, recognized here as its senior synonym.

A complete list of *Xylographus* species is given in alphabetical order. Type-locality and synonyms, if any, are given for each species. Type series and type material of its synonyms are given only for species that we could examine in museums. Syntypes of species treated in this work were almost all labeled as lectotypes and paralectotypes by John F. Lawrence in 1965, but they were not officially designated in the literature. We reexamined all specimens and preferred to maintain Lawrence's labels in most cases to avoid future inconsistencies. We designated a lectotype in cases where a single specimen was located and the author of the species name did not state whether there was one or more than one specimen in the type series. We consider a specimen to be the holotype only when the author clearly stated there was a single specimen available for description. When exact label data are listed, a backslash (\) separates individual labels. Data in square brackets were added for clarification. Remarks are provided for some species.

Taxonomic synopsis

Xylographus anthracinus Mellié, 1849

Xylographus testaceitarsis Pic, 1916, **syn. n.**

Xylographus bicolor Pic, 1916
Xylographus bostrichoides (Dufour, 1843)
    Cis cribatus Lucas, 1849
    Xylographus bostrichoides var. *aubei* Mellié, 1849
Xylographus brasiliensis Pic, 1916
    Xylographus lucasi Lopes-Andrade & Zacaro, 2003, **syn. n.**
Xylographus bynoei Blair, 1940
Xylographus ceylonicus Ancey, 1876
Xylographus contractus Mellié, 1849
Xylographus corpulentus Mellié, 1849
    Xylographus lemoulti Pic, 1916, **syn. n.**
    Xylographus richardi Mellié, 1849, **syn. n.**
Xylographus gibbus Mellié, 1849
Xylographus globipennis Reitter, 1911
Xylographus hypocritus Mellié, 1849
Xylographus madagascariensis Mellié, 1849
    Xylographus eichelbaumi Reitter, 1908, **syn. n.**
    Xylographus rufipennis Pic, 1934, **syn. n.**
    Xylographus seychellensis Scott, 1926, **syn. n.**
    Xylographus tarsalis Fåhraeus, 1871, **syn. n.**
Xylographus nitidissimus Pic, 1916
    Xylographus longicollis Pic, 1922, **syn. n.**
Xylographus perforatus Gerstaecker, 1871
Xylographus porcus Gorham, 1886
Xylographus punctatus Mellié, 1849
Xylographus ritsemai Pic, 1921
Xylographus rufipes Pic, 1930
Xylographus scheerpeltzi Nobuchi & Wada, 1956
Xylographus subopacus Pic, 1929
Xylographus subsinuatus Pic, 1916
    Xylographus rufescens Pic, 1921, **syn. n.**
Xylographus suillus Gorham, 1886
Xylographus tomicoides Reitter, 1902

Excluded species

Cis renominatus, **nom. n.**
    Xylographus dentatus Pic, 1922, not *Cis dentatus* Mellié, 1849.
Paratrichapus fultoni (Broun, 1886), **comb. n.**
    Cis fultoni Broun, 1886
Paratrichapus javanus (Pic, 1937), **comb. n.**
    Xylographus javanus Pic, 1937

Species accounts

Xylographus anthracinus Mellié, 1849

http://species-id.net/wiki/Xylographus_anthracinus

Xylographus anthracinus Mellié 1849: 222, pl. 9, fig. 17. Type-locality: Madagascar.
Xylographus testaceitarsis Pic 1916: 13., **syn. n.** Type-locality: Mahatsinjo, Madagascar.

Type series. MADAGASCAR: male lectotype (MNHN), here designated, labeled: "*Anthracinus* Dup. Madagascar.[handwritten] \ Ex-Musæo Mniszech [printed] \ [red label] LECTOTYPE *Xylographus anthracinus* Mellié [handwritten]"; 2 female paralectotypes (MNHN), labeled: "*anthracinus* (ex coll. Chev.) [handwritten] \ Mellié vidit [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus anthracinus* Mellié [handwritten]"; 2 male paralectotypes (MHNG), labeled: "Coll. Melly [printed] \ [yellow label] PARALECTOTYPE *Xylographus anthracinus* Mellié [handwritten]".

Type material of the junior synonym. MADAGASCAR: male lectotype (MNHN) of *Xylographus testaceitarsis* Pic 1916, here designated, labeled: "MAHATSINJO près Tananarive [printed] \ Type [handwritten] \ *testaceitarsis* Pic [handwritten] \ [red label] LECTOTYPE *Xylographus testaceitarsis* Pic [handwritten]"; 1 male and 3 female paralectotypes (MNHN), labeled: "MAHATSINJO près Tananarive [printed] \ [yellow label] PARALECTOTYPE *Xylographus testaceitarsis* Pic [handwritten]".

Remarks. There is no morphological difference between the lectotype of *X. anthracinus* and the lectotype of *X. testaceitarsis*. They are males of about the same size and with secondary sexual characteristic similarly developed. We have also dissected and compared sclerites of their abdominal terminalia and noted no difference.

Xylographus bicolor Pic, 1916

http://species-id.net/wiki/Xylographus_bicolor

Xylographus bicolor Pic 1916: 13. Type-locality: Mahatsinjo, Madagascar.

Type series. MADAGASCAR: male lectotype (MNHN), here designated, labeled: "MAHATSINJO près Tananarive [printed] \ Type [handwritten] \ *bicolor* Pic [handwritten] \ [red label] LECTOTYPE *Xylographus bicolor* Pic [handwritten]".

Xylographus bostrichoides (Dufour, 1843)

http://species-id.net/wiki/Xylographus_bostrichoides

Cis bostrichoides (Dufour 1843: 93). Type-locality: Vallée d'Ossan, France.
Cis cribatus Lucas 1849: 469. Junior synonym. Type-locality : Alger, Algeria.
Xylographus bostrichoides var. *aubei* Mellié 1849: 232. Junior synonym. Type-locality: Pyrénées, France.

Remarks. Unfortunately we did not find the type material of Dufour in the MNHN. We have found only specimens used by Mellié (1849) to redescribe this species and to describe its variety *aubei*, and dozens of specimens that do fit the currently accepted species limits. Müller et al. (2001) labeled one specimen deposited in MFNB as syntype of *X. bostrichoides* . However, after studying this specimen, we determined it is a member of Scolytinae (Curculionidae) and fits neither the original description by Dufour (1843) nor the redescription by Mellié (1849). Therefore, a lectotype is not designated here.

***Xylographus brasiliensis* Pic, 1916**
http://species-id.net/wiki/Xylographus_brasiliensis

Xylographus brasiliensis Pic 1916: 13. Type-locality: Rio Verde, Brazil.
Xylographus lucasi Lopes-Andrade and Zacaro 2003: 1. **syn. n.** Type-locality: Venda Nova do Imigrante, Espírito Santo, Brazil.

Type series. BRASIL: female lectotype (MNHN), here designated, labeled: "Bresil. Goyaz. Rio Verde [printed] \ *Xylographus* [handwritten] \ Type [handwritten] \ *Brasiliensis* Pic [handwritten] \ [red label] LECTOTYPE *Xylographus brasiliensis* Pic [handwritten]".

Type material of the junior synonym. See Lopes-Andrade and Zacaro (2003).

Remarks. In the description of *X. lucasi*, the authors did not have access to type specimens of *X. brasiliensis* and stated that its description was vague (Lopes-Andrade and Lawrence 2003). After we examined the available type of *X. brasiliensis*, a female located in the MNHN, we observed there is no difference between it and female paratypes of *X. lucasi*. We have located in the MNHN a male specimen collected in "Goyaz" (which may correspond to the current state of Goiás or to Tocantins), a historical specimen but not from the original type series of *X. brasiliensis*. We dissected it and compared the sclerites of abdominal terminalia to those of male paratypes of *X. lucasi*, and they are exactly the same. The species is widespread in the tropical South America and the type localities of both names are within its known range (pers. obs.).

***Xylographus bynoei* Blair, 1940**
http://species-id.net/wiki/Xylographus_bynoei

Xylographus bynoei Blair 1940: 131. Type-locality: northwest coast of Australia.

Type series. AUSTRALIA: male holoype (NHM), labeled: "[faded blue disc] N. Holl. [above] 44.4 [below] [handwritten] \ [red disc] Holotype [printed] \ *Xylographus bynoei* Blair Type det. K.G. Blair 1939 [handwritten]"; 2 male and 2 female paratypes (NHM), labeled: "[yellow disc] Paratype [printed] \ N.W. Australia pres. By B. Bynoe, R.N. Surgeon on H.M.S. Beagle. See Stokes, Voyage of Discoveries. 1846 [handwritten]"; 4 female paralectotypes (NHM), labeled: "Australia 44.4 [handwritten] \ [yellow disc] Paratype [printed]".

***Xylographus ceylonicus* Ancey, 1876**
http://species-id.net/wiki/Xylographus_ceylonicus

Xylographus ceylonicus Ancey 1876: 85. Type-locality: Point de Galle, Ceylon (Sri Lanka).

Type series. SRI LANKA: male lectotype (MNHN), here designated, labeled: "*Xylographus ceylonicus* Ancey, n. sp. Ceylan (Pointe de Galle) Types [handwritten] \ [red label] LECTOTYPE *Xylographus Ceylonicus* Ancey [handwritten]"; 6 males, 1 female and 7 specimens of undetermined gender, all paralectotypes (MNHN), labeled: "*Xylographus Ceylonicus* Ancey, n. sp. Ceylan (Pointe de Galle) Types [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus ceylonicus* Ancey [handwritten]"; 3 male and 1 female paralectotypes (MNHN), labeled: "CEYLAN [printed] \ type [handwritten] \ Syntypes [handwritten] \ Ceylan Pointe de Galles [handwritten] \ *Xylographus ceylonicus* Ancey [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus ceylonicus* Ancey [handwritten]"; 7 paralectotypes of undetermined gender (MNHN), labeled: "*Xylographus Ceylonicus* Ancey Ceylan [handwritten] \ Ex. Coll. REITTER [printed] \ [yellow label] PARALECTOTYPE *Xylographus ceylonicus* Ancey [handwritten]"; 2 paralectotypes of undetermined gender (MFNB), labeled: "*Xylographus Ceylonicus* Ancey Ceylon Ancey Type [handwritten] \ Coll. L.W. Schaufuss [printed] \ [red label] ? SYNTYPUS *Xylographus ceylonicus* Ancey 1876 labelled by MNHUB 1998 [printed] \ [yellow label] PARALECTOTYPE *Xylographus ceylonicus* Ancey [handwritten]"; 2 paralectotypes of undetermined gender (MFNB), labeled: "*Xylographus Ceylonicus* Ancey Ceylan [handwritten] \ [red label] ? SYNTYPUS *Xylographus ceylonicus* Ancey 1876 labelled by MNHUB 1998 [printed] \ [yellow label] PARALECTOTYPE *Xylographus ceylonicus* Ancey [handwritten]"; 2 paralectotypes of undetermined gender (MFNB), labeled: "*Xylographus Ceylonicus* Ancey Ceylan [handwritten] \ ex Coll. Hiller [handwritten] \ [red label] ? SYNTYPUS *Xylographus ceylonicus* Ancey 1876 labelled by MNHUB 1998 [printed] \ [yellow label] PARALECTOTYPE *Xylographus ceylonicus* Ancey [handwritten]".

***Xylographus contractus* Mellié, 1849**
http://species-id.net/wiki/Xylographus_contractus

Xylographus contractus Mellié 1849: 227, pl. 9, fig. 20. Type-locality: Brazil.

Type series. BRASIL: female lectotype (MNHN), here designated, labeled: "[green disc] *Xylographus Contractus* Bresil Lap. Cast. 72 [handwritten] \ [red label] LECTOTYPE *Xylographus contractus* Mellié [handwritten]"; 1 female paralectotype (MNHN), labeled: "[green disc] *Xylographus contractus* Bresil [unreadable] 83 [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus contractus* Mellié [handwritten]".

***Xylographus corpulentus* Mellié, 1849**
http://species-id.net/wiki/Xylographus_corpulentus

Xylographus corpulentus Mellié 1849: 225, pl. 9, fig. 19. Type-locality: Peru.
Xylographus lemoulti Pic 1916: 4. **syn. n.** Type-locality: St-Laurent du Maroni, French Guiana.
Xylographus richardi Mellié 1849: 226. **syn. n.** Type-locality: Cayenne, French Guiana.

Type series. PERU: male lectotype (MNHN), here designated, labeled: "[green label] ♂ [handwritten] \ Mellié vidit [handwritten] \ [red label] LECTOTYPE *Xylographus corpulentus* Mellié [handwritten]"; 1 female paralectotype (MNHN), labeled: "[green label] ♀ [handwritten] \ Mellié vidit [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus corpulentus* Mellié [handwritten]"; 1 female paralectotype (MNHM), labeled: "[green label] ♀ [handwritten] \ Mellié vidit [handwritten] \ *Corpulentus* Mell. (Coll. Chevrolat) [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus corpulentus* Mellié [handwritten]"; 1 male and 2 female paralectotypes (MNHN), labeled: "[green disc] *Xylographus Corpulentus* Perou Lap. Cast. 72 [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus corpulentus* Mellié [handwritten]"; 1 male paralectotype (MNHN), labeled: "[green disc] *Xylographus Corpulentus* Kunze Perou T. Cast. 83 [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus corpulentus* Mellié [handwritten]"; 1 male and 2 female paralectotypes (MNHN), labeled: "[green label] Kunze 19 [handwritten] \ [green label] *Cis Corpulentus* Kunze Perou [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus corpulentus* Mellié [handwritten]".

Type material of junior synonyms. FRENCH GUIANA: male lectotype (MNHN) of *Xylographus lemoulti* Pic, 1916, here designated, labeled: "NOVEMBRE [printed] \ [green label] GUYANE FRANÇAISE St-LAURENT du MARONI [printed] \ [green label] COLL LE MOULT [printed] \ Type [handwritten] \ [red label] LECTOTYPE *Xylographus lemoulti* Pic [handwritten]"; 3 male and 2 female paralectotypes (MNHN), labeled: "NOVEMBRE [printed] \ [green label] GUYANE FRANÇAISE St-LAURENT du MARONI [printed] \ [green label] COLL LE MOULT [printed] \ [yellow label] PARALECTOTYPE *Xylographus lemoulti* Pic [handwritten]"; 2 male and 1 female paralectotypes (MNHN), labeled: "NOVEMBRE [printed] \ GUYANE FRANÇAISE St-LAURENT du MARONI [printed] \ COLL LE MOULT [printed] \ [yellow label] PARALECTOTYPE *Xylographus lemoulti* Pic [handwritten]"; 1 male paralectotype (MNHN), labeled: "NOVEMBRE [printed] \ GUYANE FRANÇAISE St-LAURENT du MARONI [printed] \ COLL LE MOULT [printed] \ [yellow label] PARALECTOTYPE *Xylographus lemoulti* Pic [handwritten]"; 3 male and 4 female paralectotypes (MNHN), labeled: "OCTOBRE [printed] \ GUYANE FRANÇAISE St-LAURENT du MARONI [printed] \ COLL LE MOULT [printed] \ [yellow label] PARALECTOTYPE *Xylographus lemoulti* Pic [handwritten]"; 1 male and 3 female paralectotypes (MNHN), labeled: "JUIN [printed] \ [green label] GUYANE FRANÇAISE St-LAURENT du

MARONI [printed] \ [green label] COLL LE MOULT [printed] \ [yellow label] PARALECTOTYPE *Xylographus lemoulti* Pic [handwritten]"; 1 male paralectotype (MNHN), labeled: "MAI [printed] \ GUYANE FRANÇAISE St-LAURENT du MARONI [printed] \ COLL LE MOULT [printed] \ [yellow label] PARALECTOTYPE *Xylographus lemoulti* Pic [handwritten]".

Remarks. There are several type specimens of species described by Mellié deposited in historical collections of the MHNG and the MNHN. In the MHNG, these types are in the A. Melly collection, who has a surname similar to that of J. Mellié but shall not be confounded. We did not find type material of *X. richardi* in the Chevrolat collection of MNHN. We located a female specimen from Colombia in the Melly collection of MHNG named as *X. richardi*. Mellié (1849) mentioned he has examined specimens from both the Chevrolat and the Melly collections, therefore there is a possibility that this single specimen we located in the Melly collection is a syntype, but we cannot assure this. We compared this female with female paralectotypes of *X. corpulentus* and *X. lemoulti* and they are exactly the same. Mellié (1849) provided few differences between *X. corpulentus* and *X. richardi*, stating that they resemble each other "pour la taille et la forme", with *X. richardi* being more punctate. We believe the description of *X. richardi* was based on a female specimen, because the pronotal surface between punctures is described as being finely rugose. We have observed that it is common in female *Xylographus* species to have pronotal surface distinctly more rugose than that of males. The type of *X. corpulentus* was described as being black, while the one of *X. richardi* was described as reddish. It is a common variation found in *X. corpulentus*, in which teneral adults may be reddish (pers. obs.). Pic (1916b) mentioned that *X. lemoulti* differs from *X. richardi* in the coloration and pronotal shape, again a consequence of the fact that the description of *X. richardi* was based in a teneral adult female. The type-localities of *X. lemoulti* and *X. richardi* are approximately 200 Km apart and both are in the coast of French Guiana.

Xylographus gibbus Mellié, 1849

http://species-id.net/wiki/Xylographus_gibbus

Xylographus gibbus Mellié 1849: 228. Type-locality: Colombia.

Type series. COLOMBIA: female lectotype (MNHN), here designated, labeled: "[green disc] *Xylographus gibbus* Klg. Mell. Colomb. T. Cast. 72 [handwritten] \ [green label] *Cis gibbus* Klug [handwritten] \ *X. gibbus* Reiche \ MUSEUM PARIS COLL. DE MARSEUL 2842-00 [printed] \ [red label] LECTOTYPE *Xylographus gibbus* Mellié [handwritten]"; 1 male paralectotype (MHNG), labeled: "*Gibbus* Klug Colombie Klug Mellié [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus gibbus* Mellié [handwritten]".

***Xylographus globipennis* Reitter, 1911**

Xylographus globipennis Reitter 1911: 52. Type-locality: Gorbatuco, Eritrea.

Type series. See Sandoval-Gómez et al. (2011).

***Xylographus hypocritus* Mellié, 1849**
http://species-id.net/wiki/Xylographus_hypocritus

Xylographus hypocritus Mellié 1849: 221, pl. 9, fig. 16. Type-locality: Madagascar.

Type series. MADAGASCAR: male lectotype (MNHN), here designated, labeled: "*Hypocritus* Dup. Madagascar. [handwritten] \ Ex-Musæo Mniszech [printed] \ [red label] LECTOTYPE *Xylographus hypocritus* Mellié [handwritten]"; 1 female paralectotype (MNHN), labeled: "Madagascar [handwritten] \ Ex-Musæo Mniszech [printed] \ [yellow label] PARALECTOTYPE *Xylographus hypocritus* Mellié [handwritten]".

***Xylographus madagascariensis* Mellié, 1849**
http://species-id.net/wiki/Xylographus_madagascariensis

Xylographus madagascariensis Mellié 1849: 224, pl. 9, fig. 18. Type-locality: Madagascar.
Xylographus eichelbaumi Reitter 1908: 119. **syn. n.** Type-locality: Amani, Tanzania.
Xylographus rufipennis Pic 1934: 14. **syn. n.** Type-locality: Gura, Kenya.
Xylographus seychellensis Scott 1926: 10. **syn. n.** Type-locality: Mahe, Seychelles.
Xylographus tarsalis Fåhraeus 1871: 670. **syn. n.** Type-locality: Caffraria (Eastern Cape of South Africa).

Type series. MADAGASCAR: male lectotype (MNHN), here designated, labeled: "*Madagascariensis* Dup. Madagascar.[handwritten] \ Ex-Musæo Mniszech [printed] \ [red label] LECTOTYPE *Xylographus madagascariensis* Mellié [handwritten]".

Type material of junior synonyms. KENYA: female lectotype (NHM) of *Xylographus rufipennis* Pic, 1934, here designated, labeled: "[red disc]Type [printed] \ R. E, DENT GURA R, 7500 AUG 1929 [printed] \ *Xylographus rufipennis* n. sp. [handwritten] \ Pres. By Imp. Inst. Ent B. M. 1934-42. [printed] \ [red label] LECTOTYPE *Xylographus rufipennis* Pic [handwritten]"; 1 female paralectotype (MNHN), labeled: "R. E, DENT GURA R, 7500 AUG 1929 [printed] \ *Xylographus rufipennis* n. sp [handwritten] \ ex. British museum [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus rufipennis* Pic [handwritten]". SEYCHELLES: male lectotype (NHM) of *Xylographus seychellensis* Scott 1926, here designated, labeled: "[purple disc] LECTOTYPE [printed] \ Mahe, 1908-9 Seychelles Exp. [printed] \ Percy Sladen Trust Exped. Brit. Mus. 1926-246. [printed] \ *Xylographus seychellensis*, Scott TYPE. ♂. [handwritten]

\ Figured specimen [printed] (outline whole vis) [handwritten] \ TYPE [printed] \ [red label] LECTOTYPE *Xylographus seychellensis* Scott [handwritten]". SOUTH AFRICA: male lectotype (NHRS) of *Xylographus tarsalis* Fåhraeus 1871, labeled: "Caffraria [printed] \ J. Wahlb. [printed] \ ♂ [printed] \ [red label] Lectotype ♂ *Xylographus tarsalis* FÅHR. Det. Julio Ferrer 1995 [handwritten] \ [green label] Riksmuseum Stockholm [printed]"; 1 male and 1 female paralectotypes (NHRS), labeled: "Caffraria [printed] \ J. Wahlb. [printed] \ [red label] Paralectotype *Xylographus tarsalis* FÅHR. Det. Julio Ferrer 1995 [handwritten] \ [green label] Riksmuseum Stockholm [printed]". TANZANIA: female holotype (NHMW) of *Xylographus eichelbaumi* Reitter 1908, labeled: "6. [handwritten] \ Amani [printed] \ D. O. Afrika Eichelbaum'03 [printed] \ 13 April 1903 in *Fomes nigrolachatus* [handwritten] \ Amani. Deutsch Ostafr. [handwritten] \ *Xylographus eichelbaumi* m. Typ. 1907. [handwritten] \ Eichelbaumi Usambara Reitt. [handwritten] \ [red label] HOLOTYPE *Xylographus eichelbaumi* Reitt. [handwritten]".

Remarks. Scott (1926) stated he has not examined the type of *X. madagascariensis* and that he described *X. seychellensis* with some hesitation. If he had examined the known male type of *X. madagascariensis*, he would have observed that it was just slightly more elongate than the specimens he had at hand, with no other differences. Such a small difference in body elongation is expected to occur in *Xylographus* species with broad geopraphical distribution (see, for instance, the known variation in *X. globipennis*; Sandoval-Gómez et al. 2011). In order to make sure they were all conspecifics, we dissected named male *X. seychellensis* compared to the type and also the lectotype of *X. madagascariensis*, and we observed the sclerites of abdominal terminalia to be exactly the same. The lectotype of *X. tarsalis* is a male *X. madagascariensis* with weak secondary sexual characteristics. Ferrer (1997) stated that two female paralectotypes of *X. tarsalis* were deposited in NHRS. After studying the material, we have seen that they are a male and a female instead. The names *X. eichelbaumi* and *X. rufipennis* were based on females, which clearly correspond to females named *X. madagascariensis* that we examined.

Xylographus nitidissimus Pic, 1916

http://species-id.net/wiki/Xylographus_nitidissimus

Xylographus nitidissimus Pic 1916: 13. Type-locality: São Tomé, São Tomé and Príncipe. *Xylographus longicollis* Pic 1922: 8. **syn. n.** Type-locality: Dahomey, Benin.

Type series. SÃO TOMÉ AND PRÍNCIPE: male lectotype (MNHN) of *Xylographus nitidissimus* Pic 1916, here designated, labeled: "[green label] San. Thomé [printed] \ n. sp. [handwritten] \ type [handwritten] \ *nitidissimus* Pic [handwritten] \ [red label] LECTOTYPE *Xylographus nitidissimus* Pic [handwritten]"; 1 male paralectotype (MNHN), labeled: "type [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus nitidissimus* Pic [handwritten]"; 4 male and 4 female paralectotypes (MNHN), labeled: "San Thomé [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus nitidissimus* Pic [handwritten]".

Type material of the junior synonym. BENIN: female lectotype (MNHN) of *Xylographus longicollis* Pic, 1922, here designated, labeled: "? Dahomey [handwritten] \ Provenance ? [handwritten] \ *longicollis* n. sp. [handwritten] \ [red label] LECTOTYPE *Xylographus longicollis* Pic [handwritten]".

Remarks. We observed that the female lectotype of *X. longicollis* is a female of *X. nitidissimus*.

***Xylographus perforatus* Gerstaecker, 1871**
http://species-id.net/wiki/Xylographus_perforatus

Xylographus perforatus Gerstaecker 1871: 57. Type-locality: Tanzania, Zanzibar.

Type series. TANZANIA: male lectotype (MFNB), here designated, labeled: "[green label] *perforatus* Gerst.* Sansibar Cooke [handwritten] \ 56743 [printed] \ [blue label] Hist.-Coll. (Coleoptera) Nr. 56743 (1. Ex) *Xylographus perforatus* Gerst. Sansibar, Cooke Zool. Mus. Berlin [printed] \ [red label] SYNTYPUS *Xylographus perforatus* Gerstaecker 1871 labelled by MNHUB 1998 \ [red label] LECTOTYPE *Xylographus perforatus* Gerstaecker [handwritten]"; 2 male and 3 female paralectotypes (MFNB), labeled: "[blue label] Hist.-Coll. (Coleoptera) Nr. 56743 (1.-5. Ex) *Xylographus perforatus* Gerst. Sansibar, Cooke Zool. Mus. Berlin [printed] \ [red label] SYNTYPUS *Xylographus perforatus* Gerstaecker 1871 labelled by MNHUB 1998 \ [yellow label] PARALECTOTYPE *Xylographus perforatus* Gerstaecker [handwritten]"; 1 female paralectotype (MNHN), labeled: "Zanzibar, C. Cooke. [printed] \ [blue label] MUSEUM PARIS Collection Léon Fairmaire 1906 [printed] \ *Xylographus perforatus* Gerst. [handwritten] P. Lesne vid. [printed] \ [yellow label] PARALECTOTYPE *Xylographus perforatus* Gerstaecker [handwritten]"; 1 female paralectotype (MNHN), labeled: "Zanzibar, C. Cooke. [printed] \ Bragance (Para) M. de Mathan [printed] \ Kein Scolytide [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus perforatus* Gerstaecker [handwritten]"; 1 male and 1 female paralectotype (MNHN), labeled: "Zanzibar, C. Cooke. [printed] \ *Xylographus perforatus* Gerst. Zanzib. [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus perforatus* Gerstaecker [handwritten]"; 1 male and 1 female paralectotypes (MCZ), labeled: "Zanzibar, C. Cooke. [printed] \ [black disc] \ *Xylographus perforatus* 140 Gerstaecker [printed] \ [yellow label] PARALECTOTYPE *Xylographus perforatus* Gerstaecker [handwritten]".

***Xylographus porcus* Gorham, 1886**
http://species-id.net/wiki/Xylographus_porcus

Xylographus porcus Gorham 1886: 355. Type-locality: Teleman, Guatemala.

Type series. GUATEMALA: male lectotype (NHM), here designated, labeled: "[purple disc] LECTOTYPE [printed] \ Teleman, Vera Paz. Champion. [printed] \ Type.

[printed] \ *Cis, porcus* [handwritten] \ B.C.A., Col., III (2). *Xylographus porcus*. [printed] \ [red label] LECTOTYPE *Xylographus porcus* Gorh. [handwritten]"; 1 male and 2 female paralectotypes (MNHN), labeled: "Teleman, Vera Paz. Champion. [printed] \ *Xylographus porcus*, Gorh [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus porcus* Gorh. [handwritten]"; 2 female paralectotypes (MNHN), labeled: "Zapote, Guatemala. G. C. Champion. [printed] \ [yellow label] PARALECTOTYPE *Xylographus porcus* Gorh. [handwritten]"; 1 male and 2 female paralectotypes (MNHN), labeled: "Pantaleon, 700 ft. Champion. [printed] \ [yellow label] PARALECTOTYPE *Xylographus porcus* Gorh. [handwritten]".

***Xylographus punctatus* Mellié, 1849**
http://species-id.net/wiki/Xylographus_punctatus

Xylographus punctatus Mellié 1849: 230, pl. 9, fig. 21. Type-locality: Colombia.

Type series. COLOMBIA: male lectotype (MNHN), here designated, labeled: "[green label] ♂ [handwritten] \ Nouv. Grenada [handwritten] \ Coll. Chevrolat [handwritten] \ [red label] LECTOTYPE *Xylographus punctatus* Mellie [handwritten]"; 1 male and 3 female paralectotypes (MNHN), labeled: "Nouv. Grenada [handwritten] \ Coll. Chevrolat [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus punctatus* Mellie [handwritten]"; 1female paralectotype (MNHN), labeled: "Carthagene [handwritten] \ Collect. Chevrolat [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus punctatus* Mellie [handwritten]"; 1 male paralectotype (MNHN), labeled: "[green disc] *Xylographus punctatus* Mell. Colomb. T. Cast. 72 [handwritten] \ [pink disc] punctatus [handwritten] \ punctatus Chev. Colombia [handwritten] \ MUSEUM PARIS COLL DE MARSEUL [printed] \ [yellow label] PARALECTOTYPE *Xylographus punctatus* Mellie [handwritten]"; 1 male paralectotype (MNHN), labeled: "[green disc] *Xylographus punctatus* Colomb. Lap. Cast. 72 [handwritten] \ [green label] Chev^{r}. Colomb [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus punctatus* Mellie [handwritten]".

***Xylographus ritsemai* Pic, 1921**
http://species-id.net/wiki/Xylographus_ritsemai

Xylographus ritsemai Pic 1921: 7. Type-locality: Ceylon (Sri Lanka).

Type series. SRI LANKA: male lectotype (MNHN), here designated, labeled: "*Ritsemai* Pic [handwritten] \ type [handwritten] \ Ceylon. Ancey. [handwritten] \ [red label] LECTOTYPE *Xylographus ritsemai* Pic [handwritten]"; 1 male paralectotype (MNHN), labeled: "*Ritsemai* Pic [handwritten] \ type [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus ritsemai* Pic [handwritten]".

***Xylographus rufipes* Pic, 1930**
http://species-id.net/wiki/Xylographus_rufipes

Xylographus rufipes Pic 1930: 175. Type-locality: Tucumán, Argentina.

Type series. ARGENTINA: male lectotype (MNHN), here designated, labeled: "Argentina Tucumán 1 Oct. 1929 [printed] \ H. E. Box leg. [printed] \ 2705 [printed] \ *Xylographus rufipes* n. sp. [handwritten] \ [red label] LECTOTYPE *Xylographus rufipes* Pic [handwritten]"; 1 male paralectotype (NHM), labeled: "Argentina Tucumán 1 Oct. 1929 [printed] \ H. E. Box leg. [printed] \ 2709 [printed] \ *Xylographus rufipes*, Pic sp. nov. (det. Pic, per C. Bruch) [handwritten] \ Brit. Mus. 1948-460. [printed] \ [yellow label] PARALECTOTYPE *Xylographus rufipes* Pic [handwritten]".

***Xylographus scheerpeltzi* Nobuchi & Wada, 1956**
http://species-id.net/wiki/Xylographus_scheerpeltzi

Xylographus scheerpeltzi Nobuchi and Wada 1956: 53, figs. 1–2. Type-locality: Japan.

Remarks. Nobuchi and Wada (1956) described this species based on a series of 28 syntypes from five different localities in Japan. We did not have access to material of Mr. T. Nakane's collection from the Hokkaido University Museum, so a lectotype for this species is not designated here.

***Xylographus subopacus* Pic, 1929**
http://species-id.net/wiki/Xylographus_subopacus

Xylographus subopacus Pic 1929: 264. Type-locality: Democratic Republic of the Congo, Elisabethville.

Type series. DEMOCRATIC REPUBLIC OF THE CONGO: female lectotype (NHM), here designated, labeled: "[purple disc] LECTOTYPE [printed] \ BELGIAN CONGO. 18 m. S. W. of Elizabethville. 24.iii.1928. Dr. H. S. Evans. [printed] \ Pres. by Imp. Inst. Ent. Brit. Mus. 1932-147. [printed] \ [red label] LECTOTYPE *Xylographus subopacus* Pic [handwritten]"; 1 female paralectotype (MNHN), labeled: "BELGIAN CONGO. 18 m. S. W. of Elizabethville. 1928. Dr. H. S. Evans. [printed] \ *Xylographus subopacus* n. sp. [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus subopacus* Pic [handwritten]".

***Xylographus subsinuatus* Pic, 1916**
http://species-id.net/wiki/Xylographus_subsinuatus

Xylographus subsinuatus Pic 1916: 4. Type-locality: Madagascar.
Xylographus rufescens Pic 1921: 7. **syn. n.** Type-locality: Bourbon Island (Reunion).

Type series. MADAGASCAR: male lectotype (MNHN), here designated, labeled: "MADAGASCAR Plantations du Sambirano COLLECTION LE MOULT [printed] \ [red label] Coll. C [handwritten] \ Type [handwritten] \ *subsinuatus* Pic [handwritten] \ [red label] LECTOTYPE *Xylographus subsinuatus* Pic [handwritten]"; 6 males, 3 females, 17 specimens of undetermined gender, all paralectotypes (MNHN), labeled: "MADAGASCAR Plantations du Sambirano COLLECTION LE MOULT [printed] \ [red label] Coll. C [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus subsinuatus* Pic [handwritten]".

Type material of the junior synonym. REUNION: male lectotype (MNHN) of *Xylographus rufescens* Pic 1921, here designated, labeled: "Ile Bourbon n. sp. [handwritten] \ type [handwritten] \ *rufescens* Pic [handwritten] \ [red label] LECTOTYPE *Xylographus rufescens* Pic [handwritten]".

Remarks. We observed that the lectotype of *X. rufescens* is a small teneral male of *X. subsinuatus*. We dissected the types and compared the sclerites of abdominal terminalia, which are identical.

***Xylographus suillus* Gorham, 1886**
http://species-id.net/wiki/Xylographus_suillus

Xylographus suillus Gorham 1886: 355, pl. 13, figs. 21, 21a. Type-locality: Teleman, Guatemala.

Type series. GUATEMALA: male lectotype (NHM), here designated, labeled: "[purple disc] LECTOTYPE [printed] \ Type. Sp. figured [printed] \ Teleman, Vera Paz. Champion. [printed] \ *Xylographus suillus*, Gorh. [handwritten] \ B.C.A., Coll., III (2). *Xylographus suillus*, Gorh. [printed] \ [red label] LECTOTYPE *Xylographus suillus* Gorh. [handwritten]"; 2 male and 4 female paralectotypes (MNHN), labeled: "Teleman, Vera Paz. Champion. [printed] \ [pink label] In boleti attached to manaca palm [handwritten] \ *Xylographus suillus*, Gor. [handwritten] \ [yellow label] PARALECTOTYPE *Xyloraphus suillus* Gorh. [handwritten]".

***Xylographus tomicoides* Reitter, 1902**
http://species-id.net/wiki/Xylographus_tomicoides

Xylographus tomicoides Reitter 1902: 47. Type-locality: Chabarowka, Amur, Russia.

Type series. RUSSIA: male lectotype (MNHN), here designated, labeled: "Amur [handwritten] \ *tomicoides* m. 1902 [handwritten] \ [red label] LECTOTYPE *Xylographus tomicoides* Reitter [handwritten]".

Excluded species

***Cis renominatus* nom. n.**

Xylographus dentatus Pic 1922: 7. Secondary junior homonym of *Cis dentatus* Mellié, 1849. Type-locality: Republic of the Congo.

Type series. REPUBLIC OF THE CONGO: male lectotype (MNHN), here designated, labeled: "Franz. Congo [printed] \ *dentatus* n. sp. [handwritten] \ *Cis* sp. A. Kompantsev det. 2010 [handwritten] \ [red label] LECTOTYPE *Xylographus dentatus* Pic [handwritten] \ [green label] *Cis renominatus* Sandoval-Gómez, Lópes-Andrade & Lawrence nom. n."; 6 male and 2 female paralectotypes (MNHN), labeled: "Franz. Congo [printed] \ [yellow label] PARALECTOTYPE *Xylographus dentatus* Pic [handwritten] \ [green label] *Cis renominatus* Sandoval-Gómez, Lópes-Andrade & Lawrence nom. n.".

Remarks. *Xylographus dentatus* Pic, 1922 is transferred to the genus *Cis*, but *Cis dentatus* (Pic, 1922) becomes a junior secondary homonym of *Cis dentatus* Mellié, 1849. The replacement name proposed here means "renamed".

***Paratrichapus fultoni* (Broun, 1886), comb. n.**
http://species-id.net/wiki/Paratrichapus_fultoni

Cis fultoni Broun 1886: 904. Type-locality: West Taieri, New Zealand.
Xylographus fultoni (Broun, 1886) Kuschel 1990: 62.

Type series. NEW ZEALAND: male lectotype (NHM), here designated, labeled: "1614 [handwritten] \ Taieri [printed] \ New Zealand. Broun Coll. Brit. Mus. 1922-482 [printed] \ *Cis fultoni* [handwritten] \ *Rhopalodontus fultoni* Broun. K. Paviour-Smith det. 1966 [handwritten] \ [red label] LECTOTYPE *Cis fultoni* Broun [handwritten]"; 1 female paralectotype (NHM), labeled: "1614 [handwritten] \ Taieri [printed] \ New Zealand. Broun Coll. Brit. Mus. 1922-482 [printed] \ *Cis fultoni* [handwritten] \ *Rhopalodontus fultoni* Broun. K. Paviour-Smith det. 1966 [handwritten] \ [yellow label] PARALECTOTYPE *Cis fultoni* Broun [handwritten]".

***Paratrichapus javanus* (Pic, 1937), comb. n.**
http://species-id.net/wiki/Paratrichapus_javanus

Xylographus javanus Pic 1937: 304. Type-locality: Goenoeng Tangkoeban Prahoe, Java, Indonesia.
Xylographus javanus var. *rufomarginatus* Pic 1937: 304. Junior synonym. Type-locality: Goenoeng Tangkoeban Prahoe, Java, Indonesia.

Type series. INDONESIA: male lectotype (MNHN), here designated, labeled: "F. C. DRESCHER G. Tangkoeban Prahoe 4000.5000 Voet. Preanger. Java 31.x.1934 [printed] \ ex *Fomes melanopurus* Mont. [printed] \ n. sp. diffère de *X. ceylonicus* Ancey par la forme plus allongée, le thorax moins court, plus fortement rétréci en avant, les élytres sans pli huméral brillant [handwritten] \ [red label] LECTOTYPE *Xylographus javanus* Pic [printed] \ *Paratrichapus javanus* (Pic, 1937) comb. n. Sandoval-Gómez, Lopes-Andrade & Lawrence [handwritten]"; 1 male paralectotype (MNHN), labeled: "F. C. DRESCHER G. Tangkoeban Prahoe 4000.5000 Voet. Preanger. Java 31.x.1934 [printed] \ ex *Fomes melanopurus* Mont. [printed] \ [yellow label] PARALECTOTYPE *Xylographus javanus* Pic [printed] \ *Paratrichapus javanus* (Pic, 1937) comb. n. Sandoval-Gómez, Lopes-Andrade & Lawrence [handwritten]"; 1female paralectotype (MNHN), labeled: "F. C. DRESCHER G. Tangkoeban Prahoe 4000.5000 Voet. Preanger. Java 22.i.1935 [printed] \ ex *Fomes melanopurus* Mont. [printed] \ *Xylographus javanus* n. sp. [handwritten] \ [yellow label] PARALECTOTYPE *Xylographus javanus* Pic [printed] \ *Paratrichapus javanus* (Pic, 1937) comb. n. Sandoval-Gómez, Lopes-Andrade & Lawrence [handwritten]".

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RESEARCH ARTICLE

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Redescription of *Japanagromyza inferna* Spencer, first recorded from Brazil, and a key to the Neotropical species of *Japanagromyza* Sasakawa (Diptera, Agromyzidae)

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Abstract

Japanagromyza inferna Spencer is recorded for the first time from Brazil, in the North coast of the State of Rio de Janeiro, inducing galls in *Centrosema virginianum L.* (Fabaceae). The species is redescribed, with illustrations of male and female terminalia. A key to the identification of the Neotropical species of *Japanagromyza* Sasakawa is presented.

Keywords

Morphology, taxonomy, insect-plant interactions, gall-inducing, new records

Introduction

Japanagromyza Sasakawa has currently 80 known species in the world (Lonsdale 2013) and is represented by 30 in the neotropics (Martinez and Etienne 2002, Etienne and Martinez 2003, Sasakawa 2005, Boucher and Hanson 2006, Boucher 2010). Only one species has been recorded from Brazil (São Paulo), *J. macroptilivora* Esposito & Prado (Esposito and Prado 1993). Some species are known to induce galls in plants, 15 of them are associated with plants of the Fabaceae family (Benavent-Corai et al. 2005) and other species are known to induce mines in crop plants (Spencer and Stegmaier 1973).

Japanagromyza is morphologically similar to *Agromyza* Fallén and *Melanagromyza* Hendel, although its species can be recognized by the following combination of characters: halter yellow, white, uniformly dark brown or variegated on top or inside of dark knob; thorax with two pairs of dorsocentral setae (rarely three pairs, but anterior ones only a little longer than acrostichals); one pair of scutellar setae (rarely absent); fore tibia with lateral setae in the middle (see Sasakawa 2010 for a complete description of the genus).

Japanagromyza inferna Spencer was originally described from Bahamas, with no information on the host plant (Spencer and Stegmaier 1973). Spencer et al. (1992) reported this species from Guadeloupe, also with no data on the host plant. Years later Etienne and Martinez (2003) recorded from Guadalupe and Saint Christopher, inducing leaf galls on *Centrosema virginianum* L. (Fabaceae). *C. virginianum* is found throughout South America in forest scrub, "caatinga" and woodlands (Schultze-Kraft et al. 1990). Other species of Agromyzidae recorded as pests in plants of the genus *Centrosema* Benth. are *Ophiomyia centrosematis* (Meijere), *M. phaseoli* Tryon, causing damage and influencing plant growth (Lenné et al. 1990), *Japanagromyza centrosematifolii* forming mines in *C. virginianum and C. pubescens* (Etienne and Martinez 2003) and *J. centrosemae* Frost, known on *C. pubescens* (Spencer 1990).

The main aim of this paper is to present a redescription of *J. inferna*, including characters not yet described, and a key to the 30 Neotropical species of the genus *Japanagromyza*.

Material and methods

Collections were made bimonthly, from July 2011 to March 2012, in sandbanks in the North coast of the State of Rio de Janeiro (Fig. 1). The localities investigated were Arraial do Cabo, Grussaí (São João da Barra) and Saquarema (coordinates under material examined). In addition to these locations, an extra collection was made in the Marambaia sandbank, also located in Rio de Janeiro (Fig. 1).

To obtain material, branches of the plants with galls were removed and taken to the laboratory. The branches were placed in plastic pots, covered with organza and elastic for rearing and emergence of the adults. After emergence, adults were mounted on entomological pins and were deposited in the collection of Museu Nacional, Universidade Federal do Rio de Janeiro.

The terminalia were clarified in potassium 10% hydroxide for dissection under stereomicroscope and drawn using a camera lucida. Digital images of the gall, pupae and adult were prepared using a Leica MZ 16 optical microscope and the software program AutoMontage Pro by Syncroscopy. The species identification and the key to Neotropical species were based on the original descriptions. The terminology was based on Boucher 2010.

Results

***Japanagromyza inferna* Spencer, 1973**
http://species-id.net/wiki/Japanagromyza_inferna

Material examined. BRAZIL, RIO DE JANEIRO: Saquarema: 22°56'06"S, 42°4'43"W. 3 ♂, 1 ♀, 01. VI. 2012. Col. V.R. Sousa; 22°56'03"S, 42°24'16"W. 3 ♂, 3 ♀, 18. XI. 2011. Col. V.R. Sousa; 1 ♂ and 2 ♀, 19 XI 2011. Col. V.R. Sousa. Arraial do Cabo: 22°57'00"S, 42°05'05"W. 1♀, 01. VI. 2012. Col. V.R Sousa. Grussaí: 21°43'42.5"S, 41°01'46.2"W. 1♂, 29. I. 2012. Col. V.R Sousa; 21°44'36.3"S, 41°01'44.7"W. 1♂, 01. II. 2012. Col. V.R. Sousa. Marambaia: 17m 23°02'56"S, 43°37'51"W. 1 ♂, 4 ♀, 03. II. 2012. Col. V.R. Sousa. All forming galls in *Centrosema virginuanum* (L.) Benth.

Redescription. Male. (Fig. 2) - Body length: 2.5–2.6 mm. Wings length: 2.4mm.

Color. Frons black dull, paler brownish at orbits level; face dark; fronto-orbital plate and ocellar triangle shining black; lunule gray pollinose; antenna black with apex of pedicel and base of postpedicel brown; arista black; palpus black; proboscis brown with labellum paler yellow with long yellow setae; thorax black with greenish reflections; halters yellow, brown at base; calypters and fringe yellow; legs black with coppery reflections; pulvilli white; abdomen black with coppery reflections.

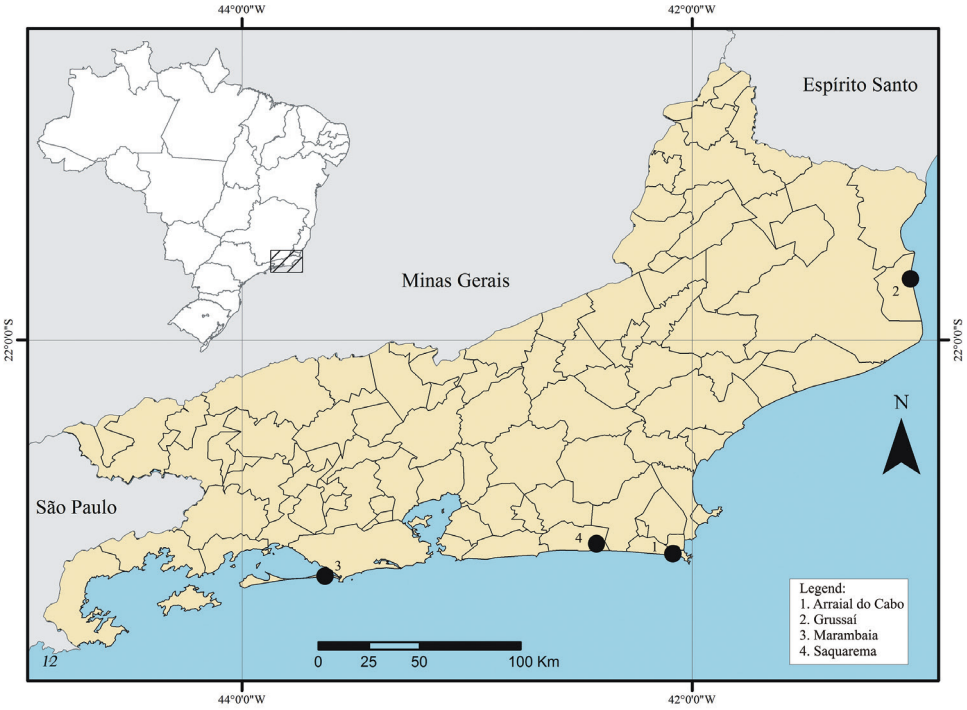


Figure 1. Map with the records localities of *Japanagromyza inferna* Spencer in Rio de Janeiro.

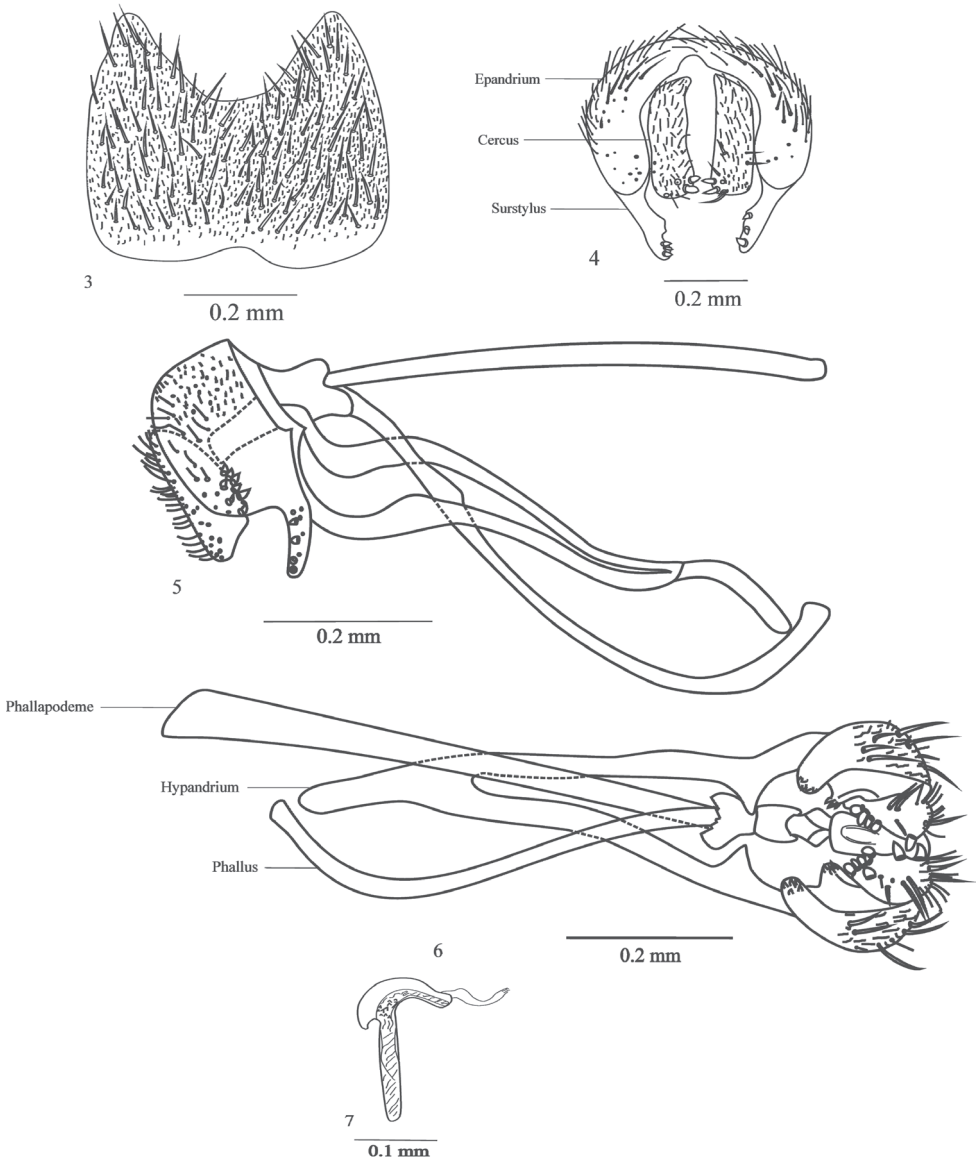
Figure 2. *Japanagromyza inferna* Spencer, male, in lateral view.

Head. Fronto-orbital setulae in 4 pairs of rows, the two upper ors longer than the lower ones, first pair inclinate and the others posteriorly directed; ocellar triangle long; ocellar setae parallel and forward directed; internal orbital seta long, parallel and divergent; external orbital seta with about half the length of the internal; third antennal segment rounded and minutely pubescent; arista long and short pubescent; gena shorter with setae; vibrissa strong and short.

Thorax. Acrostichals in 10 rows, pre-sutural pair differentiated; two postsutural dorsocentral setae; two notopleural setae; one supra-alar; one intra-alar; one post-alar weak; two prospronotals; two pairs of scutellar setae, one sub basal and one apical, similar in size; four anepisternals with second upper one long and strong; katepisternum with small setae and one long katepisternal.

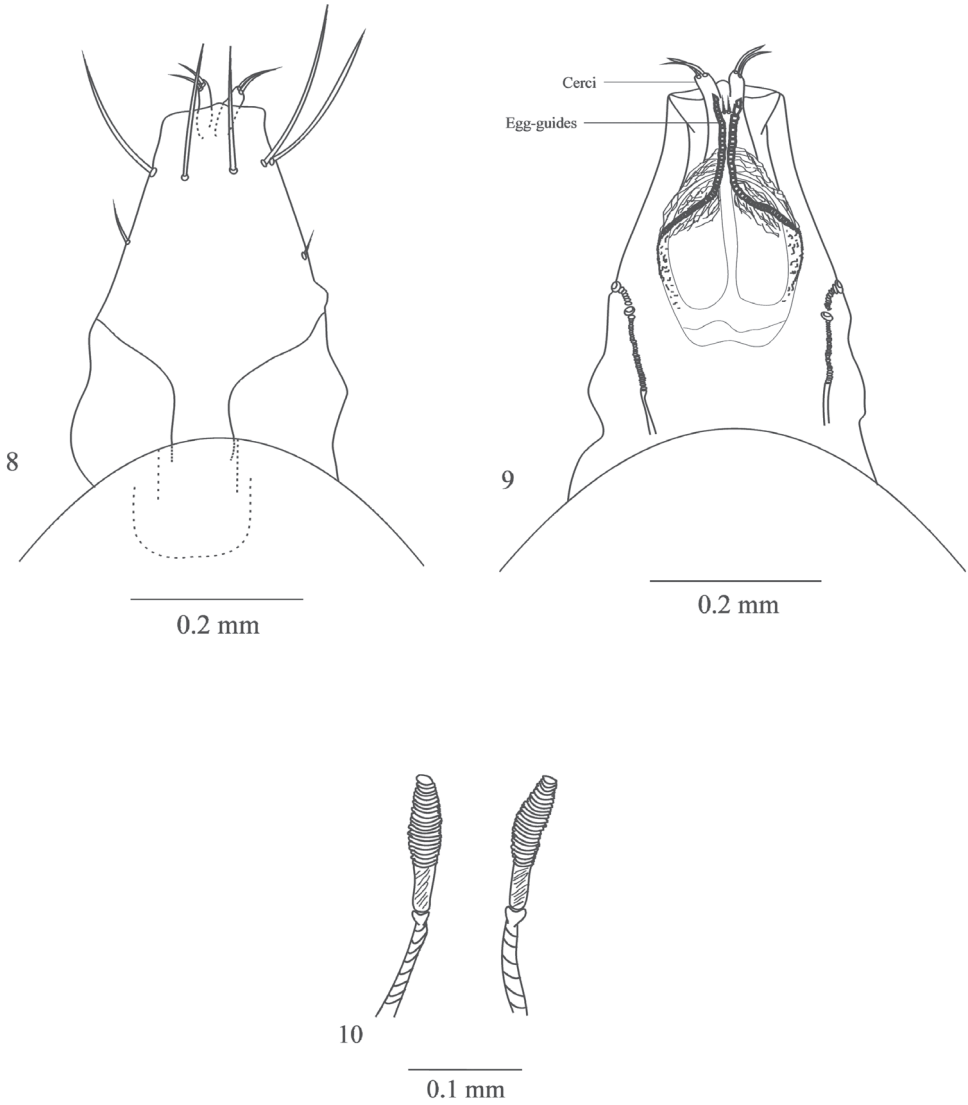
Legs. Fore tibia with one posterior supramedian seta. Mid tibia with two posterior setae inserted at middle third and one ventral apical seta. Hind tibia with one ventral apical seta.

Abdomen. Sternite 5 large with setae in all its extension (Fig. 3).



Figures 3–7. Male terminalia of *Japanagromyza inferna* Spencer **3** sternite 5 **4** epandrium, cercal plate and surstylus **5** hypandrium **6** phallapodeme, hypandrium, phallus **7** ejaculatory apodeme.

Terminalia. Epandrium with internal margin with a small median indentation and with long setae; cercal plate with 3-4 spines in the basal portion, eight lateral spines and long cilia in all extension; surstylus long, slightly curved with about four thick spines (Fig. 4). Hypandrium v-shaped (Fig. 5); aedeagus simple, long and tubular, ornamented with membranes at the basiphallus (Fig. 6); ejaculatory apodeme small, hammer-shaped, with weak spines at base (Fig. 7).



Figures 8–10. Female ovipositor of *Japanagromyza inferna* Spencer **8** dorsal view **9** ventral view **10** spermathecae.

Female. Similar to male.

Ovipositor. Dorsal view: cerci with two setae (Fig. 8). Ventral view: ninth tergite with one pair of long setae; egg-guides well sclerotized; two pairs of spiracles (Fig. 9). Spermathecae long and thin (Fig. 10).

Puparium. General color orange-brown (Fig. 11).

Host-plant. *Centrosema virginianum.* Oval gall on leaf rib. 2–5 pupae on each gall. (Fig. 12).

Distribution. Bahamas, Guadalupe, Brazil (Rio de Janeiro).

Figure 11. Pupae of *Japanagromyza inferna* Spencer in gall of the *Centrosema virginianum* L. (Fabaceae)

Figure 12. Gall of *Japanagromyza inferna* in *Centrosema virginianum* L. (Fabaceae).

Key to Neotropical species of *Japanagromyza*

1 Pre-scutelar acrostichal setae absent **2**
– Pre-scutelar acrostichal setae present **5**
2 Mesonotum distinctly greenish; two strong ors present; fringe of calypter white; male cerci without strong spines; shape of phallus as in figs 1–4 of Boucher and Hanson 2006 (Host-plant: *Lonchocarpus oliganthus*) [Costa Rica] ..... ***J. lonchocarpi* Boucher**
– Mesonotum greyish black; other combination of characters **3**
3 Calypter dark grey, margin and fringe black (Host-plant: *Polygonum* sp.) [Venezuela, U.S.A (Florida)] ..... ***J. polygoni* Spencer**
– Calypter, margin and fringe whitish or silvery white **4**
4 Abdomen greenish grey; arista bare (Host-plants: *Desmodium* sp., *D. tortuosum, D. campylocladus*) [Colombia, Equador, Peru, Venezuela, U.S.A. (Florida)] ..... ***J. desmodivora* Spencer**
– Abdomen shiny bluish black; arista plumose (Host-plant: unknown) [Peru] .. .. ***J. tingomariensis* Sasakawa**
5 Mesonotum distinctly greenish **6**
– Mesonotum greyish black **14**
6 Halter with parts brown 7
– Halter completely yellow or white **8**
7 Frons gray dusted; lunule brown; acrostichals in 8 rows (Host-plant: unknown) [Colombia] ..... ***J. ambigua* Sasakawa**
– Frons black dull, paler brownish at orbits level; lunule gray pollinose; acrostichals in 10 rows (Host-plant: *Centrosema virginianum*) [Bahamas, Guadalupe, Brazil] ..... ***J. inferna* Spencer**
8 Arista bare (Host-plant: *Macroptilium lathyroides*) [Brazil] .... ***J. macroptilivora* Esposito & Prado**
– Arista distinctly pubescence or plumose **9**
9 Frons uniformly brown (Host-plant: *Vigna luteola*) [Bahamas, Cuba, Guadalupe, La Dominica, U.S.A. (Florida)] ..... ***J. aequalis* Spencer**
– Frons black dull **10**
10 Large species; wing length 3.1 mm; mesonotum uniformly greenish (Host-plant: unknown) [Panama, Porto Rico] ..... ***J. iridescens* (Frost)**
– Smaller species; wing length 1.9 to 2.4 mm; mesonotum greenish or coppery **11**
11 Abdomen shiny greenish or faintly bluish; arista slightly pubescent (Host-plant: *Vigna luteola*) [Cuba, Barbade, Guadalupe, Dominica, Peru, Puerto Rico, Venezuela, Saint-Vicent, U.S.A. (Florida)] .... ***J. inaequalis* (Malloch)**
– Abdomen strongly shining green; arista distinctly pubescent **12**
12 Aedeagus consisting of a well-chitinized, flat basiphallus, an elongated membranous distiphallus (fig. 341, in Spencer and Stegmaier 1973); hypandrium V-shaped, with elongated hypandrial apodeme (fig. 342, in Spen-

cer and Stegmaier 1973); surstyli extending downward, with about five short bristles on inner margin (fig. 343, in Spencer and Stegmaier 1973) (Host-plant: *Rhynchosia phaseoloides*) [Antigua, Barbados, Porto Rico]..... .....***J. bennetti*** **Spencer**

– Male terminalia other that described other above.....**13**

13 Wing length 1.9 mm; aedeagus relatively short, as a membranous tubule; hypandrium with short, down-curved hypandrial apodeme (fig. 355 (A, B), in Spencer and Stegmaier 1973) (Host-plant: unknown) [Guadalupe, La Dominica] .....***J. wirthi*** **Spencer**

– Wing length 1.9 to 2.4 mm; aedeagus with basiphallus and median section uniformly but weakly chitinized, distiphallus entirely membranous; hypandrium rounded, without hypandrial apodeme (fig. 37 (A, B), in Spencer and Stegmaier 1973) (Host-plant: *Desmodium tortuosum*) [Bahamas, Costa Rica, Dominica, Guadalupe, Puerto Rico, Dominican Republic, Saint Martin, U.S.A. (Florida), El Salvador] .....***J. perpetua*** **Spencer**

14 Halter entirely brown or black.....**15**

– Halter stem white or yellow, knob white or black.....**16**

15 Fore tibia with distinct posterior setae; calypters largely brown or black (Host-plant: unknown) [Panama].....***J. orbitalis*** **(Frost)**

– Fore tibia without setae; calypters yellow with margin and fringe pale brown (Host-plant: unknown) [El Salvador].....***J. nebulifera*** **Sasakawa**

16 Calypters gray with margin and fringe black; halter with knob black; (Host-plant: unknown) [Jamaica] .....***J. jamaicensis*** **Spencer**

– Calypters yellow with margin and fringe black or yellow; halter with knob yellow or black.....**17**

17 Palpus yellow and abdomen with yellow areas **18**

– Palpus brown to black; abdomen normally shining black.....**19**

18 Frons black dull behind, brownish in front; fringe of calypters yellow; aedeagus with distiphallus as a curve tubule with small, paired processes at end (Host-plant: unknown) [Cuba, Cayman Islands, Jamaica] .....***J. maculata*** **(Spencer)**

– Frons black dull, paler, more yellowish in front; fringe of calypter dark brown; aedeagus with distiphallus large, paired terminal processes (Host-plant: unknown) [Bahamas, Guyana, Jamaica].....***J. spadix*** **(Spencer)**

19 Knob of halter dark black **20**

– Knob of halter white or yellow.....**21**

20 Arista conspicuously pubescent; mid tibiae with two posterodorsal setae (Host-plant: unknown) [Guatemala, Panama].....***J. aldrichi*** **(Frick)**

– Arista almost bare or microscopically pubescent; mid tibiae with one posterodorsal setae (Host-plant: unknown) [Guatemala]..... .....***J. approximata*** **(Frost)** (new comb. by Sasakawa 2005)

21 Fore and mid tibiae without distinct setae (Host-plant: *Centrosema pubencens*) [Panama] .....***J. centrosemae*** **(Frost)**

– Fore and mid tibiae with one or two setae differentiated.....**22**

22 Fore tibia with one anterodorsal seta and mid tibia with two strong posterodorsal setae (Host-plants: *Centrosema virginianum, Centrosema pubescens*) [Guadalupe, Saint-Christopher] ..... ***J. centrosematifolii*** **Etienne**

– Fore and mid tibiae with setae present but different disposition on anterodorsal and posterodosal **23**

23 Fringe of calypter silvery **24**

– Fringe of calypter white or yellow **26**

24 Aedeagus with long straight distiphallus; cerci without setae (fig. 40, in Spencer and Stegmaier 1973) (Host-plants: *Castanea* sp., *Quercus rubra, Quercus* spp. [Puerto Rico, Gulf of Mexico, Canada, U.S.A. (Florida)] .... ***J. viridula*** **(Coquillett)**

– Aedeagus with two coiled tubules; cerci large, with numerous strong setae **25**

25 Length of the wing about 1.75 mm; spines on cercus and surstylus not numerous (fig. 2 in Martinez 1994) (Host-plants: *Phaseolus lunatus*, *Phaseolus* sp.) [Guadalupe, Jamaica, Saint-Christopher, Saint-Martin] ... ***J. etiennei*** **Martinez**

– Length of the wing from 2.5-2.75 mm; spines on cercus and surstylus numerous (fig. 20 in Spencer 1983 (Host-plants: *Phaseolus* spp., *Phaseolus vulgaris*) [Argentina, Costa Rica, Peru, Venezuela] ..... ***J. phaseoli*** **Spencer**

26 Antennae light brown, with postpedicel darkened distally (Host-plant: unknown) [El Salvador] ..... ***J. arnaudi*** **Sasakawa**

– Antennae entirely black **27**

27 Arista bare; spines on cerci and aedeagus as in figs 9-10 of Spencer 1963 (Host-plant: unknown) [Costa Rica] ..... ***J. frosti*** **(Frick)**

– Arista pubescence or plumose; spines and aedeagus different from above .. **28**

28 Mesonotum shining black, without reflections **29**

– Mesonotum black with coppery reflections (Host-plant: unknown) [Bahamas] ..... ***J. propinqua*** **Spencer**

29 Abdomen black strongly shining; arista pubescent (Host-plant: unknown) [Colombia] ..... ***J. clausa*** **Sasakawa**

– Abdomen opaque dark brown; arista distinctly plumose (Host-plant: unknown) [Panama] ..... ***J. currani*** **(Frost)**

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RESEARCH ARTICLE

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Description and DNA barcoding of *Crematogaster fraxatrix* Forel, 1911 and two new closely related species from Cambodia and Indonesia (Hymenoptera, Formicidae)

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Abstract

Crematogaster fraxatrix Forel, 1911 and two new species, *C. chhangi* **sp. n.** and *C. simboloni* **sp. n.**, are described from Cambodia and Indonesia, respectively. DNA sequences were generated for *C. fraxarix* and the two newly described species using 3 amplications of two regions of the mitochondrial gene COI with a total of 1129 bp. The mean interspecific divergences are 9.4 % and 23.5 % for *C. fraxatrix* vs. *C. chhangi*, *C. simboloni*, respectively. DNA sequences reveal that *C. simboloni* is found to be genetically distinct from the other two species, but *C. chhangi* is not distinct from *C. fraxatrix*.

Keywords

Crematogaster fraxatrix, taxonomy, new species, lectotype designation, DNA barcoding, cytochrome *c* oxidase I

Introduction

Crematogaster fraxatrix was described by Forel (1911) based on the worker specimens from Malaysia (Borneo). This species is presently assigned to the subgenus *Crematogaster* (Blaimer 2012b). A recent molecular work re-classified the former sixteen subgenera into two larger subgenera, *Crematogaster* and *Orthocrema* (Blaimer 2012b). The subgenus *Crematogaster* is the largest, including more than 220 species, its workers generally have anteriorly broader petiole ('flared'-shape in Blaimer 2012b), but *C. ferrarii*, *C. fraxatrix* and *C. ransonneti* uniquely have the petiole broader in the middle portion among Asian *Crematogaster* fauna. The close relationship between *C. ferrarii* and *C. fraxatrix* was also suggested by a molecular phylogeny (Blaimer 2012c). However, *C. fraxatrix* can be easily distinguished by the densely sculptured mesopleuron from *C. ferrarii*, by the weakly concave metanotal groove from *C. ransonneti*, respectively. In the course of our recent examination of *Crematogaster* specimens collected from southeast Asia, two distinct species related to *C. fraxatrix* were found, which are here described as new species. Cytochrome oxidase I (COI) sequence data from *Crematogaster fraxatrix* was further compared with that of the two new species. DNA barcodes have been recently used in biodiversity studies of ant species (Smith et al. 2005), and are used as an additional and powerful method in integrative taxonomy (Schlick-Stiner et al. 2010). They can thus provide a useful reference for the identification of Asian *Crematogaster* species. Our analysis included not only in the conventional 5' DNA barcoding region, but also the 3' region of COI region. The relationship between *C. fraxatrix* and the two new species is discussed, based on morphological features and sequence divergence.

Materials and methods

Sources of material and abbreviations

Specimens were examined and/or deposited in the collections listed below. Codes for public institutions mainly follow those in Brandão (2000). Nest series samples, most of which were recently collected, are represented as colony codes, e.g., "SH12-Cam-70."

BMNH The Natural History Museum, London, U. K.
CASC California Academy of Sciences, San Francisco, CA, USA.
FRIM Forest Research Institute Malaysia, Kepong, 52109 Kuala Lumpur, Malaysia.
KUM Kyushu University, Fukuoka, Japan.
MCZC Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.
MHNG Musee d'Histoire Naturelle, Geneva, Switzerland.
MZB Museum Zoologicum Bogoriense, Cibinong, Java, Indonesia.
NHMB Naturhistorisches Museum, Basel, Switzerland.
THNHM Thailand Natural History Museum, Technopolis, Khlong Luang, Pathum Thani, Thailand.

Measurements and indices

Most observations were made using an Olympus SZX12 microscope. Images were taken using a Canon EOS 50D with a Canon MP-E 65 mm 1-5 × macro lens, then processed using Combine ZM. Measurements were made with an Olympus SZX12 stereomicroscope using micrometers. All measurements are expressed in millimeters, recorded to the second decimal place. The measurements for petiole and postpetiole follow Longino (2003).

Head Width (**HW**): Maximum width of head in full-face view, excluding the eyes.
Head Length (**HL**): Perpendicular distance from vertex margin to line tangent anteriormost projections of clypeus in full-face view.
Cephalic Index (**CI**): HW/HL × 100.
Scape Length (**SL**): Length of the first antennal segment, excluding the neck and basal condyle.
Scape Index (**SI**): SL/HW × 100.
Eye Length (**EL**): Maximum length of the compound eye.
Pronotal Width (**PW**): Maximum width of the pronotum in dorsal view.
Weber's Length of the mesosoma (**WL**): Diagonal length, measured in lateral view from the anterior margin of the pronotum (excluding the collar) to the posterior extremity of the propodeal lobe.
Propodeal Spine Length (**PSL**): measured from tip of propodeal spine to closest point on outer rim of propodeal spiracle.
Petiole Length (**PtL**): Length of the petiole in lateral view (see Longino 2003, fig. 2).
Petiole Width (**PtW**): Maximum width of petiole in dorsal view.
Petiole Height (**PtH**): Height of the petiole in lateral view (see Longino 2003, fig. 2).
Postpetiole Length (**PpL**): Length of the postpetiole in lateral view (see Longino 2003, fig. 2).
Postpetiole Width (**PpW**): Maximum width of postpetiole in dorsal view, excluding the helcium.
Petiole Height Index (**PtHI**): PtH/PtL × 100.
Petiole Width Index (**PtWI**): PtW/PtL × 100.
Postpetiole Width Index (**PpWI**): PpW/PpL × 100.
Waist Index (**WI**): PpW/PtW × 100.

Genetic analysis

Genomic DNA was extracted from tissues rich in mitochondria (e.g. legs) using a DNeasy Blood & Tissue kit (Qiagen, Maryland, USA). A 497 bp region of the mitochondrial genome, including barcoding regions of the cytochrome oxidase I (COI) was amplified via the polymerase chain reaction (PCR) using the following combinations of the primers, "LepF1" 5'-ATTCAACCAATCATAAAGATATTGG-3' and

"C_ANTMR1D-RonIIdeg_R" 5'-GGRGGRTARAYAGTTCATCCWGTWCC-3' (used only for PCR), and "MLepF1" 5'-GCTTTCCCACGAATAAATAATA-3' and "LepR1" 5'-TAAACTTCTGGATGTCCAAAAAATCA-3' (Hebert et al. 2004; Fisher and Smith 2008; Hajibabaei et al. 2006). Reactions were carried out at 10 µl volumes in a PCR Thermal Cycler MP (TaKaRa Bio Inc.) under the following conditions: a first cycle of 94°C for 2 min, followed by 5 cycles of 94°C for 40 sec, annealing at 45°C for 40 sec, and 72°C for 1min, then 36 cycles of 94°C for 40 sec, annealing at 51°C for 40 sec, and finally 72°C for 1 min for the COI. A 632 bp region of the 3' region of COI was amplified via the polymerase chain reaction (PCR) using primers "Jerry" 5'-CAACATTTATTTTGATTTTTTGG-3' and "Pat" 5'-TCCAATGCACTAATCT-GCCATATTA-3' (Simon et al. 1994). Reactions were carried out at 10 µl volumes in a PCR Thermal Cycler MP (TaKaRa Bio Inc.) under the following conditions: a first cycle of 94°C for 1 min, followed by 5 cycles of 94°C for 1 min, annealing at 48°C for 90 s, and 72°C for 90 s, then 30 cycles of 94°C for 1 min, annealing at 51°C for 90 s, and finally 72°C for 90 s for the COI.

PCR products were visualized on a 1% agarose E-Gel 96-well system (Invitrogen), and then purified with 1.0 µl of ExoSAP-IT (GE Healthcare Life Sciences). All products were sequenced in both directions (except for C_ANTMR1D-RonIIdeg_R) using BigDye Terminator v3.1 (Applied Biosystems) on an ABI 3100 Avant DNA Sequencer (Applied Biosystems) at the Faculty of Science, Kyushu University, Fukuoka. Some fragments were removed prior to alignment, due to low quality. After trimming, the 5' DNA barcoding region sequenced in this study were 497bp, therefore these sequences were not strictly DNA barcodes. Using the three primer sets, non-overlapping fragments of 244, 253 and 632 bp were sequenced respectively. DNA sequence data for eight individuals of three *Crematogaster* species were thus generated, and deposited at DNA Data Base of Japan, DDBJ (with accession numbers shown in Table 1). All data were registered in the project called: "Crematogaster ants in Asia" (CREAA) on Barcode of Life Database (BOLD). Among the three species, *C. chhangi* was sequenced from one nest series (SH12-Cam-70) from Cambodia, and *C. simboloni* was successfully sequenced only from one Krakatau specimen. Contigs were assembled using Vector NTI Advance TM ver. 11 (Invitrogen Corp.) and subsequently aligned by eye. Genetic distances were estimated using the Kimura-2-parameter (Kimura 1980) distances with MEGA 5 (Tamura et al. 2011). The phylogenetic tree was estimated using Neighbor-Joining (NJ) (Saitou and Nei 1987) in the program MEGA 5.

Results

Intraspecific variation for *C. fraxatrix* was 4.19 % on average, with a range of 0.4–6.9 %. Relatively large divergence in *C. fraxatrix* (6.2–6.9 %) was recorded when comparing Peninsular to Bornean specimens. By contrast, the divergence within Peninsular (0.7–1.2 %) or Bornean specimens (0.4–0.9 %) was low. Interspecific sequence di-

vergence was 17.1 % on average, and ranged from 8.1–24.6 % (Table 2). The lowest interspecific genetic distance occurred between specimens of *C. chhangi* and Bornean *C. fraxatrix* (8.1%).

The neighbor-joining tree (Fig. 9) shows *Crematogaster chhangi* sister to *C. fraxatrix*, with high bootstrap support (100%). Among the three species examined, *C. simboloni* was distinctly separated from the *C. chhangi* and *C. simboloni* with higher genetic divergence: 24.3 % to *C. chhangi* and 22.6 % to 24.6 % to *C. fraxatrix*. *Crematogaster chhangi* is distinguished from *C. fraxatrix* only in having an acutely developed subpetiolar process, whereas *C. simboloni* is quite different from *C. chhangi* and *C. fraxatrix* in having a densely sculptured promesonotum.

Key to species of *Crematogaster fraxatrix*-group

1 Promesonotum sculptured ..... ***C. simboloni***
– Promesonotum not sculptured, but feebly striated with longitudinal rugulae...**2**
2 Propodeal spiracles strongly flattened dorsoventrally. Subpetiolar process acutely developed..... ***C. chhangi***
– Propodeal spiracles oval in shape. Subpetiolar process weakly developed ..... ***C. fraxatrix***

Taxonomy

***Crematogaster* (*Crematogaster*) *chhangi* sp. n.**
http://zoobank.org/DDB2C024-BAE9-4A7B-9006-1C4BA647F716
http://species-id.net/wiki/Crematogaster_chhangi
Figs 1–2

Type locality. CAMBODIA: Koh Kong, 11°31'N, 103°09'E, 19.v.2012, S. Hosoishi. (SH12-Cam-70).

Type-specimens. Holotype worker: pinned. Original label: CAMBODIA, Koh Kong, 11°31'N, 103°09'E, 19.v.2012, S. Hosoishi leg., SH12-Cam-70, arboreal; deposited at THNHM.

Eight paratype workers: pinned, same data as holotype; deposited at BMNH, CASC, FRIM, KUM, MCZC, MHNG, MZB, NHMB.

Measurements and indices. HW 0.64–0.83; HL 0.64–0.75; CI 100–111; SL 0.57–0.61; SI 73–86; EL 0.13–0.16; PW 0.37–0.42; WL 0.7–0.78; PSL 0.13–0.18; PtL 0.17–0.21; PtW 0.16–0.19; PtH 0.13–0.15; PpL 0.11–0.12; PpW 0.14–0.18; PtHI 65–82; PtWI 89–100; PpWI 127–150; WI 82–95 (holotype and eight paratype workers measured).

Diagnosis. This species is similar to *C. fraxatrix*, but can be distinguished by the dorso-ventrally flattened propodeal spiracles and acutely developed subpetiolar process

Figures 1–2. *Crematogaster chhangi*. **1** lateral view **2** dorsal view of mesosoma.

in the worker caste. The COI divergence between *C. chhangi* and *C. fraxatrix* did not seem relatively high (8.1–10.8 % K2P distances) (cf. Blaimer 2012a), but the two species are clearly separated from each other by the characters shown above.

Worker description. Workers presumably monomorphic. Posterior corners of head rounded. Anterior clypeal margin slightly concave in the median portion. Compound eyes not projecting beyond lateral margins of head in full face view. Scape reaching posterior corner of head. Antennal club 3-segmented. Pronotal dorsum with distinct ridges laterally. Mesonotal dorsum with lateral ridges. Mesonotum not higher than pronotum in lateral view; forming same dorsal outline with pronotum in lateral view. Metanotal groove straight in dorsal view, deep and forming a concave region between mesonotum and propodeum. Propodeal spiracles oval, flattened dorso-ventrally, located on the lateral sides of propodeum; the horizontal diameter more than two times larger than the vertical diameter. Propodeal spines developed long, directed upward and straight. Petiole broader in the middle portion. Subpetiolar process acutely developed. Postpetiole weakly bilobed, but without longitudinal median sulcus. Petiole slightly wider than postpetiole in dorsal view. Erect pilosity sparse. Scape with abundant erect to suberect setae. Dorsal face of head with suberect setae. Clypeus with suberect setae; one pair of longer setae directed medially on anteriormost portion. Anterior clypeal margin with one single setae and one pair of longer setae, mixed with some shorter setae on the sides. Mesosoma with sparse erect setae. Fourth abdominal tergite with erect to suberect sparse setae. Dorsal surface of head generally smooth and shining, but feeble rugulae between frontal carinae; longitudinal rugulae surrounding antennal sockets and on gena. Clypeus weakly striated with longitudinal rugulae. Promesonotum striated with feeble rugulae. Lateral surface of pronotum smooth and shining. Mesopleuron sculptured. Lateral surface of propodeum generally smooth, but with feeble rugulae on the lower portion. Body color brown.

Distribution. This species is known only from the type locality of Cambodia.

Etymology. This species is dedicated to Mr. Phourin Chhang, Forestry Administration of Cambodia, who helped with field surveys in Cambodia.

***Crematogaster* (*Crematogaster*) *fraxatrix* Forel**
http://species-id.net/wiki/Crematogaster_fraxatrix
Figs 3–6

Crematogaster fraxatrix Forel, 1911: 28 Worker syntypes from MALAYSIA: Sarawak, Borneo (*Haviland*) [MHNG, NHMB, examined]. Combination in *C.* (*Acrocoelia*): Emery 1922: 151; in *C.* (*Crematogaster*): Bolton 1995: 166; Blaimer 2012b: 55.

Type material examined. MALAYSIA: Sarawak, Borneo (Haviland). Lectotype worker by present designation: top specimen of three specimens of one pin.

Other material examined. THAILAND: 5 workers, Khlong Klai Stn., Khao Nan N. P., Nakhon S. Thamarat, 13.iii.2007 (TH07-SKY-22) (Sk. Yamane); MALAYSIA: 2 workers, Ulu Gombak, Selangor, 09.iii.2009 (SH09-Mal-51) (S. Hosoishi); 2 workers, Mt. Ophir, Gunung Ledan, Johor, 11.x.2011 (SH11-Mal-47) (S. Hosoishi); 3 workers, Lambir Hill's National Park, Borneo, 21-27.ii.2009 (Y. Hashimoto).

Measurements and indices. HW 0.7–0.98; HL 0.64–0.93; CI 105–114; SL 0.58–0.68; SI 69–91; EL 0.13–0.18; PW 0.37–0.62; WL 0.69–0.95; PSL 0.13–0.21; PtL 0.18–0.25; PtW 0.18–0.25; PtH 0.13–0.17; PpL 0.11–0.15; PpW 0.16–0.23; PtHI 65–74; PtWI 94–111; PpWI 123–155; WI 83–92 (thirteen workers measured).

Diagnosis. This species is similar to *C. chhangi*, but can be distinguished by the oval-shaped propodeal spiracles and weakly developed subpetiolar process in the worker caste. Based on COI divergence, the specimens from Peninsular Malaysia were separated from the Bornean specimens with a high support value (Fig. 9). This is presumably due to lack of gene flow between the populations, but they showed no distinct morphological differences between each other. The COI divergence of 0-9.3 % (K2P distances) was recorded within *C. ranavalonae* clade in Madagascar (Blaimer 2012a). Further geographic sampling is therefore needed to determine whether the variation of 0.4–6.9 % (K2P distances) represents the intraspecific variation or includes some interspecific variation.

Worker description. Workers with weak polymorphism in size. Posterior corners of head rounded in smaller worker, but squared in larger workers. Anterior clypeal margin slightly concave in the median portion. Compound eyes not projecting beyond lateral margins of head in full face view. Scape reaching posterior corner of head. Antennal club 3-segmented. Pronotal dorsum with distinct ridges laterally. Mesonotal dorsum with lateral ridges. Mesonotum slightly higher than pronotum in larger workers in lateral view. Metanotal groove straight in dorsal view, deep and forming a concave region between mesonotum and propodeum. Propodeal spiracles dorso-ventrally oval, located on lateral sides of propodeum; the horizontal diameter slightly larger than the vertical diameter even in smaller workers. Propodeal spines long, directed upward and straight. Petiole broader in the middle portion. Subpetiolar process developed as small, blunt denticle. Postpetiole weakly bilobed, but without longitudinal median sulcus. Petiole slightly wider than postpetiole in dorsal view.

Sparsely hirsute with erect setae. Scape with abundant erect to suberect setae. Dorsal face of head with suberect setae. Clypeus with suberect setae; one pair of longer setae directed medially on anteriormost portion. Anterior clypeal margin with one single setae

Figures 3–6. *Crematogaster fraxatrix*. **3** lateral view **4** dorsal view of mesosoma **5** full face view **6** dorsal view of petiole and postpetiole.

and one pair of longer setae, mixed with some shorter setae on the sides. Mesosoma with sparse erect setae. Fourth abdominal tergite with sparse erect to suberect setae.

Dorsal surface of head generally smooth and shining, but feeble rugulae between frontal carinae; longitudinal rugulae surrounding antennal sockets and on gena. Clypeus weakly striated with longitudinal rugulae. Pronotum striated with feeble rugulae. Mesonum weakly striated with feeble rugulae. Lateral surface of pronotum smooth and shining. Mesopleuron sculptured, but the central portion relatively smooth. Lateral surface of propodeum with feeble rugulae.

Body color reddish-brown to black.

Distribution. This species is known from southern Thailand and Malaysia (Peninsular and Borneo).

***Crematogaster* (*Crematogaster*) *simboloni* sp. n.**
http://zoobank.org/FFEDDB4D-EE5D-47D6-90A3-473F11F91056
http://species-id.net/wiki/Crematogaster_simboloni
Figs 7–8

Type locality. INDONESIA: Rakata Island, Krakatau Islands, 06°09'S, 105°28'E, 11.x.2000, H. Simbolon.

Figures 7–8. *Crematogaster simboloni*. **7** lateral view **8** dorsal view of mesosoma.

Type-specimens. Holotype worker: pinned. Original label: INDONESIA, Rakata Island, Krakatau Islands, 06°09'S, 105°28'E, 11.x.2000, H. Simbolon leg., deposited at MZB.

Eight paratype workers: pinned, same data as holotype; deposited at BMNH, CASC, FRIM, KUM, MCZC, MHNG, NHMB, THNHM.

Other material examined. INDONESIA: 11 workers, Rakata Island, Krakatau Islands, 11.x.2000 (K. Ogata); 3 workers, Rakata Island, Krakatau Islands, 10.x.2000 (K. Ogata); 4 workers, Rakata Island, Krakatau Islands, 11.x.2000 (S. Matsui); 1 worker, Rakata Island, Krakatau Islands, 10.x.2000 (S. Matsui); 1 worker, Rakata Island, Krakatau Islands, 30.xii.2006 (Sk. Yamane); 1 worker, Rakata Island, Krakatau Islands, 31.xii.2006 (Sk. Yamane)

Measurements and indices. HW 0.59–0.72; HL 0.57–0.67; CI 102–108; SL 0.49–0.55; SI 75–85; EL 0.11–0.15; PW 0.34–0.39; WL 0.62–0.74; PSL 0.1–0.12; PtL 0.16–0.2; PtW 0.16–0.19; PtH 0.12–0.15; PpL 0.11–0.12; PpW 0.15–0.17; PtHI 72–82; PtWI 95–113; PpWI 125–155; WI 83–100 (fourteen workers measured).

Diagnosis. This species is similar to *C. chhangi* and *C. fraxatrix*, but can be easily distinguished from these by the sculptured promesonotum in the worker caste. The COI divergence between *C. simboloni* and *C. chhangi* (24.3 % K2P distances), as well as *C. simboloni* and *C. fraxatrix* (22.6 to 24.6 % K2P distances) were also high.

Worker description. Workers monomorphic. Posterior corners of head rounded. Anterior clypeal margin slightly concave in the median portion. Compound eyes projecting slightly beyond lateral margins of head in full face view. Scape reaching posterior corner of head. Antennal club 3-segmented. Pronotal dorsum with distinct ridges laterally. Mesonotal dorsum with lateral ridges. Mesonotum slightly higher than pronotum in lateral view. Metanotal groove straight in dorsal view, deep and forming a concave region between mesonotum and propodeum. Propodeal spines long, directed upward and straight. Propodeal spiracles oval, flattened dorso-ventrally, located on the lateral sides of propodeum, or the postero-lateral corners; the horizontal diameter slightly larger than the vertical diameter. Petiole broader in the middle portion. Subpetiolar process undeveloped. Postpetiole weakly bilobed with feeble median sulcus. Petiole as wide as postpetiole in dorsal view. Sparsely hirsute with erect setae. Scape with abundant erect to suberect setae. Dorsal face of

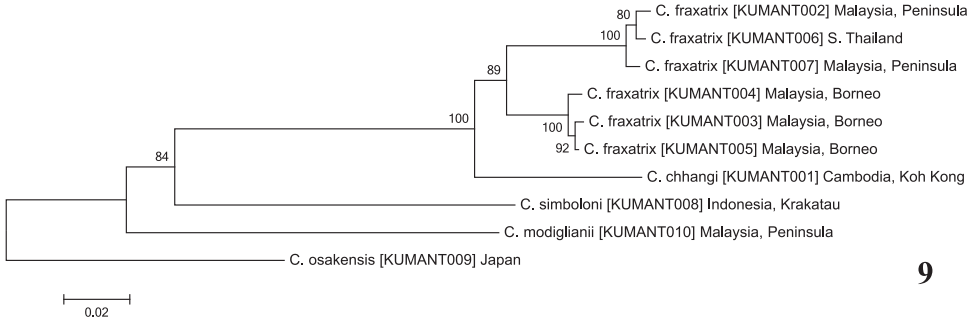

Figure 9. Neighbor-joining (Saitou and Nei 1987) tree of genetic distances (Kimura-2-parameter model (Kimura 1980), Bootstrap 1000 bootstrap replicates) of cytochrome *c* oxidase I (COI) for three *Crematogaster* species. Numbers on the nodes show the bootstrap values (>50%). Numbers in parentheses are specimen sample IDs.

Table 1. Specimen data and DDBJ accessions.

Species	Voucher specimen	Locality	DDBJ accession numbers	
			first half of COI	second half of COI
Crematogaster chhangi	KUMANT001	Cambodia, Koh Kong	AB828274, AB828377	AB828264
Crematogaster fraxatrix	KUMANT002	Malaysia, Peninsula	AB828275, AB828381	AB828265
Crematogaster fraxatrix	KUMANT003	Malaysia, Borneo	AB828276, AB828382	AB828266
Crematogaster fraxatrix	KUMANT004	Malaysia, Borneo	AB828277, AB828383	AB828267
Crematogaster fraxatrix	KUMANT005	Malaysia, Borneo	AB828278, AB828384	AB828268
Crematogaster fraxatrix	KUMANT006	S. Thailand	AB828279, AB828385	AB828269
Crematogaster fraxatrix	KUMANT007	Malaysia, Peninsula	AB828280, AB828378	AB828270
Crematogaster simboloni	KUMANT008	Indonesia, Krakatau	AB828281, AB828386	AB828271
Crematogaster osakensis	KUMANT009	Japan	AB828282, AB828379	AB828272
Crematogaster modiglianii	KUMANT010	Malaysia, Peninsula	AB828283, AB828380	AB828273

head with suberect setae. Clypeus with suberect setae; one pair of longer setae medially on anteriormost portion. Anterior clypeal margin with one single setae and one pair of longer setae, mixed with some shorter ones on the side. Mesosoma with short and sparse erect setae. Fourth abdominal tergite with few erect to suberect setae. Dorsal surface of head generally smooth and shining, but feeble rugulae between frontal carinae; longitudinal rugulae surrounding antennal sockets and on gena. Clypeus striated with longitudinal rugulae. Pronotum striated with longitudinal rugulae with the sculptured space; the longitudinal rugulae separated from anterior mesonotal margin. Mesonotum sculptured. Lateral surface of pronotum smooth and shining. Mesopleuron sculptured, but the central portion relatively smooth. Propodeal dorsum sculptured anteriorly. Lateral surface of propodeum weakly sculptured and striated with feeble rugulae. Body color brown.

Distribution. This species is known only from Indonesia (Krakatau).

Etymology. This species is dedicated to Dr. Herwint Simbolon, Research Centre for Biology, Lembaga Ilmu Pengetahuan Indonesia (The Indonesian Institute of Sciences), who collected the type material.

Table 2. Percent mitochondrial cytochrome c oxidase I (COI) sequence divergence among species of *C. chhangi*, *C. fraxatrix* and *C. simboloni*.

Species	***C. chhangi*** **[Cambodia]**	***C. fraxatrix*** **[M. Peninsula]**	***C. fraxatrix*** **[Borneo]**	***C. fraxatrix*** **[Borneo]**	***C. fraxatrix*** **[Borneo]**	***C. fraxatrix*** **[S. Thailand]**	***C. fraxatrix*** **[M. Peninsula]**
C. chhangi [Cambodia]							
C. fraxatrix [M. Peninsula]	0.105						
C. fraxatrix [Borneo]	0.082	0.069					
C. fraxatrix [Borneo]	0.082	0.067	0.009				
C. fraxatrix [Borneo]	0.081	0.067	0.004	0.007			
C. fraxatrix [S. Thailand]	0.108	0.007	0.065	0.063	0.063		
C. fraxatrix [M. Peninsula]	0.107	0.013	0.063	0.062	0.062	0.008	
C. simboloni [Krakatau]	0.243	0.241	0.227	0.23	0.226	0.246	0.241

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RESEARCH ARTICLE

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Reassessment of the taxonomic *position of Iranocypris* typhlops Bruun & Kaiser, 1944 (Actinopterygii, Cyprinidae)

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Abstract

The Iranian cave barb (*Iranocypris typhlops* Bruun & Kaiser, 1944) is a rare and endemic species of the family Cyprinidae known from a single locality in the Zagros Mountains, western Iran. This species is "Vulnerable" according to the IUCN Red List and is one of the top four threatened freshwater fish species in Iran. Yet, the taxonomic position of *I. typhlops* is uncertain. We examined phylogenetic relationships of this species with other species of the family Cyprinidae based on the mitochondrial cytochrome *b* gene. Our results show that *I. typhlops* is monophyletic and is sister taxon of a cluster formed by *Garra rufa* (Heckel, 1843) and *Garra barreimiae* (Fowler & Steinitz, 1956) within a clade that includes other species of the genus *Garra.* Based on previous molecular and morphological studies, as well as our new results, we recommend that *I. typhlops* should be transferred to the genus *Garra* Hamilton, 1822.

Keywords

Iranian cave barb, *Iranocypris typhlops*, *Garra*, phylogeny

Introduction

The Iranian cave barb (*Iranocypris typhlops* Bruun & Kaiser, 1944) is a rare and endemic species of the family Cyprinidae in the Zagros Mountains, western Iran (Mahjoorazad and Coad 2009). The distribution of the species seems to be restricted to a single cave. This species is currently recognized as "Vulnerable" according to the IUCN Red List (IUCN 2013). As such, Coad (2000) using 18 criteria that focused on distribution and habitat, found this species to be one of the top four threatened species of freshwater fishes in Iran. Zalaghi (2011) estimated the population size of the species between 353 and 625 individuals.

The species was suggested to be related to the genus *Barbus* Cuvier & Cloquet, 1816 by Bruun and Kaiser (1944) but Saadati (1977) rejected the close relationship with the genus *Barbus.* Coad (2013) proposed that the species may be related to the genus *Garra* Hamilton, 1822. More recently, Hashemzadeh Segherloo et al. (2012) provided the first molecular evidence of the species phylogeny based on the cytochrome *c* oxidase subunit I (COI) gene, which indicated that the species is phylogenetically close to the genus *Garra.* Two sympatric forms have been reported within *I. typhlops* (Sargeran et al. 2008). They are morphologically distinguished by the presence / absence of a mental disc on the ventral surface of the head (Fig. 1). The mental disc is the lower lip modified into a mental adhesive disc whose posterior margin is discontinuous with the mental region (Zhang 2005). Sargeran et al. (2008) and Hashemzadeh Segherloo et al. (2012) found morphological and molecular differentiations between the two sympatric forms, respectively. Also Hashemzadeh Segherloo et al. (2012) indicated that the two forms might represent separate species based on high intraspecific COI divergence between the two sympatric forms.

Phylogenetic studies of the cavefish populations have shown that some aspects of cavefish systematics are still debated and require molecular analyses to provide evidence on taxonomy and phylogenetic relationships. Detailed molecular studies on some cavefish species have actually shown that their taxonomic position needs a revision based on genetic evidence and that several species, whose description was based on morphological traits only, could be genetically closer to genera different from those to which they are currently assigned to (Romero and Paulson 2001, Colli et al. 2009). In this study, we used the mitochondrial cytochrome *b* (cyt *b*) gene to examine the phylogenetic relationships of the species and in this way to assess the taxonomic position of *I. typhlops.*

Study area

The Iranian cave barb is found in a water cave, the natural outlet of a subterranean limestone system of the Zagros Mountains. The stream below the cave locality is the "Ab-e Serum" which is a tributary of the Dez River, in Lorestan province. The Dez flows into the Karun River, which drains to the head of the Persian Gulf. Further locality details are given in Bruun and Kaiser (1944) and Mahjoorazad and Coad (2009). The cave is located at 33°04'39"N and 48°35'33"E (Fig. 2).

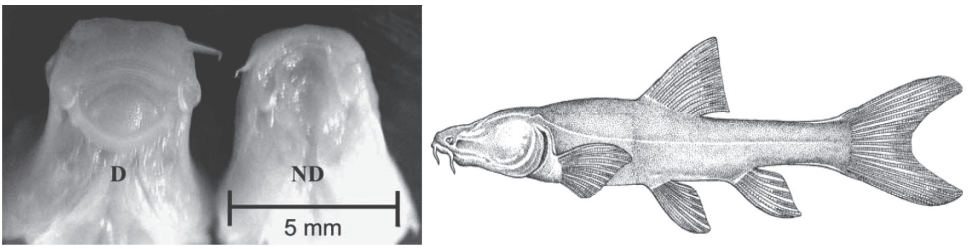

Figure 1. (Left) Ventral view of heads of putative *I. typhlops*, with (D) and without (ND) a disc, adapted from Sargeran et al. (2008); (Right) *I. typhlops,* adapted from (Coad 2013).

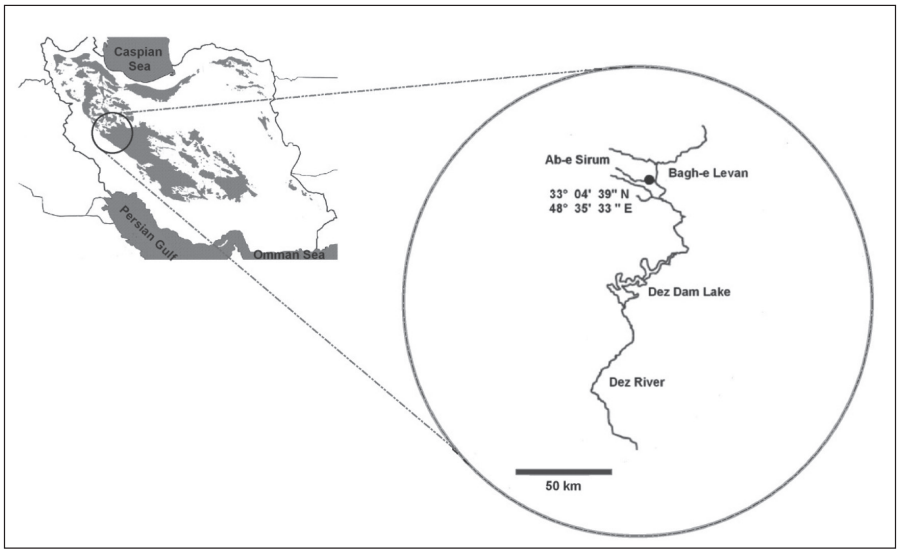

Figure 2. Location of *I. typhlops* habitat in Bagh-e Levan.

Methods

Fin-clip samples (approximately 4 mm^2) of 16 *I. typhlops* specimens (eight specimens with a mental disc and eight specimens without a mental disc) were collected from the native habitat in November 2012. The fin-clips were stored in 98% ethanol. Total DNA was extracted using the DNeasy-Tissue Kit (Qiagen, Germany) following the manufacturer's instructions. PCR amplification cyt *b* was performed using primers L15267 (R: 5'-AATGACTTGAAGAACCACCGT-3') and H16461 (F: 5'-CTTCG-GATTACAAGACC-3') (Briolay et al. 1998). The Polymerase Chain Reaction (PCR) amplification reactions were carried out in 20 µl reaction volume containing: 80 ng of genomic DNA, 1.5 units of Taq polymerase, 1× Roche Taq PCR buffer, 2.5 mM $MgCl_2$, 250 µM dNTPs and 0.25 pM of each primer. PCR reactions were conducted under the following conditions: 5 min denaturation at 94 °C; 30 cycles of 40 s at 94 °C, 35 s at 50 °C and 30 s at 72 °C and a final extension at 72 °C for 5 min. PCR products were sequenced on an ABI-3130xl sequencer using the manufacturer's protocol.

Sequences were aligned using ClustalX (Thompson et al. 1997) and manually checked for inconsistencies. The sequences were deposited in GenBank under accession numbers KF896290 to KF896300. Number of haplotypes, analysis of molecular variance (AMOVA) among and within the two sympatric forms (with and without mental disc), and Kimura two-parameter (K2P) distances (Kimura 1980), were calculated using ARLEQUIN 3.5.1.3 (Excoffier and Lischer 2010).

For the reconstruction of phylogenetic trees, cyt *b* sequences of different cyprinid species (Fig. 3) were retrieved from GenBank and aligned with the sequences of *I. typhlops*. *Myxocyprinus asiaticus* (Gill, 1878) was included as outgroup (Hashemzadeh Segherloo et al. 2012). Maximum likelihood (ML), neighbor-joining (NJ) and Bayesian analysis (BI) were used to infer phylogenetic trees. The Akaike Information Criterion, the corrected AIC and the Bayesian Information Criterion in jModeltest 2.1.3 (Posada 2008) were used to select an appropriate substitution model of DNA evolution. The GTR model of evolution with gamma shape parameter (G = 1.24) and proportion of invariable positions (I = 0.54) was the selected model according to the three criteria. ML analysis was conducted using PAUP 4.0b10 (Swofford 2003). ML tree searches were performed by heuristic searches. NJ analysis, based on K2P distances, was derived using "dnadist" and "neighbor" executables implemented in Phylip 3.6 (Felsenstein 2005). Support for nodes was assessed by nonparametric bootstrapping (1000 replicates) for ML and NJ analysis and only values > 50% were considered (Colli et al. 2009, Tang et al. 2009). Bayesian analysis was carried out using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The Markov Chain Monte-Carlo (MCMC) search was run for 10^6 generations, sampling the Markov chain every 100 generations. The first 25% (1000) trees were discarded as burn-in.

Results

After trimming the alignment, the cyt *b* gene sequences were 904 bp long and 11 haplotypes were found for the 16 sequenced samples (four haplotypes for specimens with mental disc and seven haplotypes for specimens without mental disc). AMOVA showed that 95.72% of the variation in cyt *b* sequences was attributed to differences among the two sympatric forms (Table 1). Also F_{ST} value showed significant genetic variation among the two sympatric forms. ML, NJ and BI analyses yielded phylogenetic trees with almost the same topology but the consensus tree of BI supported the relationships among species with higher posterior probabilities (Fig. 3). Genetic divergences among *I. typhlops* and 22 species of the family Cyprinidae are presented in Table 2.

Discussion

The phylogenetic trees showed that both forms of *I. typhlops* form a single clade and that this clade is a sister group of a clade comprising *Garra rufa* (Heckel, 1843) and *Garra barreimiae* (Fowler & Steinitz, 1956). These two sister clades are placed within

Table 1. Analysis of AMOVA among the two sympatric forms of *I. typhlops*.

Source of variation	d.f.	Sum of squares	Variance components	Percentage variation
Among sympatric forms	1	143.318	17.815	95.720
Within sympatric forms	14	11.160	0.7971	4.280
Total	15	154.478	18.612	100.000

F_{ST}= 0.957 (P-value= 0.0000)

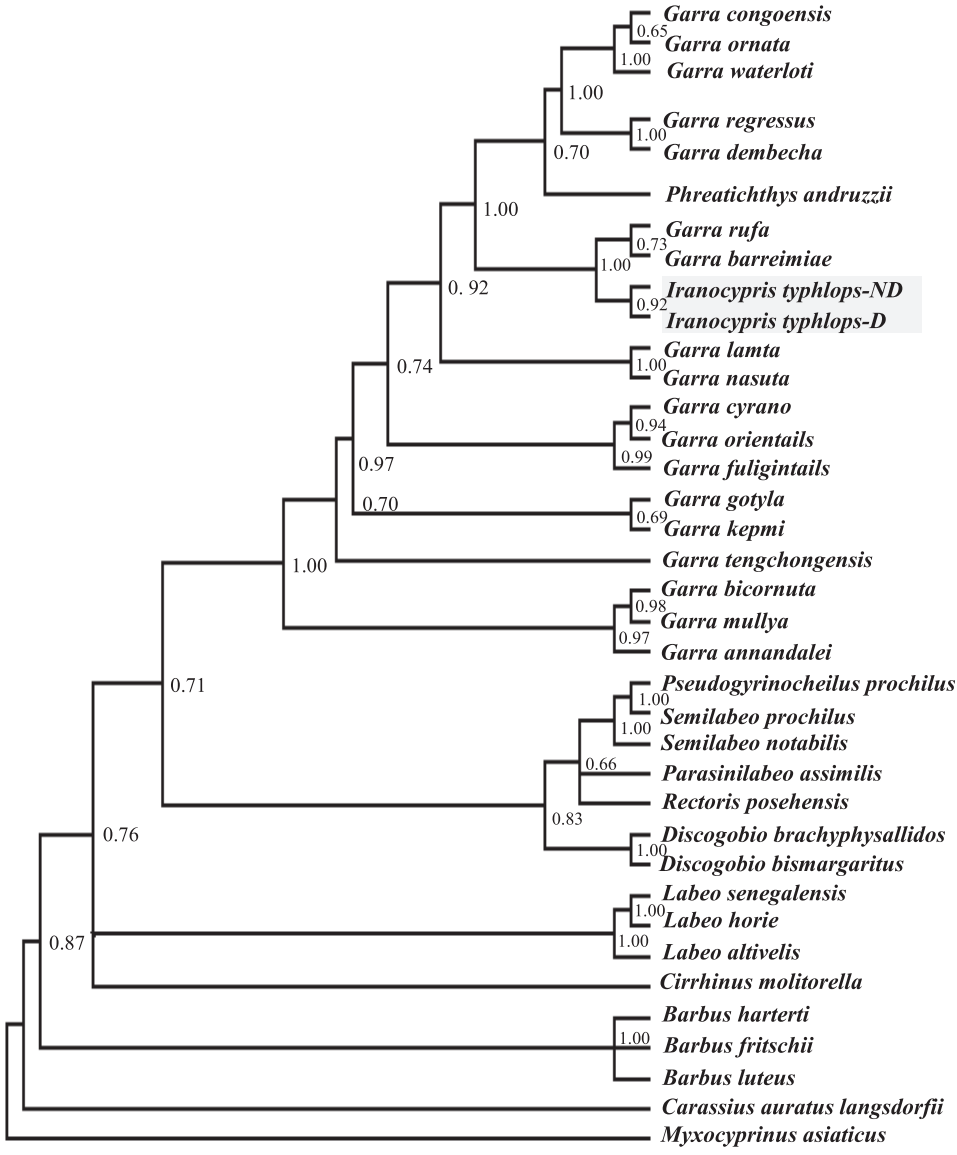

Figure 3. Phylogenetic relationships of *I. typhlops* based on cyt *b*. The posterior probability values on the branches are the results of BI. *I. typhlops-ND* = specimens without a mental disc, *I. typhlops-D* = specimens with a mental disc.

Table 2. K2P distances between *I. typhlops,* and 22 species of the family Cyprinidae based on cyt *b*.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
I. typhlops-ND (1)																							
I. typhlops-D (2)	0.041																						
G. rufa (3)	0.069	0.067																					
G. barreimiae (4)	0.086	0.083	0.083																				
G. cyrano (5)	0.119	0.099	0.125	0.131																			
G. lamta (6)	0.127	0.110	0.113	0.128	0.099																		
G. congoensis (7)	0.115	0.110	0.111	0.139	0.122	0.118																	
G. nasuta (8)	0.126	0.116	0.115	0.125	0.114	0.073	0.126																
G. fuliginosa (9)	0.131	0.121	0.133	0.158	0.086	0.101	0.131	0.111															
G. ornata (10)	0.131	0.123	0.121	0.157	0.135	0.128	0.033	0.134	0.143														
P. andruzzii (11)	0.132	0.125	0.136	0.150	0.117	0.139	0.123	0.131	0.132	0.145													
G. orientalis (12)	0.140	0.124	0.143	0.150	0.067	0.092	0.148	0.125	0.084	0.157	0.145												
G. gotyla (13)	0.143	0.129	0.133	0.160	0.120	0.122	0.135	0.129	0.121	0.151	0.166	0.116											
G. regressus (14)	0.144	0.130	0.133	0.179	0.144	0.126	0.088	0.141	0.155	0.094	0.142	0.161	0.163										
G. annandalei (15)	0.137	0.129	0.132	0.133	0.130	0.093	0.148	0.123	0.113	0.149	0.156	0.104	0.141	0.167									
G. dembecha (16)	0.146	0.132	0.134	0.179	0.146	0.128	0.089	0.143	0.156	0.096	0.143	0.163	0.165	0.001	0.167								
G. waterloti (17)	0.135	0.133	0.121	0.137	0.137	0.144	0.072	0.141	0.149	0.076	0.156	0.153	0.151	0.108	0.153	0.110							
G. kempi (18)	0.155	0.144	0.138	0.156	0.129	0.108	0.158	0.135	0.121	0.165	0.179	0.127	0.132	0.182	0.113	0.184	0.177						
G. tengchongensis (19)	0.163	0.159	0.141	0.171	0.138	0.126	0.143	0.123	0.146	0.156	0.169	0.135	0.144	0.170	0.117	0.172	0.152	0.161					
G. bicornuta (20)	0.188	0.165	0.181	0.179	0.143	0.155	0.165	0.170	0.149	0.163	0.164	0.155	0.159	0.171	0.119	0.171	0.177	0.165	0.176				
G. mullya (21)	0.173	0.162	0.165	0.181	0.149	0.158	0.173	0.162	0.158	0.184	0.181	0.143	0.162	0.206	0.151	0.206	0.173	0.162	0.152	0.146			
B. harterti (22)	0.195	0.193	0.194	0.209	0.191	0.185	0.177	0.160	0.181	0.192	0.192	0.198	0.196	0.223	0.177	0.223	0.206	0.173	0.186	0.209	0.190		
B. luteus (23)	0.203	0.193	0.198	0.218	0.195	0.180	0.193	0.175	0.191	0.201	0.197	0.212	0.203	0.233	0.201	0.233	0.226	0.189	0.199	0.244	0.204	0.066	
B. fritschii (24)	0.200	0.194	0.188	0.189	0.189	0.185	0.179	0.165	0.182	0.190	0.194	0.202	0.190	0.234	0.180	0.234	0.204	0.177	0.176	0.213	0.192	0.029	0.069

I. typhlops-ND = specimens without a mental disc, *I. typhlops-D*= specimens with a mental disc

a large clade that includes the other species of the genus *Garra,* as well as *Phreatichthys andruzzii* (Vinciguerra, 1924). Colli et al. (2009) also surveyed the phylogeny of *P. andruzzii* and *G. barreimiae* with cyt *b* and reported similar results. Therefore, these authors suggested that the taxonomic position of *P. andruzzii* should be revised, while earlier Banister (1984) had already reported that based on osteological data the genus *Phreatichthys* is closely related to *Garra*.

Bruun and Kaiser (1944) reported that *I. typhlops* is similar to the genus *Barbus.* Yet, according to the K2P distances between *I. typhlops* and species of the genera *Garra* and *Barbus* (Table 2), and the resulting phylogenetic trees, the species is most closely related to the genus *Garra* and only distantly related to the genus *Barbus.* Saadati (1977) rejected a relationship between *I. typhlops* and *Barbus* species from the Tigris River basin because of their large size differences and lack of a mental disc. Finally, our results are also similar to those of Hashemzadeh Segherloo et al. (2012), who assessed the phylogenetic position of *I. typhlops* using the mitochondrial COI gene.

We observed a mean K2P divergence of 4.1 % between the two forms of *I. typhlops*. The intraspecific divergence is higher than the mean K2P divergence reported among other fishes, *e.g.* 0.78 % for marine fishes (Zhang and Hanner 2012) and 1.1 % for freshwater fishes (Hedrick et al. 2006). Hashemzadeh Segherloo et al. (2012) also reported a similar divergence between both forms at COI. The high genetic distances between the two sympatric forms of *I. typhlops,* along with the morphological differences between the two sympatric forms of *I. typhlops* (Sargeran et al. 2008), may be due to particularities inherent to evolutionary processes in subterranean habitats (Hashemzadeh Segherloo et al. 2012).

The mental disc is the key character of species of the subfamily Labeoninae including *Iranocypris* and *Garra.* The genus *Garra* is similar to the genus *Iranocypris* in having three rows of pharyngeal teeth (Abbasi and Gharzi 2008, Coad 2013). Conversely, Bruun and Kaiser (1944) described *I. typhlops* as a new genus and a new species based on its two rows of pharyngeal teeth. Coad (2013) reported one to three teeth in the outer row, three to four teeth in the middle row and three to five teeth in the inner row. This condition, however, is also found in the genus *Garra* (typically 2, 4, 5-5, 4, 2 teeth in each row, respectively). Earlier, Sargeran et al. (2008) investigated morphometric and meristic features of *I. typhlops* to conclude that this species is similar to species of the genus *Garra.* Finally, we recommend that *I. typhlops* is transferred to the genus *Garra* and *Iranocypris* Bruun & Kaiser, 1944 is to be regarded as a species of *Garra* Hamilton, 1822.

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