

Description of *Callistethus hamus* sp. nov. (Coleoptera, Scarabaeidae, Rutelinae) from continental Southeast Asia using synchrotron to illustrate the aedeagus

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Abstract

A new species, *Callistethus hamus* Lu & Zorn, **sp. nov.**, is described from China, Laos, and Vietnam. Additionally, we used synchrotron (Shanghai Synchrotron Radiation Facility) to scan the aedeagus. The virtual 3D model of the aedeagus is reconstructed and provided.

Keywords

Anomalini, *Callistethus*, China, Laos, Vietnam, new species, Rutelinae, 3D models

Introduction

The genus *Callistethus* Blanchard, 1851, includes, to date, more than 150 species (Krajčůk 2012; Filippini 2016). Only six species and subspecies were previously recorded from China: *Callistethus auronitens* (Hope, 1835), *C. excisipennis* Lin, 1981, *C. formosanus* Kobayashi, 1987, *C. plagiicollis plagiicollis* (Fairmaire, 1886), *C. p. isidai* Miyake, 1987, and *C. stoliczkae* (Sharp, 1878). When working on anomaline speci-

mens from the IZAS collection, we found one species from Yunnan which appeared to be *Callistethus rachelae* (Arrow, 1917), originally described from Myanmar. However, after examination of the type material of this species, deposited in the NHMUK, it became apparent that there are two different species; the Yunnan species proved new to science. Additional specimens of the new species were later found also from Laos and Vietnam and the species is described herein.

Recently, Micro-CT and synchrotron radiation microtomography techniques have been used in several extant and extinct insect groups, e.g., Formicidae of Hymenoptera (Staab et al. 2018), †Alienoptera (Bai et al. 2018, 2016), Geotrupidae of Coleoptera (Bai et al. 2017), and Platycnemididae of Odonata (Steinhoff and Uhl 2015). These techniques enable a new method of acquiring the internal and external 3D morphology. In Rutelinae, the aedeagus plays a very important role in the species identification. This organ has usually a strongly three-dimensional shape in this subfamily. Therefore, sometimes it is difficult to demonstrate the precise morphology of the aedeagus using traditional line drawings or 2D photographs, although several planes could give an approximate idea of the spatial structure. Here we use synchrotron technique to provide 3D model of the aedeagus in this new species, which can provide more morphological information for the future studies of Rutelinae.

Materials and methods

The morphological terminology follows Lu et al. (2018).

The body length was measured from the apex of the clypeus to the apex of the elytra. The length of pronotum was measured in the middle in dorsal view, its width at the greatest width. The ratio of interocular width to head width was measured in dorsal view at greatest width of head and nearest interocular distance.

For observation of morphological structures, some specimens were softened by soaking the aedeagus in detergent for ca. 24 hours. Observations and dissections were carried out under an Olympus SZ61 stereomicroscope and a Zeiss Stemi 2000. The digital images were taken with a Canon 5D digital camera in conjunction with a Canon MP-E 65 mm *f*/2.8 1–5× Macro Lens, and then stacked by Helicon Focus 5.3.10. All images were edited and adjusted in Adobe Photoshop CS6 Extended. The distribution map was created by QGIS 3.4 software (QGIS Development Team). Coordinates and altitude were assigned for each locality mentioned in the text (material examined in each species). These data were used in the construction of distribution maps (see Fig. 24).

The aedeagus of one specimen was imaged using propagation phase-contrast synchrotron radiation microtomography (PPC-SR- μ CT) on the beamline 13W at the Shanghai Synchrotron Radiation Facility (SSRF). The isotropic voxel size was 3.25 μ m. The beam was monochromatised at an energy of 14 keV using a double crystal monochromator. To obtain a phase-contrast effect, we used a sample-detector distance (propagation distance) of 300 mm and 900 projections on 180°. The phase retrieval

and slice reconstruction were performed using PITRE software. Based on the obtained image stacks, three-dimensional structures of the specimen were reconstructed and virtually dissected with Amira 5.4 (Visage Imaging, San Diego, USA) (see Figs 19–23 and Appendix 1). All images were edited and adjusted in Adobe Photoshop CS6 Extended.

Type specimens of the new species are provided with one red printed label “*Callistethus hamus* sp. nov., HOLOTYPE [or] PARATYPE, Lu & Zorn, 2019”.

The material examined is housed in the following collections (curators in parenthesis):

- CZPC** Carsten Zorn private collection, Gnoien, Germany
FWPC Falei Wang private collection, Chongqing, China
IZAS Institute of Zoology, Chinese Academy of Sciences, Beijing, China (Ming Bai)
MSPC Matthias Seidel private collection, Prague, Czech Republic
NHMUK Natural History Museum, London, United Kingdom (Maxwell W. L. Barclay, Michael Geiser)
NMEC Naturkundemuseum Erfurt, Germany (Matthias Hartmann)

Taxonomy

Callistethus hamus Lu & Zorn, sp. nov.

<http://zoobank.org/03158F0F-19B4-4940-8025-649989535595>

Figs 1–9, 19–23

Type locality. China, Southern Yunnan, 23 km NW of Jinghong, vicinity of Nan Ban, Xishuangbanna [Prefecture], 22°09.49'N, 100°39.92'E, 730 m.

Material examined. Holotype (HT). CHINA • ♂; Yunnan Province, Dai Autonomous Prefecture of Xishuangbanna, Jinghong City, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 20 May 2008; A Weigel leg.; NMEC.

Paratypes. CHINA • 1 ♀; same data as the holotype • 1 ♂; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 5 Jun. 2008; A. Weigel leg.; NMEC; [Micro-CT specimen] • 1 ♂ 1 ♀; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 5 Jun. 2008; A. Weigel leg.; CZPC • 1 ♀; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 15 Jun. 2008; NMEC • 1 ♀; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 5 Jun. 2008; A. Weigel leg.; NMEC • 1 ♂; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 12 May 2008; A. Weigel leg.; NMEC • 1 ♀; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 16 May 2009; Malaise trap; L.Z. Meng leg.; IZAS IOZ(E)1966878 • 1 ♀; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National

Nature Reserve; 22°07.85'N, 100°40.12'E; 26 May 2009; Malaise trap; L.Z. Meng leg.; IZAS IOZ(E)1966879 • 1 ♀; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 22°09.49'N, 100°39.92'E; 16 May 2009; flight interception traps; L.Z. Meng leg.; IZAS IOZ(E)1966880 • 1 ♀; Yunnan Province, Xishuangbanna, Jinghong, Na Ban River Watershed National Nature Reserve; 31. Jul. 2013; FWPC • 1 ♂; Yunnan Province, Menghai, Menghun Town; 21°50.48'N, 100°23.15'E; 4 Jun. 1958; L.Y. Zheng leg.; IZAS IOZ(E)1966104 • 1 ♀; Yunnan Province, Xishuangbanna, Menghai, Menghun Town; 21°50.48'N, 100°23.15'E; 1 Jun. 1958; X.W. Meng leg.; IZAS IOZ(E)1966106 • 1 ♀; Yunnan Province, Xishuangbanna, Menghai, Menghun Town; 21°50.48'N, 100°23.15'E; 4 Jun. 1958; L.Y. Zheng leg.; IZAS IOZ(E)1966107 • 1 ♀; Yunnan Province, Xishuangbanna, Menghai, Mengzhe Town; 21°59.15'N, 100°16.02'E; 8 Jul. 1958; S.Y. Wang leg.; IZAS IOZ(E)1966105.

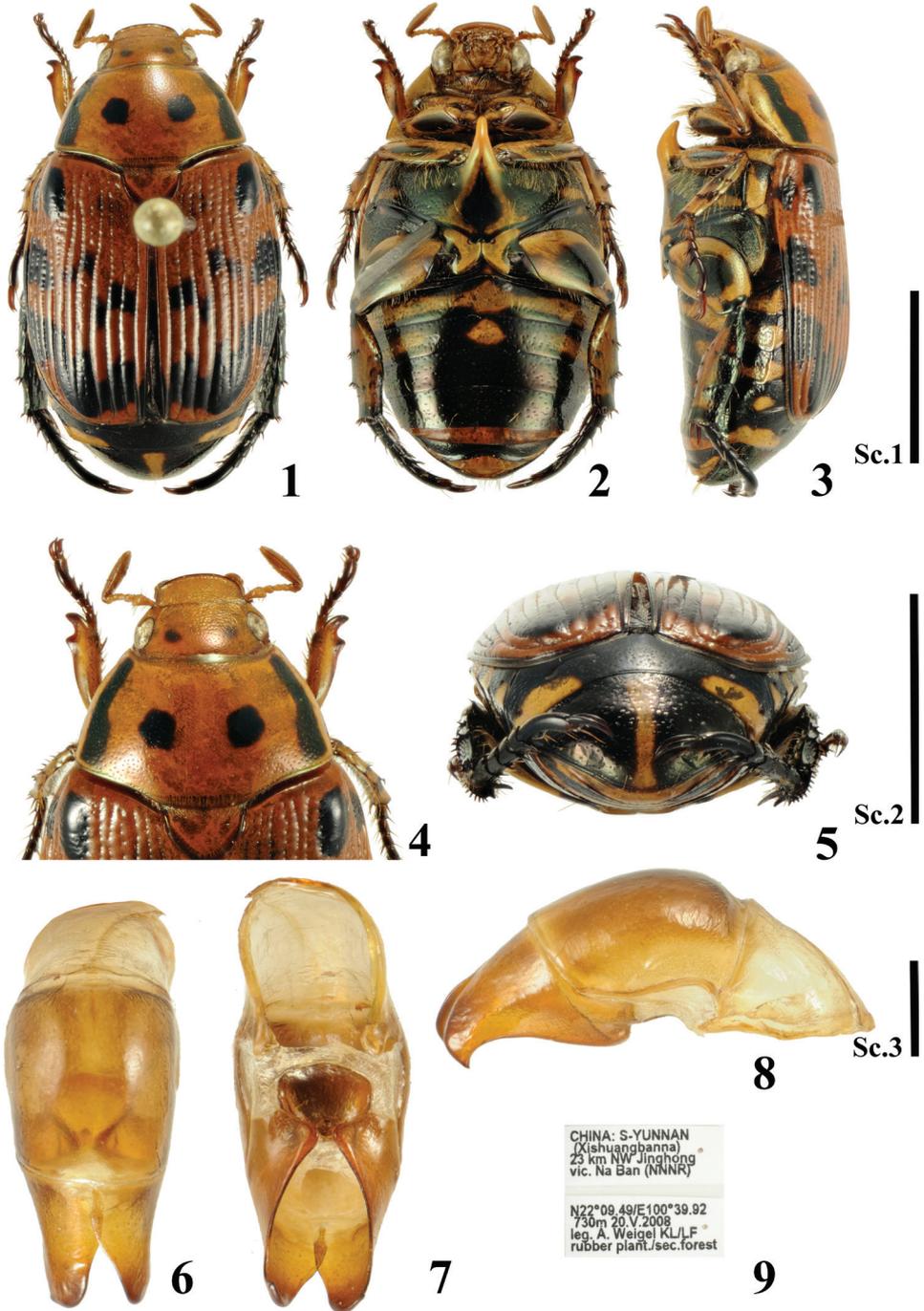
LAOS • 1 ♂; Vientiane Province, Phou Khao Khoay; 18°24.15'N, 103°02.15'E; 4–17 May 2005; P. Moravec leg.; CZPC.

VIETNAM • 4 ♂♂ 2 ♀♀; Lâm Đồng Prov., Dambri, Bảo Lâm; 11°38.42'N, 107°44.52'E; May 2017; local collector leg.; CZPC • 1 ♀; Lâm Đồng Prov., Bảo Lộc; 11°32.88'N, 107°48.46'E; March 2018; local collector leg.; CZPC • 5 ♂♂ 1 ♀♀; Lâm Đồng Prov., Bảo Lộc; 11°32.88'N, 107°48.46'E; March 2018; local collector leg.; CZPC • 1 ♂ 1 ♀; Lâm Đồng Prov., Bảo Lộc; 11°32.88'N, 107°48.46'E; March 2017; local collector leg.; MSPC.

Description of holotype (♂). Body shape elongate ovoid, convex.

Colour. Head including antenna orange-brown, with two small black spots on vertex; pronotum orange-brown with two moderately large black spots in the middle of each side; an additional longitudinal stripe near the lateral margin (not reaching anterior and posterior margins) separating the yellow sides from the orange disc (Figs 1, 4); elytra light reddish-brown with black markings as follows: an elongate spot on humeral callus, another smaller spot beneath shoulder at lateral margin, a zig-zag band of loosely connected spots crossing the middle of elytra, another zig-zag band shortly before the posterior margin (Fig. 1); propygidium black with a transverse yellow spot at the outer posterior margin (Fig. 5); pygidium black with a narrow yellow longitudinal middle line (Fig. 5); underside yellow with black markings, with slight metallic shine (Fig. 2); black macula in the middle and at extremities of femurs; various black markings present on all tibiae; pro- and mesotarsi brown, metatarsus black; major part of meso- and metaventrite black; meso-metaventral process yellow; major part of abdominal ventrites 1–3 yellow, abdominal ventrites 4–6 black with lateral yellow spot (Fig. 2).

Head. Clypeus subtrapezoidal, anterior angles rounded; anterior margin weakly reflexed, very densely, transversely rugo-punctate; frons rugo-punctate, with very shallow impression in the middle, confluent punctate at sides; vertex finely and sparsely punctate in the middle, more coarsely punctate laterally; ratio interocular width/width of head approximately 0.71; antennal club longer than antennomeres 1–6 combined (Figs 1, 4).



Figures 1–9. Holotype of *Callistethus hamus* Lu & Zorn, sp. nov. **1–3** habitus **1** dorsal view **2** ventral view **3** lateral view from left **4** head and pronotum **5** propygidium and pygidium **6–8** aedeagus **6** dorsal view **7** lateral view from right **8** ventral view **9** label. Key: Sc. 1, Scale bars: 5 mm (**1–3**); Sc. 2, 5 mm (**4, 5**); Sc. 3, 1 mm (**6–8**).

Pronotum approximately 1.89 times wider than long; sparsely and finely punctate, punctures very fine on disc, becoming gradually larger toward the sides; surface with additional micropunctures; anterior angles sub-rectangular; posterior angles obtuse; broadest at base; sides evenly curved in the middle, straight and strongly converging before anterior angles; sides very slightly sinuate near posterior angles; basal marginal line only present near posterior angles; anterior marginal line indistinct in the middle (Fig. 4).

Scutellum subtriangular, slightly broader than long, finely and sparsely punctate (Fig. 4).

Elytra regularly striate; primary striae and secondary stria of subsutural interstice sulcate in posterior half, therefore intervals flat in anterior half and gradually more convex posteriad; striae punctation distinct, coarse; 2nd and 3rd interstices each with an indistinct row of punctures; elytral surface with additional sparse micropunctuation; humeral umbone and apical protuberance rather prominent; epipleuron broad near humerus, ending approximately at the middle of elytron; posterior margins evenly, separately rounded; apico-sutural angle forming small dent (Fig. 1).

Pygidium convex; apex broadly rounded; moderately densely, coarsely punctate; apex with several long, erect brownish setae (Fig. 5).

Ventral thoracic surface smooth (Fig. 2).

Meso-metaventral process very long, reaching middle of procoxae; projecting upward in lateral view; apex acute (Fig. 3).

Abdominal ventrites with transverse row of rather sparse brown setae (usually only in lateral part); ventrites 1 and 2 carinate laterally (Figs 2, 3).

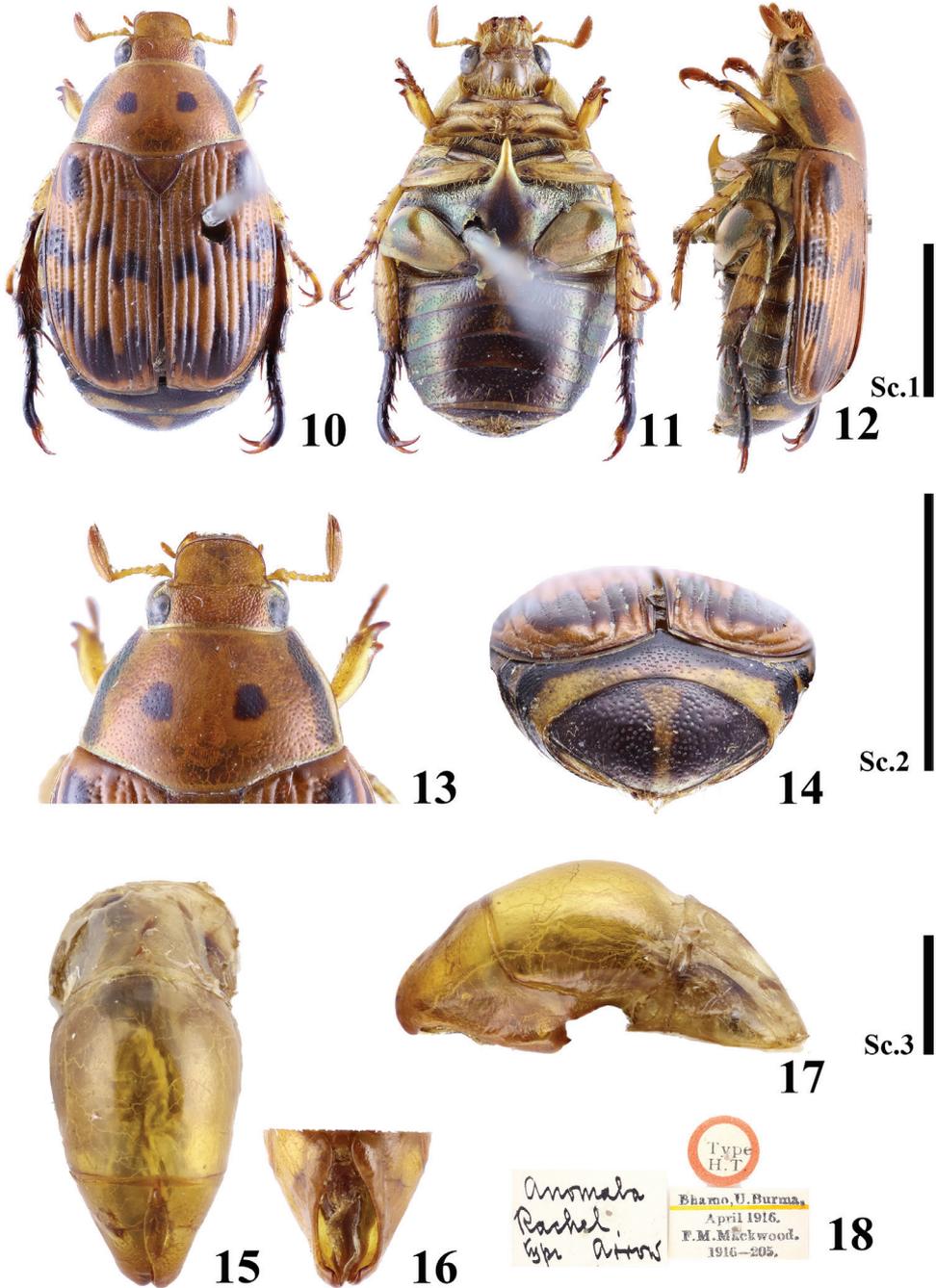
Legs. Mesofemur with two bands of long brown setae: one along anterior margin; another transverse row of punctures parallel to posterior margin. Protibia bidentate, rather slender; proximal tooth short, situated close to the rather short, weakly outwards curved apical tooth; inner spur short, articulated in opposite to proximal tooth. Metatibia strongly fusiform; protarsomere 5 shorter than tarsomeres 1–4 combined in all legs; inner protarsal claw slightly widened and deeply incised apically, lower margin with obtuse angle basally, upper branch spiniform; outer mesotarsal claw long, curved, deeply incised at apex, upper branch spiniform; metatarsal claws somewhat unequal, outer claw distinctly broader and longer than inner (Figs 1–3).

Aedeagus. Parameres short, with the hook-like apex in lateral view. See Figs 6–8.

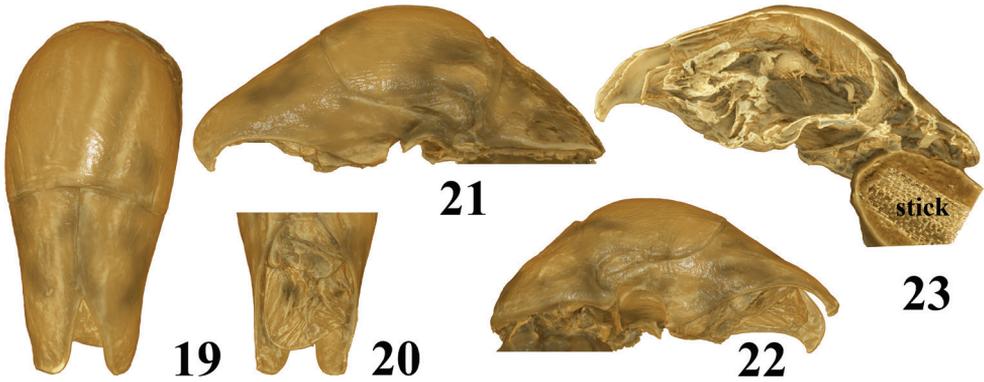
Female. Abdominal ventrites 4–6 with extensive yellow markings. Apical tooth of protibia long and somewhat spatulate; protarsus articulated slightly basally of level of proximal tooth; inner spur long; protarsomere 1 as long as combined length of protarsomeres 2–4; antennal club short, only slightly longer than antennomeres 2–6 combined.

Measurements. Total body length 11.5–14.4 mm (HT 11.8 mm), total body width 6.5–8.4 mm (HT 7.5 mm).

Morphological variability. Vertex with or without two black spots. Elytral spots vary slightly in shape and size. The extend of black markings of the ventral side variable. The secondary longitudinal rows of punctures in interstices 2 and 3 sometimes distinct until the posterior half. Shape of parameres very consistent (Figs 19–23, Appendix 1).



Figures 10–18. Holotype of *Callistethus rachelae* (Arrow, 1917). 10–12 habitus 10 dorsal view 11 ventral view 12 lateral view from left 13 head and pronotum 14 propygidium and pygidium 15–17 aedeagus 15 dorsal view 16 lateral view from right 17 ventral view 18 label. Key: Sc. 1. Scale bars: 5 mm (10–12); Sc. 2, 5 mm (13, 14); Sc. 3, 1 mm (15–17).



Figures 19–23. 3D model for aedeagus of *Callistethus hamus* Lu & Zorn, sp. nov. Paratype from China, Yunnan. These photos are screen shots of the 3D model of the aedeagus.

Differential diagnosis. *Callistethus hamus* sp. nov. resembles several other, similarly coloured South East Asian species of this genus. The reddish colour combined with black markings is also found in *C. maculatus* (Guérin-Ménéville, 1834), *C. picturatus* (Candèze, 1869), *C. rachelae* (Arrow, 1917), *C. spiniferus* (Ohaus, 1915), and *C. stolidopygus* (Ohaus, 1915). The new species is most similar to *C. rachelae*, which also has only two black spots on the pronotum, not four as all other red species (Figs 10–18). *Callistethus hamus* sp. nov. differs from *C. rachelae* primarily in the shape of the aedeagus (Figs 6–8, 15–17). The ventral margin of the parameres of *C. rachelae* is somewhat membranous and soft, but fully sclerotised in *C. hamus* sp. nov. The apices of the parameres in *C. hamus* sp. nov. are longer and more strongly curved compared to those of *C. rachelae* (Figs 15–17).

Etymology. The specific epithet refers to the hook-like shape of the parameres of the new species.

Collecting data. Specimens collected in the Naban River Watershed National Nature Reserve by Lingzeng Meng and Andreas Weigel were collected with cross-window traps in the tree canopy and malaise trap.

Distribution. China (Yunnan), Laos (Vientiane), Vietnam (Lâm Đồng) (Fig. 24).

Discussion

We found one female specimen in the collection of the IZAS, collected at Jingnan Town, Dehong prefecture, Yunnan, which could represent the first record of *C. rachelae* in China. The collecting locality is very close to the type locality of *Callistethus rachelae* in neighbouring Myanmar (“Bhamo”, see Fig. 24). This specimen differs slightly from all specimens of *C. hamus* sp. nov. in having a slightly more slender meso-metasternal process, which is consistent with the holotype of *C. rachelae* sp. nov. However, male specimens are needed to confirm the presence of *C. rachelae* sp. nov. in China.



Figure 24. Map of Southeast Asia showing distributions of *Callistethus hamus* and *Callistethus rachelae*.

In the present study the synchrotron technology was used for the first time to study a Rutelinae species. Normally, the original data of 3D information need to be present in some specific software (like Amira and Maya), which require a higher hardware configuration. Obviously it will limit the widespread use of this technology by taxonomists to some extent. In this study, we found that the structure complexity of the aedeagus of the here examined Rutelinae is relatively low and can be obtained as 3D model in pdf format containing about 20Mb (Appendix 1). It can be easily opened in Adobe Reader Pro by enabling the playing 3D content checkbox. It is possible to zoom and rotate the 3D model as desired, which can enhance details. By comparing figures 6–8 with 19–21, we are sure that this 3D model reflects the real characters. Moreover, it can improve the identification accuracy compared to 2D images by adjusting the viewing angle.

Unfortunately, in this study, internal structures like the endophallus are not clearly visible in the 3D model. The synchrotron and Micro-CT technology require a dried out specimen and are suitable for sclerotised structures, while membranous structures like the endophallus will be out of shape during the operating steps. Therefore, it will be difficult to obtain the 3D structure of the endophallus. The disadvantages of this technology include also its high cost and the fact that the procedure is time-consuming.

In summary, the synchrotron and Micro-CT technology have great potential for wide use in the taxonomy of the Rutelinae and other insect groups, because it provides

accurate morphological information of 3D structures. But the shortcomings of this technology are still obvious. Therefore, the innovation of new techniques that aid in the visualisation of microscopic anatomical structures is needed. The recently invented LED-SIM (DMD-based LED-illumination structured illumination microscopy) facilitates the acquisition of nano- and micro-3D structures of small organisms in a high-resolution format (500 nm in the XY-plane and 930 nm along the Z-axis) (Ruan et al. 2016). Recently, the second generation of LED-SIM could provide large-scale 3D imaging of insects in natural colour (Qian et al. 2019). Therefore, it is possible that the 3D imaging will be widespread in future.

Acknowledgements

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Appendix I

geomagic



Click on the image to activate the 3D Model.

Figure 1A. 3D model for aedeagus of *Callistethus hamus* Lu & Zorn, sp. nov. in pdf format.

Porongurup, a new genus of pselaphine staphylinid beetles from Western Australia (Coleoptera, Staphylinidae, Pselaphinae, Faronitae)

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Abstract

A new genus and three new species of pselaphine staphylinid beetles, supertribe Faronitae, from Western Australia are described as follows: *Porongurup* **gen. nov.** is based on *Porongurup angulatus* **sp. nov.**, with the two additional new species, *Porongurup clarkei* **sp. nov.** and *Porongurup tenuis* **sp. nov.** Illustrations of their habitus, and major diagnostic characters as well as a distribution map are included. A key to the species is provided.

Keywords

biodiversity, biogeography, Faronini, taxonomy

Introduction

The supertribe Faronitae consists of 29 genera worldwide, with 13 genera being found in Australia and New Zealand (Park and Chandler 2017). At this time only three faronite genera [*Sagola* Sharp, 1874 (9 spp.), *Logasa* Chandler, 2001 (3 spp.), and

Nornalup Park & Chandler, 2017 (3 spp.)] are known from Australia, although eastern Australia does have a complex and largely unknown fauna of Faronitae that is currently under study by J.-S. Park and colleagues. This is the second paper treating the separate and unique fauna of Faronitae from the southwestern corner of Australia, following the treatment of *Nornalup* by Park and Chandler (2017).

In the collections of Faronitae being examined as part of a treatment of the fauna of Australia, 31 specimens were segregated into three species that shared a combination of discrete diagnostic characters, with that combination separating them from all other known faronite genera and supporting the creation of a new genus. This new genus can be recognized by the following combination of characters: rostrum with linear frontal sulcus, abdominal segment VI approximately twice as long as V, and the male genitalia have the median lobe of the male genitalia elongate and narrow, culminating in an acute apex. The included species of this genus are known only from the southwestern corner of Western Australia.

The two faronite genera known from extreme southwestern Australia, *Porongurup* gen. nov. and *Nornalup*, are apparently restricted to this area, which is known as a global biodiversity hotspot that has a climatically isolated flora and fauna (Slater 1975, Coates and Atkins 2001, Hopper and Gioia 2004, Slatyer et al. 2007, Park and Chandler 2017). Members of *Porongurup* gen. nov. and *Nornalup* have been collected together in samples from this area, and apparently share the same microhabitats. However, the species of *Porongurup* have never been recorded together in samples, indicating differences in habitat preferences for these species.

Materials and methods

Thirty-one specimens were examined from the following collections: Field Museum of Natural History (**FMNH**), Chicago, Illinois, USA, and the University of New Hampshire Insect Collection (**UNHC**), Durham, New Hampshire, USA. Six specimens were mounted on permanent slides to aide in observation of the internal characters and the fine external characters that are not apparent when using a dissecting microscope. Permanent microscopic slides were prepared using the techniques described by Hanley and Ashe (2003). Terminology for the foveal system and nomenclature follows Chandler (2001). Decimal Degrees were used for the format of geographical coordinates. Holotypes are deposited in the Western Australian Museum (**WAM**), Perth, Western Australia, Australia, and paratypes are deposited in the Field Museum of Natural History, the Western Australian Museum, the Australian National Insect Collection (**ANIC**), Canberra, ACT, Australia, the University of New Hampshire Insect Collection, Durham, New Hampshire, USA, and the Chungbuk National University Insect Collection (**CBNUIC**), Cheongju, Chungbuk-do, South Korea (indicated parenthetically). Specimen label data for the holotypes is transcribed verbatim. Data from the paratypes are standardized for consistency. The map of Australia is based on an image from SimpleMappr (Shorthouse 2010), that was subsequently modified to indicate localities of the specimens.

Systematics

Porongurup gen. nov.

<http://zoobank.org/362C6CA0-A334-4897-927B-B40A527B0118>

Type species. *Porongurup angulatus* sp. nov., herein designated.

Diagnosis. Members of this genus are easily separated from other faronite genera by the following combination of characters: rostrum with linear frontal sulcus (Fig. 3A); pronotum with median antebasal foveae, outer basolateral foveae, and inner basolateral foveae; prosternum with lateral mesocoxal foveae, and median procoxal foveae (Fig. 3B); mesoventrite with lateral mesosternal foveae and promesocoxal foveae (Fig. 3C); metaventrite with lateral metasternal foveae and lateral mesocoxal foveae (Fig. 3C); length of abdominal tergite and visible sternite VI approximately twice as long as V (Fig. 1); species only known from Western Australia (Fig. 5).

Description. Small body size, 1.6–2.0 mm. Body yellowish to reddish brown. *Head.* Head with distinct narrow frontal sulcus, and distinct vertexal foveae (Fig. 4G–L). Male head triangular, broader than long, widest across eyes. Female head more rounded than that of male (Fig. 4G–L). *Thorax.* Prosternum broader than long, widest at midpoint of prosternum (Fig. 3B). *Abdomen.* Length of abdominal segment VI approximately two times longer than V (Fig. 1). *Aedeagus.* Median lobe of male genitalia slender, apically sharp and narrow. Phallobase rounded. Parameres symmetrical and slender with more than five setae apically (Fig. 2).

Etymology. *Porongurup* gen. nov. is named for Porongurup National Park, where one of the species was collected. Gender: Masculine.

Distribution. Western Australia.

Comments about secondary sexual characters. Male specimens have larger eyes than females (Fig. 4G–L). The males of *Porongurup clarkei* sp. nov. and *P. tenuis* sp. nov. have longer elytra and fully developed hind wings, but female specimens have the elytra almost half the size of those of the males, and the hind wings are reduced (Fig. 1). *Porongurup angulatus* sp. nov. has fully developed hind wings in both sexes (Fig. 1A, D).

Comments about related taxa. Based on the thoracic foveal system and the longer abdominal segment VI, *Porongurup* gen. nov. is closest to the genus *Normalup* Park & Chandler, 2017. However, all specimens of *Porongurup* gen. nov. do not have a median metasternal foveae (Fig. 3C) and have a narrow frontal sulcus (Fig. 3A). The aedeagal form is also different from that of *Normalup* in the elongate triangular shape of both the median lobe and the parameres (Fig. 2). The parameres of *Normalup* species are as wide as the median lobe, and the median lobe is parallel-sided in dorsal view (Park and Chandler 2017: fig. 4d–i).

Key to species of the genus *Porongurup* gen. nov.

- 1 Antennomere III subquadrate, as long as wide (Fig. 4A, B); female elytra longer than wide with fully developed hind-wings (Fig. 1A, D); length of

- abdominal segment VI 2.0–2.5 times as long as V (Fig. 1A, D)
 ***Porongurup angulatus* sp. nov.**
- Antennomere III rectangular, longer than wide (Fig. 4C–F); female elytra as long as wide with reduced hind-wings (Fig. 1E, F); length of abdominal segment VI approximately three times as long as V (Fig. 1B, C, E, F)..... **2**
- 2 Median lobe of male genitalia with small articulated extension apically (Fig. 2B: arrowed) ***P. clarkei* sp. nov.**
- articulated extension of median lobe of male genitalia absent (Fig. 2D: arrowed) ***P. tenuis* sp. nov.**

***Porongurup angulatus* sp. nov.**

<http://zoobank.org/69F25550-F02F-4F92-8662-201AAF05FFDC>

Figs 1A, 1D, 2A, 3, 4A–B, 4G, 4J, 5

Type material. Holotype. Australia: Western Australia (WA): 1♂, aedeagus dissected and mounted in euparal on clear plastic card, “AUSTRALIA: Western Australia: Porongurup N.P., Wansborough Walk at The Pass, 450 m, 34°40.69'S, 117°51.245'E, 6 VIII 2004, karri forest (*Eucalyptus diversicolor*), mostly young-growth; FMHD#2004-147, Berlese, leaf & log litter, A. Newton & M. Thayer 1116”. **Paratypes (N = 12; 6 males, 6 females). Australia: Western Australia:** 1♀ (FMNH, slide mounted), 40 km ESE Manjimup, Cup Road, 6 VII 1980, FMHD# 80-400, karri bark litter, S. & J. Peck; 1♂1♀ (UNHC, 1♀ slide mounted), 1♂ aedeagus dissected and mounted in Euparal on clear plastic card, Windy Harbour, 27 km S Northcliffe, 8 VII 1980, coastal scrub litter, S. & J. Peck; 1♂2♀ (FMNH, 1♀ slide mounted), Avon Valley N.P., 1.3 km from entrance, 420 m, 31°38.79'S, 116°17.94'E, 27 VII–13 VIII 2004, marri-jarrah (*Eucalyptus calophylla*-*E. marginata*) woodland; FMHD#2004-103, flight intercept trap, A. Newton & M. Thayer, 1102; 1♂ (CBNUIC, slide mounted), Walpole-Nornalup N.P., Giant Tingle Tree area, 190 m, 34°58.88'S, 116°47.42'E, 2 VIII 2004, tingle-*Allocasuarina*-karri (*Eucalyptus diversicolor*) forest; FMHD#2004-132, Berlese, leaf & log litter, Newton, Thayer, Clarke 1110; 1♂1♀ (CBNUIC), 1♂ aedeagus dissected and mounted in Euparal on clear plastic card, 54 km SE Manjimup, 22 VI/26 VI 1980, S. & J. Peck. jarrah forest litter; 1♂ (FMNH), aedeagus dissected and mounted in Euparal on clear plastic card, Pemberton, Brockman N.P., 19 VII 1980, FMHD#80-406, karri base & fungi litter, S. & J. Peck; 1♂ (FMNH), aedeagus dissected and mounted in Euparal on clear plastic card, Warren N.P., Bicentennial Tree vic., 120 m, 34°29.73'S, 115°58.62'E, 30 VII–10 VIII 2004, karri forest (*Eucalyptus diversicolor*); FMHD#2004-114, flight intercept trap, Newton, Solodovnikov, Thayer 1105; 1♀ (UNHC) Avon Valley N.P., Governor's Drive, 1.2 km from Forty-one Mile Rd., 260 m, 31°36.57'S, 116°15.04'E, 27 VII 2004, *Eucalyptus wandoo* woodland; FMHD#2004-102, Berlese, leaf & log litter, A. Solodovnikov, D. Clarke et al. 1101.

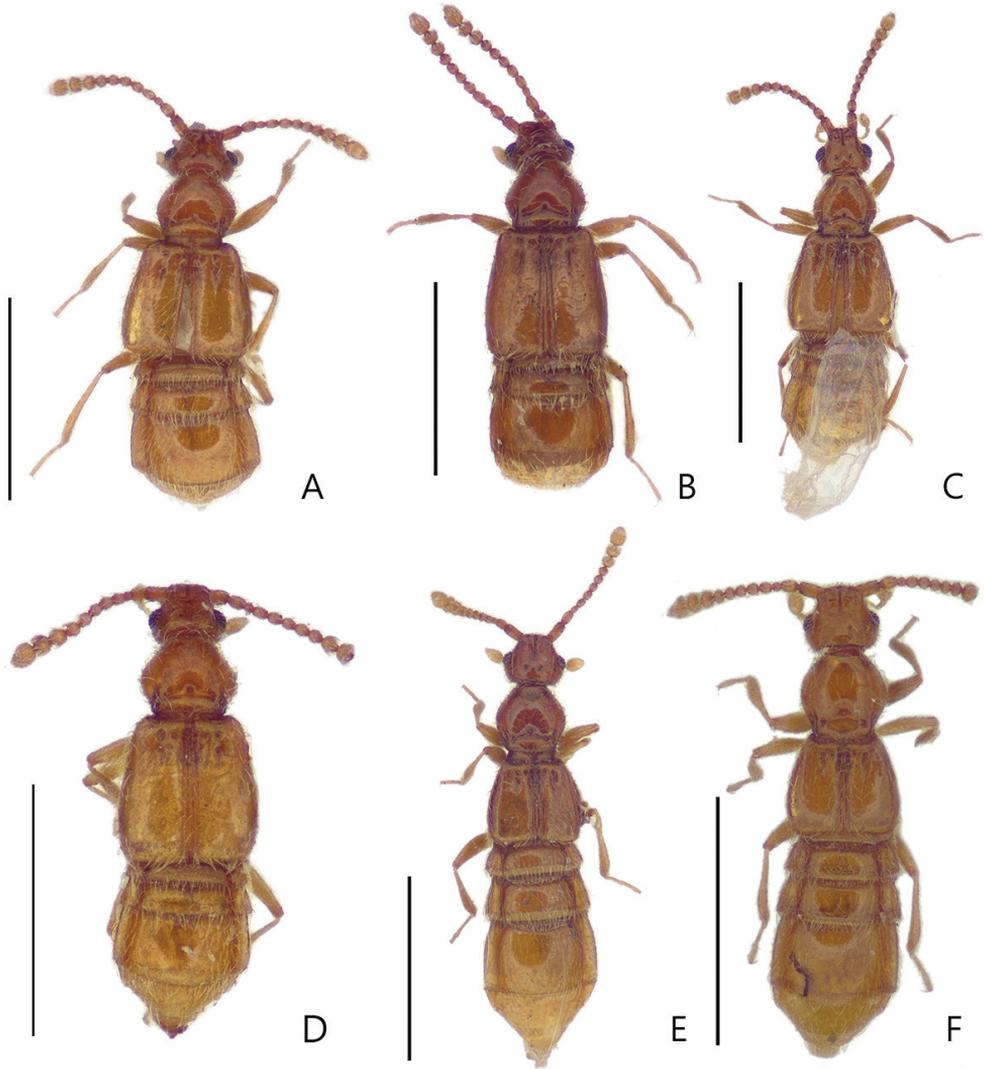


Figure 1. Male habitus photos, dorsal view **A** *Porongurup angulatus* sp. nov. **B** *P. clarkei* sp. nov. **C** *P. tenuis* sp. nov. Female habitus, dorsal view **D** *P. angulatus* sp. nov. **E** *P. clarkei* sp. nov. **F** *P. tenuis* sp. nov. Scale bar: 1 mm.

Diagnosis. This species can be distinguished from *Porongurup clarkei* sp. nov. by the smaller size of the male genitalia (Fig. 2), the subquadrate antennomere III (Fig. 4A, B), and the length of abdominal segment VI is approximately twice as long as V. This species is also separated from *Porongurup tenuis* sp. nov. by the length of abdominal segment VI being twice as long as V (Fig. 1), and the parameres of the male genitalia approximately are twice as wide as those of the other species (Fig. 2).

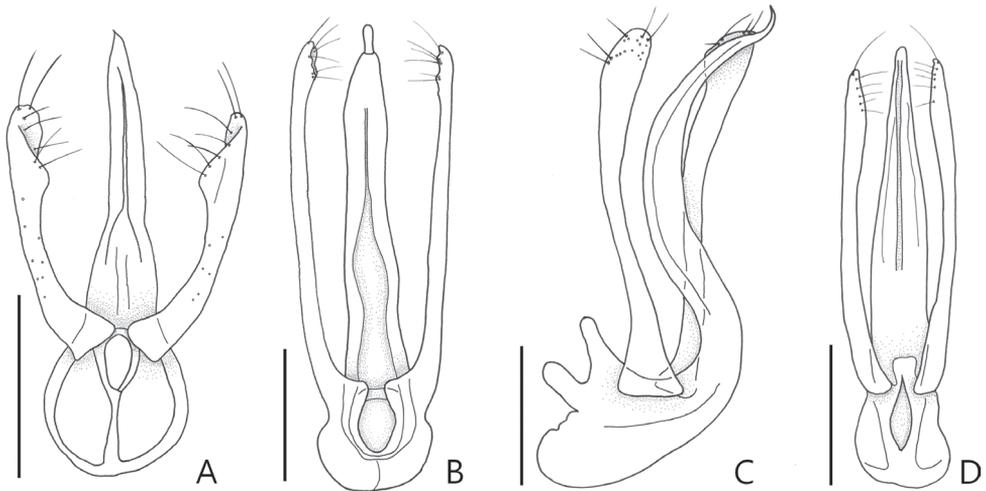


Figure 2. Male genitalia of the species of *Porongurup* gen. nov. **A** *P. angulatus* sp. nov., ventral view **B** *P. clarkei* sp. nov., ventral view **C** *P. clarkei* sp. nov., lateral view **D** *P. tenuis* sp. nov., ventral view. Scale bars: 0.1 mm.

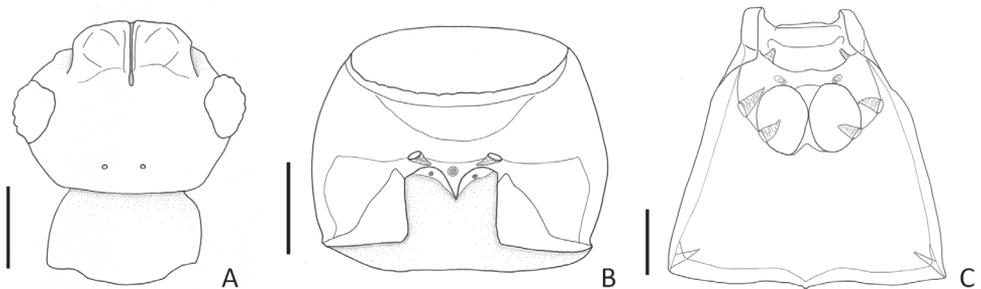


Figure 3. Generic characters of *Porongurup angulatus* sp. nov. **A** Head with frontal sulcus, dorsal view **B** Prothorax, ventral view **C** meso- metaventrite, ventral view. Scale bar: 0.1 mm.

Description. Length 1.7–1.9 mm (Fig. 1A, D). *Head.* Head in dorsal view with deep frontal sulcus and vertexal foveae (Fig. 4G). Antennomeres II longer than wide, III subquadrate, smallest of the antennomeres, IV and V longer than wide, VI and VIII as long as wide, IX and X transverse (Fig. 4A, B). *Abdomen.* Length of abdominal segment VI twice as long as V in both sexes (Fig. 1A, D). *Aedeagus.* Median lobe of male genitalia elongated triangular, lacking articulated extension at tip of apex. Phallobase rounded in dorsal view. Parameres as wide as at middle of median lobe (Fig. 2A).

Etymology. This species name refers to the sub-apically angulate parameres of the male genitalia.

Distribution. Western Australia (Fig. 5, circles).

Habitat. Specimens of this species were collected using flight intercept traps, or were taken by sifting leaf, bark, log, or fungus in *Eucalyptus* forests.

Porongurup clarkei sp. nov.

<http://zoobank.org/11556FE3-6FDF-4265-999C-C160B8F6F17E>

Figs 1B, 1E, 2B–C, 4C–D, 4H, 4K, 5

Type material. Holotype. Australia: Western Australia (WA): 1♂, aedeagus dissected and mounted in Euparal on clear plastic card, “**AUSTRALIA: Western Australia:** Stirling Range N.P., Toolbrunup Peak Track, 480–520 m, 34°23.4'S, 118°03.3'E, 5 VIII 2004, *Eucalyptus* forest & mallee; FMHD#2004-146, Berlese, leaf & log litter,

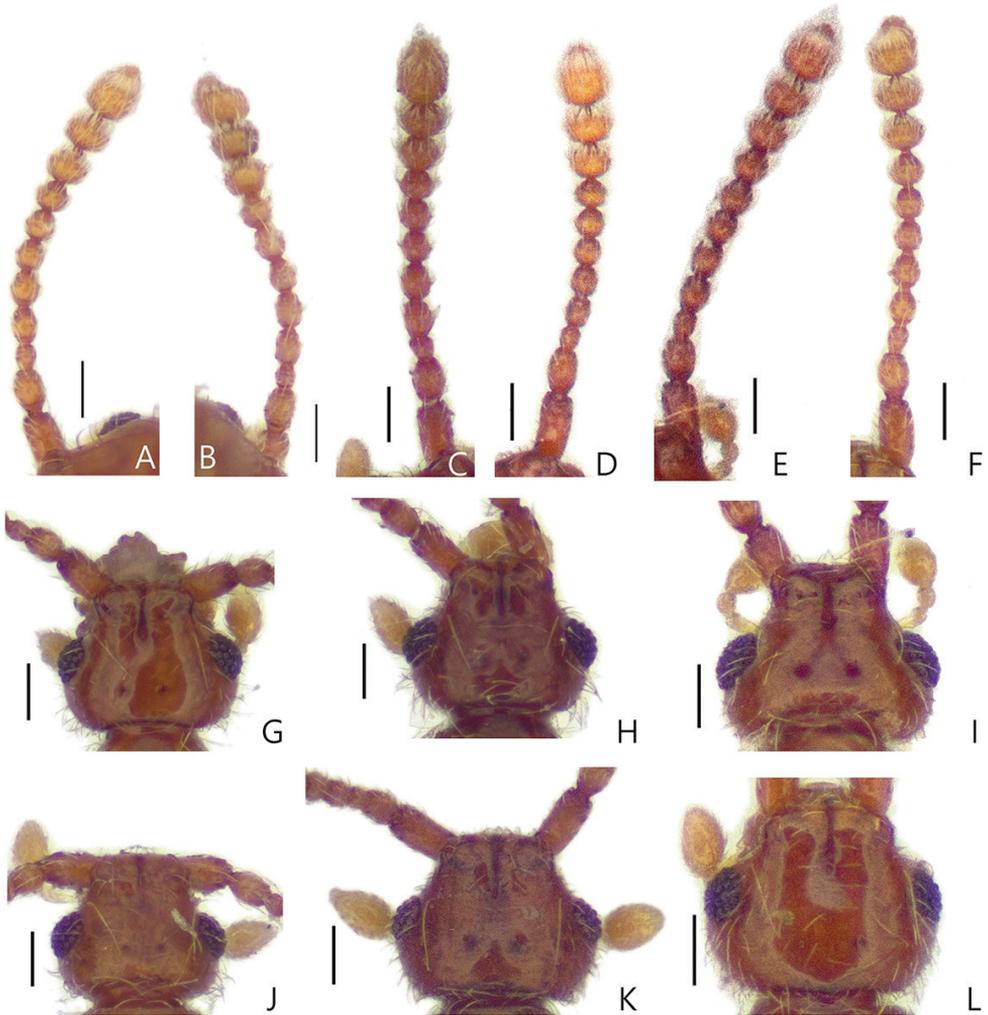


Figure 4. Antennae of *Porongurup angulatus* sp. nov. **A** male **B** female. Antennae of *P. clarkei* sp. nov. **C** male **D** female. Antennae of *P. tenuis* sp. nov. **E** male **F** female. Male head dorsal view **G** *P. angulatus* sp. nov. **H** *P. clarkei* sp. nov. **I** *P. tenuis* sp. nov. Female head dorsal view **J** *P. angulatus* sp. nov. **K** *P. clarkei* sp. nov. **L** *P. tenuis* sp. nov. Scale bar: 0.1 mm. *The distinctness and position of the vertexal foveae may be affected by the angle of view and condition of the specimens.

Clarke & Grimbacher 1115". **Paratypes** ($N = 11$; 7 males, 4 females). **Australia: Western Australia:** 1♂2♀ (FMNH, 1♀ slide mounted), Stirling Range N.P., Toolbrunup Peak Track, 430–485m, 34°23.5'S, 118°03.65'E, 5 VIII 2004, mallee *Eucalyptus*; FMHD#2004-145, Berlese, water-washed soil, 0–18 cm, D. Clarke 1114; 5♂ (UNHC), 1♂ aedeagus dissected and mounted in Euparal on clear plastic card, Porongorup N.P., Bolganup Creek, 12 VI 1980, Berlese, bark & fungi karri tree bases, S. & J. Peck; 1♂ (CBNUIC, aedeagus dissected and mounted in Euparal on clear plastic card, Stirling Range N.P., Toolbrunup Tr. WA, 10 VI 1980, Berlese, rotted logs & moss, S. & J. Peck, SBP47; 1♀ (FMNH), Stirling Range N.P., Toolbrunup Tr. WA, 10 VI 1980, Berlese, fungi on Euc. Trunks, S. & J. Peck, SBP45; 1♀ (UNHC), Porongorup N.P., Bolganup Creek, 12 VI 1980, Berlese, bark & fungi on karri tree bases, S. & J. Peck, SBP53.

Diagnosis. This species can be distinguished from *Porongurup angulatus* sp. nov. by antennomere 3 being longer than wide (Fig. 4C), and abdominal segment VI being approximately three times longer than V (Fig. 1B, E). This species is also separated from *Porongurup tenuis* sp. nov. by the median lobe of the male genitalia having a small digit at the apex (Fig. 2B: arrowed).

Description. Length 1.6–1.9 mm (Fig. 1B, E). **Head.** Head in dorsal view with both shallow frontal sulcus and vertexal foveae. Male and female antennae are almost identical in length (Fig. 4C, D). Antennomeres II longer than wide, III subquadrate and smallest, IV—VIII longer than wide, IX and X transverse (Fig. 4C, D). **Elytra.** Male elytra longer than wide, female elytra shorter, as long as wide (Fig. 1B, E). **Abdomen.** Abdominal segment VI approximately three times longer than V (Fig. 1B, E). **Aedeagus.** Median lobe of male genitalia as long as parameres with apical articulated extension. Phallobase rounded in dorsal view. Parameres symmetrical (Fig. 2B, C).

Etymology. This species is named for one of the collectors of the holotype, the staphylinid specialist Dave J. Clarke.

Distribution. Western Australia (Fig. 5, triangles).

Habitat. Specimens of this species were collected by sifting leaf, log, bark, moss or fungus litter in *Eucalyptus* forests, or were taken by Berlese funnel water-washed soil.

***Porongurup tenuis* sp. nov.**

<http://zoobank.org/04ECBDD6-4E5C-4450-BD21-12856B1D1334>

Figs 1C, 1F, 2D, 4E-F, 4I, 4L, 5

Type material. Holotype. Australia: Western Australia (WA): 1♂, aedeagus dissected and mounted in Euparal on clear plastic card, "AUSTRALIA: Western Australia: Walpole-Nornalup N.P., 1.4 km NE Mandalay Beach, 20 m, 34°59.76'S, 116°32.94'E, 3–9 VIII 2004, mixed forest on old dunes; FMHD#2004-140, flight intercept trap, A. Newton, M. Thayer, A. Solodovnikov 1112". **Paratypes** ($N = 5$; 1 male, 4 females). **Australia: Western Australia:** 1♂ (FMNH), elytra and aedeagus dissected in micro vial, Mt. Clare, 12 km, W Walpole, 20 XII 1976, JKethley, FM#76-517, Ber. #151, Euc. cornuta litter; 1♀ (FMNH), Walpole-Nornalup N.P., Anderson Rd. near Valley



Figure 5. Collection localities of *Porongurup* gen. nov. Key: *P. angulatus* sp. nov., circles; *P. clarkei* sp. nov., triangle; *P. tenuis* sp. nov., squares.

of the Giants Rd., 120 m, 34°59.48'S, 116°52.35'E, 2 VIII 2004, tingle-*Allocasuarina-karri* (*Eucalyptus diversicolor*) forest; FMHD#2004-137, Berlese, leaf & log litter, A. Newton, M. Thayer, et al. 1111; 1♀ (CBNUIC, slide mounted), Walpole-Nornalup N.P., Giant Tingle Tree area, 190 m, 34°58.88'S, 116°47.42'E, 2–9 VIII 2004, tingle-*Allocasuarina-karri* (*Eucalyptus diversicolor*) forest; FMHD#2004-130, flight intercept trap, Newton, Solodovnikov, Thayer, 1110; 2♀ (1, UNHC; 1 FMNH), Walpole N.P. Hilltop Rd., 21 VI 1980, Berlese, karri & tingle tree litter, S. & J. Peck.

Diagnosis. This species can be distinguished from *Porongurup angulatus* sp. nov. by the elytra being as long as wide (Fig. 1C, F). It also differs from *P. clarkei* sp. nov. by lacking the articulated extension at the apex of the median lobe of the male genitalia (Fig. 2D).

Description. Length 1.8–2.0 mm (Fig. 1C, F). *Head.* Head in dorsal view with both deep and narrow frontal sulcus and vertexal foveae. Male antennae longer than those of female (Fig. 4E, F). Antennomeres II longer than wide, III subquadrate and smallest, IV and V longer than wide, VI—VIII as long as wide, IX and X transverse (Fig. 4E, F). *Elytra.* Male elytra longer than wide, female elytra shorter than those of male (Fig. 1C, F). *Abdomen.* Male abdominal segment VI approximately three times as long as V, female with segment VI 1.5 times longer than V (Fig. 1C, F). *Aedeagus.*

Median lobe of male genitalia as long as parameres, apex sharp and narrow. Phallobase oval in dorsal view. Parameres symmetrical (Fig. 2D).

Etymology. This species name refers to the elongate slender parameres of the male genitalia.

Distribution. Western Australia (Fig. 5, squares).

Habitat. Specimens of this species were collected using flight intercept traps, or by sifting leaf or log litter.

Acknowledgements

This work was supported by a research grant of the Chungbuk National University in 2017, and a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201839201).

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Description of *Onthophagus humboldti* and *Uroxys bonplandi*, two new scarab beetles (Coleoptera, Scarabaeidae, Scarabaeinae) from Costa Rica, with notes on tropical mountain brachyptery and endemism

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Abstract

Two new endemic species of scarab beetles are described from Costa Rica, *Onthophagus humboldti* **sp. nov.** and *Uroxys bonplandi* **sp. nov.** *Onthophagus humboldti* **sp. nov.** is also the tenth brachypterous *Onthophagus* species to be described worldwide, representing also a case of extreme brachyptery in Onthophagini. Illustrations for both new species, as well as marking differences with closely related species are included. Maps showing the distribution of the new species, as well as the distribution of brachypterous and endemic scarab-beetle species for Costa Rica are presented and discussed. The Cordillera de Talamanca represents an area where Scarabaeinae (four genera) show very high known levels of brachypterism in Mesoamerica. A reconstruction of the montane environment in the Cordillera de Talamanca during the Last Glacial Maximum (~24 ka) is analyzed, in order to try to understand a possible historical biogeography model that might promote high levels of brachypterism in scarab-beetles. The present study supports previous proposals that brachyptery is correlated with stable environments associated with deeply incised valleys. Tropical mountain ranges are also identified as having more endemics than lowland rain forests, contradicting accepted wisdom. Lastly, a mitochondrial DNA analysis supports the existence of the *Onthophagus dicranius* and the *O. chypeatus* species-groups as two well-defined and closely related branches.

Keywords

Biogeography, boreotropical distribution, Cordillera de Talamanca, extreme brachyptery, refugia, Last Glacial Maximum, mitochondrial DNA, paleogeography

Notre imagination n'est frappée que par ce qui est grand; mais l'amoureux de la philosophie naturelle devrait également réfléchir aux petites choses.

Alexander von Humboldt

“Voyage aux régions équinoxiales du nouveau continent”, 1814

Introduction

During the last 27 years, a concerted effort has been undertaken by the first two authors in order to study the scarab beetles (Scarabaeidae: Scarabaeinae) of Costa Rica. The detailed evaluation of the specimens of this survey has yielded many new species. Currently, Scarabaeinae in Costa Rica are represented by seven tribes and 28 genera (Solís and Kohlmann 2012). This study is elevating the number of known scarab species from 182 to 184. These numbers will certainly increase in the future, as new species and new country records are discovered. We consider Costa Rica to be one of the best-known tropical countries in relation to the systematics and distribution of scarab beetles.

The discovery of these two new species of scarab beetles, one brachypterous (*Onthophagus*) and both of them endemic to the country, bring to the forefront questions regarding the existence of such interesting phenomena as brachyptery and endemism. These two mechanisms seem to be concentrated in the mountainous areas in Costa Rica. Using these new species as a model, an attempt is made to try to understand the existence of these two processes in the mountains of the tropics.

Considering the small area that Costa Rica occupies (51,100 km²), it displays a great number of brachypterous scarab-beetle species (7) from four genera (*Ateuchus*, *Canthidium*, *Cryptocanthon*, *Onthophagus*). This represents a figure of 0.014 brachypterous species / 100 km². One can compare this number with the state of Oaxaca in Mexico, an area arguably similar to Costa Rica in extension (93,952 km²) and biogeography/ecology. This Mexican state reports four brachypterous scarabaeines from two genera (*Canthidium* and *Onthophagus*) (Kohlmann in press). This would account for 0.004 brachypterous species / 100 km². These lofty numbers certainly beg the question about a possible brachyptery-generating mechanism. In order to try to explain this situation, a paleoclimatic/paleogeographic model is here developed for the Cordillera de Talamanca (Talamanca range) in Costa Rica, spanning to the Last Glacial Maximum (LGM, ~25–23 ka).

Recent biogeographical studies of these scarab beetles in Costa Rica (Kohlmann 2011; Kohlmann et al. 2007, 2010) have detected new areas of high endemism and species richness. These studies have highlighted the existence and impor-

tance of tropical mountains as areas of high biodiversity and endemism, debunking the commonly held belief that lowland tropical rain forests reign supreme on these accounts (Fogden and Fogden 1997; Valerio 2006). Obando's (2002) and Kohlmann's (2011) and Kohlmann et al. (2007, 2010) studies have concluded that it is the cloud forest, which is the most biodiverse and endemic-rich environment. The inclusion of these two new scarab-beetle species into the count continues to support the previous findings.

As mentioned previously in another paper (Solís and Kohlmann 2012), a mitochondrial DNA analysis of Costa Rican scarabaeines is being undertaken. Partial results are presented here, analyzing the relationship between the *Onthophagus dicranus* Bates species-group (Kohlmann and Solís 2001) and the *Onthophagus chlypeatus* Blanchard species-group (Zunino and Halffter 1997).

Materials and methods

Specimens studied came from the insect collection of the Museo Nacional de Costa Rica (National Museum of Costa Rica, ex INBio collection). All type material (holo- and paratypes) of both species is deposited in the same collection.

The specimens were studied using a Zeiss Stemi 2000–C stereozoom binocular microscope. Measurements were made to the nearest 0.1 mm using an ocular micrometer. Morphological nomenclature follows Kohlmann and Solís (2001) and Solís and Kohlmann (2013).

The synthetic aperture radar (SAR) image of Costa Rica, which has been used as the base map in figures 5 and 6, was downloaded from the NASA website (https://www2.jpl.nasa.gov/srtm/central_america_radar_images.html). The maps were made and edited using the QGIS geographic information system software; this program is open source on the Internet for multiple platforms (<https://qgis.org/en/site/forusers/download.html>).

We downloaded from the Internet publicly available raster-type bathymetric maps, obtained from the General Bathymetric Chart of the Oceans (GEBCO) website (https://www.gebco.net/data_and_products/gridded_bathymetry_data/). We also obtained raster elevation maps freely available from the NASA project website and the Ministry of Economy, Trade and Industry (METI) of Japan, called the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER), Global Digital Elevation Model (GDEM) (<https://asterweb.jpl.nasa.gov>). In addition, we used the commercial program Photoshop CS3 Extended Version 10.0.1 to prepare the versions for this publication.

The mitochondrial DNA information (DNA barcoding) was obtained through the methodology described by Wilson (2012) and accessible through the Barcode Data System (BOLD), the cloud-based data storage and analysis platform developed at the Center for Biodiversity Genomics in Canada (<http://boldsystems.org>).

Taxonomy

Onthophagus humboldti sp. nov.

<http://zoobank.org/C13D9441-5A22-4CA1-974F-88B6A97199AC>

Figures 1, 2a, c, e, 3a, c, 5, 6, 7, 9

Type locality. Costa Rica. Prov. Puntarenas. Buenos Aires, P.N. La Amistad. Tres Colinas.

Type deposition. Museo Nacional de Costa Rica, Santo Domingo de Heredia, Costa Rica.

Type material. Holotype male, pinned, with genitalia in a separate microvial. Original label: “Costa Rica. Provincia Puntarenas. Buenos Aires, Parque Nacional La Amistad. Tres Colinas. 2100–2200 m. 27–29 Febrero 2008. A. Solís, M. Moraga. Trampa Foso. L S 343850 565700.” “HOLOTYPE/*Onthophagus humboldti* Kohlmann, Solís, Alvarado [red printed label]”.

Other material. Paratypes. (8 males, 4 females). “Costa Rica. Provincia Puntarenas. Buenos Aires, Parque Nacional La Amistad. Tres Colinas. 2100–2200 m. 27–29 Febrero 2008. A. Solís, M. Moraga. Trampa Foso. L S 343850 565700.”

Diagnosis. Elytra as long as or shorter than pronotum (Fig. 1), due to brachyptery (Fig. 3c). Broad clypeal horn bifurcation (Fig. 2a); pygidium and apex of elytra with evident setae; clypeal margin indented at junction with clypeo-genal suture (Fig. 2e).

Description. Holotype. Male (Fig. 1), length 7.2 mm; maximum width 4.3 mm. Oval, shining reddish black. Centre of the clypeus projected forming a slender bifid horn (Figs 1, 2c); genae projected forming an angle (Fig. 2e), genal sutures almost effaced; head punctures coarse, regular, dense, becoming finer and sparser towards the center; clypeal carina absent, vertex carina substituted by two vertical asymmetric platelets, modestly developed, and obliquely oriented; eyes two times longer than wide and separated by eight times the eye width.

Pronotum (Fig. 2c) very convex, lateral margins with a small and irregular fovea, not lineal; lateral pronotal margins bordered by a deep sulcus, anterior and basal borders margined; pronotal surface reticulate and covered by dense, regular, coarse, annular, and deep punctures without setae; pronotal projection well-developed (Figs 1, 2c), forming a broad bilobed plate slightly bent downwards, with a depressed area antero-centrally, and having clear antero-lateral margins; anterior angles projected as long, slender, and curved projections (Figs 1, 2c); pronotal base with a sulcus extending forward one third its length; scutellum not visible between the base of the elytra.

Elytra convex, with clear margins and without a humeral callus; with eight well-marked striae, fine and clearly impressed and with crenulating punctures; intervals clearly punctured, punctures big and dense, not aligned, bearing short, stiff setae along the lateral and apical margins; microsculpture reticulate and regular. Wing brachypterous, measuring 0.75 mm (Fig. 3c). Pygidium moderately shiny and shagreen, margined border, with big, coarse, annular punctures bearing short and stiff setae. Aedeagus as Fig. 3a.

Mesosternum with evident annular punctures bearing no setae. Metasternum shagreen and finely punctured, more coarsely laterally, basal third with a sulcus. Abdominal segments shagreen and finely punctured.

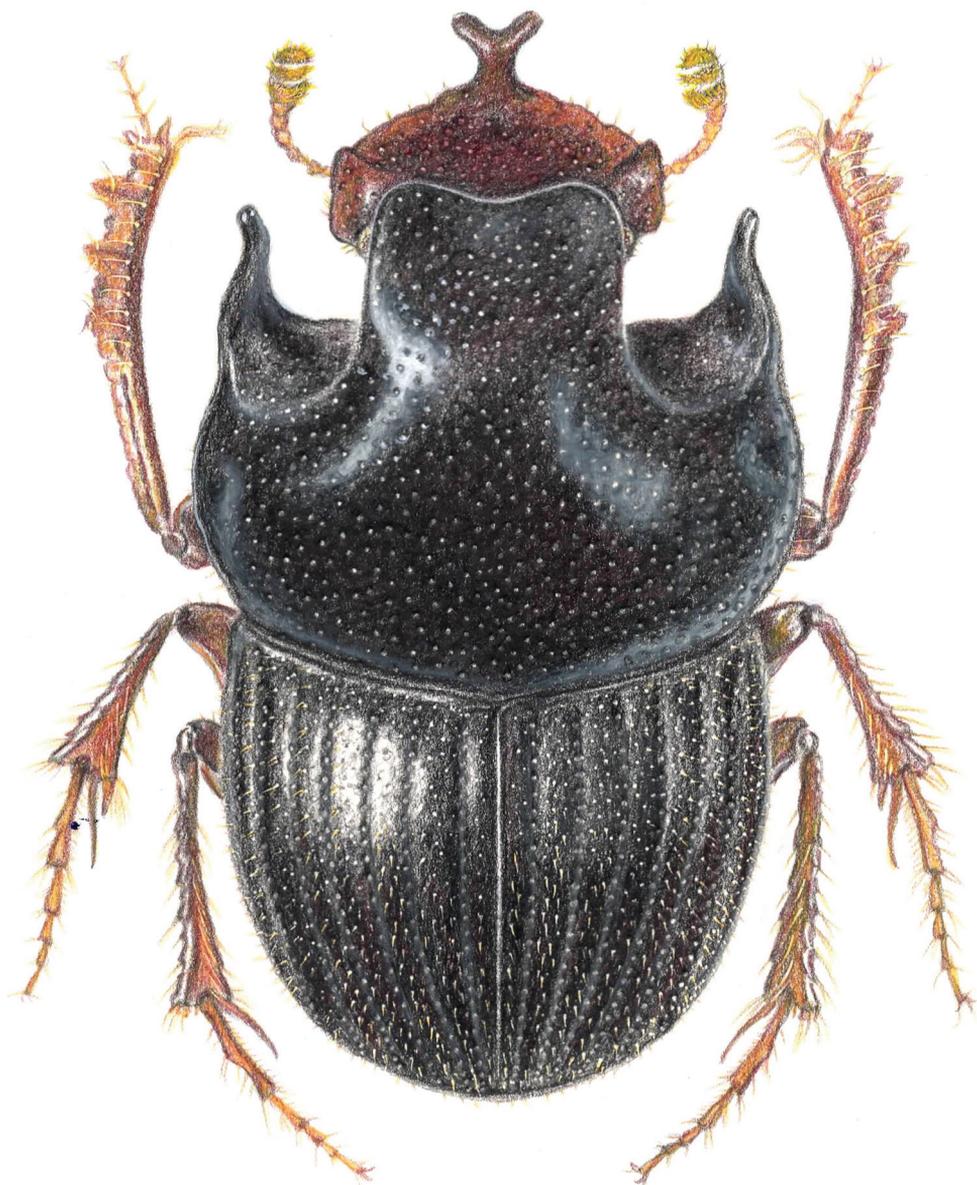


Figure 1. Dorsal drawing of a male *Onthophagus humboldti* sp. nov.

Fore femur long, slender, and punctured; meso- and metafemur short and elongate, light yellow. Fore tibia long, slender and arched (Fig. 1); with four external teeth; tibial spur elongated, straight, pointed, deflexed anteriorly, extending to second tarsal segment. Middle- and hind femur light yellow at middle.

Female, length 6.3 mm; maximum width 3.6 mm. It is similar to the male and varies in having a clypeus not forming a horn, clypeus shagreen, genae not projected as teeth, with a head frons keel, two small platelet projections at head vertex, no pronotal

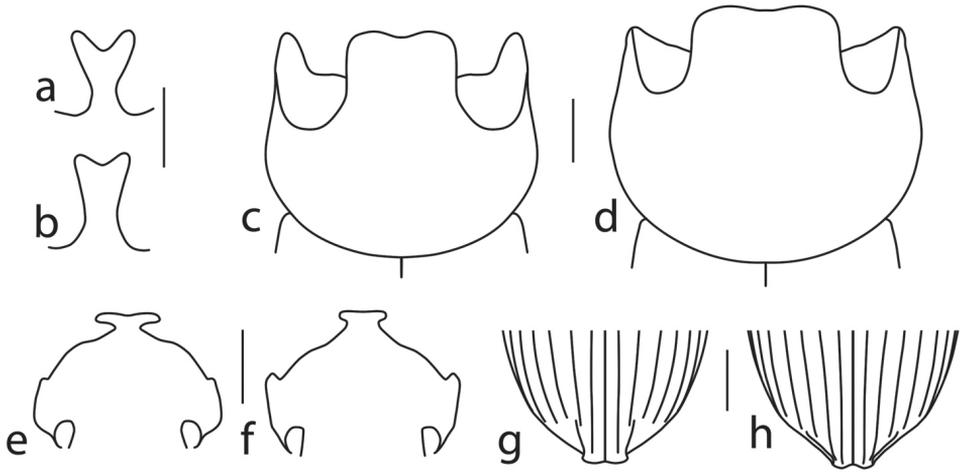


Figure 2. Drawings of the clypeal horn of **a** *O. humboldti* sp. nov. and **b** *O. micropterus*; pronotum of **c** *O. humboldti* sp. nov. and **d** *O. micropterus*; head of **e** *O. humboldti* sp. nov. and **f** *O. micropterus*; and elytral apex of **g** *U. bonplandi* sp. nov. and **h** *U. dybasi*. Scale bars: 1 mm.

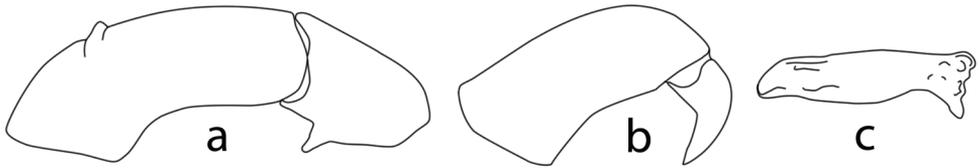


Figure 3. Aedeagi of **a** *O. humboldti* sp. nov. **b** *U. bonplandi* sp. nov. **c** brachypterous wing of *O. humboldti* sp. nov.

projection, no projected pronotal anterior angles, fore tibia short, fore femur short, last abdominal sternite broad.

Variation. Length 5.6 to 7.2 mm. Width 3.2 to 4.3 mm. Small males do not have the bifid clypeal horn, just a small erect lamella; vertex platelets forming a small projection; anterior pronotal angles not projected, pronotal projection forming a small carina. Body color varying from black to piceous red.

Etymology. This species is dedicated in honor of Friedrich Wilhelm Heinrich Alexander von Humboldt, Prussian geographer, explorer, and naturalist, commemorating the 250th anniversary of his birth. He is widely recognized for fathering the work on physical and plant geography, which laid the foundation for the development of modern biogeography.

Taxonomic considerations. Kohlmann and Solís (2001) report the existence of 39 species of *Onthophagus* for Costa Rica. This new species would increase their numbers to 40. *Onthophagus humboldti* sp. nov. belongs to the *Onthophagus dicranius* Bates species group, as defined by Kohlmann and Solís (2001).

Onthophagus humboldti sp. nov. will key out to *O. micropterus* Zunino & Halffter, 1981, in Kohlmann and Solís' key (2001). It can be easily differentiated by the following characteristics: In males clypeal horn slender at middle and very bifurcated at apex (Fig. 2a) (*O. humboldti* sp. nov.) versus broad at middle and notched at apex (Fig. 2b) (*O. micropterus*); genae projected forming an angle (Fig. 2e) (*O. humboldti* sp. nov.) versus genae projected forming a tooth (Fig. 2f) (*O. micropterus*); vertex platelets forming a carina (*O. humboldti* sp. nov.) versus a pointed projection (*O. micropterus*); anterior lateral angles of pronotum projected as long, slender, and curved projections (Fig. 2c) (*O. humboldti* sp. nov.) versus a short, curved projection (Fig. 2d) (*O. micropterus*); pronotal central forward projection well-developed, forming a broad bilobed plate slightly bent downwards (Fig. 2c) (*O. humboldti* sp. nov.) versus a bilobed plate projecting forward (Fig. 2d) (*O. micropterus*). In females: vertex platelets forming a carina (*O. humboldti* sp. nov.) versus a pointed projection (*O. micropterus*).

Geographical distribution. This species is so far only known from the area of Tres Colinas, near Buenos Aires, in the province of Puntarenas (Fig. 5). It has been collected from 2100 to 2200 m altitude in the month of February in lower montane rain forest.

Chorological affinities. *Onthophagus humboldti* sp. nov. is endemic to the Cordillera de Talamanca and is the tenth known brachypterous *Onthophagus* species to be described worldwide. A closely related species, *O. micropterus*, is also distributed in the Cordillera de Talamanca (Fig. 6), from 2100 to 3000 m altitude in tropical mountain rainforest and has been collected from October to February.

Biogeography. This species belongs to the *O. dicranius* species group, as established by Kohlmann and Solís (2001). This group of species has extra-American affinities, in which Howden and Gill (1993) indicate that the American fauna of *Onthophagus* is the result of invasive species from East Asia and that the *O. dicranius* group presents characters in common with New Guinea species. This agrees with the hypothesis originally proposed by Zunino and Halffter (1988), which points out for the supraspecific groups of American *Onthophagus*, an origin of its lineages, which in the case of the current representatives is distributed in East or Southeast Asia; and for this case, the Asian representation of the ancestral line, like the American one, has its distribution present in the humid tropics. On the other hand, the *O. dicranius* species group has its present-day center of diversity in tropical North America and relatives in South America (Zunino and Halffter 1997; Kohlmann and Solís 2001).

This situation seems to be in congruence with the boreotropical distribution hypothesis (Wang 1961; Wolfe 1975; Lavin and Luckow 1993; Xiang and Soltis 2001; Davis et al. 2002), where current flora groups show a tropical disjunct distribution, generally centered in America, Africa, and tropical Asia. This hypothesis is based on the observation of the existence of tropical broadleaf forests during the Early Paleogene (in old Stratigraphy terminology, Early Tertiary) at high latitudes in regions that are currently temperate, directed by a Late Paleocene-Early Eocene thermal maxima (ca. 52 ma, Zachos et al. 2001) and that many current angiosperm temperate taxa have evergreen relatives in subtropical rainforests (Axelrod 1966). This proposal then suggests the existence of northern bridges that were once at lower latitudes, such as the Bering

Bridge during the Early Paleogene and the North Atlantic Bridge during the Eocene, which may have served as migration routes for groups of organisms that currently present intercontinental disjunct distributions. This hypothesis suggests that a taxon with a present-day center of diversity in tropical North America, and with an early Paleogene fossil record from any region there, has a high probability of having sister-group relatives in the Paleotropics and derived relatives in South America (Lavin and Luckow 1993).

This pattern of distribution would clarify those proposed by Halffter (Halffter and Morrone 2017) for the “Mexican Transition Zone” in particular one of them, the so-called “Paleoamerican Dispersion Pattern” (Halffter 1964). This pattern of dispersion corresponds to northern taxa that arrived in North America from Eurasia, and has been subdivided by Halffter et al. (1995) into four variants, where one of them, called the “Paleoamerican Tropical Pattern”, corresponds to species found in the lowlands of the tropics and at medium altitudes, their distribution being very similar to that of the Neotropical pattern, but their affinities are with the Old World taxa. Halffter et al. (1995, 2008) placed the *Onthophagus chypeatus* and *O. dicranius* species groups of the genus *Onthophagus* within this pattern.

Actually, the groups of species mentioned above are congruent with the typical characteristics of the so-called boreotropical distribution. Therefore, the aforementioned distribution variant, the “Paleoamerican Tropical Pattern”, seems to be the same with the boreotropical distribution and it is proposed here to use the term boreotropical distribution from now on as it is a more complete and well-founded concept, besides being an older one. This pattern has been studied and characterized at very fine phylogenetic and biogeographic analysis levels in animal and plants (Lidgard and Crane 1990; Xiang and Soltis 2001; Davis et al. 2002; Feng et al. 2009; Guo et al. 2012; Ye et al. 2016).

***Uroxys bonplandi* sp. nov.**

<http://zoobank.org/E8FB3E6C-6E3B-4C5E-9550-6A4238DD70EB>

Figures 2h, 3b, 4, 5

Type locality. Costa Rica. Guanacaste. Sector Santa María, path to the cone of the Santa María, part of the Rincón de la Vieja volcanic massif, 1565 m.

Type deposition. Museo Nacional de Costa Rica, Santo Domingo de Heredia, Costa Rica.

Type material. Holotype male, pinned, with genitalia in a separate microvial. Original label: “Costa Rica. Provincia Guanacaste. Sector Santa María, Sendero a Pico Volcán Santa María. 1565 m. 2 Diciembre 2017. Col. Sergio Salas Ríos. Biocol. 10.8039N, 85.3281W.” “HOLOTYPE/*Uroxys bonplandi* Kohlmann, Solís, Alvarado [red printed label]”.

Other material. Paratypes (18 males, 25 females). “Costa Rica. *Provincia Guanacaste*. Sector Santa María, Sendero a Pico Volcán Santa María. 1565 m. 2 Diciembre 2017. Col. Sergio Salas Ríos. Biocol. 10.8039N, 85.3281W (6 males, 10 females).

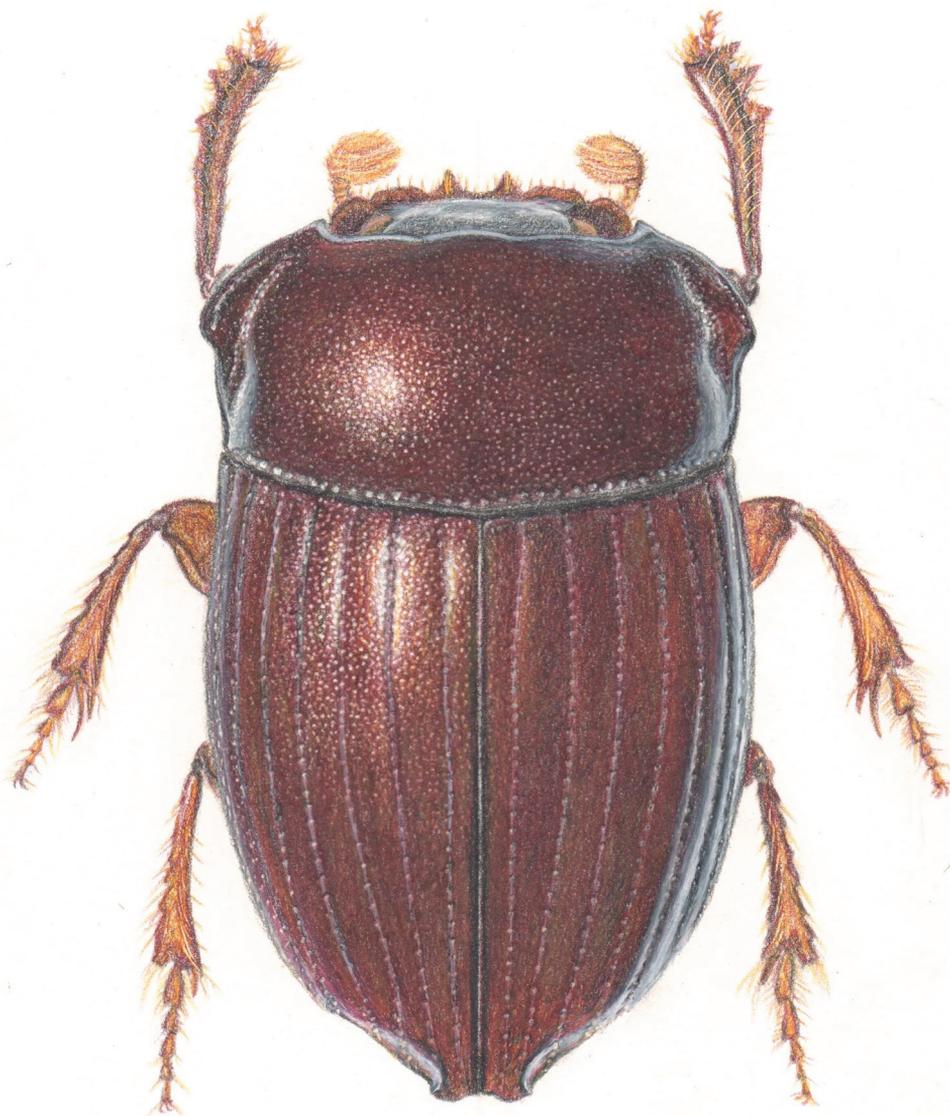


Figure 4. Dorsal drawing of a male *Uroxys bonplandi* sp. nov.

“Tilarán Bosque Nuboso Santa Elena. 1600 m. 26 Noviembre – 8 Diciembre 1999. J. Rodríguez Trampa de Luz. L N 258000 45000” (1 female). “Provincia Puntarenas. Monteverde Zona Protectora Arenal-Monteverde. Parcela Brillantes. 1500–1600 m. 17–19 Junio 2009. A. Solís, J.D. Gutiérrez. Trampa Foso. L N 252009 450981” (4 males, 2 females), “13–1600 m. 10°18'N, 84°48'W. Univ. California EAP 1991” (1 female). “Est. La Casona. 1520 m. Reserva Biológica Monteverde. N. Obando. Octubre 1991. L N 253250 449700” (2 males, 2 females), “Septiembre 1990 (1 male), 29 Nov – 17 Diciembre 1994, K. Martínez, L N 253200 449700” (2 males, 1 female).

“*Provincia Alajuela*. San Ramón. Zona Protectora Arenal-Monteverde. Parcela El Valle. 1600–1700 m. 16–18 Jun 2009. A. Solís, J.D. Gutiérrez. Trampa Foso. L N 255970 452538” (3 males, 9 females).

Diagnosis. Anterior of frons evenly convex, without carina or groove, with a dimple or transversely rugose; clypeal margin indented at junction with clypeogenal suture; dorsal ocular area twice as long as wide, distance between eyes five times eye width; pronotum evenly convex, sides angled near middle; elytral apex of the second to fourth intervals forming an oblique keel (Fig. 2h); basal sulcus of pygidium sinuate; fore tibial spur slender and deflexed distally.

Description. Holotype. Male, length 7.4 mm; maximum width 3.8 mm. Elongate oval, shining reddish black (Fig. 4). Clypeus bidentate, slightly indented immediately laterad of teeth; teeth broadly triangular and strongly reflexed (Fig. 4). Head surface with a small dimple at the center, distinct small punctures throughout. Clypeogenal suture distinct; clypeal margin distinctly indented at intersection of suture (Fig. 4); genal margins broadly rounded (Fig. 4). Frons weakly convex, with very slight, broad indentations. Dorsal ocular areas approximately twice as long as wide at posterior edge of canthus (12 to 14 facets wide at that point), distance between ocular areas approximately five times their width.

Pronotum at median angulation as wide as elytra; lateral edges of pronotum produced into prominent angles (Fig. 4), strongly sinuate on lateral view, posterior two-thirds of margin nearly vertical; pronotum weakly convex medially; surface densely covered with fine, deep punctures; median longitudinal sulcus feebly indicated in posterior third; lateral fovea in form of crenulated longitudinal deep groove three-fourths length of pronotum (Fig. 4), not extending to either anterior or posterior margin, with cluster of coarse punctures in posterior third; pronotum margined basally, with adjacent row of large longitudinal punctures (Fig. 4).

Elytron moderately convex, clearly punctate (faintly in *Uroxys dybasi* Howden & Young, 1981), humeral umbone small; striae distinct but shallow, with distinct punctures evenly spaced for most of length of each stria, seventh stria extending three-fifths length of elytron; posterior tenth of first stria furrowed; intervals flat, slightly flattened and constricted, not produced, except at the apex of the second to fourth intervals forming an oblique keel (Fig. 2h) (sharp straight keel in the third interval in *dybasi*, Fig. 2g).

Meso- and metasternum clearly punctate (faintly in *dybasi*); meso-metasternal suture medially moderately angulate anteriorly, moderately angulate laterally, three times farther from anterior margin of mesosternum than from mesocoxal cavity; metasternum swollen, with distinct median posterior depression.

Ventral abdominal segments two to five of equal length medially, each only slightly shorter medially than sixth; sixth slightly longer laterally than medially; anterior margins with small punctures (big crenulated punctures in *dybasi*). Pygidium strongly convex, faintly punctate, twice as wide as long; sulcus surrounding disc deep basally, shallow elsewhere; margin formed of same width apically and laterally; sulcus basally very slightly arcuate toward apex on each side of midline.

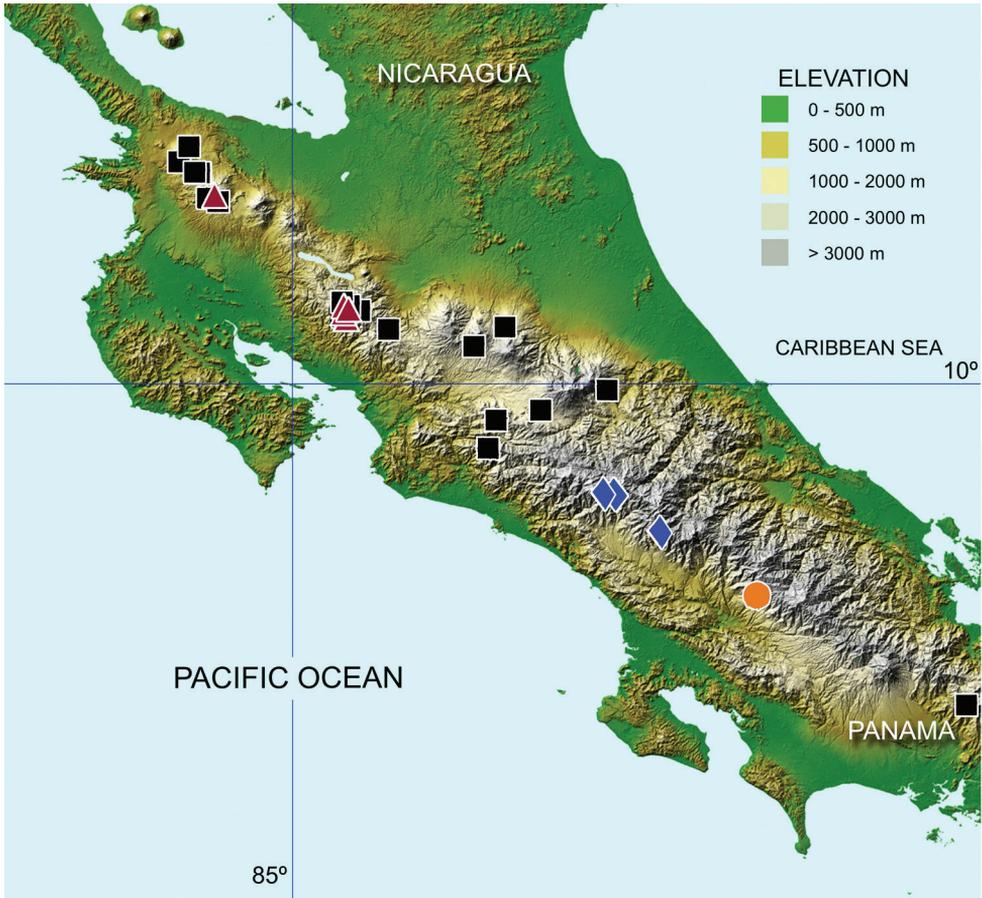


Figure 5. Known distribution of *O. humboldti* sp. nov. (orange circle) and *U. bonplandi* sp. nov. (red triangle). The distribution of the proposed sister species of these new taxa is also depicted, *O. micropterus* (blue rhombus) and *U. dybasi* (black square).

Fore tibia elongate with inner margin broadly curved (Fig. 4); outer margin with three teeth in apical third, teeth approximately equidistant, basal tooth somewhat reduced and more broadly triangular (Fig. 4); apex of fore tibia with short, narrow, rounded, deflexed projection at inner corner, projection approximately half length of tibial spur. Tibial spur elongated, straight, pointed, extending to fourth tarsal segment. Fore femur gradually tapering distally; middle femur with a faint ventral posterior triangular projection at apical third (evident projection in *dybasi*); hind femur with a well-developed ventral posterior swelling at apical third; posterior margin of hind trochanter continuous with posterior margin of femur.

Female, length 6.9 mm; maximum width 3.6 mm. It is similar to the male and varies in having a rugose clypeus, lateral edges of pronotum produced into less prominent angles. Elytral apex without oblique keels. Fore femur and fore tibia not as long. Middle and hind femur without a projection or swelling at apical third.



Figure 6. Known distribution of brachypterous Scarabaeinae in Costa Rica.

Variation. Length 5.7 to 7.6 mm. Width 3.2 to 4.1 mm. The center of the head might have a small dimple and/or also a slight transverse rugosity.

Etymology. This species is dedicated in honor of Aimé Jacques Alexandre Goujaud Bonpland, French naturalist, physician, and botanist, member of the scientific expedition that accompanied Humboldt to Spanish America.

Taxonomic considerations. Solís and Kohlmann (2013) report the existence of 12 species of *Uroxys* for Costa Rica. This new species would increase their numbers to 13. Due to its great similarity, we here propose that *Uroxys bonplandi* sp. nov. represents the sister species of *U. dybasi* Howden & Young, 1981.

Uroxys bonplandi sp. nov. will key out to *U. dybasi* in Solís and Kohlmann's (2013) key. It can be easily differentiated by the following characteristics: *Uroxys bonplandi* sp. nov. is consistently bigger (5.7 to 7.6 mm) than its sister species (4.3 to 5.6 mm), *U. dybasi*. It can also be separated by the clear punctures in thorax and elytra in *bonplandi* sp. nov. (faint in *dybasi*). In males: elytral apex of the second to fourth intervals forming an oblique keel in *bonplandi* sp. nov. (Fig. 2h) (sharp straight keel in the third interval in

dybasi, Fig. 2g), meso- and metasternum clearly punctate on *bonplandi* sp. nov. (faintly in *dybasi*), anterior margins of ventral abdominal segments with small punctures in *bonplandi* sp. nov. (big crenulated punctures in *dybasi*), and middle femur with a faint ventral posterior triangular projection at apical third in *bonplandi* sp. nov. (evident projection in *dybasi*).

Geographical distribution. *Uroxys bonplandi* sp. nov. has been collected so far in the Cordillera de Guanacaste and the Cordillera de Tilarán (Fig. 5). It is a mountain species distributed from 1520 to 2200 m of altitude and has been collected in the following life-zones: wet tropical forest (premontane transition), lower montane rain forest, lower montane wet forest, premontane rainforest, and premontane wet forest. It has been collected from June to February.

Chorological affinities. *Uroxys bonplandi* sp. nov. coincides with *U. dybasi* in being distributed along the Guanacaste and Tilarán mountain ranges. (Fig. 5) This last species has been also reported from mountain forests from Panama in the Cordillera de Chiriquí and in Costa Rica in the Cordillera Central and Talamanca (Fig. 5), being distributed between 600 and 1700 m and collected throughout the whole year. *U. bonplandi* sp. nov. represents also the first known endemic species of *Uroxys* for Costa Rica.

Another related species is *Uroxys tacanensis* Delgado & Kohlmann, 2007, known only from its type locality, the Tacaná volcano, at the border of Mexico and Guatemala, living in cloud forest at 2000 m altitude (Delgado and Kohlmann 2007). No other species of this group has been yet collected in the intermediate areas. They are all montane species.

Discussion

Brachyptery

As mentioned above, *O. humboldti* sp. nov. is a brachypterous species. There are also another two brachypterous species of *Onthophagus* in Costa Rica: *O. inediapterus* Kohlmann & Solís, 2001 (*Onthophagus dicranius* Bates line) and *O. micropterus* Zunino & Halffter, 1981 (*Onthophagus dicranius* Bates line) (Kohlmann and Solís 2006). Other six brachypterous species are also known for Mexico: *O. brachypterus* Zunino & Halffter, 1997 (*O. landolti* Harold group); *O. chilapensis* Gasca-Álvarez, Zunino & Deloya, 2018 (*O. chevrolati* Harold group); *O. gilli* Delgado & Howden, 2000 (*O. chevrolati* Harold group); *O. inflaticollis* Bates, 1886–1889 (*O. chevrolati* Harold group); *O. pedester* Howden & Génier, 2004 (*O. landolti* Harold group); and *O. zapotecus* Zunino & Halffter, 1988 (*O. landolti* Harold group) (Kohlmann and Solís 2006; Gasca-Álvarez 2018). All the aforementioned species live in areas of old geological emergence: Cordillera de Talamanca and Sierra Madre del Sur (Kohlmann and Solís 2006). Outside the American continent there is only one brachypterous *Onthophagus* species known from Australia, living in vine scrub in arid areas, *O. apterus* Matthews, 1972 (Matthews 1972). All the American species have in common that they inhabit

humid montane forests, a habitat considered as stable by most ecologists. It is interesting to note that *Onthophagus* brachyptery in Costa Rica is confined to the *Onthophagus dicranius* line, whereas in Mexico it is confined to the *O. landolti* and *O. chevrolati* species lines. This would suggest a line independent morphological convergence to similar ecological/historical conditions.

In relation to wing reduction, Zunino and Halffter (1988) described the most extreme case then known in Onthophagini with the example of *O. zapotecus*, where the wing does not show any trace of venation and the wing length to body length ratio has a value of 0.156. On the other hand, *O. micropterus* presents a ratio of 0.205 (Zunino and Halffter 1981), whereas *O. humboldti* sp. nov. has a ratio of 0.107. This ratio represents at present the most extreme case known so far of wing reduction in onthophagine scarab beetles. The wing does not show any trace of wing venation. The elytra are not fused together, but are strongly interlocked. This species shows also narrowed elytral humeri, as well as shortened elytra as had been already observed by Darlington (1936) for carabid beetles and Scholtz (2000) for scarab beetles. Contrary to the observation made by Scholtz (2000) and Scholtz et al. (2009) that flightless scarabs have reduced eyes with a smooth margin, *O. humboldti* has no such condition; however, as indicated by Scholtz et al. (2009), this species has a rounded body shape.

Accepted wisdom has proposed that in Scarabaeoidea the evolution of flightlessness is related to temperate highland forests in the tropics; arid environments, such as deserts; temperate forests at low latitudes in the southern hemisphere; islands; termite nests; and cold regions (Zunino and Halffter 1988; Génier 2000; Scholtz 2000). In montane environments, where *Onthophagus*, and other brachypterous genera, such as *Ateuchus*, *Canthidium*, and *Cryptocanthon* live in Costa Rica (Fig. 6), flight is apparently non-essential. According to Darwin's classical explanation (Darwin 1859), known as "Darwin's factor" (Darlington 1971), the presence of strong mountain winds could drag flying individuals towards unfavourable habitats for their survival (Zunino and Halffter 1981, 1988). This last explanation is strongly contested by Roff (1990), because it does not correctly take into account scale issues.

Scholtz et al. (2009) propose that flightlessness increases in scarab beetles with altitude in temperate forests in the tropics (which are considered to be a stable habitat), being rare in lowland tropical forests. A similar process has been observed in carabids, where brachypterous species also predominate in montane areas (Darlington 1971); as well with passalid beetles in tropical humid montane forests (Mac Vean and Schuster 1981). This seems to be the case for the present study, where all brachypterous species are montane, no species having been found in the lowlands so far. This would support Roff's (1990) original hypothesis that flightlessness increases with altitude.

Wagner and Liebherr (1992) present an analysis of flightlessness in insects, indicating that around 10 % of temperate Coleoptera show this characteristic. Based on our current tally, 184 Scarabaeinae taxa have been listed for Costa Rica; of these, seven species are brachypterous (Fig. 6), thus representing 3.8 % of flightless scarab beetles. The low brachyptery percentage found in Costa Rica would support the hypothesis that insect and scarab-beetle flightlessness increases with latitude (Roff 1990; Scholtz et al. 2009).

At present, a very much accepted hypothesis that tries to explain the origin of this phenomenon is the one given by Lawrence et al. (1991), where they propose that wing brachyptery may have a selection value in insects that have adopted a sedentary, cryptic or a parasitic way of life, or that live in mountain, island or high latitude habitats. Another explanation for this situation, and the one we follow and expand here, is the one proposed by Kavanaugh (1985). Kavanaugh suggests that macroptery represents the ancestral (plesiotypic) condition among beetles and that brachyptery has evolved independently many times among Coleoptera and other pterygote insects. Such a widespread phenomenon requires explanation. Brachyptery is a major factor contributing to restricted distributions and it usually does not progress to a stage where the wing rudiment is actually absent (Kavanaugh 1985). However, Frolov (pers. comm., 2019) has observed that in Orphninae (Scarabaeidae) two genera are completely apterous. Brachyptery has also been suggested as a factor that promotes speciation (Mayr 1963; Hackman 1964).

It is clear that the distribution of brachypterous forms is not random, certain patterns are repeated. In North and Central America no brachypterous Scarabaeinae are known from the lowlands, alpine regions, or from rodent nests. They are only known from the mountains in Costa Rica, especially the Talamanca range and the Sierra Madre del Sur in Mexico. In all cases these flightless species live in humid montane forests, spanning an altitudinal distribution that goes from 1100 to 3000 m. Flightlessness in scarabaeines is confined so far in Mesoamerica to small-sized genera, like *Onthophagus* (9), *Canthidium* (4), *Cryptocanthon* (2), and *Ateuchus* (1), in descending order of known species number. On the other hand, brachyptery seems to be confined in South American Scarabaeinae to eight medium-sized species of the genus *Dichotomius*, out of 170 described taxa, where this condition has evolved independently, at least four times in this genus (Nunes and Vaz-de-Mello 2013, 2016). However, this genus does not show a clear attachment to a particular ecological environment, because the different brachypterous species have been collected ranging from tree sand dune habitats to riparian forests (Nunes and Vaz-de-Mello 2016). According to models developed by Roff (1994), a dominant brachyptery can spread if the cost of being macropterous and habitat stability are important. In other words, regarding the last point, habitat stability is a key factor favoring the loss of flight (Roff 1990, 1994).

If it is generally accepted that the occurrence of brachyptery reflects long-term stability of habitats (Roff 1990, 1994), then one could propose that the occurrence of such forms in particular geographical areas is an indication of long-term stability for these regions as well, especially if this pattern is repeated by different taxa in the same area. This train of thought has been used for recognizing areas of long-term occupation, such as glacial refugia in carabids by Lindroth (1979) in Scandinavia and by Kavanaugh (1985) in Canada.

If one would plot the geographical ranges of the fore mentioned brachypterous scarab-beetles on a map, coincident occurrence of such taxa is apparent. The pattern that emerges is one in which one particular area stands out, the Cordillera de Talamanca (Fig. 6). All four known brachypterous genera and six species are concentrated

in this range. All other known areas boast one to two genera and species and would probably represent minor centers or areas of subsequent colonization. These data would strongly suggest that the Cordillera de Talamanca has served as a center for both long-term survival and differentiation in this group of beetles, acting as a stable area. The Cordillera de Talamanca is the highest mountainous area in Costa Rica, and the highest range in Central America, reaching 3820 m altitude, and thus acting as a possible built-in buffer for residents against sudden and dramatic climate change. If the climate were to change rapidly and drastically, montane species could be able to move a short distance up or down in elevation, tracking their required microclimate, whereas lowland organisms would have to move far greater distances north or south in order to achieve the same result.

Lachniet and Seltzer (2002) and Vázquez-Selem and Lachniet (2017) analyzed the effects of the last glaciation on the Cordillera de Talamanca and estimated that the last local glacial maximum (LLGM) for the Cerro Chirripó occurred at 21–18 ka with a depression of the equilibrium line altitude (ELA) or snow line of ~1500 m in relation to the modern regional ELA of 4900–5100 m, thus representing a LLGM temperature reduction on the order of ~8–9 °C. However, a more recent research reevaluation by Potter et al. (2019) estimates the age of the LLGM at 25–23 ka for the Cerro Chirripó with a reconstructed ELA depression of 1317–1536 m and an associated cooling of ~7–9 °C. Palinological studies have indicated that during the last glacier interval (50–15.6 ka) with temperatures 7–8 °C cooler than today the treeless páramo extended down to 2100 m altitude, whereas it is distributed from 3300 to 3819 m at present (Islebe and Hooghiemstra 1997; Horn 2007). At the end of the last deglaciation (15.6–13 ka), the upper forest limit rose to 2700–2800 m (3100–3300 m present-day forest limit of subalpine tropical rain/elfin cloud forest), indicating a temperature increase of up to 4.6 °C (Islebe and Hooghiemstra 1997; Horn 2007). Subsequently, the upper forest limit dropped 300–400 m from 13.1 to 11.2 ka indicating a temperature decline of 2–3 °C (Horn 2007). From 12.3 to 11.2 ka the glaciers retreated above 3500 m and the subalpine tropical rain forest was gradually replaced by mountain rain forest as the forest limit and temperatures rose toward present-day values (Islebe and Hooghiemstra 1997; Horn 2007).

Figure 7 shows the present-day distribution of *Onthophagus humboldti* sp. nov. and *O. micropterus* and lines indicate proposed localities depressed by 1500 m (14 km in straight line) generated by the last glacial maximum, ~25–23 ka, in the Talamanca Cordillera. All mountain systems are depicted 150 m lower than present day height, considering a generalized continental uplift of 1 mm/year and an estimated sea level descent, due to glaciation, of 120 m. Interestingly, the glacially depressed localities are not only to be found at the base of the mountain system but also within the Valle de El General (Valley of the General or The General's Valley), surrounded and probably climatically protected by the embracement of this very long valley (Kohlmann et al. 2002), that possibly dampened the cooling effect of the glaciation. Solano Quintero and Villalobos Flores (2001) did a climatic-geographic regionalization of Costa Rica and they found this intermontane valley to differ from the rest of the southern Pacific

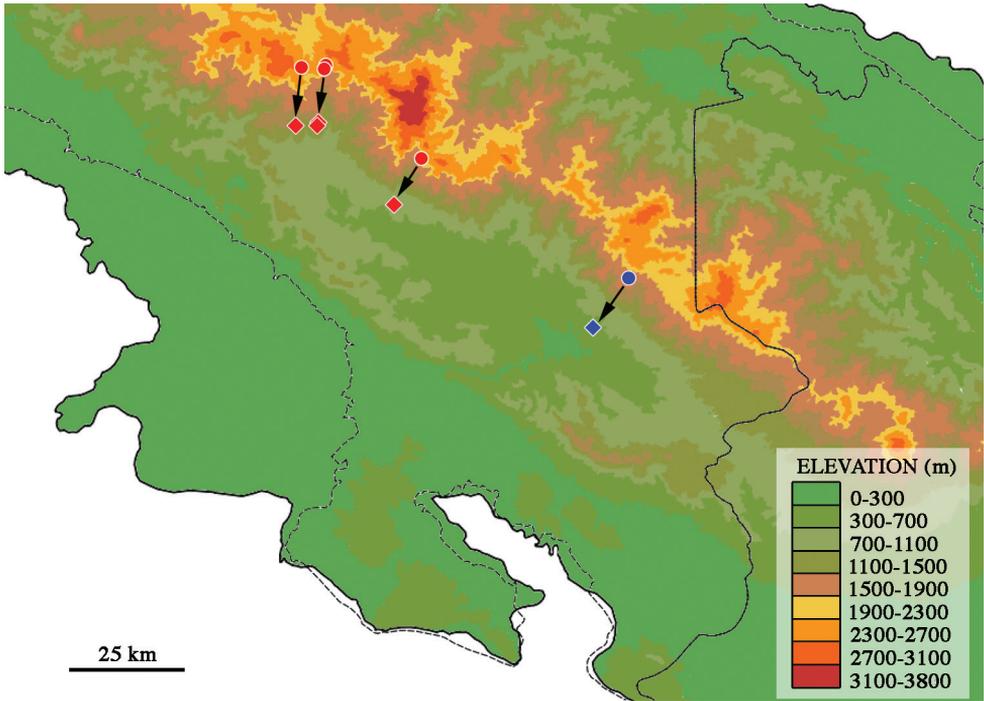


Figure 7. Present day distribution of *Onthophagus humboldti* sp. nov. (blue dot) and *O. micropterus* (red dot) and lines indicating proposed localities (rhombi) depressed by 1500 m (14 km in straight line) generated by the last glacial maximum, ~25–23 ka, in the Cordillera de Talamanca. All mountain systems are 150 m lower than present day height and an estimated sea level descent of 120 m is depicted. Dotted black lines represent present-day sea levels.

area in relation to having very homogeneous lower rainfall values (3050 m total annual precipitation) and a longer dry period (three months).

We propose here that the Valle de El General might have acted as a refugium for the brachypterous species during the last glacial maximum (LGM). This valley would then resemble what has been called a cryptic refugium (Stewart and Lister 2001; Stewart et al. 2010), a refugium situated at different latitudes or longitudes than would normally be expected, often resembling climatic islands in which conditions differ favourably from the surrounding areas. According to the classification proposed by Stewart et al. (2010), the Valle de El General would fit the description of being a glacial southern refugium, representing an accepted low-latitude refugium for temperate species during a glacial phase. Stewart et al. (2010) also indicate that one characteristic of cryptic northern refugia is that they are sheltered in habitats located in deeply incised valleys that provide microclimates for temperate species, which is precisely the case under study here. Stewart et al. (2010) do not present any examples in their study of cryptic glacial southern refugia (only cryptic interglacial southern refugia), so that the example of the Valle de El General could represent the first one reported of its kind. Finally, because the Valle de El General represents a small area (~1850 km²), and in ac-

cordance with island biogeography tenets (MacArthur and Wilson 1967) that indicate that because of low population size and a limited food base in small areas, this could explain that mostly small-sized (brachypterous and non-brachypterous) scarab species and not big-sized ones, could have had patches of suitable habitats to live in during glacial periods which seems to be the case for the present study, all reported brachypterous scarab beetle species in Costa Rica are small-sized. Coincidentally, six of the known seven brachypterous scarab beetle species are also distributed around the Valle de El General. This area is occupied by montane forests, representing incredibly biodiverse and more species-rich environments than lowland tropical forests in Costa Rica (Kohlmann et al. 2007, 2010; Kohlmann 2011). This high concentration of brachypterous species in these montane forests contradicts Scholtz (2000) and Scholtz et al. (2009) proposal that relatively species-poor, environmentally stable habitats, lacking complex biotic interactions, like temperate forests on tropical mountains, contribute toward flightlessness. Costa Rican montane forests are decidedly species-rich and thus most probably also having complex biotic interactions.

The Valle de El General must have been formed by the uplift of the Cordillera de Talamanca and the Fila Costeña (Costeña range), a process that began about 7 million years ago and accelerated during the last 4 million years, triggered by the arrival of the Coco submarine range (an extinct volcanic range, also known as Cocos Ridge) in the Pacific and by the compression of the microplate of Panama in the Caribbean. The Arenal depression (tectonic graben) must have originated less than 2 million years ago, although there is no better estimate. While the Valle Central (Central Valley) is of a more recent formation and its age goes back to less than half a million years (Alvarado et al. 2007; Alvarado and Gans 2012; Alvarado and Cárdenes 2016). The absence of endemic brachypterous species in the Valle Central can be a product of never having been present or that they were exterminated due to the cataclysmic volcanism that occurred several times in the last 800 ka, the last large one 322 ka ago, with the formation of pyroclastic flows (pyroclastic density currents), which destroyed everything in hundreds of square kilometers (burning ash clouds with a temperature > 600 °C, pyroclastic deposits with thickness between 10 and > 80 m); the last major event of this kind occurred some 322 ka, covered an extension of at least 820 km² in the Valle Central and neighboring areas, as well as a distance of up to 80 km from the eruptive source (Pérez et al. 2006; Alvarado and Gans 2012). The cataclysmic and paroxysmal volcanism of this type (pyroclastic density currents or ignimbrites) has been absent in the last few million years both in the Valle de El General and in the Arenal tectonic depression, where, coincidentally, the only other known endemic brachypterous species (*Cryptocanthon lindemanae*) that does not live around the Valle de El General Area is present.

Endemicity

Areas of endemism (AE) are fundamental areas in the analyses of biogeography and are defined as areas of non-random distributional congruence among taxa, whose bio-

geographical history probably shared common factors such as geological, ecological, or evolutionary processes (Harold and Mooi 1994; Morrone 2008). Important questions regarding the AE are its distribution and defining the major ecological/evolutionary factors (climatic/elevational gradients, geographic isolation, topographical heterogeneity) that affect the distribution of these areas. A quick glance at the distribution map of the new species and their closely related taxa (Fig. 5), as well as the map published by Kohlmann et al. (2007), showing the distribution of the endemic Costa Rican scarab beetle species (Fig. 8), shows a clear non-random distribution of the AE.

The mapping of the AE's (Fig. 8) clearly identified mountain ranges as important centres of endemism. Endemism seems to be higher in the tropical mountains than in the tropical lowlands of Costa Rica. Similar results have been found in other areas of the world. Noroozi et al. (2018) have identified in the mountains of Iran using Asteraceae that patterns of endemic richness and areas of endemism identify mountain ranges as main centers of endemism, likely due to high environmental heterogeneity and strong geographic isolation among and within mountain ranges. Noroozi et al. (2018) also found that endemic richness and degree of endemism are positively related to topographic complexity and elevational range. The proportion of endemic taxa at a certain altitude (percent endemism) is not congruent with the proportion of total surface area at this elevation, but is higher in mountain ranges. While the distribution of endemic richness (*i.e.*, number of endemic taxa) along an altitudinal gradient was hump-shaped peaking at mid-elevations, the percentage of endemism gradually increased with elevation. Sosa and Loera (2017) have shown that endemism of Mexican monocot geophytes was highest in montane regions (Mexican Trans-Volcanic Belt) and Millar et al. (2017) demonstrated that angiosperm endemism was highest in the mountains of New Zealand's South Island. To very similar results came Buirra et al. (2017) in relation to the vascular flora of the Iberian Peninsula. McDonald and Colding (1995) found that endemic mountain fynbos flora (represented by shrubs with short-distance seed dispersal) are over-represented in high altitude wet habitats, where almost twice the number occur than expected on the basis of area occupied by these habitats. Finally, Scherson et al. (2014) found that coastal ranges in southern Chile have acted as glacial refugia for ancient flora during the Quaternary, showing a higher endemism than expected by chance.

Noroozi et al. (2018) ask themselves the question, if endemics have higher numbers in the mountains than in the lowlands. This seems to be certainly the case in tropical Costa Rica for scarab-beetles (Scarabaeinae, Dynastinae) and monocot plants (Araceae, Arecaceae, Bromeliaceae) (Kohlmann et al. 2007, 2010; Kohlmann 2011), and as shown by other references, also around the world. High environmental heterogeneity and strong geographic isolation among and within mountain ranges seems to be a very plausible explanation. The effect of mountain systems as possible glacial refugia seems to also play an important role. So, as indicated in the previous section, because of simple geographical distance, mountains allow for small linear displacements that still maintain the same ecology, thus allowing a cenocron to stay concentrated in a small area; whereas, lowland species have to travel greater linear distances, hence presenting a much more extended distribution of endemic species.

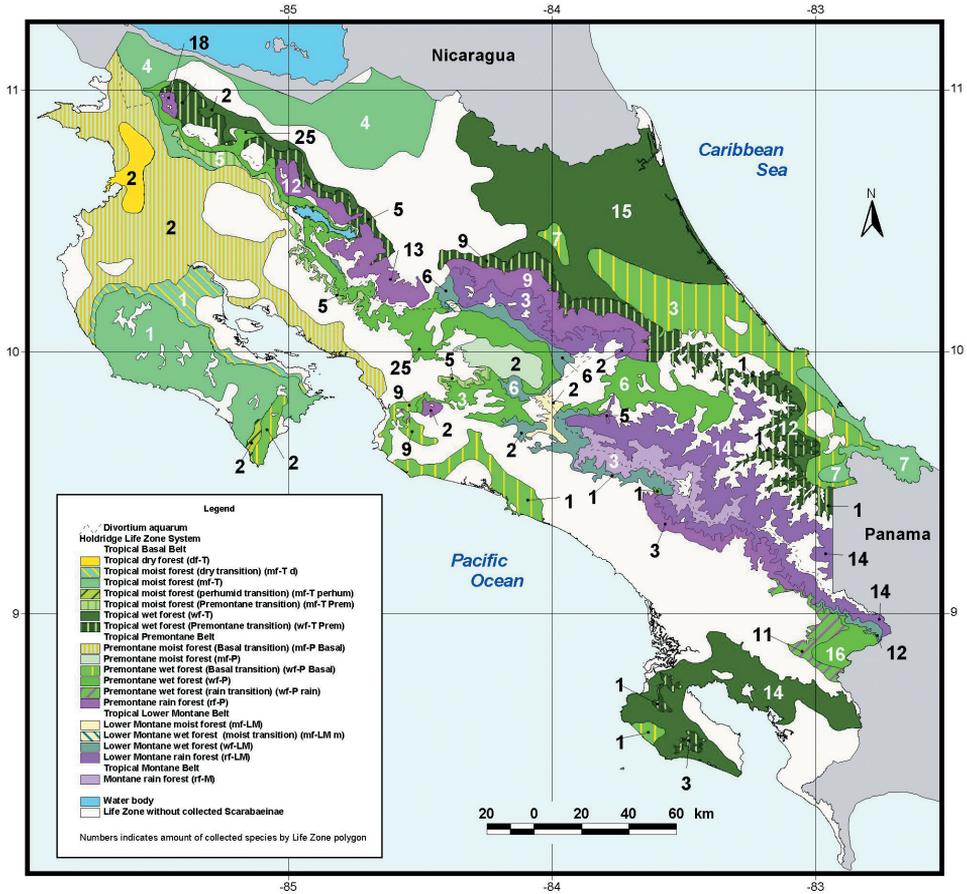


Figure 8. Map showing Costa Rican scarabaeine endemic numbers as distributed by life-zones (taken from Kohlmann et al. 2007).

DNA mitochondrial analysis

An analysis of the cytochrome c-oxidase I (COI) for both new species was undertaken. The Bar Code Index Number (BIN) for each species is: *Onthophagus humboldti*, BOLD: ABA7524 and *Uroxys bonplandi*, BOLD: ABA3722. Results are clearly distinct, whereas the value registered for the *Onthophagus* pair gives an average Kimura-2-parameter [K2P] value of 6.35 % with a maximum distance of 10.6 %, the amount of DNA difference for the *Uroxys* pair is of only 3.3 %. *Uroxys* results stay in line with other similar ones calculated for a group of Caribbean-Pacific scarab-beetle sister-species pairs separated by the Cordillera de Talamanca which started its emergence around 7 million years ago; *Phanaeus pyrois* Bates, 1887 and *P. malyi* Arnaud, 2002 ([K2P]= 3.8 %) and *Phanaeus beltianus* Bates, 1887 and *P. changdiazi* Kohlmann and Solís 2001 ([K2P] = 3.0%), which show basically a similar amount of mitochondrial DNA difference (Solís and Kohlmann 2012). These average values are similar to

the ones that Johns and Avise (1998) found (K2P difference) of 3.5 % in 47 pairs of bird sister species and divergences greater than 2% in 98% of vertebrate sister species.

However, the value shown for the *Onthophagus* sister-pair looks higher ([K2P] = 6.35 %). This is interesting if we consider that this pair is formed by flightless species and the geographical nearness between them, 52 km in a straight line (Fig. 5). It would seem therefore that a limited dispersal capacity tends to favour differentiation as Mayr had already suggested (1963). Another possible explanation is that this species pair represents an old clade, as is known that nucleotide substitutions accumulate through time (older clades tend to accumulate more substitutions).

This last explanation is concordant with the previous results shown in the brachyptery section, where it is suggested that the Cordillera de Talamanca has been an area of long-term stability, thus allowing the continuous and uninterrupted presence of clades. In general, areas with a preponderance of brachypterous populations represent areas of older populations (Lindroth 1979; Kavanaugh 1985). Volcanologically, the Cordillera de Talamanca and the Fila Costeña have been very stable during the last 9 and 3.5 million years, respectively. During these last 5 million years the Valle de El General starts to acquire its present geomorphological configuration due to the formation of the two aforementioned mountain systems (Alvarado and Gans 2012; Alvarado and Cárdenes 2016).

In a very interesting study of phylogenetics and biogeography of the genus *Onthophagus* inferred from mitochondrial genomes, Breeschoten et al. (2016) found that all New World species of *Onthophagus* form a monophyletic group. This study found an origin of the Onthophagini from an Afrotropical ancestral stock in the Eocene and a subsequent spread to the Americas via the oriental region at about 20–24 Ma. New World Onthophagini started diversifying around the Miocene (20 Ma).

Among the American Onthophagini that Breeschoten et al. (2016) studied, *O. clypeatus* Blanchard, 1843 from the tropical forests of Colombia to Bolivia (0–1000 m) and *O. rhinolophus* Harold, 1869 from tropical forests (0–1500 m) of Mexico to Guatemala, were included. They estimated a phylogenetic branching process between both of them at ca. 3 Ma (95 % HPD: 1.6–4.4 Ma). At that time and according to paleogeographic reconstructions of Alvarado and Cárdenes (2016), Costa Rica had already emerged. The Cordillera de Talamanca began to rise fast after 3.5 Ma with an estimated uplift rate of 1 mm/year (Miyamura 1975) and creating an uplifted area where montane species could start to evolve.

Onthophagus clypeatus Blanchard, 1846 and *O. rhinolophus* Harold, 1869, are part of the *clypeatus* species group and considered to be closely related to the *dicranius* species group (Zunino and Halffter 1997; Kohlmann and Solís 2001). These species are therefore related to *O. humboldti* sp. nov. and *O. micropterus*, and are also distributed in tropical mountain forests. This closeness might suggest a similar phylogenetic branching process of the Costa Rican species in the Cordillera de Talamanca around 3 Ma. One could suggest that after this relatively old speciation process the gradual evolution of flightlessness took place in a subsequent, much more recent time, where the orographic scenario was more akin to the present-day one. This scenario would be then in accordance with the aforementioned estimations of the rise of the Cordillera de

Talamanca, as well as with the proposal of Sanmartín et al. (2001), where they found that in the western Nearctic animal diversification and animal species richness increased in the later part of the Neogene and early Quaternary (2.56 Ma), whereas they consider unlikely elevated speciation rates during the Pleistocene. However, Kohlmann et al. (2018) studying scarab beetles of the genus *Geotrupes* (Coleoptera: Geotrupidae), have found evidence that the last glacial maxima in the mountains of Oaxaca, Mexico (18–15 ka, but in Costa Rica around 24 ka), could explain very recent speciation processes, where speciation can be described as incipient, generating very closely related taxa with small taxonomic differences.

We present in Figure 9 a cytochrome c-oxidase I (COI-5P) mitochondrial DNA sequence-based Bold Taxon ID tree of the species group to which *O. humboldti* sp. nov. belongs. The Taxon ID Tree is a functionality of the BOLD System, that allows for the generation of dendrograms from sequencing using the neighbor-joining algorithm.

BOLD uses neighbor-joining trees which group sequences together by the number of amino acid or nucleotide differences. The arrangement of the specimens in the tree is based on sequence similarities, with the sequences that are most similar placed closer together on the tree, and with the branch length indicating the degree of similarity. The percentage of similarity between sequences can be measured against the legend (line), where the longer the branch the more disparity between the sequences. It is often expected that specimens of the same species have more similar sequences and cluster closer together than specimens from different species.

This figure is part of a more general analysis done for the genus *Onthophagus* in Costa Rica. All four depicted taxa are mountain species distributed in the mountains of Costa Rica and Panama. *O. humboldti* sp. nov. seems to be a closely related species of *O. dorsipilulus* Howden & Gill, 1993, a species distributed in the Cordillera de Talamanca and the Cordillera de Chiriquí in Costa Rica and Panama from 1400 to 1800 m altitude and would seem to be its ecological equivalent at a slightly lower altitudinal belt. On the other hand, *O. micropterus*, also distributed in the Cordillera de Talamanca, seems to be the sister species of *O. quetzalis* Howden & Gill, 1993, a taxon distributed in the neighboring Cordillera de Tilarán and Guanacaste. The DNA mitochondrial analysis neatly recovers the formation of this cluster belonging to the *O. dicranus* species-group (Kohlmann and Solís 2001). The nearest species cluster to this last group is also included (Fig. 9), where all seven taxa belong to the *O. clypeatus* species-group (Zunino and Halffter 1997), as defined by Kohlmann and Solís (2001). The formation of these two well-defined, but closely related branches seems to support the proposal forwarded by Kohlmann and Solís (2001) that they are effectively two different groupings and not a single one, as proposed by Zunino and Halffter (1997).

Finally, *Onthophagus* having around 2200 valid species (Schoolmeesters 2016) and being a hyperdiverse and ecologically plastic genus, does not conform with Roff's (1990) proposal that lineage size should favor the evolution of flightlessness. At present, only ten brachypterous species of *Onthophagus* are known: six in North America, three in Central America, and one in Australia. As a comparison, the genus *Dichotomius* Hope, 1838 has around 170 described species with eight brachypterous taxa (Nunes and Vaz-de-Mello 2013, 2016), all of them living in South America.

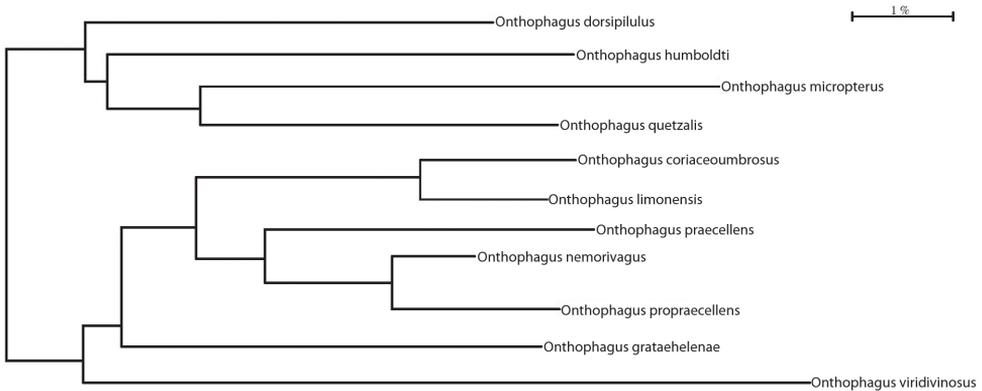


Figure 9. A cytochrome c-oxidase I (COI-5P) mitochondrial DNA sequence-based BOLD taxon ID tree of the nearest species of *O. humboldti* sp. nov.

Conclusions

The study of mountain biology retains all of its actuality and relevance. This study on tropical mountain brachyptery and endemism falls in line with what Humboldt had already discovered (Humboldt 1805), which is the vertical progression of climate and vegetation in a mountain that explains the distribution and ecology of a species, as beautifully demonstrated by his drawing of plant distribution on the Chimborazo volcano published in his *Essai sur la géographie des plantes* (Humboldt 1805). This drawing can be considered as a veritable scientific epiphany in the case of Humboldt, so that it allowed him to connect all plant species according to their altitude and latitude. In other words, Humboldt could now establish the correlation between similar ecosystems in any part of the world. “Alles ist Wechselwirkung” (Everything is interaction) wrote Humboldt (1803) later in August 1803 in one of his diaries, while travelling in the Valley of Mexico. Everything is organically connected through multiple natural correlations. He could not have been more correct. This is Humboldt’s essence; he was far in advance of his time.

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Checklist of the ichthyofauna of the Rio Negro basin in the Brazilian Amazon

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Abstract

This study presents an extensive review of published and unpublished occurrence records of fish species in the Rio Negro drainage system within the Brazilian territory. The data was gathered from two main sources: 1) literature compilations of species occurrence records, including original descriptions and revisionary studies; and 2) specimens verification at the INPA fish collection. The results reveal a rich and diversified ichthyofauna, with 1,165 species distributed in 17 orders (+ two incertae sedis), 56 families, and 389 genera. A large portion of the fish fauna (54.3% of the species) is composed of small-sized fishes < 10 cm in standard length. The main groups are Characiformes (454 species; 39.0%), Siluriformes (416; 35.7%), Gymnotiformes (105; 9.0%), and Cichliformes (102; 8.8%). The species composition differs between the main aquatic environments, such as: main channel (159 species), lakes (296), tributary rivers (596), small streams (234), seasonal beaches (186), and rapids (41). Part of the ichthyofauna is shared with adjacent basins, such as the Orinoco, rivers of the Guiana Shield, lower Solimões/Amazonas and upper Amazonas, which contributes to the remarkable ichthyofaunal diversity of the basin. A high rate of species endemism was observed in Characidae (24), Loricariidae (18), Cichlidae (18) and Callichthyidae (18), totalling 156 species (13.4%) endemic to the basin. An estimation of the species richness for the Rio Negro basin, considering 23 published references, resulted in 1,466 and 1,759 species (Jackknife 1 and 2, respectively), which seems reasonable when considering the large number of morphotypes left out of the present list and the low sampling effort in many areas of the basin. The results presented herein provide an additional tool for environmental managers and decision makers for conservation purposes of one of the richest and most well-preserved sub-basins of the Rio Amazonas system.

Keywords

Blackwater, conservation, diversity, freshwater fish, ichthyofaunal survey

Introduction

The Amazon basin is the largest hydrographic system in the world, with a total of 6,869,000 km² (Goulding et al. 2003), and harbors the richest freshwater ichthyofauna of the planet, predominantly composed of species of the Superorder Ostariophysi (85%) (Roberts 1972; Lowe-McConnell 1987; Reis et al. 2003, 2016). This richness comprises numerous evolutionary lineages, resulting from the interaction of multi-layered geological patterns associated with vicariant and dispersal agents (Lundberg et al. 1998; Dagosta and de Pinna 2017). The distribution of the fish species in the Amazon sub-basins and adjacent drainage systems is complex, and amounts to numerous distributional overlaps and superlative degrees of biogeographic congruence (Dagosta and de Pinna 2017). Currently, more than 2,700 fish species have been formally described for the Amazon basin (Reis et al. 2016; Dagosta and de Pinna 2019), but recent estimates suggest that this number exceeds 3,000 species (Carvalho et al. 2007). However, this number possibly will increase significantly with the addition of ichthyofaunal inventories in poorly sampled areas; with the accumulation of taxonomic reviews; and with the use of novel information derived from molecular and other analytical tools. The true species richness in the Amazon basin may rise to approximately 4,000–5,000 species in the next 40 to 70 years, if the description rate remains at the current level (see Ota et al. (2015) for an estimative for Siluriformes; and Birindelli and Sidlauskas (2018) for freshwater fishes in Neotropical region).

The Rio Negro is one of the main tributaries of the Amazon basin, with a drainage area of approximately 696,808 km² that is mostly (80%) contained in Brazilian territory (Goulding et al. 2003). Its extension from the headwaters located in Colombia and Venezuela and its mouth on the Rio Amazonas/Solimões is of approximately 2,250 km (Goulding et al. 2003). The Rio Negro has an average discharge of 28,000 m³ per second, corresponding to ca. 15% of the discharge of the Rio Amazonas at its mouth, being the fifth largest river in volume of water of the world (Goulding et al. 2003). The water level of the Rio Negro follows the rainfall regime of the region and shows a great variation throughout the year, with a difference of 10 - 12 m between the peak of flood period and the lowest level at the end of the dry period (as measured in Manaus Municipality; Irion et al. 1997). This sub-basin is characterized by geologically ancient (Paleozoic) and quite leached sediments, in which sandy, acidic and nutrient-poor soils prevail (Sioli 1950; Fittkau et al. 1975; Küchler et al. 2000). Moreover, the Rio Negro waters are characterized by the presence of large amounts of dissolved organic carbon in the form of humic and fulvic acids, contributing to the acidity of the water and to its reddish color (that looks black in deep waters), despite its high transparency (Fittkau et al. 1975; Junk and Furch 1985; Goulding et al. 1988). Consequently, the Rio Negro presents highly acidic waters (pH < 5.0), with low suspended sediment load (corresponding to approximately only 2–3% of the amount of suspended solids transported by Rio Solimões, for instance Fisher 1978), remarkably poor in nutrients

(especially cations) and low values of electrical conductivity (as low as 8–13 $\mu\text{S}/\text{cm}$), which may represent extreme ecological conditions for many species of animals and plants (Fittkau et al. 1975; K uchlera et al. 2000).

Those supposedly harsh limnological conditions, however, do not constitute a limiting factor for the diversity of fishes, and the Rio Negro waters harbor one of the most diversified ichthyofaunas in the world. So far, Goulding et al. (1988) carried out the most complete inventory of the ichthyofauna of that basin, recording approximately 450 fish species, but pointing out the possible presence of up to 700 species. At the time of that publication the estimated fish richness for the Rio Negro was superior to the encountered in all the European rivers (233 species), in the Mississippi/Missouri River in the USA (375 species) (L ev eque et al. 2008), or in all the Argentinian territory (339 species) (L opez et al. 2002), and being comparable to that of the entire Congo/Zaire River basin in Equatorial Africa (Skelton 2001).

Despite this outstanding richness, the fish fauna inhabiting blackwater rivers is poorly studied when compared to that of white-water rivers in the Amazon (Kramer et al. 1978; Goulding 1980; Bayley 1998; Junk et al. 1997; Barthem and Goulding 1997; Saint Paul et al. 2000; Junk et al. 2007; Zuanon et al. 2007; Queiroz et al. 2013; Ohara et al. 2015). One of the first surveys of the Rio Negro ichthyofauna was performed by the British naturalist Alfred Russel Wallace between 1850 and 1852. However, the preserved specimens were destroyed by a fire and the consequent shipwreck of the ship carrying the sampled material when on the way to Europe, leaving only the illustrations of ca. 180 fish species that were donated to the Museum of Natural History of London; such pictorial records were later found in the museum archives and published in a book by Toledo-Piza Ragazzo (2002). In addition to the seminal work of Goulding et al. (1988), more recent studies have provided complementary information regarding the Rio Negro fish fauna, which include ecological studies of the main river channel (Chao et al. 2001; Thom e-Souza and Chao 2004; Ferreira et al. 2007; Rapp Py-Daniel et al. 2017), lakes and flooded forest areas (igap os) (Garcia 1993; Saint Paul et al. 2000; Soares and Yamamoto 2005; Ferreira et al. 2007; Noveras et al. 2012; Yamamoto et al. 2014; Farias et al. 2017; Beltr ao and Soares 2018), seasonal beaches and rapids (Lima et al. 2005; Ferreira et al. 2007), streams and small marginal ponds (Kn oppel 1970; Silva 1993, 1995; Mendon a et al. 2005; Pazin et al. 2006; Anjos and Zuanon 2007; Zuanon et al. 2015; Beltr ao and Soares 2018), interfluvial swamps (Chao and Prada-Pedrerros 1995), flooded Savannah-like areas (Ferreira et al. 2007), as well as ichthyofaunal inventories of specific tributaries (Henderson and Walker 1986; Chao and Prada-Pedrerros 1995; Zuanon et al. 1998; Lima et al. 2005; Ferreira et al. 2007; Zuanon et al. 2008; Kemenes and Forsberg 2014; Rapp Py-Daniel et al. 2017). This study aimed to make a comprehensive survey of the fish species present in the Rio Negro basin, as well as to analyze the fish diversity associated to the different aquatic environments present in the basin (Goulding et al. 1988). The compilation of the available information (both published and in databanks) presented herein provides a general picture of the distribution of fish species in the Rio Negro basin by habitat types; allows an appreciation of the species description rate in the basin along time; and provides estimates of the total fish species richness for the basin.

Materials and methods

Fish species occurrence records for the Rio Negro basin were obtained from two main sources of information. First, a compilation of the records of species originally described based on specimens collected in the Rio Negro basin or that included that basin in their distribution range was made based in the catalogues of Reis et al. (2003), Buckup et al. (2007), Ferraris-Jr (2007), and Ferraris-Jr et al. (2017). Additional information from taxonomic revisions and species descriptions from 2003 to 2019 were also included: Armbruster (2003), Britto and Lima (2003), Costa (2003a, 2003b), Vari and Lima (2003), Costa (2004a, 2004b), Crampton et al. (2004), Hrbek et al. (2004), Lehmann and Reis (2004), Malabarba (2004), Zanata and Toledo-Piza (2004), Zarske et al. (2004), Ferraris-Jr et al. (2005), Kullander and Ferreira (2005), Lundberg and Akama (2005), Reis et al. (2005), Sabaj (2005), Sousa and Rapp Py-Daniel (2005), Vari et al. (2005), Zanata and Lima (2005), Armbruster and Page (2006), Bührnheim and Malabarba (2006), Ferreira and Lima (2006), Kullander and Ferreira (2006), Mautari and Menezes (2006), Mattox et al. (2006), Menezes (2006), de Santana and Nogueira (2006), Zarske and Géry (2006), Parisi et al. (2006), Lundberg and Cox-Fernandes (2007), Bührnheim and Malabarba (2007), Buitrago–Suárez and Burr (2007), Ferraris-Jr (2007), Lucena (2007), Rodriguez and Reis (2007), Staeck and Schindler (2007), Zarske and Géry (2007), Armbruster (2008), Birindelli et al. (2008), Orti et al. (2008), Sabaj and Birindelli (2008), Sabaj et al. (2008), Sidlauskas and Vari (2008), Rapp Py-Daniel and Fichberg (2008), Rocha et al. (2008a, 2008b), Thomas and Rapp Py-Daniel (2008), Birindelli and Britski (2009), de Santana and Vari (2009), Lima and Sousa (2009), Lima et al. (2009), Marinho (2009), Marinho and Lima (2009), Oyakawa and Mattox (2009), Paixão and Toledo-Piza (2009), Sullivan (2009), Vari and Ferraris-Jr (2009), Bertaco and Carvalho (2010), Carvalho et al. (2010), de Santana and Vari (2010), Lucena and Malabarba (2010), Marinho (2010), Marinho and Langeani (2010), Peixoto and Wosiacki (2010), Ribeiro and Rapp Py-Daniel (2010), Römer et al. (2010), Arbour and López-Fernández (2011), Birindelli et al. (2011), Schindler and Valdesalici (2011), Kullander (2011), Marshall et al. (2011), Maxime et al. (2011), Melo et al. (2011), Netto-Ferreira et al. (2011), de Pinna and Kirovsky (2011), de Santana and Crampton (2011), Sousa and Birindelli (2011), Birindelli and Zuanon (2012), Birindelli et al. (2012), Loeb (2012), Rocha et al. (2012), Andrade et al. (2013), Costa et al. (2013), Costa and Bragança (2013), Marinho et al. (2013), Mattox et al. (2013), Peixoto et al. (2013), Roberts (2013), Birindelli (2014), Britzki et al. (2014), Burns et al. (2014), Calegari et al. (2014), Coutinho and Wosiacki (2014), Cox-Fernandes et al. (2014), Ivanyisky and Albert (2014), Melo and Vari (2014), Menezes and Lucena (2014), Ota et al. (2014), Toledo-Piza et al. (2014), Vari and Calegari (2014), Zarske (2014), Cox-Fernandes et al. (2015), de Oliveira et al. (2015), Kullander and Varella (2015), Marinho et al. (2015), Ottoni (2015), Silva-Oliveira et al. (2015), Hernández-Acevedo et al. (2015), Peixoto et al. (2015), Walsh et al. (2015), Carvalho et al. (2016), Henschel (2016), Ota et al. (2016), Ray and Armbruster-

er (2016), Tan and Armbruster (2016), Tan et al. (2016), Tencatt and Britto (2016), Tencatt and Ohara (2016), Britski and Birindelli (2016), Bernt and Albert (2017), Burns et al. (2017), Evans et al. (2017), Ferraris-Jr et al. (2017), Fontenelle and Carvalho (2017), Sabaj and Hernández (2017), Ribeiro et al. (2017), Marinho and Menezes (2017), Melo and Oliveira (2017), de Pinna et al. (2017), Bernt et al. (2018), Bragança (2018), Caires and Toledo-Piza (2018), Henschel et al. (2018), Mateussi et al. (2018), Silva and Rapp Py-Daniel (2018), Urbanski et al. (2018), and Reia and Benine (2019). Information was also collected from publications focusing on biogeographic analyses and large taxonomic fish inventories in specific areas or sub-basins of the Rio Negro drainage system: Knöppel (1970), Henderson and Walker (1986), Goulding et al. (1988), Barletta (1995), Chao and Prada-Pedrerros (1995), Silva (1995), Zuanon et al. (1998), Saint-Paul et al. (2000), Chao (2001), Mortati (2004), Thomé-Souza and Chao (2004), Soares and Yamamoto (2005), Lima et al. (2005), Anjos and Zuanon (2007), Ferreira et al. (2007), Galuch (2007), Zuanon et al. (2008), Noveras et al. (2012), Kemenes and Forsberg (2014), Zuanon et al. (2015), Farias et al. (2017), Rapp Py-Daniel et al. (2017), Beltrão and Soares (2018), and Beltrão et al. (2018).

The second main source of information was composed by a thorough survey for all species that were collected in the Rio Negro basin and that have lots deposited in the INPA Fish Collection, which were personally verified by the authors. Morphotypes of nearly 40 new, undescribed species that were considered unequivocally distinct from the known species in their respective genera (mostly based on recently reviewed genera) were also included in the list. An update of the taxonomic nomenclature and geographic distribution of the species (at river-basin scale) was made using the published studies of Reis et al. (2003), Ferraris-Jr (2007), Ferraris-Jr et al. (2017), Dagosta and de Pinna (2019), and the FishBase online catalog (www.fishbase.org).

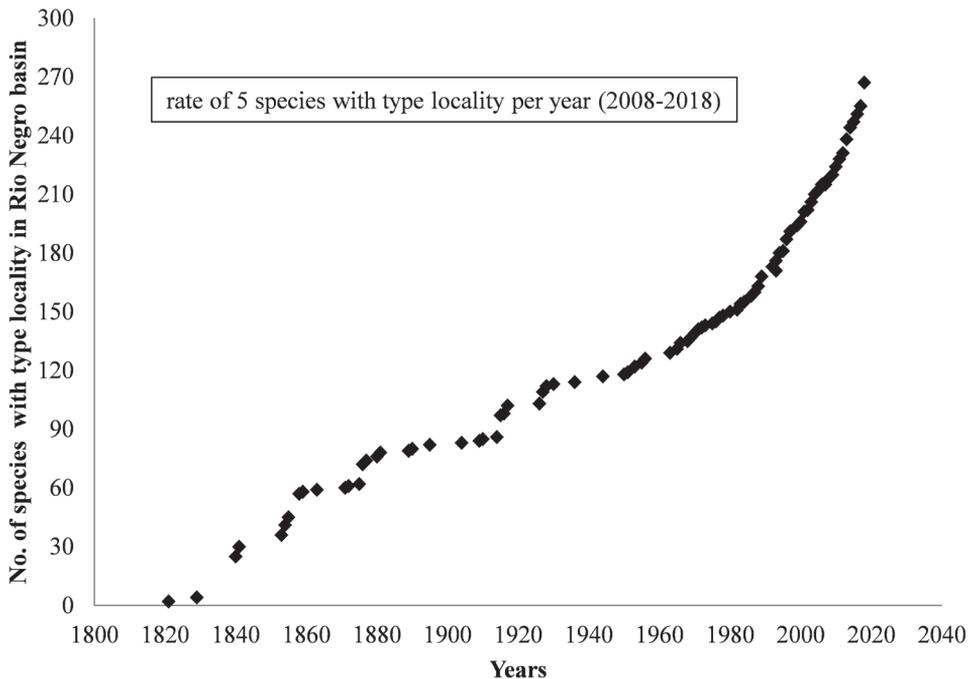
Exotic species that were introduced purposely or accidentally by fish farming or aquarium releases in impacted urban streams in Manaus, which have vouchers in the fish collections of INPA and the Federal University of Amazonas (UFAM), were also included in the list.

To provide an overview of the amount of described species originating from the Rio Negro basin, a species accumulation curve was constructed based on published information since the year 1821. Additionally, based on the 23 aforementioned ichthyofaunistic published studies, an estimative of total species richness in the Rio Negro basin was done using Jackknife 1 and 2 (Magurran 1988) using Past 3.0 statistical package (Hammer et al. 2001).

The species list presented herein follows the taxonomic classification of Betancur et al. (2017), with the orders arranged following the systematic/phylogenetic organization of the latter, whereas families, genera and species are presented in alphabetical order. Regarding the distribution of each species, the information regarding habitat use/preference was obtained from the literature and/or from labels of preserved voucher specimens in fish collections. Finally, a map of the Rio Negro basin with the sampling localities mentioned in the bibliographic and collection sources mentioned above is presented (Figure 1).

Table 1. Number of families, genera and species for each order of fishes recorded in the Rio Negro basin.

Order	Families	Genera	Species
Myliobatiformes	1	3	9
Osteoglossiformes	2	2	3
Clupeiformes	2	7	13
Cypriniformes (not native)	1	1	1
Characiformes	20	127	454
Siluriformes	12	165	416
Gymnotiformes	5	27	105
Batrachoidiformes	1	1	1
Gobiiformes	1	2	4
Anabantiformes (not native)	1	1	1
Synbranchiformes	1	1	3
Pleuronectiformes	1	4	5
Beloniformes	1	3	6
Cyprinodontiformes	2	7	26
Cichliformes	1	30	102
incertae sedis: Ovalentaria (Polycentridae)	1	2	2
Tetraodontiformes	1	1	1
incertae sedis: Eupercaria (Sciaenidae)	1	4	12
Lepidosireniformes	1	1	1
17 (+2 incertae sedis)	56	389	1165

**Figure 2.** Cumulative curve of valid fish species numbers described from type localities in the Rio Negro basin between 1821 and 2019, based on the catalogs of Reis et al. (2003) and Buckup et al. (2007), and species descriptions published after those compilations.

pared to just 36 species described between 1958 and 1987). The sharp increase in the species descriptions rate with time lends support to the provided estimate of total fish richness for the Rio Negro basin lying between 1,466 and 1,759 species (Jackknife 1 and 2, respectively).

The composition of fish species in the Rio Negro ranges from very large species to miniature forms (sensu Weitzman and Vari 1988). Among the largest fishes are the giant or goliath catfishes *Brachyplatystoma filamentosum* and *B. capapretum* (Siluriformes: Pimelodidae), which can reach approximately 2.8 m TL and more than 140 kg, the pirarucu *Arapaima gigas* (Osteoglossiformes: Arapaimidae) with approximately 3.0 m ST and 200 kg. On the other hand, miniature species include those recently described *Leptophilypnion fittkai* (Gobiiformes: Eleotridae) with the maximum length of 9.2 mm SL, *Priocharax nanus* (13.0 mm SL) and *Cyanogaster noctivaga* (20.0 mm SL) (Characiformes: Characidae) and *Scoloplax baskini* (Siluriformes: Scoloplacidae) with 14.4 mm SL. In fact, a large portion of the Rio Negro fauna (634 species, 54.4%) have small or very small maximum sizes (< 10 cm of SL). Most these species are Characiformes of the families Characidae, Crenuchidae, Lebiasinidae, Iguanodectidae and Curimatidae (265 species), catfishes (Loricariidae, Callichthyidae, Heptapteridae, Trichomycteridae, Doradidae, Auchenipteridae, Aspredinidae, and Cetopsidae, 224 species), dwarf cichlids Cichliformes (41 species), and Cyprinodontiformes (Poeciliidae and Rivulidae, 26 species) (Suppl. material 1).

Two genera of small Characidae (*Cyanogaster* and *Tucanoichthys*), two genera of Hypopomidae (*Procerusternarchus* and *Racenisia*), one genus each of Tarumaniidae (*Tarumania*), Trichomycteridae (*Glanapteryx*), and Loricariidae (*Niobichthys*) are only found in the Rio Negro basin. Small-sized endemic species belonging to genera with wide distribution in the Amazon and Orinoco basins were also recorded, e.g., *Astyanax ajuricaba*, *Creagrutus tuyuka*, *Jupiaba poekotero*, *Knodus tiquiensis*, *Hyphessobrycon paepkei*, *Priocharax nanus*, *Tyttobrycon xeruini* (Characidae), *Microsternarchus brevis* (Hypopomidae), *Hypopygus cryptogenes* (Rhamphichthyidae), *Brachyglanis nocturnus* (Heptapteridae), *Scoloplax baileyi*, *S. dolicholophia* (Scoloplacidae), and *Polycentrus jundia* (Polycentridae) (Suppl. material 1).

This compilation shows that 156 species (13.4%) are probably endemic to the Rio Negro drainage, for example, *Potamotrygon wallacei* (Potamotrygonidae), *Osteoglossum ferreirai* (Osteoglossiformes), *Brittanichthys myersi*, *Tucanoichthys tucano* (Characidae), *Corydoras adolfi*, *C. tukano*, *C. burgessi*, *C. crimmeni*, *C. imitador* (Callichthyidae), *Physopyxis cristata* (Doradidae), *Brachyglanis nocturnus*, *Brachyrhamdia rambarrani* (Heptapteridae), *Laimosemion kirovskyi*, *L. amanapira*, and *L. uakti* (Rivulidae) (Suppl. material 1).

Among the species found in the Rio Negro, 436 (37.4%) have a wide distribution in the Amazon basin and in the adjacent watersheds, such as the Rio Orinoco basin, rivers of the Guianas shield (including the Essequibo, rivers of the Suriname and French Guiana, and the headwaters of rivers draining to the Rio Amazonas basin), and in Rio Tocantins basin, and inhabit a wide range of environments (cf. Dagosta and de Pinna 2019). This group is represented mainly by medium to large-sized species of

the genera *Pellona* (Pristigasteridae), *Leporinus* (Anostomidae), *Acestrorhynchus* (Acestrorhynchidae), *Boulengerella* (Ctenoluciidae), *Curimata*, *Curimatella* (Curimatidae), *Cynodon*, *Hydrolycus* (Cynodontidae), *Hemiodus* (Hemiodontidae), *Serrasalmus* (Serrasalmidae), *Brachyplatystoma*, *Hypophthalmus*, *Pimelodus*, *Pseudoplatystoma* (Pimelodidae), *Apteronotus*, *Sternarchogiton*, *Sternarchorhamphus* (Apteronotidae), *Gymnotus* (Gymnotidae), *Brachyhyopomus* (Hypopomidae), and *Eigenmannia* (Sternopygidae) (Suppl. material 1). Some of these species carry out long-distance migrations (mainly in the reproductive period) that may reach thousands of kilometers, like the giant catfishes of the genus *Brachyplatystoma* (Pimelodidae).

At least 118 species (10.1%) are exclusively shared by the Negro and Orinoco basins, such as *Potamotrygon schroederi* (Potamotrygonidae), *Acestrorhynchus grandoculis* and *Heterocharax leptogrammus* (Acestrorhynchidae), *Pseudanos varii* (Anostomidae), *Creagrutus ephippiatus*, *C. phasma*, *C. runa*, *Hemigrammus bleheri*, *Hyphessobrycon diancistrus*, *H. epicharis*, *Paracheirodon axelrodi*, *P. simulans* (Characidae), *Elachocharax geryi* and *E. mitopterus* (Crenuchidae), *Curimatopsis evelynae* (Curimatidae), *Copella eigenmanni*, *Nannostomus anduzei* and *N. marilynae* (Lebiasinidae), *Corydoras crypticus*, *C. melini* (Callichthyidae), *Apistogramma uaupesi*, and *Dicrossus filamentosus* (Cichlidae) (Suppl. material 1).

A portion of the fish fauna recorded for the Rio Negro (89 species; 7.6%) is also known to occur in the clear water rivers of the Guiana Shield (Guyana, Suriname and French Guiana). Many were originally described for this region, and some also occur in the clear water rivers of the Brazilian Central Plateau. This group of species includes *Leporinus desmotes*, *L. nigrotaeniatus* (Anostomidae) *Moenkhausia hemigrammoides*, *M. lata* (Characidae), *Nannostomus beckfordi* (Lebiasinidae), *Corydoras potaroensis* (Callichthyidae) and *Mesonauta guyanae* (Cichlidae) (Suppl. material 1). Another 223 species (19.1%) are common inhabitants of Central Amazonian white water lowlands (várzeas), among them *Ilisha amazonica*, *Pellona castelnaeana* (Pristigasteridae), *Laemolyta proxima*, *Leporinus klausewitzi*, *Rhytiodus agenteofuscus*, *R. microlepis* (Anostomidae), *Brycon melanopterus* (Bryconidae), *Potamorhina altamazonica*, *P. latior*, *P. pristigaster*, *Psectrogaster rutiloides* (Curimatidae), *Semaprochilodus insignis*, *S. taeniurus*, *Prochilodus nigricans* (Prochilodontidae), *Anodus elongatus* (Hemiodontidae), *Mylossoma albiscopum*, *M. aureum*, *Metynnix luna* (Serrasalmidae), *Triportheus albus* (Triportheidae), *Aequidens pallidus*, *Geophagus altifrons*, *Satanoperca acuticeps*, *Uaru amphiacanthoides* (Cichlidae), and *Plagioscion montei* (Sciaenidae) (Suppl. material 1).

At least 61 species (5.2%) found in the Rio Negro are also distributed in tributaries of upper Amazonas near the Peruvian and Colombian borders with Brazil, indicating a possible zoogeographical relationship between these two parts of the Amazon Basin (cf. Ruokolainen et al. 2018), for example, *Hyphessobrycon erythrostigma*, *Petitella georgiae*, *Priocharax pygmaeus*, *Copeina guttata*, *Corydoras arcuatus*, *C. rabauti*, *Cetopsis parma*, *Myoglanis koepckei*, *Nemuroglanis lanceolatus*, and *Rhabdolichops nigrimans* (Suppl. material 1). Another 36 species (3.1%) have very wide geographic distribution, occurring also in the Paraná-Paraguay, La Plata and even the Rio São Francisco basins, i.e., *Pygocentrus nattereri*, *Serrasalmus maculatus* (Serrasalmidae), *Ossancora punctata*, *Oxy-*

doras niger, *Pterodoras granulosus* (Doradidae), *Sorubim lima*, *Sorubimichthys planiceps* (Pimelodidae), *Scoloplax dicra* (Scoloplacidae), *Pseudotylorus angusticeps* (Belonidae), *Astronotus crassipinnis*, *Mesonauta festivus* (Cichlidae), and *Hoplerythrinus unitaeniatus* (Erythrinidae) also occurs in coastal drainages in southeastern Brazil and *Callichthys callichthys* (Callichthyidae) widespread in South America (Suppl. material 1).

Six species (0.5%) are exotic and invasive, possibly introduced by fish farmers, ornamental fish breeders, or even discarded by amateur aquarists in currently polluted streams draining the urban area of Manaus, in the lowermost stretch of Rio Negro near its confluence with Rio Solimões: *Danio rerio* (Southeast Asia), *Oreochromis niloticus* (Africa), *Poecilia reticulata* (northern South America), *Trichopodus trichopterus* (South-east Asia), *Xiphophorus hellerii*, and *X. maculatus* (Central American countries).

Despite the presence of dozens of species identified only to genus-level in the surveyed lists, those could not be unambiguously considered as different taxonomic entities. Conversely, during this survey, representatives of 40 species representing unequivocally undescribed species were discovered among collection specimens and included in the present list, representing 3.4% of the listed species richness and probably do not represent an exact amount of undescribed species in the Rio Negro basin.

Aquatic habitats and their fish faunas

Main river channel

The ichthyofauna of the main channel of the Rio Negro was accessed mostly by bottom trawl net sampling in six areas, including the mouth bay immediately upstream from Manaus, Rio Cuieiras, Rio Jufari, the confluence with Rio Branco, the lower Rio Branco, and nearby the city of Barcelos. The fish fauna is represented by at least 159 species distributed in 8 orders (+ the incertae sedis Eupercaria) and 24 families, mostly represented by benthic fishes. Siluriformes was dominant with 82 species (51.6%) in seven families, followed by Gymnotiformes with 28 species (17.6%) in three families, Characiformes with 20 species (12.6%) in six families, Eupercaria (Sciaenidae) with ten species (6.3%), and Cichliformes with seven species (4.4%) in one family. Four other orders were represented by 12 species (7.5%) (Figure 3, Suppl. material 1).

The most diverse genera in main channel of the Rio Negro were *Leptodoras* (7 species), *Eigenmannia*, *Rineloricaria*, *Trachydoras* (5 spp. each) *Pachyurus*, *Rabdolichops* (4 spp. each), *Hypophthalmus*, *Microschemobrycon*, *Plagioscion*, *Potamotrygon*, and *Sternarchella* (3 spp. each) (Suppl. material 1). Among the 159 species captured in the main channel, 18 were present in at least three of the six sampled areas: *Brachyplatystoma filamentosum*, *Hypophthalmus edentatus*, *Pimelodus blochii*, *Pimelodina flavipinnis*, *Platystomatichthys sturio*, *Pinirampus pirinampu* (Pimelodidae), *Pimelodella cristata* (Heptapteridae), *Pseudoloricaria laeviuscula*, *Pecklotia vittata* (Loricariidae), *Trachydoras microstomus* and *Opsodoras morei* (Doradidae), *Adontosternarchus clarkae*,

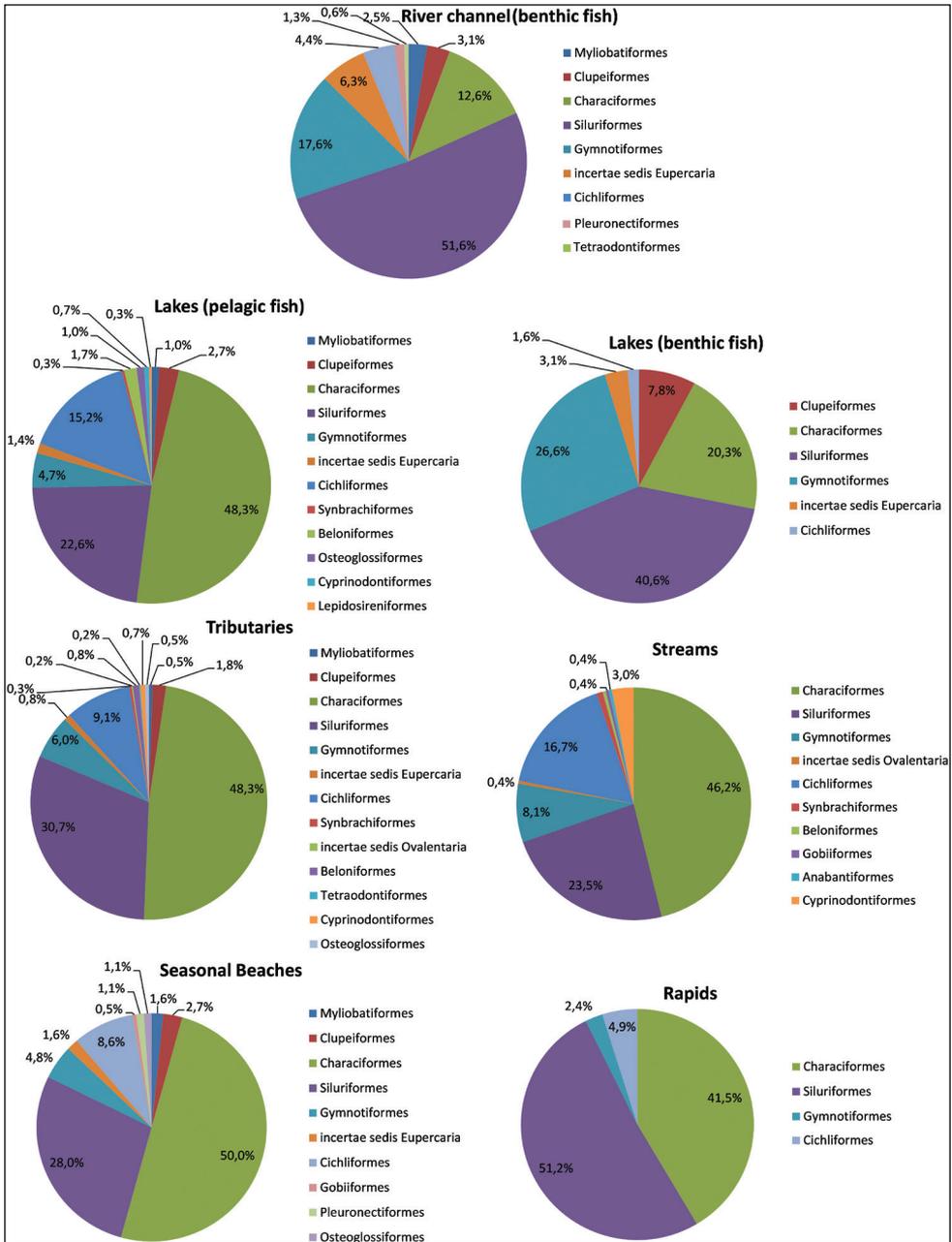


Figure 3. Taxonomic characterization (proportions of species by order) of fish assemblages found in different aquatic habitats of Rio Negro, Amazon Basin, Brazil.

Sternarchogiton nattereri and *S. porcinum* (Aptereronotidae), *Steatogenys elegans* (Rhamphichthyidae), *Distocyclus conirostris* (Sternopygidae), *Pachypops fourcroyi*, and *Pachyurus schomburgkii* (Sciaenidae).

Floodplain lakes

A total of 296 fish species (eleven orders + Eupercaria), distributed in 40 families, was sampled in five lakes of the lower Rio Negro, four lakes in the middle Rio Negro, and five lakes in the middle and lower Rio Branco. Representatives of the Characiformes were the richest with the largest number of families and species (17 families, 143 species, 48.3%), followed by Siluriformes (7 families, 67 species, 22.6%), Cichliformes (one family, 45 species, 15.2%), and Gymnotiformes (5 families, 14 species, 4.7%). Another seven orders (+ Eupercaria) were represented by nine families and 27 species (9.1%) (Figure 3, Suppl. material 1).

The lake fish fauna was generally sampled by gill nets that selectively catch medium- to large -sized fishes of both migratory and sedentary habits. However, small-sized fishes (88 species of up to 10 cm SL adult size) were sampled in the margins of the lakes, including representatives of the genera *Moenkhausia* (7 spp.), *Hemigrammus* (6 spp.), *Brynopops*, *Nannostomus* (4 spp. each), *Copella*, *Centromochlus*, *Tatia* (3 spp. each), and at least two species of dwarf cichlids (*Apistogramma* spp.) (Suppl. material 1).

Approximately 30 medium- to large-size species were captured in most sampled lakes: *Ilisha amazonica* and *Pellona flavipinnis* (Pristigasteridae), *Metynnys hypsauchen* (Serrasalminidae), *Boulengerella lucius* (Ctenolociidae), *Laemolyta taeniata* (Anostomidae), *Anodus elongatus*, *Hemiodus immaculatus* (Hemiodontidae), *Semaprochilodus insignis*, *S. taeniurus* (Prochilodontidae), *Triportheus angulatus* (Triportheidae), *Curimata vittata*, *Potamorhina altamazonica*, *P. latior* and *Cyphocharax abramoides* (Curimatidae), *Raphiodon vulpinus* (Cynodontidae), *Acestrorhynchus falcirostris* and *A. microlepis* (Acestrorhynchidae), *Serrasalmus rhombeus* and *S. elongatus* (Serrasalminidae), *Pimelodus blochii*, *Hypophthalmus edentatus*, *H. fimbriatus* and *H. marginatus* (Pimelodidae), *Age-neiosus lineatus* (Auchenipteridae), *Cichla temensis*, *Geophagus altifrons*, *G. proximus* and *Uaru amphiacanthoides* (Cichlidae), and *Plagioscion squamosissimus* (Scianidae).

Benthic ichthyofauna in a black water lake

So far, only one lake (Lago do Prato) in the lower Rio Negro basin has been thoroughly sampled for benthic fish using bottom trawl nets (Garcia (1993); specimens deposited in the INPA Fish Collection), resulting in 64 species in five orders (+ Eupercaria) and 20 families. Siluriformes was the richest order with six families and 26 species (40.6%), followed by Gymnotiformes (3 families and 17 species, 26.6%) and Characiformes (seven families and 13 species, 20.3%). Two other orders (Clupeiformes and Cichliformes) plus Eupercaria were represented by eight genera and eight species (Figure 3, Suppl. material 1). Siluriformes species (Pimelodidae: 11 spp., Doradidae: 7 spp.) and Gymnotiformes (Apterodontidae: 9 spp.; Sternopogidae: 7 spp.) were present in all periods of the hydrological cycle (Suppl. material 1).

Tributary rivers

Ichthyofaunal surveys and taxonomic inventories were made for the Jaú and Unini rivers (Jaú National Park), Carabinani and Paduari rivers (Rio Negro State Park – North Sector),

Igarapé Tarumã-Açú near Manaus (all located in the lower Rio Negro), as well as for the rivers Branco, Jufari, Cuaru, Anapixi, Atauí, and some of the large tributaries of the middle Rio Negro (Igarapé Rei and Igarapé Zamula), and for the Rio Tiquié in the upper Rio Negro, resulting in 596 species. The ichthyofauna was dominated by species of Characiformes (17 families, 288 species; 48.3%), Siluriformes (12 families, 183 species, 30.7%), Cichliformes (one family, 54 species, 9.1%), and Gymnotiformes (5 families, 36 species, 6.0%) (Figure 3). Another seven orders (plus Eupercaria and Ovalentaria) were represented by ten families and 35 species (5.9%) (Suppl. material 1).

The most representative families in terms of species richness were: Characidae (103 species), Loricariidae and Cichlidae (54 each), Auchenipteridae (29), Serrasalmidae and Pimelodidae (28, each), and Anostomidae and Curimatidae (26 each), which corresponded to 58.5% of the species (Suppl. material 1). The most representative genera in these environments were: *Hemigrammus* (18 species), *Moenkhausia* (17), *Leporinus* (14), *Crenicichla* (13), *Hemiodus* (11), *Jupiaba* and *Corydoras* (10 spp. each), *Serrasalmus* (9), and *Characidium* (8), together representing 18.4% of the species found (Suppl. material 1).

Small upland forest streams

Two hundred thirty-four species belonging to seven orders of 36 families were recorded in streams. The ichthyofauna of this kind of environment is dominated by small Characiformes that correspond to 46.2% of the captured species (13 families, 108 species), followed by 23.5% of Siluriformes (10 families, 55 species), 16.7% of Cichliformes (one family, 39 species), and 8.1% of Gymnotiformes (5 families, 19 species), but also including some species (5.5%) of Cyprinodontiformes, Synbranchiiformes, Gobiiformes, Anabantiformes, Beloniformes, and Ovalentaria (seven families and 13 species in total) (Figure 3 and Suppl. material 1). Some of the most common and/or abundant fishes in 1st to 3rd order streams include species of *Pyrrhulina*, *Copella* (Lebiasinidae), *Hyphessobrycon*, *Hemigrammus*, *Moenkhausia* (Characidae), *Bryconops* (Iguanodectidae), *Crenuchus*, *Characidium* (Crenuchidae), *Erythrinus*, *Hoplias* (Erythrinidae), *Helogenes* (Cetopsidae), *Hypopygus*, *Steatogenys* (Hypopomidae), *Aequidens*, *Apistogramma* (Cichlidae), and *Microphylipnus* (Eleotridae).

Seasonal beaches

Sampled beaches in Rio Branco and in the lower Rio Negro revealed the presence of 186 species distributed in eight orders and 33 families. The Characiformes were the richest group (16 families and 93 species, 50.0%), followed by Siluriformes (seven families and 52 species, 28.0%), Cichliformes (one family and 16 species, 8.6%), and Gymnotiformes (three families and nine species, 4.8%) (Figure 3). Five other orders (+ Eupercaria) were also present: Myliobatiformes, Osteoglossiformes, Clupeiformes, Gobiiformes, Pleuronectiformes, and Eupercaria (Sciaenidae) (Figure 3, Suppl. material 1). Some families stood out in terms of species richness in beaches: Characidae (38 spp.),

Cichlidae (16 spp.), Hemiodontidae (12 spp.), Loricariidae and Trichomycteridae (11 spp. each), and Doradidae and Auchenipteridae (9 spp. each). The most representative genera in the beaches were *Moenkhausia* (10 species), *Hemiodus* (9), *Hemigrammus* (6), *Boulengerella*, *Bryconops*, and *Cyphocharax* (4 spp. each) (Suppl. material 1).

Rapids

In the rapids of the upper Rio Branco (middle Rio Negro) and Rio Tiquié (upper Rio Negro), 41 fish species of four orders and nine families were recorded. Siluriformes predominated (3 families and 21 species, 51.2%), followed by Characiformes (4 families and 17 species, 41.5%), Cichliformes (1 family and 2 species), and Gymnotiformes (1 family and 1 species) (Figure 3, Suppl. material 1). In those rapids the Loricariidae (10 species), Heptapteridae (9 spp.), Crenuchidae and Serrasalminidae (5 spp. each), and Anostomidae (4 spp.) were the richest families. The most representative genera found in the rapids were *Characidium* and *Peckoltia* (3 species each) (Suppl. material 1).

Discussion

This study presents an extensive, updated compilation of occurrence records of fish species for the Rio Negro basin, one of the most important tributaries of the Rio Amazonas. Overall, 1,165 species have confirmed occurrences in the basin, and the estimated total fish richness may reach 1759 species. This remarkably high species richness is far from adequately known, and the rate of species descriptions for the basin does not show signs of stabilization.

The compiled species richness characterizes the Rio Negro ichthyofauna as one of the richest and most diversified in the Amazon Basin and in the world, shouldering the recently disclosed fish richness for the Rio Madeira in southwestern Brazilian Amazon (Queiroz et al. 2013). However, differently from the Rio Madeira, there were no concentrated efforts to survey the Rio Negro fish fauna, which points out to a potential increase of fish species records for the basin in the future. Moreover, there is a strong unbalance in the accumulated sampling effort (and, consequently, in the recorded amount of species) between the relatively well-sampled lower portion (between Manaus and the confluence with the Unini and Jaú rivers) and the mid and upper courses of the Rio Negro close to the borders with Colombia and Venezuela (pers. obs.). This knowledge and sampling effort discrepancies do not allow a more comprehensive comparison of fish assemblages among sub-regions of the basin.

Despite the wide distribution areas of many fishes in the basin, most species are confined to specific aquatic environments, such as the main channel of large rivers, tributary rivers of different water types, lakes, shallow banks and beaches, small upland forest streams, interfluvial swamps and rapids stretches. Analyzing the taxonomic composition of the fish fauna by environment, it was verified that ostariophysans dominates in most types of habitats, but with differences reflecting the ecological particularities of each

group. In the deep waters of the main river channel, Siluriformes (catfishes) and Gymnotiformes (electric fishes) dominate the assemblages (Chao 2001; Thomé-Souza and Chao 2004; Ferreira et al. 2007; Rapp Py-Daniel et al. 2017). Thomé-Souza and Chao (2004), studying the ichthyofauna of the channels of the Negro and Branco rivers, verified that these environments presented differences in composition, species richness and abundance throughout the hydrological periods, which was presumed to be the result of predation and migration. The dominance of electric fishes was also observed in a large black water lake in the Anavilhanas archipelago surveyed by bottom trawl net samplings (Garcia 1993). Although little is known in terms of their natural history, representatives of the Gymnotiformes seem to represent a large biomass in deep river channels and to be of great importance as food source for many species of large catfish (Barthem and Goulding 1997; Barbarino-Duque and Winnemiller 2003; Cox-Fernandes et al. 2004).

In lakes and tributary rivers, migratory species of Characiformes and Siluriformes presented the highest frequency and abundance (Zuanon et al. 1998; Saint-Paul et al. 2000; Lima et al. 2005; Soares and Yamamoto 2005; Zuanon et al. 2008; Yamamoto et al. 2014). Many species occurring in these environments are also common and abundant in white water floodplains of the Solimões/Amazonas system, and in other tributaries of the Amazon Basin (Ferreira 1993; Rapp Py-Daniel et al. 2007; Lima and Caires 2011), and are of great commercial importance (Goulding 1980; Junk et al. 1983; Bayley 1983, 1998, Saint-Paul et al. 2000; Ruffino et al. 2006; Soares et al. 2007). In addition, many of the small-sized species recorded for lakes and tributaries are also of great importance for the ornamental fish trade, especially in the middle and upper Rio Negro (Chao et al. 2001; Anjos et al. 2009).

In small upland forest streams that are not influenced by the seasonal flood pulse, small characins and catfishes are common (Mendonça et al. 2005; Espírito-Santo et al. 2009; Zuanon et al. 2015), but their local abundance is usually low because of the low aquatic productivity of those oligotrophic streams (Anjos 2014). The fish communities that inhabit these environments are among the most diversified and least known in the Amazon, mainly due to difficulties of access to the such water bodies far inland in the forest. In general they are small species that usually do not exceed 10 cm in standard length. Those fishes, for the most part, do not make long migrations, and spend almost their entire life in the same system or habitats. Because they are primarily dependent on allochthonous forest material, these species are highly sensitive to changes in the surrounding environment (Lowe-McConnell 1987; Silva 1993; Walker 1995; Beltrão 2007; Beltrão et al. 2018).

Streams disturbed by deforestation and pollution in the urban areas of large cities such as Manaus are occupied by non-native species such as the tilapia *Oreochromis niloticus*, three-spot gourami *Trichopodus trichopterus*, guppy *Poecilia reticulata*, swordfishes *Xiphophorus hellerii* and *X. maculatus* and the zebra fish *Danio rerio* (Beltrão 2007). Allochthonous species from nearby white-water floodplains also invade such heavily polluted urban streams. Such species dominates or even completely replace the fish fauna in impacted streams, and investigations have found evidence of established populations, a situation not yet recorded in intact streams to date (Beltrão 2007).

Water types, habitat diversity and hydrological connectivity

Historical and geomorphological factors certainly determined to a great extent the differences observed in the fish fauna within the sub-basins of the Amazon basin (Goulding et al. 1988; Lundberg et al. 1998; Albert et al. 2011; Dagosta and de Pinna 2017, 2019), but the relative importance of these factors still needs to be properly evaluated. However, ecological factors are also determinant to the composition and abundance of fish assemblages in any hydrographic system. Among the several factors that influence the structure of fish assemblages of the Amazon are the water type and the diversity of habitats (Junk et al. 1997; Lundberg et al. 1998). Blackwater rivers such as the Rio Negro show a very low autochthonous (aquatic) productivity, because they originate in extremely poor soils with low nutrient contents (Fittkau et al. 1975; Goulding et al. 1988; Worbes 1997; Küchlera et al. 2000), which results in low values of fish density and biomass (Saint-Paul et al. 2000). However, such low productivity does not imply in a low fish diversity, since black water rivers may have comparable or even higher fish diversity than white water systems of similar sizes (Goulding et al. 1988; Saint-Paul et al. 2000). Therefore, differences in water chemistry, sediment loads and nutrient contents among the tributaries of the Rio Negro add heterogeneity to the basin and allow the coexistence of a huge diversity of fishes in a basin-scale (e.g., Ferreira et al. 2007).

The high fish diversity in the Rio Negro basin can also be explained by the enormous variety of habitats available for fish, such as the flooded forests (igapós), fluvial channels, lakes, streams, marginal ponds, temporary beaches, rapids, waterfalls and various types of interfluvial swamps. In the middle Rio Negro, for example, there are large floodplains located between the cities of Barcelos and Santa Isabel do Rio Negro as well as in the lower portion of the Rio Branco basin, in Roraima State. These extensive interfluvial regions become flooded during the rainy seasons and, in some areas, may remain flooded even during periods of extreme drought, due to the high level of the water table and the presence of hydromorphic soils (Fittkau et al. 1975; Goulding et al. 1988; Marshall et al. 2011; Vale et al. 2014). These areas harbor many species of small fish, several of them of great importance for the ornamental fish trade (Chao et al. 2001; Anjos et al. 2009).

The connectivity of the Rio Negro with large adjacent basins is another factor that contributes to the high richness in the basin. In fact, due to its peculiar geographic position, the Rio Negro has been historically interconnected to other rivers with different types of water (Winemiller and Willis 2011), which may constitute (or have constituted) important ichthyofaunistic interchange zones between basins. A preliminary analysis of the distribution of the species present in three adjacent basins (Rio Negro – Rio Orinoco – Rio Branco-Essequibo River in Guiana shield) reveals that at least 227 species are shared among these basins, indicating the importance of historical or current hydrological connectivity for the ichthyofaunal similarity of these basins. One of the most representative instances is the current connection with the Orinoco River by the Casiquiare and other adjacent channels (Winemiller et al. 2008; Winemiller and Willis 2011). Other drainage connections have been pointed out, involving clear rivers of the Guiana Shield through the Rio Branco/Rupununi interfluvial plains (Ferreira

et al. 2007; Winemiller and Willis 2011), as well as white water rivers of the upper Amazonas, such as the Caquetá-Japurá rivers through headwater capture events in the past (Ruokolainen et al. 2018). The headwaters of the Rio Quiúni, a tributary of the middle Rio Negro, in periods of great floods present turbid waters possibly due to the overflow of white water from the Rio Japurá (H. Beltrão, personal observation), which may constitute another active route for fish interchange among basins. Other possible connections exist between the rivers that flow to the Lago Amaná in the middle Rio Solimões/Amazonas and the headwaters of the Rio Unini in the right bank of the lower Rio Negro (Zuanon et al. 1998). These different connections possibly have helped maintaining - and may have incremented - the fish diversity of the Rio Negro basin.

Dagosta and de Pinna (2017) analyzing the biogeography of fishes in sub-basins of the Amazon Basin, considered the Rio Negro and Rio Branco as two distinct biogeographic regions, despite the latter constituting a tributary of the former. In fact, of the 156 species considered exclusive to the Rio Negro, 115 occur only in that river, 29 only in Rio Branco, and just 12 are shared by the two drainage basins. However, nearly 65% of the species that occur in the Rio Branco are shared with the Rio Negro basin.

There is a direct relation between a river basin area and its fish species richness (Hugueny et al. 2010), which is supported by the general species-area theory (e.g., Rosenzweig 1995). However, the Rio Negro basin shows a species richness that is much higher than expected by its drainage area, holding the greatest fish species richness (1,165 spp.) so far recorded for a river in the Brazilian territory and surpassing the 1,062 species recorded for the Rio Madeira (Dagosta and de Pinna 2019). It's noteworthy that such huge fish diversity is contained in an area of 696,808 Km² which corresponds to half of the area of the Rio Madeira basin (1,380,000 km²). The Rio Negro fish richness is remarkably higher than that of the Rio Tocantins basin with 757,000 km² and 705 species (Lima and Caires 2011; Dagosta and de Pinna 2019), and also proportionally much higher than that of the Rio Xingu basin with 504,277 Km² and 502 species (Camargo et al. 2004; Dagosta and de Pinna 2019). However, it should be noted that differences in the accumulated sampling effort may partially explain the remarkably lower species richness recorded for some of the above-mentioned basins. Conversely, the fish richness in the Rio Negro basin is comparable to that of the Rio Orinoco basin in Venezuela with 1,002 species (Reis et al. 2016) but for a basin area of 1,212,000 km².

Besides the high number species richness, some taxonomic groups apparently show a higher rate of endemism in Rio Negro than in other adjacent drainage basins. For example, 17 of the 31 species of *Corydoras* and 12 of 20 species of *Apistogramma* found in Rio Negro are endemic. Other examples are the cyprinodontiform genera *Anablepsoides* and *Laimosemion*, with 13 species found in Rio Negro and at least 10 endemic species. Representatives of these groups generally are small-sized species that are sedentary and with low active dispersal capacity (such as the small Characidae, Callichthyidae and Loricariidae) or display strong territorial behavior (i.e., small cichlids of the genus *Apistogramma*). These characteristics may favor speciation events at relatively small spatial scales (e.g., de Oliveira et al. 2009), resulting in discontinuous patterns of occurrence and possibly contributing to a high endemism rate in the Rio Negro basin.

Final considerations and perspectives

The results presented in this compilation show that in recent years considerable progress has been achieved regarding the knowledge of the fish diversity in the Rio Negro basin, but also point out to important information gaps that still need to be addressed. It is necessary to investigate the taxonomic identity of many forms with uncertain status and, in the case of new species, to describe them, which may still take a long time (e.g., Ota et al. 2015). This may allow the identification of rare or threatened species that will eventually need protection policies.

Additionally, the fish diversity in hard-to-reach areas in the basin should be better sampled. Studies in the upper Rio Negro (Lima et al. 2005) revealed the presence of taxa that had never been found in Rio Negro (i.e., representatives of *Tometes* and *Utiaritchthys*; Serrasalminidae) and the discovery of at least 30 possible new species, several of which were eventually described (Britto and Lima 2003; Zanata and Lima 2005; Ferreira and Lima 2006; Lima et al. 2009; Marinho and Lima 2009; Lima and Sousa 2009; Lima and Soares 2018; Lehmann et al. 2018). Research should be encouraged in the regions of the upper and middle Rio Negro, particularly in the smaller tributaries near the borders between Brazil, Venezuela and Colombia.

The list of 1,165 fish species presented herein for the Rio Negro basin represents a 259% increase in the number of species documented by Goulding et al. (1988) (450 species). This improvement in knowledge resulted from an increase of the ichthyofaunal surveys along the Rio Negro basin, but also from an accelerated rate of species descriptions in recent years. Despite this improvement in the knowledge about the fish fauna in the basin. A considerable number of species still await to be inventoried, discovered or formally described, as already pointed out by Bohlke et al. (1978), Ota et al. (2015) and Birindelli and Sidlauskas (2018) regarding the neotropical ichthyofauna.

Information about the Rio Negro basin and its biota has been gathered for ca. 230 years, since the pioneer naturalists reports by Alexandre Rodrigues Ferreira (1784–87), Alexander Von Humboldt (1800) and Alfred Russel Wallace (1850–52). Samples that have been deposited in collections and/or museums for many years and that were not properly identified were recently described as new species (e.g., *Leptophilypnion fittkaui* Robert, 2013 and *Polycentrus jundia* Coutinho & Wosiacki, 2014). Revision of many genera or problematic groups (e.g., *Moenkhausia*) have revealed species still unknown to science, including some occurring in Rio Negro (Marinho 2009; Marinho et al. 2015). More recently, a new fish family (Tarumaniidae) has been discovered in a stream of the lower Rio Negro (Igarapé Tarumã-Mirim), very close to Manaus, the largest city in the Brazilian Amazon, *Tarumania walkeri* de Pinna, Zuanon, Rapp Py-Daniel & Petry, 2017, a very surprising discovery for an ichthyofauna that has been surveyed for more than two centuries.

Although the Rio Negro basin is apparently very well preserved (< 1% of its area is deforested, accumulated in 2000–2013; Ricardo et al. 2015), environmental changes due to human activities, such as deforestation, habitat loss, pollution and introduction of non-native species, may jeopardize the rich fish fauna of this drainage basin, as reported for several freshwater ecosystems around the world (Revenge et al.

1998, 2005; Revenga and Kura 2003; Dudgeon et al. 2006). Therefore, concentrated conservation efforts should be directed to the Rio Negro basin and its biota ahead of anthropic action, preserving one of the most species-rich and environmentally unique areas of the planet.

This study honors the late Dr. Javier Alejandro Maldonado-Ocampo, a very good friend and former Subject Editor of ZooKeys, for his important contribution to the knowledge of the fish diversity, taxonomy and evolution in the Amazon basin.

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Supplementary material I

Checklist of the species found in the different environments of the Rio Negro basin

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Data type: species data

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Morphology and phylogenetic analysis of two new deep-sea species of *Chrysogorgia* (Cnidaria, Octocorallia, Chrysogorgiidae) from Kocebu Guyot (Magellan seamounts) in the Pacific Ocean

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Abstract

Two new species of *Chrysogorgia* Duchassaing & Michelotti, 1864 collected from Kocebu Guyot in the Magellan seamounts of the Pacific Ocean are described and illustrated: *Chrysogorgia ramificans* **sp. nov.** collected from a depth of 1831 m and *Chrysogorgia binata* **sp. nov.** collected from a depth of 1669 m. *Chrysogorgia ramificans* **sp. nov.** belongs to the *Chrysogorgia* “group A, Spiculosae” with rods distributed in body wall and tentacles, and *C. binata* **sp. nov.** belongs to the “group C, Squamosae typicae” with rods and/or spindles not present but only scales. *Chrysogorgia ramificans* **sp. nov.** differs from congeners by its main stem with 2/5R branching sequence at the bottom forming two large bottlebrush-shaped branches with 1/3R branching sequence at the top. *Chrysogorgia binata* **sp. nov.** is similar to *C. scintillans* Bayer & Stefani, 1988, but differs by its larger polyps, larger sclerites in the body wall, and different scales in the upper part of polyps. The mtMutS genetic distances between *C. ramificans* **sp. nov.** and *C. binata* **sp. nov.** and congeners are in the range of 0.33%–2.28% and 0.33%–2.94%, respectively, while the intraspecific distances are in the range of 0–0.16%. Molecular phylogenetic analysis indicates that *C. ramificans* **sp. nov.** is clustered with *C. monticola* Cairns, 2007 and *C. binata* **sp. nov.** is clustered with *C. chryseis* Bayer & Stefani, 1988, both with high support indicating close relationships.

Keywords

Anthozoa, *Chrysogorgia ramificans* sp. nov., *Chrysogorgia binata* sp. nov., gorgonian, phylogeny, taxonomy

Introduction

Within the gorgonian family Chrysogorgiidae, the genus *Chrysogorgia* Duchassaing & Michelotti, 1864 is the largest and most common group, distributed worldwide including the Antarctic, ranging from 100 m to 3860 m water depth. In some colonies it is characterized by a spiralling main axis that branches sympodially giving off secondary branches that subdivide dichotomously, resulting in a bottle-brush colony shape. In others, the sympodial main axis does not spiral, resulting in fan-like planar or bi-flabellate colonies (Pante and Watling 2011; Cordeiro et al. 2015). To date, *Chrysogorgia* contains 70 species (Cairns 2018). Among them, 45 species are found only from the Pacific, 17 species only from the Atlantic and 7 only from the Indian Ocean (Cairns 2001, 2007, 2018; Pante and Watling 2011; Cordeiro et al. 2015). *Chrysogorgia flexilis* Wright & Studer, 1889 occurs in both the Pacific and Indian Oceans (Wright and Studer 1889; Cairns 2001).

Based on the presence of rods or scales in the body wall and tentacles, Versluys (1902) divided *Chrysogorgia* species into three groups, which were summarized by Cairns (2001) as following: “group A, Spiculosae” (rods and/or spindles in body wall and tentacles) with 38 species, “group B, Squamosae aberrantes” (rods and/or spindles in tentacles but not in body wall) with 13 species, and “group C, Squamosae typicae” (rods and/or spindles not present; only scales) with 18 species. More recently, Cordeiro et al. (2015) described the species *C. upsilonia*, which possesses spindles in the body wall but not in the tentacles, and classified it as “group D, Spiculosae aberrantes”. The separation of four groups was further recognized by Cairns (2018).

During the investigation of the Magellan seamount benthic diversity in the tropical Western Pacific, we obtained two golden gorgonians from the Kocebu Guyot using a remotely operated vehicle (ROV). Based on morphological and phylogenetic analyses, both species proved to be new species of *Chrysogorgia* and are described as *C. ramificans* sp. nov. and *C. binata* sp. nov., respectively. Their genetic distances and phylogenetic relationships within *Chrysogorgia* are discussed.

Materials and methods

Specimen collection and morphological examination

Specimens were obtained by the ROV *FaXian* (Discovery) from the Kocebu Guyot in the Magellan seamounts in the tropical Western Pacific during the cruises of the R/V *KeXue* (Science) in 2018 (Fig. 1). The specimens were photographed in situ before sampled, photographed onboard, and then stored in 75% ethanol after collection. Small branches were cut off and stored at -80 °C for molecular study.

The morphological terminology follows Bayer et al. (1983). A stereo dissecting microscope was used to examine the general morphology and anatomy. The sclerites of the polyps

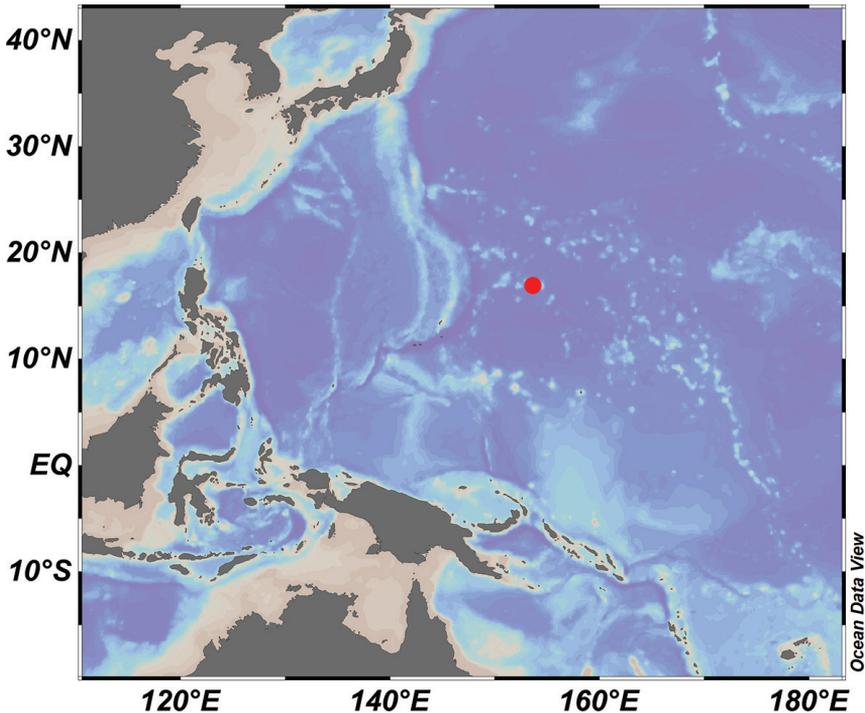


Figure 1. Sampling site in the Kocebu Guyot in the Western Pacific Ocean.

and branches were isolated by digestion of the tissues in sodium hypochlorite, and then were washed with deionized water repeatedly. To investigate the structure of polyps and sclerites, they were air-dried and mounted on carbon double adhesive tape and coated for scanning electron microscopy (SEM) observation. SEM scans were obtained and the optimum magnification was chosen for each kind of sclerites by using TM3030Plus SEM at 5 kV.

The type specimens (registration numbers: MBM286307 and MBM286346) of the two new species have been deposited in the Marine Biological Museum of Chinese Academy of Sciences (MBMCAS) at Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China.

DNA extraction and sequencing

Total genomic DNA was extracted from the polyps of each specimen using the TIANamp Marine Animal DNA Kit (Tiangen Bio. Co., Beijing, China) following the manufacturer's instructions. PCR amplification for the mitochondrial genomic region 5'-end of the DNA mismatch repair protein – *mutS* – homolog (mtMutS) was conducted using primers AnthoC-orMSH (5'-AGGAGAATTATTCTAAGTATGG-3'; Herrera et al. 2010) and Mut-3458R (5'-TSGAGCAAAGCCACTCC-3'; Sánchez et al. 2003). PCR reactions were performed using I-5 2 × High-Fidelity Master Mix DNA polymerase (TsingKe Biotech, Beijing, China). The amplification cycle conditions were as follow: denaturation at 98 °C for 2 min, followed

by 32 cycles of denaturation at 98 °C for 20 s, annealing at 50 °C for 20 s, extension at 72 °C for 15 s, and a final extension step at 72 °C for 2 min. PCR purification and sequencing were performed by TsingKe Biological Technology (TsingKe Biotech, Beijing, China).

Genetic distance and phylogenetic analyses

All the available mtMutS sequences of *Chrysogorgia* spp. and the out-group species from related chrysogorgiid genera were downloaded from GenBank, and those without associated publications or named *Chrysogorgia* sp. were omitted from the molecular analyses (see Table 2 and Fig. 8). The sequences were aligned using MAFFT v.7 (Katoh and Standley 2013) with the G-INS-i algorithm. Genetic distances, calculated as uncorrected “*p*” distances within each species and among species, were estimated using v.6 (Tamura et al. 2013).

For the phylogenetic analyses, only one sequence was randomly selected from the conspecific sequences without genetic divergence (see Table 2). The evolutionary model GTR+G was the best-fit model for mtMutS, selected by AIC as implemented in jModeltest2 (Darriba et al. 2012). Maximum likelihood (ML) analysis was carried out using PhyML-3.1 (Guindon et al. 2010). For the ML bootstraps, we consider values < 70% as low, 70–94% as moderate and ≥ 95% as high following Hillis and Bull (1993). Node support came from a majority-rule consensus tree of 1000 bootstrap replicates.

Bayesian inference (BI) analysis was carried out using MrBayes v3.2.3 (Ronquist and Huelsenbeck 2003) on CIPRES Science Gateway. Posterior probability was estimated using four chains running 10,000,000 generations sampling every 1000 generations. The first 25% of sampled trees were considered burn-in trees. For the Bayesian posterior probabilities, we consider values < 0.95 as low and ≥ 0.95 as high following Alfaro et al. (2003). The accession numbers of the mtMutS sequences are listed next to the species names in the phylogenetic tree (Fig. 8).

Results

Class Anthozoa Ehrenberg, 1834

Subclass Octocorallia Haeckel, 1866

Order Alcyonacea Lamouroux, 1812

Suborder Calcaxonia Grasshoff, 1999

Family Chrysogorgiidae Verrill, 1883

Genus *Chrysogorgia* Duchassaing & Michelotti, 1864

***Chrysogorgia ramificans* sp. nov.**

<http://zoobank.org/DF4284E7-CC5E-4AE7-94C8-4E84366387E9>

Figs 2, 3

Holotype. MBM286307, station FX-Dive 174 (17°29.93'N, 153°14.69'E), Kocebu Guyot, depth 1831 m, 8 April 2018. GenBank accession number: MK431863.

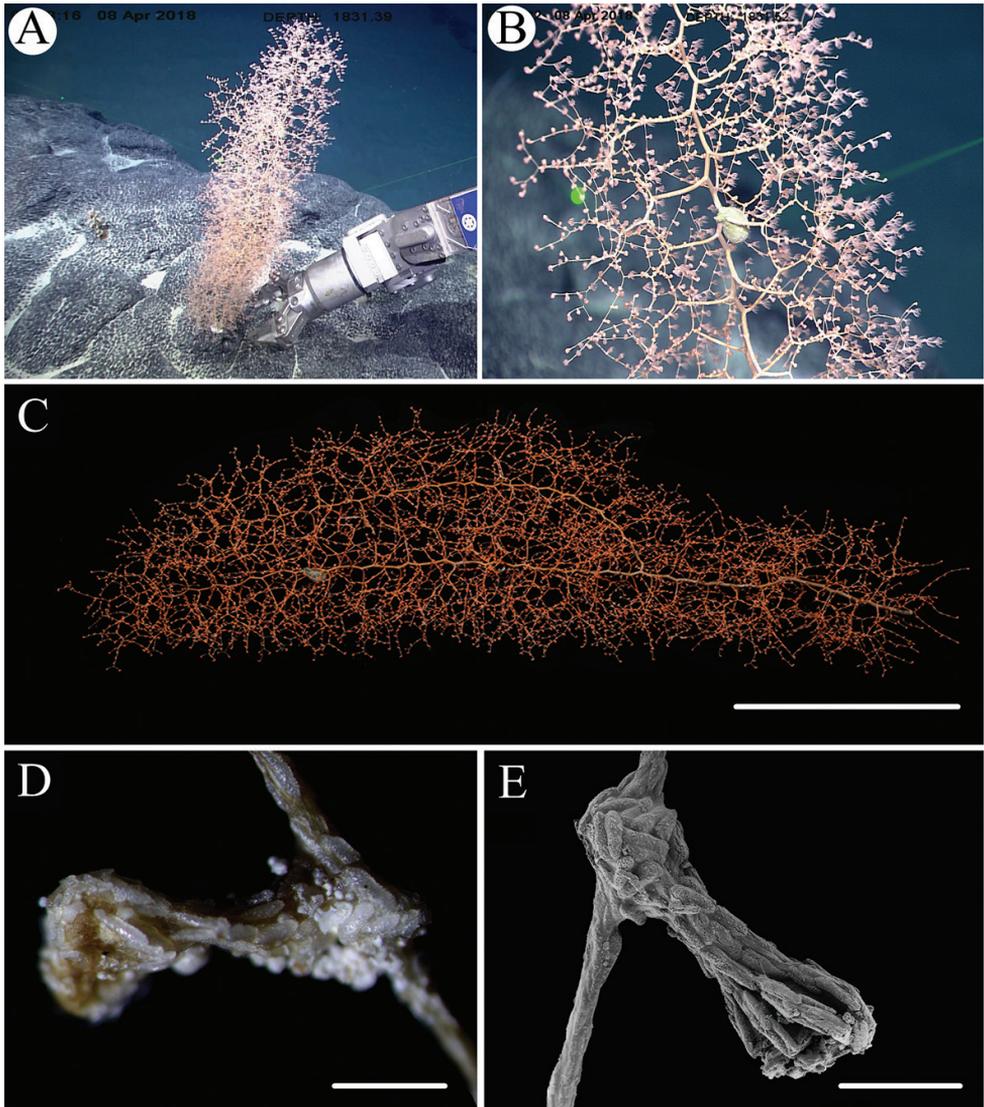


Figure 2. External morphology and polyps of *Chrysogorgia ramificans* sp. nov.: **A** The holotype in situ **B** Close-up of branches and polyps in situ **C** The colony after collection **D** A single polyp under light microscope **E** Single polyp under SEM. Scale bars: 20 cm (**C**); 1 mm (**D, E**).

Diagnosis. *Chrysogorgia* (tertiary “group A, Spiculosae” – rods or spindles in the tentacles and the body wall) with a short basal stem leading to a bottlebrush-shaped main stem, giving of a single major branch also bottlebrush-shaped. Minor branches subdivided dichotomously, up to fourth order, with the first branch internode 20–30 mm long. Branching sequence 1/3R in two large branches and 2/5R in the basal stem. Polyps 2–4 mm tall with a thin neck. Sclerites of polyp body of large and thick rods and spindles with many warts. Small scales and rods in tentacles with many warts. Scales in coenenchyme elongate with irregular edges and a few warts.

Description. Specimen about 73 cm long with the holdfast not recovered. Main stem forming two large bottlebrush-shaped branches whose axis has a brown metallic luster. The larger branch is 49 cm long and the other 45 cm long. The basal stem about 24 cm long and 4 mm in diameter (Fig. 2C). Branching sequences differing from bottom to top, 2/5R in the basal stem and 1/3R in the two large branches. Branches subdivided dichotomously, up to fourth order, the first branch internodes 20–30 mm long, with the terminal branchlets up to 41 mm. Distance between adjacent branches 8–12 mm, and orthostiche intervals about 30 mm in the two large branches and 50 mm at the bottom. Polyps thin, about 2–4 mm long and 1.0–1.5 mm wide at bases, with a long neck less than 1 mm wide. Two to four polyps on the first internodes, up to eight on terminal branchlets (Fig. 2D). No polyps on main axis internodes.

Rods and spindles of base of polyp body wall large and thick, rarely branched, with many warts on surface, and measuring 247–628 × 109–180 μm, with an average of 430 × 136 μm (Figs 2D, 3C). Rods and spindles longitudinally arranged in the polyp neck extending to the rachis of tentacles, all covered with many warts, and measuring 95–520 × 25–96 μm, with an average of 304 × 54 μm (Fig. 3A). Scales of pinules small with coarse edges and many warts on surface, and measuring 114–214 × 29–49 μm, with an average of 146 × 36 μm (Fig. 3B). Scales elongated and flat in coenenchyme with dentate edges and a few warts, and measuring 139–553 × 35–87 μm, with an average of 267 × 61 μm (Fig. 3D).

Etymology. The Latin adjective *ramificans* (branching) refers to the ramous structure of the stem.

Distribution. Found only from the Kocebu Guyot with water depth of 1831 m.

Habitat. Colony attached to a rocky substrate with a small, oval-shaped holdfast (Fig. 2A).

Remarks. *Chrysogorgia ramificans* sp. nov. differs from all known congeners by its main stem, with 2/5R branching sequence, forming two large bottlebrush-shaped branches with 1/3R branching sequence (Cairns 2001, 2018; Pante and Watling 2011). The new species belongs to the *Chrysogorgia* “group A, Spiculosae”, in which it mostly resembles *C. monticola* Cairns, 2007. However, *C. ramificans* sp. nov. differs from *C. monticola* by the much longer interval of adjacent branches (8–12 mm vs. 4–5 mm), longer first internode of branch (20–30 mm vs. 12 mm), larger polyps (2–4 mm vs. 1.1 mm in height), much wider rods (109–180 μm vs. 50–80 μm) with thick ends and warty surface in the body walls (vs. with thin ends and usually smooth surface), and small and unbranched rods at the tentacle base (vs. large and lobed) (Cairns 2007).

Within the group A, *Chrysogorgia ramificans* sp. nov. is also similar to *C. arborescens* Nutting, 1908, *C. tuberculata* Cordeiro et al., 2015 and *C. terasticha* Versluys, 1902. However, the new species differs from *C. arborescens* by its much longer interval of adjacent branches (8–12 mm vs. 3 mm), the higher number of polyps in the distal branchlets (up to 8 vs. 2), and usually regular sclerites (vs. irregular) (Nutting 1908). It differs from *C. tuberculata* by the larger orthostiche intervals (30–50 mm vs. 8–23 mm), rods with numerous coarse warts (vs. spindles with acute and sparse

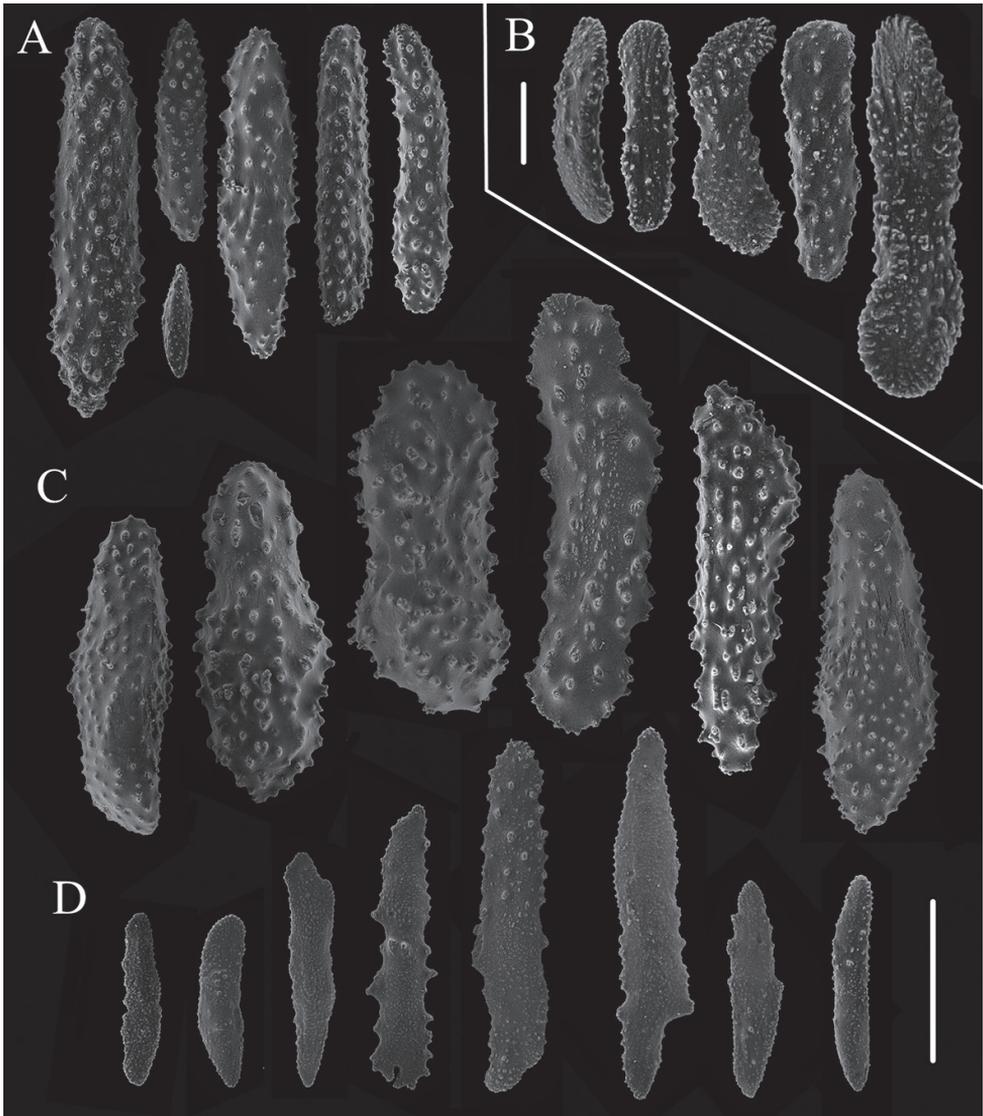


Figure 3. Sclerites of *Chrysogorgia ramificans* sp. nov. **A** Sclerites of polyp neck extending to the rachis of tentacles **B** Sclerites in the pinnules **C** Sclerites at the body base **D** Sclerites of coenenchyme. Scale bars: 200 μm (**A**, **C**, **D**); 50 μm (**B**).

warts), rods present in tentacles (vs. only scales), and scales in coenenchyme with inconspicuous warts (vs. prominent) (Cordeiro et al. 2015). The new species can be easily distinguished from *C. terasticha* by its branching sequence (1/3R at top and 2/5R at bottom vs. 1/4L), larger orthostiche intervals (30–50 mm vs. no more than 8 mm), no nematozooids in coenenchyme (vs. many), larger polyps (2–4 mm vs. no more than 1.6 mm), larger rods with various ends (vs. smaller with rounded ends), and the absence of scales at the polyp base (vs. presence) (Versluys 1902).

***Chrysogorgia binata* sp. nov.**

<http://zoobank.org/D9FCB01F-49B7-4BBA-B3F0-D40026DB6519>

Figs 4–7; Table 1

Holotype. MBM286346, station FX-Dive 173 (17°28.69'N, 153°09.95'E), Kocebu Guyot, depth 1669 m, 7 April 2018. GenBank accession number: MK431862.

Diagnosis. *Chrysogorgia* (“group C, Squamosae typicae”) with a biflabellate colony and a short main stem. Polyps 3–5 mm tall. Scales smooth and thin in the basal part of polyps body with various shape, up to 1 mm long. Scales in the upper part of polyps of various shapes, converged to form an inconspicuous and blunt point at the base of a naked tract below each tentacle. Scales bluntly lancet-shaped, often with numerous coarse granules, longitudinally arranged around both sides of each naked tract. Scales irregular and coarse, usually with lobed edges in the back of tentacles. Scales of coenenchyme slipper-shaped with a medial contraction. Nematozooids absent from coenenchyme.

Description. Specimen with two attached individuals of the crustacean genus *Galathea* Fabricius, 1793 (Fig. 4C). Main stem short with a principal bifurcation, forming two roughly parallel, fans (Fig. 4A, F). Calcareous holdfast small and white, about 7 mm in diameter (Fig. 4F). Major branches of each fan subdivided dichotomously or sympodially. Specimen about 16 cm long and 15 cm wide with a brilliant golden axis, and the stem about 1.5 mm in diameter at base (Fig. 4C). Internodes about 5–9 mm long, each with a single polyp except the terminal twigs, which may sometimes have two polyps. Polyps large and orange in situ, about 3–5 mm tall by 1–2 mm wide, with sclerites forming an inconspicuous blunt point at the base of a naked tract below each tentacle (Figs 4D, 5A). Terminal polyps usually with a long and narrow body (Fig. 4E).

In the basal part of the polyp body, the sclerites comprise transversally arranged, large, smooth scales. They represent a variety of shapes, a few with broad marginal lobes, length by width measuring 216–936 × 58–283 μm, with an average of 549 × 166 μm, (Figs 5B, 6A). There are also scales in the upper part of polyps of various shapes, sometimes thick and with a medial contraction, often sharp at one end, broad and lobed at the other, which combine to form an inconspicuous and blunt point at the base of a naked tract below each tentacle; measuring 275–635 × 77–254 μm, with an average of 451 × 151 μm (Figs 5B, 6B). Above these points are irregular, elongate or lancet-shaped scales mostly with coarse granules on surface, that are arranged longitudinally around the sides of each naked tract. The scales measure 337–650 × 45–85 μm with an average 431 × 70 μm (Figs 5B, 7A). The scales in the back of tentacles are coarse, of various shapes, mostly flat and lobed, and densely and transversally arranged, measuring 88–352 × 19–149 μm, with an average of 183 × 55 μm (Figs 5A, 7B). The scales in the pinnules are curved at an obtuse angle, and are sometimes thick with a few lobes on their edges; measuring 87–196 × 19–34 μm, with an average of 152 × 27 μm (Fig. 7D). The scales of the coenenchyme are generally slipper-shaped, some elongate elliptical, occasionally with indentations in their edges, and measure 138–361 × 40–87 μm, with an average of 222 × 56 μm (Fig. 7C). The coenenchyme is thin with no nematozooids.

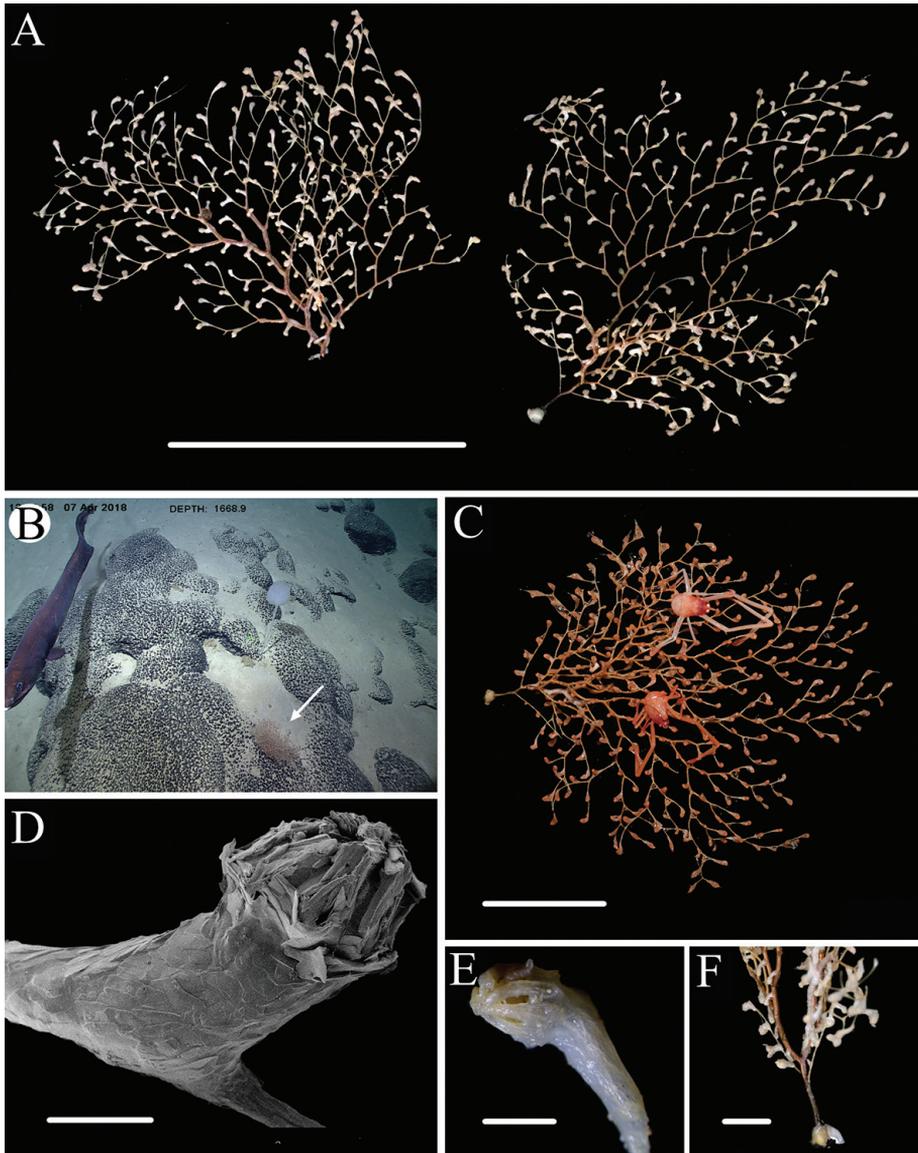


Figure 4. External morphology of the holotype and polyps of *Chrysogorgia binata* sp. nov. **A** Two planar fans of the colony after fixation **B** The holotype (arrow) in situ. Laser dots spaced at 33 cm used for measuring dimensions **C** The colony after collection **D** A single polyp under SEM **E** Single terminal polyp under light microscope **F** Short trunk with the first bifurcation of branches after fixation. Scale bars: 10 cm (**A**); 5 cm (**C**); 1 mm (**D, E**); 1 cm (**F**).

Etymology. The Latin adjective *binatus* (binate) refers to the biflabellate structure of the species.

Distribution. Found only from the Kocebu Guyot in the Magellan seamounts with water depth of 1669 m.

Table 1. Comparison of *Chrysozorgia* species with planar structure. “–” means missing data.

Characters/ Species	<i>C. binata</i> sp. nov.	<i>C. chryseis</i>	<i>C. desbonni</i>	<i>C. electra</i>	<i>C. pinnata</i>	<i>C. scintillans</i>	<i>C. stellata</i>	<i>C. upsilonia</i>
Group type	C	B	A	C	A	C	B	D
Colony shape	biflabellate	biflabellate	biflabellate	biflabellate	flabellate	biflabellate or multiflabellate	multiflabellate	flabellate
Internode length (mm)	5–9	5	3–4	6–12	3–3.5	6–7	8–10	4–30
Polyp height (mm)	3–5	up to 2	up to 2.8	1.75–2.00	up to 2.8	up to 2.75	2–4	up to 4
Eight points beneath the tentacles	short and blunt	long and sharp	inconspicuous	inconspicuous	inconspicuous	short and blunt	long and sharp	inconspicuous
Sclerites in body wall	scales various shape with low and broad marginal lobes	scales terete, tapering smoothly toward pointed ends with weak and broad marginal lobes	spindles often curved, somewhat flattened	scales elongate with narrow prominent marginal lobes	rods elongate with flattened tips	scales various shape with low and broad marginal lobes	scales terete with broad marginal lobes	spindles tuberculate
Maximum length of scale in body wall (mm)	0.93	0.7	0.75	0.6	0.56	0.65	1.1	0.67
Sclerites in tentacles	scales	rods and scales	rods and scales	scales	rods	scales	rods and scales	scales
Maximum length of rods in tentacles (mm)	0.65	0.3	0.24	–	0.21	–	0.5	0.16
Scale shape in coenenchyme	mainly slipper shape	various shape with prominent marginal lobes	elongate, warty with irregular margins	elongate, tapered with prominent marginal lobes	relatively smooth with finely serrate edges	mainly slipper shape	elongate with more or less marginal lobes	with serrate margins
Nematozooids on stem and large branches	absent	absent	–	absent	–	absent	conspicuous	–
Distribution	Western Pacific	Western Pacific	Western Atlantic	Western Pacific	Eastern Pacific	Central and Eastern Pacific	Central Pacific	South Atlantic
References	Present study	Bayer and Stefani 1988	Cairns 2001	Bayer and Stefani 1988	Cairns 2007	Bayer and Stefani 1988, Cairns 2018	Nutting 1908, Bayer and Stefani 1988	Cordeiro et al. 2015

Habitat. Colony attached to a rocky substrate with a small holdfast (Fig. 4B).

Remarks. Within the known species of *Chrysozorgia*, seven species mainly possess a planar structure (Table 1). Among these, including our specimen, *C. desbonni* Duchassaing & Michelotti, 1864 and *C. pinnata* Cairns, 2007 belong to the *Chrysozorgia* “group A, Spiculosae”; *Chrysozorgia chryseis* Bayer & Stefani, 1988 and *C. stellata* Nutting, 1908 belong to the “group B, Squamosae aberrantes”. *Chrysozorgia binata* sp. nov.; *C. electra* Bayer & Stefani, 1988 and *C. scintillans* Bayer & Stefani, 1988 belong to the “group C,

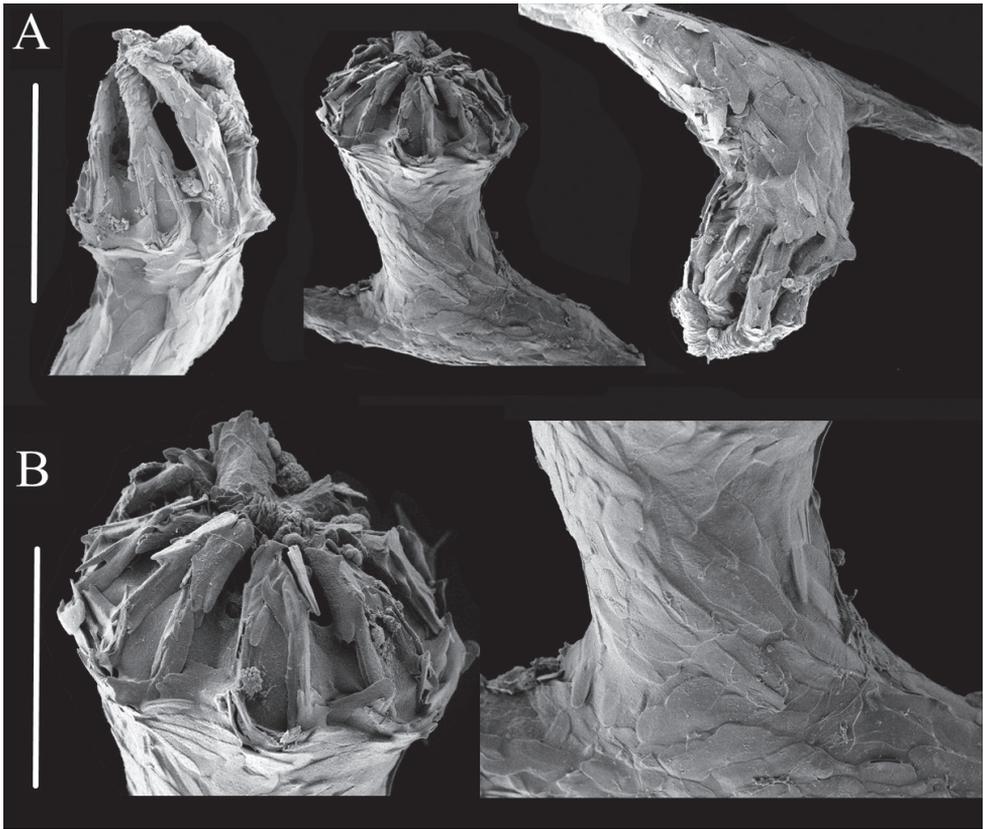


Figure 5. Polyps of *Chrysogorgia binata* sp. nov. **A** Three polyps under SEM **B** Head and body of one polyp under SEM. Scale bars: 2 mm (**A**); 1 mm (**B**).

Squamosae typicae”. The species *C. upsilonia* Cordeiro, Castro & Pérez, 2015 belongs to the “group D, Spiculosae aberrantes”. Based on the arrangement of the sclerites, *Chrysogorgia binata* sp. nov. can easily be distinguished from the species in groups A, B and D.

Both *Chrysogorgia binata* sp. nov. and *C. electra* have a biflabellate colony. However, the new species differs from the latter by its larger polyps (3–5 mm vs. generally 1.75–2.00 mm in height), eight short and blunt points beneath the tentacles (vs. inconspicuous), scales of various shapes with low and broad marginal lobes in the body wall (vs. elongate with narrow prominent marginal lobes), scales mainly slipper-shaped in coenenchyme (vs. elongate tapered with prominent marginal lobes) (Bayer and Stefani 1988). *Chrysogorgia binata* sp. nov. differs from *C. scintillans* by its larger polyps (3–5 mm vs. up to 2.8 mm in height), larger sclerites in the body wall (maximum length 0.93 mm vs. 0.65 mm), scales in the upper part of polyps (irregular and usually with sharp end vs. regular and usually with smooth end), scales around the sides of each naked tract (lancet-shaped and usually with coarse granules vs. twisted, flat and often lobed) (Bayer and Stefani 1988, Cairns 2018).

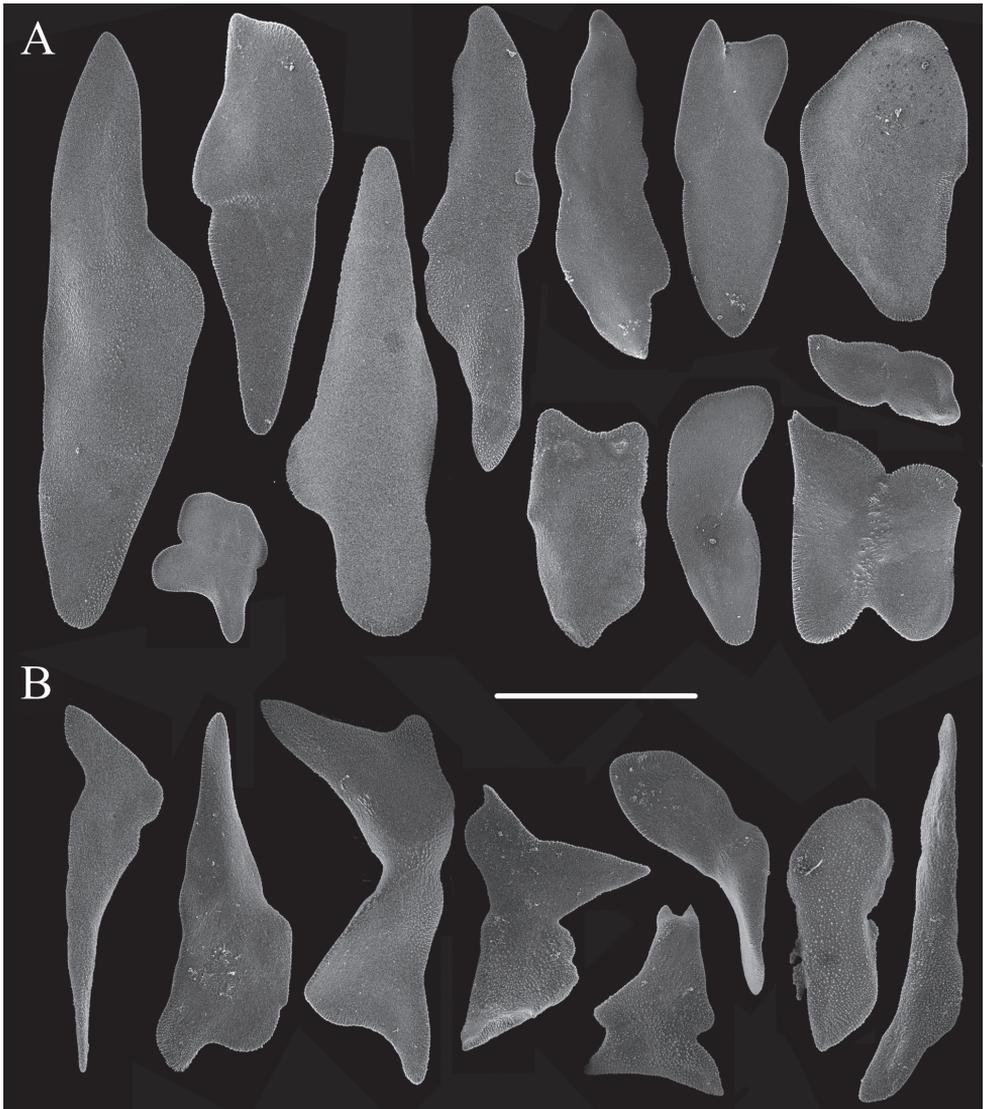


Figure 6. Sclerites of *Chrysogorgia binata* sp. nov. **A** Sclerites in the basal part of the polyp body **B** Sclerites in the point at the base of a naked tract below each tentacle. Scale bar: 300 μ m (all at the same scale).

Genetic distance and phylogenetic analyses

Two mtMutS sequences of the two new species were obtained and deposited in GenBank, and the accession number and the length are as follows: MK431863, 695 bp for *C. ramificans* sp. nov.; and MK431862, 690 bp for *C. binata* sp. nov. The alignment datasets each comprised 649 nucleotide positions. The mtMutS genetic distances among the species of *Chrysogorgia* range from 0.16% to 2.94%, while the intraspecific distances within *C. binata* sp. nov., *C. tricaulis*, *C. artospira*, *C. averta*, *C. abludo* and

Table 2. Interspecific and intraspecific uncorrected pairwise distances at mtMutS of species of *Chrysogorgia* and *Radicipes*.

	Species/ populations	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	<i>Chrysogorgia ramificans</i> sp. nov. MK431863	–													
2	<i>C. binata</i> sp. nov. MK431862	2.28%	–												
3	<i>C. cf. stellata</i> JN2279201	2.12%	0.16%	–											
4	<i>C. tricaulis</i> JN227990, JN227991, JN227998, GQ180123–31, EU268056	0.82%	1.79%	1.63%	0										
5	<i>C. artospira</i> GQ180132-5, GQ353317	0.65%	1.63%	1.47%	0.16%	0									
6	<i>C. artospira</i> GQ868346	0.82%	1.79%	1.63%	0.33%	0.16%	–								
7	<i>C. averta</i> KC788265, GQ180136	0.98%	1.96%	1.79%	0.49%	0.33%	0.49%	0							
8	<i>C. abludo</i> GQ180139, JN227999	1.47%	2.45%	2.28%	0.98%	0.82%	0.98%	1.14%	–						
9	<i>C. abludo</i> GQ180138	1.96%	2.94%	2.77%	1.47%	1.31%	1.47%	1.63%	0.49%	–					
10	<i>C. chryseis</i> DQ297421, JN227992	2.28%	0.49%	0.33%	1.79%	1.63%	1.79%	1.96%	2.45%	2.94%	–				
11	<i>C. pinnata</i> JN227988	0.65%	1.63%	1.47%	0.16%	0.00%	0.16%	0.33%	0.82%	1.31%	1.63%	–			
12	<i>C. monticola</i> JN227989	0.33%	2.28%	2.12%	0.82%	0.65%	0.82%	0.98%	1.47%	1.96%	2.28%	0.65%	–		
13	<i>Radicipes stonei</i> MG986912	2.28%	2.61%	2.45%	1.79%	1.63%	1.79%	1.96%	2.45%	2.94%	2.61%	1.63%	2.28%	–	
14	<i>Radicipes gracilis</i> JN227987	1.79%	2.12%	1.96%	1.31%	1.14%	1.31%	1.47%	1.96%	2.45%	2.12%	1.14%	1.79%	1.14%	–

C. chryseis are in the range 0–0.16% (Table 2). Thus, there is no distinct barcoding gap between the intra- and interspecific distances. The genetic distances between the new species *C. ramificans* sp. nov. and the known sequences of the congeners range from 0.33%–2.28%, and those between *C. binata* sp. nov. and the congeners are in the range of 0.33%–2.94% (Table 2).

The ML and BI phylogenetic trees of the mtMutS gene were nearly identical in topology and thus were combined into a consensus tree with both support values (Fig. 8). All the *Chrysogorgia* species were separated into two main groups (Fig. 8). Group I includes *C. binata* sp. nov., *C. cf. stellata* and *C. chryseis*, and Group II contains the subclades *C. ramificans* sp. nov. + *C. monticola*, *C. artospira*, *C. pinnata*, *C. averta*, *C. abludo*, *C. tricaulis* and *C. monticola*.

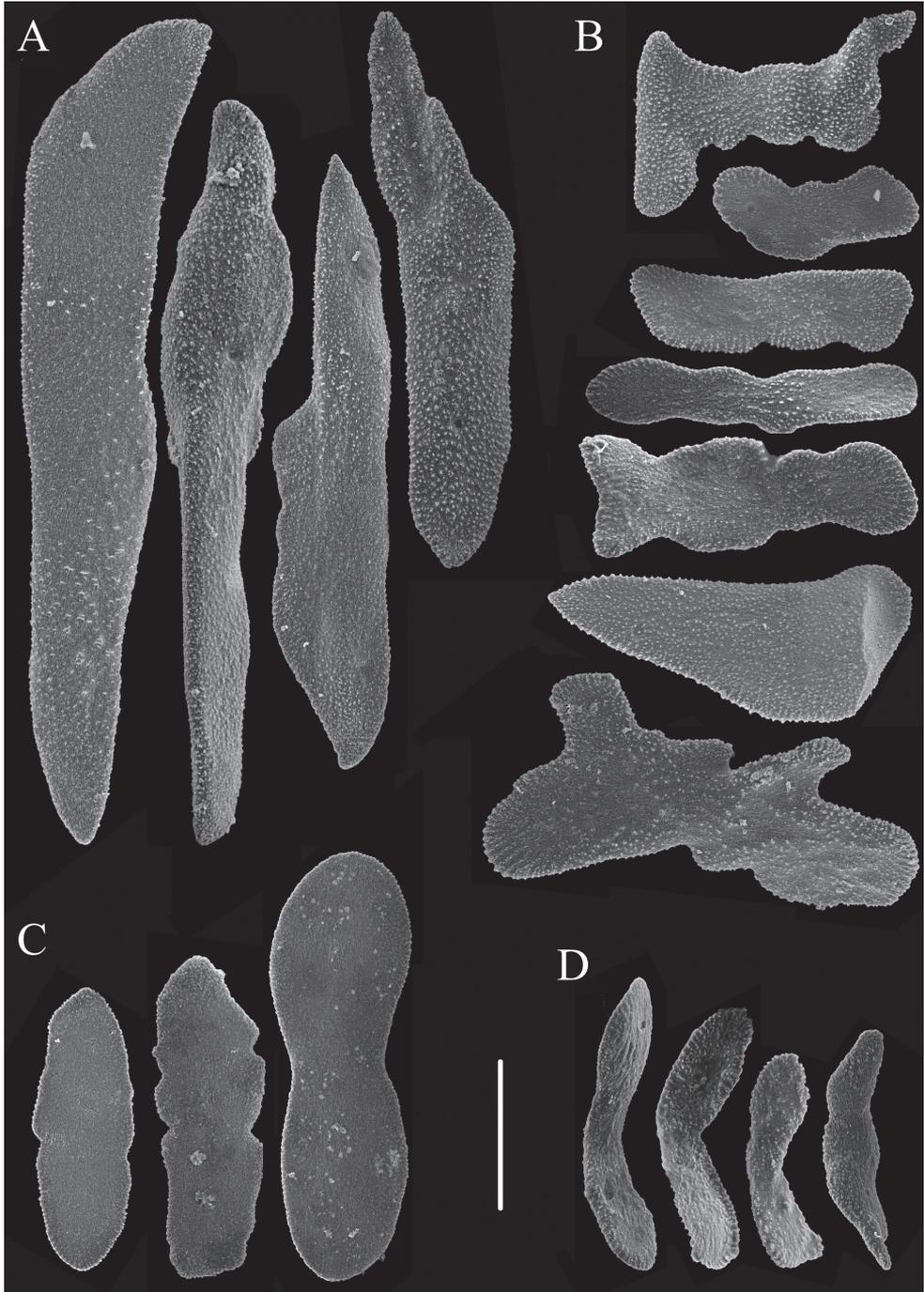


Figure 7. Sclerites of *Chrysogorgia binata* sp. nov. **A** Sclerites around the sides of each naked tract **B** Sclerites in the back of tentacles **C** Sclerites of the coenenchyme **D** Sclerites in pinnules. Scale bar: 100 μ m (all at the same scale).

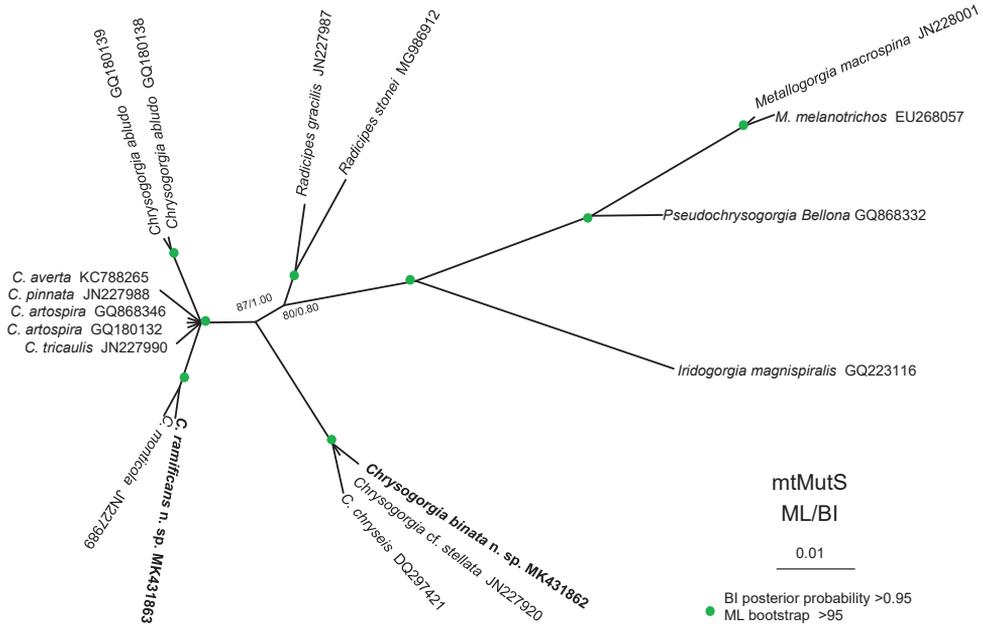


Figure 8. Unrooted maximum likelihood (ML) tree inferred from the mtMutS sequences of *Chrysogorgia* and related genera and species. Numbers at the nodes represent ML and Bayesian inference (BI) support values, respectively. Newly sequenced species are in bold.

Discussion

Chrysogorgia ramificans sp. nov. mostly resembles *C. monticola* Cairns, 2007, which is also strongly supported by the phylogenetic tree and their genetic distance. However, the two species can be easily separated, as discussed above. In the phylogenetic trees, *C. binata* sp. nov., *C. cf. stellata* Bayer & Stefani, 1988 and *C. chryseis* Bayer & Stefani, 1988 formed a single clade with high support, indicating their close relationships (Fig. 8). However, *C. binata* sp. nov. belongs to the *Chrysogorgia* “group C, Squamosae typicae”, while *C. chryseis* belongs to “group B, Squamosae aberrantes” (Table 1; Bayer and Stefani 1988). Bayer and Stefani (1988) also reported a specimen they recorded as *C. cf. stellata* Nutting, 1908, which was based only on some detached branches. *Chrysogorgia binata* sp. nov. differs from *C. cf. stellata* by its larger polyps (3–5 mm vs. about 2 mm), many elongate or lancet-shaped scales below the tentacle base (vs. one or two rods with coarse granules), short, squarish or polygonal scales in the polyp body wall (vs. narrow and long), regular slipper-shaped scales in coenenchyme (vs. relatively irregular) (Bayer and Stefani 1988). No sequences are available for *Chrysogorgia stellata* Nutting, 1908, a species possessing a multilabellate colony form, while *C. binata* sp. nov. is bilabellate. The new species differs from *C. stellata* also in the shorter and more blunt points beneath the tentacles (vs. long and sharp), various shapes of scales in the upper part of the body wall (vs. only a single slenderly elongate shape), and generally slipper-shaped scales in coenenchyme (vs. elongate with more lobed margin).

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Anastatus Motschulsky (Hymenoptera, Eupelmidae): egg parasitoids of *Caligula japonica* Moore (Lepidoptera, Saturniidae) in China

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Abstract

Four species of *Anastatus* Motschulsky (Hymenoptera, Eupelmidae, Eupelminae) are newly reported as egg parasitoids of the Japanese giant silkworm, *Caligula japonica* Moore and, as an alternate laboratory host, the Chinese oak silk moth, *Antheraea pernyi* (Guérin-Méneville) (Lepidoptera, Saturniidae) in China. The four species, *A. fulloi* Sheng & Wang, 1997, *A. gansuensis* Chen & Zang, **sp. nov.**, *A. japonicus* Ashmead, 1904, and *A. meilingensis* Sheng, 1998, were reared initially from eggs of *C. japonica* collected in Gansu, Jilin and Liaoning provinces and subsequently cultured in the laboratory on eggs of *A. pernyi*. An illustrated key to differentiate females of the four species, and males of some of the species is provided. Key features are illustrated, both sexes of the new species are described, and diagnoses of females of the other species are given.

Keywords

Antheraea pernyi, Chalcidoidea, natural enemy, new species, parasitic wasp, taxonomy

Introduction

The Japanese giant silkworm, *Caligula japonica* Moore, 1862 (Lepidoptera, Saturniidae), is a widely distributed indigenous species in China, being reported previously from both Palaearctic (Heilongjiang, Jilin, Liaoning, Hebei, Shandong, Shaanxi) and Oriental (Chongqing, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangxi, Sichuan, Taiwan, Yunnan, Zhejiang) provinces (Qiao et al. 2014), as well as from Japan, North Korea and Russia (Li et al. 2009). Host plants include 38 species belonging to 30 genera in 20 families, including walnut (*Juglans regia* L., 1753), ginkgo (*Ginkgo biloba* L., 1771), chestnut (*Castanea mollissima* Bl., 1851), plum (*Prunus* spp. L., 1753), apple (*Malus pumila* Mill., 1768), sumac (*Toxicodendron vernicifluum* (Stokes) F. A. Barkley, 1937), pear (*Pyrus* spp. L., 1753) and persimmon (*Diospyros kaki* Thunb., 1780) (Yang et al. 2008; Liu and Feng 2013). In China, its most serious economic impact is as a defoliator of walnut and chestnut trees, causing millions of U.S. dollars of damage annually (Qiao et al. 2014). Currently, chemical pesticides are the most commonly used method to control this pest. Although some biological control techniques have been attempted, such as spraying the fungus *Beauveria bassiana* (Bals.-Criv.) Vuill., 1912, these have yielded unsatisfactory results (Liu and Luo 2008). Therefore, development of safe and effective methods for biological control of this pest is needed on an urgent basis.

Prior to the present study, seven species of Chalcidoidea (Hymenoptera) have been reported as parasitoids of *C. japonica* (sometimes cited under the combination *Dicthyoploca japonica*), including a single species of Chalcididae (*Kriechbaumerella hofferi* (Bouček, 1952)), another of Encyrtidae (*Ooencyrtus dicthyoplocae* Sharkov, 1995) and Eulophidae (*Aprostocetus brevipedicellus* Yang & Cao, 2015), two species of Eupelmidae (*Mesocomys albitarsis* (Ashmead, 1904) and *M. kalinai* Ozidikmen, 2011) and of Trichogrammatidae (*Trichogramma chilonis* Ishii, 1941 and *T. dendrolimi* Matsumura, 1926) (Noyes 2019). Of these, *T. chilonis* and *T. dendrolimi* as well as a *Kriechbaumerella* sp. are also reported as parasitoids of the Chinese oak silk moth, *Antheraea pernyi* (Guérin-Méneville, 1855) (Lepidoptera, Saturniidae) (Noyes 2019).

Species of *Anastatus* Motschulsky (Eupelmidae, Eupelminae) are mostly primary parasitoids of the eggs of other insects, although a few have been reared as secondary parasitoids (hyperparasitoids) of Lepidoptera eggs through Scelioninae (Platygasstroidea, Platygastridae) and Ichneumonidae (Ichneumonoidea) primary parasitoids, and some have been reared from Coleoptera larvae and Diptera puparia (Gibson 1995; Noyes 2019). Currently, about 150 valid species of *Anastatus* are described, making it the second largest genus of Eupelminae after *Eupelmus* Dalman (Noyes 2019). Seventeen described species are reported from China, all from the subgenus *A.* (*Anastatus*). Of these, Noyes (2019) listed 13 species from mainland China plus one other species (*A. formosanus* Crawford, 1913) separately from Taiwan. However, Noyes (2019) did not include *A. kashmirensis* Mathur, 1956, reported by Hu et al. (2011) or *A. acherontiae* Narayanan, Subba Rao & Ramachandra, 1960 and *A. gastropachae* Ashmead, 1904, reported by Yang et al. (2015), from mainland China. Although the *Anastatus*

fauna of China has yet to be revised comprehensively, Yang et al. (2015) provided a key and imaged the females of *A. acherontiae*, *A. gastropachae*, and *A. japonicus* Ashmead, 1904, and Peng et al. (2017) provided an illustrated key to females of the six species (*A. dexingensis* Sheng & Wang, 1997, *A. flavipes* Sheng & Wang, 1997, *A. fulloi* Sheng & Wang, 1997, *A. shichengensis* Sheng & Wang, 1997, *A. huangi* Sheng & Yu, 1998, and *A. meilingensis* Sheng, 1998) described originally from China by Sheng and coauthors. Because of the extreme sexual dimorphism that characterizes Eupelminae (Gibson 1986, 1995), species recognition within the subfamily, including in *Anastatus*, has historically been based mostly on females and there is no key to the males that includes any Chinese species.

Several species of *Anastatus* have been used for biological control of various insect pests in China and elsewhere (Stahl et al. 2018), including *A. japonicus* in China against the litchi stink bug, *Tessaratoma papillosa* Stål, 1864 (Hemiptera, Tessaratomidae) (Li et al. 2014), and more recently *A. bifasciatus* (Geoffroy, 1785) in Europe against the invasive brown marmorated stink bug, *Halyomorpha halys* Stål, 1855 (Hemiptera, Pentatomidae) (Hancock et al. 2018; Judith et al. 2018). As part of studies to discover potentially new biological control agents of *C. japonica* in China, members of the Institute of Biological Control, Jilin Agricultural University, collected eggs of this species in Gansu, Jilin and Liaoning provinces and reared these in the laboratory for parasitoids. This resulted in what we provisionally identify as two species of *Mesocomys* Cameron (Eupelmidae, Eupelminae), four species of *Anastatus*, and one species of *Aprostocetus* Westwood (Eulophidae, Tetrastichinae). Here we report on the species of *Anastatus* reared from *C. japonica* eggs, which were cultured subsequently in the laboratory on the eggs of an alternate host, *A. pernyi*. Based on females reared in our surveys we differentiated four morphospecies of *Anastatus* that we identify as three previously described species, *A. fulloi*, *A. japonicus* and *A. meilingensis*, plus one species new to science. Here we describe that new species. The culturing of the other three described species also provided fresh specimens of both sexes. We therefore present an illustrated key to both sexes of the four species of *Anastatus* reared as egg parasitoids of *C. japonica*, except for the males of *A. fulloi* and *A. japonicus* that we cannot reliably differentiate.

Material and methods

Surveys and rearing

Surveys and rearing of specimens on which this study is based were conducted by Yong-Ming Chen, Lian-Sheng Zang and Asim Iqbal. Surveys were conducted in three provinces in China that were infested with *C. japonica*, one province in north-central China (Gansu, Kangxian County, 33°26'33.81"N, 105°41'52.10"E in January to March, 2017 and 2018) and two provinces in north-eastern China (Jilin, Changchun City, 43°48'29.09"N, 125°24'14.03"E in July to August, 2017, and Liaoning, Benxi

County, 41°14'56.40"N, 124°28'55.06"E in March, 2017). The overwintering egg masses collected in the field surveys were placed into glass tubes and the opening of each tube covered with a small piece of cloth. The glass tubes were kept in a rearing chamber kept $25 \pm 1^\circ \text{C}$ and $70 \pm 5\% \text{RH}$, with a 14 L: 10 D photoperiod until the wasps emerged. The sex ratio = was calculated as the number of females/(number of females + number of males). Emerging wasps were then used to establish colonies at Jilin Agricultural University using fresh and healthy unfertilized eggs of *A. pernyi* that were obtained by dissecting female abdomens as per Li et al. (2018).

Taxonomy

Random samples of reared colony individuals were preserved in 75% ethanol for further use, with specimens used for morphological study and collection deposition either air dried and point-mounted at JLAU or critical-point dried from ethanol and point-mounted at CNC. Species identifications were made using the keys and illustrations provided by Kalina (1981), Narendran (2009), Yang et al. (2015) and Peng et al. (2017), plus examination of type and other identified material in CNC, FAFU and USNM. The description of the new species is based on specimens reared in the laboratory from the eggs of *A. pernyi* that were observed and measured in micrometers using a Keyence VHX-2000 digital microscope at JLAU or with a Nikon SMZ 1500 binocular microscope with an ocular grid having 100 divisions at the CNC. Macro-photographs and plates of illustrations of all treated species were made at the CNC using a Leica DMC5400 20-megapixel camera attached to a Leica Z16 APO motorized macrocope. A Leica, 100-watt halogen light source was used to illuminate specimens for the descriptions whereas three Leica KL2500 LCD fiber optic light sources fitted with 250-watt cold light reflector lamps were used to photograph the specimens. To reduce glare, the halogen light source was filtered through a piece of translucent Mylar tracing acetate taped to the microscope objective, whereas the fiber optic light sources were filtered through a polystyrene foam dome. Because of this, color illustrated in the plates of illustrations may differ somewhat from the described color. Serial images were combined with Zerene Stacker and digitally retouched as necessary using Adobe Photoshop to enhance clarity. Imaged specimens bear a unique 'CNC Photo 2019-x' number label, of which "x" is cited between parentheses in the figure captions to indicate the specimen imaged. All imaged specimens are in the Canadian National Collection of Insects and Arachnids.

Morphological and sculptural terms follow Gibson (1995, 1997, 2011) and Gibson and Fusu (2016). Attributes of both sexes of the new species are described under four separate headings-color, setation, structure, and sculpture, except female fore wing color and setal patterns are described together because the features are strongly correlated. For descriptive purposes, the terms 'dark' and 'pale' are used as general terms relative to one another, with dark referring from dark brown to black, often

also with variably conspicuous metallic lusters, and pale referring to light brown to orange or yellow. The fore wing is subdivided into two regions, the 'basal' and 'discal' regions. The basal region of a macropterous individual is the region basal to the basal fold behind the costal cell whereas the discal region is apical to that fold; in brachypterous females, the basal region is the more hyaline and less setose basal region and the discal region is the more densely setose apical region. Interpretation of the folds and regions of the basal region of the fore wing follows Gibson (2004). Abbreviations used in the text or plates of figures are: **clv** = clava, **cbr** = remnant of hyaline cross band of fore wing, **flx** = flagellomere 1–8, **Gtx** = gastral tergite 1–6, **LOL** = minimum distance between anterior and posterior ocellus, **MPOD** = maximum diameter of posterior ocellus, **mps** = multiporous plate sensilla, **MV** = marginal vein, **OOL** = minimum distance between posterior ocellus and inner orbit, **POL** = minimum distance between posterior ocelli, **pdl** = pedicel, **PMV** = postmarginal vein, **STV** = stigmal vein, **syn** = syntergum. Measurement of scape length does not include the radicle. Width of antennal segments does not include the length of projecting setae (Gibson 1989, 2011). Total body length excludes the ovipositor. Type material of the new species and voucher specimens of the other three treated species of *Anastatus* have been deposited in the following institutions as listed in the species treatments:

- AICF** Al. I. Cuza University, Iași, Romania, Lucian Fusu collection;
BMNH The Natural History Museum, Department of Entomology, London, England;
CNC Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, Ottawa, ON, Canada;
FAFU Biological Control Research Institute, Fujian Agriculture and Forestry University, Fuzhou, China;
JLAU Institute of Biological Control, Jilin Agriculture University, Changchun, China;
IZCAS Institute of Zoology, Chinese Academy of Sciences, Beijing, China;
USNM National Insect Collection, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

Results

Survey and rearing

From the cultures established from the surveys in Gansu Province, seven species were ultimately identified as egg parasitoids of *C. japonica*, belonging to two families and three genera (*Anastatus* (4 spp.) and *Mesocomys* (2 spp.) and *Aprostocetus* (1 sp.)). In 2017, three species were reared, *A. gansuensis* sp. nov., *M. albitarsis* (Ashmead) and *M. trabalae* Yao, Yang & Zhao, 2009. In 2018, all seven species were reared and identified as *A. fulloi*, *A. gansuensis*, *A. japonicus*, *A. meilingensis*, *M. albitarsis*, *M. trabalae*,

and *Aprostocetus brevipedicellus*. The proportion of females to males under natural field conditions was 0.75, 1.00, 0.89, 0.75, 0.66, 0.66, and 0.77, respectively, for the seven species. In 2017 in Jilin Province only *A. japonicus* was reared, whereas in Liaoning province *A. fulloi* and *M. albitarsis* were reared. Their sex ratios were 0.82, 0.73, and 0.68, respectively. All seven species were reared successfully on the eggs of the alternative host, *Antheraea pernyi*, under laboratory conditions.

Systematics

Anastatus (*Anastatus*) *Motschulsky*

Anastatus Motschulsky, 1859: 116. Type species: *Anastatus mantoidae* Motschulsky, by monotypy.

Anastatus (*Anastatus*); Gibson 1995: 105, 111.

Remarks. For a complete listing of the extensive generic synonymy of *Anastatus* see Gibson (1995) and Noyes (2019). Bouček (1977) recognized two subgenera in *Anastatus*, the nominate subgenus and *A.* (*Cladanastatus*) Bouček, 1979). Currently, the nominate subgenus comprises all presently described species except for the type species of *A.* (*Cladanastatus*). Females and males of *Anastatus* and *A.* (*Anastatus*) can be distinguished from those of other genera of Eupelminae using the key by Gibson (1995).

Key to species of *A.* (*Anastatus*) reared from *Caligula japonica* eggs in China

- 1 Female 1
- Male 5
- 2(1) Brachypterous, fore wings extending only to about posterior margin of Gt1 (Fig. 6A–E), with discal region densely setose with slightly lanceolate dark setae except for distinct region of orangish setae medially behind marginal vein and often a very slender, sometimes interrupted remnant of a hyaline cross band distally (Fig. 6E: cbr) *A. meilingensis* Sheng
- Macropterous, fore wings extending to apex of gaster (Figs 1A, 2B, 5A) and discal region with broad hyaline cross band with white setae extending across wing behind marginal vein (Figs 1E, 3A, 5E) 3
- 3(2) Acropleuron often dark anteriorly to about level of base of fore wing but variably paler, light brown to orangish or yellowish over at least posterior half (Fig. 5D) in contrast to dark mesonotum (Fig. 5C) *A. japonicus* Ashmead
- Acropleuron entirely dark and not contrasting in color with mesonotum or at most somewhat paler anteriorly only near prepectus, the paler region not nearly extending to level of base of fore wing (Figs 1D, 2F) 4

- 4(3) Procoxa pale, orangish to yellowish (Fig. 2F), similar in color to lateral surface of pronotum but much lighter than posternum; concave part of mesoscutal medial lobe medially setose with white setae for width only about equal to width of bare region on either side (Fig. 2E); mesotarsus, excluding tarsal pegs, uniformly pale (Fig. 3E); fore wing hyaline cross band with apical margin more strongly V-like angulate than basal margin (Fig. 3A) and usually with a few isolated dark setae medially within hyaline band (Fig. 3C).....
*A. gansuensis* Chen & Zang, sp. nov.
- Procoxa dark, much darker than lateral surface of pronotum and similar in color to prosternum (Fig. 1D); concave part mesoscutal medial lobe setose with white setae for almost entire width (Fig. 1C); mesotarsus with basal two tarsomeres obviously darker than more apical tarsomeres over at least dorsal and posterior surfaces (Fig. 3F, G); fore wing hyaline cross band with apical and basal margins similarly curved and without isolated dark setae within band (Fig. 1E).....*A. fulloi* Sheng & Wang
- 5(1) Clava at least as long as combined length of previous three funiculars (Figs 1H, 5H)..... *A. fulloi* Sheng & Wang and *A. japonicus* Ashmead
- Clava shorter than combined length of previous three funiculars (Figs 4H, 6H)..... **6**
- 6(5) Metatibia mostly similarly dark as femur, pale basally for distance only about equal to own apical width or about one-quarter or less length of tibia (Fig. 4B)*A. gansuensis* Chen & Zang, sp. nov.
- Metatibia with about basal half pale and apical half darker, though lighter brown than femur (Fig. 6F)*A. meilingensis* Sheng

Species treatments

***Anastatus (Anastatus) fulloi* Sheng & Wang**

Fig. 1A–H

Anastatus fulloi Sheng & Wang in Sheng et al., 1997: 61–62, figs 14, 15; holotype (JLAU), examined.

Anastatus fulloi; Peng et al. 2017: 10–13, figs 19–27.

Diagnosis. Female. Macropterous (Fig. 1A); fore wing with broad hyaline cross band behind marginal vein with similarly curved basal and apical margins so band uniformly wide, and without isolated dark setae medially (Fig. 1E). Mesosoma (excluding legs) dark except prepectus and lateral, vertical surface of pronotum entirely or mostly pale (Fig. 1A–D); procoxa dark, similar in color to prosternum and acropleuron (Fig. 1D). Mesoscutum with posterior concave portion almost entire setose (Fig. 1C). Mesotarsus with basal two tarsomeres infuscate over at least dorsal and posterior surfaces, obviously darker than subsequent two tarsomeres (Fig. 3F, G). Antenna with at least apical funicular slightly transverse and previous one or two funiculars subquadrate (Fig. 1B).

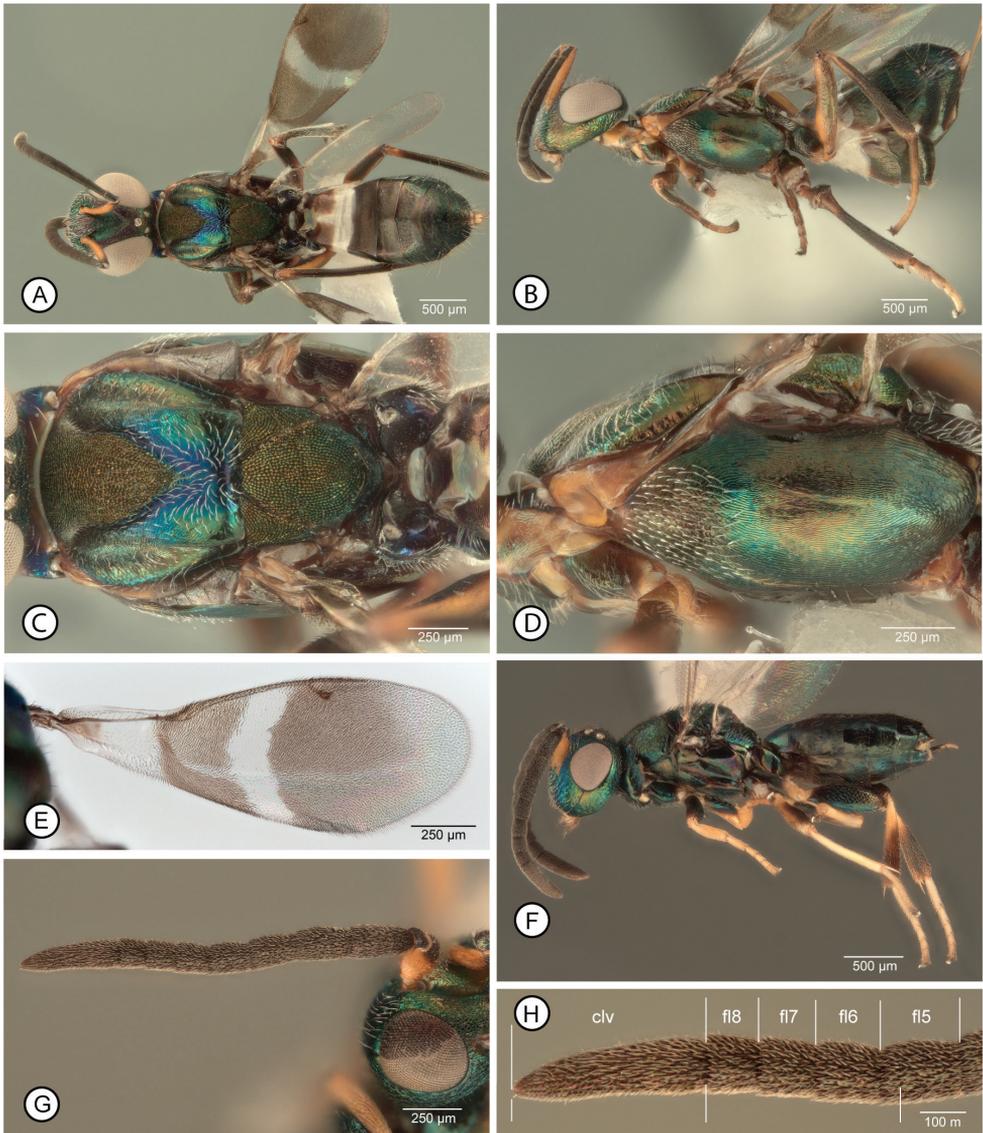


Figure 1. *Anastatus fulloi* **A–E** female: **A** dorsal habitus (13) **B** lateral habitus (12) **C** dorsal mesosoma (13) **D** lateral mesosoma (12) **E** fore wing (14). **F–H** male: **F** lateral habitus (22) **G** antenna (32) **H** clava and apical three funiculars (32) (three lower bars indicate length of clava compared to combined length of apical funiculars). Abbreviations: clv = clava, flx = flagellomere number.

Male. Structure plus color, setal and sculptural patterns (Fig. 1F) similar to those described for *A. gansuensis* except clava at least about as long as combined length of fl6–fl8 + 0.25 apical length of fl5 (Fig. 1G, H), and sometimes as long as combined length of fl5–fl8, with fl8 quadrate to slightly transverse, fl7 quadrate to slightly longer than wide, and fl6 and fl5 longer than wide (Fig. 1H).

Distribution. *Anastatus fulloi* (Genbank accession no. MK604241) was described originally from Jiangxi Province from two localities (Wuyi Mountains and Meiling) (Sheng et al. 1997) as detailed by Peng et al. (1997). We reared it in the field from the following two new localities: **Gansu Province:** Longnan City, collected 23.I.2018, Yong-Ming Chen (1♀, 1♂ AICF; 1♀, 1♂ BMNH; 19♀, 21♂ CNC; 5♀, 3♂ FAFU; 12♀, 7♂ IZCAS; 1♀, 1♂ USNM). **Liaoning Province:** Benxi, Manchu Autonomous Co., Benxi City, Tai Shan Forest Farm, 23.IV.2017, Yong-Ming Chen.

Hosts. The type series was reared from the eggs of the yellow spotted stink bug, *Erthesina fullo* (Thunberg, 1783) (Hemiptera, Pentatomidae). Here we newly report it as an egg parasitoid of the lepidopteran hosts *C. japonica* and, in the laboratory, *A. pernyi*.

Remarks. Of the species we reared from *C. japonica*, females of *A. fulloi* are most similar to those of *A. gansuensis* because they are fully winged (cf. *A. meilingensis*) and have a dark acropleuron that does not contrast with the mesonotum (cf. *A. japonicus*). However, females differ from those of *A. fulloi* in color, setal and structural features, as given in the key to species and diagnoses.

Sheng et al. (1997, fig. 14) and Peng et al. (2017, figs 25, 26) described and illustrated the flagellar structure of males from the type series of *A. fulloi* reared from *E. fullo*; they have the clava at least as long as the previous four segments combined. However, the males we reared with females from *A. pernyi* eggs that we identify as *A. fulloi* have the clava quite obviously shorter than the combined length of the four preceding funiculars (Figs 1G, H), being about as long as the combined length of fl6–fl8 plus about the apical quarter (Fig. 1H) to half of fl5. The difference between the flagellar structures observed between our males and those of the type series could be explained by populational or host correlated differences or, perhaps, indicate two cryptic species that are more host-taxon restricted than is currently considered for *A. fulloi*. Regardless, compared to males of *A. gansuensis* (Fig. 4H) and *A. meilingensis* (Fig. 6H), males of *A. fulloi* (Fig. 1H) have somewhat short apical funiculars so that the clava relative to the combined length of the funiculars is greater. Because of similarity in their flagellar structures (cf. Figs 1H, 5H) we cannot currently reliably differentiate *A. fulloi* from *A. japonicus* males.

***Anastatus (Anastatus) gansuensis* Chen & Zang, sp. nov.**

<http://zoobank.org/B1EC8F11-A663-4F1B-B6E2-189224691C5F>

Figs 2A–I, 3A–G, 4A–H

Type material. *Holotype* ♀ (FAFU), Shanchacun, Yuntai Town, Kangxian County, Gansu Province, China, 01.III.2017, Yong-Ming Chen. *Allotype* ♂ (FAFU), same data as holotype (from subsequently established colony on *A. pernyi*).

Additional paratypes. Same data as holotype (2♀, 1♂ FAFU). Gansu Province, Kang Co., Longnan City, collected 23.I.2018, Yong-Ming Chen, ex. *Caligula japonica* Moore egg, lab reared on eggs of *Antheraea pernyi* (Guérin-Méneville) (Lep: Saturniidae) (2♀, 1♂ AICF; 2♀, 1♂ BMNH; 67♀, 18♂ CNC; 5♀, 3♂ FAFU; 6♀ IZCAS; 2♀, 1♂ USNM).

Diagnosis. Female. Macropterous (Fig. 2B); fore wing with broad hyaline cross band behind marginal vein and with apical margin more distinctly angulate (V-like) than basal margin such that length of hyaline band along marginal vein about twice medial length of hyaline band (Fig. 3A), and usually with a few isolated dark setae within band medially (Fig. 3C). Mesosoma (excluding legs) dark except prepectus and pronotum or at least lateral, vertical surface of pronotum, pale (Fig. 2A, B, E, F); procoxa pale, similar in color to lateral surface of pronotum (Fig. 2F). Mesoscutum with posterior concave portion setose only medially, width of setose region about equal to width of bare region on either side (Fig. 2E). Mesotarsus (excluding tarsal pegs) with at least basal four tarsomeres similarly pale (Fig. 3C). Antenna with all funiculars (fl2–fl8) longer than wide (Fig. 2G–I).

Male. Legs with all femora mostly to entirely dark, mesotibia entirely pale, and metatibia mostly dark, pale basally for a distance at most equal to about apical width or about one-quarter length of tibia (Fig. 4B). Flagellum (Fig. 4G) with clava distinctly shorter than combined length of fl6–fl8, with at least fl6 and fl7 obviously longer than wide (Fig. 4H).

Description. Female (habitus, Fig. 2A, B). Body length 2.2–3.9 mm.

Color. Head (Fig. 2C, D) mostly metallic green to bluish-green but with variably extensive and distinct coppery to reddish-violaceous lusters on at least parascrobal region and interantennal prominence (Fig. 2C) and sometimes on frontovertex, including ocellar triangle (Fig. 2D). Maxillary and labial palpi dark brown. Antenna with scape mostly pale, yellowish (Fig. 2G), except variably extensively darker brown and sometimes with slight metallic luster dorsoapically (Fig. 2C); pedicel and flagellum dark brown (Fig. 2G–I).

Pronotum mostly pale (Fig. 2E, F) except black posterolaterally anterior each spiracle, and concave, dorsomedial part often variably darker brownish (Fig. 2A, E); propleuron similarly pale as lateral surface of pronotum but prosternum dark. Mesonotum (Fig. 2E) mostly dark with metallic green to bluish-green lusters similar to head except convex part of mesoscutal medial lobe and/or scutellar-axillar complex anteriorly usually with slight coppery to reddish lusters, and concave posteromedial part of mesoscutum more distinctly blue to purple adjacent to medially dark violaceous to black setose part (Fig. 2E). Prepectus similarly pale as lateral surface of pronotum (Fig. 2F). Tegula dark brown. Mesopleurosternum mostly dark with greenish luster except variably distinctly paler anterodorsally near prepectus, the paler region at most extending to or slightly beyond level of base of tegula, and strigose medial part of acropleuron usually with slight coppery to reddish-violaceous lusters under some angles of light (Fig. 2F). Front leg with coxa, trochanter and trochantellus similarly pale as lateral surface of pronotum and prepectus; femur with anterior and/or dorsal surfaces and tibia with anterior surface similarly pale as coxa, but otherwise variably dark brown; tarsus with at least apical tarsomere brown but basal four tarsomeres pale to variably dark brown. Middle leg with trochanter and trochantellus at least ventrally dark; femur with dorsal surface longitudinally dark at least posteriorly, but ventral surface and usually dorsal surface anteriorly paler, and more whitish-translucent, anteroapically; tibia dark dorsally but paler basally, ventrally, and apically except for dark apical pegs; mesotibial spur

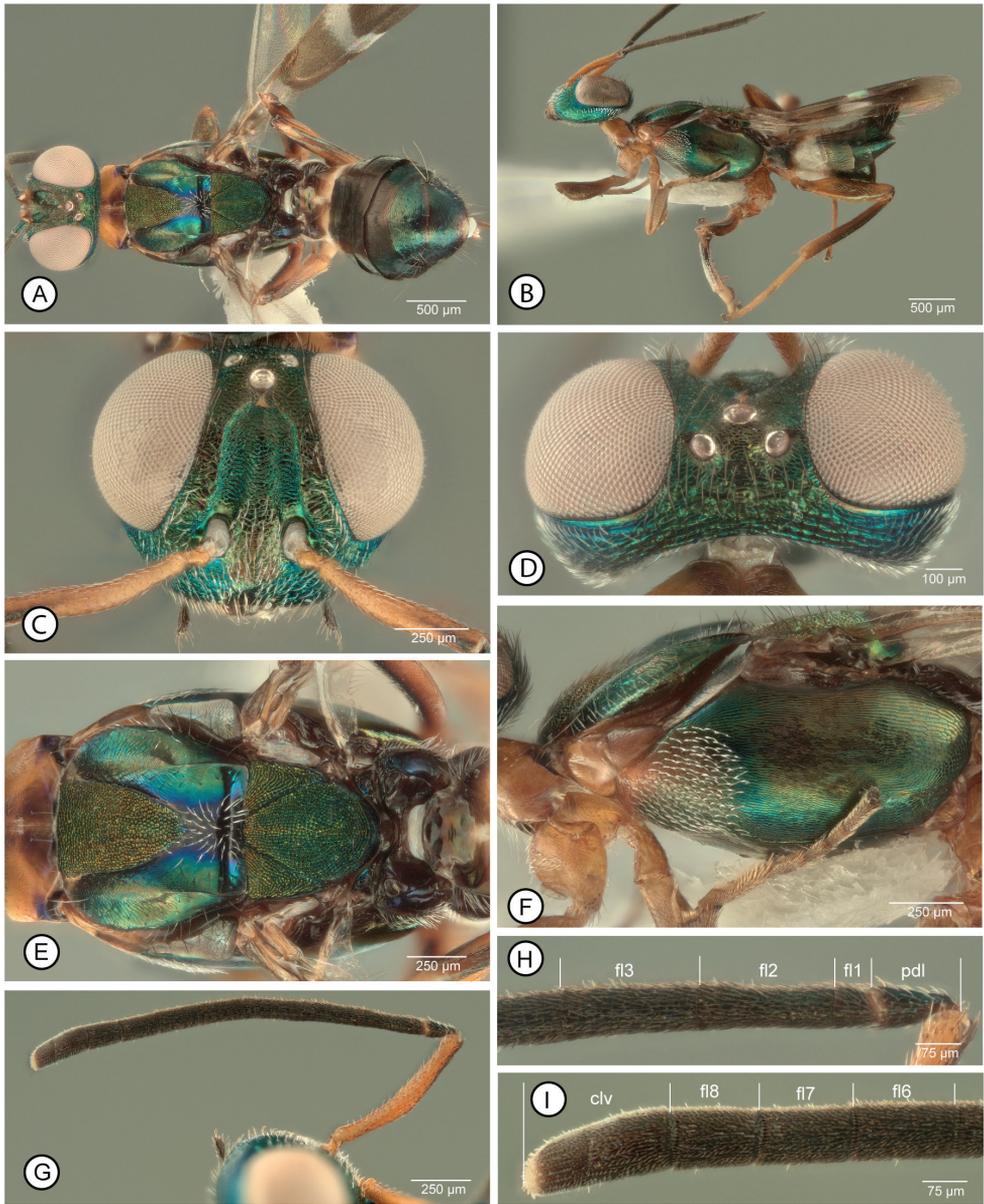


Figure 2. *Anastatus gansuensis*, female **A** dorsal habitus (9) **B** lateral habitus (7) **C** head in frontal view (10) **D**, same in dorsal view (9) **E** mesosoma in dorsal view (9) **F** same in lateral view (7) **G** antenna (10) **H** pedicel and basal three funiculars (10) **I** clava and apical three funiculars (10). Abbreviations: clv = clava, flx = flagellomere number; pdl = pedicel

and mesotarsus similarly pale, yellowish-white to white, except for dark pegs (Fig. 3E). Hind leg with at least ventral surface of trochanter and trochantellus dark; femur with dorsal and ventral surfaces paler than longitudinally dark anterior and posterior surfaces; tibia with dorsal surface dark but paler basally, apically and ventrally; tarsus with

at least apical tarsomere brown and often one or two basal tarsomeres variably distinctly brownish. Macropterous, with fore wing extending beyond gastral apex when folded over dorsum (Fig. 2B); fore wing (Fig. 3A) with basal region mostly hyaline but brownish basally, with dark setae posteriorly along vanal and cubital areas and apically within basal brownish part (Fig. 3B), and hyaline part with whitish-translucent setae over at least about posterior half bare but behind length of SMV from level of base of parastigma; discal region (Fig. 3A) brownish from base of parastigma to level slightly beyond apex of PMV but hyaline towards wing apex and with complete hyaline cross band behind MV; hyaline cross band with white hair-like setae except for a few isolated dark setae within band medially (Fig. 3C), with apical margin reaching base of STV along leading margin and noticeably angulate compared to more evenly curved basal margin such that length of hyaline band along MV is about twice medial length (Fig. 3A); brownish region basal to hyaline cross band with slightly lanceolate dark brown setae except often for variably distinct, more orangish region of lanceolate setae behind parastigma and base of MV anterior to medial fold (when apparent, orangish region of setae reaching parastigma and base of MV, but at least narrowly separated by dark brown setae from basal cell basally and hyaline cross vein apically, Fig. 3B), and with entirely dark setae beyond hyaline cross band, the setae slightly lanceolate behind venation but more hair-like apically within more hyaline part. Metanotum and propodeum (Fig. 2E) dark brown or with slight metallic purple luster.

Gaster (Fig. 3D) mostly dark but syntergal flange and ovipositor sheaths pale, yellowish; in dorsal view Gt1 brown basally and variably extensively white apically and Gt2 translucent-hyaline so with sub-basal whitish region (Fig. 3D), but in lateral view basal two sternites entirely white (Fig. 2B); Gt3–Gt5 dark brown or with slight violaceous luster, and Gt6 and syntergum except for flange with green to bluish luster (Fig. 3D).

Setation. Head with slightly lanceolate, whitish-translucent setae on lower face, interantennal prominence, lower parascrobal region (Fig. 2C) and gena behind eye (Fig. 2B, D), and with dark hair-like setae on upper parascrobal region and frontovortex (Fig. 2D).

Pronotum mostly bare but with a few long, bristle-like setae in transverse line anterior to spiracle and similar seta medially on either side of mediolongitudinal groove (Fig. 2E). Mesonotum (Fig. 2E) with convex anteromedial mesoscutal lobe, uniformly, inconspicuously setose with whitish hair-like setae, outer convex surface of lateral lobe laterally with 1–2 rows of somewhat longer whitish setae, and posteromedial concave part of mesoscutum with more conspicuous, very slightly lanceolate white setae medially, the region of white setae about as wide as bare region on either side of band and not extending to line of dark hair-like setae on inner inclined surface of lateral lobe near dorsally carinate posteromedial part of lateral lobe; scutellar-axillar complex bare, except for dark setae laterally on axillae and posterolaterally on scutellum, the posteriormost setae somewhat longer and more bristle-like than others. Mesopleurosternum sparsely setose with whitish-translucent hair-like setae ventrally and more densely, uniformly setose with very slightly lanceolate whitish-translucent setae anterolaterally to level about equal with apex of tegula; acropleuron bare (Fig. 2F). Propodeum bare except for a few slightly lanceolate white setae along anterior margin lateral of spiracle and along extreme lateral margin (Fig. 2E).

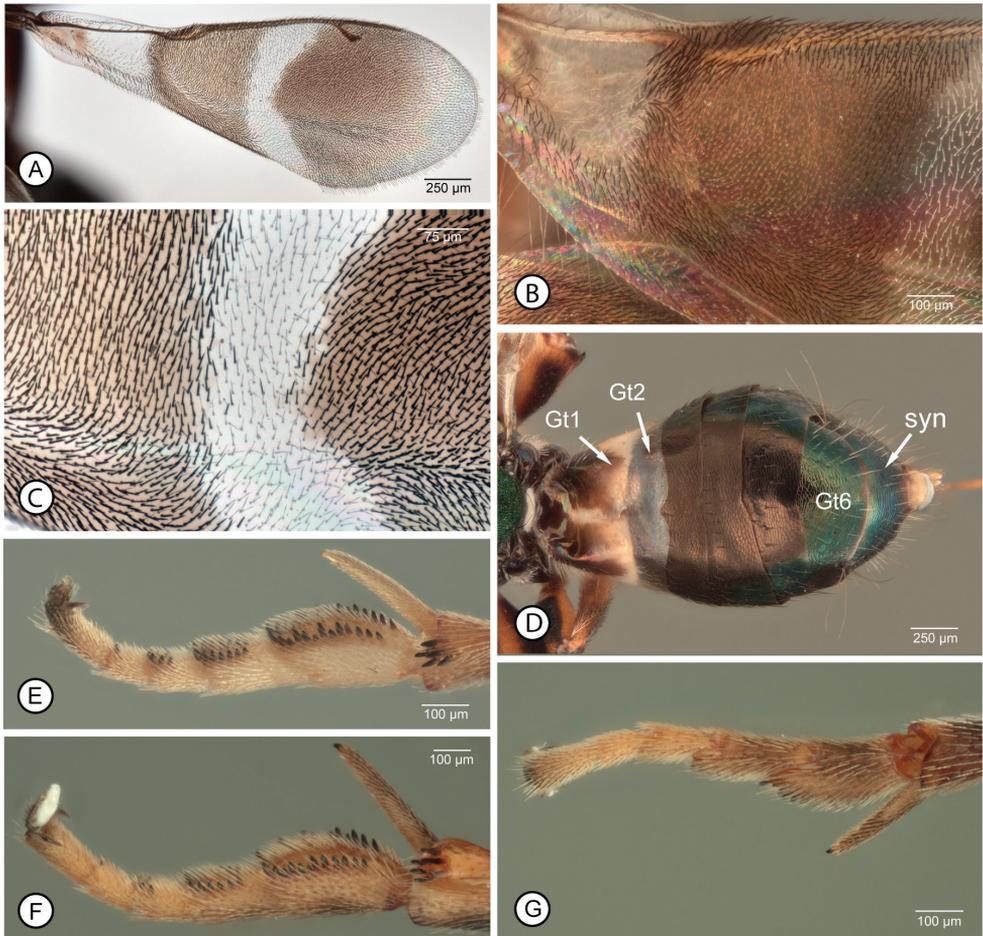


Figure 3. *Anastatus* spp., female **A–E** *A. gansuensis*: **A** fore wing (8) **B** basal half of fore wing (29) **C** enlargement of medial part of wing disc (8) **D** gaster in dorsal view (9) **E** mesotarsus and apex of mesotibia, in anterior view (7). **F, G** *A. fulloi*, mesotarsus and apex of mesotibia (12): **F** in anterior view **G** in posterodorsal view. Abbreviations: Gtx = gastral tergite number, syn = syntergum.

Structure. Head in frontal view (Fig. 2C) about 1.3× wider than high; in dorsal view (Fig. 2D) width about 2× length and about 3.5× minimum distance between eyes, with vertex gradually curved to occiput without margin or carina; in lateral view about 1.6× higher than long; eye height about 2× length of malar space; OOL: LOL: POL: MPOD (holotype) = 3: 6: 11.4: 6.2; scrobal depression (Fig. 1C) with abrupt to carinate margins laterally but dorsally margins less distinct and not complete below anterior ocellus, separated medially by short, lighter colored line or vertical ridge below ocellus, with indistinctly delimited dorsal margin separated from anterior ocellus by distance slightly less than longitudinal diameter of ocellus. Antenna (holotype) with relative length (width) of scape = 43(7); pedicel = 10(5); fl1–fl8 = 4(5), 13(6), 15(6), 16(7), 14(7), 14(7.5), 12(8), 11(8); clava 23(9).

Mesoscutum (Fig. 2E) slightly wider than long, with anterior, convex part of medial lobe V-shaped with slightly sinuate to slightly outcurved sides convergent posteriorly for about $0.7\times$ medial length of mesoscutum; lateral lobe carinately margined over almost posterior half; medial length of scutellar-axillar complex, including frenal area, about as long as maximum width with scutellum about $1.25\times$ as long as own width, Fore wing about $2.7\times$ as long as maximum width; smv: mv: pmv: stv (holotype) = 51: 70: 33: 10. Propodeum typical of genus, bowtie-like (Fig. 2E). Profemur with ventral margin evenly arched, without abrupt angulation or denticle within about apical third, and with 2 or 3 dorsoapical pegs. Mesotibia with apical patch of 3–6 pegs (Fig. 3E). Mesotarsus with single row of pegs on either side of basal three tarsomeres and single peg apically on either side of penultimate tarsomere (Fig. 3E).

Gaster with ovipositor sheaths at least slightly protruding beyond syntergal flange (Fig. 3D), but for distance up to about $2\times$ length of flange.

Sculpture. Head with vertex and interocellar triangle transversely alutaceous to alutaceous-strigose, upper parascrobal region and frons to posterior ocelli on either side of anterior ocellus more mesh-like coriaceous to coriaceous-imbricate or coriaceous-granular, and lower half of parascrobal region reticulate-rugulose; lower face reticulate to reticulate-rugose; interantennal prominence reticulate-imbricate; scrobal depression mostly reticulate-imbricate.

Mesoscutum with convex anterior part of medial lobe uniformly punctate-reticulate, posteromedial concave part of medial lobe smooth and shiny, and lateral lobes mostly finely mesh-like coriaceous to alutaceous, with sculpture of inner inclined surfaces more distinct lateral of anteromedial lobe and much finer to smooth and shiny posteriorly near scutellar-axillar complex. Scutellar-axillar complex uniformly, similarly punctate-reticulate as convex part of medial lobe. Mesopleurosternum with anterolateral region finely mesh-like coriaceous but dorsally bare part near tegula more alutaceous-coriaceous; acropleuron mostly longitudinally strigose, more minutely centrally, sculpture dorsally and ventrally more longitudinally strigose-coriaceous and posteriorly mesh-like coriaceous with larger cell size.

Gaster (Fig. 3D) with Gt1 and Gt2 dorsally almost smooth, only obscurely and very finely mesh-like coriaceous, Gt3–Gt5 more distinctly, somewhat transversely, coriaceous-reticulate with sculpture defined by slightly raised lines, and Gt6 and syntergum more transversely coriaceous-alutaceous to mesh-like coriaceous.

Male (habitus, Fig. 4A, B). Body length 2.0–2.2 mm.

Color. Head with frontal surface (Fig. 4C) mostly green to bluish-green, frontoververtex variably extensively dark or with slight coppery to reddish-violaceous lusters (Fig. 4A, C). Maxillary and labial palpi dark brown. Antennae with scape mostly yellow, but dorsoapically dark with variably distinct metallic green luster (Fig. 4G); pedicel dark except ventral surface usually paler; flagellum uniformly dark such that mps not conspicuous from surrounding cuticle (Fig. 4G).

Mesosoma (Fig. 4A, B, D) dark with mostly green to bluish-green lusters, though scutellar-axillar complex (Fig. 4A) and dorsellum (Fig. 4E) dark with coppery to reddish-violaceous lusters similar to frontoververtex, propodeum (Fig. 4E) more distinctly

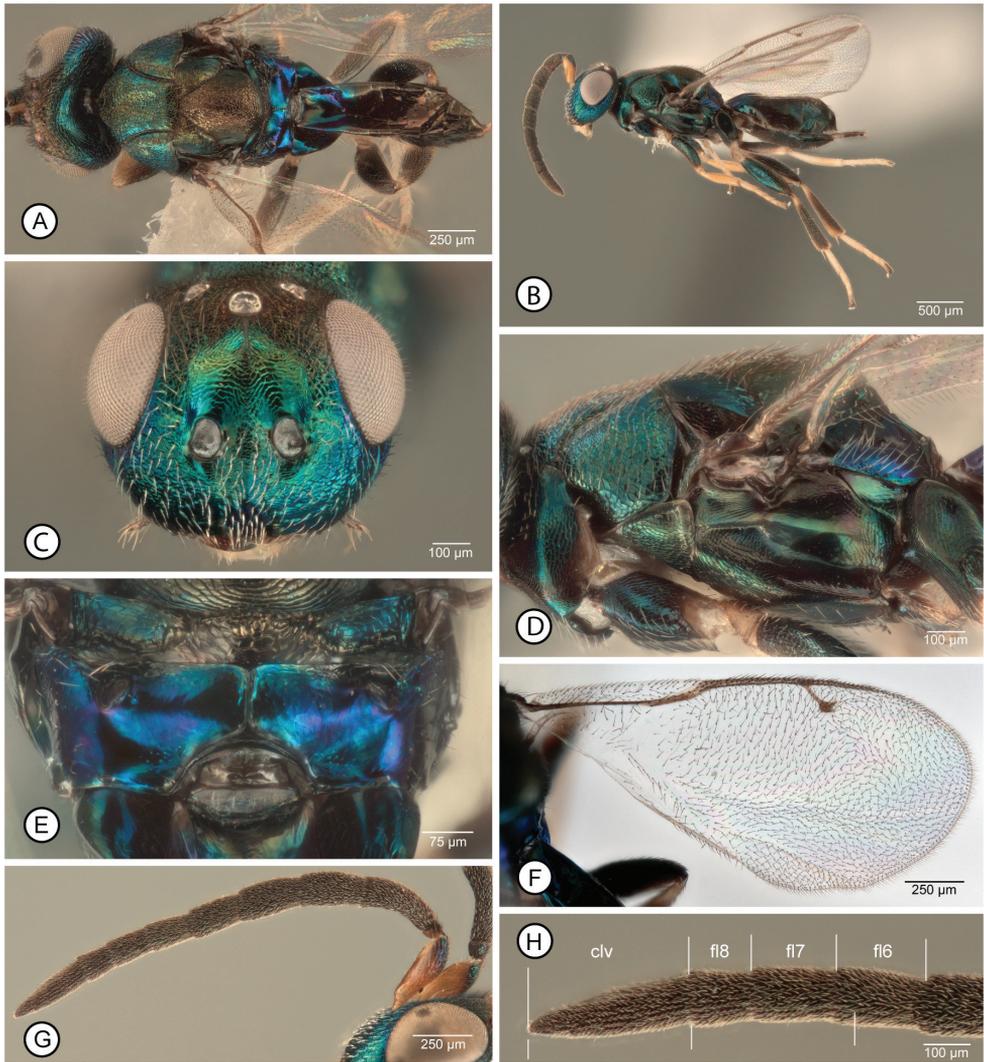


Figure 4. *Anastatus gansuensis*, male **A** dorsal habitus (17) **B** lateral habitus (16) **C** head in frontal view (19) **D** mesosoma in lateral view (16) **E** propodeum (17) **F** fore wing (17) **G** antenna (18) **H** pedicel and basal three funiculars (18) (three lower bars indicate length of clava compared to combined length of apical funiculars). Abbreviations: clv = clava, flx = flagellomere number.

blue to purple, and mesepisternum posteriorly near metapleuron usually more violaceous (Fig. 4D). Front leg with coxa, trochanter and most of femur dark, but following pale: trochantellus, femur apically, tibia except for dark mesotibial apical pegs, and tarsus except pale often apical tarsomere variably darker brown. Middle leg color pattern similar to front leg except femur often somewhat more extensively pale apically or dorsoapically along anterior margin and apical tarsomere more distinctly dark, but mesotibial spur similarly pale as basal four tarsomeres. Hind leg mostly dark but

at least ventral surface of trochantellus, tibia basally for distance less than one-third length of tibia, and basal four tarsomeres pale (Fig. 4B). Fore wing entirely hyaline with yellowish to yellowish-brown venation and dark, hair-like setae.

Gaster mostly dark brown but with variably distinct metallic green luster dorsobasally on Gt1 (Fig. 4B).

Setation. Head with very slightly lanceolate white setae on lower face, interantennal prominence and parascrobal region ventrally, but more hair-like dark setae on parascrobal region dorsally and frontovertex (Fig. 4C), and hair-like white setae on gena. Mesonotum with dark hair-like setae (Fig. 4D), the setae slightly longer and more bristle-like on scutellum. Fore wing (Fig. 4F) cubital cell ventrally with 3–4 rows of setae along entire length and dorsally with line of setae along entire leading margin and at least a partial second row behind first row; basal cell and disk entirely setose except for slender speculum along length of basal fold from parastigma to medial fold, though this variably obscured by setae on ventral surface at least toward parastigma.

Structure. Head in frontal view (Fig. 4C) about 1.2× wider than high, in dorsal view (Fig. 4A) about 2.1× wider than long, and in lateral view (Fig. 4B) about 1.8× higher than long; OOL: LOL: POL: MPOD (allotype) = 1.0: 1.6: 4.0: 1.6. scrobal depression (Fig. 4C) shallow, arch-like, with inclined lateral surfaces extending obscurely to anterior ocellus, but flat surface of depression above toruli usually separated from anterior ocellus by pale, yellowish line almost equal in length to longitudinal diameter of ocellus. Antenna (Fig. 4G) with scape almost 3× as long as maximum width; pedicel subglobular, as long as wide; length of pedicel + flagellum about 2.4× head width; flagellum densely microsetose with numerous rows of densely packed mps (Fig. 4G); fl1 strongly discoidal, not evident between scape and fl2; fl2 dorsoventrally flattened so as to be wider than high and in lateral view distinctly curved with ventral surface concave, but subsequent flagellomeres increasingly subcylindrical and straight (Fig. 4G); length(width) of fl2–fl8 and clava in lateral view (allotype): 24(10), 17(10), 17(10), 14(10), 14(10), 13(10), 10(10), 44(11) (Fig. 4G, H).

Structure of mesosoma and gaster (Fig. 4A–D) typical for the genus. Fore wing (Fig. 4F) 2.2–2.3× as long as wide; SMV: MV: PMV: STV (allotype) = 20: 11: 8.3: 4.7. Protibia with two dorsoapical spicules. Mid leg with spur about as long as basitarsus.

Sculpture. Head with vertex reticulate-rugose to transversely reticulate-strigose; frons and parascrobal regions mesh-like coriaceous to granular, lower face shallowly reticulate to reticulate-imbricate.

Mesoscutum (Fig. 4A) with medial lobe usually more coarsely reticulate-rugulose anteriorly, but at least about posterior two-thirds mesh-like reticulate except sculpture variably extensively finer laterally near notauli and sometimes posteriorly near transscutal articulation, and lateral lobe mostly mesh-like coriaceous; scutellar-axillar complex with dorsal surface of axilla variably distinctly reticulate-rugulose, but larger inclined lateral surface obliquely alutaceous-strigose, and scutellum usually at least slightly reticulate anteromedially but more mesh-like coriaceous to coriaceous-imbricate laterally and posteriorly. Propodeum (Fig. 4E) with complete medial carina; plical region smooth and shiny or at most with extremely obscure mesh-like coriaceous sculpture.

Distribution. *Anastatus gansuensis* (Genbank accession no. MK373759) originally described from China (Gansu Province).

Biology. Solitary endo-parasitoid of the eggs of *C. japonica* in the field and, at least in the laboratory, of *A. pernyi*. *Caligula japonica* has one generation a year and overwinters in the egg stage; its egg-laying period is from late August to late October in northwestern China (Qiao et al. 2014). In the winter of 2017 were collected a total 283 egg masses of *C. japonica* from Gansu Province and reared them in the laboratory (T: $25\pm 1^\circ$ C, RH: $70\pm 5\%$, L: D=14h: 10h). Of these, 12 egg masses (4.2%) were parasitized by *A. gansuensis*. All the emerged wasps of *A. gansuensis* were females. These were subsequently offered the eggs of *A. pernyi* in the laboratory as an alternative host for propagation, which led to the discovery that *A. gansuensis* is thelytokous parthenogenetic. When unmated females were reared on *A. pernyi* eggs in the laboratory both sexes were produced in a highly female-biased sex ratio (sex ratio about 113:1, ♀:♂ = 5987:53; $N=29$). There can be 12–14 generations in one year, with one generation completed in 28–33 days under a $25\pm 1^\circ$ C temperature and $70\pm 5\%$ humidity regime. The average longevity and fecundity of a female ($N=29$) was 67 (37–93) days and 303 (68–506) eggs, respectively, when fed with honey water (honey: water = 3:7). The results of our rearing experiments indicate that this species has potential as a biological control agent for suppression of *C. japonica*.

Etymology. Derived from the name of the province in China from which the type series was originally collected through rearing.

Remarks. Because of their dark acropleuron, females of *A. gansuensis* key most closely to females of *A. fulloi* in Peng et al. (2017), but the two are readily differentiated by the color, setal and structural features as given in the key and diagnoses. Females key to *A. tenuipes* Bolivar y Peltain, 1925 in Narendran (2009), in part because the apical margin of the hyaline cross band is angulate and all funiculars are longer than wide in both, but *A. tenuipes* females are readily differentiated by their pale mesosoma. Similarly, depending on the interpretation of whether or not the scrobal depression “by far” does not reach the anterior ocellus, *A. gansuensis* females could key to *A. tenuipes* in Kalina (1981) or to *A. splendens* Nikol’skaya, 1952 and *A. brevicaudus* Kalina, 1981. However, in contrast to the key features of Kalina (1981), the mesoscutum is slightly wider than long (Fig. 2E), unlike that keyed for *A. splendens* and the anterior convex portion of the mesoscutal medial lobe is triangular with gradually convergent sides (Fig. 2E), unlike that keyed for *A. brevicaudus*. Furthermore, females of both *A. brevicaudus* (Kalina 1981, fig. 14) and *A. splendens* have at least the apical two funiculars subquadrate to slightly transverse and the acropleuron pale to coppery.

Two features of the fore wing of female *A. gansuensis* are not apparent in all females: the presence of isolated dark setae within the hyaline cross band medially (Fig. 3C) and a region of more orangish setae behind the parastigma and base of the marginal vein anterior to the medial fold (Fig. 3B). Although the latter feature is distinctive it is not unique to *A. gansuensis*, being possessed also by the brachypterous females of *A. meilingensis* (Fig. 6E) and by some females we recognize as *A. japonicus*, but not by *A. fulloi* females.

As noted under *A. fulloi*, a flagellar structure differentiates males of both *A. gansuensis* and *A. meilingensis* from those of *A. fulloi* and *A. japonicus*. Males of the former

two species have the clava distinctly shorter than the combined length of the three apical funiculars (Figs 4H, 6H) whereas males of the latter two species have the clava at least as long and sometimes distinctly longer than the combined length of the three apical funiculars (Figs 1H, 5H).

Anastatus (Anastatus) japonicus Ashmead

Fig. 5A–H

Anastatus japonicus Ashmead, 1904: 153; syntypes (USNM), examined.

Anastatus bifasciatus disparis Ruschka, 1921: 265. Synonymy by Bouček 1977: 124–124.

Anastatus disparis; Burgess 1929: 574, new status for *Anastatus bifasciatus disparis*.

Anastatus japonicus; Yang et al. 2015: 163–164, fig. 84.

Diagnosis. Female. Macropterous (Fig. 5A, B); fore wing with broad hyaline cross band behind marginal vein with similarly curved basal and apical margins so band uniformly wide, and without isolated dark setae medially (Fig. 5E). Mesosoma (excluding legs) with mesonotum, prosternum and usually acropleuron anteriorly dark, but at least about posterior two-thirds of acropleuron, pronotum except for dark spot anterior to each spiracle (Fig. 5B, D), prepectus (Fig. 5D), and tegula at least basally (Fig. 5C) contrastingly paler; procoxa, except often in part laterally (Fig. 5D), similarly pale as pronotum and acropleuron posteriorly; mesotarsus with all tarsomeres similarly pale yellowish to white or with basal two tarsomeres only inconspicuously darker infuscate in part (Fig. 5B). Mesoscutum with posterior concave part comparatively broadly setose but white setae not attaining lateral margins (Fig. 5C). Antenna with at least apical funicular slightly transverse and previous one or two funiculars subquadrate.

Male. Structure as well as color, setae and sculptural patterns (Fig. 5F) similar to those described for *A. gansuensis* except clava at least about as long as combined length of fl6–fl8 (Fig. 5H), with fl8 and fl7 slightly transverse to quadrate and fl6 and fl5 slightly longer than wide (Fig. 5H).

Distribution. *Anastatus japonicus* (Genbank accession no. MK604240) was reported previously from China (Beijing, Fujian, Guangdong, Guangxi, Hong Kong, Jiangsu, Jilin, Liaoning, Shaanxi, Shandong) by several authors (Han et al. 1999; He 2001; He et al. 2001). We reared it in the field from the following localities: **Gansu Province:** Kang Co., Longnan City, collected 23.I.2018, Yong-Ming Chen (1♀, 1♂ AICF; 1♀, 1♂ BMNH; 21♀, 16♂ CNC; 5♀, 3♂ FAFU; 7♀, 6♂ IZCAS; 1♀, 1♂ USNM). **Jilin Province:** Changchun City, Jilin Agricultural University, 20.VII.2017, Yong-Ming Chen (1♀, 1♂ AICF; 1♀, 1♂ BMNH; 33♀, 17♂ CNC; 1♀, 1♂ USNM).

Extralimital distribution listed for *A. japonicus* by Noyes (2019) includes at least one country from all biogeographic regions except the Neotropical. It is widely distributed throughout the entire Palaearctic region where it is native.

Hosts. Noyes (2019) lists *A. japonicus* as a parasitoid of over 15 host species in two families of Hemiptera (Alydidae, Pentatomidae) and five families of

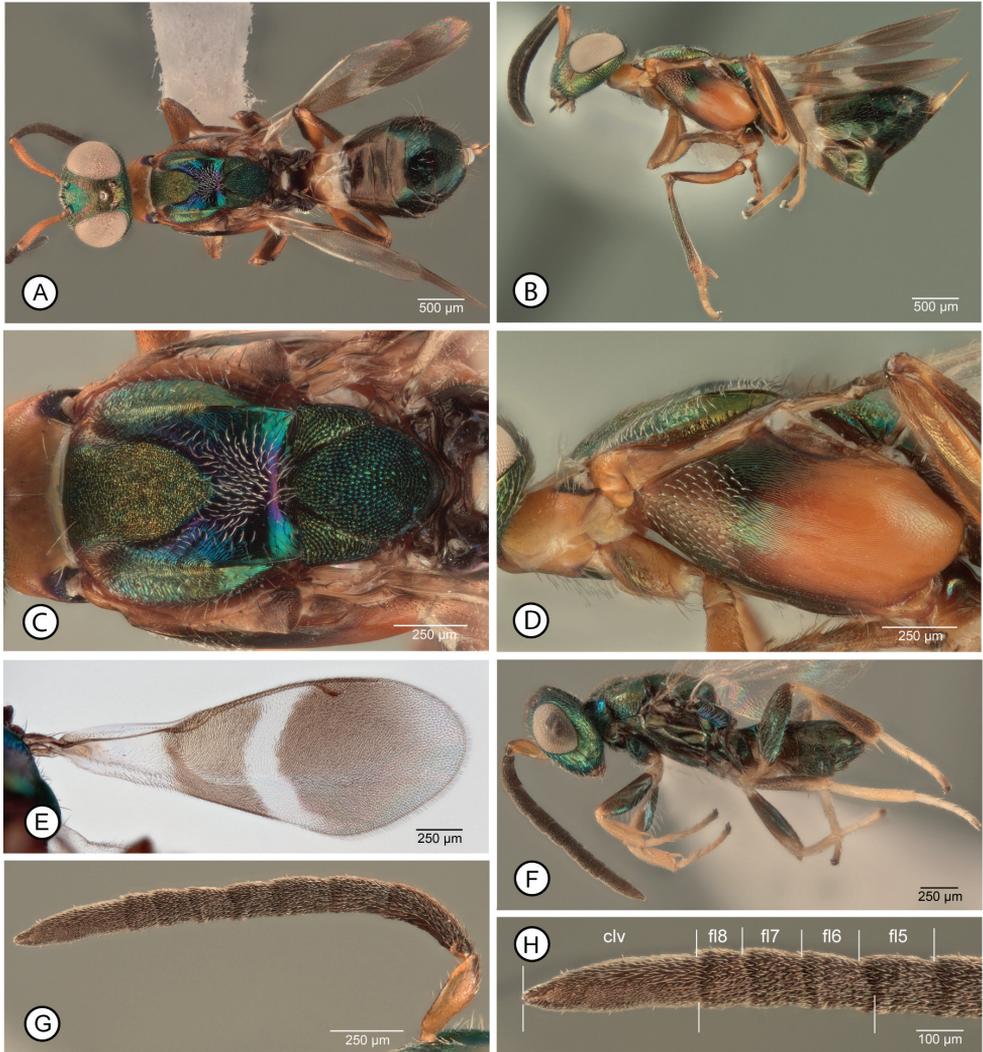


Figure 5. *Anastatus japonicus* **A–E** female: **A** dorsal habitus (6) **B** lateral habitus (4) **C** mesosoma in dorsal view (6) **D** same in lateral view (4) **E** fore wing (5). **F–H** male: **F** lateral habitus (25) **G** antenna (25) **H** clava and apical three funiculars (25) (three lower bars indicate length of clava compared to combined length of apical funiculars). Abbreviations: clv = clava, flx = flagellomere number.

Lepidoptera (Lymantriinae (Erebidae), Lasiocampidae, Notodontidae, Papilionidae, Saturniidae), sometimes as a hyperparasitoid through Braconidae and Encyrtidae primary parasitoids (Peck 1963; Kochetova 1968). It was reared in China previously on *A. pernyi* (Wu et al. 2000) and it has been utilized for biocontrol of the litchi stink bug, *T. papillosa*, being identified in the literature most commonly either as *Anastatus* sp. (Lu and Yang 1983; Lin and Lin 1998) or as *A. japonicus* (Xin and Li 1989, 1990; Tang et al. 1993; Xian et al. 2008; Li et al. 2017). Here we newly report it as an egg parasitoid of *C. japonica*.

Remarks. Females of *A. japonicus* are most easily distinguished from two species with macropterous females reared from *C. japonica* by the acropleuron being extensively paler, lighter brown to yellowish (Fig. 5D), in contrast with its dark mesonotum (Fig. 5C). Females of *A. fulloi* and *A. gansuensis* either have the acropleuron entirely dark (Fig. 1D, 2F), similar in color to the mesonotum, or paler anteriorly only near the base of the prepectus (Fig. 2F).

As noted under *A. fulloi*, males of *A. japonicus* and *A. fulloi* are differentiated from those of *A. gansuensis* and *A. meilingensis* by a longer clava relative to the combined length of the apical funiculars. This is because in *A. fulloi* (Fig. 1H) and *A. japonicus* (Fig. 5H) the apical funiculars are somewhat shorter compared to those of *A. gansuensis* (Fig. 4H) and *A. meilingensis* (Fig. 6H). Currently, we cannot reliably differentiate *A. japonicus* from *A. fulloi* males.

Anastatus (Anastatus) meilingensis Sheng

Fig. 6A–H

Anastatus meilingensis Sheng, 1998: 5–6, fig. 1; holotype (JLAU), examined.

Anastatus meilingensis; Peng et al. 2017: 16–18, figs 34–40.

Diagnosis. Female. Brachypterous, fore wing extending only to about level of posterior margin of Gt1 when body uncoerced (Fig. 6A, B); basal 0.6× (basal region) hyaline and much more sparsely setose than densely setose apical 0.4× (discal region) (Fig. 6C); basal region with slightly lanceolate dark setae extending from discal region for half-length within slender vana region and with white or mostly white hair-like setae immediately anterior to venal fold and at least narrowly along base of discal region, though often bare behind submarginal vein; discal region uniformly covered with slightly lanceolate dark setae except for conspicuous region of orangish setae medially and often variably conspicuous but slender, sometimes interrupted remnant of hyaline cross band apically (Fig. 6: cbr), with orangish setae extending at least to leading margin, including part of marginal vein, though sometimes not to posterior margin of wing so as to be enclosed by dark setae basally and apically and sometimes posteriorly; submarginal vein, including parastigma, extending slightly beyond basal region and partly orangish marginal vein extending most of length of discal region, without differentiated stigmal or postmarginal veins. Mesosoma (excluding legs) with prosternum dark but pronotum mostly pale except for dark spot anterior to each spiracle (Fig. 6C) and posterolateral, vertical surface (panel) often brown to dark (Fig. 6D); prepectus (Fig. 6D) and tegula (Fig. 6C) at least basally pale; mesonotum (Fig. 6C) with scutellar-axillar complex and mesoscutum medially dark, but mesoscutal lateral lobes with at least outer, inclined surfaces partly to mostly pale; acropleuron mostly pale except dark anteriorly in region ventral to tegula (Fig. 6D); procoxa, except often in part laterally, similarly pale as at least anterolateral surface of pronotum and most of acropleuron (Fig. 6D); mesotarsus with all tarsomeres similarly pale yellowish to white (Fig. 6B). Mesoscutum

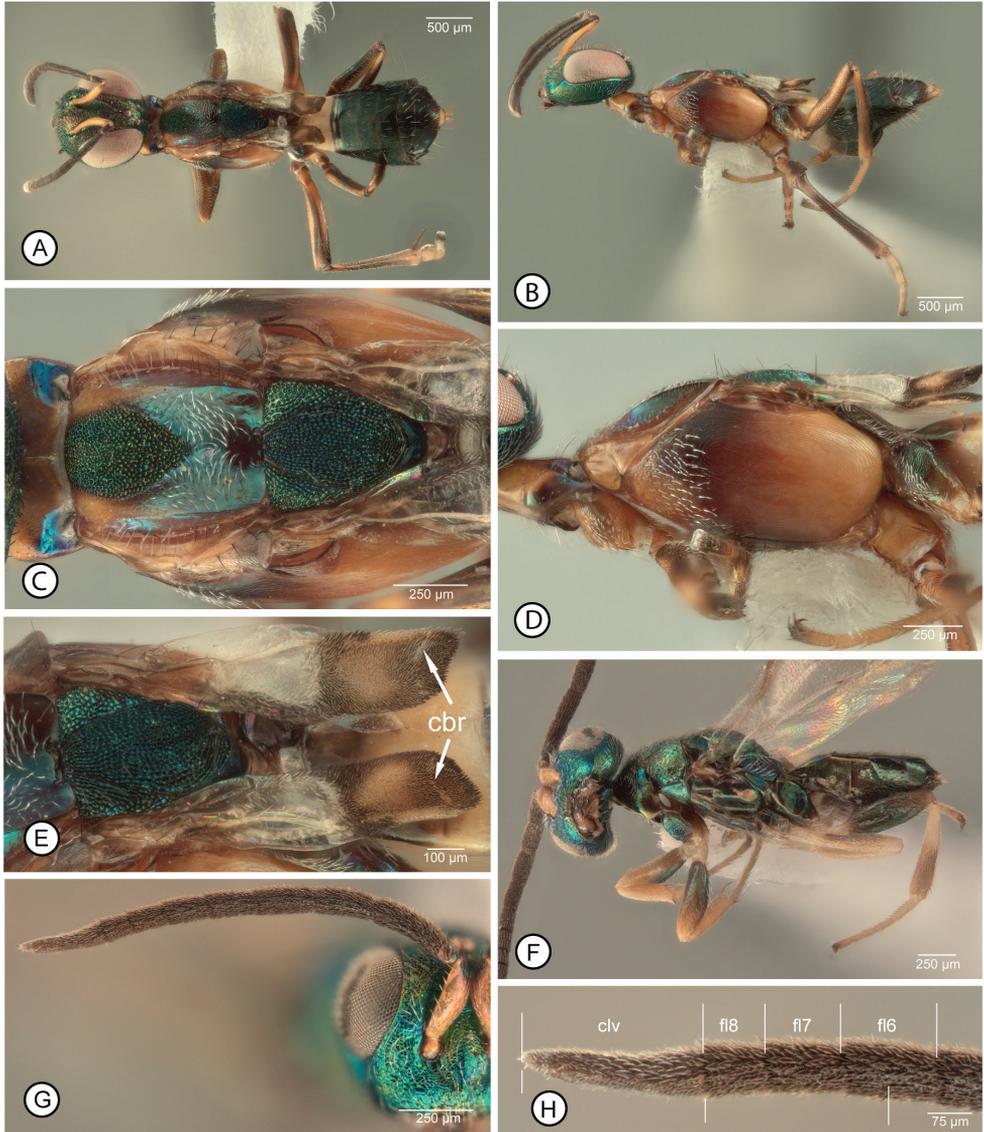


Figure 6. *Anastatus meilingensis* **A–E** female (2): **A** dorsal habitus **B** lateral habitus **C** dorsal mesosoma **D** lateral mesosoma **E** fore wing, dorsal view. **F–H** male (19): **F** lateral habitus **G** antenna **H** clava and apical three funiculars (three lower bars indicate length of clava compared to combined length of apical funiculars). Abbreviations: cbr = remnant of hyaline cross band, clv = clava, flx = flagellomere number.

with posterior concave part completely setose with white setae (Fig. 6C). Antenna with at least apical funicular subquadrate and previous two funiculars only slightly longer than wide.

Male. Structure as well as color, setae and sculptural patterns (Fig. 6F–H) similar to those described for *A. gansuensis* except about basal half of metatibia pale and apical half lighter brown, lighter in color than respective femur (Fig. 6F).

Distribution. *Anastatus meilingensis* (Genbank accession no. MK604242) was described originally from Jiangxi Province from three localities (Shangrao, Yushan, and Meiling, Nanchang) as detailed by Peng et al. (2017). We reared *A. fulloi* in the field from the following new locality: **Gansu Province:** Kang Co., Longnan City, 23.I.2018, Yong-Ming Chen (2♀, 2♂ AICF; 2♀, 2♂ BMNH; 58♀, 36♂ CNC; 5♀, 3♂ FAFU; 15♀, 12♂ IZCAS; 2♀, 2♂ USNM).

Hosts. Originally described reared from the eggs of the pine moth, *Dendrolimus punctatus* Walker, and of the Simao pine moth, *D. kikuchii* Matsumura (Lepidoptera, Lasiocampidae) (Sheng and Yu 1988). Here we newly report it as an egg parasitoid of *C. japonica* and, in the laboratory, of *A. pernyi*.

Remarks. Of the species we reared from *C. japonica*, females of *A. meilingensis* are readily distinguished because they are the only ones that are brachypterous. The fore wing color pattern, with the discal region having a distinct region of orangish setae behind the venation and often also a slender remnant of a hyaline cross band more apically (Fig. 6E: cbr), is unique among described *Anastatus* species with brachypterous females. However, except for the reduced hyaline cross band, the setal pattern is similar to that described for *A. gansuensis*. Females of one other described *Anastatus* species from China, those of *A. huangi* Sheng & Yu (1998), are also brachypterous. However, the reduced fore wings of female *A. huangi* have a color pattern more similar to macropterous females, having a distinct hyaline cross band with white, hair-like setae behind the marginal vein (Peng et al. 2017, fig. 32).

Although Sheng and Yu (1998) reared a single male as part of the type series of *A. meilingensis* this male was not found by Peng et al. (2017). The line drawn figure of the antenna stated as that of a male by Sheng and Yu (1998, fig. 4) is obviously that of a female because of the presence of a visible, anelliform fl1, and a three segmented clava. Of the species treated, males of *A. meilingensis* are mostly similar to those of *A. gansuensis* because both have a clava that is distinctly shorter than the combined length of the apical funiculars (Figs 4H, 6H). However, based on our reared material males of *A. meilingensis* have a different metatibial color pattern, the metatibia being more extensively pale basally and lighter brown apically than for males of *A. gansuensis* (cf. Figs 4B, 6F).

Discussion

Our study newly demonstrates that *Anastatus fulloi*, *A. gansuensis*, *A. meilingensis* and *A. japonicus* are natural egg parasitoids of *Caligula japonica* and thus are potential biological control agents of this pest in China. Further, all four species were reared successfully on the eggs of the alternate host, *A. pernyi* in the laboratory, as for some *Trichogramma* species (Zhang et al. 2017; Li et al. 2018), which demonstrates the possibility of mass-rearing the species for augmentative release against *C. japonica*. However, further study is required to evaluate their parasitism and possible competition in the field for biological control efficacy, as well as any effects of adaptation on *A. pernyi* eggs upon field-release.

All *Anastatus* species previously reported have been considered to have a haplo-diploid reproductive mechanism, which requires fertilization of the female by a male for female production (Noyes 2019). However, by culturing unfertilized females on *A. pernyi* eggs we discovered that *A. gansuensis* is a thelytokous parthenogenetic species, which implies huge reproductive potential of the female wasps for laboratory experiments and biocontrol. In order to use most effectively a biological control agent within a mass production system, the production of female individuals is clearly desirable because this is the sex responsible for reducing the pest population in the field. It remains to be demonstrated whether thelytokous parthenogenesis is more common in *Anastatus* than for just *A. gansuensis*.

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Taxonomic revision of the endemic Cameroonian freshwater crab genus *Louisea* Cumberlidge, 1994 (Crustacea, Decapoda, Brachyura, Potamonautidae), with descriptions of two new species from Nkongsamba and Yabassi

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Abstract

The taxonomy of the freshwater crab genus *Louisea* Cumberlidge, 1994, is reviewed based on type material and newly obtained specimens from three different localities in southwestern Cameroon. The genus is endemic to Cameroon and previously included two species: *L. edeaensis* (Bott, 1969) (type species) from Lake Ossa wetland complex (altitudes below 400 m asl) and *L. balsa* (Bott, 1959) from Kumba and Mt. Manengouba (altitudes above 1300 m asl). Here two new species of *Louisea* are described based on morphological and/or genetic data: *L. nkongsamba* **sp. nov.** from the Nlonako Ecological Reserve (1000–1400 m asl) in the sub-montane zone and *L. yabassi* **sp. nov.** from Yabassi in the lowlands. A redescription and amended diagnostic features of *L. edeaensis* and *L. balsa* are provided, and the genus diagnosis is updated to accommodate all four species. An identification key is also provided for the species of *Louisea*. A tree of phylogenetic relationships based on three mtDNA loci (COI, 12S rRNA, and 16S rRNA) supports the taxonomic revision, and indicates speciation of *Louisea* species along an altitudinal gradient, but further phylogenetic analyses are needed to understand whether this can lend support to the hypothesis that there is a montane centre of speciation along the Cameroon Volcanic Line. The phylogenetic tree also shows that *Buea* Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019 and *Potamonemus* Cumberlidge & Clark, 1992 are sister genera that may be derived from the *Louisea* lineage.

Keywords

Cameroon, Crustacea, identification key, new species, phylogeny, Potamoidea, redescription, taxonomic revision

Introduction

The freshwater crab genus *Louisea* Cumberlidge, 1994, was established by Cumberlidge (1994) to accommodate two species from Cameroon. The first of these, *L. edeaensis* (Bott, 1969), was originally described by Bott (1969) as *Globonautes macropus edeaensis* Bott, 1969 from a single male specimen from Edea, Cameroon collected in 1910. The second species is *L. balssi* (Bott, 1959), which was described by Bott (1959) as *Globonautes balssi* based on specimens from Kumba, Cameroon collected between 1900 and 1910. This taxon was later treated by Bott (1970) as the subspecies *Globonautes macropus balssi* (Bott, 1970). Cumberlidge (1999) found that the genus *Globonautes* Bott, 1959, is endemic to the Upper Guinea Forests from Liberia to Guinea, and that *Louisea* is endemic to the Lower Guinea Forests in southwestern Cameroon. Both *L. edeaensis* and *L. balssi* were assessed as endangered (EN) under the International Union for Conservation of Nature (IUCN) Red List protocols, and it was thought at that time that both these species might even be extinct (IUCN 2003, Cumberlidge 2008a, 2008b, Cumberlidge et al. 2009). However, our recent biotic surveys in Cameroon (in August 2015 and May 2017) have led to the rediscovery of *L. edeaensis* from lowland forests below 400 m asl (Bedimet Island of the Lake Ossa wetland complex), and of *L. balssi* from high altitude forests above 1300 m asl (Mt. Manengouba) (Mvogo Ndongo et al. 2017a, 2018). The molecular data from the fresh specimens established the validity of the genus *Louisea*, and enabled the resolution of longstanding questions surrounding the phylogenetic relationships of the *Louisea* that was definitively assigned to the subfamily Potamonautinae Bott, 1970 (Mvogo Ndongo et al. 2017c).

The rediscoveries of *L. edeaensis* and *L. balssi* prompted renewed extensive systematic surveys of the lowland and upland zones in the tropical rainforests of southwestern Cameroon in 2017 and 2018, which resulted in the recognition of a new species of *Louisea* from the submontane zone of Mt. Nlonako (1000–1400 m asl). This new species is described here based on morphological and genetic data. A second new species of *Louisea* is also recognised from Yabassi in the lowlands of southwestern Cameroon based on the re-examined specimens from the Museum für Naturkunde, Berlin, Germany (ZMB) that had previously been identified as belonging to *Louisea* (Cumberlidge 1994, 1999, Mvogo Ndongo et al. 2017a). The genus *Louisea* is revised here to include four species, *L. edeaensis* is redescribed, and the diagnosis of *L. balssi* is amended. The taxonomic revision of *Louisea* is based on a unique combination of characters of the carapace, thoracic sternum, chelipeds, and male first gonopods. It is also supported by original data on the phylogenetic relationships of *Louisea* and the other species of freshwater crabs found during our surveys of the rainforest ecosystems of southwestern Cameroon. The present molecular study used three mitochondrial genes (COI, 12S rRNA, and 16S rRNA) but included only three of the four species of *Louisea* (because no fresh specimens of the

new species from Yabassi could be collected). Also included in the phylogenetic tree and species of the two other genera found in southwestern Cameroon: *Buea* Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019 and *Potamonemus* Cumberlidge & Clark, 1992.

Materials and methods

Sampling

A series of field surveys of freshwater decapods undertaken in southwestern Cameroon from 2015 to 2018 focused on biodiversity hotspots that had been previously identified in 2011 by Conservation International for other freshwater taxa (Fig. 1). Crabs were hand-caught from puddles near small permanent streams, from under fallen leaves, and from their burrows sited close to water bodies. Specimens of *Louisea* sp. (ZMB Crust. 21575) collected from Yabassi, Cameroon between 1900 and 1910 held in the Museum für Naturkunde were re-examined here.

Morphological analyses

All measurements (in mm) were taken with digital callipers. The terminology used follows Cumberlidge (1999), and the classification follows Ng et al. (2008). Characters of the gonopods, carapace, thoracic sternum, chelipeds, third maxillipeds, and mandibles were examined in detail, and photographs were taken using a Leica microscope (model Z16A POA), and LAS V4 and Helicon Focus 6.7.1 software. Post processing of the images was undertaken using Adobe Photoshop CC5. The type specimens of the two new species and the freshly collected specimens of *Louisea* species are deposited in the Museum für Naturkunde, Berlin, Germany (**ZMB**). Other material is deposited in the Institute of Fisheries and Aquatic Sciences, University of Douala at Yabassi (**IFAS**), the Senckenberg Museum, Frankfurt, Germany (**SMF**), the Zoological Institute Museum, Hamburg, Germany (**ZIM**), the Naturhistorisches Museum Wien, Austria (**NHMW**), and the Zoologische Sammlung des Bayerischen Staates, München, Germany (**ZSBS**).

The following abbreviations are used:

a	pleonal (abdominal) segment or pleomere;
a5/a6	sulci between adjacent pleomeres;
asl	above sea level;
CW	carapace width measured at widest point;
CL	carapace length measured along medial line from anterior to posterior margin;
CH	carapace height measured at maximum height of cephalothorax;
e	episternite;
FW	front width measured along anterior frontal margin between inner angles of orbits;

G1	male first gonopod;
G2	male second gonopod;
p2–p5	pereiopods 2–5 or walking legs 1–4;
SS	subterminal segment of G1 or G2;
s4/e4	(s4/e4, s5/e5, s6/e6, s7/e7) episternal sulci between adjacent thoracic sternites and episternites;
s	thoracic sternite;
s1/s2	(s1/s2, s2/s3, s4/s5, s5/s6, s6/s7) sternal sulci between adjacent thoracic sternites;
TA	terminal article of G1 or G2;
TS	terminal segment of mandibular palp.

Molecular analysis

Genomic DNA was extracted from a tissue sample of up to 25 mg cut from the pereiopod muscle of 70% ethanol-preserved specimens using the Qiagen DNeasy Blood & Tissue kit.

Polymerase chain reaction (PCR) was used to amplify three mitochondrial gene fragments, a ~ 638 bp region of the 16S ribosomal RNA gene (16S) using primers 16L29 and 16HLeu or 16H10 (Schubart 2009), a ~ 594 bp region of the 12S ribosomal RNA gene (12S) using primers 12L4 and 12H2 (Schubart 2009), and a 648 bp fragment of the Cytochrome Oxidase subunit I gene (COI) using primers COL6a (Schubart 2009) and COH1b (Schubart 2009), COH6 (Schubart and Huber 2006), or CO1a (Palumbi et al. 1991). PCR was performed in 25 µl volumes containing 1× Taq buffer, 1.5 mM MgCl₂, 200 µM each dNTP, 1 U Taq polymerase, ca. 50–100 ng DNA and ddH₂O up to volume. After an initial denaturation step of 4 min at 94 °C, cycling conditions were 35 cycles at 94 °C for 30 s, 45 °C for 60 s, and 72 °C for 90 s, with a final elongation step of 5 min at 72 °C. The same primers were used in PCR and sequencing.

PCR products were sent to Macrogen Europe for purification and cycle sequencing of both strands of each gene. The sequences obtained were proofread manually using Chromas and aligned with Bioedit. Results from these genes were concatenated into a single alignment that was then converted into a Nexus file with FaBox (Villesen 2007). The best evolutionary model was determined with jModeltest v.2.1.7 (Darrriba et al. 2012) based on the Akaike information criterion (Posada and Buckley 2004) and resulted in the GTR+I+G (COI), GTR+G (16S) and HKY+G (12S) models. The phylogenetic reconstruction was conducted with Maximum Likelihood (ML) using the software RAxML (Stamatakis 2006) under GTR + (I) + G model of sequence evolution. Bayesian Inference (BI) was performed to infer phylogeny by using MrBayes v. 3.2.2 (Huelsenbeck and Ronquist 2001). The MCMC was run with four independent chains for 10,000,000 generations, samplefreq = 500, and burnin = 10,001. Analyses were conducted separately to test for topology congruence. The trees were drawn to scale, with branch lengths measured as the number of substitutions. All sequences generated for this study have been uploaded to GenBank (Table 1).

Table 1. Species of *Louisea*, *Buea*, *Potamonemus* and outgroups included in the molecular analyses. The newly-presented data are given in bold.

Species	Locality	Museum number	Reference study	GenBank accession number		
				CO1	12S rRNA	16S rRNA
<i>L. nkongsamba</i> sp. nov.	Nlonako	ZMB Crust. 31618	Present	MN188072	MN217386	MN217393
<i>L. nkongsamba</i> sp. nov.	Nlonako	ZMB Crust. 31620	Present	MN188065	MN217387	MN217394
<i>L. balssi</i> (CW 16.2 mm)	Manengouba	ZMB Crust. 30319	Present	MN188071	MN217385	MN217392
<i>L. balssi</i> (CW 14.8 mm)	Manengouba	ZMB Crust.29628	Present	MN188070	MN217384	MN217391
<i>L. edeaensis</i> (CW 17.5 mm)	Lake Ossa	LZUY 15-3 (T351-30)	Mvogo Ndongo et al. 2017c	KY964474	KY964479	KY964472
<i>L. edeaensis</i> (CW 16.15 mm)	Lake Ossa	ZMB Crust. 30335	Present	MN188068	–	MN217395
<i>Buea</i> sp.1	N.P. Korup	ZMB Crust. 30321	Present	MN188069	MN217388	MN217396
<i>Buea</i> sp.2	N.P. Bakossi	ZMB Crust. 30325	Present	MN188066	MN217389	MN217397
<i>B. asylos</i>	Buea and Kumba	NHM 1994.588-591	Daniels et al. 2015	KP640489	KP640410	KP640453
<i>Potamonemus</i> sp.	N.P. Bakossi	ZMB Crust. 30327	Present	MN188067	MN217390	MN217398
<i>P. mambilorum</i>	southwest Cameroon	NHM 1991.183	Daniels et al. 2015	–	KP640409	KP640452
<i>P. sachsi</i>	southwest Cameroon	NMU09.04.1983	Daniels et al. 2002	–	AY803490	AY803530
<i>Afrithelphusa monodosa</i>	Guinea	NMU 25.IV.2005.C	Daniels et al. 2015	KP640469	KP640386	KP640430
<i>Globonautes macropus</i>	Guinea	NMU VII. 1988	Daniels et al. 2015	–	KP640391	KP640435

LZUY: Zoological Collection of the Laboratory of Zoology, University of Yaounde 1, Cameroon; NHM: Natural History Museum, London, UK; NMU: Northern Michigan University Museum, USA; ZMB: Museum für Naturkunde, Berlin, Germany.

Systematic accounts

Infraorder Brachyura Latreille, 1802

Superfamily Potamoidea Ortmann, 1896

Family Potamonautidae Bott, 1970

Subfamily Potamonautinae Bott, 1970

Genus *Louisea* Cumberlidge, 1994

Globonautes Bott 1959: 995, pl. 1, figs 1–6; 1969: 359; 1970: 23.

Louisea Cumberlidge 1994: 123; 1999: 226; Ng et al. 2008: 169 (list).

Type species. *Globonautes macropus edeaensis* Bott, 1969, by original designation; gender feminine.

Diagnosis. Amended from Cumberlidge (1994, 1999). Carapace ovoid, high (CH/FW 1.28–2.12, $N = 57$) with faint urogastric groove (Figs 2a–d, 3a–d). Postfrontal crest detectable (either prominent or faint), but meeting anterolateral margins of carapace (Fig.

7a–d). Exorbital, intermediate teeth small, but detectable; epibranchial tooth minute, almost undetectable (Figs 4, 7a–d). Medial inferior margin of merus of cheliped with large jagged tooth one-third from distal margin, followed by numerous distinct smaller teeth decreasing in size proximally (Fig. 9a–d). Third maxilliped exopod completely lacking flagellum; third maxilliped ischium with vertical groove (Fig. 14a–d). Mandibular palp 2 segmented; terminal segment (TS) bilobed, with large anterior lobe ($0.5\text{--}0.8 \times$ TS length) (Fig. 15a–d). G1 highly stout, distinctly sinuous; terminal article (TA) short, about one quarter length of subterminal segment (SS) ($TA/SS\ 0.22\text{--}0.29$), directed outwards at 45° angle to longitudinal axis of G1; SS, inverted funnel-shaped, proximally distinctly broad, abruptly narrow, slim, distal two-thirds tube-like; G1 SS stout, tapering slightly from wide basal margin to relatively wide distal margin ($0.6 \times$ SS basal margin), dorsal face with broad dorsal membrane (maximum width $0.1 \times$ SS length) at TA/SS junction (Figs 11a–d, 12a–d). G2 TA long ($TA/SS\ 0.40\text{--}0.44$), flagellum-like, almost as long as G2 SS (Fig. 13a–d). Small species (CW 14–22 mm in adults).

Distribution. *Louisea* is endemic to southern Cameroon (Cumberlidge 1994, 1999) (Fig. 1). *Louisea edeaensis* is known from Yaounde, Edea, and the Lake Ossa faunal reserve, while *L. balssi* is known from the Bakossi region at Barombi Mbo near Kumba and from Manengouba Ecological Reserve (Cumberlidge 1994, 1999, Mvogo Ndongo et al. 2017a, 2018). *Louisea nkongsamba* sp. nov. is known from Mt. Nlonako Ecological Reserve at Nkongsamba, while *Louisea yabassi* sp. nov. is from Yabassi.

Remarks. Cumberlidge (1994, 1999) provided the diagnostic characters of *Louisea* based mainly on the male holotype of *L. edeaensis*, because *L. balssi* was only known then from a juvenile male. The revision of the diagnostic characters for this genus is based on our examinations of adult males of all four species included here (*L. edeaensis*, *L. balssi*, *L. nkongsamba* sp. nov., and *L. yabassi* sp. nov.). The amended character descriptions are also used to compare *Louisea* with other potamonautid genera.

The bilobed terminal segment of the mandibular palp of *Louisea* is unusual, and it sets this genus apart from most genera in the Potamonautinae that typically possess a simple mandibular palp (i.e., with no additional anterior lobe) (Cumberlidge 1999; Cumberlidge et al. 1999; Cumberlidge and Reed 2003). This is true for *Erimetopus* Rathbun, 1894 and *Platythelphusa* A. Milne-Edwards, 1887, and all but one species of *Potamonautes* MacLeay, 1838 [*P. brincki* (Bott, 1960) being the exception], most species of *Sudanonautes* Bott, 1955 [except for *S. floweri* (de Man, 1901) and *S. orthostylis* Bott, 1955], and most species of *Liberonautes* Bott, 1955 (Cumberlidge 1999). It should be noted that in those potamonautine species without a simple mandibular palp, the anterior lobe is little more than a hard ledge at the junction between the segments, rather than a true lobe shape (Cumberlidge 1999). The terminal segment of the mandibular palp of *Louisea* is distinctly bilobed (with an anterior lobe $0.6 \times$ the terminal segment length) and is superficially similar to the mandibular palps of *Afrithelphusa* Bott, 1969 and *Globonautes* (the two West African genera assigned to the Deckeniinae Ortmann, 1897, but the mandibular palp of each of the latter two genera has a larger anterior lobe that is subequal to the posterior lobe (Fig. 15a–d; Cumberlidge 1999: fig. 48A–C).

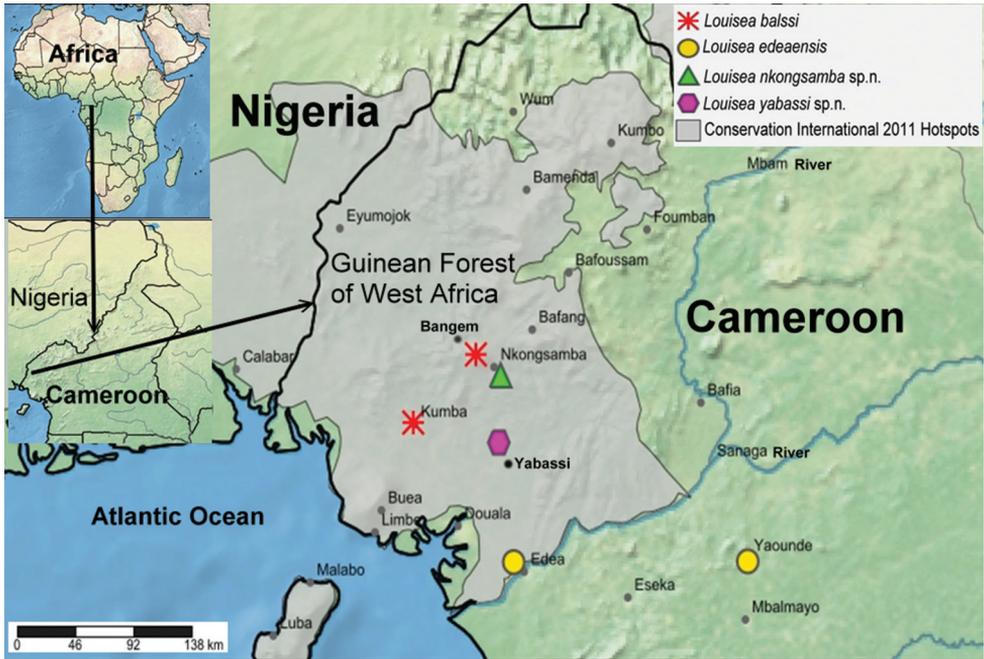


Figure 1. Collection localities of the four species of *Louisea* in Cameroon.

The lack of a flagellum on the exopod of the third maxilliped in *Louisea* is rarely seen in other species of the Potamonautinae, and most of the members of this subfamily typically possess a long flagellum on the third maxilliped exopod (Cumberlandidge 1999). The exceptions to this are the species of *Buea* and *Potamonemus*, and *Liberonautes grandbassa* Cumberlandidge, 1999 and *L. lugbe* Cumberlandidge, 1999 (Cumberlandidge and Clark 1992; Cumberlandidge 1993, 1999; Cumberlandidge et al. 2019). The lack of a flagellum on the exopod of the third maxilliped of *Louisea* is also shared with species of the Deckeniinae (*Afrithelphusa*, *Globonautes*, and *Madagapotamon humberti* Bott, 1965) (Cumberlandidge 1999; Cumberlandidge et al. 2008). *Louisea* can also be distinguished from the West African Deckeniinae genera *Afrithelphusa* and *Globonautes* by characters of the gonopods (G1 TA shape and G2 TA length), the presence or absence of an intermediate tooth between the exorbital and epibranchial teeth (Cumberlandidge 1999), and by molecular evidence (Daniels et al. 2015; Mvogo Ndongo et al. 2017c).

Louisea edeaensis (Bott, 1969)

Figs 2a, 3a, 4c, 5a, 6a, 7a, 8a, b, 9a, 10a, 11a, 12a, 13a, 14a, 15a

Globonautes macropus edeaensis Bott 1969: 360; 1970: 24, pl. 1, figs 3–5, pl. 26, fig. 8; Cumberlandidge 1987: 2215, table 2.

Louisea edeaensis Cumberlidge 1994: 124, fig. 1, table 1; 1997: 577; 1999: 227, 5300, 54–57, figs 46F, 47E, 48E, 49F, 51F, 52F, 53DD, 54–57, 62F, 68F, table IX, plate 3; Ng et al. 2008: 169 (list); Cumberlidge et al. 2009: 6; Mvogo Ndongo et al. 2017a: 273, figs 1–3; 2017c: 440, fig. 1.

Material examined. CAMEROON. Holotype: adult male (CW 22.5 mm), Edea, Jan 1910, coll. Riggenbach (ZSBS 1118/1). Adult male, 2 adult females (CWs 19.4, 17.5 mm), Yaounde, 1907, coll. Haberer (NHMW 1877). See Table 2 for details of the material examined from Lake Ossa.

Diagnosis. Amended from Cumberlidge (1994, 1999); Mvogo Ndongo et al. (2017a). Carapace smooth, urogastric groove faint (Figs 2a, 3a, 4c). Postfrontal crest faint, complete, meeting anterolateral margin behind intermediate tooth (Fig. 7a). Ex-orbital, intermediate teeth small, low, distinct (Figs 2a, 3a, 4c, 7a). Third maxilliped ischium with distinct vertical groove (Fig. 14a). Terminal segment (TS) of mandibular palp bilobed, with large distinct anterior lobe ($0.6 \times$ terminal segment length) (Fig. 15a). Major cheliped dactylus relatively stout, straight, with two large teeth (one proximal, one medial) (Fig. 8a); cheliped propodus with four large teeth (three proximal, one distal); cheliped carpus inner margin with long, broad distal tooth followed by slim, smaller proximal tooth (Fig. 10a). G1 TA short (TA/SS = 0.3), directed outward at 45° angle to longitudinal axis of G1 SS, proximally broad, distal two-thirds narrowing abruptly to form slim tube (Figs 11a, 12a). G1 SS tapering slightly from broad basal margin to relatively wide distal margin ($0.6 \times$ SS basal margin), dorsal face with broad dorsal membrane (maximum width $0.1 \times$ SS length) at TA/SS junction (Fig. 11a). G2 TA long (TA/SS = 0.40), flagellum-like, almost as long as G2 SS (Fig. 13a). Mature between CWs 14–23 mm.

Re-description. Amended from Cumberlidge (1994, 1999). Carapace ovoid, high, slightly arched (CH/FW 1.41, $N = 22$), wide (CW/FW 3.14, $N = 22$), smooth; postfrontal crest poorly defined, completely crossing carapace, meeting anterolateral margins of carapace behind intermediate tooth (Fig. 7a); mid-groove broad, shallow, epigastric crests poorly defined (Fig. 7a); external orbital tooth small, low; epibranchial tooth reduced to granule, almost undetectable (Figs 4a, 7a); intermediate tooth on anterolateral margin between external orbital, epibranchial teeth small but detectable (Figs 4a, 7a); anterolateral margin lined by small granules (Figs 4a, 7a); posterolateral margin continuous with anterolateral margin, curving inward; posterior margin of carapace wide ($2/3$ CW). Carapace branchiostegal wall with 2 sutures dividing it into 3 parts (Fig. 6a); longitudinal suture beginning at respiratory opening dividing suborbital, subhepatic regions from pterygostomial region (Fig. 6a); vertical suture beginning at epibranchial tooth curving sharply down to meet longitudinal suture, marked by row of granules (Figs 6a, 7a). Sternal sulcus s2/s3, deep, complete, s3 lacking depression (Fig. 5a); s3/s4 reduced to 2 short, distinct notches on each side of sternum (Fig. 5a); episternal sulci s4/e4, s5/e5, s6/e6 faint or missing, s7/e7 complete (Fig. 5a). Mandibular palp 2 segmented; terminal segment (TS) bilobed, anterior lobe $0.6 \times$ length of terminal segment (Fig. 15a, d). Third maxillipeds (Fig. 6a) filling entire buccal cavern, except for transversely oval efferent

Table 2. Morphometric analysis and collection data of specimens ($N = 22$) of *Louisea edeaensis* from Cameroon (Lake Ossa; 3°48'56.1"N, 10°03'18.5"E; 90 m a.s.l.). All measurements are given in mm.

Specimens	CW	CL	CH	FW	CW/ FW	CL/FW	CH/FW	FW/CL	Coll. Date	Museum
1 ad♂	17.5	13.5	6.8	5.8	3.01	2.32	1.17	0.43	P.A.M.N 10.07. 15	ZMB Crust. 26930
2 ad♂	16.15	12.70	7.80	5.10	3.17	2.49	1.53	0.40	P.A.M.N 15.01. 16	ZMB Crust. 30335
3 ad♂	15.60	13.1	7.90	4.80	3.25	2.73	1.64	0.37	P.A.M.N 15.01. 16	IFAS-001
4 ad♂	14	11.03	6.5	4.5	3.11	2.51	1.44	0.39	P.A.M.N 15.01. 16	ZMB Crust. 30335
5 ad♂	15.35	12.15	8.30	5	3.07	2.43	1.66	0.41	P.A.M.N 10.07. 15	ZMB Crust. 30319
6 ad♀	19.90	15.2	7.7	5.9	3.37	2.57	1.30	0.38	P.A.M.N 10.07. 15	LZUY 15-2 (IFAS-002)
7 ad♀	17.5	13.30	6.6	5.3	3.30	2.50	1.24	0.39	P.A.M.N 11.11. 16	LZUY 15-2 (IFAS-002)
8 ad♀	17.0	13.2	6.30	5.2	3.26	2.53	1.21	0.39	P.A.M.N 11.11. 16	LZUY 15-2 (IFAS-002)
9 ad♀	14.80	11.30	7.2	4.9	3.02	2.30	1.46	0.43	P.A.M.N 15.01. 16	LZUY 15-3 (T351-30)
10 ad♀	17.30	13.80	9.80	5.90	2.90	2.33	1.66	0.42	P.A.M.N 10.07. 15	LZUY 15-2 (IFAS-002)
11 ad♀	14.6	11.2	7.1	4.90	2.97	2.28	1.44	0.43	P.A.M.N 15.01. 16	LZUY 15-1 (IFAS-003)
12 ad♀	18.90	13.89	10.50	6.01	3.14	2.31	1.74	0.43	P.A.M.N 15.01. 16	ZMB Crust. 30335
13 ad♀	14.10	11.20	7	4.7	3.0	2.38	1.48	0.41	P.A.M.N 10.07. 15	IFAS-004
14 sd♀	13	10.50	5.80	4	3.25	2.62	1.45	0.38	P.A.M.N 10.07. 15	LZUY 15-3 (IFAS-005)
15 sd♂	11.80	10.09	5.80	4	2.95	2.52	1.45	0.39	P.A.M.N 11.11. 16	IFAS-004
16 sd♂	11.70	9.89	6	4	2.92	2.47	1.5	0.40	P.A.M.N 11.11. 16	IFAS-004
17 sd♂	12.40	9.80	5.7	4	3.1	2.45	1.42	0.40	P.A.M.N 11.11. 16	IFAS-004
18 sd♂	12.00	9.5	5.6	4	3	2.37	1.4	0.42	P.A.M.N 10.07. 15	LZUY 15-1 (IFAS-003)
19 sd♀	13.60	10.01	6.80	4.15	3.27	2.41	1.63	0.41	P.A.M.N 11.11. 16	IFAS-004
20 sd♀	12.80	9.8	6	4	3.2	2.45	1.5	0.40	P.A.M.N 11.11. 16	IFAS-004
21 sd♀	11.60	10	5.5	3.8	3.05	2.63	1.44	0.38	P.A.M.N 11.11. 16	IFAS-004
22 sd♀	11.01	9.50	5.40	3.8	2.89	2.5	1.42	0.40	P.A.M.N 11.11. 16	LZUY 15-4 (IFAS-005)
Mean	14.02	11.07	6.36	4.51	2.96	2.45	1.41	0.40	–	–

Key: P.A.M.N: Pierre A. Mvogo Ndongo; ad: adult; sd: subadult.

respiratory openings in superior lateral corners; ischium with distinct vertical groove (Fig. 14a); exopod lacking flagellum (Fig. 14a).

Male chelipeds greatly unequal, right cheliped larger than left cheliped (Figs 3a, 6a). Dactylus, propodus of right (major) cheliped slim, elongated; fixed finger (propodus) with 4 large pointed teeth (3 proximal, 1 distal); movable finger (dactylus) relatively stout, straight, with 2 large teeth (1 proximal, 1 medial) (Fig. 8a). Dactylus, propodus of left (minor) cheliped slender, with small teeth on occluding margins (Figs 2a, 3a). Medial inferior margin of cheliped merus with large jagged distal tooth angled outward at 90°, followed by numerous distinct smaller teeth decreasing in size proximally (Figs 6a, 9a; see Mvogo Ndongo et al. 2017a: fig. 1B). Cheliped carpus inner margin with 2 large pointed teeth, distal tooth long, broad with pointed tip; proximal tooth longer than distal tooth (Fig. 10a). Walking legs (p2–p5) slender, p4 longest, p5 shortest; dactyli (p2–p5) tapering to point, each bearing rows of downward-pointing sharp bristles, p5 dactylus shortest (Figs 2a, 3a).

Male pleon triangular, telson (a7) rounded at distal margin (Fig. 5a). G1 TA short (TA/SS 0.3), directed outward at 45° angle to longitudinal axis of G1 SS, proximally distinctly broad, abruptly narrow, slim and tube-like at distal two-thirds (Figs 11a, 12a). G1 SS tapering slightly from broad basal margin to relatively wide distal margin (0.6 × SS basal margin); dorsal face with broad dorsal membrane (maximum width 0.1 × SS length) at TA/SS junction (Fig. 11a); ventral face with raised triangular flap extending halfway across segment forming roof of chamber for G2, flap tapering di-



Figure 2. Four species of *Louisea* endemic to southwestern Cameroon, whole animal, dorsal view. **a** Largest adult male (CW 17.5 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 26930) (missing right cheliped) **b** largest adult male (CW 16.2 mm) of *L. balsi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) (missing left p5) (Mvogo Ndongo et al. 2018: fig. 1', www.mapress.com/j/zit) **c** largest adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) (missing left p2–p5, and right cheliped and p2, p3) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 8 mm (**a**), 9 mm (**b**), 8.30 mm (**c**), 17 mm (**d**).

agonally from broad base to narrow point at SS/TA junction (Fig. 12a). G2 TA long (TA/SS = 0.40), flagellum-like, almost as long as G2 SS (Fig. 13a); G2 SS wide at base, tapering sharply to long, thin process with raised rim at junction with TA (Fig. 13a). Mature between CW 14–23 mm.

Remarks. The description and diagnosis of *L. edeaensis* by Cumberlandidge (1994) was based on characters of specimens from Edea and Yabassi. Mvogo Ndongo et al. (2017a) updated these characters following the discovery of a large series of *L. edeaensis* from Bedimet Island in Lake Ossa in August 2015. These specimens included only one adult male that agreed well with the diagnostic characters of the holotype from Edea, and with other specimens from Yaounde and Yabassi. Nevertheless, the morphological variations raised by Cumberlandidge (1994) that distinguished the specimens from Yabassi from those from Yaounde, Edea, and Lake Ossa still remained. The specimens of Lake Ossa examined in this study included five adult males whose morphological characters are consistent with those from Edea and Yaounde, but different from the specimens from Yabassi. This resulted in the present re-description of *L. edeaensis*. Differences between *L. edeaensis* and its congeners are given below under general remarks.



Figure 3. Four species of *Louisea* endemic to southwestern Cameroon, whole animal, dorsal view. **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) (missing left p4) **b** second largest adult male (CW 14.8 mm) of *L. balssi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) (missing left p4 and right p2) **c** subadult male, paratype (CW 13.8 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) (missing left p2–p4, and right cheliped and p2–p5) **d** second largest adult male (CW 18.38 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 12.42 mm (**a**), 10 mm (**b**), 8.42 mm (**c**), 11.63 mm (**d**).

Louisea balssi (Bott, 1959)

Figs 2b, 3b, 4b, 5b, 6b, 7b, 8c, d, 9b, 10b, 11b, 12b, 13b, 14b, 15b

Globonautes balssi Bott 1959: 999, fig. 7; Cumberlidge 1987: 2210; 1994: 127, figs 2 a, b, 3 (j–l only), tables 1–2 (ZIM K 3506 only).

Globonautes macropus balssi Bott 1970: 25, pl. 1, figs 6–8.

Louisea balssi Cumberlidge 1999: 231, figs 53EE, 54–57, 62G, 68F, tables V, X–XIII, pl. 4 (not figs 46G, 48F, 49G, 52G, table IX); Ng et al. 2008: 169 (list); Mvogo Ndongo et al. 2018: 400.

Material examined. CAMEROON. Holotype: juvenile male (CW 12.5 mm), Barombi Mbo [formerly Johann Albrechtshöhe (Government Station Johann Albrecht Mountain), Barombi Station] (4.666686N, 9.392042E), 323 m asl, 10 September 1909, coll. Carl Rathke (ZIM K3506). Paratypes: 3 adult females (CWs 22.0, 21.0, 21.0 mm) (ovigerous), subadult female (CW 13.5 mm), Barombi Mbo [formerly Johann Albrechtshöhe (Government Station Johann Albrecht Mountain), Barombi Station] (4.666686N, 9.392042E), 323 m asl, 10 September 1909, coll.

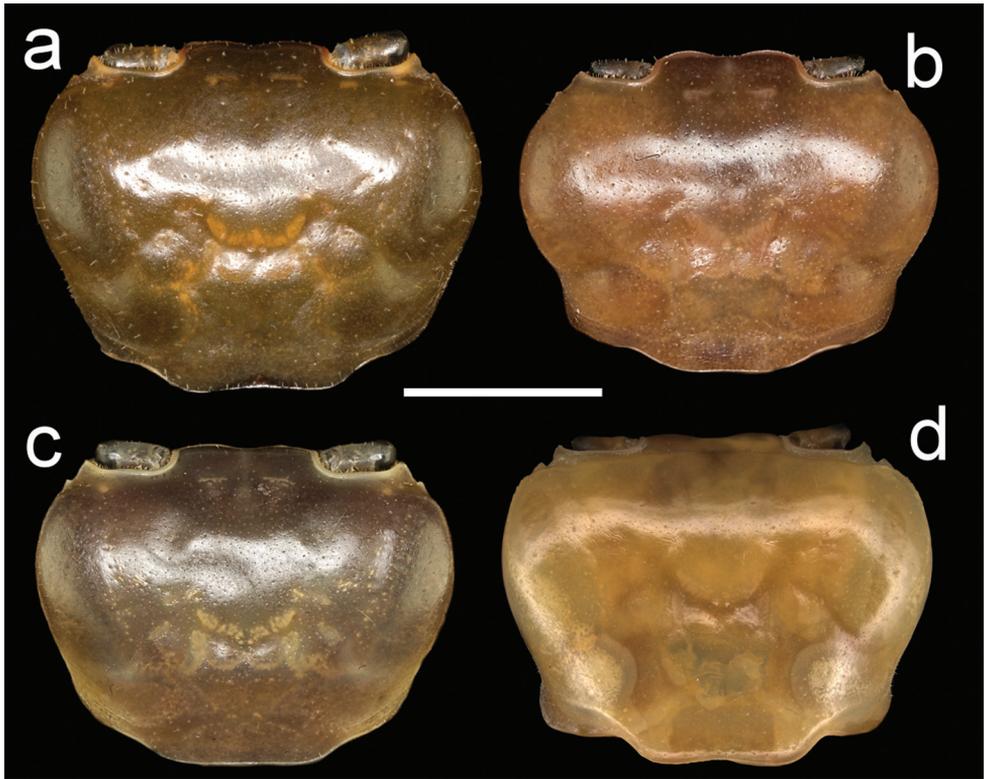


Figure 4. Four species of *Louisea* endemic to southwestern Cameroon, carapace, dorsal view. **a** Largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618) **b** second largest adult male (CW 14.8 mm) of *L. balsi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) **c** second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **d** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575). Scale bars: 9.1 mm (**a**), 7.25 mm (**b**), 7.90 mm (**c**), 8.62 mm (**d**).

Carl Rathke (SMF 5093, donated by ZIM K3506). Other material examined is given in Table 3.

Diagnosis. Amended from Cumberlidge (1994, 1999); Mvogo Ndongo et al. (2018). Carapace smooth, urogastric groove faint; postfrontal crest faint, complete, meeting anterolateral margin behind intermediate tooth (Fig. 3b); exorbital, intermediate teeth small, low, distinct (Figs 4b, 7b). Mandibular palp 2 segmented; terminal segment (TS) bilobed, with large distinct anterior lobe $0.5 \times$ terminal segment length (Fig. 15b). Third maxilliped ischium with distinct vertical groove (Fig. 14b). Episternal sulci $s4/e4$, $s5/e5$, $s6/e6$ faint or missing, $s7/e7$ complete (Fig. 5b). Major cheliped dactylus relatively slender, highly arched enclosing oval interspace when closed, with two large teeth (one proximal, one medial) (Fig. 8b); propodus of major cheliped with two large proximal teeth, large medial tooth, small distal tooth (Fig. 8b); cheliped carpus inner margin with long, broad distal tooth, slim subequal proximal tooth (Fig. 10b); cheliped merus medial inferior margin with large jagged distal tooth followed by six

Table 3. Morphometric analysis and collection data of specimens ($N = 8$) of *Louisea balsi* from Cameroon (S.R, Man. Man's Crater Lake; 5°01'56.9"N, 9°49'37.8"E; 1,958 m a.s.l.). All measurements are given in mm.

Specimens	CW	CL	CH	FW	CW/FW	CL/FW	CH/FW	FW/CL	Coll. Date	Museum
1 adult ♂	16.2	11.8	7.1	5.5	2.94	2.14	1.29	0.46	P.A.M.N 14.03.17	ZMB Crust. 30319
2 adult ♂	14.8	10.7	6.1	5.1	2.90	2.09	1.19	0.47	P.A.M.N 14.03.17	ZMB Crust.29628
3 adult ♂	14.3	10.5	6.0	5.0	2.86	2.10	1.20	0.47	P.A.M.N 14.03.17	LZUY 20 (IFAS-005)
4 adult ♂	13.3	9.7	5.6	4.6	2.89	2.11	1.21	0.47	P.A.M.N 14.03.17	LZUY 20 (IFAS-005)
5 adult ♀	14.8	10.9	6.4	4.7	3.14	2.31	1.36	0.43	P.A.M.N 14.03.17	ZMB Crust. 30319
6 subadult ♂	11.1	8.6	4.8	4.2	2.64	2.04	1.14	0.48	P.A.M.N 14.03.17	LZUY 20 (IFAS-005)
7 subadult ♂	12.7	9.2	5.7	4.3	2.95	2.13	1.32	0.46	P.A.M.N 14.03.17	LZUY 20 (IFAS-005)
8 subadult ♀	11.2	8.2	4.8	4.0	2.80	2.05	1.20	0.48	P.A.M.N 14.03.17	LZUY 20 (IFAS-005)
Mean	13.6	10	5.8	4.7	2.89	2.12	1.23	0.47	–	–

Key: P.A.M.N: Pierre A. Mvogo Ndongo



Figure 5. Four species of *Louisea* endemic to southwestern Cameroon, thoracic sternites (s1–s8) and pleonal segments (a4–a7). **a** Second largest adult male (CW 16.1 mm) of *L. edaeensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balsi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) (missing left p5) ('Mvogo Ndongo et al. 2018: fig. 2c', www.mapress.com/j/zt) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 12.42 mm (**a**), 9 mm (**b**), 8.30 mm (**c**), 17 mm (**d**).

distinct smaller teeth decreasing in size proximally (Fig. 9b). G1 TA short (TA/SS 0.3), directed outwards at 45° angle to longitudinal axis of G1 SS, with distinct longitudinal groove, proximally distinctly broad, abruptly narrow, slim and tube-like at distal two-thirds (Figs 11b, 12b). G1 SS tapering slightly from wide basal margin to relatively

wide distal margin ($0.6 \times$ SS basal margin); dorsal face with broad dorsal membrane (maximum width $0.1 \times$ SS length) at TA/SS junction (Fig. 11b). G2 TA long (TA/SS = 0.40), flagellum-like, almost as long as G2 SS. Mature between CW 13–17 mm.

Redescription. A re-description of *L. balssi* is given in Mvogo Ndongo et al. (2018). Additional character state descriptions are added here in the light of the new material to further distinguish between *L. balssi* and its congeners.

Remarks. Specimens of *L. balssi* are known only from Kumba and Mt. Manengouba in southwestern Cameroon. The morphological features of *L. balssi* are mainly defined from the adult male specimens collected from Mt. Manengouba (Mvogo Ndongo et al. 2018). The only available specimens from Kumba are sub-adults whose gonopod, sternal, and cheliped characters are not fully developed, which makes them less useful for taxonomic studies (Cumberlidge 1999). Further systematic surveys at the type locality near Kumba are needed to resolve this taxonomic problem but current social issues in this part of Cameroon preclude such surveys. Distinctions between *L. balssi* and its congeners are given below under general remarks.

***Louisea yabassi* sp. nov.**

<http://zoobank.org/FA6DE8AD-B833-415C-95C7-47287F3C6158>

Figs 2c, 3c, 4d, 5c, 6c, 7c, 8e, f, 9c, 10c, 11c, 12c, 13c, 14c, 15c

Common name: Yabassi freshwater crab

Material examined. CAMEROON. Holotype: adult male (CW 18.11 mm, CL 12.78 mm, CH 8.30 mm, FW 6.29 mm; CW/FW 2.88, CL/FW 2.03, CH/FW 1.32, FW/CL 0.49, FW/CW 0.34), Yabassi, 10 September 1909, coll. Rigggenbach (ZMB Crust. 21575). Paratype: subadult male (CW 13.82 mm, CL 10.61 mm, CH 6.25 mm, FW 5.01 mm; CW/FW 2.75, CL/FW 2.11, CH/FW 1.24, FW/CL 0.47, FW/CW 0.36), same data as holotype (ZMB Crust. 21575).

Diagnosis. Carapace smooth, urogastric groove faint; postfrontal crest distinct, prominent, complete, meeting anterolateral margin behind intermediate tooth (Fig. 3c); exorbital, intermediate teeth large, triangular; epibranchial tooth undetectable (Figs 4c, 7c). Vertical sulcus on carapace branchiostegal wall curving backward to meet anterolateral margin at epibranchial tooth (Fig. 6c). Mandibular palp bi-segmented; terminal segment (TS) bilobed, with large distinct anterior lobe $0.6 \times$ terminal segment length (Fig. 15c). Third maxilliped ischium with distinct vertical groove (Fig. 14c). Episternal sulci $s4/e4$, $s5/e5$, $s6/e6$ faint or missing, $s7/e7$ complete (Fig. 5c). Major cheliped dactylus highly arched enclosing oval interspace when closed, with five large teeth (one small distal, two large medial, two small proximal) (Fig. 8e); propodus of major cheliped with two large proximal teeth, large medial tooth, small distal tooth (Fig. 8e); cheliped carpus inner margin with long, broad distal tooth, relatively narrow, subequal proximal tooth (Fig. 10c). G1 TA short (TA/SS 0.22), directed outwards at 45° angle to longitudinal axis of G1 SS, with distinct longitudinal groove proximally distinctly broad, abruptly narrow, slim and tube-like at distal two-thirds (Figs 11c,



Figure 6. Four species of *Louisea* endemic to southwestern Cameroon, whole animal, ventral view. **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** second largest adult male (CW 14.8 mm) of *L. balsi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 11.63 mm (**a**), 9.61 mm (**b**), 9.50 mm (**c**), 11.60 mm (**d**).

12c). G1 SS tapering slightly from broad basal margin to relatively wide distal margin ($0.5 \times$ SS basal margin); dorsal face with broad dorsal membrane (maximum width $0.1 \times$ SS length) at TA/SS junction (Fig. 11c). G2 TA long (TA/SS 0.44), flagellum-like, almost as long as G2 SS (Fig. 13c). Mature at CW 19 mm.

Description. Carapace ovoid, flat (CH/FW 1.28, $N = 2$), wide (CW/FW 2.8), smooth, urogastric groove distinct; front wide (FW/CW 0.35, $N = 2$), deflexed, anterior margin straight; postfrontal crest distinct, prominent, completely crossing carapace, meeting anterolateral margin of carapace behind intermediate tooth (Fig. 4d); exorbital, intermediate teeth large, triangular; epibranchial tooth undetectable (Figs 4c, 7c). Carapace branchiostegal sidewall with vertical, longitudinal sutures dividing it into three regions (suborbital, subhepatic, pterygostomial) (Fig. 6c); longitudinal suture beginning at respiratory opening, curving backward across sidewall dividing suborbital- and subhepatic regions from pterygostomial region (Fig. 6c); vertical sulcus on carapace branchiostegal wall curving backward to meet anterolateral margin at epibranchial tooth (Fig. 6c), dividing suborbital from subhepatic regions (Fig. 6c).

Mandibular palp bi-segmented; terminal segment (TS) bilobed, with large distinct anterior lobe $0.6 \times$ terminal segment length (Fig. 15c). Third maxilliped exopod completely lacking flagellum; ischium with distinct vertical groove (Fig. 14c). Sternal



Figure 7. Four species of *Louisea* endemic to Southwestern Cameroon, carapace, frontal view. **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** second largest adult male (CW 14.8 mm) of *L. balssi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 7.20 mm (**a**), 6.60 mm (**b**), 8.06 mm (**c**), 8.70 mm (**d**).

sulcus s2/s3 prominent, completely crossing sternum; s3/s4 incomplete, reduced to 2 short lateral notches (Fig. 5c). Episternal sulci s4/e4, s5/e5, s6/e6 faint or missing, s7/e7 complete (Fig. 5c).

Male chelipeds unequal, right chelipeds larger than left cheliped; fingers slim, elongated. Right (major) cheliped dactylus relatively stout, straight, with five large teeth (one small distal, two large medial, two small proximal); fixed finger (pollex) with five large teeth (one distal, four proximal) (Fig. 8e); dactylus and propodus of left cheliped straight, with small teeth on occluding margin (Fig. 8f); cheliped carpus inner margin with two pointed teeth, distal tooth large with pointed tip, proximal tooth smaller (Fig. 10c); medial inferior margin of cheliped merus with large jagged distal tooth followed by numerous distinct smaller teeth (Fig. 9c).

Male pleon triangular, sides not indented with small setae; telson (a7) rounded at distal margin (Fig. 5c). G1 TA short (TA/SS 0.22), directed outwards at 45° angle to longitudinal axis of G1 SS, with distinct longitudinal groove on ventral face, proximally distinctly broad, abruptly narrow, slim and tube-like at distal two-thirds (Figs 11c, 12c). G1 SS tapering slightly from broad basal margin to relatively wide distal margin (0.5 × SS basal margin); ventral face of with raised triangular flap extending halfway across segment forming roof of chamber for G2, flap tapering diagonally from broad base to narrow point at SS/TA junction (Fig. 12c); dorsal face with broad dorsal

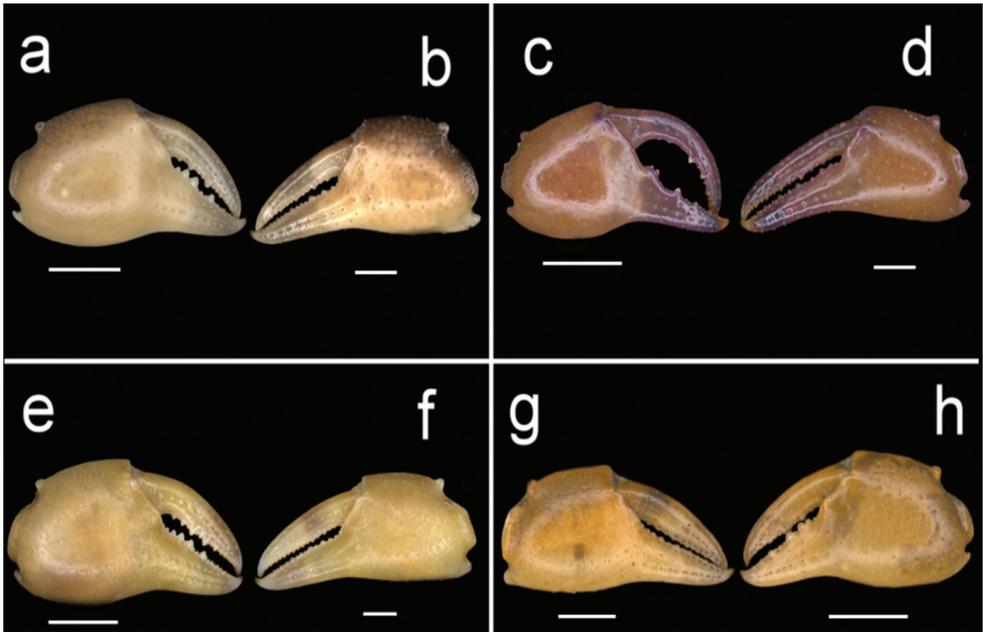


Figure 8. Four species of *Louisea* endemic to Southwestern Cameroon, frontal view of right and left chela. **a, b** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **c, d** largest adult male (CW 16.2 mm) of *L. balsi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) (missing left p5) ('Mvogo Ndongo et al. 2018: fig. 3a, b', www.mapress.com/j/zt) **e, f** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **g, h** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 5 mm (**a, c, e, g**), 2 mm (**b, d, f**), 10 mm (**h**).

membrane (maximum width $0.1 \times$ SS length) at TA/SS junction (Fig. 11c). G2 TA long (TA/SS 0.44), flagellum-like, almost as long as G2 SS (Fig. 13c). Mature at CW 19 mm.

Color in life. Unknown.

Type locality. Yabassi, littoral region of Cameroon.

Etymology. The new species is named for Yabassi in south-western Cameroon, where it was collected in 1909. The species epithet is a noun in apposition.

Habitat. *Louisea yabassi* sp. nov. is known only from Yabassi, a humid area of the coastal rain forest of southwestern Cameroon. It is possible that this species is also present in the Ebo forest near Yabassi, which is one of the largest remaining tracts of lowland and submontane rainforest in the area.

Remarks. Characters of the carapace and chelipeds of adult male specimens from Yabassi assigned to *L. edeaensis* by Cumberlandidge (1994) and by Mvogo Ndongo et al. (2017a) proved to be inconsistent with the holotype from Edea. Re-examination of these specimens supported the hypothesis that specimens from Edea and those from Yabassi belong to two different species: *L. edeaensis* (Edea, Yaounde, and Lake Ossa) and *L. yabassi* sp. nov. (Yabassi). The differences between *L. yabassi* sp. nov. and its congeners are discussed below under general remarks.



Figure 9. Four species of *Louisea* endemic to southwestern Cameroon, right cheliped merus. **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balssi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) (missing left p5) (Mvogo Ndongo et al. 2018: fig. 3d', www.mapress.com/j/zt) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 5 mm (**a, c**), 2 mm (**b**), 10 mm (**d**).

***Louisea nkongsamba* sp. nov.**

<http://zoobank.org/141A1FD3-DF3B-4E84-9296-5AA5A26A3B68>

Figs 2d, 3d, 4a, 5d, 6d, 7d, 8g, h, 9d, 10d, 11d, 12d, 13d, 14d, 15d

Common name: Nkongsamba freshwater crab

Material examined. CAMEROON. Holotype: adult male (CW 20 mm, CL 14.85 mm, CH 8.4 mm, FW 6.6 mm), Littoral Region, Mount Nlonako Ecological Reserve (locality 1) (4.91046N, 9.976332E), 1,237 m asl, 23 May 2018, coll. P.A. Mvogo Ndongo (ZMB Crust. 31618). Paratype: adult male (CW 18.38 mm, CL 13.32 mm, CH 8.13 mm, FW 6.34 mm), Littoral Region, Mount Nlonako Ecological Reserve (locality 2) (4.91343N, 9.98500E), 1,176 m asl, 23 May 2018, coll. P.A. Mvogo Ndongo (ZMB Crust. 31620). Other material examined is listed in Table 4.

Diagnosis. Carapace smooth, urogastric groove faint; postfrontal crest faint, complete, meeting anterolateral margin behind intermediate tooth (Fig. 7d); exorbital, intermediate teeth large, triangular; epibranchial tooth undetectable (Figs 4d, 7d). Verti-

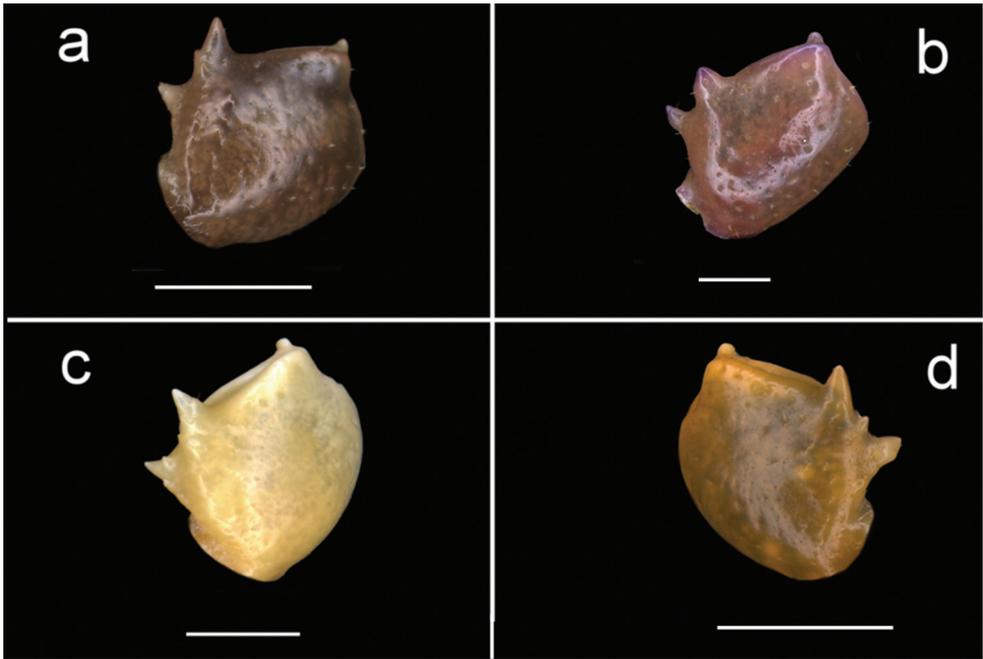


Figure 10. Four species of *Louisea* endemic to southwestern Cameroon, right cheliped carpus. **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balsi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) ('Mvogo Ndongo et al. 2018: fig. 3c', www.mapress.com/j/zt) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkong-samba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 5 mm (**a, c**), 2 mm (**b**), 10 mm (**d**).

cal sulcus on carapace branchiostegal wall meeting anterolateral margin at intermediate tooth (Fig. 6d). Mandibular palp bi-segmented; terminal segment (TS) bilobed, with large distinct anterior lobe $0.8 \times$ terminal segment length (Fig. 15d). Third maxilliped exopod completely lacking flagellum; ischium with distinct vertical groove (Fig. 14d). Episternal sulci $s4/e4$, $s5/e5$, $s6/e6$, $s7/e7$ complete (Fig. 5d). Major cheliped dactylus relatively stout, straight, not arched, enclosing long thin interspace when closed, with small distal tooth (Fig. 8g); propodus of major cheliped with three large teeth (proximal, medial, distal) (Fig. 8g); cheliped carpus inner margin with large, broad pointed distal tooth, robust subequal proximal tooth (Fig. 10d); cheliped merus medial inferior margin with large jagged distal tooth followed by several distinct smaller teeth (Fig. 9d). G1 TA short (TA/SS 0.22), directed outwards at 45° angle to longitudinal axis of G1 SS, with distinct longitudinal groove on ventral face, proximally distinctly broad, abruptly narrow, slim and tube-like at distal two-thirds (Figs 11d, 12d). G1 SS tapering slightly from broad basal margin to relatively wide distal margin ($0.5 \times$ SS basal margin); dorsal face with broad dorsal membrane (maximum width $0.1 \times$ SS length) at TA/SS junction (Fig. 11d). G2 TA long (TA/SS 0.44), flagellum-like, almost as long as G2 SS (Fig. 13d). Mature at CW 20 mm.

Table 4. Morphometric analysis and collection data of specimens ($N = 27$) of *Louisea nkongsamba* sp. nov. from Cameroon. All measurements are given in mm.

Specimens	CW	CL	CH	FW	CW/FW	CL/FW	CH/FW	FW/CL	Coll. Date	Museum
1 ad ♂ ¹	20	14.85	8.4	6.6	3.03	2.25	1.27	0.44	P.A.M.N 23.05.18	ZMB Crust. 31618
2 ad ♂ ²	18.38	13.32	8.13	6.34	2.89	2.1	1.28	0.47	P.A.M.N 25.05.18	ZMB Crust. 31620
3 ad ♂ ¹	18.36	13.30	8.12	6.17	2.97	2.15	1.31	0.46	P.A.M.N 23.05.18	ZMB Crust. 31618
4 ad ♂ ³	17.01	12.30	7.53	5.56	3.05	2.21	1.35	0.45	P.A.M.N 26.05.18	ZMB Crust. 31619
5 ad ♂ ³	17.27	12.80	7.55	5.50	3.14	2.32	1.36	0.42	P.A.M.N 26.05.18	ZMB Crust. 31619
6 ad ♂ ⁴	18.40	13.52	8.56	5.90	3.11	2.29	1.45	0.32	P.A.M.N 27.05.18	ZMB Crust. 31621
7 ad ♂ ⁴	17.61	13.15	7.80	6.08	2.89	2.16	1.28	0.34	P.A.M.N 27.05.18	ZMB Crust. 31621
8 ad ♂ ⁴	17.37	12.89	7.73	5.60	3.10	2.30	1.38	0.43	P.A.M.N 27.05.18	ZMB Crust. 31621
9 ad ♂ ¹	18.05	12.84	8	6.16	2.93	2.08	1.29	0.47	P.A.M.N 25.05.18	ZMB Crust. 31618
10 ad ♂ ¹	16.75	12.50	7.71	5.77	2.90	2.16	1.33	0.46	P.A.M.N 23.05.18	ZMB Crust. 31618
11 ad ♂ ¹	16.01	11.68	7.04	5.31	3.01	2.19	1.32	0.45	P.A.M.N 23.05.18	ZMB Crust. 31618
12 ad ♂ ¹	16.90	12.20	7.81	5.32	3.17	2.29	1.46	0.43	P.A.M.N 23.05.18	ZMB Crust. 31618
13 ad ♂ ¹	15.24	11.47	6.41	5.42	2.81	2.11	1.18	0.47	P.A.M.N 23.05.18	ZMB Crust. 31618
14 ad ♂ ²	15.55	11.60	6.93	5.40	2.87	2.14	1.28	0.46	P.A.M.N 25.05.18	ZMB Crust. 31620
15 ad ♂ ²	16.50	12.19	7.47	5.60	2.94	2.17	1.33	0.45	P.A.M.N 25.05.18	ZMB Crust. 31620
16 ad ♀ ²	19.72	14.76	9.12	7.03	2.80	2.1	1.29	0.47	P.A.M.N 25.05.18	IFAS-005
17 ad ♀ ²	17.10	13.03	8.28	5.96	2.86	2.18	1.38	0.45	P.A.M.N 25.05.18	IFAS-005
18 ad ♀ ¹	16.15	11.66	7.10	5.62	2.87	2.07	1.26	0.48	P.A.M.N 23.05.18	IFAS-005
19 ad ♀ ²	15.04	11.10	6.86	5	3	2.22	1.37	0.45	P.A.M.N 25.05.18	IFAS-005
20 ad ♀ ²	14.73	10.80	6.25	4.80	3.06	2.25	1.30	0.44	P.A.M.N 25.05.18	IFAS-005
21 ad ♀ ²	14.56	10.9	6.08	5.15	2.82	2.11	1.18	0.47	P.A.M.N 25.05.18	IFAS-005
22 sd ♂ ²	13.49	10.33	6.20	4.45	3.03	2.32	1.39	0.43	P.A.M.N 25.05.18	IFAS-006
23 sd ♂ ²	13.76	10.54	5.85	4.35	3.16	2.42	1.34	0.41	P.A.M.N 25.05.18	IFAS-006
24 sd ♂ ²	13.33	10.30	6.10	4.25	3.13	2.42	1.43	0.41	P.A.M.N 25.05.18	IFAS-006
25 sd ♂ ²	12.87	10.08	5.86	4.17	3.08	2.41	1.40	0.41	P.A.M.N 25.05.18	IFAS-006
26 sd ♂ ²	13.14	10.09	5.91	4.60	2.85	2.19	1.28	0.45	P.A.M.N 25.05.18	IFAS-006
27 sd ♂ ²	12.91	9.94	5.64	4.70	2.74	2.11	1.20	0.47	P.A.M.N 25.05.18	IFAS-006
Mean	16.15	12.00	7.20	5.46	2.95	2.19	1.32	0.45	–	–

Key: P.A.M.N: Pierre A. Mvogo Ndongo; ad: adult; sd: subadult. Location: ¹ Nlonako site 1; 4°54'44.8"N, 9°58'50.2"E; 1211 m a.s.l.; ² Nlonako site 2; 4.91343° N, 9.98500° E; 1176 m a.s.l.; ³ Nlonako site 3; 4°53'30.5"N, 9°59'12.1"E; 938 m a.s.l.; ⁴ Nlonako site 4; 4°54'56.4"N, 9°59'41.8"E ASL: 1392 m a.s.l.

Description. Carapace ovoid, moderately high (CH/FW 1.32, $N = 27$), wide (CW/FW 2.95, $N = 27$), texture smooth, urogastric groove distinct. Front wide (FW/CW 0.34, $N = 27$), deflexed, anterior margin straight (Figs 2d, 3d, 4a, 7d). Postfrontal crest faint but complete, ends meeting anterolateral margins at epibranchial teeth (Fig. 7d); mid-groove faint, shallow; epigastric crests poorly defined (Fig. 7d). Exorbital, intermediate teeth large, triangular, epibranchial tooth small but detectable (Figs 4a, 7d). Anterolateral margin of carapace lined by small granules (Figs 4a, 7d); posterolateral margin curving inward, continuous with anterolateral margin (Fig. 7d); posterior carapace margin about 2/3 CW. Carapace branchiostegal wall with longitudinal, vertical sutures dividing sidewall into three parts (Fig. 6d). Longitudinal sulcus beginning at respiratory opening, curving backward across sidewall, dividing suborbital- and subhepatic regions from pterygostomial region (Fig. 6d); vertical sulcus on sidewall marked by row of granules, meeting anterolateral margin at intermediate tooth (Fig. 6d), divid-



Figure 11. Four species of *Louisea* endemic to southwestern Cameroon, right G1 dorsal view (a–c), left G1 dorsal view (d). **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balsi* from Man’s Crater Lake Manengouba (ZMB Crust. 30319) (‘Mvogo Ndongo et al. 2018: fig. 5a’, www.mapress.com/j/zt) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 2 mm (a, c, d), 1 mm (b).

ing suborbital- from subhepatic regions (Figs 6d, 7d). Sternal sulcus s2/s3, deep, ends not meeting side margins of sternum (Fig. 5d); s3 with distinct central depression; s3/s4 reduced to two short lateral notches (Fig. 5d). Episternal sulci s4/e4, s5/e5, s6/e6, s7/e7 complete (Fig. 5d). Mandibular palp bi-segmented; terminal segment (TS) bilobed, with large distinct anterior lobe $0.8 \times$ terminal segment length (Fig. 15d). Third maxilliped (Fig. 6d) filling entire buccal cavern, except for transversely oval efferent respiratory openings in superior lateral corners; exopod completely lacking flagellum; ischium with distinct vertical groove (Fig. 14d).

Major cheliped dactylus relatively stout, straight, not arched enclosing long, thin interspace when closed, with small proximal tooth (Fig. 8g); propodus of major cheliped with three large teeth (proximal, medial, distal) (Fig. 8g); cheliped carpus inner margin with large, broad, pointed distal tooth, robust, subequal proximal tooth (Fig. 10d). Walking legs (p2–p5 slender, p4 longest, p5 shortest; dactyli (p2–p5) tapering to point, each bearing rows of downward-pointing sharp bristles, p5 dactylus shortest (Figs 2d, 3d).

Male pleon triangular, telson (a7) with rounded distal margin (Fig. 5d). G1 TA short (TA/SS 0.22), directed outwards at 45° angle to longitudinal axis of G1 SS, with distinct longitudinal groove on ventral face, proximally distinctly broad, abruptly narrow, slim and tube-like at distal two-thirds (Figs 11d, 12d). G1 SS tapering slightly from

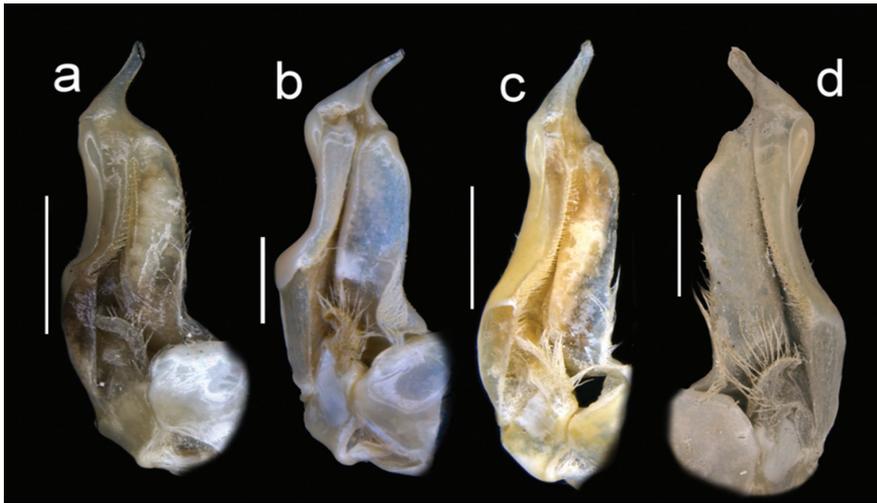


Figure 12. Four species of *Louisea* endemic to southwestern Cameroon, right G1 ventral view (a–c), left G1 ventral view (d). **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balsi* from Man’s Crater Lake Manengouba (ZMB Crust. 30319) (Mvogo Ndongo et al. 2018: fig. 5b) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 2 mm (a, c, d), 1 mm (b).

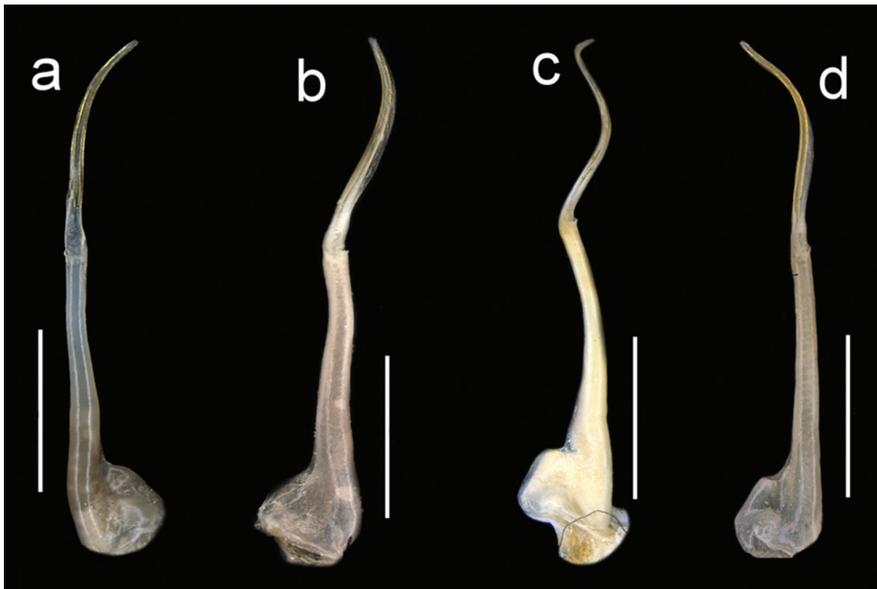


Figure 13. Four species of *Louisea* endemic to southwestern Cameroon, G2 **a** second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balsi* from Man’s Crater Lake Manengouba (ZMB Crust. 30319) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 2 mm (a–d).

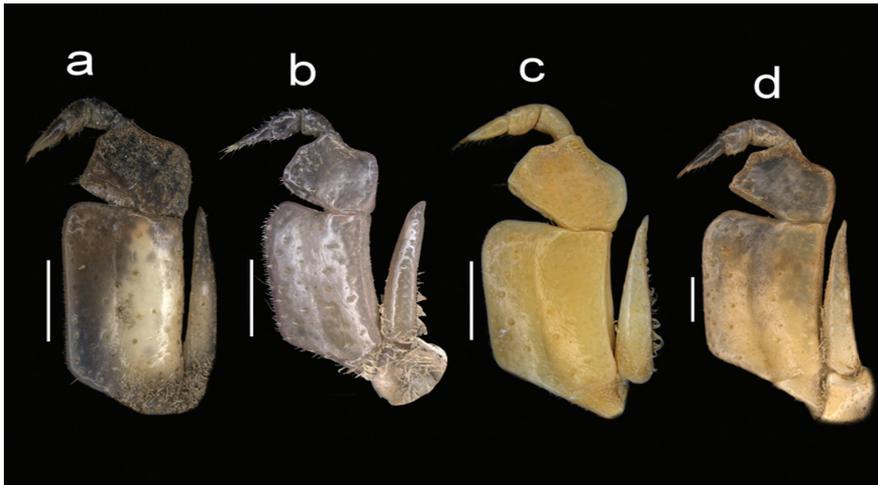


Figure 14. Four species of *Louisea* endemic to southwestern Cameroon, left third maxilliped. **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balssi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) ('Mvogo Ndongo et al. 2018: fig. 4a', www.mapress.com/j/zt) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 2 mm (**a–d**).

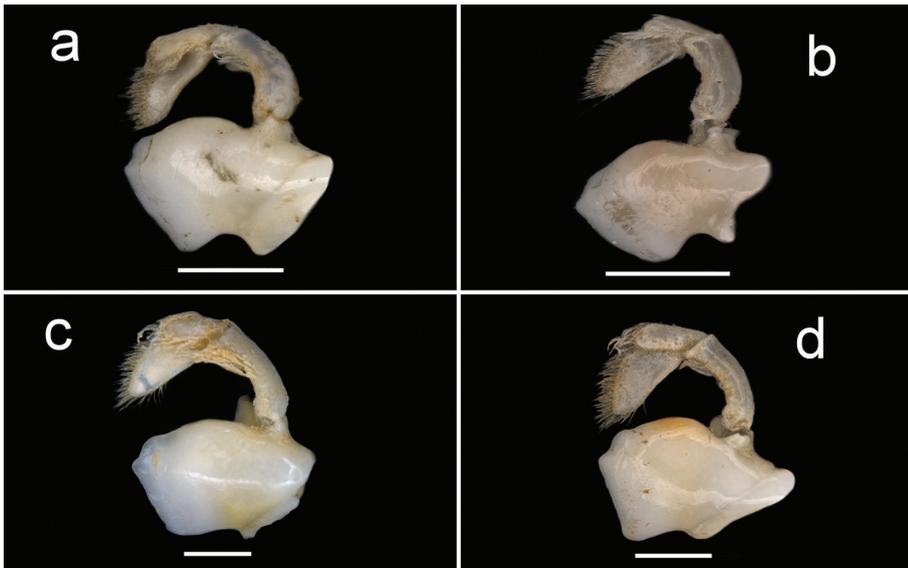


Figure 15. Four species of *Louisea* endemic to southwestern Cameroon, ventral view of right mandible. **a** Second largest adult male (CW 16.1 mm) of *L. edeaensis* from Lake Ossa wetland complex (ZMB Crust. 30335) **b** largest adult male (CW 16.2 mm) of *L. balssi* from Man's Crater Lake Manengouba (ZMB Crust. 30319) **c** adult male, holotype (CW 18.1 mm) of *L. yabassi* sp. nov. from Yabassi (ZMB Crust. 21575) **d** largest adult male, holotype (CW 20.0 mm) of *L. nkongsamba* sp. nov. from Mt. Nlonako (ZMB Crust. 31618). Scale bars: 1 mm (**a, c**), 2 mm (**b, d**).

broad basal margin to relatively wide distal margin ($0.5 \times$ SS basal margin); dorsal face with broad dorsal membrane (maximum width $0.1 \times$ SS length) at TA/SS junction (Fig. 11d). G2 TA long (TA/SS 0.44), flagellum-like, almost as long as G2 SS (Fig. 13d).

Color in life. Specimens of *L. nkongsamba* sp. nov. have a dark brown or green carapace and walking legs.

Type locality. Nlonako Wildlife Reserve, Nkongsamba, littoral region of Cameroon.

Etymology. The new species is named for Nkongsamba, the closest town to the type locality. The species epithet is a noun in apposition.

Habitat. *L. nkongsamba* sp. nov. is known only from Nlonako Wildlife Reserve, one of the threatened tropical rainforest habitats in the littoral region of Cameroon.

Remarks. *L. nkongsamba* sp. nov. possesses numerous characters that link it to *L. edeaensis*, *L. balssi*, and *L. yabassi* sp. nov. Differences between these species are discussed below under general remarks.

General remarks

The generic characters of *Louisea* proposed by Cumberlidge (1994, 1999) were based on *L. edeaensis* and a subadult specimen of *L. balssi*. These characters are therefore reassessed here in the light of recently-rediscovered populations of *L. edeaensis* and *L. balssi* that included adult males of both species (Mvogo Ndongo et al. 2017a, 2018), and the two new species from Yabassi and Mt. Nlonako described here. All these four species are assigned to *Louisea* because they share the emended diagnostic characters for the genus presented here, and three of them at least form a monophyletic group (Fig. 16). The main characters that differentiate the four species are based on the cheliped dactylus; the inner margin teeth on the cheliped carpus; the intermediate tooth between exorbital and epibranchial teeth; the postfrontal crest; sternal segment S3; sternal sulcus S2/S3; the anterior lobe on the TS of the mandibular palp; and the G1 TA length. Some of these characters are shared by two of the four species. The differences between the four species of *Louisea* are given in Table 5.

Discussion

The highlands of southwestern Cameroon are part of the continental segment of the Cameroon Volcanic Line (CVL) that includes Mount Cameroon, Mt. Manengouba, Mt. Nlonako, Mt. Lefo, Mt. Oku, and the Ngaoundere Plateau (Burke 2001; Zimkus 2009). The CVL crosses a significant portion of the West African forest biodiversity hotspot, which is remarkably species rich and has a high rate of endemism (Stuart 1986; Lawson 1993; Bowden and Andrews 1994; Stattersfield et al. 1998; Myers et al. 2000; Lovett and Taplin 2004; Herrmann et al. 2005). Although the freshwater crab fauna of southwestern Cameroon is also species-rich and has a high number of endemic species (Cumberlidge et al. 2019), most parts of this part

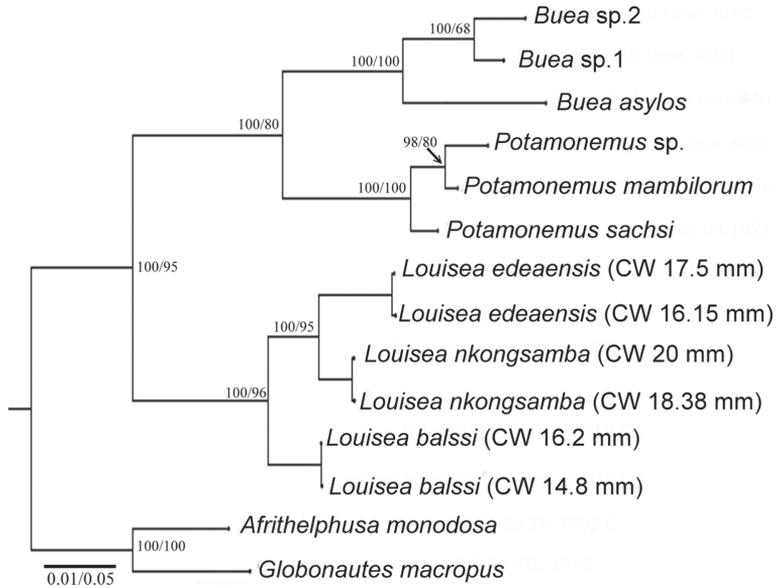


Figure 16. ML tree topology for the freshwater crab taxa from Cameroon included in this study derived from mtDNA sequences corresponding to three loci (partial 16S rRNA, COI and 12S rRNA genes). BI and ML statistical values (%) on the nodes indicate posterior probabilities and bootstrap support, respectively.

Table 5. Differences between the four species of the genus *Louisea*.

Characters	<i>L. nkongsamba</i> sp. nov.	<i>L. yabassi</i> sp. nov.	<i>L. balssi</i>	<i>L. edeaensis</i>
Major cheliped dactylus shape	Stout, straight (Fig. 8h)	Stout, straight (Fig. 8e)	Slender, highly arched (Fig. 8c)	Stout, straight (Fig. 8a)
Major cheliped propodus dentition	3 large teeth (Fig. 8h)	5 large teeth (Fig. 8e)	2 large proximal teeth (Fig. 8c)	4 large teeth (Fig. 8a)
Cheliped carpus inner margin teeth	Distal larger than proximal (Fig. 10d)	Both large and pointed (Fig. 10c)	Both large and pointed (Fig. 10b)	Distal larger than proximal (Fig. 10a)
Intermediate tooth between exorbital and epibranchial teeth	Large and triangular (Figs 4a, 7d)	Large and triangular (Figs 4d, 7c)	Faint, barely detectable (Figs 4b, 7b)	Small but distinct (Figs 4c, 7a)
Postfrontal crest	Faint (Figs 4a, 7d)	Prominent, clearly defined (Figs 4d, 7c)	Faint (Figs 4b, 7b)	Faint (Figs 4c, 7a)
Sternal sulcus s2/s3	Deep, ends not meeting side margins of sternum (Figs 5d, 6d)	Deep, ends meeting side margins of sternum (Figs 5c, 6c)	Deep, ends meeting side margins of sternum (Figs 5b, 6b)	Faint and shallow, ends meeting side margins of sternum (Figs 5a, 6a)
Sternal segment s3	With distinct central depression (Figs 5d, 6d)	No depression (Figs 5c, 6c)	No depression (Figs 5b, 6b)	No depression (Figs 5a, 6a)
Size of anterior lobe on terminal segment (TS) of mandibular palp	Large (0.8× TS length) (Fig. 15d)	Medium (0.6× TS length) (Fig. 15c)	Small (0.5× TS length) (Fig. 15b)	Medium (0.6× TS length) (Fig. 15a)
G1 TA length	Short (0.22× G1 length) (Fig. 11d)	Medium (0.27× G1 length) (Fig. 11c)	Medium (0.28× G1 length) (Fig. 11b)	Medium (0.29× G1 length) (Fig. 11a)

of the country have remained largely unsurveyed for freshwater crabs until recently (Mvogo Ndongo et al. 2017a, 2017b, 2017c, 2018). Recent biotic surveys of the freshwater crab fauna in southwestern Cameroon coupled with the re-examination of museum specimens have led to the discovery or rediscovery of a number of taxa, raising the number of species from Cameroon to 17, and the number of genera to five (Cumberlidge et al. 2019). However, the two rediscovered species *Louisea edeaensis* and *L. balssi* and the two new species of *Louisea* described here are all from new localities, because attempts to find additional specimens of *Louisea* were not successful at Yabassi, Edea, Yaounde and Kumba, localities where they were collected between 1900 and 1910. It is possible that the populations of *Louisea* in these four locations have been extirpated because they were last found in 1910, and there has been considerable human population expansion and urban development in these areas since then. This is especially unfortunate because the specimens from Yabassi have a problematic taxonomic history, but the larger series of specimens of all species in this genus now available enables us to recognise the specimens from Yabassi as a new species, *L. yabassi* sp. nov.

Our phylogenetic analyses (Fig. 16) based on 1801 base pairs of three mitochondrial genes (combined COI, 16S RNA, 12S RNA) found strong BI and ML support for the continued recognition of the genus *Louisea* with a well-supported clade that includes *L. edeaensis*, *L. balssi*, and *L. nkongsamba* sp. nov. We were not able to extract DNA from the available specimens of *L. yabassi* sp. nov., and this species was therefore not included in the tree. It has been suggested that the montane regions on the Cameroon Volcanic Line act as centres of speciation, as has been reported by Zimkus (2009) for puddle frogs from Mt. Oku. Our data indicate that the highland dwelling species *L. balssi* is the sister group to the two other species of *Louisea* found at lower altitudes, which indicates that speciation may have happened along an altitudinal gradient. However, further phylogenetic analyses are needed to establish whether this can lend support to the hypothesis that CVL is a montane centre of speciation. It is interesting to note that our phylogenetic tree also recognizes for the first time two distinct lineages within the genus *Buea* and one lineage within *Potamonemus* (Mvogo Ndongo et al. in prep). *Buea* and *Potamonemus* are sister genera and are apparently derived from the well-established diversified lineages of *Louisea*, but a phylogenetic work that includes all known genera assigned to the Potamonautinae is needed to test this hypothesis.

Revised key to the species of the genus *Louisea* Cumberlidge, 1994

- 1 Postfrontal crest prominent, clearly defined (Figs 4d, 7c) ***L. yabassi* sp. nov.**
- Postfrontal crest faint (Figs 4a–c, 7a, b, d)..... 2
- 2 Dactylus of male major cheliped relative slender, highly arched (Fig. 8c).....
..... ***L. balssi***
- Dactylus of male major cheliped relative stout, straight (Fig. 8a, h)..... 3

- 3 Sternal segment s3 lacking central depression, sternal sulcus s2/s3 relatively shallow, ends meeting side margins of sternum (Figs 5a, 6a); anterior lobe on terminal segment of mandibular palp relatively small, 0.6× TS length (Fig. 15a); G1 terminal article relatively long (TA/SS 0.3) (Fig. 11a).... *L. edeaensis*
- Sternal segment s3 with distinct central depression, sternal sulcus s2/s3 deep, ends not meeting side margins of sternum (Figs 5d, 6d); anterior lobe on terminal segment of mandibular palp relatively large, 0.8× TS length (Fig. 15d); G1 terminal article relatively short (TA/SS 0.2) (Fig. 11d).....
*L. nkongsamba* sp. nov.

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