RESEARCH ARTICLE



Three new species of the sea fan genus Leptogorgia (Octocorallia, Gorgoniidae) from the Gulf of California, Mexico

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Academic editor: J. Reimer Received 30 January 2020 Accepted 6 November 2020 Published 12 February 2021	
http://cookank.com/DCC50010.2C74.6605.0E4E.B2E25EE60C84	

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Citation: Hernández O, Gómez-Gutiérrez J, Sánchez C (2021) Three new species of the sea fan genus *Leptogorgia* (Octocorallia, Gorgoniidae) from the Gulf of California, Mexico. ZooKeys 1017: 1–20. https://doi.org/10.3897/ zookeys.1017.50619

Abstract

Three new sea fan species of *Leptogorgia* were discovered during multiple scuba diving expeditions along the Gulf of California coast and islands. *Leptogorgia iridis* **sp. nov.** is distributed in the southern region of the gulf (Mexican Province), inhabiting tropical rocky reefs of the Islas Marías Archipelago (Nayarit) and Bahía Banderas (Jalisco). This species has small colonies (< 7 cm height) with at least five clearly distinct chromotypes. *Leptogorgia martirensis* **sp. nov.** was found exclusively on the rocky reefs of San Pedro Mártir and San Esteban Islands located in the northern region of the Gulf of California (northern region of Cortez Province). *Leptogorgia emrici* **sp. nov.** is distributed from the south to the northern region of the Gulf of California (Cortez Province), inhabiting substrates of rocky reefs, sandy and pebbly sea floors. Comprehensive ecological diving expeditions to identify and classify octocorals in the Mexican Pacific (1995–2019) indicate that *L. iridis* **sp. nov.** and *L. martirensis* **sp. nov.** are likely to be micro-endemics and *L. enrici* **sp. nov.** is endemic to the Gulf of California, which defines their currently known biogeographic distribution ranges.

Keywords

Alcyonacea, chromotypes, Cnidaria, gorgonians, microendemism, rocky reef

Introduction

The family Gorgoniidae Lamouroux, 1812 includes mostly species of three genera in the eastern Pacific: Pacifigorgia Bayer, 1951 with anastomosed branching as the main diagnostic character, Eugorgia Verrill, 1868 with the presence of double disk capstans and Leptogorgia Milne Edward & Haime, 1857; which, in contrast to the previous two genera, does not have a single diagnostic genus morphological feature (Williams et al. 2004; Breedy et al. 2009). Verrill (1868) separated species of the genera *Eugorgia* from Leptogorgia because Eugorgia species have double disk capstan sclerites. Leptogorgia taxonomic classification is based on several morphological characters that might be present or absent in the genera *Pacifigorgia* and or *Eugorgia* (Breedy and Guzman 2002; Breedy et al. 2009). Branching and colony growth patterns and types of sclerites are required morphological diagnostic traits to identify and distinguish among Leptogorgia species (Breedy and Guzman 2007). The lack of a single diagnostic taxonomic character in the genus Leptogorgia causes uncertainties in the taxonomic classification of species in this highly morphologically diverse genus, which currently includes 103 nominal species and seven species assigned as nomen dubium worldwide (Cordeiro et al. 2020). This taxonomical problem is particularly accentuated by multiple species having wide interspecific and intraspecific variability of morphotypes and chromotypes. The molecular evidence strongly suggests that genus *Leptogorgia* has deep genetic divergence among morphologically similar species, with multiple genetic geographically restricted lineages (Poliseno et al. 2017; Soler-Hurtado et al. 2017a, b; Olvera et al. 2018; Silvestri et al. 2019).

There are 30 extant *Leptogorgia* species recorded along the American Pacific coast, with *Leptogorgia waltonae* Olvera, Hernández, Sánchez & Gómez-Gutiérrez, 2018 being the latest species described in the Mexican Pacific (Olvera et al. 2018). Here we describe three new species of the genus *Leptogorgia* discovered in the Gulf of California during extensive ecological diving expeditions to identify and classify octocorals in the Mexican Pacific (1995–2019). Therefore, these three new *Leptogorgia* species increase the total number of nominal *Leptogorgia* species to 33 for the American Pacific and 20 for the Mexican Pacific.

Materials and methods

Approximately 500 quantitative monitoring transects, each one covering an area of 30 m², were surveyed during extensive annual ecological expeditions located along the peninsular coast and at 25 islands of the Gulf of California (1995–2019), Islas Marías Archipelago (2010 and 2018), Bahía Banderas (2013) and Bahía Magdalena (2013–2014) (Fig. 1). Several octocoral colonies were collected during those monitoring surveys for taxonomic purposes. A total of 35 colonies of *Leptogorgia iridis* sp. nov., 25 colonies of *Leptogorgia martirensis* sp. nov. and 42 colonies of *Leptogorgia enrici* sp. nov. were collected during scuba diving between 2–55 m depths. All

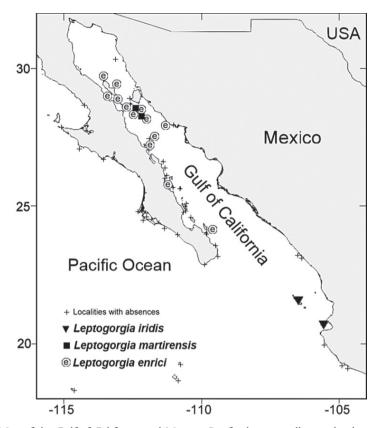


Figure I. Map of the Gulf of California and Mexican Pacific showing collection localities of the three new species of *Leptogorgia*.

specimens were dried or preserved in 96% ethanol. A portion of each colony was macerated in sodium hypochlorite to extract the sclerites, washed several times with distilled water and preserved in 96% ethanol for further microscopic analyses. Sclerites were air-dried and attached to aluminum stubs with double adhesive bands. They were coated with gold using a sputter coater (Polaron E5100) in an argon atmosphere and observed under a Hitachi S-3000 N scanning electron microscopy (SEM) at 20 kV. The sclerite morphological traits were compared with sclerites of fourteen nominal Leptogorgia species distributed along the tropical eastern Pacific (Table 1) using original taxonomic descriptions (Breedy and Guzman 2007; Horvath 2011; Breedy et al. 2012; Olvera et al. 2018) and taxonomical analyses from octocoral specimens from the institutional collection of Universidad Autónoma de Baja California Sur (Proyecto Fauna Arrecifal: PFA). Species identification and morphological comparisons among Leptogorgia species were assessed following the standard techniques and nomenclature used by Verrill (1868), Breedy and Guzman (2007), Breedy et al. (2009, 2012), and Horvath (2011) (Table 1). We used standard taxonomic terminology and criteria to describe the three new species (Bayer et al.

Table 1. Comparison of morphological characters of <i>Leptogorgia iridis</i> sp. nov, <i>Leptogorgia martirensis</i> sp. nov. and <i>Leptogorgia enrici</i> sp. nov. with fourteen other <i>Leptogorgia</i> nominal species distributed along the Mexican Pacific and Gulf of California of specimens collected from 2002–2016 and from other regions of the Eastern Pacific reported in Breedy and Guzman (2007), Horvath (2011), Breedy et al. (2012), and Soler-Hurtado et al. (2017b). All morphological measurements are given in mm. All the taxonomical characters are based on the holotypes or lectotypes except the color of the colony and the sclerites of several species from the Gulf of California that are based on the characteristics of extra specimens. Branching type: bra-str = branches/strands, di = dichotomous, irr = irregularly, lb = laterally branched, pd = pseudodichotomous, pi = pinnate. Polyp distribution rows: irr = irregular, spar = sparsely. Color: am = ambar, br = brownish, brr = brownish red, hy = hyaline, o = orange, r = red, y = yellow, pi = pink, pu = purple, w = white. Numerically dominant types of sclerites: C = capstan, O = oval, S = spindles.	omparisol tominal s fic reportu mm. All t ornia thau ornia thau l = pseudu = orange,	n of morp pecies dis ed in Bree the taxonu t are basec odichotor r = red, y	bhological stributed edy and C omical ch 1 on the c nous, pi = yellow,	characte along the duzman (aracters a haracteris pinnate. pi = pinl	gical characters of <i>Leptogorgia rirdis</i> sp. nov., <i>Leptogorgia martirensis</i> sp. nov. and <i>Leptogorgia enrici</i> sp. nov. with fourteen other ted along the Mexican Pacific and Gulf of California of specimens collected from 2002–2016 and from other regions of the d Guzman (2007), Horvath (2011), Breedy et al. (2012), and Soler-Hurtado et al. (2017b). All morphological measurements l characters are based on the holotypes or lectorypes except the color of the colony and the sclerites of several species from the he characteristics of extra specimens. Branching type: bra-str = branches/strands, di = dichotomous, itr = irregularly, lb = laterally pi = pinnate. Polyp distribution rows: itr = irregular, spar = sparsely. Color: am = ambar, br = brownish, brr = brownish red, hy low, pi = pink, pu = purple, w = white. Numerically dominant types of sclerites: C = capstan, O = oval, S = spindles.	<i>rgia ir</i> acific a vath (2 the ho specim ibution le, w =	<i>idis</i> sp. n nd Gulf 011), Bru lotypes c lens. Brau n rows: ir white. N	lov., <i>L</i> of Ca eedy e or lectu nchinę r = irru	<i>eptogorgia</i> ulifornia o t al. (2011 otypes exc g type: bra egular, spa egular, spa rically don	<i>martirensi</i> f specimer 2), and Sol 2), an	<i>is</i> sp. nov as collect ler-Hurta lor of the iches/stra ly. Color: es of scle	. and <i>Lepto</i> ed from 201 ido et al. (21 2 colony and nds, di = di am = amba rites: C = ca	orgia en 02-201(017b). A 1 the scl thotomc t, br = b pstan, C	<i>rici</i> sp. no 5 and fron dl morphc erites of se ous, irr = ir rownish, l rowal, S	v. with f n other n ological sp veral sp regularly orr = bro	ourteen o regions o measuren ecies fron ecies fron s, lb = lato wnish ree les.	f the f the nents r the rally f, hy
Species	Colony growth	Branching Terminal type branches diameter	Terminal Polyp branches distribution diameter rows	Polyp distribution rows	Polyp mound elevation	Capstan length	Spindles length s _l	Bent spindles	Crosses	Anthocodial Dominant sclerites sclerites	Dominant sclerites	Color of colony	Bicolor colonies d	Number of solid chromotypes	Bicolor A sclerites	Bicolor Anthocodial Color sclerites sclerites ring color	Color ring
L. iridis sp. nov.	planar	lb	1–2	2	slightly raised	0.06	0.07	no	0.05×0.05	rod	υ	pu/y,pu, r, w, y	yes	5	yes	pu, r, w, y	no
L. martirensis sp. nov	bushy	qI	7	1–2	prominent	0.05	ou	ou	оп	rod	C	y, p, br	ou	ŝ	yes	0, I, W	no
L. enrici sp. nov.	planar	qI	1.5	1, irr	ou	0.06	0.11	yes	0.06×0.06	rod	C	o, pu, y, w, y/pu	yes	4	no	o, y, pu	no
L. alba	flabellate	irr/di	I	2	slightly raised, flat	90.0	0.18	yes	I	rod	S	×	no	1	no	no	no
L. chilensis	lank, bushy	/ irr/di	2.8	spar	flat	0.08	0.12	ou	0.06×0.06	Rod, biscuit	C-S	0	ou	1	no	0	no
L. clavata	I	pi	I	irr	slightly raised	0.075	0.10	ou	I	rod	C	г	ou		no	pi	no
L. cuspidata	bushy	irr/pd	3.25-4	crowd	I	0.09	0.13	yes	I	rod	C	pu/y	yes	1	yes	у	yes
L. diffusa	bushy, arborescent	-ql	ĉ	1–2	prominent	0.09	0.15	yes	I	por	S	0	no	1	ou	0	ou
L. ena	cluster	lb	2–3	in	slightly raised	0.086	0.108	no	0.05×0.07	rod-platelet	C	pu, y, pu/y	yes	3	yes	pu, y	no
L. exigua	bushy	irr/pi	3-4	crowd	slightly raised	0.10	0.13	ou	I	I	U	brr/y	ou	1	yes	I	no
L. filicrispa	strands	bra-str	0.5-1	2	prominent	0.08	0.11	ou	I	rod	I	pi	ou	1	no	pi	no
L. flexilis	bushy	inr/di	1.0-1.5	4-5	flat	0.09	0.09	ou	I	rod	U	r/br	ou	1	yes	r	no
L. labiata	flabellate	inr/pi	2	4	prominent	0.08	0.1	ou	I	rod	U	pi/y	yes	1	yes	у	yes
L. laxa	planar	in:/di	1.0-1.5	2	slightly raised	0.08	0.18	ou	I	rod	I	w	no	1	ou	ou	no
L. manabiensis	planar	inr/pi	1.9	spar	slightly raised	0.08	0.14	yes	I	rod	S	pi	no	1	ou	no, hy	no
L. pumila	bushy	irr/pi	2,3	2, spar	raised	0.1	0.15	yes	0.08×0.06	rod	I	pu, pi	no	1	no	am	ou
L. rigida	bushy, arborescent	īd .	2–3	3-4	slightly raised	0.08	0.12	NO	0.04×0.04	rod	0 C	nd	Ю	1	ou	pi	оп

1983; Calvo and Breedy 2002; Breedy et al. 2009, 2013; Breedy and Guzmán 2007, 2013). All the holotypes and paratypes were deposited in the Smithsonian National Museum of Natural History (**NMNH**).

Systematics

Class Anthozoa Ehrenberg, 1834 Subclass Octocorallia Haeckel, 1866 Order Alcyonacea Lamouroux, 1812 Suborder Holaxonia Studer, 1887 Family Gorgoniidae Lamouroux, 1812 Genus *Leptogorgia* Milne Edward & Haime, 1857

Leptogorgia iridis sp. nov.

http://zoobank.org/38587E54-6E24-4949-BCF5-61FBB2982023 Figures 3, 8A, B

Material examined. *Holotype.* NMNH-1638551: dry María Magdalena Island (south west rocky point), Islas Marías Archipelago, Nayarit, Mexico (21°25.267'N, -106°24.900'W), 10 m depth, 15 November 2010, collector Carlos Sánchez. *Paratypes.* NMNH-1638552: dry María Madre Island (southern rocky point), Islas Marías Archipelago, Nayarit, Mexico (21°32.391'N, -106°31.877'W), 8 m depth, 18 November 2010, collector Carlos Sánchez. NMNH-1638553: dry María Madre Island (southwest rocky point, Islas Marías Archipelago, Nayarit, Mexico (21°32.391'N, -106°31.877'W), 8 m depth, 18 November 2010, two colonies connected by single holdfast, collector Carlos Sánchez. NMNH-1638554: dry, María Madre Island (southwest rocky point), Islas Marías Archipelago, Nayarit, Mexico (21°25.267'N, -106°24.900'W), 10 m depth, 15 November 2010, collector Carlos Sánchez. NMNH-1638555: dry colony from El Faro de Cabo Corrientes, Bahía Banderas, Jalisco, Mexico (20°24.553'N, -105°41.708'W), 2013, collector Carlos Sánchez.

Type locality. Islas Marías Archipelago is located in the southern region of the Gulf of California, Mexico (21°25.267'N, -106°24.900'W) near the continental shelf-break about 158 km southwest of Mazatlán, Sinaloa and 106 km northwest of Bahía Banderas, Nayarit (Fig. 1).

Holotype colony description. Colony shows lateral branching and planar growth of 7 cm height and 8.1 cm width. Holdfast is 5 mm diameter and arises the main steam 2.1 cm length and 2 mm diameter, subdividing into two main branches (Fig. 2A). The longer branch grows up to 2.8 cm length and 2 mm diameter before dividing into secondary and further branches 1–2 mm in diameter. The smaller branches are 4 mm length and 1 mm diameter before subdividing and growing downward. The main stem shows three alternating and broken pinnula with a brownish nude axis of 0.5 mm diameter. Secondary and terminal twigs have blunt tips arising at 45° angles and of > 2 mm

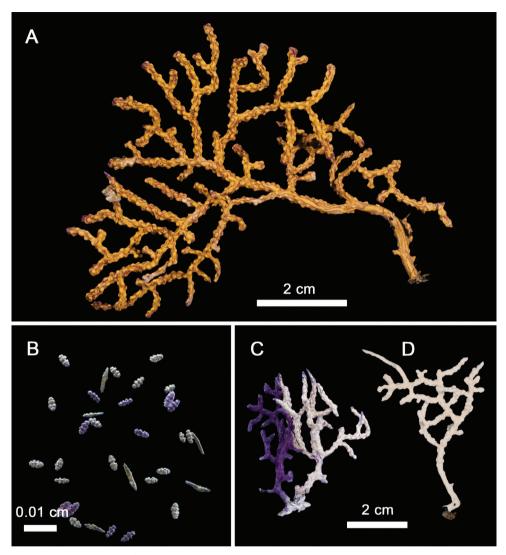


Figure 2. *Leptogorgia iridis* sp. nov. **A** holotype NMNH-1638551 **B** holotype anthocodial and coenenchymal sclerites **C** paratype NMNH-1638553, two colonies with different color connected with a single holdfast **D** paratype NMNH-1638554, monochromatic white chromotype.

diameter. The entire colony is yellow and deep purple, forming longitudinal bicolor striped patterns along the branches from the base to the tips of the colony (Fig. 2A). Polyp mounds are oval 1.0×0.5 mm, slightly raised by 1 mm with polyp rings, arranged in two rows along with the entire colony, except on the lower half of the stem.

Holotype sclerites. Coenenchymal sclerites of *Leptogorgia iridis* sp. nov. holotype are mostly bright yellow or purple and few of them are bicolor or white (Fig. 2B). Dominant sclerites are capstans (0.06 mm length and 0.04 mm width) (Fig. 3A). Spindles are scarce (0.07 mm length and 0.03 mm width), slightly tuberculate, of white color with pale orange in the middle (Fig. 3B). Crosses measure up to 0.05 mm

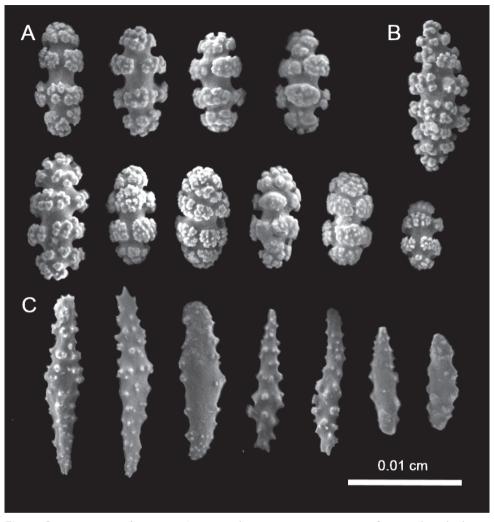


Figure 3. *Leptogorgia iridis* sp. nov., Scanning Electron Microscopy images of coenenchymal sclerites from the holotype NMNH-1638551 **A** capstans **B** spindle **C** anthocodial rod sclerites.

length and 0.05 mm width. Anthocodial sclerites are long rods of < 0.1 mm length and 0.02 mm width with acute ends and lobed margins (Fig. 3C).

Morphological variations. *Leptogorgia iridis* sp. nov. paratypes differ from the holotype in branch diameter and coloration. The morphotypes have a wide range of colorations due to the different proportion of sclerite colors and coenenchymal sclerite arrangement (Figs 2A–D, 8A, B). There are four solid sclerite colorations: yellow, red, purple, and white or with a gradient of colorations among them, including bicolor chromotypes. A colony may have one or two sclerite colors, but their proportion and combination may create different patterns in the colony's appearance. The holotype has yellow and purple sclerites with a longitudinal color arrangement giving the colony a bicolor (yellow and purple) appearance (Fig. 2A, B). The paratype NMNH-1638553 also

has a mixing of sclerites, one colony has the major sclerite proportion of purple compared to white, and the other colony has a major proportion of white compared to purple sclerites, and both colonies have a scrambled sclerite arrangement giving the colonies their coloration (Fig. 2C). However, in several specimens, such as paratype NMNH-1638554, the colony and sclerite coloration is white (Fig. 2D). Colony growth forms of *L. iridis* sp. nov. have relatively low variability. The only different morphotype, so far collected exclusively at Bahía Banderas, Jalisco, were colonies with similar coloration patterns to the holotype, but with relatively thicker branches (up to 4 mm diameter).

Diagnosis. Purple and red *Leptogorgia iridis* sp. nov. have quite similar colony shapes. Both *L. iridis* sp. nov. chromotypes resemble the color of *Leptogorgia obscura* Bielschowsky, 1929 and *Leptogorgia parva* Bielschowsky, 1929. However, *L. obscura* has small anthocodial rods with blunt ends and *L. parva* has anthocodial rods with conspicuous lobed margins, which are absent in *L. iridis* sp. nov. Additionally, *L. obscura* and *L. parva* have only one known chromotype, and their terminal branches have acutely pointed ends. In contrast, *L. iridis* sp. nov., has long anthocodial rods with acute ends and no lobed margins, showing up to five solid colony chromotypes and terminal branches with blunt ends.

Habitat and distribution. The distribution of *Leptogorgia iridis* sp. nov. covers part of the Central Tropical Mexican Pacific (Mexican Province in Brusca and Wallerstein 1979 and Hasting 2000) from Bahía Banderas, Jalisco to Islas Marías Archipelago Nayarit, Mexico (Fig. 2). *Leptogorgia iridis* sp. nov. grows on shallow rocky reefs < 20 m depth. Purple colonies were mostly observed in shallow waters < 5 m depth, the bicolor colonies mostly at 7–8 m depth, and yellow colonies mostly observed at 10–20 m depth. *Leptogorgia iridis* sp. nov. shares habitat with *Leptogorgia ena* Breedy, Abeytia & Guzman, 2012, *Leptogorgia rigida* Verrill, 1864, *Leptogorgia alba* (Duchassaing & Michelotti, 1864), *Pacifigorgia arenata* (Valenciennes, 1846), *Pacifigorgia agassizii* (Verrill, 1864), *Pacifigorgia media* (Verrill, 1864), *Pacifigorgia stenobrochis* (Valenciennes, 1846), *Muricea austera* Verrill, 1869, and *Heterogorgia papillosa* Verrill, 1870.

Etymology. *Leptogorgia iridis* sp. nov. is named from the Latin word *iridis*, which means "rainbow" due to the large number of chromotypes observed in the colonies. Large numbers of chromotypes are one of the main diagnostic characteristics of this novel tropical species.

Leptogorgia martirensis sp. nov.

http://zoobank.org/2F4C8356-9EF8-4772-A607-CBCC74032DB8 Figures 1, 4, 5, 8C, D

Material examined. *Holotype.* NMNH-1638556: dry, Cueva Refugio, San Pedro Mártir Island, Sonora, Mexico (28°22.297'N, -112°19.040'W), 1 m depth, 16 July 2010, collector Carlos Sánchez. *Paratypes.* NMNH-1638557: dry, Cueva Refugio, San Pedro Mártir Island, Sonora, Mexico (28°22.297'N, -112°19.040'W), 1 m depth, 16 July 2010, collector Carlos Sánchez; NMNH-1638558: dry, Cueva Refugio, San

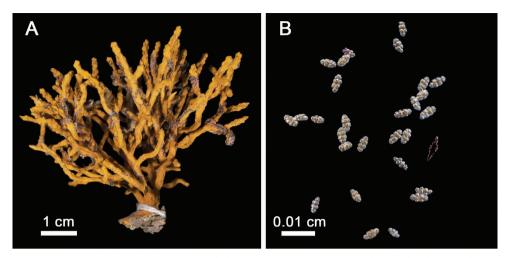


Figure 4. *Leptogorgia martirensis* sp. nov. **A** holotype NMNH-1638556 **B** anthocodial and coenenchymal sclerites.

Pedro Mártir Island, Sonora, Mexico (28°22.297'N, -112°19.040'W), 1 m depth, July 16, 2010, collector Carlos Sánchez; NMNH-1638559: San Pedro Mártir Island, Sonora, Mexico (28°22.818'N, -112°18.4422'W), 20 m depth, 16 July 2010, collector Carlos Sánchez.

Type locality. Cueva Refugio, San Pedro Mártir Island, Sonora, Mexico is one of the furthest offshore islands in the Gulf of California (part of midriff islands at the upper Gulf) where volcanic rocky reefs predominate. San Pedro Mártir Island is a UNESCO "Islas del Golfo de California" Biosphere Reserve (Fig. 1).

Holotype colony description. A yellow colony with bushy and dense growth with multiple and irregular brownish lines (Fig. 4A). The colony is 6.1 cm in length and 8.1 cm in width. The holdfast is irregular, 14 mm × 11 mm from which the short main stem rises (2 mm length, 4 mm diameter). The colony has four main branches up to 11 mm length and 3 mm diameter. The main branches subdivide into multiple secondary branches (up to 31 mm length, 2 mm diameter). Terminal twigs are flat and short (12 mm length, 2 mm width) with acute ends. The general pattern of upward ramification is lateral at 45° angle. Polyp mounds are oval and prominent, forming one or two rows at each side of the branches with 0.5 mm height, 2 mm length, and 1 mm width with elongated calyces. The colony has several specimens of unidentified dried ophiuroids (< 2 mm oral disc diameter) attached to the branches (Fig. 4A).

Holotype sclerites. The coenenchymal sclerites are exclusively capstans (Figs 4B, 5A). There is no evidence of other types of sclerites being present in any other section of the colony. The capstans reach 0.05 mm long and 0.03 mm wide (Fig. 5A), their color is pale yellow, pink, red or bicolor yellow-red, but the predominant color is pale yellow (90%). The anthocodial sclerites are lobed rods with acute or blunt ends up to 0.1 mm length and 0.03 mm width in the center (Fig. 5B, C). They are bicolor white-red, red, orange or white. The red chromotype is predominant (70% of observed colonies) (Fig. 4B).

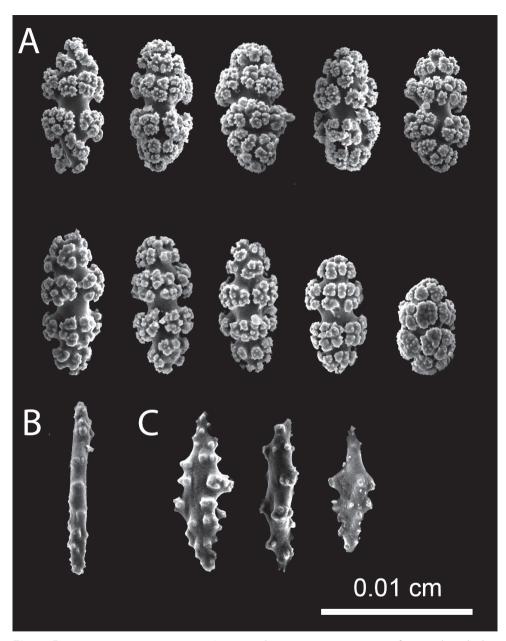


Figure 5. *Leptogorgia martirensis* sp. nov., Scanning Electron Microscopy images of coenenchymal sclerites from the holotype NMNH-1638556 **A** capstans **B** rod lateral view **C** anthocodial rod sclerites.

Morphological variations. *Leptogorgia martirensis* sp. nov. colonies show three chromotypes: purple, yellow and brown (Figs 4A, 8C, D). The colony color depends on the proportion of the dominant color of the coenenchymal sclerites, but in a few cases the colonies show a brown chromotype when the color proportion of sclerites is approximately 50% purple and 50% yellow.

Diagnosis. The colony growth, size and polyp mounds of *Leptogorgia martirensis* sp. nov. are similar to those of *Leptogorgia aequatorialis* Bielschowsky, 1929, *Leptogorgia obscura* and *Leptogorgia parva*. However, these three species each have only one chromotype (purple, pink, and orange, respectively), and all these species have spindles in their coenenchyme up to 0.1 mm length, while *L. martirensis* sp. nov. has three chromotypes and no spindles in the coenenchyme.

Habitat and distribution. The micro-endemic *Leptogorgia martirensis* sp. nov. is only recorded in rocky shallow waters (up to 10 m depth), and low abundance, at San Pedro Mártir and San Esteban Islands, Sonora. The islands are located in the northern Gulf of California (as part of the Cortez Province according to Brusca and Wallerstein 1979, Hasting 2000), and are the most isolated islands in the gulf (Fig. 1). The Cortez Province is associated with the lowest winter sea superficial temperature (SST 15 °C), the widest annual range of SST (15–30 °C), high marine productivity, and harbor a unique macroinvertebrate community, dominated by endemic octocorals of the genus *Muricea* (Ulate et al. 2016). *Leptogorgia martirensis* sp. nov. shares its habitat with *Muricea austera* Verrill, 1869, *Muricea plantaginea* (Valenciennes, 1846), *Muricea* spp., *Psammogorgia teres* Verrill, 1868, and *Eugorgia excelsa* Verrill, 1868.

Etymology. *Leptogorgia martirensis* sp. nov. takes its name from the collection site San Pedro Mártir Island.

Leptogorgia enrici sp. nov.

http://zoobank.org/A3DE39AC-113D-436E-834B-4084F5B6F44F Figures 1, 6, 7, 8E–G

Material examined. *Holotype.* NMNH-1638560: dry, San Esteban Island (northwest rocky point), Sonora, Mexico (28°43.564'N, -112°36.799'W), 24 m depth, *in situ* temperature 19 °C, 01 November 1999, collector Carlos Sánchez. *Paratypes.* NMNH-1638561: dry, San Esteban Island (northwest rocky point), Sonora, Mexico (28°43.564'N, -112°36.799'W), 24 m depth, *in situ* temperature 19 °C, 01 November 1999, collector Carlos Sánchez; NMNH-1638562: dry, San Esteban Island (northwest rocky point), Sonora, Mexico (28°43.564'N, -112°36.799'W), 24 m depth, *in situ* temperature 19 °C, 03 November 1999, collector Carlos Sánchez; NMNH-1638563: dry, San Pedro Nolasco Island (south rocky point), Sonora, Mexico (27°57.094'N, -111°22.001'W), 30 m depth, 20 October 1999, collector Carlos Sánchez.

Type locality. San Esteban Island is part of the midriff islands at the upper Gulf of California, and is the 15th largest island in Mexico by area (40 km²), and has predominantly volcanic rocky reefs. San Esteban Island is a UNESCO "Islas del Golfo de California" Biosphere Reserve (Fig. 1)

Holotype colony description. A bright yellow colony with planar growth and lateral branching (Fig. 6A, B). The colony is 15.3 cm high and 115 cm wide. The colony has a 9 mm diameter holdfast attached to a rock of small size $(14 \text{ mm} \times 11 \text{ mm})$ of biogenic origin from which emerges the main stem of 15 mm length and 2 mm diameter. The stem has longitudinal grooves. From the stem arise two main branches: one of

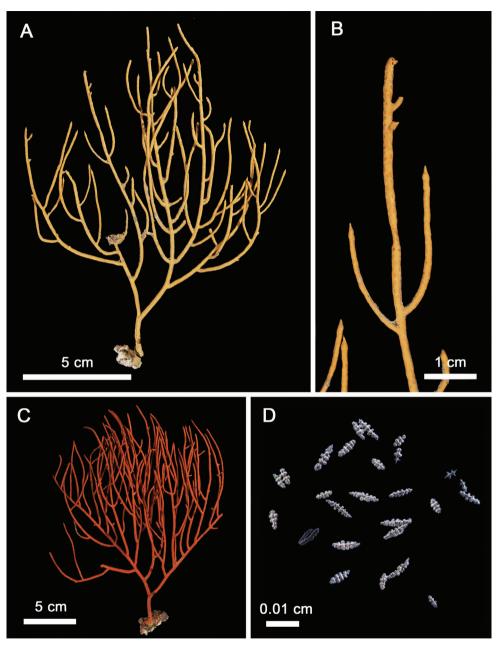


Figure 6. *Leptogorgia enrici* sp. nov. **A** holotype NMNH-1638560 **B** close up of terminal twig **C** orange chromotype colony **D** anthocodial and coenenchymal sclerites.

35 mm length and 2 mm diameter and the other of 117 mm length and 2 mm diameter. From these branches arise multiple secondary laterally growing branches. The terminal branches measure 20–30 mm long, 1.5 mm diameter, and have sharp points (Fig. 6B). The polyp mounds are oval of 1 mm length and 0.5 mm width. Mounds are

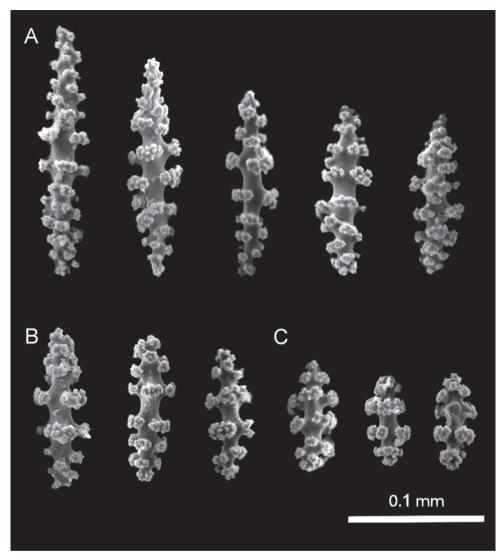


Figure 7. *Leptogorgia enrici* sp. nov., Scanning Electron Microscopy images of coenenchymal sclerites from the holotype NMNH-1638560 **A** acute spindles **B** dull spindles **C** capstans.

slightly evident with no elevation and are arranged irregularly or in rows on each side of all branches but not the stem.

Holotype sclerites. The dominant type of sclerites is capstans of 0.06 mm length and 0.03 mm width (Fig. 7C). There are abundant long spindles up to 0.11 mm long and 0.02 mm thick, which may or may not be slightly curved at the tips (Fig. 7A, B). Crosses are unusual, of 0.06 mm \times 0.06 mm diameter (not shown). Anthocodial sclerites are mostly small yellow rods of up to 0.05 mm length and 0.01 mm width, these anthocodial sclerites have smooth edges and blunt tips (Fig. 6D). Long rods are also present, but in considerably low proportion.

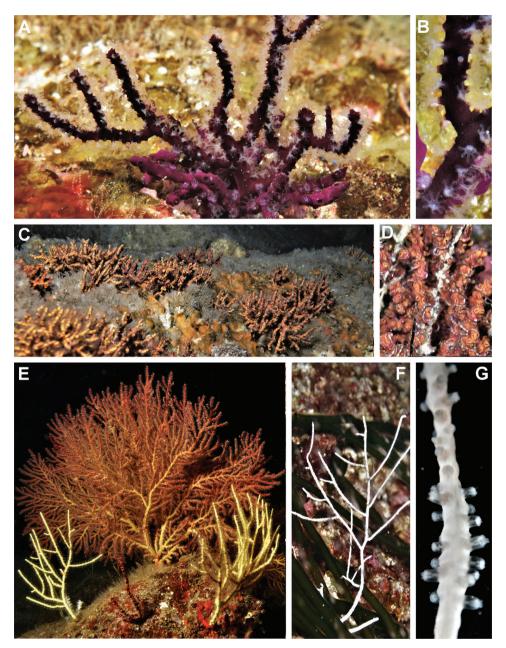


Figure 8. Three new species of sea fans, colonies *in situ*, underwater images **A** *Leptogorgia iridis* sp. nov., San Juanito Island, Piedra El Morro, Islas Marías Archipelago, 10 m depth, 23 November 2010, a deep purple colony, at the base a small red wine colony of *Leptogorgia ena* **B** *Leptogorgia iridis* sp. nov., polyps close up **C** *Leptogorgia martirensis* sp. nov., Cueva Refugio, San Pedro Mártir Island, Sonora, 2–3 m depth, 16 July 2010 into the cave several small colonies **D** *Leptogorgia martirensis* sp. nov., colony close up **E** *Leptogorgia enrici* sp. nov., Los Choros, BC, 25 m depth, 10 July 2009, two yellow colonies, a large colony of *Eugorgia multifida* in the background **F** *Leptogorgia enrici* sp. nov., El Bajo Sur, Cerralvo Island, BCS, 30 m depth, 23 June 2006, white colony **G** *Leptogorgia enrici* sp. nov., polyps close up. Photographs by Carlos Sánchez.

Morphological variations. *Leptogorgia enrici* sp. nov. has arborescent and planar forms of colony growth. The planar colony is the more common morphotype. *Leptogorgia enrici* sp. nov. has four solid colony colorations: yellow (Figs 6A, B, 8E), orange (Fig. 6C), purple, and white (Fig.8F, G) plus a rare bicolor colony (yellow with purple rings around the calices). The sclerites of the coenenchyme always have the

same coloration as the colony. Diagnosis. The purple chromotype of Leptogorgia enrici sp. nov. is morphologically similar to the thin and planar morphotype of Leptogorgia rigida; however, both species differ completely in the form of their sclerites. The coenenchyme sclerites of L. rigida consist mainly of robust capstans with short waists, double heads and spheres (absent in L. enrici sp. nov.), while the sclerites of L. enrici sp. nov. are mainly thin capstans and long and spindle sclerites; spindles are absent in L. rigida. These two species are distributed in different habitats: L. rigida in shallow areas (<10 m depth) attached to rocky reefs, typically inhabiting areas with strong currents or wave action and even in the cracks of rocks, while L. enrici sp. nov. is found in rocky reefs, sandy or pebble beds at depths usually < 20 m depth. The morphology of *L. enrici* sp. nov. is similar in the type of branching and colony color to Leptogorgia chilensis (Verrill, 1868) and Leptogorgia flexilis (Verrill, 1868). However, these three species are distinct because L. enrici sp. nov. has colonies with planar growth and four solid chromotypes (yellow, orange, purple and white) and has many long spindles. Leptogorgia chilensis and *L. flexilis* show arborescent growth typically with branches very close to each other. Each species has a single colony chromotype (L. chilensis is orange and L. flexilis is red) and spindle sclerites are present in low proportions, with blunt tips rather than the long spindles with pointed tips observed in L. enrici sp. nov. The long and acute spindles in L. enrici sp. nov., are only comparable in size to the spindles of Leptogorgia alba and Leptogorgia manabiensis Soler-Hurtado, Megina, Machordom & López-González, 2017 (Soler-Hurtado et al. 2017b). However, these long spindles are the dominant type in L. alba and L. manabiensis, they are broad with acute ends and crowded tubercles. The dominant type of sclerites of L. enrici sp. nov. are capstans, the spindles are thin with blunt tips and with sparse tubercles. The anthocodial rods of *L. alba* and L. manabiensis are flat, long and have scalloped margins; while the anthocodial rods of L. enrici sp. nov. are mostly short with lobed margins and blunt tips.

Habitat and distribution. *Leptogorgia enrici* sp. nov. is endemic to the Gulf of California (Cortez Province according to the biogeographic regions of Brusca and Wallerstein 1979 and Hasting 2000). *Leptogorgia enrici* sp. nov.'s highest densities are concentrated at the northern Gulf of California (northern Cortez sub-province) (Fig. 1), associated with the lowest winter sea surface temperature (SST, 15 °C), the widest annual range of SST (15–30 °C), and high marine productivity (Ulate et al. 2016). *Leptogorgia enrici* sp. nov. inhabits substrates of rocky reefs, or pebbly and shell seafloor habitats surrounded by sand, in shallow waters (5–40 m depth), but most frequently between 20–40 m. *Leptogorgia enrici* sp. nov. may also be distributed in deeper waters.

Leptogorgia enrici sp. nov. occurs in low densities scattered on the reefs (< 1 colony 100 m²) and never clustered in several colonies. Marine ecological censuses carried out

during 2009, 2010 and 2018 showed *L. enrici* sp. nov. is distributed at the Mid-Rift Archipelago of the Gulf of California (Ángel de la Guarda, Partida, Salsipuedes, Las Ánimas, San Lorenzo, San Esteban, San Pedro Mártir, Tortuga and San Marcos) and at the coast of Baja California peninsula (Los Choros). *Leptogorgia enrici* sp. nov. has been collected with scuba at 40 m in the central and southern Gulf of California (Isla Danzante and Isla Cerralvo). *Leptogorgia enrici* sp. nov. shares its habitat with *Muricea* spp., *Muricea plantaginea* (Valenciennes, 1846), *Muricea austera* Verrill, 1869, *Muricea fruticosa* Verrill, 1869, *Eugorgia aurantiaca* (Horn, 1861), *Psammogorgia teres* Verrill, 1868, and *Heterogorgia papillosa* Verrill, 1870.

Etymology. *Leptogorgia enrici* sp. nov. is named in honor of Dr. Enric Sala, a National Geographic Explorer-in-Residence actively engaged in the exploration, research, and science communication to advance ocean conservation. Enric Sala is a passionate enthusiast of marine life and the conservation of Mexican seas who actively collaborates to generate marine biodiversity knowledge. He founded and leads the National Geographic's Pristine Seas project that has conducted 30 expeditions in the world, creating 22 no-take large marine reserve (~5 million km² of no-fishing zones).

Discussion

We discovered three new species of the genus *Leptogorgia* in the Gulf of California, adding biodiversity information on the Eastern Pacific Ocean. Although sea fans are the most abundant benthic macroinvertebrates in the rocky reefs of the Gulf of California (Ulate et al. 2016), their taxonomic identities and geographic delimitations in the Gulf of California have been historically poorly studied (Hernández 2014). Ten out the 30 nominal *Leptogorgia* species known for the Eastern Pacific have been described between 2000–2018 (Bayer 2000; Horvath 2011; Breedy et al. 2012; Soler-Hurtado et al. 2017b; Olvera et al. 2018). Bayer (1981) estimated that about 50% of the sea fan species distributed in the Eastern Pacific were then unknown. This study helps to lessen the knowledge gap of total species for *Leptogorgia* via the description of these three new species.

The description of *Leptogorgia iridis* sp. nov., *Leptogorgia martirensis* sp. nov. and *Leptogorgia enrici* sp. nov. increases the number of nominal *Leptogorgia* species currently known in the Mexican Pacific to 20 (Verrill 1868; Bayer 1981; Breedy and Guzman 2005, 2007; Hovart 2011; Breedy et al. 2012; Olvera et al. 2018). Even though Linnean morphological taxonomy remains an integral approach to describing species, the artificial grouping of the genus "*Leptogorgia*" will change soon based on mitogenomic molecular evidence that suggests that genus "*Leptogorgia*" is a polyphyletic taxon with multiples generic geographically restricted lineages (Soler-Hurtado et al. 2017a; Poliseno et al. 2017). The Eastern Pacific "*Leptogorgia*" species are not the oldest nominal assignation and share an immediate common ancestor with the species included to *Eugorgia* and *Pacifigorgia* genera (Soler-Hurtado et al. 2017a; Poliseno et al. 2017).

The discovery of these three new octocoral species was possible because the highest population density of each species was found in relatively isolated marine areas with exceptionally restricted access (Islas Marías Archipelago was a federal penitentiary) or at isolated offshore islands (San Pedro Mártir Island) with access for general public only through touristic trips. However, those locations do not qualify as pristine habitats and they may already be impacted or will be impacted in the near future. The lack of research effort and the small population size of these three new *Leptogorgia* species explain why these species have been overlooked at the Islas Marías Archipelago and oceanic islands of the Gulf of California. The lack of knowledge of *Leptogorgia enrici* sp. nov. is because this species is distributed below 30–40 m deep, often on sandypebble sea floors where previous research efforts has been few. Our quantitative, historical, and systematic invertebrate monitoring program has been, so far, focused on fauna from rocky reefs in <20 m depth (Hernández 2014; Ulate et al. 2016; Olvera et al. 2018), thus leaving deeper depths unexplored.

Leptogorgia martirensis sp. nov. has been observed and sampled only from rocky reefs from San Pedro Mártir Island (type locality) and San Esteban Island. We observed this species in low density, and only in cavities or caves formed in the island's rocky reefs. We have been carrying out systematic scuba-diving monitoring during 2008–2019 in at least 50 locations at seven islands located close to San Pedro Mártir, providing strong evidence that, except for San Esteban Island, these islands do not harbor colonies of *L. martirensis* sp. nov. The distribution records of these new species compared with our ecological marine census data at extensive locations along the Pacific coast of Mexico (Breedy and Guzman 2005, 2007; Breedy et al. 2012; Ulate et al. 2016) provide strong evidence that these species are micro-endemic to the Gulf of California. Most islands in the Gulf of California have endemic terrestrial fauna (Álvarez-Castañeda and Ortega-Rubio 2003; Brusca et al. 2005), and this may extend to marine species based on our findings. We show evidence that sea fan micro-endemism also exists in aquatic insular habitats of the Gulf of California, such as Islas Marías Archipelago, San Pedro Mártir and San Esteban Islands, similar to that proposed for the Islas Revillagigedo Archipelago (Olvera et al. 2018).

Acknowledgements

We thank Octavio Aburto (SIO-UCSD) and Exequiel Ezcurra (UC Riverside) for their facilities for the collection of invertebrates at Islas Marías Archipelago (IMA) during November 2010 and 2016. We thank scientists and crew of the liveaboard "Rocío del Mar" for their valuable help during the scuba diving collection. Instituto Nacional de Ecología (INE), Mexico, Fondo Mexicano and an anonymous donor funded the IMA research cruise. We are grateful for research collection facilities and permission to Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT) and Comisión Nacional de Áreas Naturales Protegidas (CONANP), Mexico (Oficio NUM. F00-0526). We thank Secretaría de Gobernación de México and Secretaría de Marina for their logistic facilities. Universidad Autónoma de Baja California Sur and Centro Interdisciplinario de Ciencias Marinas (Instituto Politécnico Nacional, SIP-IPN 2010–2020) provided additional financial support. This research was conducted as part of the Universidad Autónoma de Baja California Sur, Proyecto Fauna Arrecifal (UABCS-PFA). UABCS Fauna Reef Conservation Research Programs is funded by the grants: SEMARNAT-CONACYT 2004-01-445 "Biogeography and molecular systematics of sea fans and soft corals (Cnidaria: Octocorallia) in Mexico's Pacific and the Gulf of California"; CONABIO JF190/2013 "Inventory of sessile marine life in the Pacific islands of Baja California Sur"; UC-MEXUS "An ecological and economic baseline for the Revillagigedo Archipelago Biosphere Reserve, Mexico" (2006), UABCS-SCRIPPS-CBMC "Ecological Monitoring ProMARES", and Pristine Seas of National Geographic Society (2016). All these research projects were carried out at Revillagigedo Archipelago (2006, 2016), Islas Marías Archipelago (2010), Bahía Banderas (2013), and Gulf of California (1998-2018) expeditions. J.G.-G. is a COFAA-IPN, EDI-IPN, and SNI fellow.

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RESEARCH ARTICLE



Four new species of *Closterocerus* Westwood (Hymenoptera, Eulophidae) from China, with a key to Chinese species

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Academiceditor:K.vanAchterberg Received19December2020 Accepted20January2021 Published12February2021
http://zoobank.org/B2C3AC49-9B63-42F6-8FC1-8C38A6DFE92C

Citation: Li M-R, Li C-D (2021) Four new species of *Closterocerus* Westwood (Hymenoptera, Eulophidae) from China, with a key to Chinese species. ZooKeys 1017: 21–36. https://doi.org/10.3897/zookeys.1017.62256

Abstract

Four new species of *Closterocerus* Westwood, *C. rectisulcus* **sp. nov.**, *C. shaanxiensis* **sp. nov.**, *C. separatus* **sp. nov.** and *C. unifasciatus* **sp. nov.** are described from China, each with a distinct pattern on the fore wings, and belonging to subgenus *Closterocerus*. A key to all species of the genus *Closterocerus* in China is provided.

Keywords

Chalcidoidea, Entedoninae, natural enemy, parasitoid wasp, taxonomy

Introduction

Closterocerus Westwood (Hymenoptera, Eulophidae, Entedoninae), contains 74 valid species worldwide, but only four species have been recorded from China (Noyes 2020). Hansson (1994) divided the Nearctic species into two subgenera, *Achrysocharis* Girault and *Closterocerus* Westwood according to whether the pedicel was compressed or not. Species of subgenus *Closterocerus*, with a more or less compressed pedicel provided with dorsal and ventral edges, include one Holarctic species (*C. trifasciatus* Westwood), seven Nearctic species (*C. brachyphagus* Hansson, *C. cincinnatus* Girault, *C. cinctipennis* Ashmead, *C. nitidus* Hansson, *C. ruforum* (Krausse), *C. tau* Girault, *C. utahensis* Crawford) and one Palearctic species (*C. lyonetiae* (Ferrière)). Additional species from other

parts of the world possibly belong to the subgenus *Closterocerus*, but the descriptions lack the character Hansson (1994) used to characterize the subgenus.

In the present paper, we describe four new species of the subgenus *Closterocerus* s. str. from China, each with a distinct pattern on the fore wing, and a key to all species of the genus *Closterocerus* distributed in China is provided.

Material and methods

Specimens were collected by sweeping, and were dissected and mounted in Canada Balsam on slides following the method of Noyes (1982), or mounted on a card. Photos were taken with a digital CCD camera attached to an Olympus BX51 compound microscope or Aosvi AO-HK830-5870T digital microscope. Measurements were made using an eye-piece reticle, or using the ruler tool in Adobe Photoshop 2020.

Terminology follows the Hymenoptera Anatomy Consortium (2020), and the following abbreviations are used:

F1–5	flagellomeres 1–5;	PMV	postmarginal vein;
HE	height of eye;	POL	minimum distance between poste-
MS	malar space;		rior ocelli;
MV	marginal vein;	SMV	submarginal vein;
OOL	minimum distance between a pos-	STV	stigmal vein;
	terior ocellus and corresponding	WM	width of mouth opening.
	eye margin;		

All type material is deposited in the insect collections at Northeast Forestry University (**NEFU**), Harbin, China.

Taxonomy

Key to Chinese species of genus Closterocerus (females)

1	Pedicel and flagellum not compressed, and all flagellomeres longer than wide
	(fig. 1A in Yang and Luo 1994); fore wing without distinct infuscate trans-
	verse band, only with a brown spot around STV (fig. 1C in Yang and Luo
	1994) <i>C. litchii</i> (Yang & Luo, 1994)
_	Pedicel and flagellum compressed, and at least 1-2 flagellomeres wider than
	long or quadrate (e.g. Figs 5, 31); fore wing with 1–3 distinct infuscate bands
	(Figs 7, 25, 33) (<i>Closterocerus</i> s. str.) 2
2	Scape slightly compressed, widest in middle part; F3 wider than F2
	(Fig. 31) C. unifasciatus Li & Li, sp. nov.
_	Scape strongly compressed, widest at apex; F3 at most as wide as F2, usually
	narrower (Fig. 5)

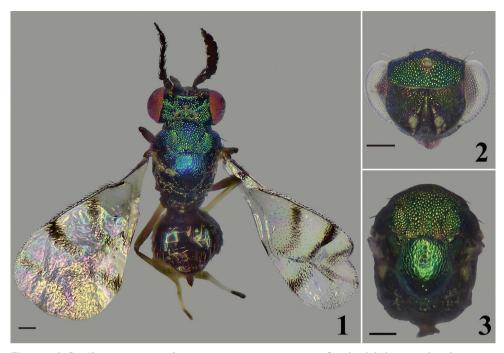
3	Frontal sulcus V-shaped (Figs 20, 22); infuscate transverse band at apex of
	fore wing not V-shaped4
_	Frontal sulcus straight (Figs 2, 4); infuscate transverse band at apex of fore
	wing V-shaped (Fig. 7)7
4	Mesoscutellum distinctly convex, metascutellum small, predominantly hid-
	den under mesoscutellum C. cincinnatus Girault, 1916
-	Mesoscutellum distinctly flat, metascutellum larger, not hidden under mesos-
	cutellum (Figs 21, 32)5
5	Fore wing, between cubital setal line and hind margin of wing with a longi- tudinal infuscation (Fig. 25); head in frontal view about 1.4 times as wide as
	high, nearly oval (Fig. 20)
_	Fore wing, between cubital setal line and hind margin of wing hyaline, with-
	out the longitudinal infuscation; head in frontal view about 1.65 times as
	wide as high, nearly triangular (Fig. 2)
6	F2 distinctly wider and longer than F1; median part of midlobe of mesoscu-
	tum usually differently colored from lateral parts of midlobe; median part of
	mesoscutellum usually with a differently colored longitudinal band
	C. trifasciatus Westwood, 1833
_	F2 similar to F1; mesoscutum and mesoscutellum always unicolorous
7	Propodeal plica absent, spiracular sulcus present (Fig. 6); transverse V-shaped
	band at apex of fore wing dark and distinct (Fig. 7)
	C. rectisulcus Li & Li, sp. nov.
_	Propodeal plica present, spiracular sulcus absent; transverse V-shaped band at
	apex of fore wing much paler and obscure (Fig. 14)
	C. shaanxiensis Li & Li, sp. nov.

Closterocerus rectisulcus Li & Li, sp. nov.

http://zoobank.org/9DD5A3E2-25AF-4EBD-BB29-56AFDC94F088 Figs 1–9

Type material. *Holotype:* \bigcirc [NEFU; on slide], CHINA, Heilongjiang Province, Shangzhi City, Maoershan, 04.VIII. 2016, Si-Zhu Liu, Ye Chen and Hai-Yan Wang, sweeping. *Paratypes:* $4\bigcirc$ [1 on slide, 3 in alcohol], same data as holotype; $2\bigcirc$ [in alcohol], CHINA, Liaoning Province, Anshan City, Mountain Qian Shan, 23.VI.2015, Hui Geng, Si-Zhu Liu, Zhi-Guang Wu and Yan Gao, sweeping; $1\bigcirc$ [in alcohol], CHINA, Shaanxi Province, Ankang City, Ningshan County, Guanghuojie Town, 03.VIII.2015, Ye Chen and Chao Zhang, sweeping; $2\bigcirc$ [1 on card, 1 in alcohol], CHINA, Shaanxi Province, Ankang City, Ningshan County, Chengguan Town, Huoditang Forestry Station, 11.VIII.2015, Ye Chen and Chao Zhang, sweeping.

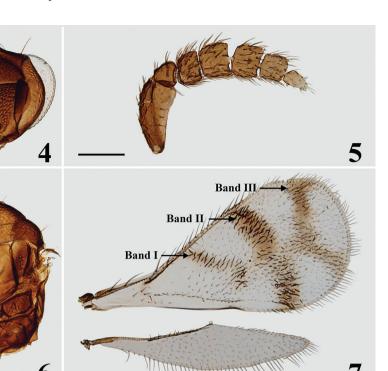
Diagnosis. Frontal sulcus straight, reaching eye margins; fore wing with three infuscate transverse bands (Fig. 7): band I V-shaped, with upper arm longer and more distinct than lower arm; band II obscure medially or nearly interrupted; band



Figures 1–3. *Closterocerus rectisulcus* Li & Li, sp. nov., paratypes, females **I** habitus in dorsal view, on card **2** head, frontal view, before slide-mounting **3** mesosoma and petiole, before slide-mounting. Scale bars: 100 μm.

III V-shaped, with upper arm slightly longer than lower arm; the three bands are nearly connected in the middle; stigmal hairline absent, radial cell setose; below base of cubital setal line with five setae in a row; propodeum with a short median carina delimited by a transverse carina posteriorly; propodeal plica absent; spiracular sulcus present.

Description. Female. Length 0.9–1.3 mm. Scape and pedicel dark brown to black. Flagellum dark brown to pale brown, becoming gradually paler distad. Eyes red, ocelli pale brown. Vertex and frons above frontal sulcus golden-green to gold-en-blue. Frons below frontal sulcus golden-yellow to golden-green. Mandibles pale brown. Pronotal collar, mesoscutum, mesoscutellum and axillae golden-green in dorsal view, golden-blue in lateral view. Metascutellum and propodeum dark bronze. Fore wing (Fig. 7) with three infuscate transverse bands: band I (below the middle of MV) V-shaped, with upper arm longer and more distinct than lower arm; band II (below STV), obscure medially or nearly interrupted; band III (at apical margin of fore wing) V-shaped, with upper arm slightly longer than lower arm; the three bands are nearly connected in middle. All coxae, femora and tarsal claws dark brown (metacoxae darker, nearly black); trochanters pale brown; protibiae and mesotibiae mainly pale yellow, slightly pale brown basally; metatibiae dark brown; pro- and mesotarsi pale yellow with last tarsomere pale brown; metatarsi pale yellow with last tarsomere dark brown. Metasoma dark brown with weak metallic bluish-green to bronze reflections.



 $\frac{6}{8} = \frac{7}{9}$

Figures 4–9. *Closterocerus rectisulcus* Li & Li, sp. nov., holotype, female, on slide: **4** head, frontal view **5** antenna **6** mesosoma and petiole **7** fore and hind wings **8** metasoma without petiole **9** legs, from left to right: fore, mid and hind leg. Scale bars: 100 μm.

Head (Figs 2, 4), in frontal view 1.4 times as wide as high. Sculpture on vertex and frons above frontal sulcus nearly with the same sized meshes. POL : OOL = 7 : 5. Frontal sulcus straight, reaching eye margins; inner eye margins slightly concave in lower part. Antennal scrobes join on frontal sulcus. Malar sulcus absent, but with a curved transverse carina near clypeus, extending to lower eye margins. Clypeus not delimited. HE : MS : WM about 4.8 : 1.0 : 3.3. Antenna (Fig. 5) inserted slightly above level of lower margin of eyes. Scape reticulate, extremely compressed, and expanded from base to apex, about 2.1 times as long as wide. Pedicel moderately compressed compared

to the extremely compressed scape, slightly shorter than wide (ca 4 : 5). Flagellum extremely compressed; F1–3 wider than long, F2 longer and wider than F1 and F3; F4 quadrate; F5 small, tapering distad, with terminal spine shorter than the segment.

Mesosoma (Figs 3, 6). Pronotum, mesoscutum, mesoscutellum, axillae and posterior part of propodeum with reticulate sculpture, meshes nearly of same size (but wider on mesoscutellum). Metascutellum with irregular rugae. Pronotum transverse, invisible in dorsal view. Median area of mesoscutum with two pairs of setae. Notauli curved in anterior part, and indicated posteriorly by depression. Mesoscutellum 0.97 times as long as wide. Axillae slightly advanced forwards in front of level of anterior margin of mesoscutellum. Mesoscutum and mesoscutellum slightly convex. Metascutellum about 1/3 as long as median length of propodeum. Propodeum with a short median carina delimited by a transverse carina posteriorly. Propodeal plica absent, spiracular sulcus present. Fore wing (Fig. 7) about twice as long as wide, without stigmal hairline, radial cell setose. Speculum closed below. Ratio length of: SMV : MV : PMV : STV about 3:6:1:1. Cubital setal line straight and completely extending to base of MV. Below base of cubital setal line with five setae in a row. Hind wing (Fig. 7) about 4.5 times as long as wide. Legs (Fig. 9) with all coxae reticulate on outer surface; ventral margin of pro- and metafemur with six and ten long setae respectively; mesotibial spur about 0.9 times as long as corresponding basitarsus.

Metasoma (Fig. 8) ovate; petiole short, pyriform; ovipositor exserted beyond apex of metasoma.

Male. Unknown.

Host. Unknown.

Etymology. The name refers to the straight frontal sulcus in this species (*rectus* is Latin for straight).

Distribution. China (Heilongjiang, Liaoning, Shaanxi provinces).

Remarks. Closterocerus rectisulcus sp. nov. is similar to *C. orientalis* Yefremova & Kriskovich, 1996 because they share a similar pattern of the fore wing according to the description. The new species differs as follows: pedicel slightly shorter than, or at most as long as wide (longer than wide in *C. orientalis*); mesoscutellum approx. as long as wide (three times as long as wide in *C. orientalis*); mesotibial spur 0.9 times as long as corresponding basitarsus (1.7 times as long as corresponding basitarsus in *C. orientalis*).

Closterocerus shaanxiensis Li & Li, sp. nov.

http://zoobank.org/4FBDD51E-135A-4D06-837F-27019927CF25 Figs 10–18

Type material. *Holotype*: ♀ [NEFU; on slide], CHINA, Shaanxi Province, Ankang City, Ningshan County, Chengguan Town, Huoditang Forestry Station, 11.VIII.2015, Ye Chen and Chao Zhang, sweeping. *Paratype*: 1♀ [on card], CHINA, Shaanxi Province, Ankang City, Ningshan County, Chengguan Town, Huoditang Forestry Station, 09.VIII.2015, Ye Chen and Chao Zhang, sweeping.



Figure 10. *Closterocerus shaanxiensis* Li & Li, sp. nov., holotype, female, habitus in dorsal view, before dissection. Scale bar: 200 µm.

Diagnosis. Frontal sulcus straight, reaching eye margins; fore wing with band I V-shaped, with upper arm much longer and darker than lower arm, the lower arm obscure; band II distinct and interrupted medially; band III V-shaped, obscure, much paler than band I and II, with upper arm slightly longer than lower arm; the three bands are separated from each other; fore wing without stigmal hairline, radial cell setose; below base of cubital setal line with nine setae in a row; propodeal plica present, spiracular sulcus absent.

Description. Female. Length 1.1–1.3 mm. Scape and pedicel dark brown to black. Flagellum dark brown to pale brown, becoming gradually paler distad. Eyes red, ocelli pale brown to red. Vertex and frons above frontal sulcus golden-green to golden-blue. Frons below frontal sulcus mainly golden-yellow with golden-green tinge. Pronotal collar, mesoscutum, mesoscutellum and axillae golden-green to golden-yellow in dorsal view, golden-blue in lateral view. Metanotum and propodeum brown, without metallic reflections, except the median part of metascutellum concolorous with mesoscutellum. Fore wing (Fig. 16) with band I V-shaped, with upper arm much longer and darker than lower arm, the lower arm much obscure; band II distinct and interrupted medially; band III V-shaped, obscure, much paler than the band I and II, with upper arm slightly longer than lower arm; the three are separated from each other. Legs with all coxae, femora and tarsal claws dark brown; trochanters pale brown; protibiae main-



Figures 11–18. *Closterocerus shaanxiensis* Li & Li, sp. nov., holotype, female, on slide (except **11** and **14**, which are photographed before slide-mounting): **11** head, frontal view **12** mandibles **13** antenna **14**, **15** mesosoma and petiole **16** fore and hind wings **17** metasoma without petiole **18** legs, from left to right: fore, mid and hind leg. Scale bars: 100 μm.

ly pale yellow, pale brown basally; mesotibiae with basal half pale brown and apical half pale yellow; metatibiae dark brown; tarsi pale yellow with last tarsomere pale brown to brown. Metasoma brown with rather weak reflections.

Head (Figs 11, 12), in frontal view 1.45 times as wide as high. Sculpture on vertex and frons above frontal sulcus nearly with the same sized meshes. POL : OOL = 6:5. Frontal sulcus straight, reaching eye margins; inner eye margins slightly concave in lower part. Antennal scrobes join on frontal sulcus. Malar sulcus absent, but with a curved transverse carina near clypeus, extending to lower margin of eyes. Clypeus not delimited. HE : MS : WM about 3.9:1.0:2.2. Antenna (Fig. 13) inserted slightly above level of lower margin of eyes. Scape reticulate, extremely compressed, and expanded from base to apex, about 2.2 times as long as wide. Pedicel moderately

compressed compared to the extremely compressed scape, slightly longer than wide (about 9 : 8). Flagellum extremely compressed; F1 wider than long; F2 and F3 quadrate; F4 slightly longer than wide; F2 widest and longest and gradually tapering from F2 to F5; F5 with terminal spine shorter than the segment.

Mesosoma (Figs 14, 15). Pronotum, mesoscutum, mesoscutellum and axillae with reticulate sculpture, meshes nearly of same size. Metascutellum with irregular rugae. Pronotum transverse, invisible in dorsal view. Median area of mesoscutal midlobe with two pairs of setae. Notauli curved in anterior part, and indicated posteriorly by depression. Mesoscutellum approx. as long as wide. Axillae slightly advanced forwards in front of level of anterior margin of mesoscutellum. Mesoscutum and mesoscutellum slightly convex. Metascutellum about 3/5 as long as median length of propodeum. Propodeum without reticulate sculpture, with a short median carina delimited by a transverse carina posteriorly. Propodeal plica present, spiracular sulcus absent. Fore wing (Fig. 16) about twice as long as wide, without stigmal hairline, radial cell setose. Speculum closed below. Ratio length of: SMV : MV : PMV : STV about 4 : 8 : 1 : 1. Cubital setal line straight and completely extending to base of MV. Below base of cubital setal line with nine setae in a row. Hind wing (Fig. 16) about 4.5 times as long as wide. Legs (Fig. 18) normal.

Metasoma (Fig. 17) ovate; petiole short, pyriform; ovipositor exserted beyond apex of metasoma.

Male. Unknown.

Host. Unknown.

Etymology. The specific name is derived from the name of the collection locality, Shaanxi Province.

Distribution. China (Shaanxi Province).

Remarks. Closterocerus shaanxiensis sp. nov. is similar to *C. rectisulcus* sp. nov., but differs as follows: fore wing below base of cubital setal line with nine setae in a row (five in *C. rectisulcus*); propodeum smooth, without reticulate sculpture (with reticulate sculpture posteriorly in *C. rectisulcus*); propodeal plica present (absent in *C. rectisulcus*); spiracular sulcus absent (present in *C. rectisulcus*). The pattern on the fore wing and color of the metasoma are also different from that in *C. rectisulcus*: band III obscure, much paler than band I and II; the three bands are separated from each other (in *C. rectisulcus*, band III distinct, only slightly paler than band I and II; the three bands are nearly connected in the middle); metasoma brown (dark brown in *C. rectisulcus*).

Closterocerus separatus Li & Li, sp. nov.

http://zoobank.org/6C889A04-1340-47E0-9513-5672DE233418 Figs 19–27

Type material. *Holotype*: \bigcirc [NEFU; on slide], CHINA, Heilongjiang Province, Hegang City, Wuzhishan Park, 22.VII.2020, Ming-Rui Li, sweeping. *Paratypes*: $2\bigcirc$ [on slides], same data as holotype; $2\bigcirc$ [on slides], CHINA, Heilongjiang Province, Hegang

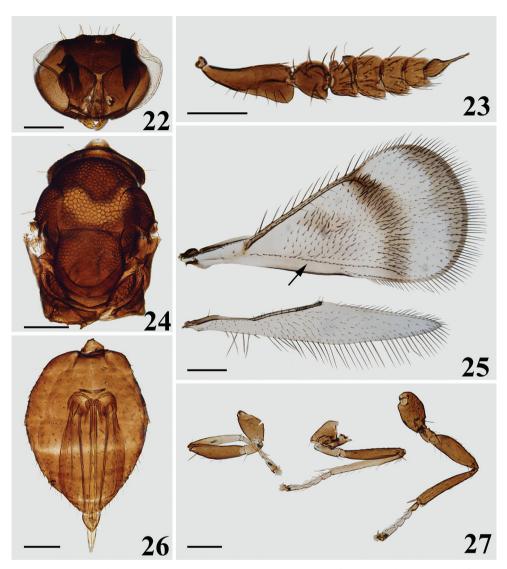


Figures 19–21. *Closterocerus separatus* Li & Li, sp. nov., holotype, female **19** habitus in lateral view, before dissection **20** head, frontal view, before slide-mounting **21** mesosoma, before slide-mounting. Scale bars: 100 μm.

City, Beishan Park, 11.VII.2020, Ming-Rui Li, sweeping; 1♀ [in alcohol], CHINA, Hebei Province, Chengde City, Mountain Wu Ling, 16.VII.2017, Guang-Xin Wang and Wen-Jian Li, sweeping.

Diagnosis. Female. Head, in frontal view, oval, 1.4 times as wide as high; scape strongly compressed, and extremely expanded distad, 2.4 times as long as wide; fore wing with band I replaced by an obscure infuscate cloud; band II obvious, reaching hind margin of fore wing; band III extended along apical margin, obvious, slightly protruded inwards medially; the cloud and two bands are separated from each other; between cubital setal line and the hind margin of fore wing with a longitudinal infuscation; ventral margin of metafemur with six long setae.

Description. Female. Length 1.06–1.15 mm. Antennae dark brown. Eyes and ocelli dull red. Frons golden-yellow to golden-green. Vertex golden-green in dorsal view, golden-blue in lateral view and frontal view. Mandibles pale brown. Pronotal collar, mesoscutum, mesoscutellum, axillae and metascutellum golden-green in dorsal view, golden-blue in lateral view. Propodeum, mesopleuron and metapleuron brown, dark brown to black. Legs with all coxae, femora and tarsal claws dark brown; pro- and mesotrochanters pale brown, metatrochanters dark brown; protibiae mainly pale yellow, pale brown basally; mesotibiae mainly pale yellow; metatibiae dark brown; all tarsi pale yellow, but first segment of metatarsi dark brown. Fore wing (Fig. 25) with band I replaced by an obscure infuscate cloud; band II obvious, reaching hind margin; band III extending along apical margin, obvious, slightly protruded inwards medially; the



Figures 22–27. *Closterocerus separatus* Li & Li, sp. nov., holotype, female, on slide: **22** head, frontal view **23** antenna **24** mesosoma **25** fore and hind wings, arrow shows longitudinal infuscation between cubital setal line and the hind margin **26** metasoma **27** legs, from left to right: fore, mid and hind leg. Scale bars: 100 μ m.

cloud and two bands are separated from each other; between cubital setal line and the hind margin of fore wing with a longitudinal infuscation. Metasoma dark brown with weak metallic green to blue reflections.

Head (Figs 20, 22), oval in frontal view, 1.4 times as wide as high. Meshes of reticulate sculpture on vertex and frons below frontal sulcus smaller than that on frons above frontal sulcus. POL : OOL = 5 : 4. Frontal sulcus V-shaped, reaching eye margins; inner eye margins concave medially. Antennal scrobes join on the frontal sulcus. Malar sulcus absent, but with a curved transverse carina near clypeus, extending to lower margin of eyes. Clypeus not delimited. HE : MS : WM about 4.0 : 1.0 : 2.0. Antenna (Fig. 23) inserted above level of lower margin of eyes. Scape strongly compressed, and extremely expanded distad, 2.4 times as long as wide. Pedicel moderately compressed compared to the extremely compressed scape, nearly as long as wide. Flagellum extremely compressed; F1–4 wider than long, F2 slightly larger than F1 and F3; F3 wider than F4; F5 small, almost oval, with terminal spine long and nearly as long as the segment.

Mesosoma (Figs 21, 24). Pronotum, mesoscutum, axillae and mesoscutellum (except posterior margin) with reticulate sculpture, meshes nearly of same size. Metascutellum and propodeum smooth. Along pronotal collar with four strong setae. Median area of midlobe of mesoscutum with three pairs of setae. Notauli curved in anterior part, and indicated posteriorly by depression. Mesoscutellum as long as wide. Axillae slightly advanced forwards in front of level of anterior margin of mesoscutellum. Mesoscutum and mesoscutellum rather flat. Metascutellum large, about half median length of propodeum. Propodeum without any carina in middle part. Fore wing (Fig. 25) twice as long as wide, with a stigmal hairline, radial cell bare. Speculum nearly elongate-triangular, closed below. Ratio length of: SMV : MV : PMV : STV about 5 : 11 : 1 : 2. Cubital setal line straight and completely extending to base of MV. Hind wing (Fig. 25) narrow, about 6.2 times as long as wide. Legs (Fig. 27) normal, with all coxae reticulate on outer surfaces; ventral margin of metafemur with six long setae.

Metasoma (Fig. 26). Ovate; petiole short, pyriform; ovipositor exserted beyond apex of metasoma.

Male. Unknown.

Host. Unknown.

Etymology. The name refers to the separated distal two cross bands of fore wing (*separatus* is Latin for separate).

Distribution. China (Heilongjiang, Hebei provinces).

Remarks. Closterocerus separatus sp. nov. is similar to C. africanus Waterston, 1925, C. cruy (Girault, 1918) and C. mirabilis Edwards & La Salle, 2004, according to the original descriptions. They share the following characters with the new species: fore wing with an infuscate cloud and two infuscate bands; between cubital setal line and the hind margin of fore wing with a longitudinal infuscation; but the new species differs from C. africanus by having the head 1.4 times as wide as high in frontal view (1.6 times in C. africanus); ventral margin of metafemur with six long setae (ten setae in C. africanus); the area between infuscate cloud and band II of fore wing hyaline (slightly infuscate in C. africanus). The new species differs from C. cruy and C. mirabilis in having the infuscate cloud and two bands on the fore wing separated from each other (the cloud is distinctly connected with band II, and band III is nearly connected with band II in C. mirabilis; band II is distinctly connected to the cloud and bands III in C. cruy and C. mirabilis).

Closterocerus unifasciatus Li & Li, sp. nov.

http://zoobank.org/72189C1E-6C3D-4044-9242-F12985AE1701 Figs 28–35

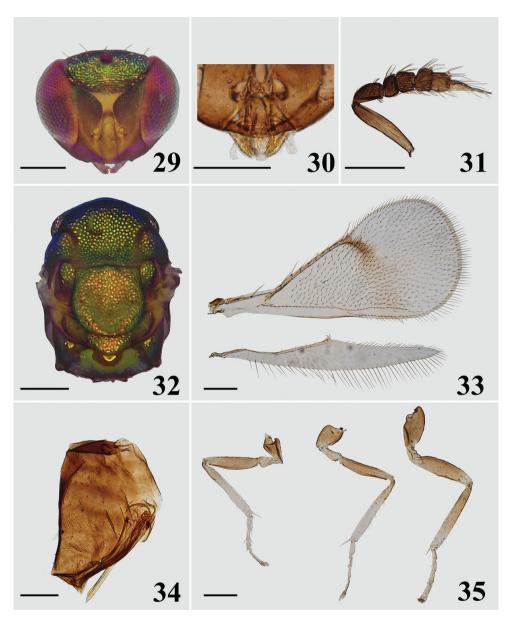
Type material. *Holotype:* \bigcirc [NEFU; on slide], CHINA, Liaoning Province, Anshan City, Mountain Qian Shan, 25.VI.2015, Hui Geng, Si-Zhu Liu, Zhi-Guang Wu and Yan Gao, sweeping. *Paratypes:* $1\bigcirc$ [on slide], same data as holotype; $2\bigcirc$ [1 on slide and 1 in alcohol], CHINA, Heilongjiang Province, Yichun City, Dailing District, Liangshui Forestry Station, 29.VII.2015, Xin-Yu Zhang, Si-Zhu Liu and Xing-Yue Jin, sweeping; $1\bigcirc$ [on card], CHINA, Liaoning Province, Anshan City, Mountain Qian Shan, 23.VI.2015, Hui Geng, Si-Zhu Liu, Zhi-Guang Wu and Yan Gao, sweeping.

Diagnosis. Face near clypeus with a curved, nearly V-shaped transverse carina; clypeus delimited laterally; F4 widest; fore wing with band I absent; band II becoming paler and wider posteriorly; band III extending along apical margin, much obscure (nearly imperceptible on slide); propodeum smooth and shiny, without any carina, spiracular sulcus present.

Description. Female. Length 0.9–1.0 mm. Scape with basal 3/5 pale brown and remainder part brown; pedicel dark brown. Funicle dark brown, clava dark brown to



Figure 28. *Closterocerus unifasciatus* Li & Li, sp. nov., holotype, female, habitus in lateral view, before dissection. Scale bar: 200 µm.



Figures 29–35. *Closterocerus unifasciatus* Li & Li, sp. nov., holotype, female, on slide (except **29** and **32**, which are photographed before slide-mounting): **29** head, frontal view **30** mandibles **31** antenna **32** mesosoma **33** fore- and hind wings **34** metasoma, lateral view **35** legs, from left to right: fore, mid and hind leg. Scale bars: 100 μm.

pale brown, becoming gradually paler from F3 to F5. Eyes and ocelli dull red. Vertex and frons above frontal sulcus golden-green, golden-blue, or golden-red. Frons below frontal sulcus golden-yellow to golden-green. Gena brown without metallic reflection. Mandibles pale brown. Pronotal collar, mesoscutum, mesoscutellum, axillae and propodeum golden-green, occasionally golden-red, in dorsal view, golden-blue in lateral view. Legs with pro- and mesocoxae pale brown, metacoxae brown; all femora and metatibiae brown, except about apical 2/7 of metatibiae pale yellow; pro- and mesotibiae mainly pale yellow, except basal part pale brown; all tarsi pale yellow and claws dark. Fore wing (Figs 28, 33) with band I absent; band II becoming paler and wider posteriorly; band III extending along apical margin of fore wing, much obscure (nearly imperceptible on slide). Metasoma concolorous with mesosoma, except median part of tergum 2 and 5, whole tergum 4, brown, with weak metallic reflections.

Head (Figs 29, 30), in frontal view 1.2 times as wide as high. Vertex and frons with irregular sculpture. POL : OOL = 15 : 8. Frontal sulcus V-shaped, reaching eye margins; inner eye margins hardly concave. Antennal scrobes join on frontal sulcus. Subtorular sulci present and long. Malar sulcus absent, but with a curved, nearly V-shaped transverse carina near clypeus, extending to near lower margin of eyes. Clypeus delimited laterally. HE : MS : WM about 4.3 : 1.0 : 2.0. Antennae (Fig. 31) inserted above level of lower margin of eyes. Scape compressed, 4.8 times as long as wide. Pedicel moderately compressed compared to the compressed scape, approx. twice as long as wide. Flagellum compressed, with two funicular segments and three claval segments. F1–F3 wider than long; F2 slightly wider and longer than F1; F3, widest of all segments of antenna; F4 quadrate; F5 tapering distad, with terminal spine long and as long as the segment.

Mesosoma (Fig. 32). Pronotum, mesoscutum, mesoscutellum, axillae and metascutellum with reticulate sculpture, meshes nearly of same size. Median area of midlobe of mesoscutum with two pairs of setae. Notauli curved in anterior part, and indicated posteriorly by depressions. Mesoscutellum approx. as long as wide. Axillae slightly advanced forwards in front of level of anterior margin of mesoscutellum. Mesoscutum and mesoscutellum flat. Metascutellum about 3/4 as long as median length of propodeum. Propodeum smooth, without any carina and plica, spiracular sulcus present. Fore wing (Fig. 33) slightly more than twice as long as wide, with a stigmal hairline, radial cell bare. Speculum closed below. Ratio length of: SMV : MV : PMV : STV about 5 : 10 : 1 : 2. Cubital setal line straight and completely extending to base of MV. Hind wing (Fig. 33) much narrow, about seven times as long as wide. Legs (Fig. 35) normal.

Metasoma (Fig. 34). Ovate; petiole short, pyriform; ovipositor exserted beyond apex of metasoma.

Male. Unknown.

Host. Unknown.

Etymology. The name refers to the single cross band in the fore wing (*uni* is Latin for one, single and *fasciatus* is Latin for banded).

Distribution. China (Heilongjiang, Liaoning provinces).

Remarks. *Closterocerus unifasciatus* shares with *C. brachyphagus* Hansson, 1994 the distinct transverse band below the STV in the fore wing. The new species differs in having the mesoscutellum reticulate (smooth, without any trace of reticulation in *C. brachyphagus*); propodeum smooth, without any carina (with a weak median carina in *C. brachyphagus*) and fore wing with speculum closed below (open in *C. brachyphagus*).

Acknowledgements

We are grateful to Drs Ye Chen, Hui Geng, Si-Zhu Liu, Hai-Yan Wang, Mr Guang-Xin Wang, Mr Wen-Jian Li, Mr Zhi-Guang Wu, Mr Chao Zhang, Miss Yan Gao, Miss Xin-Yu Zhang and Miss Xing-Yue Jin for specimen collection. We are also grateful to Prof. Christer Hansson for providing references when we did this research and providing valuable comments on manuscript as a reviewer. Prof. Kees van Achterberg helped the authors a lot to improve the manuscript.

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RESEARCH ARTICLE



Tiger beetles (Coleoptera, Cicindelidae) of Northern Mindanao region (Philippines): checklist, distributional maps, and habitats

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Academic editor: R. Vermeulen Received 13 March 2019 Accepted 12 January 2021 Published 12 February 20	21
http://zoobank.org/390FEA39-DEBA-4406-B99F-BC6625821960	

Citation: Acal DAP, Wiesner J, Nuńeza OM, Jaskuła R (2021) Tiger beetles (Coleoptera, Cicindelidae) of Northern Mindanao region (Philippines): checklist, distributional maps, and habitats. ZooKeys 1017: 37–75. https://doi.org/10.3897/zookeys.1017.34500

Abstract

The knowledge about tiger beetle fauna of the Northern Mindanao region (Philippines) is summarized based on literature data and new records. Thirty species classified in ten genera (*Tricondyla, Neocollyris, Protocollyris, Therates, Prothyma, Heptodonta, Thopeutica, Lophyra, Calomera,* and *Cylindera*) were documented from the area (56% of tiger beetle fauna of Mindanao and 21% of Philippine species). Twelve species were noted from Northern Mindanao region for the first time, including five taxa, *Neocollyris speciosa, Calomera angulata, Cylindera minuta, Lophyra striolata tenuiscripta,* and *Thopeutica virginea,* not recorded from Mindanao before. Distribution maps for all recorded species and the first photographs of habitats for some species in Mindanao and/or in the Philippines are provided. Eight species (27% of recorded fauna) were noted from riverine habitats while 18 tiger beetles (60%) were typical forest taxa; in the case of four species, their habitats in Northern Mindanao region are not known.

Keywords

Endemic species, *Calomera*, *Cylindera*, diversity, distribution, *Heptodonta*, identification key, *Lophyra*, *Neocollyris*, *Protocollyris*, *Prothyma*, *Therates*, *Tricondyla*

Introduction

Tiger beetles (Coleoptera: Cicindelidae) are a beetle family (López-López and Vogler 2017; Duran and Gough 2020) of more than 2850 species distributed world-wide, but with the larger number of taxa occurring in tropical regions (Cassola and Pearson 2000; Wiesner 2020). With 162 taxa (including 144 species) actually known from the country the tiger beetle fauna of the Philippines is recognized as one of the most diverse in the world (Cabras et al. 2016a; Dheurle 2016, 2019; Zettel and Pangantihon 2017; Zettel and Wiesner 2018; Anichtchenko and Medina 2019, 2020; Medina et al. 2019, 2020; Görn 2020). Moreover, it can be characterized by high percentage of endemic species as more than 85% of Cicindelidae are noted only from this country (Cassola and Pearson 2000; Cabras et al. 2016a; Dheurle 2016, 2019; Zettel and Pangantihon 2017; Zettel and Wiesner 2018; Anichtchenko and Medina 2019, 2020; Medina et al. 2019, 2020; Görn 2020). Moreover, it can be characterized by high percentage of endemic species as more than 85% of Cicindelidae are noted only from this country (Cassola and Pearson 2000; Cabras et al. 2016a; Dheurle 2016, 2019; Zettel and Pangantihon 2017; Zettel and Wiesner 2018; Anichtchenko and Medina 2019, 2020; Medina et al. 2019, 2020c; Görn 2020), with particular species often noted only on single islands (Cabras et al. 2016a). It can be expected that such high diversity values results from both geographical location of the country in the tropical region as well as the occurrence of a large number of geographically isolated islands that influence the evolution of endemic species.

Based on previous studies, ten genera and 54 species of tiger beetles were recorded on Mindanao Island: *Tricondyla* Latreille, 1822 (7 species), *Protocollyris* Mandl, 1975 (3 species), *Neocollyris* Horn, 1901 (12 species), *Therates* Latreille, 1817 (4 species), *Prothyma* Hope, 1838 (6 species), *Heptodonta* Hope, 1838 (2 species), *Calomera* Motschulsky, 1862 (3 species), *Lophyra* Motschulsky, 1859 (1 species), *Thopeutica* Chaudoir, 1861 (8 species), and *Cylindera* Westwood, 1831 (4 species) (Wiesner 1992; Cassola 2000, 2011; Naviaux 1994, 2002; Cassola and Ward 2004; Dheurle 2015, 2019; Cabras et al. 2016a; Anichtchenko and Medina 2019; Görn 2020; Medina et al. 2019, 2020c).

Although more than 50 tiger beetle species are known as occurring on Mindanao Island (36% of Philippine fauna), for many of these taxa only single records are known (Cabras et al. 2016a). Hence, little is known about the general distribution of many species and from many regions no data are available. As Philippine tiger beetle fauna includes many endemic taxa, lack of distributional data often do not allow estimates of species ranges, abundance, or habitat selection and as a consequence, also their threats. As degradation of wildlife both in the Philippines and Mindanao is significant due to different human activities (e.g., deforestation for agricultural land, human pressures because of overpopulation; Lasco et al. 2008; Navarrete et al. 2018), even simple faunistic data may play an important role in the conservation of this beetle group.

In the present paper we focus on the region of Northern Mindanao where no regular studies on tiger beetle species were previously done. As a result, the first checklist of Cicindelidae occurring in this region as well as distributional maps for all known species occurring in the area, and photographs of habitats for the 12 recorded taxa are provided. This paper may serve as a baseline for further studies on this beetle family not only in the Northern Mindanao but also in other regions in the country.

Materials and methods

Northern Mindanao geographically lies within latitude 7°15' to 9°15'N and longitude 123°30' to 125°30'E, is bound on the north by the Bohol Sea, on the west by Zamboanga provinces, on the east by Agusan and Davao provinces, on the south by Lanao del Sur and Cotabato. The whole region covers a total land area of 19,279.60 km² and more than 60% of the region's area are classified as forest land. Geologically, this region is formed of a combination of coastal areas, rivers, falls, volcanoes, highlands with flat terrain, rugged and faulted mountains with rich soil, abundant minerals, and agricultural resources. Since the region is located outside the typhoon belt area, rainfall is evenly distributed throughout the year (Dejarme-Calalang and Colinet 2014; Bouquet 2017). Northern Mindanao comprises five provinces: Bukidnon, Camiguin, Lanao del Norte, Misamis Occidental, and Misamis Oriental (Figure 1). Regular studies were done in sixteen sampling sites in the Northern Mindanao region, including riverine areas, secondary forests, and coastal areas (Table 1, Figures 2-4), and most of the tiger beetle material used in this study was collected using entomological hand nets during field work in 2017–2019. Some additional data were provided from earlier studies, including published data (Wiesner 1988; Naviaux 1994, 2002; Cassola 2000, 2011; Dheurle 2015, 2017; Cabras et al. 2016a; Görn 2020).

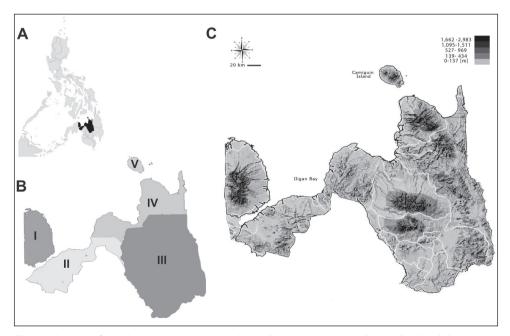


Figure 1. Map of Northern Mindanao region **A** in relation to entire Mindanao island and the remaining Philippine archipelago **B** administrative provinces (I – Misamis Occidental, II – Lanao del Norte, III – Bukidnon, IV – Misamis Oriental, V – Camiguin) **C** detailed physical map.

Site	Sampling site	GPS coordinates
1.	Barangay Bulokbulok, Municipality of Mambajao, Camiguin Island, Camiguin Province	9°15'9"N, 124°42'31"E
2.	Looc River, Barangay Mainit, Municipality of Catarman, Camiguin Island, Camiguin Province	9°10'30"N, 124°40'44"E
3.	Sagay River, Barangay Bonbon, Municipality of Sagay, Camiguin Island, Camiguin Province	9°6'18"N, 124°43'57"E
4.	Barangay Bura, Municipality of Catarman, Camiguin Island, Camiguin Province	9°10'4.7"N, E124°39'23"E
5.	Barangay Poblacion, Municipality of Mambajao, Camiguin Island, Camiguin Province	9°13'24"N, 124°41'47"E
6.	Barangay Umagus, Municipality of Lagonglong, Misamis Oriental Province	8°48'11"N, 124°48'53"E
7.	Cabulaway River, Municipality of Balingasag, Misamis Oriental Province	8°46'9"N, 124°48'2"E
8.	Barangay Kalasungay, Malaybalay City, Bukidnon Province	8°11'28"N, 125°5'54"E
9.	Barangay Can-ayan, Malaybalay City, Bukidnon Province	8°11'31"N, 125°9'13"E
10.	Barangay Bonbonon, Iligan City, Lanao del Norte Province	8°15'56"N, 124°18'37"E
11.	Tubod River, Barangay Merilla, Iligan City, Lanao del Norte Province	8°12'17"N, 124°15'24"E
12.	Barangay Esperanza, Municipality of Bacolod, Lanao del Norte Province	8°10'12"N, 124°0'22"E
13.	Barangay Mati, Municipality of Bacolod, Lanao del Norte Province	8°9'4"N, 124°0'57"E
14.	Barangay San Isidro, Municipality of Sinacaban, Misamis Occidental Province	8°17'5"N, 123°47'5"E
15.	Barangay San Lorenzo, Municipality of Sinacaban, Misamis Occidental Province	8°17'10"N, 123°41'43"E
16.	Mt. Agad-Agad, Iligan City, Lanao del Norte Province	8°12'49.34"N, 124°16'9.66"E
17.	Mimbilisan Protected Landscape, Misamis Oriental Province	8.94884N, 124.86517E
18.	Municipality of Lopez Jaena, Misamis Occidental Province	8°33'00"N, 123°46'00"E

Table 1. Sampling sites in Northern Mindanao region were regular studies were done in years 2017 and/or 2018.

Material is currently deposited in the authors' collections:

- **JWC** J. Wiesner Collection (Wolfsburg, Germany);
- **RJC** R. Jaskuła Collection (Łódź, Poland).

Checklist of tiger beetles of Northern Mindanao

Family Cicindelidae Latreille, 1802 Subfamily Cicindelinae Latreille, 1802 Tribus Collyridini Brullé, 1834 Subtribus Tricondylina Naviaux, 1991 Genus *Tricondyla* Latreille, 1822

The genus *Tricondyla* is represented in the Philippine islands by 14 species, including seven recorded from Mindanao Island (Naviaux 2002; Cabras et al. 2016a).

Subgenus Tricondyla Latreille, 1822

Tricondyla (*Tricondyla*) *aptera punctipennis* Chevrolat, 1841 Figures 4B, 5A, 6A

General distribution. Subspecies known from Indonesia and Philippines. In the Philippines it was found in Sibuyan, Samar, Cebu, and Mindanao; in Mindanao Island it was recorded only in the Northern Mindanao region (Cabras et al. 2016a).

Literature data for Northern Mindanao. Bukidnon province: Impasung-ong (Cabras et al. 2016a).

Material examined. Bukidnon Province: Mt. Kitanglad, 28.VII.1990, 1∂ 1♀, ex coll. Y. Nishiyama (JWC); [no detailed locality], 1977, 1∂, leg. R. Lumawig (JWC); Lanao del Norte province: Mt. Agad-agad, 8°12'49.34"N, 124°16'9.66"E, 470 m a.s.l., 19.11.2018, 1∂, leg. R. Jaskuła et D.A.P. Acal (RJC).

Habitat. Forest species found on vertical and fallen tree trunks, sometimes in forest floor; occasionally also outside the forest (but close to the trees).

Remarks. Larva of nominal subspecies was described by Trautner and Schawaller (1996), who observed it hunting during night period in the bark of tree (e.g., *Samanea saman*).

Tricondyla (*Tricondyla*) *elongata* Horn, 1906

Figure 6B

General distribution. Species endemic to the Philippines, where it was recorded from Luzon, Visayas, and Mindanao; in Mindanao recorded only in Northern Mindanao, Davao, and Bangsamoro Autonomous Region in Muslim Mindanao regions (Cabras et al. 2016a, b; Marohomsalic et al. 2021).

Literature data for Northern Mindanao. Bukidnon province: Lantapan and Impasung-ong (Cabras et al. 2016a); Misamis Occidental province: Mt. Malindang (Cabras et al. 2016a); Misamis Oriental province: Mt. Balatucan-Lumot (Cabras et al. 2016a).

Material examined. Bukidnon province: Mt. Kitanglad, 28.VII.1990, $1^{\circ}_{\circ} 2^{\circ}_{\circ}$, ex coll. Y. Nishiyama (JWC); Mt. Kitanglad, 10.2014, $9^{\circ}_{\circ}_{\circ}^{\circ} 5^{\circ}_{\circ}^{\circ}_{\circ}$, leg. N. Mohagan (JWC); Lanao del Norte province: Mount Agad-agad, 8°12'49.34"N, 124°16'9.66"E, ca. 470 m a.s.l., 11.2019, 1 ex., leg. J. Ebina, M. L. Lumontod, G. C. Café (RJC); Dodiongan Falls, Iligan City – Barangay Bonbonon, 8.271457N, 124.314140E, 47 m a.s.l., 11.2019, 1 ex., leg. R. Jaskuła (RJC); Tinago Falls, Iligan City – Barangay Ditucalan, 8.159820N, 124.185460E, 11.2019, 1 ex., leg. Ł. Trębicki (RJC); Misamis Oriental province: Cagayan de Oro, Malasag forest, 23.05.1978, 1 $^{\circ}_{\circ}$, leg. A. Bandinelli (JWC).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks. Marohomsalic et al. (2021) recorded native and invasive tree species having extrafloral nectaries as favorite hunting areas for this species in the human-disturbed habitats.

Tricondyla (Tricondyla) gracilis Naviaux, 2002

Figure 6C

General distribution. Species endemic to Philippines where it was found only in Mindanao and Romblon islands (Naviaux 2002; Cabras et al. 2016a); in Mindanao known from Davao and Northern Mindanao regions (Naviaux 2002).

Literature data for Northern Mindanao. Misamis Oriental province: Malasag forest Cagayan de Oro (Naviaux 2002).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks.

Subgenus Stenotricondyla Naviaux, 2002

Tricondyla (*Stenotricondyla*) *cyanipes* Eschscholtz, 1829 Figure 6D

General distribution. Species endemic to the Philippines where it was found in Luzon, Leyte, Sibuyan, and Mindanao; in Mindanao recorded only in Northern Mindanao region (Cabras et al. 2016a).

Literature data for Northern Mindanao. Misamis Oriental province: Gingoog City (Cabras et al. 2016a).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks.

Tricondyla (Stenotricondyla) cavifrons Schaum, 1862

Figure 6E

General distribution. Species endemic to the Philippines where it was noted from Balabac, Mindanao, and Palawan; in Mindanao it was recorded only from Bangsamoro Autonomous Region in Muslim Mindanao (Marohomsalic et al. 2021), this is the first record from Northern Mindanao region.

Material examined. Bukidnon province: Mt. Kitanglad, 10.2014, 1 \bigcirc , leg. N. Mohagan (JWC); Mt. Kitanglad, 11–12.2014, 2 \bigcirc 1 \bigcirc , leg. N. Mohagan (JWC); Lanao del Norte province: Mount Agad-agad, 8°12'49.34"N, 124°16'9.66"E, ca. 470 m a.s.l., 11.2019, 3 exx., leg. J. Ebina, M. L. Lumontod, G. C. Café (RJC).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks. Marohomsalic et al. (2021) recorded native and invasive tree species having extrafloral nectaries as favorite hunting areas for this species.

Genus Neocollyris Horn, 1901

The genus *Neocollyris* is represented in the Philippines by 29 species, including 12 recorded from Mindanao Island (Naviaux 1994; Cabras et al. 2016a).

Subgenus Neocollyris Horn, 1901

Neocollyris (Neocollyris) albitarsis (Erichson, 1834)

Figure 6F

General distribution. Species endemic to the Philippines, noted from Homonhon, Luzon, Mindanao, and Palawan; in Mindanao it was recorded only from Northern

Mindanao and Bangsamoro Autonomous Region in Muslim Mindanao region (Cabras et al. 2016a; Marohomsalic et al. 2021).

Literature data for Northern Mindanao. Bukidnon province: Impasung-ong (Cabras et al. 2016a).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks and leaves of different bush species.

Neocollyris (Neocollyris) brevicula Naviaux, 1994

Figure 7A

General distribution. Species endemic to Philippines, known from Basilan, Mindanao, and Samar island (Cabras et al. 2016a); in Mindanao recorded only in Northern Mindanao (Naviaux 1994).

Literature data for Northern Mindanao. Lanao del Norte province: Municipality of Balo-i, Barangay Momungan (Naviaux 1994).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks and leaves of different bush species.

Neocollyris (Neocollyris) emarginata (Dejean, 1825)

Figure 7B

General distribution. Species noted from Borneo, Indonesia, Malaysia, and Philippines, where it was found in Mindanao and Palawan; in Mindanao recorded only in Northern Mindanao region (Cabras et al. 2016a).

Literature data for Northern Mindanao. Bukidnon province: Impasug-ong (Cabras et al. 2016a).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks and leaves of different bush species.

Subgenus Heterocollyris Naviaux, 1995

Neocollyris (Heterocollyris) affinis (Horn, 1892) Figure 7C

General distribution. Species endemic to the Philippines where it was recorded in Bohol, Leyte, Luzon, Mindanao, Panay, and Samar; in Mindanao found only in Northern Mindanao region (Cabras et al. 2016a).

Literature data for Northern Mindanao. Bukidnon province: Impasug-ong (Cabras et al. 2016a); Misamis Oriental province: Balatucan-Lumot (Cabras et al. 2016a).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks and leaves of different bush species.



Figure 2. Habitats of tiger beetles from Northern Mindanao region: *Calomera mindanaoensis* (**A–G**) (sites 1–7), *Cylindera discreta elaphroides* (**H**) (site 8), *C. minuta* (**G**) (site 7), *C. mouthiezi* (**H**) (site 8) (descriptions of sites in Table 1; photographs DAPA).

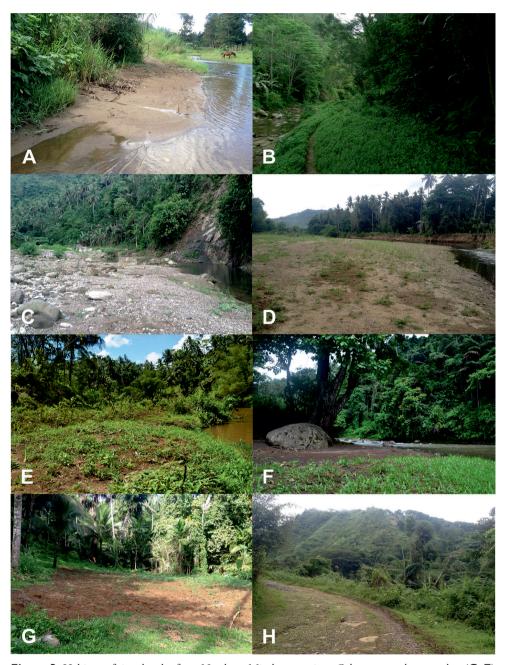


Figure 3. Habitats of tiger beetles from Northern Mindanao region: *Calomera angulata angulata* (C–E) (sites 10–12), *C. lacrymosa* (C, E) (sites 10, 12), *C. mindanaoensis* (C–F, H) (sites 10–13, 15), *Cylindera discreta elaphroides* (A, C–E) (sites 9–12), *C. minuta* (A, C–E) (sites 9–12), *C. viduata* (G) (site 14), *Lophyra striolata tenuiscripta* (G) (site 14), *Therates coracinus coracinus* (B) (site 10) (descriptions of sites in Table 1; photographs A, C–H DAPA, B RJ).

Neocollyris (Heterocollyris) similior (Horn, 1893)

Figures 4D, 7D

General distribution. Species endemic to Philippines where it was recorded only from Mindanao (Naviaux 1994; Cabras et al. 2016; Marohomsalic et al. 2021).

Material examined. Misamis Oriental province: Mimbilisan Protected Landscape, 8.94884N, 124.86517E, 501 m a.s.l., 18.07.2017, 1⁽²⁾, leg. O. Bagona (RJC); Lanao del Norte province: Dodiongan Falls, Iligan City – Barangay Bonbonon, 8.271457N, 124.314140E, 47 m a.s.l., 11.2019., 1 ex., leg. M. L. Lumontod (RJC).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks and leaves of different bush species.

Neocollyris (Heterocollyris) speciosa (Schaum, 1863)

Figure 7E

General distribution. Species endemic to the Philippines, where it was noted only from Luzon, Mindoro (Cabras et al. 2016a), and Mindanao (new record).

Material examined. Bukidnon province: Mt. Kitanglad, 11–12.2014, 2♀♀, leg. N. Mohagan (JWC).

Habitat. Forest, arboreal species found on vertical and fallen tree trunks and leaves of different bush species.

Genus Protocollyris Mandl, 1975

Protocollyris mindanaoensis (Mandl, 1974)

Figure 7F

General distribution. Species endemic for Philippines where it was noted only from Mindanao Island (Cabras et al. 2016a) from Northern Mindanao region (Naviaux 1994).

Literature data for Northern Mindanao. Lanao del Norte province: Momungan [actually Barangay Momungan in Municipality of Balo-i] (Naviaux 1994).

Habitat. Forest, arboreal species.

Tribe Cicindelini Latreille, 1802 Subtribus Theratina Horn, 1910

Genus Therates Latreille, 1817

The genus *Therates* is represented in the Philippine islands by six species, including four or five recorded from Mindanao Island; three of them have been noted from the Northern Mindanao region by Cabras et al. (2016a).

Therates coracinus coracinus Erichson, 1834

Figures 3B, 4D, 8A

General distribution. Subspecies known from Indonesia, Moluccas, and Philippines, where it was recorded in Balabac, Leyte, Luzon, Mindanao, Mindoro, Negros, Palawan, Panay, Romblon, and Samar; in Mindanao recorded from Davao, Northern Mindanao, and Soccsksargen regions (Wiesner 1988; Cabras et al. 2016a, b; Cabras and Wiesner 2016; Pepito et al. 2020).

Literature data for Northern Mindanao. Bukidnon province: Impasug-ong (Cabras et al. 2016a).

Habitat. Forest species noted on tree trunks and leaves.

Remarks. When disturbing, actively fast flying among trees; during flight shows bright orange abdomen coloration. Taxonomical status of both subspecies of *Therates coracinus* noted in Mindanao (spp. *coracinus* and ssp. *fulvescens* Wiesner, 1988) should be revised including molecular data as they probably represent separate species or synonyms.

Therates fasciatus (Fabricius, 1801)

Therates fasciatus fasciatus (Fabricius, 1801)

Figures 4D, 8B

General distribution. Subspecies known from Indonesia and Philippines. In the Philippines recorded only from Mindanao and Palay islands (Cabras et al. 2016a); from Mindanao Island it was known only from Davao and Northern Mindanao regions (Cabras et al. 2016b).

Literature data for Northern Mindanao. Bukidnon province: [no detailed locality] (Wiesner 1988).

Material examined. Bukidnon province: [no detailed locality], 1977, 1^Q, leg. R. Lumawig (JWC). **Camiguin province:** Camiguin Island, Municipality of Catarman, Mt. Timpoong-Hibok-Hibok Natural Monument, Mt. Hibok-Hibok 11.2019, 2 exx.,

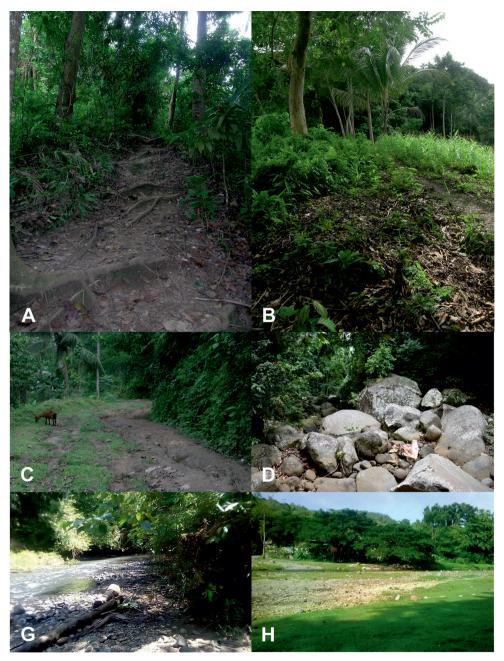


Figure 4. Habitats of tiger beetles from Northern Mindanao region: *Calomera mindanaoensis* (**C**) (site 16) (**E**, **F**) (sites 18–19), *Prothyma heteromallicollis heteromallicollis* (**A**) (site 16), *Neocollyris similior* (**D**) (site 17), *Therates coracinus coracinus* (**D**) (site 17), *T. fasciatus fasciatus* (**D**) (site 17), and *Tricondyla aptera punctipennis* (**B**) (site 16) (descriptions of sites in Table 1; photographs **A–C** RJ, **D** O. Bagona, **E** A.B. Lapore, **F** C. Torres).

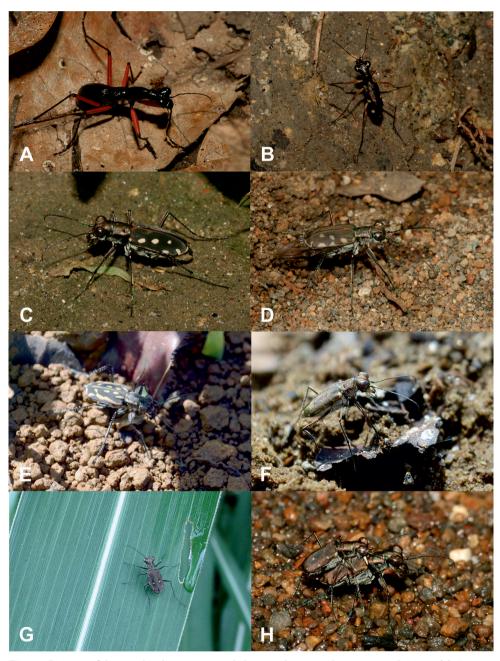


Figure 5. Some of the tiger beetle species recorded in Northern Mindanao region: **A** *Tricondyla* (*Tricondyla*) *aptera punctipennis* **B** *Prothyma* (*Symplecthyma*) *heteromallicollis heteromallicollis* **C** *Calomera mindanaoensis* **D** *C. lacrymosa* **E** *Lophyra* (*Spilodia*) *striolata tenuiscripta* **F** *Cylindera* (*Eugrapha*) *minuta* **G**, **H** *C.* (*Ifasina*) *discreta elaphroides* (photographs **A–D**, **H** RJ, **E–G** DAPA).

leg. R. Jaskuła, D. A. P. Acal (RJC); **Lanao del Norte province:** Dodiongan Falls, Iligan City – Barangay Bonbonon, 8.271457N, 124.314140E, 47 m a.s.l., 11.2019, 11 exx., leg. R. Jaskuła, D.A.P. Acal, J. Ebina, M. L. Lumontod (RJC); near Sikyop Cave, Iligan City – Barangay Lawlawon, 8.246627N, 124.422387E, 11.2019, 2 exx., leg. R. Jaskuła (RJC); **Misamis Oriental province:** Mimbilisan Protected Landscape 8.94884N, 124.86517E, 501 m a.s.l., 18.07.2017, 13 exx., leg. O. Bagona (RJC); Bolyok Falls, Barangay Lubilan, Naawan Municipality, 11.2019, 1 ex., leg. R. Jaskuła (RJC).

Habitat. Forest species noted on tree trunks and leaves.

Therates fasciatus pseudolatreillei Horn, 1928

Figure 8B

General distribution. Subspecies endemic to Philippines where it was recorded from Leyte, Mindanao, and Mindoro; in Mindanao noted only from Northern Mindanao and Soccsksargen regions (Wiesner 1988; Cabras et al. 2016a; Pepito et al. 2020).

Literature data for Northern Mindanao. Bukidnon province: Impasug-ong (Cabras et al. 2016a); Lanao del Norte province: "Mai-nit" (actually Barangay Mainit) (Wiesner 1988); Misamis Occidental province: Malindang Range (Cabras et al. 2016a).

Material examined. Bukidnon province: Mt. Talemo, 30.06.1977, 1 \Diamond , leg. M. Sato (JWC); **Lanao del Norte province:** Barangay Mainit (between Iligan City and Cagayan de Oro), 17–20.08.1978, 2 \Diamond \Diamond 2 \Diamond \Diamond , leg. Cabides et Lobin (JWC); **Misamis Oriental province:** Gingoog, 8.5N, 125.0E, 04.1984, 6 \Diamond \Diamond 4 \Diamond \Diamond (JWC).

Habitat. Forest species noted on tree trunks and leaves.

Remarks. Taxonomical status of all four subspecies of *Therates fasciatus* noted in Mindanao (spp. *fasciatus* (Fabricius, 1801), spp. *quadrimaculatus* Horn, 1895, spp. *pseudolatreillei* Horn, 1928, and ssp. *flavohumeralis* Mandl, 1964) should be revised including molecular data as probably at least some represent separate species or synonyms.

Therates fulvipennis Chaudoir, 1848

Therates fulvipennis bidentatus Chaudoir, 1861

Figure 8C

General distribution. Subspecies known from Indonesia and Philippines (Basilan and Mindanao islands); from Mindanao noted from Northern Mindanao region only (Wiesner 1988; Cabras et al. 2016a).

Literature data for Northern Mindanao. Lanao del Norte province: "Ma-Init" [actually Barangay Mainit] (Wiesner 1988).

Material examined. Bukidnon province: Mt. Kitanglad, 10.2014, $15 \Im \Im \Im \Im$, leg. N. Mohagan (JWC); **Lanao del Norte province:** Barangay Mainit (between Iligan City and Cagayan de Oro), 17-20.08.1978, $5\Im \Im \Im \Im$, leg. Cabides et Lobin (JWC).

Habitat. Forest species noted on tree trunks and leaves.

Therates fulvipennis everetti Erichson, 1834

Figure 8C

General distribution. Subspecies endemic to the Philippines, where it was recorded from Dinagat, Luzon, Mindanao, Negros, and Panay; in Mindanao it was recorded in Davao, Northern Mindanao, and Soccsksargen regions (Cabras et al. 2016a, b).

Literature data for Northern Mindanao. Bukidnon province: Impasug-ong (Cabras et al. 2016a).

Habitat. Forest species noted on tree trunks and leaves.

Remarks. Taxonomical status of all three subspecies of *Therates fulvipennis* noted in Mindanao (spp. *bidentatus* Chaudoir, 1861, ssp. *fulvipennis* Chaudoir, 1848, and spp. *everetti* Erichson, 1834) should be revised including molecular data as probably at least some of them represent separate species or synonyms.

Subtribus Dromicina Thomson, 1859

Genus Prothyma Hope, 1838

The genus *Prothyma* is represented in the Philippine islands by 12 species, including six recorded from Mindanao Island (Cabras et al. 2016a).

Prothyma (Symplecthyma) heteromallicollis heteromallicollis Horn, 1909 Figures 4A, 5B, 8D

General distribution. Species endemic to the Philippines, where it was recorded on Luzon and Mindanao till now (Cabras et al. 2016a); in Mindanao it has been noted from Davao (Cabras et al. 2016b) and Northern Mindanao (this publication) regions.

Material examined. Bukidnon province: Mt. Kitanglad, 11–12.2014, $3\overset{\circ}{\circ}\overset{\circ}{\circ}$ 1 $\overset{\circ}{\circ}$, leg. N. Mohagan (JWC); Lanao del Norte province: Mount Agad-agad, 8°12'49.34"N, 124°16'9.66"E, 470 m a.s.l., 18.10.2018, 10 exx., leg. D. A. P. Acal (DAC), 19.11.2018, $2\overset{\circ}{\circ}\overset{\circ}{\circ}$, leg. R. Jaskuła et D. A. P. Acal (RJC); Misamis Oriental province: Mimbilisan Protected Area, 8.94884N, 124.86517E, 501 m a.s.l., 18.07.2017, 1 $\overset{\circ}{\circ}$, leg. O. Bagona (RJC).

Habitat. Species found on shaded forest paths and forest litters.

Remarks. Some individuals were observed resting on undershrub leaves along forest trails.

Genus Heptodonta Hope, 1838

The genus *Heptodonta* is represented in the Philippine islands by five species, including two recorded from Mindanao Island (Cabras et al. 2016a; Görn 2020).

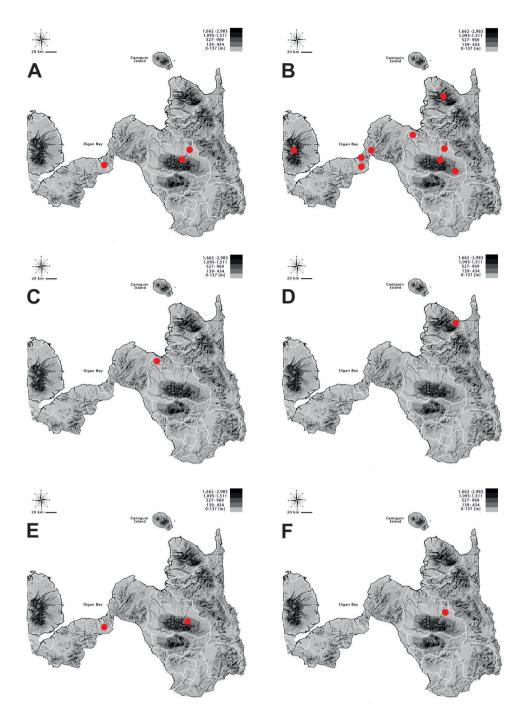


Figure 6. Distribution of **A** *Tricondyla* (*Tricondyla*) *aptera punctipennis* **B** *T*. (*T*.) *elongata* **C** *T*. (*T*.) *gracilis* **D** *T*. (*Stenotricondyla*) *cyanipes* **E** *T*. (*Stenotricondyla*) *cavifrons*, and **F** *Neocollyris* (*Neocollyris*) *albitarsis* in Northern Mindanao region.

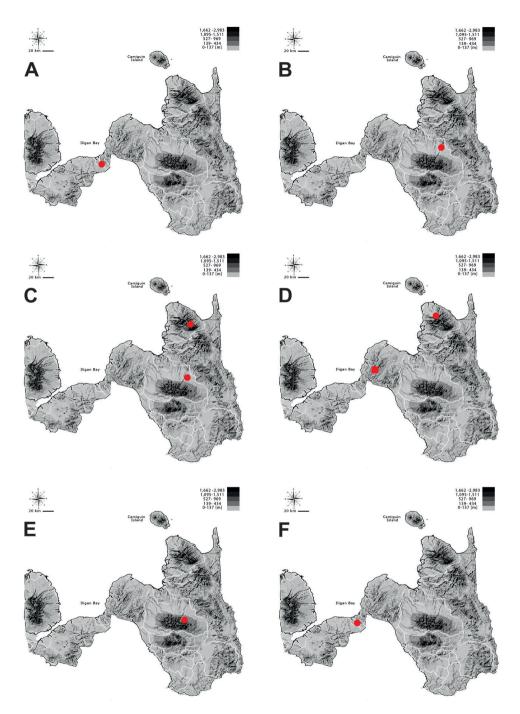


Figure 7. Distribution of **A** *Neocollyris* (*Neocollyris*) brevicula **B** *N*. (*N*.) emarginata **C** *Neocollyris* (*Heterocollyris*) affinis **D** *N*. (*Heterocollyris*) similior **E** *N*. (*Heterocollyris*) speciosa, and **F** *Protocollyris* mindanaoensis in Northern Mindanao region.

Heptodonta nigrosericea (W. Horn, 1930)

Figure 8E

General distribution. Species endemic to the Philippines, where it has been noted on Mindanao and Panay islands only; in Mindanao it was noted in Davao, North Mindanao, and Soccsksargen regions (Cabras et al. 2016a; Cabras and Wiesner 2016; Görn 2020; Medina et al. 2020c; Medina 2020; Pepito et al. 2020).

Literature data for Northern Mindanao. Bukidnon province: Impasug-ong (Cabras et al. 2016a: noted as *Heptodonta lumawigi* Wiesner, 1980); Mt. Kintanglad (Görn 2020); Kabanglasan [= Cabanglasan] (Görn 2020); Intavas (Görn 2020); Silipon (Görn 2020); **Misamis Occidental province:** Mt. Malindang Range (Cabras et al. 2016a: noted as *H. lumawigi* Wiesner, 1980); **Misamis Oriental province:** Mt. Balatucan-Lumot (Cabras et al. 2016a: noted as *H. lumawigi* Wiesner, 1980); Gingoog (Görn 2020).

Material examined. Bukidnon province: Mt. Kintanglad, 10.2014, 533599, leg. N. Mohagan (JWC); [no detailed locality], 1977, 19, leg. R. Lumawig (JWC); **Misamis Oriental province:** Gingoog, 8.5N, 125.0E, 04.1984, 73399 (JWC).

Habitat. Species noted in shaded areas on river banks.

Remarks. Heptodonta lumawigi is a junior synonym of this species (Görn 2020).

Subtribe Cicindelina Latreille, 1802

Genus Calomera Motschulsky, 1862

The genus *Calomera* is represented in the Philippine islands by five species, including four recorded from Mindanao Island (Cabras et al. 2016a). Three of them have been noted from the Northern Mindanao region.

Calomera angulata angulata (Fabricius, 1798)

Figures 3C-E, 8F

General distribution. India, Nepal, Sri Lanka, Thailand, Pakistan, Afghanistan, Cambodia, Vietnam, Laos, Taiwan, Malaysia, Indonesia, China; in the Philippines the species was recorded only from Luzon (Cabras et al. 2016a) and Mindanao (new record).

Material examined. Lanao del Norte province: Iligan City – Barangay Merila, 8°12'17"N, 124°15'24"E, 18 m a.s.l., 05–07.2017, $15 \stackrel{\circ}{\circ} \stackrel{\circ}{\circ} 8 \stackrel{\circ}{\circ} \stackrel{\circ}{\circ}$, 15.12.2018, $8 \stackrel{\circ}{\circ} \stackrel{\circ}{\circ} 3 \stackrel{\circ}{\circ} \stackrel{\circ}{\circ}$, leg. D. A. P. Acal (DAC); Iligan City – Barangay Bonbonon, 8.265458N, 124.310138E, 47 m a.s.l., 05–07.2017, $3 \stackrel{\circ}{\circ} \stackrel{\circ}{\circ} 1 \stackrel{\circ}{\circ}$, leg. D. A. P. Acal (DAC); Municipality of Bacolod, Barangay Esperanza, 8°10'12"N, 124°0'22"E, 27 m a.s.l., 05–07.2017, $1 \stackrel{\circ}{\circ}$, leg. D. A. P. Acal (DAC); Iligan City – Barangay Puga-an, sandy bank of Puga-an River, 8°13'21.3"N, 124°15'52.0"E, 29.10.2018, $3 \stackrel{\circ}{\circ} \stackrel{\circ}{\circ} \stackrel{\circ}{\circ} \stackrel{\circ}{\circ}$, leg. C. Torres (RJC); Iligan City – Barangay Puga-an, sandy bank of Puga-an River, 8°13'29.6"N,

55

124°15'57.8"E, 29.10.2018, $3\overset{\circ}{\circ} \overset{\circ}{\circ} 6 \bigcirc \bigcirc$, leg. C. Torres (RJC); Iligan City – Barangay Puga-an, rocky bank of Puga-an River, 8°13'31.5"N, 124°16'08.6"E, 29.10.2018, $2\overset{\circ}{\circ} \overset{\circ}{\circ}$, leg. C. Torres (RJC); 29.08.2018, $1\overset{\circ}{\circ} 1\bigcirc$, leg. D. A. P Acal (DAC); Iligan City – Barangay Tipanoy, Tubod River, 8°11'38.12"N, 124°15'25.38"E, 20 m a.s.l., 29.08.2018, $1\overset{\circ}{\circ}$, leg. D. A. P. Acal (RJC); Iligan City – Barangay Baraas, Tubod River, 8°12'40.23"N, 124°14'53.25"E, 12 m a.s.l., 17.07.2018, $4\overset{\circ}{\circ} \overset{\circ}{\circ} 1\bigcirc$, leg. D. A. P. Acal (RJC); Barangay Merila, Iligan City, 15.12.2018, $8\overset{\circ}{\circ} \overset{\circ}{\circ} 3\bigcirc \bigcirc$, leg. D. A. P. Acal (RJC).

Habitat. The species occurs on sandy river banks exposed to direct sunlight (pers. obs.). Remarks. First records both from Northern Mindanao region and entire Mindanao Island. This species was observed to co-occur with *Calomera mindanaoensis*, *C. lacrymosa*, *Cylindera discreta elaphroides*, and *C. minuta*.

Calomera cabigasi Cassola, 2011

Figure 9A

General distribution. Species endemic to Philippines where it was found only in Mindanao (Northern Mindanao region) (Cassola 2011; Cabras et al. 2016a).

Literature data for Northern Mindanao. Bukidnon province: Impasug-ong (Cassola 2011); Misamis Oriental province: Gingoog City (Cassola 2011).

Habitat. Species noted from river banks.

Calomera lacrymosa (Dejean, 1825)

Figures 3C, E, 5D, 9B

General distribution. Species endemic to Philippines where it was found in greater part of the country, including Balabac, Bucas Grande, Homonhon, Luzon, Palawan, Mindanao, Mindoro, and Sibuyan; in Mindanao Island it was noted from Davao and Northern Mindanao regions (Cabras et al. 2016a, b).

Literature data for Northern Mindanao. Lanao del Norte province: Iligan City, Barangay Tipanoy (Cassola 2000; Cabras et al. 2016a); Misamis Oriental province: Municipality of Tagoloan, Tagoloan River (Cassola 2000; Cabras et al. 2016a).

Material examined. Lanao del Norte province: Iligan City – Barangay Bonbonon, 8.265458N, 124.310138E, 47 m a.s.l., 05–07.2017, 40 exx., leg. D. A. P. Acal (DAC); Municipality of Bacolod, Barangay Esperanza, 8°10'12"N, 124°0'22"E, 27 m a.s.l., 05–07.2017, 210 exx., leg. D. A. P. Acal (DAC), 13.12.2018, $43^{\circ}3^{\circ}$, leg. R. Jaskuła (RJC); Iligan City – Barangay Puga-an, sandy bank of Puga-an River, 8°13'21.3"N, 124°15'52.0"E, 10.29.2018, $43^{\circ}3^{\circ}5^{\circ}\oplus^{\circ}$, leg. C. Torres (RJC); Iligan City – Barangay Puga-an River, 8°13'29.6"N, 124°15'57.8"E, 10.29.2018, $33^{\circ}3^{\circ}$, leg. C. Torres (RJC); Iligan City – Barangay Puga-an, rocky bank of Puga-an River, 8°13'31.5"N, 124°16'08.6"E, 10.29.2018, $73^{\circ}3^{\circ}1^{\circ}$, leg. C. Torres (RJC); Iligan City – Barangay Puga-an, 8°13'30.73"N, 124°16'6.18"E, 21 m

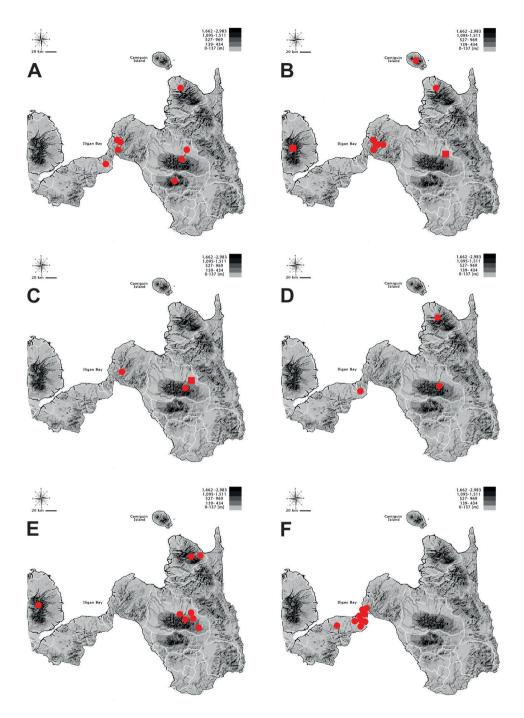


Figure 8. Distribution of A Therates coracinus coracinus B Therates fasciatus fasciatus (circle) and T. fasciatus pseudolatreillei (square) C Therates fulvipennis bidentatus (cirle) and T. fulvipennis everetti (square)
D Prothyma (Symplecthyma) heteromallicollis heteromallicollis E Heptodonta nigrosericea, and F Calomera angulata angulata in Northern Mindanao region.

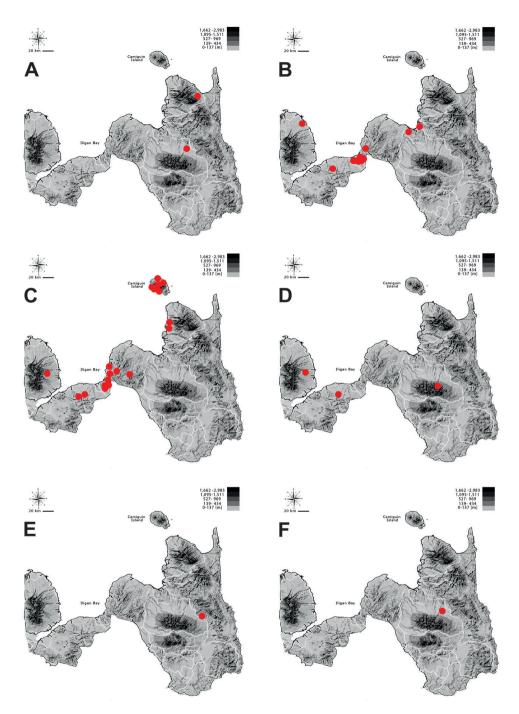


Figure 9. Distribution of **A** Calomera cabigasi **B** C. lacrymosa **C** C. mindanaoensis **D** Lophyra (Spilodia) striolata tenuiscripta **E** Thopeutica (Thopeutica) angulihumerosa, and **F** T. (T.) darlingtonia in Northern Mindanao region.

a.s.l., $3\sqrt[3]{3}$, leg. D. A. P. Acal (RJC); Iligan City – Barangay Tipanoy, Tubod River, 8°11'38.12"N, 124°15'25.38"E, 20 m a.s.l., 29.08.2018, 1 \bigcirc , leg. D. A. P. Acal (RJC); Iligan City – Barangay Merila, 8°12'17"N, 124°15'24"E, 13.12.2018, 18 m a.s.l., $4\sqrt[3]{3}$, $4\bigcirc$. leg. D. A. P. Acal (RJC); Iligan City – Barangay Bonbonon, 8°16'11.69"N, 124°17'16.11"E, 11 m a.s.l., 04.11.2018, 40 exx., leg. D. A. P. Acal (DAC); Barangay Merila, Iligan City, 15.12.2018, $4\sqrt[3]{3}$, $4\heartsuit \bigcirc$, leg. D. A. P. Acal (RJC); **Misamis Oriental province:** Cagayan de Oro City, Malasag Cugman, Mapawa Nature Park, 8°26'5.93"N, 124°42'12.40"E, 334 m a.s.l., 20.08.2017, $7\sqrt[3]{3}$, 1 \bigcirc , leg. O. Bagona (RJC); **Misamis Occidental province:** Municipality of Lopez Jaena, 8°33'00"N, 123°46'00"E, 11.2018, $11\sqrt[3]{3}$, $4\heartsuit \bigcirc$, leg. A. B. Lapore (RJC).

Habitat. The species occurs on sandy river banks (pers. obs.).

Remarks. At least in some areas *Calomera lacrymosa* seems to occur sympatrically or even syntopically with *C. mindanaoensis* (pers. obs.). *C. lacrymosa* was recently noted as a host of *Hexathrombium* parasitic mites (Acari: Microtrombidiidae) (Acal et al. in press).

Calomera mindanaoensis (Cassola, 2000)

Figures 2A-G, 3C-F, H, 4C, 5C, 9C

General distribution. Species endemic to Philippines where it was found in Mindanao (Cassola 2000; Cabras et al. 2016a, b; Cabras and Wiesner 2016) and Camiguin islands (new record); till now in Mindanao it was recorded in Davao, Northern Mindanao, Soccsksargen, and Zamboanga Peninsula regions (Cassola 2000; Cabras et al. 2016a, b; Cabras and Wiesner 2016; Pepito et al. 2020).

Literature data for Northern Mindanao. Bukidnon province: S. Vicente, 20 km S Cagayan de Oro (Cassola 2000); Impasug-ong (Cabras et al. 2016a); Lanao del Norte province: Iligan City, Tipanoy (Cassola 2000); Misamis Oriental province: Tagoloan River, Tagoloan (Cassola 2000).

Material examined. Camiguin province: Camiguin Island: Municipality of Mambajao, Barangay Bulok-bulok, 9°15'9"N, 124°42'31"E, 7 m a.s.l., 05–07.2017, 27 \bigcirc 22 \bigcirc \bigcirc , leg. D. A. P. Acal (DAC); Municipality of Mambajao, Barangay Poblacion, 9°13'24"N, 124°41'47"E, 229 m a.s.l., 17.06.2017, 6 \bigcirc 1 \bigcirc , leg. D. A. P. Acal (DAC); Municipality of Sagay, Sagay River, 28 m a.s.l., 08.07.2017, 23 \bigcirc 8 \bigcirc \bigcirc , leg. D. A. P. Acal (DAC); Municipality of Catarman, Barangay Mainit, Looc River, 9°10'30"N, 124°40'44"E, 278 m a.s.l., 05–07.2017, 60 \bigcirc 3 1 \bigcirc \bigcirc , leg. D. A. P. Acal (DAC); Municipality of Catarman, Barangay Mainit, Looc River, 9°10'30"N, 124°40'44"E, 278 m a.s.l., 05–07.2017, 60 \bigcirc 3 1 \bigcirc \bigcirc , leg. D. A. P. Acal (DAC); Municipality of Catarman, Barangay Bura, 9°10'4.7"N, 124°39'23"E, 143 m a.s.l., 17.06.2017, 3 \bigcirc 4 \bigcirc \bigcirc , leg. D. A. P. Acal (DAC); Tuasan Falls, Looc River, Barangay Bonbon, Municipality of Catarman, 9.176009N, 124.679768E, 11.2019, 12 exx., leg. D. A. P. Acal, R. Jaskuła (RJC); **Lanao del Norte province:** Barangay Mainit (between Iligan City and Cagayan de Oro), 17–20.08.1978, 1 \bigcirc 1 \bigcirc , leg. Cabides et Lobin (JWC); Municipality of Bacolod, Barangay Mati, 8°9'4"N, 124°0'57"E, 53 m

a.s.l., 05–07.2017, 27 d 17 9, leg. D. A. P. Acal (DAC); Municipality of Bacolod Barangay Esperanza, 8°10'12"N, 124°0'22"E, 27 m a.s.l., 05–08.2017, 67 3 2499, leg. D. A. P. Acal (DAC), 13.12.2018, 3∂∂ 1♀, leg. R. Jaskuła (RJC); Iligan City – Barangay Bonbonon, 8.265458N, 124.310138E, 47 m a.s.l., 05–08.2017, 50♂♂ 23♀♀, leg. D. A. P. Acal (DAC); Iligan City – Barangay Merila, 8°12'17"N, 124°15'24"E, 18 m a.s.l., 20.06.2017, $26 \stackrel{\diamond}{\land} 4 \stackrel{\circ}{\ominus} \stackrel{\circ}{\Box}$, leg. D. A. P. Acal (DAC); Iligan City – Barangay Puga-an, sandy bank of Puga-an River, 8°13'21.3"N, 124°15'52.0"E, 10.29.2018, 1∂ 1♀, leg. C. Torres (RJC); Iligan City – Barangay Tipanoy, Tubod River, 8°11'38.12"N, 124°15'25.38"E, 20 m a.s.l., 28.09.2018, 13, leg. D. A. P. Acal (DAC); Iligan City - Barangay Rogongon, Sitio Lawlawon, 8°14'51.13N, 124°25'25.31"E, 359 m a.s.l., 10.03.2019, 8 exx., leg. D. A. P. Acal (DAC); Misamis Occidental province: Municipality of Sinacaban, Barangay San Lorenzo, 8°17'10"N, 123°41'43"E, 800 m a.s.l., 06.09.2017, 5 \bigcirc 4 \bigcirc \bigcirc , leg. D. A. P. Acal (DAC); Misamis Oriental province: Municipality of Balingasag, 8°46'9"N, 124°48'2"E, 86 m a.s.l., 07.2017, 433 299, leg. D. A. P. Acal (DAC); Municipality of Lagonglong, 8°48'11"N, 124°48'53"E, 90 m a.s.l., 05–08.2017, 59♂♂ 29♀♀, leg. D. A. P. Acal (DAC); Municipality of Lugait, Barangay Upper Talacogon, river bank, 8°20'47.04"N, 124°16'58.80"E, 11.07.2018, 2රී රී, leg. V. M. Mirabueno (RJC).

Habitat. The species was recorded as the most opportunistic according to habitat type among all Cicindelidae presented in this paper, found on sandy river banks, forest paths, coastal area, and unused compost pit near the river (pers. obs.).

Remarks. At least in some areas co-occur with *C. lacrymosa. C. mindanaoensis* was recently noted as a host of *Hexathrombium* (Acari: Microtrombidiidae) parasitic mites (Acal et al. – in press). This species was also observed resting on undershrub plants along the trails during rainy season.

Genus Lophyra Motschulsky, 1859

The genus *Lophyra* is represented in the Philippine islands by one species, known to occur also in Mindanao Island (Cabras et al. 2016a). **Subgenus** *Spilodia* **Rivalier**, **1961**

Lophyra (Spilodia) striolata tenuiscripta (Fleutiaux, 1893) Figures 3G, 5E, 9D

General distribution. Subspecies known only from Indonesia and Philippines, in the second country noted only in Palawan (Cabras et al. 2016a) and Mindanao (new record).

Material examined. Bukidnon province: Mt. Kitanglad, 11–12.2014, 1∂, leg. N. Mohagan (JWC); Lanao del Norte province: Municipality of Bacolod, Barangay Mati, 8°9'4"N, 124°0'57"E, 53 m a.s.l., 06.2017, 26 exx., leg. D. A. P. Acal (DAC); Misamis Occidental province: Municipality of Sinacaban, Barangay San Isidro, 8°17'5"N, 123°47'5"E, 269 m a.s.l., 05–07.2017, 78 exx., leg. D. A. P. Acal (DAC).

Habitat. Collected along the trails of coconut field (Municipality of Bacolod, Lanao del Norte) and from cultivated corn and ginger field (Municipality of Sinacaban, Misamis Occidental) (Acal – pers. obs.).

Remarks. Lophyra striolata is a polytypic species with wide distribution in nearly the whole Oriental region (Cassola, 2000). Four subspecies currently are known from the Philippines (ssp. striolata (Illiger, 1800), spp. dorsolineolata (Chevrolat, 1845), spp. tenuiscripta (Fleutiaux, 1893), and spp. uniens (Horn, 1896)) (Cabras et al. 2016a) but at least in some cases their distribution and taxonomical status should be clarified as few subspecies were noted from the same areas.

Genus Thopeutica Chaudoir, 1861

The genus *Thopeutica* is the largest tiger beetle genus in the Philippines with 31 species classified in two subgenera described to date, including 27 species in subgenus *Thopeutica* s. str. and four species in subgenus *Philippiniella* (Cabras et al. 2016a, Medina et al. 2019, 2020c). *Thopeutica* is geographically restricted to Sulawesi and the Philippines and is one of the most exclusive genera since all except two species know from the country seem to be restricted to only one island or to very few islands (Cassola and Zettel 2006).

Thopeutica (Thopeutica) angulihumerosa (Horn, 1929) Figure 9E

General distribution. Species endemic to the Philippines, where it was recorded from Leyte, Mindanao, and Samar; according to Cabras et al. (2016a) only general distributional data from Mindanao for this species were known, this is the first record from Northern Mindanao region.

Material examined. Bukidnon province: Barangay Kalasungay, 8°11′28″N, 125°5′54″E, 770 m a.s.l., 14.06.2017, 1♀, leg. D.A.P. Acal (DAC).

Habitat. The only specimen from Northern Mindanao studied was collected in a shaded riverine area.

Thopeutica (*Thopeutica*) *darlingtonia* Cassola & Ward, 2004 Figure 9F

General distribution. Species endemic to the Philippines, where it was recorded from Luzon and Mindanao; in Mindanao recorded till now only from Northern Mindanao region (Cabras et al. 2016a).

Literate data from Northern Mindanao region. Bukidnon province: Impasugong (Cabras et al. 2016a).

Habitat. No detailed data on habitat specificity in Northern Mindanao region but most probably occurring along rivers in shaded areas.

Thopeutica (Thopeutica) milanae Wiesner, 1992

Figure 10A

General distribution. Species endemic to the Philippines, where it was recorded from Leyte, Luzon (?), Mindanao, and Samar; in Mindanao noted only from Northern Mindanao region (Cabras et al. 2016a).

Literature data from Northern Mindanao region. Bukidnon province: Impasug-ong (Cabras et al. 2016a).

Habitat. No detailed data on habitat specificity in Northern Mindanao region but most probably occurring along rivers in shaded areas.

Thopeutica (Thopeutica) virginea (Schaum, 1860)

Figure 10B

General distribution. Species endemic to Philippines where it was recorded only from Luzon (Cabras et al. 2016a) and Mindanao (new record) islands.

Material examined. Bukidnon province: Mt. Kitanglad, 11 –12.2014, 4 $\stackrel{>}{\circ}$ $\stackrel{<}{\circ}$ $\stackrel{<}{\circ}$, leg. N. Mohagan (JWC).

Habitat. No detailed data on habitat specificity in Northern Mindanao region but most probably occurring along rivers in shaded areas.

Genus Cylindera Westwood, 1831

The genus *Cylindera* is represented in the Philippine islands by 22 species, including five recorded from Mindanao Island (Cabras et al. 2016a).

Subgenus Eugrapha Rivalier, 1950

Cylindera (Eugrapha) minuta (Olivier, 1790) Figures 2G, 3A, C–E, 5F, 10C

General distribution. Species recorded till now from Bangladesh, Brunei, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Thailand, and Vietnam (Wiesner 1992). According to Cabras et al. (2016a) in the Philippines it is known only on the basis of general distributional data; here we present the first records of this species from Mindanao.

Material examined. Bukidnon province: Malaybalay City, Barangay Can-ayan, 8°11'31"N, 125°9'13"E, 653 m a.s.l, 15.06.2017, 1 $\stackrel{\circ}{\rightarrow}$, leg. D.A.P. Acal (DAC); **Lanao del Norte province:** Iligan City – Barangay Bonbonon, 8.265458N, 124.310138E, 47 m a.s.l., 06.2017, 2 $\stackrel{\circ}{\rightarrow}$ 2 $\stackrel{\circ}{\rightarrow}$ eg. D. A. P. Acal (DAC); Iligan City – Barangay Merila, Tubod River, 8°12'17"N, 124°15'24"E, 18 m a.s.l., 05–07.2017, 108 $\stackrel{\circ}{\rightarrow}$ 60 $\stackrel{\circ}{\rightarrow}$ eg. D. A. P. Acal (DAC); Iligan City – Barangay Merila, Tubod River, 8°12'17"N, 124°15'24"E, 18 m a.s.l., 05–07.2017, 108 $\stackrel{\circ}{\rightarrow}$ 60 $\stackrel{\circ}{\rightarrow}$ eg. D. A. P. Acal (DAC); Iligan City – Barangay Merila, Tubod River, 8°12'17"N, 124°15'24"E, 18 m a.s.l., 05–07.2017, 108 $\stackrel{\circ}{\rightarrow}$ 60 $\stackrel{\circ}{\rightarrow}$ eg. D. A. P. Acal (DAC); Iligan City – Barangay Merila, 12 eg. D. A. P. Acal (DAC); Ilig

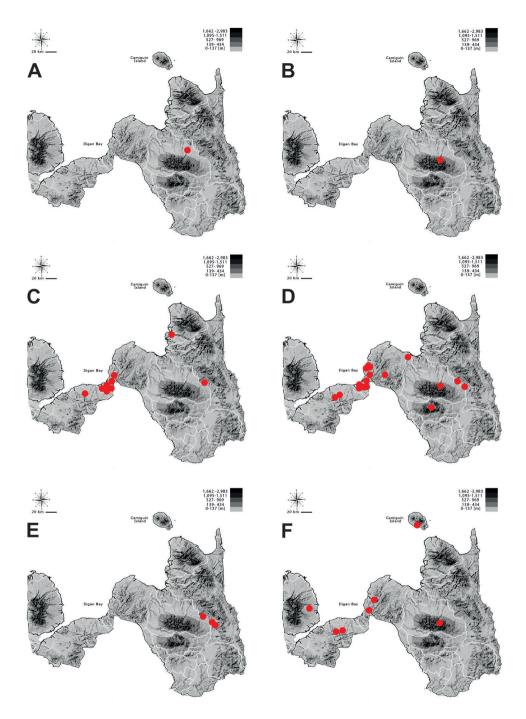


Figure 10. Distribution of **A** Thopeutica (T.) milanae **B** T. (Thopeutica) virginea **C** Cylindera (Eugrapha) minuta **D** C. (Ifasina) discreta elaphroides **E** C. (Ifasina) mouthiezi, and **F** C. (Ifasina) viduata in Northern Mindanao region.

Puga-an, rocky bank of Puga-an River, 8°13'31.5"N, 124°16'08.6"E, 10.29.2018, $3\overline{\checkmark}$ 2Q Q, leg. C. Torres (RJC); Iligan City – Barangay Baraas, Tubod River, 8°12'40.23"N, 124°14'53.25"E, 12 m a.s.l., 17.07.2018, 11 $\overline{\checkmark}$ $\overline{\circlearrowright}$ 6Q Q, leg. D. A. P. Acal (RJC); Iligan City – Barangay Tubod, Tubod River, 8°13'12.12"N, 124°14'56.00"E, 9 m a.s.l., 30 exx., leg. D. A. P. Acal (DAC); Municipality of Bacolod, Barangay Esperanza, 8°10'12"N, 124°0'22"E, 27 m a.s.l., 06.2017, 1 $\overline{\checkmark}$, leg. D. A. P. Acal (DAC); Iligan City – Barangay Puga-an, 8°13'30.73"N, 124°16'6.18"E, 21 m a.s.l., 18.10.2018, 2 $\overline{\circlearrowright}$ $\overline{\circlearrowright}$, leg D. A. P. Acal (RJC); **Misamis Oriental province:** Municipality of Balingasag, Cabulaway River, 8°46'9"N, 124°48'2"E, 86 m a.s.l, 06–08.2017, 44 $\overline{\circlearrowright}$ 34Q Q, leg. D. A. P. Acal (DAC).

Habitat. Species occurs on sandy river banks (pers. obs.).

Remarks. This species was observed to co-occur with *Calomera mindanaoensis*, *C. lacrymosa*, *C. angulata*, and/or *Cylindera discreta elaphroides* (pers. obs.).

Subgenus Ifasina Jeannel, 1946

Cylindera (Ifasina) discreta elaphroides (Doktouroff, 1882)

Figures 2H, 3A, C-E, 5G-H, 10D

General distribution. Subspecies endemic to the Philippines, recorded till now from Leyte, Mindanao, Palawan, Samar (Cabras et al. 2016a), and Cebu (Cabrera et al. 2019); in Mindanao noted from Davao (Cabras et al. 2016a, b; Cabras and Wiesner 2016) and Northern Mindanao regions (new records).

Material examined. Bukidnon province: Mt. Kitanglad, 11–12.2014, 1^Q, leg. N. Mohagan (JWC); Malaybalay City, Barangay Kalasungay, 8°11'28"N, 125°5'54"E, 770 m a.s.l., 06-08.2017, 47 exx., leg. D. A. P. Acal (DAC); Malaybalay City, Barangay Can-ayan, 8°11'31"N, 125°9'13"E, 653 m a.s.l., 06–08.2017, 226 exx., leg. D.A.P. Acal (DAC); Mt. Kalatungan, Sitio Bato, Municipality of Maramag, 11.2019., 2 exx., leg. R. Jaskuła, D. A. P. Acal (RJC); Lanao del Norte province: Iligan City – Barangay Bonbonon, 8.265458N, 124.310138E, 47 m a.s.l., 06.2017, 5 exx, leg. D. A. P. Acal (DAC); Iligan City - Barangay Merila, Tubod River, 8°12'17"N, 124°15'24"E, 18 m a.s.l., 05.2017, 3 exx., leg. D. A. P. Acal (DAC); Iligan City - Barangay Baraas, Tubod River, 8°12'40.23"N, 124°14'53.25"E, 12 m a.s.l., 17.07.2018, 299, leg. D. A. P. Acal (RJC); Iligan City - Barangay Tubod, Tubod River, 8°13'12.12"N, 124°14'56.00"E, 9 m a.s.l., 17.07.2018, 1 ex., leg. D.A.P. Acal (DAC); Iligan City - Barangay Puga-an, sandy bank of Puga-an River, 8°13'21.3"N, 124°15'52.0"E, 10.29.2018, 9 \bigcirc 2 \bigcirc 2 \bigcirc , leg. C. Torres (RJC); Iligan City – Barangay Puga-an, sandy bank of Puga-an River, 8°13'29.6"N, 124°15'57.8"E, 10.29.2018, 3 8, leg. C. Torres (RJC); Iligan City – Barangay Tipanoy, Tubod River, 8°11'38.12"N, 124°15'25.38"E, 20 m a.s.l., 29.08.2018, 4 d d 1 Q, leg. D. A. P. Acal (RJC); Municipality of Bacolod, Barangay Esperanza, 8°10'12"N, 124°0'22"E, 27 m a.s.l., 05.2017, 1 ex., leg. D. A. P. Acal (DAC), 13.12.2018, bank of river, 200, leg. R. Jaskuła (RJC); Municipality of Bacolod, Barangay Mati, 8°9'4"N, 124°0'57"E, 53 m a.s.l., 13.12.2018, 233, leg. R. Jaskuła (RJC); Iligan City - Barangay Rogongon, Sitio Lawlawon, 8°14'51.13N, 124°25'25.31"E, 359 m a.s.l., 10.03.2019, 10 exx., leg. D. A. P. Acal (DAC); **Misamis Oriental province:** Cagayan de Oro City, Malasag Cugman, Mapawa Nature Park, 8°26'5.93"N, 124°42'12.40"E, 334 m a.s.l., 20.08.2017, $9 \stackrel{<}{\circ} \stackrel{<}{\circ} 7 \stackrel{\bigcirc}{\circ} \stackrel{<}{\circ}$, leg. O. Bagona (RJC); Municipality of Lugait, Barangay Upper Talacogon, river bank, 8°20'47.04"N, 124°16'58.80"E, 08.11.2018, $2\stackrel{<}{\circ} \stackrel{<}{\circ} 2 \stackrel{\bigcirc}{\circ} \stackrel{<}{\circ}$, leg. V. M. Mirabueno (RJC); 01.12.2018, $4\stackrel{<}{\circ} \stackrel{<}{\circ} 1 \stackrel{\bigcirc}{\circ}$, leg. V. M. Mirabueno (RJC); Municipality of Lugait, Barangay Lower Talacogon, river bank, 8°20'47.04"N, 124°16'58.80"E, 03.12.2018, $2\stackrel{<}{\circ} \stackrel{<}{\circ} 1 \stackrel{\bigcirc}{\circ}$, leg. V. M. Mirabueno (RJC); Municipality of Lugait, Barangay Lower Talacogon, river bank, 8°20'47.04"N, 124°16'58.80"E, 03.12.2018, $2\stackrel{<}{\circ} \stackrel{<}{\circ} 1 \stackrel{\bigcirc}{\circ}$, leg. V. M. Mirabueno (RJC); Municipality of Lugait, Barangay Lower Talacogon, river bank, 8°20'47.04"N, 124°16'58.80"E, 03.12.2018, $2\stackrel{<}{\circ} \stackrel{<}{\circ} 1 \stackrel{\bigcirc}{\circ}$, leg. V. M. Mirabueno (RJC); Municipality of Lugait, Barangay Aya-Aya, river bank, 8°20'00.16"N, 124°18'36.77"E, 07.11.2018, $2\stackrel{<}{\circ} \stackrel{<}{\circ}$, leg. V. M. Mirabueno (RJC).

Habitat. A riverine tiger beetles species recorded on sandy bank (per. obs.).

Remarks. Although *C. discreta elaphroides* is active mainly during sunlight hours on the river banks, it was also noted as species actively hunting during heavy rain on vertical surfaces (Cabrera et al. 2019). Some specimens were also observed resting on the leaves of *Pennisetum* sp. along the river (pers. obs.).

Cylindera (Ifasina) mouthiezi Dheurle, 2015

Figures 2H, 10E

General distribution. Species endemic to Philippines (Cabras et al. 2016) where it has been recorded only from Mindanao, where it was found only from Davao and Northern Mindanao regions till now (Dheurle 2015, 2017; Cabras et al. 2016b).

Literature data from Northern Mindanao region. Bukidnon province: Cabanglasan (Dheurle 2015, 2017); Panamokan (Dheurle 2017).

Material examined. Bukidnon province: Cabanglasan, 06.2014, 1♂ (JWC); Panamokan, 06.2014, 1♀ (JWC); Barangay Kalasungay, 8°11′28″N, 125°5′54″E, 770 m a.s.l., 14.06.2017, 8 exx., leg. D. A. P. Acal (DAC, RJC).

Habitat. All specimens known for us from Northern Mindanao region were collected in a shaded riverine area.

Remarks. This species co-occurs with *Cylindera discreta elaphroides* and *Thopeutica angulihumerosa*.

Cylindera (Ifasina) viduata (Fabricius, 1801)

Figures 3G, 10F

General distribution. Species recorded from Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Papua New Guinea, Philippines, Thailand, Vietnam; in the Philippines is was noted from the following islands: Leyte, Mindanao, Palawan, and Tawi-tawi; in Mindanao recorded only on the basis of general information (Cabras et al. 2016a); here we provide the first records from Northern Mindanao region.

Material examined. Bukidnon province: Mt. Kitanglad, 11–12.2014, 1², leg. N. Mohagan (JWC); **Camiguin province:** Camiguin Island, Municipality of Sagay,

Barangay Bonbon, Sagay River, 28 m a.s.l., 08.07.2017, 233, leg. D. A. P. Acal (DAC); Lanao del Norte province: Iligan City, Barangay Bonbonon, 8.265458N, 124.310138E, 47 m a.s.l., 29.05.2017, 3332299, leg. D. A. P. Acal (DAC); 11.2019, 1ex., leg. R. Jaskuła (RJC); Iligan City – Barangay Esperanza, 8°10'12"N, 124°0'22"E, 27 m a.s.l., 08.06.2017, 3999, leg. D. A. P. Acal (DAC); Municipality of Bacolod, Barangay Mati, 8°9'4"N, 124°0'57"E, 53 m a.s.l., 05–07.2017, 223313999, leg. D. A. P. Acal (DAC), 13.12.2018, 2337, leg. D. A. P. Acal (DAC); Misamis Occidental province: Municipality of Sinacaban, Barangay San Isidro, 8°17'5"N, 123°47'5"E, 269 m a.s.l., 05–07.2017, 833799, leg. D. A. P. Acal (DAC); Misamis Oriental province: Mambuntan Falls, Barangay Lubilan, Naawan Municipality, 8.412300N, 124.351642E, 11.2019, 2exx., ad lucem, leg. R. Jaskuła (RJC).

Habitat. Species noted from trails along riverine areas, river banks, cultivated corn and ginger fields (pers. obs.).

Remarks. This species co-occurs with *Lophyra striolata* in agricultural fields (pers. obs.).

Provisional key to tiger beetle species known to occur in the Northern Mindanao Region

1	Metepisternum narrow, with grooves anteriorly; mesepisternum strongly elon-
	gated2
_	Metepisternum relatively broad, without anterior grooves; mesepisternum usually
	short
2	Outer margin of mandible without tooth; labrum 6-dentate; humeral angles of
	elytra and hind wings absent
	3 Tribe Collyridini Brullé, 1834, Subtribe Tricondylina Naviaux, 1991
_	Outer margin of mandible with tooth; labrum 7-dentate; humeral angles and
	hind wings present
	7 Tribe Collyridini Brullé, 1834, Subtribe Collyridina Brullé, 1834
3	Base of interocular cavity at same level as neck; no distinct transverse line between
	neck and occiput; pronotum never both long and subrectangular; body length
	from 13 to 28 mm
_	Base of interocular cavity slightly higher than neck or not exactly on same exten-
	sion; presence of a distinct transverse mark between neck and occiput; temples
	abruptly shaped from dorsal view; body length less than 17 mm, habitus very
	slender6
	Genus Tricondyla Latreille, 1822, Subgenus Stenotricondyla Naviaux, 2002
4	Very robust species with body length 19–24 mm; elytral sculpture may be almost
	smooth or granular
	Tricondyla (Tricondyla) aptera punctipennis Chevrolat, 1841
_	Smaller and narrower species with body length 15-20 mm; sculpture covering
	entire elytra (but decreasing at apex) or posterior half part almost smooth5

5	Elytral sculpture deeper and covering the entire surface but decreasing at apex;
	median lobe of aedeagus with tip particularly acute
_	Elytral sculpture not regularly distributed with the posterior half almost smooth;
	median lobe of aedeagus with tip less acute
	Tricondyla (Tricondyla) elongata Horn, 1906
6	Generally smaller species (usually between 13 and 15.5 mm), aedeagus not longer
	than 2.5 mm
_	Larger species, usually with body length between 14 and 17 mm; aedeagus at least
	3 mm long Tricondyla (Stenotricondyla) cavifrons Schaum, 1862
7	Labrum very short; body smaller than 9 mm, slender; sculpture of elytra shallow,
	dense and uniform
	Genus Protocollyris Mandl, 1975, Protocollyris mindanaoensis (Mandl, 1974)
_	Labrum longer
8	Generally smaller and slender species (between 9.5 and 13.5 mm)
_	Larger and robust species with body length between 17.5 and 23 mm
	11 Genus Neocollyris Horn, 1901, Subgenus Heterocollyris Naviaux, 1995
9	Antennae short, reaching basal half of pronotum; color dark, not bright blue
/	Neocollyris (Neocollyris) brevicula Naviaux, 1994
_	Antennae longer, reaching base of pronotum; color bright blue, sometimes with
_	violet reflections
10	Vertex dilated behind eyes; pronotum short; tip of aedeagus rounded
10	
_	Vertex not dilated behind eyes, pronotum longer; tip of aedeagus acute
_	
11	Pronotum strongly constricted in front; aedeagus sigmoid in lateral view
11	
	Pronotum lesser constricted in front; aedeagus not sigmoid in lateral view12
12	e e
12	Elytral sculpture dense and fine, less creased near suture
_	
12	
13	Galea of maxilla reduced, one segmented; 4 th tarsal segment very shortened, with
	5 th segment inserted toward the middle of its upper side; labrum long
_	Galea of maxilla two-segmented; 4 th tarsal segment rarely shortened, 5 th segment
1/	always inserted apically; labrum often short
14	Clypeus with two sensitive hairs
-	Clypeus without sensitive hairs
15	Elytra completely shiny black
_	Elytra black with brownish maculation

16	Metasternum yellow Therates fulvipennis everetti Erichson, 1834
_	Metasternum black Therates coracinus coracinus Erichson, 1834
17	Black maculation of elytra does not reach the furrow behind the basal hump
_	Black maculations of elytra covers the furrow behind the basal hump
	Therates fasciatus pseudolatreillei Horn, 1928
18	Head, pronotum, pro- and mesosternum, base of abdomen and elytra glabrous
	19 Tribe Cicindelini Latreille, 1802, Subtribe Prothymina Horn, 1906
-	Either head, pronotum, pro- and mesosternum, base of sternum or base of elytra setose
	20 Tribe Cicindelini Latreille, 1802, Subtribe Cicindelina Latreille, 1802
19	Body ventrally almost entirely glabrous except for fringe of setae on free lateral
	margin of hind coxae, elytra immaculate
_	Body ventrally entirely glabrous
	Prothyma (Symplecthyma) heteromallicollis heteromallicollis Horn, 1909
20	Flagellum of inner sac of aedeagus coiled in a sagittal plane
_	Flagellum complexly coiled on both sides of the inner sac
21	Elytra with complete humeral lunule
	<i>Cylindera (Eugrapha) minuta</i> (Olivier, 1790)
_	Humeral lunule absent, himeral maculations, if present, split in two dots
22	Elytra without any humeral maculations
_	Humeral maculations present
23	Humeral maculations consists of a small posthumeral dot only, humeral dot ab-
	sent Cylindera (Ifasina) discreta elaphroides (Doktouroff, 1882)
_	Humeral maculations constist of a large humeral and a large posthumeral dot
24	Flagellum of inner sac of aedeagus with more than four windings
_	Flagellum with less than four windings
25	Prothorax mostly glabrous, setation restricted to pleurosternal suture or along the
	anterior margin
_	Pronotum with lateral margins and/or lateral angles variably setose27
26	Labrum with 10–14 submarginal setae; females without elytral mirror
-	Labrum with 8 submarginal and mesal setae; females with elytral mirror spot
27	Elytra with humeral callus; prosternum distinctly longer than wide
-	Elytra without humeral callus; prosternum slightly longer than wide

28	Labrum with more than ten marginal setae
_	Labrum with four to eight marginal setae Genus Lophyra Mots-
	chulsky, 1859, Lophyra (Spilodia) striolata tenuiscripta (Fleutiaux, 1893)
29	Elytral maculation consists of humeral and apical lunule marginal band and mid-
	dle band, which all are more or less connected with one another; female elytra
	expanded laterally Calomera angulata angulata (Fabricius, 1798)
_	Elytral maculation consists of apical lunule and five or six dots; female elytra are
	not expanded laterally30
30	Elytra with apical lunule and five dots (humeral, subhumeral, submarginal, discal
	and another submarginal one below the other submarginal dot); elytra velvety black,
	without visible punctuation throughout
_	Elytra with apical lunule and six dots (as mentioned above, plus an upper discal
	dot); elytra with visible punctuation
31	Elytra greenish or bluish, with blue green punctuation; aedeagus short, with a
	tiny hook shaped tip
_	Elytra darker, nearly velvety black, punctuation nearly not visible in apical half of
	elytra; aedeagus longer, ending in a long straight apical beak

Discussion

Tiger beetle fauna of Northern Mindanao vs. fauna of the entire island and country

Thirty species (including two with two subspecies each) classified in ten genera are actually known from Northern Mindanao region (Table 2, Figures 5-9), which constitute 56% of Cicindelidae fauna of Mindanao and 21% of the Philippines. Three of these species are known as endemics of Mindanao Island (Neocollyris similior, Calomera cabigasi, and Cylindera (Ifasina) mouthiezi), one more (Calomera mindanaoesis) is restricted only to Mindanao and Camiguin islands, while 22 taxa occur only in the Philippines (Cabras et al. 2016a). Among the recorded taxa, Neocollyris speciosa, Calomera angulata, Cylindera minuta, and Lophyra striolata tenuiscripta, Thopeutica virginea were noted for the first time from Mindanao island, and moreover, an additional seven species had not been reported from Northern Mindanao region before (Tricondyla cavifrons, Neocollyris similior, Prothyma heteromallicollis heteromallicollis, Thopeutica angulihumerosa, Cylindera discreta elaphroides, C. mouthiezi, C. viduata). The highest number of taxa was noted from Bukidnon (24 species, 80% of Northern Mindanao fauna) Misamis Oriental (15 species, 50% of fauna), and Lanao del Norte provinces (14 species, 47% of fauna). Misamis Occidental and Camiguin provinces were characterized by 23% and 7% of fauna respectively (Table 2). The number of recorded Cicindelidae taxa seems to depend on the surface area of the province, as Bukidnon is the largest

Species	Pr	Provinces of Northern Mindanao region				
-	Bukidnon	Camiguin	Lanao	Misamis	Missamis	
		0	del Norte	Occidental	Oriental	
Tricondyla (Tricondyla) aptera punctipennis Chevrolat, 1841	+		+			
Tricondyla (Tricondyla) elongata Horn, 1906	+		+	+	+	
Tricondyla (Tricondyla) gracilis Naviaux, 2002					+	
Tricondyla (Stenotricondyla) cyanipes Eschscholtz, 1829					+	
Tricondyla (Stenotricondyla) cavifrons Schaum, 1862	+		+			
Neocollyris (Neocollyris) albitarsis (Erichson, 1834)	+					
Neocollyris (Neocollyris) brevicula Naviaux, 1994			+			
Neocollyris (Neocollyris) emarginata (Dejean, 1825)	+					
Neocollyris (Heterocollyris) affinis (Horn, 1892)	+				+	
Neocollyris (Heterocollyris) similior (Horn, 1893)			+		+	
Neocollyris (Heterocollyris) speciosa (Schaum, 1863)	+					
Protocollyris mindanaoensis (Mandl, 1974)			+			
Therates coracinus coracinus Erichson, 1834	+		+		+	
Therates fasciatus fasciatus (Fabricius, 1801)	+	+	+		+	
Therates fasciatus pseudolatreillei Horn, 1928	+		+	+	+	
Therates fulvipennis bidentatus Chaudoir, 1861	+		+			
Therates fulvipennis everetti Erichson, 1834	+					
Prothyma (Symplecthyma) heteromallicollis heteromallicollis Horn, 1909	+		+		+	
Heptodonta nigrosericea (W. Horn, 1930)	+			+	+	
Calomera angulata angulata (Fabricius, 1798)			+			
Calomera cabigasi Cassola, 2011	+				+	
Calomera lacrymosa (Dejean, 1825)			+	+	+	
Calomera mindanaoensis (Cassola, 2000)	+	+	+	+	+	
Lophyra (Spilodia) striolata tenuiscripta (Fleutiaux, 1893)	+		+	+		
Thopeutica (Thopeutica) angulihumerosa (Horn, 1929)	+					
Thopeutica (Thopeutica) darlingtonia Cassola et Ward, 2004	+					
Thopeutica (Thopeutica) milanae Wiesner, 1992	+					
Thopeutica (Thopeutica) virginea (Schaum, 1860)	+					
Cylindera (Eugrapha) minuta (Olivier, 1790)	+		+		+	
Cylindera (Ifasina) discreta elaphroides (Doktouroff, 1882)	+		+		+	
Cylindera (Ifasina) mouthiezi Dheurle, 2015	+					
Cylindera (Ifasina) viduata (Fabricius, 1801)	+	+	+	+	+	
Total	25	3	18	7	16	

one and Camiguin is the smallest area. Since not all types of habitats were studied in particular provinces, additional tiger beetle species inhabiting in Northern Mindanao region are expected to be found with more extensive fieldwork in the future.

Tiger beetles and their habitats

Among Cicindelidae taxa recorded in Northern Mindanao region both epigeic (*Calomera, Cylindera, Heptodonta, Lophyra, Prothyma, Thopeutica*) and arboreal (*Therates, Neocollyris, Protocollyris, Tricondyla*) species were noted. Most of the epigeic species are recognized as riverine tiger beetles (all *Calomera* and most *Cylindera* except *C. viduata*, as well as *Heptodonta nigrosericea, Lophyra, Thopeutica*) occurring on sandy and sunny banks or on shaded banks of medium and large rivers. Among the epigeic Cicindelidae only *Cylindera viduata, Prothyma heteromallicollis heteromallicollis*, and

some Lophyra striolata tenuiscripta were noted as forest beetles occupying different sandy areas. Our data from Northern Mindanao region confirm observations both from other regions of Mindanao (Cabras et al. 2016b; Cabras and Wiesner 2016; Medina 2020; Medina et al. 2020; Pepito et al. 2020) and different parts of the world including e.g., some regions of North America (Pearson et al. 1997), Africa (Jaskuła 2015; Jaskuła et al. 2015; Jaskuła and Rewicz 2015; Jaskuła and Płociennik 2020), Asia (Dangalle et al. 2014) or Europe (Jaskuła 2011; Jaskuła et al. 2019), as tiger beetles are known to prefer riverine habitats not only because of adequate water and food resources but also for protection from predators and human disturbances (Bhargav and Unival 2008). In many regions of the world the highest diversity and species richness of epigeic tiger beetles are noted mostly on lowland areas that had a variety of habitats such as coastal areas, river banks, grasslands, and sand dunes attractive for tiger beetles (e.g., Pearson and Cassola 1992; Pearson et al. 1997; Jaskuła 2011, 2015; Dangalle et al. 2014; Jaskuła et al. 2019; Jaskuła and Płociennik 2020). On the other hand, in the tropical regions large number of Cicindelidae are typical arboreal taxa (e.g., Wiesner 1992; Pearson and Vogler 2001; Moravec 2007; Dangalle 2018) and large forests, especially natural ones, are characterized by high species diversity of such tiger beetles. In Northern Mindanao region, where more than 60% of its entire area is classified as forest land, 48% of all recorded Cicindelidae taxa are noted as arboreal taxa (Table 2). This number is expected to increase in the future as several additional arboreal species are known from other parts of Mindanao, including areas located close to the administrative border of Northern Mindanao region (Cabras et al. 2016a). Moreover, in case of some species, only general distributional data from Mindanao are known (e.g., Protocollyris okajimai Mandl, 1982, Neocollyris rugei Horn, (1892) N. erichsoni (Horn, 1892), N. chaudoiri (Horn, 1892) (Naviaux 1994)), it cannot be excluded that some of these taxa were collected (and actually inhabit) in the Northern Mindanao region. On the other hand it is necessary to note that many areas in Mindanao, including Northern Mindanao region, are under large impacts of human activities, and as a result many tiger beetle habitats are regularly destroyed. Forest destruction, including deforestation in all sorts and forms such as illegal logging, mining, agricultural expansion, quarrying, over-extraction of plant biota for fuel and other domestic uses, or conversion of land into human settlement are among the rampant problems in the area (Magdalena 1996; Carandang et al. 2012).

Conclusions

Present data on diversity and distribution of Cicindelidae of Northern Mindanao region clearly suggest that the area (especially riverine habitats and forests) is unique for tiger beetle fauna which includes a significant number of both species endemic to Mindanao and to the entire country. Moreover, the lack of data on Cicindelidae in many areas in Mindanao Island and in the country is evident, and for many species, only single records are known. As the region is characterized by a large mosaic of still poorly explored habitats (e.g., forests in the mountains, upper parts of river systems), and more than 12% of all species noted from Mindanao were discovered and described as new for science only during last two decades, it should be expected that future studies will provide many new and important distributional data and probably will describe new Cicindelidae taxa.

Acknowledgements

We would like to thank Obed Bagona, Annaly B. Lapore, Vera Marie Mirabueno, Charity Torres, and Łukasz Trębicki who made possible to study material of Cicindelidae collected by them; Obed Bagona and Annaly B. Lapore also provided photographs of tiger beetle habitats. The first author would like to thank to the following persons for their kind help during MSc fieldwork: Penny Acal, Kieth Jay Acal, Angel Luz M. Lesondra. The following persons helped DAPA and RJ to collect insect material during the Agad-Agad and Naawan trips: Noville Jay Ebina, Christine Jean Abapo, Gypsie Claudine Café, Norjanah Campong, Marco Luis Lumontod, Grapesy Violet, Angeleth Taotao, Jamalia Macatanong, Fatimah Radiamodah, Sitty Adna Camama, Jonaisah Abdullah, Nurhana Sabaani, Eddie Modejar, Radonna Jessah Christy Maandig, and during the Bukidnon and Camiguin trips: Elbert B. Caballero, Philip Noel Banaag, Rico Ray T. Mendoza. Finally, we would like to thank to our guides and cooks in Bukidnon Province, Mt. Kalatungan: Jose A. Sabares, Joenel A. Sabares, Nelia A. Sabares; Mt. Kitanglad: Don Espiñosa, Bong Espiñosa, Thelma Aatoque; and in Camiguin Island: Gloria D. Abian, Johner C. Abian, Hilda Lsserna. Permissions to conduct field collections were obtained from the Department of Environmental and Natural Resources (DENR-Region 10) through a Wildlife Gratuitous Permits no. R10 2017-23, no. R10 2019-81, and Wildlife Export permit no. R10-2018-02, Wildlife Gratuitous Permit no. R10 2019-48 (Mt. Kalatungan Range Natural Park), Wildlife Gratuitous Permit no. R10 2019-89 (Camiguin Island), Wildlife Gratuitous Permit no. R10 2019-88 (Bukidnon, Mt. Kitangland Range National Park). The Department of Science and Technology – Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP) is gratefully acknowledged by DAPA for all financial support during the collection of materials and the fellowship through the Research Enrichment Program (Sandwich Program). Two visits in the Philippines of RJ were possible due to financial support by the Faculty of Biology and Environmental Protection, University of Lodz, Poland. Finally, we would like to thank Thorsten Assmann (Germany), Andrey Matalin (Russia), and Rikjan Vermeulen (The Netherlands) for their valuable comments to the first version of the manuscript.

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RESEARCH ARTICLE



The first record of the genus *Laemostenus* from China, with descriptions of two new species from the Himalaya (Carabidae, Sphodrini, Sphodrina)

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Academic editor: B.M. Kataev Received 26 November 2020 Accepted 27 January 2021 Published 12 February 2021
http://zoobank.org/09CCA8F4-5E25-4DD6-B37F-FEF66B0E809D

Citation: Zhu P, Shi H, Liang H (2021) The first record of the genus *Laemostenus* from China, with descriptions of two new species from the Himalaya (Carabidae, Sphodrini, Sphodrina). ZooKeys 1017: 77–88. https://doi.org/10.3897/zookeys.1017.61383

Abstract

The genus *Laemostenus* is recorded from China for the first time, and two new species are described: *L. (Pristonychus) zhentangensis* **sp. nov.** (type locality: Dinggyê County, Xizang), and *L. (P.) zhamensis* **sp. nov.** (type locality: Nyalam County, Xizang). The relationships of these new species are briefly discussed.

Keywords

brunneus species group, ground beetle, new record, Pristonychus, taxonomy, Xizang

Introduction

The genus *Laemostenus* Bonelli, 1810, containing 14 subgenera and more than 200 described species, is widely distributed in the Western Palaearctic Region (Europe, North Africa, West and Central Asia, and Himalaya) (Casale 2017). It differs from the other genera of Sphodrina by the combination of the following characters: tarsomeres 2–5 pubescent dorsally, antennomere 3 glabrous, interval 3 of the elytra without dorsal pores, and labrum with six setae on its anterior margin.

Pristonychus Dejean, 1828 was established as a genus for *Carabus terricola* Herbst, 1784 but was subsequently reduced to a subgenus of *Laemostenus* (Casale 1988). It differs from other subgenera mainly by the inner side of metatibia having a dense brush of setae at the apex. The subgenus *Sphodroides* Schaufuss, 1863 from North Africa and West Asia also shares this character, but it can be distinguished from *Pristonychus* by the more strongly protruding shoulder angles between the basal and lateral margins of the elytra. Casale (1988) treated and illustrated 48 species of this subgenus and divided them into 11 species groups. Subsequently, a few species were demoted to subspecies, while some new species were described, mainly from Europe and West Asia (Vereschagina and Kabak 1997; Nitzu 1998; Casale and Vigna Taglianti 1999; Guéorguiev 2002; Guéorguiev 2003; Casale and Wrase 2012). To date, subgenus *Pristonychus* contains 56 species from the western Palaearctic Region to the Himalaya.

During our recent expeditions to Xizang, two specimens of Sphodrina were collected from Zhêntang and Zham towns in the valleys of the south Himalaya near the border with Nepal. They can be readily recognized as species of the genus *Laemostenus* due to the pubescence on the dorsal sides of tarsomeres 2–5. They both belong to the *brunneus* species group of the subgenus *Pristonychus* according to Casale's work (1988) and represent two different new species. These are the first records of the genus *Laemostenus* from China.

The primary purpose of this paper is to record the genus *Laemostenus* from China and describe two new species. In addition, the relationships of these new species are briefly discussed. For the new species, complete descriptions, illustrations, and a distribution map are provided.

Materials and methods

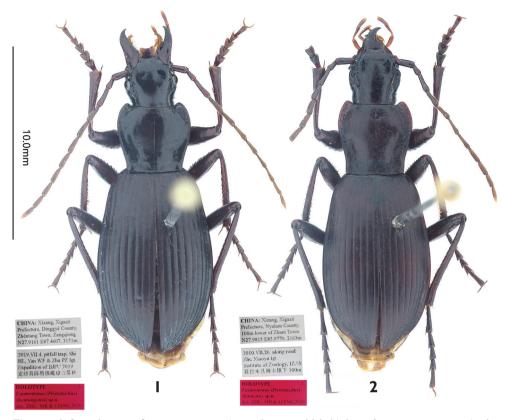
Specimens examined during our study are deposited in the Institute of Zoology, Chinese Academy of Sciences, Beijing, China (**IZAS**). Labels are cited verbatim.

Abbreviations for measurements used in the paper are as follows: body length (**BL**) was measured from the apical margin of the labrum to the elytral apex; body width (**BW**) was measured across the elytral greatest width (**EW**). Pronotum width (**PW**) was measured across its greatest width; basal width of pronotum (**PBW**) was measured along its basal margin; pronotum length (**PL**) was measured along its median line. Elytra length (**EL**) was measured along the suture from the base of the scutellum to the elytra apex.

Taxonomy

Laemostenus (Pristonychus) zhentangensis sp. nov. http://zoobank.org/DDFF30AE-D552-44F4-9B82-3F7E4DDDDC5D Figs 1, 3–5, 9–11

Type locality. China, Xizang: Dinggyê (27.9161°N, 87.4607°E), altitude 3151 m.



Figures 1, 2. Holotypes of *Laemostenus* spp. (general view and labels) 1 *L. zhentangensis* sp. nov. (male, Xizang, China, IZAS) 2 *L. zhamensis* sp. nov. (male, Xizang, China, IZAS).

Type material. *Holotype:* male (IZAS), body length 15.6 mm, pin mounted, with genitalia dissected and glued on cardboard pinned under the specimen; labeled: "CHINA: Xizang, Xigazê Prefecture, Dinggyê County, Zhêntang Town, Zangqiong, 27.9161°N 87.4607°E, 3151 m"; "2019.VII.4, pitfall trap, Shi HL, Yan WF & Zhu PZ lgt. Expedition of BJFU 2019. 定结县陈塘镇藏琼云雾林"; "HOLOTYPE d' *Laemostenus (Pristonychus) zhentangensis* sp. n. des. ZHU, SHI & LIANG 2020" [red label].

Diagnosis. Body dark brown. Head slightly narrow. Eyes small, slightly prominent laterally; tempora oblique, as long as eyes. Elytra with lateral margins straight near sutural angles; sutural angles acute. Parascutellar pores present. Ventral side of profemora smooth, with one seta on posterior margin, without tooth on anterior margin. Mesotibiae faintly curved in male. Meso- and metatibiae with a dense brush of reddish-yellow setae in apical half. Metatrochanters reniform, not elongate. Apical lamella of median lobe short, length half its basal width, apex slightly truncate. Right paramere strongly curved (the angle between basal and apical portions near 90°), distinctly widened at middle, strongly narrowed to apex, apex very thin.

Comparison. This new species belongs to the *brunneus* species group sensu Casale (1988) for (1) reniform metatrochanters, not elongate in shape; (2) the ventral side of profemora smooth or at most with a small tooth on anterior margin; (3) eyes small, not very prominent; (4) body dark brown, without metallic luster; and (5) metatibiae usually curved in males at least.

Among this species group, the new species is most similar to *Laemostenus* (*Pristonychus*) arthuri (Morvan, 1982) and *L*. (*P.*) migliaccioi (Casale, 1982), both from Nepal, sharing the ventral side of profemora with one or two setae on posterior margin and the subcordate pronotum. The new species differs from them by the narrower head, the slightly larger eyes, and the slightly truncate apical lamella of the aedeagus. The apical lamella of the aedeagus is rounded in *L*. (*P.*) arthuri and emarginate in *L*. (*P.*) migliaccioi, and both species have a more globular head with smaller eyes.

Description (male). BL = 15.6 mm, BW = 5.9 mm. **Body** (Fig. 1) dark brown, without metallic luster; antennomeres 4–11, labial and maxillary palpi, and apex of mouthparts brown to light brown; venter reddish brown. Head, base of pronotum, and elytra with strong isodiametric microsculpture; disc of pronotum with slightly transverse microsculpture.

Head (Fig. 3) medium in width. Vertex smooth; frontal impressions reduced to two small pits in front of eyes, shallow but distinct; anterior margin of labrum emarginate, with four setae; eyes small, slightly prominent laterally; tempora oblique, as long as eyes; two pairs of supraorbital setae present; antennae long and slender, extended to half of elytra.

Pronotum (Fig. 4) subcordate, wider than long, PW/PL = 1.16, widest near anterior quarter; apical margin weakly concave, its width subequal to basal margin; sides distinctly converged to base (PW/PBW = 1.32), faintly sinuate before posterior angles, with two pairs of setae, at widest points of pronotum and at posterior angles, respectively; basal margin nearly straight; anterior angles rounded, clearly projecting forwards; posterior angles forming distinct obtuse angles; disc gently convex, with some shallow transverse wrinkles; median line fine but clearly defined, reaching anterior and posterior borders; basal foveae shallow and wide, extending beyond middle of pronotum, without punctures or wrinkles.

Elytra elongate, EL/EW = 1.61, slightly dilated towards apex, widest at posterior third; lateral margins straight before sutural angles, sutural angles acute (Fig. 5); basal margins straight; shoulders moderately oblique; shoulder angles between basal ridges and lateral margins forming obtuse angles; humeral teeth very small, not pointed; striae shallow, impunctate; parascutellar striae well developed, short, located between suture and stria 1; parascutellar pores present; intervals feebly convex, interval 3 without setigerous pores, interval 7 with two setigerous pores near apex; umbilicate series composed of 20 or 21 setigerous pores, sparser in middle. Hind wings reduced.

Venter. Propleuron, mesepisternum, and metepisternum smooth. Mesosternum not denticulate in front of mesocoxae. Metepisternum long and narrow. All abdominal sternites with a few shallow wrinkles laterally, without ambulatory setae.



Figures 3–8. Morphological features of *Laemostenus* spp. 3–5 *L. zhentangensis* sp. nov. 6–8 *L. zhamensis* sp. nov. 3, 6 head 4, 7 pronotum 5, 8 sutural angle of elytron.

Legs long and slender; ventral side of profemora smooth, with one seta on posterior margin, without tooth on anterior margin; protibiae with sparse pubescence on apices; mesotibiae faintly curved (in male); meso- and metatibiae inner sides with a dense brush of reddish-yellow setae in apical half; metatrochanters reniform; tarsi elongate and narrow; metatarsomere 1 sparsely pubescent dorsally; claws smooth on internal margin. Protarsomeres 1–3 distinctly dilated and with ventral adhesive vestiture in male.

Male genitalia. Median lobe (Fig. 9) short and stout, distinctly bent ventrally; apical orifice very long, stretching from basal bulb to apical lamella, slightly narrowed in middle; in dorsal view, left and right margins of median lobe both straightly converged to apex and rounded to base; apical lamella short, length half its basal width, apex slightly truncate; in lateral view, ventral margin straight, not expanded in the middle; apex slightly thickened; left paramere (Fig. 11) large and rounded, apical membranous filament small; right paramere (Fig. 10) markedly styloid, strongly curved (the angle between basal and apical portions near 90°), distinctly widened at middle and strongly narrowed to apex, apex very thin.

Female unknown.

Distribution and habitat. This species is only known from Zhêntang Town, Dinggyê County, Xizang, China (Fig. 15). The only specimen was caught by pitfall trap in a cloudy forest at 3151 m a.s.l. (Fig. 16).

Etymology. The new species is named for its type locality, Zhêntang Town.

Remarks. There is an unusual character in this new species: only four setae are present on the anterior margin of labrum instead of six, but they are irregularly arranged (Fig. 3), leaving gaps for the 2nd and 6th (from the left to right) of the normally six setae presenting in the genus. Among all Sphodrina of the world, only *Miquihuana rhadiniformis* Barr, 1982, a cavernicolous ground beetle from Mexico, has four setae present on the anterior margin of labrum, and these are evenly arranged (Barr 1982; Casale 1988). Considering the other characters and geographical distance, it is obvious that these two species have no close relationship. It is presumed that the absence of the two setae on the anterior margin of labrum is probably an individual variation rather than a specific character.

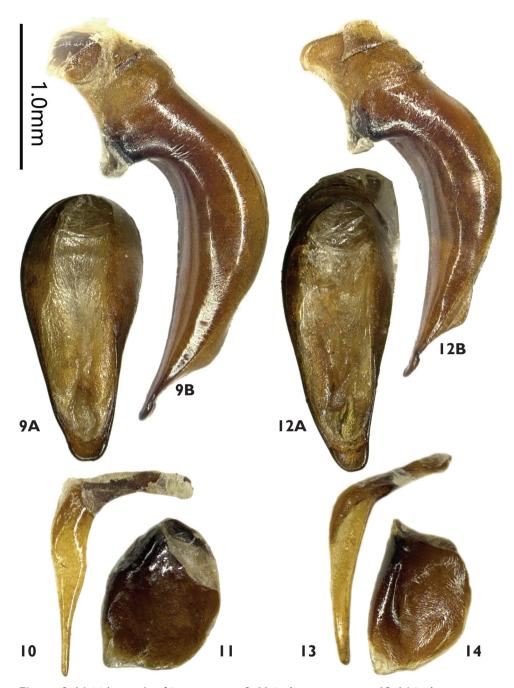
Laemostenus (Pristonychus) zhamensis sp. nov.

http://zoobank.org/53D17D2A-239B-4079-81A5-5DF57DD4569A Figs 2, 6–8, 12–14

Type locality. China, Xizang: Nyalam (27.9815°N, 85.9770°E), altitude 2163 m.

Type material. *Holotype*: male (IZAS), body length 15.9 mm, pin mounted, with genitalia dissected and glued on cardboard pinned under the specimen; labeled: "CHINA: Xizang, Xigazê Prefecture, Nyalam County, 100 m lower of Zham Town, 27.9815°N 85.9770°E, 2163m"; "2010.VII.26, along road, Zhu Xiaoyu lgt., Institute of Zoology, IZAS 聂拉木县樟木镇下100 m"; "HOLOTYPE *& Laemostenus (Pristonychus) zhamensis* sp. n. des. ZHU, SHI & LIANG 2020" [red label].

Diagnosis. Body dark brown. Head medium in width. Eyes very small, hardly prominent laterally; temporae slightly swollen, twice as long as eyes. Elytra with lateral



Figures 9–14. Male genitalia of *Laemostenus* spp. 9–11 *L. zhentangensis* sp. nov. 12–14 *L. zhamensis* sp. nov. 9, 12 median lobe of aedeagus A dorsal view B left lateral view 10, 13 right paramere 11, 14 left paramere.

margins distinctly sinuate near sutural angles; sutural angles rounded. Parascutellar pores absent. Ventral side of profemora smooth, with one seta on posterior margin, without tooth on anterior margin. Mesotibiae faintly curved in males. Meso- and

metatibiae inner sides with a dense brush of reddish yellow setae in apical half. Metatrochanters reniform, not elongate. Apical lamella of median lobe short, length half its basal width, apex slightly truncate, somewhat rounded. Right paramere strongly curved (the angle between basal and apical portions near 120°), slightly widened at middle and slightly narrowed apically, apex moderately thin.

Comparison. This new species also belongs to the *brunneus* species group, as does the previous new species.

It is distinguishable from most species of this group by the absence of the parascutellar pores on the elytra. There are three other species in this species group which have this character: *Laemostenus (Pristonychus) tentiobtusus* (Morvan, 1979), *L. (P.) brunneus* (Hope, 1831), and *L. (P.) pseudobrunneus* Casale, 1981, from India and Nepal. *Laemostenus (P.) zhamensis* sp. nov. differs from the first by the ventral side of profemora not having a tooth on the anterior margin, and it differs from the latter two species by the narrower and not globular head and the shallow and impunctate striae of the elytra.

Description (male). BL = 15.9 mm, BW = 5.5 mm. *Body* (Fig. 2) dark brown, without metallic luster; labial and maxillary palpi and apex of mouthparts light brown; venter light brown. Head and pronotum with weak isodiametric microsculpture, elytra with strong isodiametric microsculpture.

Head (Fig. 6) medium in width. Vertex smooth; frontal impressions reduced to two small pits in front of eyes, which are shallow but distinct; anterior margin of labrum emarginate, with six setae; eyes very small, hardly prominent laterally; tempora slightly swollen, twice as long as eyes; two pairs of supraorbital setae present; antennae long and slender, extending to basal one-third of elytra.

Pronotum (Fig. 7) narrow, width subequal to length, PW/PL = 1.03, widest near anterior quarter; apical margin nearly straight, its width subequal to basal margin; sides distinctly converged to base (PW/PBW = 1.23), moderately sinuate before posterior angles, with two pairs of setae, at widest points of pronotum and at posterior angles, respectively; basal margin almost straight; anterior angles rounded, distinctly projecting forward; posterior angles forming distinct right angles; disc gently convex, smooth; median line fine but clearly defined, not reaching anterior and posterior borders; basal foveae deep and wide, extending to middle of pronotum, without punctures and wrinkles.

Elytra elongate, EL/EW = 1.65, slightly dilated towards apex, widest at posterior third; lateral margins distinctly sinuate near sutural angles; sutural angles rounded (Fig. 8); basal ridges straight; shoulders strongly oblique; shoulder angles between basal and lateral margins forming obtuse angles; humeral teeth very small, not pointed; striae shallow, impunctate; parascutellar striae well developed, short, located between suture and stria 1; parascutellar pores absent; intervals feebly convex, interval 3 without setigerous pores, interval 7 with one setigerous pore near apex; umbilicate series composed of 16 or 17 setigerous pores, very sparser in middle. Hind wings reduced.



Figure 15. Distribution map of Laemostenus spp. L. zhentangensis sp. nov. (blue); L. zhamensis sp. nov. (red).

Venter. Propleuron, mesepisternum, and metepisternum smooth. Mesosternum not denticulate in front of mesocoxae. Metepisternum slightly longer than wide. All abdominal sternites with a few shallow wrinkles laterally, without ambulatory setae.

Legs long and slender; ventral side of profemora smooth, with one seta on posterior margin, without tooth on anterior margin; protibiae with sparse pubescence on apices; mesotibiae faintly curved (in male); meso- and metatibiae with a dense brush of reddish yellow setae in apical half of their inner sides; metatrochanters reniform, not elongate; tarsi elongate and narrow; metatarsomere 1 sparsely pubescent dorsally; claws smooth on internal margin. Protarsomeres 1–3 (in male) distinctly dilated and with ventral adhesive vestiture.

Male genitalia. Median lobe (Fig. 12) short and stout, distinctly bent ventrally; apical orifice very long, stretching from basal bulb to apical lamella, not narrowed in middle; in dorsal view, left and right straightly converged to apex and rounded to base; apical lamella short, length half its basal width, apex slightly truncate, somewhat rounded; in lateral view, ventral margin straight, not expanded at the middle; apex slightly thickened, faintly bent ventrally at tip; left paramere (Fig. 14) large and round, apical membranous filament small; right paramere (Fig. 13) markedly styloid; strongly curved (the angle between basal and apical portions near 120°), slightly widened in middle, slightly narrowed apically, apex moderately thin.

Female unknown.



Figures 16, 17. Habitats in the type localities of *Laemostenus* spp. 16 Zangqiong, Zhêntang Town, Dinggyê County, Xigazê, Xizang, China; locality of *L. zhentangensis* sp. nov. 17 Nyalam County, 100 m below Zham Town, Xigazê, Xizang, China; locality of *L. zhamensis* sp. nov.

Distribution and habitat. This species is only known from Zham Town, Nyalam County, Xizang, China (Fig. 15). The only specimen was caught along road during day in a cloudy forest at 2163 m a.s.l. (Fig. 17).

Etymology. The new species is named for its type locality, Zham Town.

Discussion

There are many lineages of the genus *Laemostenus* in the Himalaya, representing different species groups of the subgenera *Pristonychus* and *Laemostenus*. The two new species from southern Xizang belong to the *brunneus* species group, subgenus *Pristonychus*, according to Casale (1988). This species group previously contained seven Himalayan species that all have been treated and illustrated by Casale (1988). The two new species share many characters, such as the slightly narrow head, the smooth claws, and smooth ventral side of profemora, without seta or tooth on posterior margin, but differ from each other in the shape of the pronotum, the presence or absence of parascutellar pores, and the form of the elytral apices.

Acknowledgements

We wish to thank Prof. Zong Shixiang (Beijing Forestry University, BJFU), Ms Ren Lili (BJFU), Mr Zhao Bin (Forestry and Grassland Administration of the Tibet Autonomous Region), and Mr Jin Pengfei (Forestry and Grassland Administration of Xigazê Prefecture) for their support and assistance during our expeditions in Xizang. We are grateful to Mr Yan Weifeng (BJFU), Mr Ge Sixun (BJFU) and Mr. Zhu Xiaoyu (Ningbo, Zhejiang) for their help in collecting specimens for the study. Thanks also to Dr Ilya Kabak (Saint Petersburg), Dr Joachim Schmidt (Rostock), Dr. Borislav Guéorguiev (Sofia), and Dr Achille Casale (Milan) for their comments during the review process. This work was supported by grants from the the Biodiversity Survey and Assessment Project of the Ministry of Ecology and Environment, China (grant no. 2019HJ2096001006), National Natural Science Foundation of China (grant no. 31970400), and the Key Collaborative Research Program of the Alliance of International Science Organizations (grant no. ANSO-CR-KP-2020-04).

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RESEARCH ARTICLE



Re-description of the loach species Leptobotia citrauratea (Teleostei, Botiidae), with the description of L. brachycephala from southern Zhejiang Province, China

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Academic editor: S. Kullander Received 11 August 2020 Accepted 22 January 2021 Published 12 February 20	21

Citation: Guo D-M, Zhang E (2021) Re-description of the loach species *Leptobotia citrauratea* (Teleostei, Botiidae), with the description of *L. brachycephala* from southern Zhejiang Province, China. ZooKeys 1017: 89–109. https://doi. org/10.3897/zooKeys.1017.57503

Abstract

Leptobotia citrauratea (Nichols, 1925), a loach species, originally described from Dongting Lake, was recently rehabilitated, based on the examination of the holotype and non-topotypical specimens. Several field surveys conducted from 2016 to 2019 in Zhejiang, Jiangxi and Hunan Provinces, P.R. China, yielded many specimens of *Leptobotia* which were initially identified as *L. citrauratea*. Molecular and morphological analyses of these specimens demonstrated that two distinct species are involved. One was identified as *L. citrauratea*, represented by specimens from both the Poyang and Dongting Lake (type locality) systems in Jiangxi and Hunan Provinces, and the other species is described as *L. brachycephala*, represented by specimens from the Ou-Jiang and Qu-Jiang, two coastal rivers of Zhejiang Province, China. *Leptobotia brachycephala* resembles *L. citrauratea* and *L. micra* in having a row of orange dots or an orange stripe along the dorsal mid-line of the body, extending from the nape to the caudal-fin base – a unique character in *Leptobotia. Leptobotia brachycephala* differs from *L. citrauratea* and *L. micra* Bohlen & Šlechtová, 2017, in caudal-fin shape and pelvic-fin insertion and proportional measurements including caudal-fin length, head length, predorsal length and anal-fin length. Its species status was further corroborated by position in a molecular phylogenetic analysis, based on the mitochondrial cyt b gene and its minimum uncorrected p-distance (2.9%) from congeneric species.

Keywords

Biodiversity, Cypriniformes, morphology, phylogeny, taxonomy

Introduction

The loach genus *Leptobotia* was erected by Bleeker (1870) with the simultaneouslydescribed *Leptobotia elongata* (Bleeker, 1870) as type species by monotypy. The genus is distinguished from other genera of the family Botiidae by the presence of a simple suborbital spine beneath the eye (Tang et al. 2008). Sixteen species are currently included in *Leptobotia* (Kottelat 2012; Bohlen and Šlechtová 2016, 2017). The majority of these species are known from southern China, mainly in the Yangtze River (= Chang-Jiang) and Pearl River (= Zhu-Jiang) Basins and coastal rivers of southern Zhejiang Province. Two species – *L. flavolineata* Wang, 1981 and *L. orientalis* Xu, Fang & Wang, 1981 – occur in northern China (Tang et al. 2008; Kottelat 2012; Bohlen and Šlechtová 2017).

Nichols et al. (1925) described *Leptobotia citrauratea* from the Dongting Lake system in Hunan Province, China. Chen (1980) considered specimens of *L. citrauratea* to be juveniles of *L. elongata*. The synonymy of *L. citrauratea* with *L. elongata* was generally accepted by subsequent researchers (Kottelat 2004, 2012). However, Nalbant (2002) regarded *L. citrauratea* to be a valid species. Bohlen and Šlechtová (2017), based on examination of the holotype and non-topotypical specimens from the Poyang Lake system in Jiangxi Province, southern China, recognised *L. citrauratea* as a valid species. A row of orange dots or an orange stripe along the dorsal mid-line distinguishes it from *L. elongata*.

Several field surveys conducted by us from 2016 to 2019 in Zhejiang, Jiangxi and Hunan Provinces, yielded many specimens of *Leptobotia* with a row of orange dots or an orange stripe along the dorsal mid-line and orange or yellowish-brown lateral portion, by which they were initially identified as *L. citrauratea*. These specimens were recovered in two distinct lineages in a phylogenetic analysis, based on the mitochondrial cytochrome b (cyt b) gene sequences. Morphological analysis also indicated that two distinct species are involved. One of them was identified as *L. citrauratea*, represented by specimens sampled from the Poyang and Dongting Lake systems. The other species is an undescribed species represented by specimens from the Ou-Jiang and Qu-Jiang in Zhejiang Province. The present study aims to provide a re-description of *L. citrauratea*, based on fresh topotypical specimens and the formal description of the undescribed species.

Materials and methods

Specimens were either initially fixed in 10% formalin and then transferred to 70% ethanol for morphological examination or preserved in 95% ethanol for DNA extraction. Seventy-three specimens from the three species (*L. citrauratea*, *L. elongata* and *L. brachycephala*) were used for morphometric analysis. Voucher specimens are kept in the ichthyological collection of the Museum of Aquatic Organisms at the Institute of Hydrobiology (**IHB**), Chinese Academy of Sciences, Wuhan City, Hubei Province, China.

Twenty-five measurements (Tables 1, 2) were taken from 22 specimens of *Leptobotia elongata* collected from the upper Chang-Jiang Basin in Sichuan Province; 29 specimens of *L. citrauratea* from the Gan-Jiang (an effluent of Poyang Lake) and Dongting Lake; and 22 specimens of *L. brachycephala* from the Ou-Jiang and Qu-Jiang. Measurements were taken point to point with digital calipers directly linked to a datarecording computer and data recorded to the nearest 0.1 mm. All measurements and counts were made on the left side of each specimen, following the methods of Kottelat (2001) and Xin et al. (2009). The lateral head length and measurements of other parts of the body were given as percentages of the standard length (SL) and measurements of parts of the head were expressed as proportions of the lateral head length (HL). Morphometric variation was analysed with Principal Component Analysis (PCA) in Past v.1.89 (Hammer et al. 2009). The PCA was made with log-transformed measurement data to a tenth of a millimetre in a covariance matrix and without rotation.

Genomic DNA was extracted from fin clips stored in ethanol using the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing) with the recommended protocol. The cyt b gene was amplified by primers L14724 (GACTTGAAAAACCACCGTTG) and H15915 (CTCCGATCTCCGGATTACAAGAC) adopted from Xiao et al. (2001), with 1 μ l of each primer, 1 μ l template DNA, 12.5 μ l Master mix Taq (Beijing TsingKe Biotech Co. Ltd.) and 9.5 μ l double distilled water (dd H₂O) for a total reaction volume of 25 μ l. The thermocycling conditions were as follows: initial denaturation for 4 min at 94 °C, denaturation for 50 s at 94 °C, annealing for 50 s at 55 °C and extension for 1 min at 72 °C. After 34–35 cycles, the final extension was done at 72 °C for 10 min and the PCR product was preserved at 4 °C. Sequencing was carried out by the Tianyihuiyuan Biotechnology Company.

A total of 98 cyt b sequences were generated from 12 species of *Leptobotia*. These sequences were used for phylogenetic analysis together with five sequences from two congeneric species (*L. posterodorsalis* Lan & Chen, 1992 and *L. elongata*) and two sequences serving as outgroup (*Parabotia fasciata* Dabry de Thiersant, 1872 and *P. liji-angensis* Chen, 1980) downloaded from GenBank (Table 3).

The sequences were aligned utilising MAFFT version 7 (Katoh and Standley 2013) and ends trimmed, for a total alignment length of 1060 bp. The genetic distance, based on the uncorrected p-distance model (Kumar et al. 2016), was calculated with MEGA 7.0. DNASP v.5 was utilised to filter the haplotype (Librado and Rozas 2009).

PhyloSuite (Zhang et al. 2020) was used for phylogenetic analyses. The selection of the best-fit model of nucleotide evolution based on Akaike's Information Criterion was performed in ModelFinder (Kalyaanamoorthy et al. 2017). MrBayes 3.2.6 (Ronquist et al. 2012) was utilised for Bayesian analysis with the selected model: GTR+I+G+F, applying the optimal nucleotide evolution model and the MCMC method with four chains (three hot chains and one cold chain) running simultaneously for 6,000,000 generations to calculate posterior probability. Trees were sampled for every 1000 cycles. The initial 25% of sampled data were discarded as burn-in. Sufficient mixing of the chains was regarded to be reached when the average standard deviation of split frequencies was below 0.01.

Taxonomy

Leptobotia citrauratea Nichols, 1925

(Fig. 1a–c)

Botia citrauratea Nichols, 1925: 5 [Tungting [now Dongting] Lake, Hunan Province *Leptobotia elongata*: Chen, 1980: 14 (no localities). Kottelat, 2004:15 (no localities); 2012:16 (no locality)

Leptobotia citrauratea: Nalbant, 2002: 316 (no localities). Bohlen & Šlechtová, 2017: 90 (Nanchang City, Jiangxi Province)

Material examined. *Leptobotia citrauratea*: AMNH 8402, holotype, 50 mm SL; China: Hunan Province: Dungting Lake (photograph examined); collected by Clifford H. Pope, 29 December 1921; IHB 2017100260-65, 201801026314, 201711016295, 201711015676, 201711015673-74, 201711015715-16, 201711016204, 201707028880, 201711015718, 201707028888, topotypes, 17 specimens, 47.0–65.3 mm SL; China: Hunan Province: Nanxian County: Dongting Lake; 29°2'29"N, 112°18'22"E; collected by C.T An, November 2017; IHB 2017056850-60, 2017056862, 12 specimens, 33.3–42.7 mm SL; China: Jiangxi Province: Nanchang City: Gan-Jiang, an effluent of Poyang Lake; 28°32'12"N, 115°49'24"E; collected by D.M. Guo, November 2019.

Diagnosis. Leptobotia citrauratea shares with *L. micra* and *L. brachycephala* the unique presence of a row of orange spots or an orange stripe along the dorsal mid-line of the body, extending from the nape to the caudal-fin base. It differs from *L. micra* and *L. brachycephala* by having a deeply forked (vs. emarginate) caudal fin (length of median rays 1.7–2.3 times in length of upper lobe vs. 1.3–1.4 in *L. micra* and 1.2–1.5 in *L. brachycephala*), pelvic fin inserted slightly posterior or inferior (vs. slightly anterior in *L. brachycephala*) to the dorsal-fin origin, a longer head (22.5–26.8% SL vs. 18.4–22.8% SL in *L. brachycephala*) and a shorter predorsal distance (51.4–56.3% SL vs. 58.1–59% SL in *L. micra*) (Table 2, Fig. 2).

Description. Morphometric data for specimens examined in Tables 1, 2. See Fig. 1 for lateral and dorsal view of body. Body slender, strongly compressed laterally, with maximum depth at dorsal-fin origin. Predorsal body profile slightly convex. Ventral profile of head slightly concave or straight; ventral profile of body almost straight or slightly concave from pectoral-fin insertion to anal-fin origin and slightly convex from anal-fin origin to caudal-fin base. Lateral line nearly complete, extending along midlateral of body. Cheek and trunk covered with minute scales.

Head short, compressed laterally, length greater than maximum body depth. Snout slightly concave in lateral view, slightly shorter than postorbital head. Eye small, dorsolateral, in upper half of head; diameter less than interorbital space. Mouth inferior, with opening laterally extended to vertical through anterior margin of nostril. Buttonlike fleshy protrusion in gular region absent. Two rostral barbels at tip of snout. Maxillary barbel in corner of mouth, reaching beyond vertical through posterior margin of nostrils, not or just approaching to level of anterior margin of eye. Simple suborbital spine ventral to anterior margin of eye, reaching posterior margin of eye.

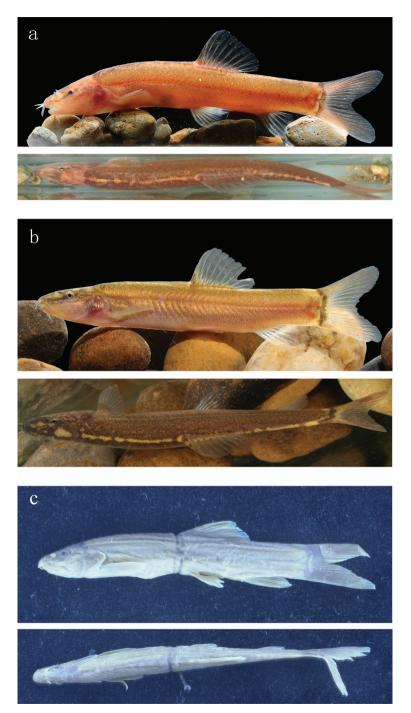


Figure 1. Lateral (upper) and dorsal (lower) view of *L. citrauratea* for freshly-caught specimens **a** IHB 2017100265, topotype, 53.0 mm SL, China, Hunan Province, Yiyang City, Nanxian County, Dongting Lake **b** IHB 2017056850, 38.1 mm SL, China, Jiangxi Province, Nanchang City, Gan-Jiang **c** AMNH 8402, holotype, 50 mm SL, China, Hunan Province, Dungting (presently Dongting) Lake (photos by Department of Ichthyology, American Museum of Natural History).

		L. citra	uratea		L. ela	ongata	L. bra	chycephala	sp. nov.
	Dongting L	ake (n = 17)	Poyang La	Poyang Lake (n = 12)		Chang-Jiang (n = 22)		Paratype	s (n = 21)
	Range N	fean±SD	Range M	lean±SD	Range Mean±SD			Range	Mean±SD
SL (mm)	47.0-65.3	52.9±4.2	33.3-42.7	37.5±2.6	97.8-272.0	$180.2{\pm}52.1$	63.9	43.7-66.8	56.1±5.7
Morphometric data									
% of SL									
Body depth	14.8-18.8	16.7 ± 1.4	15.7-19.2	17.2 ± 1.0	17.3-23.7	20.0 ± 1.7	12.9	11.1-15.8	$12.8 {\pm} 1.1$
Body width at dorsal origin	8.7-12.5	10.5 ± 1.1	9.7-12.4	$11.4 {\pm} 0.8$	8.3-14.9	11.4 ± 1.5	7.6	6.3–9.7	8.3±0.9
Head length	22.5-25.6	$24.2 {\pm} 0.8$	23.8-26.8	25.4 ± 0.8	24.4-31.4	$27.8 {\pm} 1.8$	19.9	18.4-22.8	20.6 ± 1.1
Dorsal-fin length	14.9-18.0	16.9±0.9	13.7-16.8	$14.9 {\pm} 1.0$	16.3-22.0	18.7±1.6	10.6	9.0-11.6	10.3 ± 0.7
Pectoral-fin length	14.8-21.1	17.0 ± 2.0	13.8-17.5	15.6 ± 1.0	14.4-18.2	$16.8 {\pm} 0.9$	12.8	9.6-14.2	11.9 ± 1.1
Pelvic-fin length	12.7-16.9	13.7±1.1	12.1-14.3	13.3±0.6	13.3-16.3	14.9 ± 0.7	10.2	9.1-12.6	10.6 ± 0.9
Anal-fin length	14.7-17.7	16.3±0.8	13.0-15.9	14.2±0.9	15.8-20.2	18.0 ± 1.2	9.3	8.1-11.4	$10.0 {\pm} 0.8$
Upper caudal-lobe length	23.6-26.9	25.5±0.9	23.6-26.7	$25.1 {\pm} 1.0$	23.1-30.0	25.9 ± 2.1	18.4	15.2-20.8	17.9±1.5
Median caudal-ray length	11.5-13.7	12.4±0.6	13.2-15.0	14.2±0.5	9.6-13.1	10.7 ± 1.0	13.0	11.5-15.0	13.0±0.9
Caudal-peduncle length	12.1-15.8	13.9±0.9	12.5-14.2	13.3±0.5	13.8-17.1	15.5±0.9	17.3	14.6-20.0	17.7±1.6
Caudal-peduncle depth	10.4-12.9	$11.6 {\pm} 0.7$	11.3-13.5	12.3±0.7	10.9-13.8	12.1±0.7	11.5	10.3-14.4	11.7 ± 1.1
Caudal-peduncle width	2.1-3.9	$2.9 {\pm} 0.7$	3.2-4.2	3.7 ± 0.3	2.6-4.8	3.6 ± 0.6	2.8	1.7-4.3	3.1 ± 0.8
Predorsal length	51.4-56.3	54.0 ± 1.2	52.8-55.3	53.9±0.7	54.5-58.8	56.5±1.2	51.7	49.9–54.7	52.7±1.6
Prepectoral length	21.7-26.8	23.7±1.1	23.4-25.5	24.5±0.6	25.1-31.6	28.3±1.8	19.7	18.9-23.4	20.5 ± 1.1
Prepelvic length	53.2-59.0	56.5±1.7	52.3-56.0	54.4±1.2	56.0-61.5	57.8±1.6	49.2	47.9-53.1	50.9±1.5
Preanal length	76.7-81.4	79.0±1.3	75.0-81.3	78.1±1.6	75.9–79.8	77.4±1.1	74.6	70.2-76.9	73.8±1.6
Vent to anal distance	6.8-10.3	8.9±0.9	6.7-8.3	7.5±0.6	6.1-10.1	8.4±1.1	10.4	7.4-10.7	9.3±0.9
Pelvic to anal distance	19.9-25.7	22.9±1.8	20.2-23.6	21.4±1.1	16.5-23.0	19.3±1.5	24.4	20.3-24.6	22.0±1.3
% of HL									
Head depth at nape	56.4-63.1	59.3±1.6	55.0-62.8	58.8±2.0	53.6-64.2	57.4±2.8	51.8	49.0-56.8	52.8±2.6
Head depth at eye	42.3-48.4	45.8±2.1	43.6-49.7	47.0±1.8	40.5-48.5	43.7±2.1	38.6	37.7-45.8	41.8±2.7
Snout length	35.4-43.9	39.6±2.3	35.1-42.5	38.4±2.0	35.7-42.6	38.4±2.1	36.2	35.6-41.8	38.2±2.3
Postorbital head length	48.5-54.8	51.6-2.0	50.8-55.2	52.5±1.6	54.5-61.9	57.8±1.9	55.2	52.4-60.0	56.2-2.6
Eye diameter	9.2-12.1	10.3±1.0	9.3-10.8	10.0±0.5	4.2-8.4	6.0 ± 1.1	11.4	9.1-12.2	10.4 ± 0.9
Interorbital width	14.5-22.8	17.3±2.4	16.1–19.6	17.7±1.1	12.3-17.7	14.8 ± 1.5	17.2	11.7-17.8	15.1±2.1

Table 1. Morphometric measurements for three species of *Leptobotia*: *L. citrauratea*, *L. elongata* and *L. brachycephala*.

Table 2. Major diagnostic characters amongst three species with a continuous or discontinuous orange line along the dorsal mid-line of the back. Data utilised for *L. micra* are from Bohlen and Šlechtov (2017).

Character	L. brachycephala sp.nov. (n = 22)	L. citrauratea (n = 29)	<i>L. micra</i> (n = 5)		
Colour on the back of body	A continuous or discontinuous	A row of rounded orange spots	A row of rounded orange spots		
	orange line along dorsal mid-line	along the dorsal mid-line	along the dorsal mid-line		
Dorsal-fin origin	Slightly posterior to pelvic-fin	Slightly anterior to or superior to	Slightly posterior to or superior to		
	insertion	pelvic-fin insertion	pelvic-fin insertion		
Caudal-fin shape	Emarginate with rounded lobes;	Strongly forked, with broadly	Moderately forked, with broadly		
	median rays 1.2-1.5 times as long	pointed lobes; median rays	pointed lobes; median rays		
	as upper lobe.	1.7–2.3 times as long as upper	1.3–1.4 times as long as upper		
		lobe.	lobe.		
Predorsal length	49.9–54.7	51.4-56.3	58.1-59.0		
Body depth (% SL)	11.1–15.8	14.8–19.2	15.3–18.3		
Head length (% SL)	18.4–22.8	22.5-26.8	23.6–25.9		
Upper caudal-lobe length (% SL)	15.2-20.8	23.6-26.9	18.7–23.9		
Caudal-peduncle length (% SL)	14.6-20.0	12.1–15.8	11.8–13.5		
Dorsal-fin length (% SL)	9.0–11.6	13.7–18.0	11.0–14.6		
Anal-fin length (% SL)	8.1–11.4	13.0-17.7	15.3–16.8		

Fin rays flexible. Dorsal fin with 4 unbranched and 8 branched rays; distal margin slightly concave; origin slightly anterior to or superior to pelvic-fin insertion and closer to caudal-fin base than to snout tip. Pectoral fin with 1 unbranched and 10–11

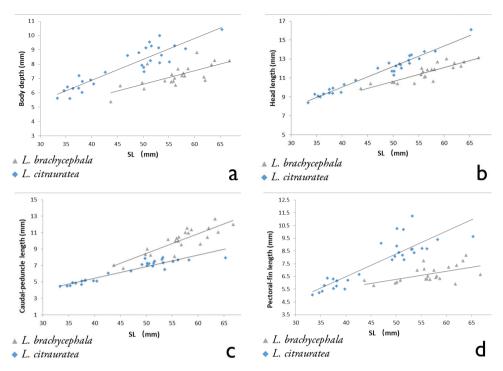


Figure 2. Relationship between body depth and SL (**a**), between head length and SL (**b**), between caudal-peduncle length and SL (**c**) and between pectoral-fin length and SL (**d**) for two closely-related species: *L. brachycephala* (gray triangle) and *L. citrauratea* (light blue diamond).

branched rays, tip of depressed fin extending about midway between pectoral-fin and pelvic-fin insertion. Pelvic fin with 1 unbranched and 7 branched rays, reaching about half of distance between pelvic-fin insertion and anal-fin origin and just reaching anus. Anus closer to anal-fin insertion than pelvic-fin insertion. Anal fin with 3 unbranched and 5 branched rays, tip of depressed fin not extending to caudal-fin base; distal margin slightly concave. Caudal fin strongly forked, median fin rays 1.7–2.3 times as long as lobes; upper and lower lobes broadly pointed and almost equal in length and shape.

Colouration. In freshly-collected specimens, ground colour of head and body yellowish-brown or orange; lateral head and flank faintly peppered with dark grey flecks. Dorsal side of head and body dark with some rounded light orange spots usually fused to form an orange stripe extending along mid-line of dorsum from nape to caudal-fin base. Anterior to orange spots or light stripe, an orangish stripe present between eye and nape. Faint dark grey stripe extending from snout tip to anterior margin of eye. Grey bar, similar in width to eye diameter, present on caudal-fin base. In some specimens, caudal fin hyaline, in others with dark grey stripes. Single row of faint dark grey stripes present in dorsal fin.

In specimens preserved in formalin, ground colour slightly faded, not presenting vivid yellowish-brown or orange, but becoming whitish-grey and peppered with dark flecks. Dorsum and head darkened. Orange spots along mid-line of dorsum white.

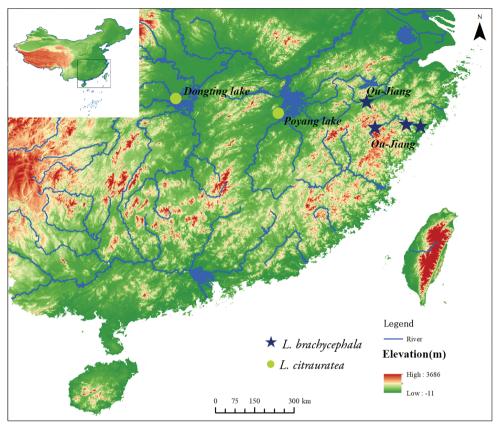


Figure 3. Collection localities of *L. brachycephala* (dark blue star) and *L. citrauratea* (light green dot).

Dorsal, pectoral, pelvic and anal fins greyish-yellow at base with white distal margins. Caudal fin dusky.

Geographical distribution and habitat. Leptobotia citrauratea is known from Dongting Lake in Hunan Province and the Gan-Jiang, discharging into Poyang Lake, in Jiangxi Province, southern China (Fig. 3). The specimens here described were collected in deep and slow-running water with mixed substrate. Syntopic fish species included Saurogobio dabryi Bleeker, 1871, Parabotia banarescui (Nalbant, 1965) and Hemibarbus maculatus Bleeker, 1871.

Leptobotia brachycephala sp. nov. http://zoobank.org/4A74D0D5-5A3B-4458-8227-B67A591A4143 (Fig. 4a, b)

Holotype. IHB 201909037510, 63.9 mm SL; China: Zhejiang Province: Qingtian County: a stream discharging into the Ou-Jiang; 28°10'20"N, 120°12'51"E; collected by E Zhang and D.M. Guo, 8 October 2018.

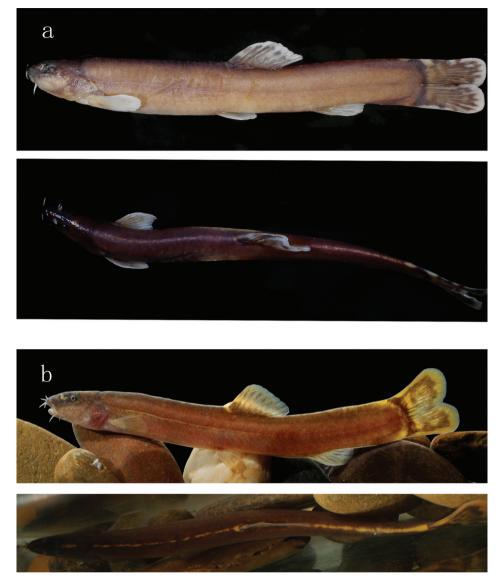


Figure 4. Lateral (upper) and dorsal (lower) view of body for *L. brachycephala*: **a** IHB 201909037510, holotype, 63.9 mm SL, China, Zhejiang Province, Lishui City, Qingtian County, Ou-Jiang, kept in formalin preservative after capture **b** IHB 2017056867, paratype, 62.5 mm SL, freshly collected from Qu-Jiang at Kecheng District, Quzhou City, Zhejiang Province.

Paratypes. IHB 2017056858, 2017056869-80, 13 specimens, 54.2–66.8 mm SL; China: Zhejiang Province: Quzhou City: a stream flowing into the Qu-Jiang; 28°57′6″N, 118°51′15″ E; collected by D.M. Guo, 15 December 2019.

Non-types examined. IHB 64VI410-15, 930138-39, 8 specimens, 43.7–60.5 mm SL; China: Zhejiang Province: Longquan City: a stream flowing into the Ou-Jiang; 28°4'12"N, 119°6'54"E; collected in 1964 and 1983.

Diagnosis. Leptobotia brachycephala, together with L. citrauratea and L. micra, is distinguished from all other congeneric species by the presence (vs. absence) of a row of orange dots or an orange stripe extending along the dorsal mid-line of the body from the nape to the caudal-fin base (Fig. 4a, b: lower). It differs from L. citrauratea and L. micra in having an emarginate (vs. forked) caudal fin with two rounded (vs. broadly pointed) lobes (Figs 1a–c, 4a, b: upper), a shorter head (18.4–22.8% SL vs. 22.5–26.8% SL in L. citrauratea and 23.6–25.9% SL in L. micra), a slender caudal peduncle (14.6–20.0% SL vs. 12.1–15.8% SL in L. citrauratea and 11.8–13.5% SL in L. micra), a shorter dorsal fin (9.0–11.6% SL vs. 13.7–18% SL in L. citrauratea and 11.0–4.6% SL in L. micra) and a shorter anal fin (8.1–11.4% SL vs. 13.0–17.7% SL in L. citrauratea and 15.3–16.8% SL in L. micra) (Table 2, Fig. 2).

Description. Morphometric data given in Tables 1, 2. See Fig. 4 for lateral and dorsal view of body. Body slender, strongly compressed laterally, with greatest depth at dorsal-fin origin. Dorsal profile of head rising progressively from tip of snout to nape, from there to caudal-fin base nearly straight. Ventral profile of head slightly concave; ventral profile of body almost straight or slightly concave. Lateral line nearly complete, extending along mid-lateral body to terminate in median caudal-fin rays. Cheek and trunk covered with some minute scales.

Head short, compressed laterally, longer than maximum body depth. Snout slightly obtuse in lateral view, slightly shorter than postorbital head. Eye small, dorsolateral, in upper half of head; diameter less than interorbital width. Mouth inferior, with opening laterally extended to vertical through anterior margin of nostril. Button-like structures in gular region absent; no median incisions in lower lip. Two rostral barbels at tip of snout. Maxillary barbel in corner of mouth, not reaching to level of anterior margin of eye. Simple suborbital spine ventral to anterior margin of eye, not or just reaching posterior margin of eye.

Fin rays flexible. Dorsal fin with 4 unbranched and 8 branched rays; distal margin slightly concave; origin slightly posterior to pelvic-fin insertion and closer to caudal-fin base than to tip of snout. Pectoral fin with 1 unbranched and 10–11 branched rays, not extending to midway from pectoral-fin to pelvic-fin insertion. Pelvic fin with 1 unbranched and 7 branched rays, not extending to halfway to anal-fin origin or not reaching anus; vent closer to anal-fin origin than to pelvic-fin insertion. Anal fin with 3 unbranched and 5 branched rays, not reaching caudal-fin base; distal margin slightly concave; origin closer to pelvic-fin insertion than to caudal-fin base. Caudal fin emarginate or shallowly forked, length of median fin rays 1.3–1.5 times in length of upper lobe; caudal-fin lobes rounded; upper and lower ones almost equal in length and shape.

Colouration. In freshly-caught specimens, ground colour of head and body brownish-yellow; darker in upper half of head, but lighter in lower half of head and ventral side of body. A continuous or discontinuous orange stripe along mid-line of dorsum from nape to caudal-fin base, becoming more conspicuous towards caudal-fin base. Anterior to orange stripe, a short orange stripe present between eye and anterior margin of nape. A dark grey stripe on basal portion of dorsal fin and one stripe on dorsal fin. A dark grey band at caudal-fin base. Some irregular black stripes on caudal



Figure 5. Type locality of *L. brachycephala*: fast-flowing clear water with mixed substrate including pebbles, gravels and boulders.

fin with hyaline distal edge. Distinct stripes absent from other fins. Specimens stored in formalin with ground colour of head and body pale brown. Discontinuous or continuous white line along dorsal mid-line of body also faded.

Geographical distribution and habitat. *Leptobotia brachycephala* is known only from the Ou-Jiang and Qu-Jiang, two coastal rivers of southern Zhejiang Province, China (Fig. 3). Type specimens were caught in fast-flowing clear water with mixed substrate including pebbles, gravels and boulders (Fig. 5). Syntopic species included Sarcocheilichthys parvus Nichols, 1930, *Acrossocheilus wenchowensis* Wang, 1935, *Cobitis sinensis* Sauvage & Dabry de Thiersant, 1874 and *Rhinogobius giurinus* (Rutter, 1897).

Explanation of name. The specific epithet is a Latin version of the Greek words $\beta \varrho \alpha \chi \dot{\upsilon} \varsigma$ (short) and $\varkappa \epsilon \varphi \alpha \lambda \dot{\alpha}$ (head), with reference to the short head; to be treated as a noun in apposition.

Genetic comparisons. A total of 50 unique haplotypes were detected amongst the 103 cyt b sequences of species of *Leptobotia* (Table 3). The fragment contained 784 conserved sites, 276 variable sites, 233 parsimony informative sites and 43 singleton sites. The average frequency of four nucleotides of *L. citrauratea* was A = 27.8%, T = 27.8%, C = 30.3% and G = 14.1%. The intraspecific genetic distance, calculated for sampled species of *Leptobotia* with more than one haplotype, varied from 0.1% to 0.8%. *Leptobotia citrauratea* is separated from other congeneric species by high genetic divergences of 2.9% to 10.5%; its intraspecific genetic distance was 0.4%. The genetic

Species L. brachycephala L. brachycephala L. brachycephala L. brachycephala	201909034355	Sampling location Wenzhou, Zhejiang Prov.	0.1	Haplotype		
L. brachycephala L. brachycephala		weinenous Eneplang 1100	Ou-Jiang	H1	MT747394	This study
L. brachycephala	201909034354	Wenzhou, Zhejiang Prov.	Ou-Jiang	H1	MT747394	This study
	201909034353	Wenzhou, Zhejiang Prov.	Ou-Jiang	H1	MT747394	This study
v 1	201909034352	Wenzhou, Zhejiang Prov.	Ou-Jiang	H1	MT747394	This study
L. brachycephala	IHB2017056869	Quzhou, Zhejiang Prov.	Qu-Jiang	H1	MT747394	This study
L. brachycephala	IHB2017056870	Quzhou, Zhejiang Prov.	Qu-Jiang	H1	MT747394	This study
L. brachycephala	IHB2017056871	Quzhou, Zhejiang Prov.	Qu-Jiang	H1	MT747394	This study
L. brachycephala	IHB20181010544	Qingtian, Zhejiang Prov.	Ou-Jiang	H1	MT747394	This stud
L. brachycephala	201909034349	Wenzhou, Zhejiang Prov.	Ou-Jiang	H2	MT747395	This stud
L. brachycephala	IHB20181010537	Qingtian, Zhejiang Prov.	Ou-Jiang	H3	MT747396	This stud
L.tientainensis	IHB2017056861	Jingdezhen, Jiangxi Prov.	Rao-He	H1	MT747348	This stud
L.tientainensis	IHB2017056836	Jingdezhen, Jiangxi Prov.	Rao-He	H1	MT747348	This stud
L.tientainensis	IHB2017056835	Jingdezhen, Jiangxi Prov.	Rao-He	H1	MT747348	This stud
L.tientainensis	IHB2017056834	Jingdezhen, Jiangxi Prov.	Rao-He	H1	MT747348	This stud
L.tientainensis	IHB2017056833	Jingdezhen, Jiangxi Prov.	Rao-He	H1	MT747348	This stud
L.tientainensis	201909034356	Linhai, Zhejiang Prov.	Ling-Jiang	H1	MT747348	This stud
L.tchangi	IHB2018099882	Shaoxing, Zhejiang Prov.	Cao'e-Jiang	H1	MT747349	This stud
L.tchangi	IHB2018099861	Shaoxing, Zhejiang Prov.	Cao'e-Jiang	H1	MT747349	This stud
L.tchangi	201909034361	Shaoxing, Zhejiang Prov.	Cao'e-Jiang	H1	MT747349	This stud
L.tchangi	IHB2018099848	Quzhou, Zhejiang Prov.	Qu-Jiang	H2	MT747350	This stud
L.tchangi	IHB201904029046	Hangzhou, Zhejiang Prov.	Qiantang-Jiang	H2	MT747350	This stud
L.tchangi	IHB201904029045	Hangzhou, Zhejiang Prov.	Qiantang-Jiang	H2	MT747350	This stud
L.tchangi	201904028852	Hangzhou, Zhejiang Prov.	Qiantang-Jiang	H2	MT747350	This stud
L.tchangi	IHB2018099847	Quzhou, Zhejiang Prov.	Qu-Jiang	H3	MT747351	This stud
L.tchangi	IHB2018099846	Quzhou, Zhejiang Prov.	Qu-Jiang	H4	MT747352	This stud
L.tchangi	IHB2018099845	Quzhou, Zhejiang Prov.	Qu-Jiang	H4	MT747352	This stud
L.tchangi	IHB2018099844	Quzhou, Zhejiang Prov.	Qu-Jiang	H5	MT747353	This stud
L.tchangi	IHB201904029047	Hangzhou, Zhejiang Prov.	Qiantang-Jiang	H6	MT747354	This stud
L.tchangi	IHB201904029044	Hangzhou, Zhejiang Prov.	Qiantang-Jiang	H6	MT747354	This stud
L.taeniops	IHB2017056865	Nanchang, Jiangxi Prov.	Gan-Jiang	H1	MT747355	This stud
L.taeniops	IHB2017056864	Nanchang, Jiangxi Prov.	Gan-Jiang	H2	MT747356	This stud
L.taeniops	201711015714	Yiyang, Hunan Prov.	Zi-Shui	H2	MT747356	This stud
L.taeniops	201711015711	Yiyang, Hunan Prov.	Zi-Shui	H2	MT747356	This stud
L.taeniops	201711015710	Yiyang, Hunan Prov.	Zi-Shui	H2	MT747356	This stud
L.taeniops	2017101867	Yuanjiang, Hunan Prov.	Zi-Shui	H2	MT747356	This stud
L.taeniops	2017101866	Yuanjiang, Hunan Prov.	Zi-Shui	H2	MT747356	This stud
L.taeniops L.taeniops	201711010290	Yiyang, Hunan Prov.	Zi-Shui	H2	MT747356	This stud
L.taeniops	201711010026	Yiyang, Hunan Prov.	Zi-Shui	H2	MT747356	This stud
L.taeniops	IHB2017056863	Nanchang, Jiangxi Prov.	Gan-Jiang	H3	MT747357	This stud
L.taeniops	201711015671	Yiyang, Hunan Prov.	Zi-Shui	H3	MT747357	This stud
L.taeniops	201801016175	Yiyang, Hunan Prov.	Zi-Shui	H4	MT747358	This stud
L.taeniops	2017101865	Yuanjiang, Hunan Prov.	Zi-Shui	H5	MT747359	This stud
L. rubrilabris	201904028870	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H1	MT747360	This stud
L. rubrilabris	201904028867	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H2	MT747361	This stud
L. rubrilabris	201904028858	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H2	MT747361	This stud
L. rubrilabris	201904028866	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H3	MT747362	This stud
L. rubrilabris L. rubrilabris	201904028865	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H4	MT747363	This stuc
L. rubrilabris L. rubrilabris	201904028863	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H5	MT747364	This stud
L. ruortuoris L.punctata	201909037450	Baise, Guangxi Prov.	Zhu-Jiang	H1	MT747365	This stud
punctata L.punctata	018099887	Liuzhou, Guangxi Prov.	Zhu-Jiang Zhu-Jiang	H1	MT747365	This stud
_		ě				
L.punctata I. punctata	201909037448	Baise, Guangxi Prov. Baise, Guangxi Prov.	Zhu-Jiang Zhu-Jiang	H2 H2	MT747366 MT747366	This stud This stud
L.punctata L.punctata	201909037447	Baise, Guangxi Prov. Baise, Guangxi Prov	Zhu-Jiang Zhu-Jiang	H2 H3	MT747366 MT747367	This stuc This stuc
L.punctata I. punctata	201909037446	Baise, Guangxi Prov. Baise, Guangxi Prov.	Zhu-Jiang Zhu-Jiang	H3 H4	MT747367 MT747368	This stud This stud
L.punctata I. punctata	201909037445	Baise, Guangxi Prov.	Zhu-Jiang Zhu Jiang	H4	MT747368 MT747368	This stud
L.punctata I. punctata	018099886	Liuzhou, Guangxi Prov.	Zhu-Jiang Zhu Jiang	H4	MT747368 MT747369	This stud
L.punctata	018099885 IHB2018099886	Liuzhou, Guangxi Prov. Liuzhou, Guangxi Prov.	Zhu-Jiang Zhu-Jiang	H5 H1	MT747369 MT747370	This stud This stud

Table 3. Species included in this analysis with specimen voucher, sampling location and basin, haplotype and GenBank accession number; the haplotype with * means downloaded from GenBank.

Species	Specimen voucher	Sampling location	Basin	Haplotype	GenBank no.	Source
L. pellegrini	IHB2018099885	Liuzhou, Guangxi Prov.	Zhu-Jiang	H2	MT747371	This study
L. pellegrini	IHB2018099884	Liuzhou, Guangxi Prov.	Zhu-Jiang	H2	MT747371	This study
L. pellegrini	IHB2018099840	Liuzhou, Guangxi Prov.	Zhu-Jiang	H2	MT747371	This study
L. pellegrini	IHB2018099839	Liuzhou, Guangxi Prov.	Zhu-Jiang	H2	MT747371	This study
L. pellegrini	201909034360	Liuzhou, Guangxi Prov.	Zhu-Jiang	H2	MT747371	This study
L. pellegrini	201909034359	Liuzhou, Guangxi Prov.	Zhu-Jiang	H2	MT747371	This study
L. pellegrini	201909034358	Liuzhou, Guangxi Prov.	Zhu-Jiang	H2	MT747371	This study
L. microphthalma	201904028856	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H1	MT747372	This study
L. microphthalma	201904028850	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H1	MT747372	This study
L. microphthalma	201904028855	Neijiang, Sichuan Prov.	Upper Chang-Jiang	H2	MT747373	This study
L. microphthalma	IHB2016105308	Leshan, Sichuan Prov.	Min-Jiang	H2	MT747373	This study
L. microphthalma	IHB2016105306	Leshan, Sichuan Prov.	Min-Jiang	H2	MT747373	This study
L. microphthalma	IHB2016105311	Leshan, Sichuan Prov.	Min-Jiang	H3	MT747374	This study
L. microphthalma	IHB2016105307	Leshan, Sichuan Prov.	Min-Jiang	H3	MT747374	This study
L. microphthalma	IHB2016105310	Leshan, Sichuan Prov.	Min-Jiang	H4	MT747375	This study
L. microphthalma	IHB2016105309	Leshan, Sichuan Prov	Min-Jiang	H5	MT747376	This study
L. hengyangensis	2017042831	Hengyang, Hunan Prov	Xiang-Jiang	H1	MT747377	This study
L. hengyangensis	2017042828	Hengyang, Hunan Prov	Xiang-Jiang	H2	MT747378	This study
L. guilinensis	2015040820	Guilin, Guangxi Prov	Zhu-Jiang	H1	MT747379	This study This study
L.guilinensis	2015040820	Guilin, Guangxi Prov	Zhu-Jiang Zhu-Jiang	H1	MT747379	This study This study
L.guilinensis L.guilinensis	2015040812	Guilin, Guangxi Prov	Zhu-Jiang Zhu-Jiang	H1	MT747379	This study This study
L.guilinensis	2015040811	0	Zhu-Jiang Zhu-Jiang	H1	MT747379	This study This study
L.guilinensis L.guilinensis	2015040805	Guilin, Guangxi Prov	-	H1	MT747379	,
0		Guilin, Guangxi Prov	Zhu-Jiang	H1 H2		This study
L.guilinensis L.guilinensis	2015040818	Guilin, Guangxi Prov	Zhu-Jiang Zhu Jiang	H2 H2	MT747380	This study This study
0	2015040815	Guilin, Guangxi Prov	Zhu-Jiang Zhu Jiang	H2 H3	MT747380	,
L.guilinensis	2015040813	Guilin, Guangxi Prov	Zhu-Jiang		MT747381	This study
L.guilinensis	2015040808	Guilin, Guangxi Prov	Zhu-Jiang	H4	MT747382	This study
L. elongata	IHB2018059216	Leshan, Sichuan Prov	Min-Jiang	H1	MT747383	This study
L. elongata	IHB2018059215	Leshan, Sichuan Prov	Min-Jiang	H2	MT747384	This study
L. elongata	SCULE007	Unknown		H2*	NC018764	GenBank
L. elongata	Unknown	Luzhou, Sichuan Prov	Upper Chang-Jiang	H2*	AY625715	GenBank
L. elongata	IHB2018059214	Leshan, Sichuan Prov	Min-Jiang	H3	MT747385	This study
L. elongata	201904028849	Leshan, Sichuan Prov	Min-Jiang	H4	MT747386	This study
L. elongata	IAPG A214	Unknown		H5*	AY887779	GenBank
L. elongata	Unknown	Unknown		H6*	KY307845	GenBank
L. citrauratea	IHB2017056860	Nanchang, Jiangxi Prov	Gan-Jiang	H1	MT747387	This study
L. citrauratea	IHB2017056859	Nanchang, Jiangxi Prov	Gan-Jiang	H2	MT747388	This study
L. citrauratea	IHB2017056858	Nanchang, Jiangxi Prov	Gan-Jiang	H3	MT747389	This study
L. citrauratea	201711016295	Nanxian, Hunan Prov	Donngting Lake	H3	MT747389	This study
L. citrauratea	201711015674	Nanxian, Hunan Prov	Donngting Lake	H3	MT747389	This study
L. citrauratea	IHB2017056857	Nanchang, Jiangxi Prov	Gan-Jiang	H4	MT747390	This study
L. citrauratea	201711016297	Nanxian, Hunan Prov	Donngting Lake	H5	MT747391	This study
L. citrauratea	201711016296	Nanxian, Hunan Prov	Donngting Lake	H6	MT747392	This study
L. citrauratea	201711015675	Nanxian, Hunan Prov	Donngting Lake	H6	MT747392	This study
L. citrauratea	201711015716	Nanxian, Hunan Prov	Donngting Lake	H7	MT747393	This study
L. posterodorsalis	Unknown	Xiaoxi, Hunan Prov	Yuan-Jiang	H1*	MH922928	GenBank
P. fasciata	Unknown	Unknown		$H1^*$	AY625710	GenBank
P. lijiangensis	Unknown	Chenxi, Hunan Prov	Yuan-Jiang	H1*	AY625713	GenBank

distance of *L. brachycephala* versus congeneric species ranged from 2.9% to 10.6%; its intraspecific genetic distance was 0.1% (Table 4).

In the Bayesian 50% majority consensus tree, samples of *L. brachycephala* formed a well-supported (100% pp) lineage and so did those of both *L. citrauratea* and *L. elongata*. *L. citrauratea* was distantly allied to *L. elongata*, but robustly supported by 100% pp to be sister to *L. brachycephala* (Fig. 6).

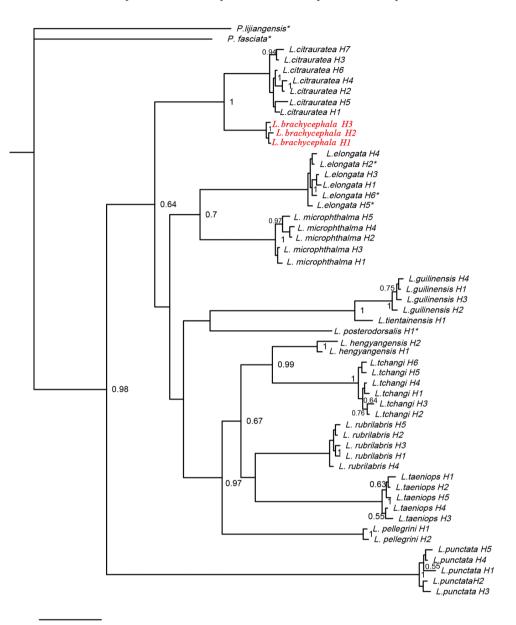
Species	Intraspecific	1	2	3	4	5	6	7	8	9	10	11	12
1 L. tientainensis	n/c												
2 L. tchangi	0.003	0.078											
3 L. taeniops	0.003	0.081	0.058										
4 L. rubrilabris	0.002	0.077	0.057	0.055									
5 L. punctata	0.004	0.106	0.104	0.111	0.096								
6 L. pellegrini	0.002	0.077	0.064	0.073	0.061	0.107							
7 L. microphthalma	0.004	0.067	0.069	0.067	0.061	0.092	0.077						
8 L. hengyangensis	0.008	0.069	0.041	0.057	0.047	0.098	0.059	0.059					
9 L. guilinensis	0.002	0.018	0.086	0.086	0.080	0.110	0.080	0.069	0.079				
10 L. elongata	0.002	0.073	0.071	0.073	0.066	0.096	0.067	0.053	0.067	0.073			
11 L. citrauratea	0.004	0.079	0.075	0.069	0.062	0.105	0.076	0.061	0.069	0.080	0.071		
12 L. posterodorsalis	n/c	0.066	0.069	0.074	0.068	0.104	0.069	0.070	0.066	0.069	0.068	0.065	
13 L. brachycephala	0.001	0.076	0.072	0.071	0.064	0.106	0.065	0.064	0.066	0.078	0.067	0.029	0.060

Table 4. Genetic distances of the cyt b gene computed by MEGA 7 amongst 13 analysed species of *Leptobotia*.

Table 5. Loadings of morphological traits on the first three principal components. Variables in bold indicate higher loading values.

Variable	PC1	PC2	PC3
Standard length	0.224	-0.150	-0.079
Body depth	0.164	0.155	0.060
Body width at dorsal origin	0.163	0.123	0.287
Head length	0.169	0.050	-0.002
Head depth at nape	0.165	0.173	0.108
Head depth at eye	0.148	0.150	0.112
Caudal-peduncle length	0.288	-0.445	-0.100
Caudal-peduncle depth	0.206	-0.138	-0.026
Caudal-peduncle width	0.169	-0.237	0.862
Dorsal-fin length	0.197	0.375	-0.025
Pectoral-fin length	0.187	0.241	-0.118
Pelvic-fin length	0.176	0.144	-0.124
Anal-fin length	0.199	0.381	0.014
Upper caudal-lobe length	0.162	0.259	-0.031
Predorsal length	0.218	-0.122	-0.082
Prepectoral length	0.181	0.029	-0.025
Prepelvic length	0.228	-0.039	-0.030
Preanal length	0.220	-0.075	-0.052
Vent to anal-fin origin	0.325	-0.242	-0.216
Pelvic-fin insertion to anal-fin origin	0.256	-0.130	-0.059
Snout length	0.186	0.076	0.072
Postorbital head length	0.170	-0.035	-0.087
Eye diameter	0.180	0.035	-0.023
Interorbital width	0.156	0.209	0.099
Median caudal-ray length	0.147	-0.168	-0.119
Cumulative variance (%)	64.6	19.6	7.4

Comparative morphometrics. In the Principal Component Analysis of specimens of *L. citrauratea* from Dongting Lake and the Gan-Jiang and *L. brachycephala* from the Ou-Jiang and Qu-Jiang, the first three components explained 91.60% of the total variance, of which 64.58%, 19.61% and 7.41% were explained, respectively by PC 1, PC 2 and PC 3 (Table 5). In the scatterplot of PC 2 and PC 3 loadings (Fig. 7), specimens of *L. citrauratea* and *L. brachycephala* constituted two distinct clusters separated on the



0.02

Figure 6. Bayesian Inference tree inferred from the cyt b gene for 13 analysed species of *Leptobotia*. Nodal numbers are posterior probability values greater than 50%

PC 2 axis. Six characters with main loading on this axis were caudal-peduncle length, anal-fin length, dorsal-fin length, upper caudal-lobe length, pectoral-fin length and vent to anal-fin distance. Except for the last character, all of them exhibited differences in the morphometric comparisons. Table 2 and Fig. 2 show the main morphological characters.

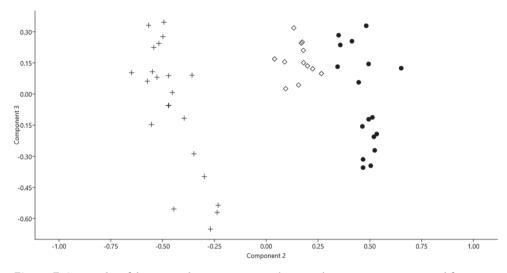


Figure 7. Scatter plot of the principal components II and principal components III, extracted from morphometric data of *L. brachycephala*: Ou-Jiang (plus symbol). *L. citrauratea*: Dongting Lake (black circle) and Gan-Jiang (white diamond).

Discussion

Nichols et al. (1925) described the colour of *L. citrauratea* as "purplish brown; yellowish below". This colouration is shared with freshly-caught specimens of this species from the Dongting Lake (type locality) and Poyang Lake systems (Fig. 1). Our examination of these topotypical specimens and a photograph of the holotype (AMNH 8402) (Fig. 1c) confirmed Bohlen and Šlechtová's (2016) observation that *L. citrauratea* has some round orange dots or an orange stripe along the dorsal mid-line of the body. There were no significant differences in morphometric measurements and meristic counts detected between specimens from the Dongting and Poyang Lake systems (Table 1).

Chen (1980) synonymised *L. citrauratea* with *L. elongata*, a species found in the mid-upper Chang-Jiang Basin, but without examination of their type specimens or even reference to topotypical specimens. This classification had been widely accepted by subsequent researchers until 2002 when Nalbant recognised *L. citrauratea* as valid. Morphological data, provided in this study, indicated that there were distinct variations between these two species. Topotypical specimens examined of *L. citrauratea* possessed a small-sized body of up to 70.0 mm SL, while the body size of *L. elongata* attained a length of 97.8 to 272.0 mm SL for available specimens caught from the upper Chang-Jiang Basin. The specimen of the species, caught by Fang (1936), reached up to 500 mm in total length. *Leptobotia citrauratea* has a series of small orange spots or an orange stripe along the dorsal mid-line of the body from the nape to the caudal-fin base, a yellowish-brown or orange ground colour of head and body and no black band crossing the dorsum (Fig. 1). This is contrast to *L. elongata* which, in light of its original account and our observation on specimens collected from the upper Chang-Jiang Basin, has a

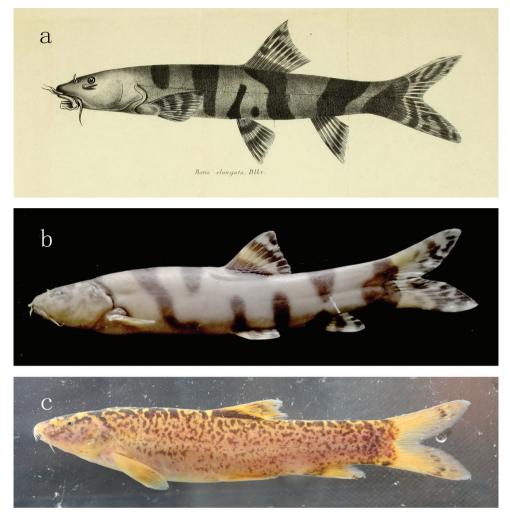


Figure 8. a a copy of Bleeker' (1870) illustration of *L. elongata* **b** lateral view of body for *L. elongata* in IHB 2018059214, 175.83 mm SL, China, Sichuan Province, Leshan City, upper Chang-Jiang Basin, kept in formalin preservative after capture **c** lateral view of body for *L. taeniops* in IHB 2017110267, 100.2 mm SL, freshly caught from Dongting Lake at Nanxian County, Yiyang City, Hunan Province, South China.

body colouration of many wide brown and transverse bands (Fig. 8a, b). Additionally, *L. citrauratea* differs from *L. elongata* in having larger eyes (diameter 9.2–12.1% HL vs. 4.2–8.4% HL; see Table 1) and pelvic fin not or just reaching (vs. exceeding) the anus. Our molecular analysis also showed that *L. citrauratea* had a 7.1% interspecific genetic distance with *L. elongata* (Table 4) and that these two species constituted two independent lineages distantly related in the phylogenetic tree, based on the cyt b gene (Fig. 6). It is thus concluded here that *L. citrauratea* is a species distinct from *L. elongata* and confined only to the mid-lower Chang-Jiang Basin (the Dongting and Poyang Lake Basins); *L. elongata* is actually an endemic species of the upper Chang-Jiang Basin.



Figure 9. Ventral view of the mouth of two species **a** *L. rubrilabris*, IHB 581-032, 150.2 mm SL, China, Chongqing City, Banan District, upper Chang-Jiang **b** *L. brachycephala*, IHB 2017056872, Paratype, 66.8 mm SL, China, Zhejiang Province, Quzhou City, Kecheng District, Qu-Jiang. FP = fleshy protrusion.

Nichols et al. (1925) recognised three species of *Botia* from the Dongting Lake system: one previously-described species as *Botia rubrilabris* Dabry de Thiersant, 1872 and two new species, *B. purpurea* and *B. citrauratea*. These three species were later referred to *Leptobotia* where *B. purpurea* were synonymised with *L. taeniops* (Sauvage, 1878) (Chen 1980; Kottelat 2004, 2012). The latest report on the distribution of *L. rubirilabris* (Dabry de Thiersant, 1872) in this Lake was Anonymous (1980) who caught a single specimen of 80.0 mm SL. This specimen, in light of their description, has a button-like fleshy protrusion in the gular area, a character diagnostic for *L. rubirilabris* within this genus (Chen 1980); thus, it is conspecific with this species. Recent field surveys, conducted by us from 2014 to 2018 in Dongting Lake, yielded no specimens of this species. Likely, it was extirpated in this Basin. *Leptobotia citrauratea* mainly differs from *L. rubirilabris*, caught from the upper Chang-Jiang Basin (its type locality), in having a shorter (vs. longer) suborbital spine just reaching (vs. far beyond) the posterior margin of the eye and no button-like fleshy protrusion in the gular area (vs. present) (Fig. 9).

The specific status of *L. brachycephala* was confirmed by its morphological and genetic distinction with closely-related congeneric species (Tables 2–4). The PCA results showed that specimens of this species from southern Zhejiang Province formed a cluster, distinct from the one formed by specimens of *L. citrauratea* from the Dongting and Poyang Lake systems (Fig. 7). Although *L. brachycephala* was robustly supported by 100% pp to be sister to *L. citrauratea*, their interspecific genetic distance was 2.9%.

The seventeen species currently included in *Leptobotia* can be subdivided into six groups, based on their body colourations. The first one is only composed of one species *L. taeniops* that has a unique body colouration of some irregular purplish-brown stripes in the shape of a worm on the flank, hence resulting in a marbled or vermiculated pattern (Fig. 8c). The second group, represented by *L. punctata* Li, Li & Chen, 2008, has a lot of irregularly-organised white spots on the flank, giving a reticulated pattern. The third group, including *L. pellegrini* Fang, 1936, *L. elongata*, *L. hengyangensis* Huang & Zhang, 1986, *L. tchangi* Fang, 1936 and *L. rubrilabris*, is characterised by having a body colouration of some broad brown-black blotches on the body or saddles on the

dorsum. The fourth group is formed by the following four species: *L. posterodorsalis*, *L. bellacauda* Bohlen & Šlechtová, 2016, *L. tientainensis* (Wu, 1930) and *L. microph-thalma* Fu & Ye, 1983, all of them having a plain brown body and no other colour formation. The fifth group has some rounded light orange spots extending along the mid-line of the dorsum from nape to caudal-fin base and three species are included in this group: *L. micra*, *L. citrauratea* and *L. brachycephala*. The last group has narrow bands on the body or mid-line of the back and three species can be referred to this group: *L. guilinensis* Chen, 1980, *L. orientalis* and *L. flavolineata*.

Comparative material

- Leptobotia elongata: IHB 3609, 58199, 64V2269-70, 4 specimens, 189.6–224.7 mm SL, upper Chang-Jiang Basin at Yichang City, Hubei Province; IHB 2018059214-16, 3 specimens, 170.0–176.5 mm SL, upper Chang-Jiang Basin at Leshan City, Sichuan Province; IHB 790197, 790198-99, 3 specimens, 87.8–167.4 mm SL, upper Chang-Jiang Basin at Luzhou City, Sichuan Province; IHB 790349, 1 specimens, 161.0 mm SL, upper Chang-Jiang Basin at Yibin City, Sichuan Province; IHB 201909035604, 1 specimens, 272.0 mm SL, upper Chang-Jiang Basin at Liangshan Yi Autonomous Prefecture, Sichuan Province; IHB 585410, 501244, 73V1466, 420457, 590452-54, 580451, 8 specimens, 116.8–236.7 mm SL, upper Chang-Jiang Basin at Chongqing City; IHB 201909035958, 201909035934, 2 specimens, 100.7–110.7 mm SL, upper Chang-Jiang Basin at Zhaotong City, Yunnan Province.
- *Leptobotia taeniops*: IHB 2017100254-59, 2017110267, 7 specimens, 43.6–112.9 mm SL, middle Chang-Jiang Basin at Yiyang City, Hunan Province; 201807020856, 201807028170, 2 specimens, 61.2–82.4 mm SL, middle Chang-Jiang Basin at Yueyang City, Hunan Province;
- *Leptobotia rubrilabris*: IHB 581-032, 2 specimens, 150.2 mm SL, Dongting Lake at Nanxian County, Yiyang City, Hunan Province, South China;
- *Leptobotia tientainensis*: IHB 74VI3347-50, 4 specimens, 70.5–91.2 mm SL, Ling-Jiang at Taizhou City, Zhejiang Province;
- *Leptobotia guilinensis*: IHB 2015040803, 1 specimen, 70.3 mm SL, Zhu-Jiang at Guilin City, Guangxi Province;
- *Leptobotia tchangi*: IHB 201904029044, 1 specimen, 80.4 mm SL, Qiantang-Jiang at Hangzhou City, Zhejiang Province;
- *Leptobotia pellegrini*: IHB 2018099839, 1 specimen, 103.2 mm SL, Zhu-Jiang at Liuzhou City, Guangxi Province;
- *Leptobotia hengyangensis*: IHB 2017042831, 2017042828, 2 specimens, 99.7–102.8 mm SL, Xiang-Jiang at Hengyang City, Hunan Province;
- *Leptobotia microphthalma*: IHB 2016105306, 1 specimen, 78.3 mm SL, upper Chang-Jiang Basin at Leshan City, Sichuan Province.
- Data for *L. bellacauda* and *L. micra* were taken from Bohlen and Šlechtová (2016), (2017).

Acknowledgements

Our sincere thanks go to Chang-Ting An, Xiao Chen, Wei-Han Shao, Zi-Tong Wang and Hao-Jun Chen for their kind assistance with collecting samples. In addition, we express our deepest gratitude to Liang Cao and Shu-Qing Deng for their help with laboratory analyses. This study was funded by two special funds of Biodiversity Survey, Monitoring and Assessment (2017HB2096001006 and 2019HB2096001006).

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RESEARCH ARTICLE



Resurrection of the Butterfly-winged Comber, Serranus papilionaceus Valenciennes, 1832 (Teleostei, Serranidae) and its phylogenetic position within genus Serranus

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Academic editor: N. Bogutskaya Received 12 November 2020 Accepted 9 January 2021 Published 12 February 202	l
http://zoobank.org/F678A836-A14F-4851-8ECD-926D8C0061B7	

Citation: Vella A, Vella N, Acosta-Díaz C (2021) Resurrection of the Butterfly-winged Comber, *Serranus papilionaceus* Valenciennes, 1832 (Teleostei, Serranidae) and its phylogenetic position within genus *Serranus*. ZooKeys 1017: 111– 126. https://doi.org/10.3897/zookeys.1017.60637

Abstract

The family Serranidae is represented by 92 genera and 579 valid species, with the genus *Serranus* Cuvier, 1816, containing 30 species. In this study, specimens of Butterfly-winged Comber, *Serranus papilionaceus* Valenciennes, 1832, were collected from the Canary Islands and compared morphologically and genetically to Painted Comber, *Serranus scriba* (Linnaeus, 1758), from the Mediterranean Sea. Morphological differences, especially in the colour banding pattern, were corroborated by genetic differences in mitochondrial (COI and ND2) and nuclear (Rhod and PTR) markers. The mitochondrial DNA markers revealed a high level of divergence and no shared haplotypes between the two species (interspecific divergence: COI 4.31%; ND2 8.68%), and a phylogenetic analysis showed that these two species are closely related sister species sharing common ancestry. This study is therefore offering to resurrect *S. papilionaceus* Valenciennes, 1832 as a valid species increasing the number of eastern Atlantic *Serranus* species to 11. This should direct new species-specific research, including its population conservation status assessment across its distribution.

Keywords

Butterfly-winged Comber, Canary Islands, Mediterranean Sea, Painted Comber, phylogeny, taxonomy

Introduction

The family Serranidae, which is composed of sea basses and groupers, has three subfamilies Anthiadinae, Epinepheline, and Serraninae, which are represented by 579 valid species in 72 genera (Parenti and Randall 2020). The genus *Serranus* (Perciformes, Serranidae) has 30 small reef-associated species (Iwamoto and Wirtz 2018; Horton et al. 2020), with the most recent additions to the genus *Serranus* being *S. aliceae* Carvalho Filho & Ferreira, 2013 from the Southwest Atlantic Ocean and *S. pulcher* Wirtz & Iwamoto, 2016, *S. drewesi* Iwamoto, 2018, and *S. inexpectatus* Wirtz & Iwamoto, 2018 from the Eastern Central Atlantic Ocean (Carvalho Filho and Ferreira 2013; Wirtz and Iwamoto 2016; Iwamoto and Wirtz 2018).

Most of the species belonging to this genus are recorded in the coastal regions of the Atlantic Ocean, with 14 species occurring in the Western Atlantic and 10 species within the Eastern Atlantic. In addition to these, another six species inhabit the Eastern Pacific Ocean while another two occur in the Indian Ocean (Iwamoto and Wirtz 2018). Both the Mediterranean Sea and the Canary Islands are known to host the same four *Serranus* species (Dooley et al. 1985; Bauchot 1987): the Blacktail Comber, *S. atricauda* Günther, 1874, which in the Mediterranean is restricted to the western basin; the Comber, *S. cabrilla* (Linnaeus, 1758); the Brown Comber, *S. hepatus* (Linnaeus, 1758); and the Painted Comber, *S. scriba* (Linnaeus, 1758). However, analysing photos of specimens commonly identified as *S. scriba* collected off the Canary Islands, we noticed distinct differences in colour pattern when compared to the specimens from the Mediterranean Sea, including Malta (Fig. 1), as was already observed by Iwamoto and Wirtz (2018). These notable differences led us to further investigate the species' identity using morphological and genetic analyses, while reviewing first descriptions and museum specimens to accurately identify the species in question.

Materials and methods

Morphological analyses of sampled specimens

Specimens were collected by local coastal fishermen during regular fishing activities in the Canary Islands using traps for fish (n = 12) and Malta during shoreline fishing (n = 15) between 2016 and 2017 (Figs 1, 2; Suppl. material 1: Table S1). The total length of each specimen was measured to the nearest millimeter using electronic callipers, and tissue samples were taken from each specimen. All the specimens collected from Malta and five specimens collected from the Canary Islands were morphologically examined following Heemstra and Randall (1993) and Zorica et al. (2010), where various body parts were measured to the nearest millimeter using electronic callipers and counts of the soft and hard rays in the dorsal, pectoral, pelvic, anal, and caudal fins were counted.

DNA and phylogenetic analyses

Total genomic DNA was extracted using GF-1 DNA Extraction Kit (Vivantis Technologies, Malaysia) and used as a template for amplifying two mitochondrial DNA (mtDNA) genes [cytochrome oxidase c subunit 1 gene (COI); and NADH dehydrogenase subunit 2 gene (ND2)], and two nuclear DNA (nDNA) genes [rhodopsin gene (Rhod); and si:ch211-105n9.1-like protein, hypothetical protein LOC564097 (PTR)]. The amplification protocols were carried out following literature in Table 1. The PCR products were sequenced using their respective forward and reverse primers through 3730XL Genetic Analyzer (Applied Biosystems, USA).

Sequences were trimmed and the complimentary sequences of each individual were assembled using Geneious R10 (Kearse et al. 2012). Sequences were manually checked for consistencies. The final sequences were deposited in GenBank under accession numbers: MW439283–MW439309 (COI); MW447416–MW447496 (ND2; Rhodo; PTR) (Suppl. material 1: Table S2)

The mtDNA haplotype diversity and nucleotide diversity indices for both species were calculated via Arlequin v. 3 (Excoffier and Lischer 2010), while the intraspecific and interspecific *p*-distance was measured using DnaSP (Rozas et al. 2017). Parsimony haplotype networks were constructed via TCS (Clement et al. 2000) to analyse the association between the various haplotypes identified during this study. For nDNA genes, Geneious R10 (Kearse et al. 2012) was used to identify single nucleotide polymorphism between the two species.

To evaluate the phylogenetic relationship of the resurrected taxon to that of other *Serranus* species, COI sequences were imported from BOLD (Ratnasingham and Hebert 2007) and GenBank (NCBI 2020). Further details of these sequences are included in Suppl. material 1: Table S3. All the COI sequences were aligned using ClustalW (Thompson et al. 1994) and the sequences were trimmed to 578 bp representing the smallest homologous sequence for this data set. The phylogenetic relationships between different *Serranus* species were evaluated through the construction of a phylogenetic tree using neighbour-joining analysis and the *p*-distance model (Collins et al. 2012; Srivathsan and Meier 2012; Collins and Cruickshank 2013). This analysis was conducted via MEGA v. 7 (Kumar et al. 2016) using 1000 bootstraps.

Gene	Primers	(5' to 3')	Reference
COI	FishF1	TCAACCAACCACAAAGACATTGGCAC	Ward et al. (2005)
	FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	
ND2	ND2-MetF	AAGCTYTTGGGCCCATACC	Vella et al. (2017)
	ND2-TrpR	AGCTTTGAAGGCTTTTGGTYT	
Rhod	193F	CNTATGAATAYCCTCAGTACTACC	Chen et al. (2003)
	1039R	TGCTTGTTCATGCAGATGTAGA	
PTR	Ptr_F458	AGAATGGATWACCAACACYTACG	Chenhong et al. (2007)
	Ptr_R1248	TAAGGCACAGGATTGAGATGCT	

Table I. A list of the primers and amplification protocols used to amplify the genes used in this study.

Historical material

The following museum specimens' photographs and describing manuscript diagrams were analysed carefully, comparing the historic image evidence, when available, with the photographs taken from specimens sampled for this study.

Descriptions of *S. scriba* and its possible synonyms were reviewed given the close resemblance between *S. scriba* and the species being resurrected. Relevant scientific descriptions evaluated for this purpose included: *Perca scriba* described by Linnaeus (1758: 292) and Linnaeus (1764: 86); *Perca marina* described by Brünnich (1768: 63); *Holocentrus fasciatus* described by Bloch (1790: 86); *Holocentrus marocannus* described by Bloch and Schneider (1801: 320); *Holocentrus argus* described by Spinola (1807: 372); *Serranus scriba* described by Cuvier and Valenciennes (1828: 214); and *Serranus papilionaceus* described by Valenciennes (1832: 471).

Holotypes

Serranus scriba originally described as *Perca scriba* Linnaeus 1758; NRM 442; holotype status follows Fernholm and Wheller (1983) and Parenti and Randall (2020) • *Holocentrus marocannus* Bloch and Schneider 1801; Morocco; ZMB5531 (left skin); a junior synonym to *Serranus scriba* as noted in Cuvier and Valenciennes (1828), Gunther (1859) and Parenti and Randall (2020).

Syntype

Serranus papilionaceus Valenciennes 1832; Gorée, Senegal, Atlantic Ocean; two specimens MNHN-IC-0000-7279.

Other material

Serranus scriba; Algeria; two specimens MNHN-IC-0000-7129 • *Serranus papilionaceus* (labelled as *Serranus scriba*); Dakar, Senegal; collected in 1896; MNHN-IC-1896-0389 • *Serranus papilionaceus* (labelled as *Serranus scriba*); Cap Blanc, Baie du Levrier, Mauritania; four specimens MNHN-IC-1999-1053.

Results

Based on the currently used literature and morphological keys (Bauchot 1987; Lloris and Rucabado 1998; Iwamoto and Wirtz 2018), all specimens collected and analysed during this study (Suppl. material 1: Table S1) fit the body shape description of *S. scriba sensu lato*; however, genetic data revealed divergence between the two forms. Based on the type specimens and descriptions evaluated in this study, we assigned the specimens collected from Malta to *S. scriba* in its traditional usage based on Cuvier and Valenciennes (1828). These specimens matched the museum specimens NRM 442 and MNHN-IC-0000-7129 (Suppl. material 1: Figs S1a, S2b). We assign the specimens collected from the Canary Islands to the resurrected taxon *S. papilionaceus*, as described by Valenciennes (1832) and matching the syntype specimens MNHN-IC-0000-7279 (Suppl. material 1: Fig. S2a) and museum specimens MNHN-IC-1896-0389 and MNHN-IC-1999-1053.

Morphology

Meristic counts and measures

Meristic counts (Table 2) indicated a general overlap between the counts of the two species investigated, with only the anal soft fin rays counts showing a difference (7 in *S. scriba* and 8 in *S. papilionaceus*). Though the meristic measures (Table 3) overlapped between the two species, the specimens investigated also showed a longer average dorsal fin length in *S. papilionaceus* than in *S. scriba* (46.3% TL \pm 1.1 and 41.4% TL \pm 1.4, respectively). Extended morphometric studies on *S. papilionaceus* covering a wider distribution and life-stages are required to corroborate the significance of these differences. Consequently, the most important identifying morphological characteristic feature between the two species would be their body colouration patterns described below.

Table 2. Meristic counts for *Serranus scriba*, its synonyms¹ (Parenti and Randall 2020) and *Serranus papilionaceus*. Values in brackets represent the mean and SD for counts that have a range. ² Questionable locality (Fernholm and Wheller 1983).

Species	References			Meristic counts	6		Locality
name	(sample size)	Dorsal	Pectoral	Ventral	Anal	Caudal	
Perca scriba	Linnaeus, 1758	10 / 15	13	1/5	3/7	15	America (Linnaeus, 1764) ²
Perca marina ¹	Brünnich, 1768	10 / 16	13	1/5	3/8	15	Mediterranean
Holocentrus fasciatus ¹	Bloch, 1790	10 / 15	13	1/5	3/7	16	
Holocentrus marocannus ¹	Bloch & Schneider, 1801	10 / 16	15	1/5	3/7	18	Morocco
Serranus scriba	Cuvier & Valenciennes, 1828	10 / 14	13	1/5	3/7	17	Mediterranean: France, Malta, Italy and Egypt
Serranus scriba	Zorica et al. 2010 (n = 253)	10 / 14–17 (15.01 ±2.04)	12–16 (14.10 ±1.15)	1 / 4-6 (4.97 ±0.49)	3 / 7-8 (7.03 ±0.82)	15-18 (16.59 ±1.36)	Trogir, Turkey
Serranus scriba	current work (n = 15)	10 / 15	13–14 (13.27 ±0.46)	1/5	3/7	15–17 (16.67 ±0.62)	Malta
Serranus papilionaceus	Valenciennes, 1832	10 / 15	16	1/5	3/8	17	Gorée, Senegal
Serranus papilionaceus	current work (n = 5)	10 / 15–16 (15.40 ±0.55)	14–15 (14.60 ±0.55)	1/5	3/8	17	Canary Islands

Meristic measures	Serran	us papilio	naceus	Ser	ranus scri	ba	Ser	ranus scri	ba	
	Ca	Canary Islands			Malta			Turkey		
	curre	current study $(n = 5)$		curren	current study $(n = 15)$			Zorica et al. 2010 (n = 253)		
	range	mean	SD	range	mean	SD	range	mean	SD	
Total length (mm)	187-245	216.6	±26.3	101-205	131.8	±30.9	71-200	110	±17.0	
Standard length	82.9-85.7	84.5	±1.2	82.6-88.3	85.3	±1.6	74.7–94.3	84.3	± 1.8	
Head length	28.9-33.9	31.6	± 2.0	31.8-35.6	33.8	± 1.1	16.8-40.0	33.5	±1.6	
Preocular head length	8.9-10.0	9.4	± 0.4	8.0-10.2	9.4	±0.6				
Eye diameter	4.8-5.3	5.1	±0.3	4.9-6.8	6.2	±0.6				
Postocular head length	14.3-17.6	16.0	±1.5	15.8-18.8	17.1	± 0.8				
Predorsal distance	28.6-32.9	30.7	±1.6	31.2-35.9	33.7	±1.3	28.9-43.5	33.6	±1.9	
Preanal distance	52.4-56.7	55.2	±1.6	53.0-59.2	56.4	±1.9	48.3-64.8	55.3	±1.9	
Maximum body height	24.2-25.8	25.1	±0.6	22.9-28.7	25.5	±1.5	16.8-47.1	27.9	±2.6	
Minimum body height	10.7-11.7	11.3	± 0.4	9.0-10.2	9.7	±0.3				
Length of dorsal fin	45.3-47.6	46.3	± 1.1	39.8-44.1	41.4	± 1.4	31.0-47.4	40.9	± 1.4	
Length of anal fin	12.7-15.3	14.0	± 1.1	12.0-14.2	12.9	±0.7	7.5-15.5	12.2	±1.3	
Length of pectoral fin	18.4-23.0	21.1	± 1.8	21.1-23.8	22.0	± 0.8	17.4-27.8	22.5	± 1.4	
Length of ventral fin	17.6-21.6	19.3	±1.6	15.6-17.4	16.6	±0.5	10.3-21.0	17.6	± 1.4	
Length of caudal fin	16.3-18.6	17.0	±0.9	11.7-17.4	14.7	±1.6	5.7-18.4	15.7	± 1.4	

Table 3. External measures of *Serranus papilionaceus* and *Serranus scriba* expressed as a percentage of the total length.

Colour patterns

The S. scriba (Fig. 1) specimens analysed in this study had nine dorso-ventral brown bands. The first band was observed to originate dorsally over the head and fade ventrally just behind the preopercular area. The second band, starting in front of the origin of the dorsal fin, was found to touch the back of the operculum and end at the origin of the pectoral fin. The third, shorter band originating at the first hard spine of the dorsal fin, fades away ventrally. The fourth and fifth band originating at the membrane between the fourth and the seventh hard spine of the dorsal fin and fade away ventrally. They are usually paired up and at times are nearly overlapping. The sixth and seventh band originating at the membrane between the eighth hard spine and the first soft spine of the dorsal fin, may at times be fused, and fade away ventrally into a bluish-violet blotch located over the abdomen area. The eighth and ninth bands are much wider than the former bands, appearing as two fused bands, especially when they fork out ventrally. These two wider bands originate from the soft spines of the dorsal fin and fade away ventrally as they approach the anal fin. A brown banding pattern on the tail peduncle is not always present. The pectoral fins, pelvic fins, anal fin, and tail peduncle together with the tail, are brownish-orange. A faint bluish colouration on the outer side of the pelvic fins and anal fin membrane is present. The pelvic fins, anal fin, dorsal fin, tail peduncle, and tail have red-orange spots, while at the tip of each hard spine of the dorsal fin there is a small, red-orange-coloured membrane. Yellowish thin vertical striations are visible on the abdominal area but quickly fade away ventrally. The head area is reddish-brown with vermiculation. The head area below the eye is light coloured, while the area above the eye has a darker red-brown colouration. A longitudinal brown stripe runs through the eye area.



Figure 1. A-C Serranus papilionaceus D-F Serranus scriba. Photos include details of specimens representing both species (underwater photography by Mr. P. Schembri, BICREF NGO). Scale bars: 1 cm (A-C, E, F).

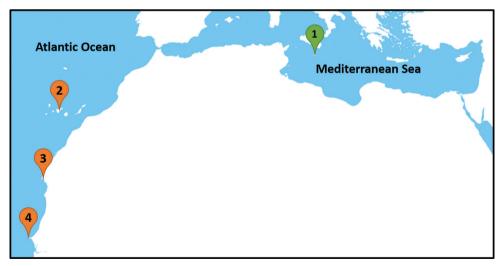


Figure 2. A map showing: the sampling locations for this study [1 – Malta, 2 – Canary Islands]; and the locations from where *Serranus papilionaceus* was recorded [2 – Canary Islands (Barker-Webb and Berthelot 1836; current study); 3 – Mauritania (MNHN-IC-1999-1053); 4 – Gorée, Senegal (Valenciennes, 1832; MNHN-IC-0000-7279; MNHN-IC-1896-0389)].

The banding pattern of the S. papilionaceus (Fig. 1) specimens examined in this study was composed of two wide dorso-ventral brown bands, at times each appearing as composed of multiple fused bands. The first anterior brown band, occurring between the head area and the membrane of the eighth hard spine of the dorsal fin, fades ventrally at the abdomen and was found to be longer than the bands noted in S. scriba. This brown band is followed by a lighter cream-coloured band originating between the last few hard spines and the first few soft spines of the dorsal fin. This light-coloured band ends with a blue-violet patch at the abdomen. The latter was not present in all specimens we examined, and when present, it was less conspicuous than that found on S. scriba. This light-coloured band is followed by another dorso-ventral, wide, brown band originating from the soft spines of the dorsal fin and which appears as being composed of multiple fused bands. A brown banding pattern is also visible on the tail peduncle. The pectoral fins, pelvic fins, and tail, are brownish-orange. The anal fin and the membrane of the soft-rays of the dorsal fin have red-orange spots that are more pronounced than those of S. scriba and are separated by bluish lines. The tip of each hard spine of the dorsal fin has a small, red-orange-coloured membrane. The head area of S. papilionaceus is brownish as opposed to the red-brown of S. scriba. In S. papilionaceus, the head area below the eye is lighter in colour than the dorsal area of the head. The vermiculation on the head is more pronounced in S. papilionaceus than in S. scriba, and as for the former species, the lighter patterns contrast more against the darker brown colouration. In S. papilionaceus, the brown longitudinal stripe that runs through the eye area is not as conspicuous as that of S. scriba.

Most of the vivid colourations noted on both *S. scriba* and *S. papilionaceus* are mostly visible on live specimens, and some of the details are lost once the individuals die; however, the main brown bands tend to remain visible for a longer time and remain persistent in some old museum preserved specimens (Suppl. material 1: Fig. S2).

Genetic investigations

Genetic divergence

The COI and ND2 data produced three and six haplotypes for the specimens collected from the Canary Islands (n = 12), while five and nine haplotypes were identified amongst the specimens collected from Malta (n = 15) for each respective gene (Table 4; Fig. 3). The intraspecific divergence for COI of the analysed specimens from Malta was

Table 4. The number of haplotypes noted (*hap.*), the haplotype diversity (*h*) and the nucleotide diversity (π) at each gene for *Serranus papilionaceus* and *Serranus scriba*.

mtDNA gene	Serranus papilionaceus				riba	
	hap	b	π	hap	b	π
COI (621 bp)	3	0.621 ±0.087	0.0011 ±0.0010	5	0.476 ±0.155	0.0009 ± 0.0009
ND2 (846 bp)	6	0.758 ± 0.122	0.0031 ± 0.0020	9	0.924 ± 0.044	0.0045 ± 0.0027
concatenated (1467 bp)	7	0.833 ± 0.100	0.0023 ± 0.0014	11	0.952 ± 0.040	0.0029 ± 0.0017

0.09%, while on incorporating similar publicly available data for *S. scriba* from other areas of the Mediterranean (n = 41; Suppl. material 1: Table S3), the intraspecific divergence increased to 1.12%, with all sequences clustering together on the same branch of the phylogenetic tree (Fig. 4). For the specimens collected from the Canary Islands, the intraspecific divergence on COI was 0.11%. The genetic divergence between the two collections was found to be 4.31% (Table 5), that is four times larger than the intraspecific divergence, exceeding the species boundary delimitation of fish species (Ward et al. 2005). This led to the confirmation that specimens belonged to two different species. This large interspecific genetic difference was also noted on the ND2 gene where the intraspecific divergence was 0.45% and 0.31% for *S. scriba* and *S. papilionaceus*, respectively, while the interspecific divergence was 8.68% (Table 5), again surpassing the intraspecific divergence differences noted on the ND2 gene in other fish species by Naylor et al. (2012) and Vella et al. (2017). The haplotype networks of this mtDNA data show that the two species form two distinct clades, with no overlapping haplotypes (Fig. 3).

These results were further corroborated by both nuclear markers, where species specific SNPs were noted (Table 6). This data therefore confirms that genetically the specimens collected from the Canary Islands belong to a different species from those collected from Malta.

Phylogeny

All the species of *Serranus* found in the Mediterranean and the Canary Islands (*S. atricauda, S. cabrilla, S. hepatus, S. papilionaceus*, and *S. scriba*) share the same ancestral branch of the phylogenetic tree (Fig. 4), with each species forming its own distinct clade. This phylogenetic tree placed *S. scriba* and *S. papilionaceus* as sister species to each other.

mtDNA gene	Intraspecific o	Interspecific divergenc	
	Serranus papilionaceus	Serranus scriba	
COI (621 bp)	0.70 (0.11%)	0.53 (0.09%)	26.77 (4.31%)
ND2 (846 bp)	2.62 (0.31%)	3.79 (0.45%)	73.43 (8.68%)
concatenated (1467 bp)	3.32 (0.23)	4.33 (0.30%)	100.20 (6.83%)

Table 5. Intraspecific and intraspecific average number of pairwise differences (percentage differences) for the mtDNA loci of *Serranus papilionaceus* and *Serranus scriba*.

Table 6. SNPs noted for the nDNA loci of *Serranus papilionaceus* and *Serranus scriba* (^a species-specific differences).

nDNA gene				Rhod				P	ΓR
Position	30	204	225ª	232	273	628ª	690ª	160ª	205ª
Serranus	C(n = 11)	C(n = 15)	G(n = 12)	T(n = 9)	C(n = 9)	A $(n = 15)$	C(n = 15)	T(n = 12)	G (<i>n</i> = 12)
papilionaceus	C/T $(n = 1)$			$\mathrm{C/T}\left(n=3\right)$	C/T $(n = 3)$				
Serranus	C(n = 15)	C(n = 14)	T $(n = 15)$	C(n = 15)	C(n = 15)	G(n = 15)	T $(n = 15)$	C(n = 15)	A $(n = 15)$
scriba		C/T $(n = 1)$							

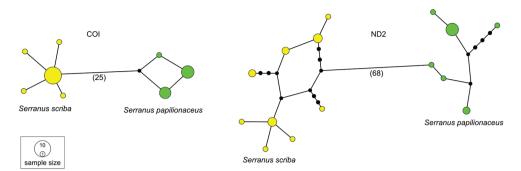
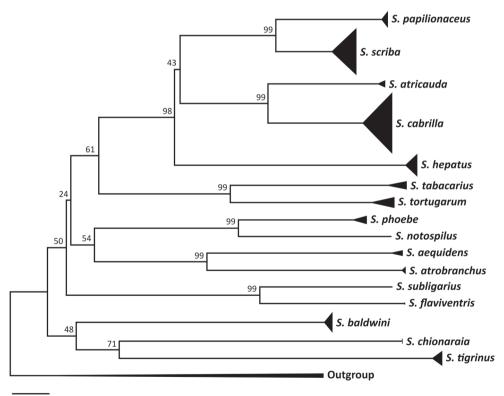


Figure 3. Two parsimony haplotype networks representing the genetic relationship between *Serranus papilionaceus* (Canary Islands) and *Serranus scriba* (Malta) for the COI and ND2 data (The haplotype frequencies are proportional with the area of the circle. The numbers in the brackets represent the number of interspecific substitutions, while the black circles represent inferred putative haplotypes within species that were not observed during this study).



0.01

Figure 4. A neighbour-joining phylogenetic tree using p-distance. 186 COI sequences (578 bp each) representing 16 different *Serranus* species were utilized (Suppl. material 1: Table S3). The numbers near nodes represent the bootstrap values.

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Vernacular name

We suggest Butterfly-winged Comber (Le Serran à Ailes de Papillon) as a common name of the species referring to its scientific name and the first description by Valenciennes (1832).

Distribution

Specimens of *S. papilionaceus* used in this work were collected from the coastal waters of the Canary Islands (Eastern Atlantic Ocean), the same archipelago where the species was recorded nearly two centuries ago by Barker-Webb and Berthelot (1836). The original description of Valenciennes (1832) also places this species at the island of Gorée off the coast of Senegal. Museum specimen MNHN-IC-1896-0389 reconfirms this species at Senegal, while species occurs also along the coast of this country (Fig. 2). The species might be more widely distributed along the western African coast and, therefore, more studies are required to better evaluate its distribution.

Discussion

Mitochondrial DNA data show that the genetic distance between the Mediterranean specimens of *S. scriba* and the ones collected from waters of the Canary Islands exceeded the intraspecific genetic distance (COI: 4.31%; ND2: 8.68%; Figs 3, 4) for both genes (Ward et al. 2005; Naylor et al. 2012; Vella et al. 2017), confirming that the specimens from these two different sampling sites belong to two different species. The morphological colour and banding pattern difference between these two species support the genetic distinction found.

Serranus scriba was first described by Linnaeus (1758: 292) as Perca scriba. This description is fairly brief and lacks the locality; however, specimen NRM 442 from the Swedish Museum of Natural History (Suppl. material 1: Fig. S1a) is believed to be the holotype for *P. scriba* (Fernholm and Wheller 1983; Parenti and Randall 2020). In *Museum Adolphi Friderici* volume 2, Linnaeus (1764: 86) updated the description of *Perca scriba* and included some more details on the colouration of the species, namely reticulations and undulations over the head and brownish transverse bands, relating it to the Mediterranean *S. scriba*. However, in this account Linnaeus (1764: 86) indicated America as its area of distribution (Fernholm and Wheller 1983). Cuvier and Valenciennes (1828) made a detailed description of *S. scriba*, providing an illustration of the species (plate 28; Suppl. material 1: Fig. S1b), as well as indicating its distribution as including the coast of Provence (France), Malta, Naples (Italy), and Alexandria (Egypt). This description matches the two museum specimens MNHN-

IC-0000-7129 (Suppl. material 1: Fig. S2b) and the specimens collected from Malta for the current study (Fig. 1). We therefore keep the widely accepted *S. scriba* nomenclature here in reference to the Mediterranean species sampled and genetically investigated in this study.

After reviewing the possible junior synonyms for S. scriba (Fernholm and Wheller 1983; Smith-Vaniz 2015; Horton et al. 2020; Parenti and Randall 2020), it was noted that our specimens from the Canary Islands matched the description of S. papilionaceus (Valenciennes, in Cuvier and Valenciennes 1832: 471). Serranus papilionaceus was first collected from Gorée Island, Senegal, by M. Rang and described by Valenciennes (1832: 471), and it is represented by two extant syntypes in the Muséum National d'Histoire Naturelle (MNHN-IC-0000-7279; Suppl. material 1: Fig. S2a). Our findings corroborate the second account for this species as described by Barker-Webb and Berthelot (1836), who indicated that S. papilionaceus occurs in the Canary Islands. In the current study, we also reviewed the description of H. marocannus Bloch & Schneider, 1801, as Peters (1865) suggested that this is synonymous to S. papilionaceus Valenciennes, 1832. However, given that the original description (Bloch and Schneider 1801: 320) and the quality of the type specimen (ZMB5531) lack the morphological details required to synonymise the two, we consider *H. marocannus* to be a junior synonym of S. scriba as noted by Cuvier and Valenciennes (1828), Gunther (1859), and Parenti and Randall (2020).

Historic records show that *S. papilionaceus* was accepted as a valid species name for a couple of decades (Barker-Webb and Berthelot 1836; Gunther 1859). However, by 1890, studies on specimens from the Canary Islands were considered as the variant *S. scriba* var. *papilionaceus* (Dooley et al. 1985). As a result, the Butterfly-winged Comber had lost its status as a distinct species. The similarity between the two species was reported since the first description of *S. papilionaceus*, with Barker-Webb and Berthelot (1836) stating that the two species, considered here, were very similar and could be easily misidentified. Nonetheless, the banding pattern, especially of live specimens is the most distinctive morphological feature to differentiate between the two species. Additionally, the results of our genetic analyses confirm the genetic divergence between these two species.

The resurrection of *S. papilionaceus* adds another species to the genus *Serranus*, genetically sister to *S. scriba* (Fig. 4). This calls for new studies that look into the geographical distribution of both *S. papilionaceus* and *S. scriba*. Online searches for *S. scriba* indicate that the morphological details belonging to *S. papilionaceus* are present in photos that have originated from different coastal regions along the North-Eastern Atlantic Ocean, and we cannot exclude the possibility of potential range overlap of the two species in certain geographical areas.

Though *Serranus* species have been listed as Least Concern (Smith-Vaniz 2015; IUCN 2020), the conservation status of the now recognised distinct species, *S. papilionaceus*, needs to be evaluated. This is especially so when one considers that the Canary Islands had already listed their *Serranus* species as threatened some time ago (Bonnet and Rodriguez 1992; Tuset et al. 2005) and fishing pressures have not decreased.

Conclusion

Genetic diversity investigation results and different body colour patterns observed have led us to resurrect *S. papilionaceus* Valenciennes 1832 as a species distinct from *S. scriba* (Linnaeus, 1758). Phylogenetic analysis confirmed that these two species are sister species within the genus *Serranus* and that their interspecific genetic divergence is four times larger than the usual intraspecific divergence in fish species. The resurrection of *S. papilionaceus* also emphasizes the required new research to understand the distinct distributions, threats, and conservation management of these species.

Acknowledgements

The research disclosed in this publication has been funded through the BioCon_Innovate Research Excellence Grant from the University of Malta awarded to AV. The authors would like to thank several fishers from Malta and the Canary Islands, who have supported this research and assisted in specimen collection. A special thanks goes to Jonathan Pfliger from the Muséum National d'Histoire Naturelle (France), Edda Aßel from Museum für Naturkunde – Leibniz Institute for Evolution and Biodiversity Research (Berlin, Germany), and Bo Delling and Anders Silfvergrip from the Swedish Museum of Natural History (Stockholm, Sweden) for providing the authors with the required information and photos of museum specimens. Additionally, thanks are also due to the scuba diver Patrick Schembri from the NGO BICREF for providing photography of live specimens from Malta. The three scientific reviewers of this manuscript are appreciated for their valuable feedback.

This study did not require ethical approval as it made use of dead specimens and tissue samples collected from specimens caught by local fishermen during regular fishing activities.

DNA sequences generated in this study have been deposited in GenBank. Details of the specimens and the associated accession numbers are included in the Suppl. material 1: Table S2.

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Supplementary material I

Tables S1–S3, Figures S1, S2

Authors: Adriana Vella, Noel Vella, Carolina Acosta-Díaz

Data type: tables and images

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Link: https://doi.org/10.3897/zookeys.1017.60637.suppl1

RESEARCH ARTICLE



A new species of the freshwater crab genus Potamonemus Cumberlidge & Clark, 1992 (Crustacea, Potamonautidae) endemic to the forested highlands of southwestern Cameroon, Central Africa

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Academic editor: C. Magalhães Received 21 November 2020 Accepted 12 January 2021 Published 15 February 2020	21
http://zoobank.org/36B25C08-43C8-4622-9189-B4F692651CFB	

Citation: Mvogo Ndongo PA, von Rintelen T, Cumberlidge N (2021) A new species of the freshwater crab genus *Potamonemus* Cumberlidge & Clark, 1992 (Crustacea, Potamonautidae) endemic to the forested highlands of southwestern Cameroon, Central Africa. ZooKeys 1017: 127–141. https://doi.org/10.3897/zooKeys.1017.60990

Abstract

A new species of freshwater crab of the genus *Potamonemus* Cumberlidge & Clark, 1992 is described from Mount Manengouba Reserve and Bakossi National Park in the tropical rainforests of southwestern Cameroon, Central Africa. *Potamonemus man* **sp. nov.** is recognized by characters of the carapace and chelipeds. In addition, a phylogenetic analysis based on partial sequences of three mitochondrial DNA genes (COI, 12S rRNA, and 16S rRNA) that included representatives of all other freshwater crab genera found in Cameroon recovered each of the new species as a distinct lineage. A diagnosis and illustrations of the new species are provided, and it is compared to the other species of *Potamonemus*. Brief notes are provided on the ecology of the new species and the two other species of *Potamonemus*. An identification key to the species of *Potamonemus* is provided. The conservation status of the genus is discussed.

Keywords

Afrotropical region, conservation, identification key, mtDNA, taxonomy

Introduction

Potamonemus Cumberlidge & Clark, 1992 is one of five genera of freshwater crabs currently known from Cameroon. The other four species are Buea Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019, Louisea Cumberlidge, 1994, Potamonautes MacLeay, 1838, and Sudanonautes Bott, 1955 (Cumberlidge 1987, 1989, 1993a, b, c, 1994a, b; 1999; Cumberlidge and Boyko 2001; Cumberlidge and Clark 1992; Cumberlidge et al. 2019; Mvogo Ndongo et al. 2017a, b, c, 2018, 2019, 2020). Potamonemus was originally established as a monotypic genus for P. mambilorum Cumberlidge & Clark, 1992, and in the following year two more species were described, namely P. asylos Cumberlidge, 1993 and P. sachsi Cumberlidge, 1993. Subsequent phylogenetic analyses of the Afrotropical freshwater crab fauna (Daniels et al. 2006, 2015) supported the close relationship between P. mambilorum and P. sachsi but indicated that P. asylos formed a separate genetic lineage from these two species. Recently, P. asylos was assigned to a new genus, Buea Cumberlidge, Mvogo Ndongo, Clark & Daniels, 2019. Mvogo Ndongo et al. (2020) recently described three additional Buea species, including B. bangem Mvogo Ndongo, von Rintelen, Tomedi-Tabi & Cumberlidge, 2020, B. mundemba Mvogo Ndongo, von Rintelen & Cumberlidge in Mvogo Ndongo, von Rintelen, Tomedi-Tabi and Cumberlidge 2020, and B. nlonako Mvogo Ndongo, von Rintelen & Cumberlidge in Mvogo Ndongo, von Rintelen, Tomedi-Tabi and Cumberlidge 2020.

Cumberlidge et al. (2019) and Mvogo Ndongo et al. (2020) established that *Buea* is endemic to southwestern Cameroon and that *Potamonemus* is a more widely distributed genus, with a range including southwestern Cameroon and eastern Nigeria. Cumberlidge et al. (2019) revised the diagnostic characters of *Potamonemus* as a 2-segmented mandibular palp lacking a lobe or anterior flap at the junction between the two segments, a G1 with a slim, outward-curving and elongated TA (TA/SS 0.63) that lacks marginal setae and tapers to a pointed tip, and a G2 with a remarkably short TA (TA/SS 0.13). The three protected areas surveyed in the present study for freshwater decapods are located in a region of southwestern Cameroon which has been recognised as a biodiversity hotspot for several other freshwater taxa.

Extensive systematic surveys carried out from 2017 to 2020 in the lowland and upland zones of the tropical rainforests of southwestern Cameroon resulted in the collection of several new taxa, including a new species of *Potamonemus*. The purpose of the present study is to describe this new species using an integrative approach based on morphological characters and molecular genetic data and to provide a key to the three species now assigned to this genus. The molecular analysis based on three partial mitochondrial genes (COI, 12S rRNA, and 16S rRNA) also recovers the three species as separate genetic lineages within *Potamonemus*. For all the species treated in this study we also provide notes on their ecology and conservation.

Materials and methods

Sampling

Field surveys of freshwater decapods were conducted in 2017 at Mount Manengouba Reserve and Bakossi National Park, and in 2018 and 2020 at Nlonako Ecological Reserve. Survey transects were made in each study area. Crabs were collected from small rivers using nylon fishnets and dip nets, and near small permanent streams where crabs were found in puddles, under fallen leaves, under stones, and in burrows. The amount of disturbance of the habitat and the various threats to freshwater organisms, including to freshwater crabs, were evaluated during structured discussions with local people.

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), LAS V4, and Helicon Focus 6.7.1 software. Post processing of the images was undertaken using Adobe Photoshop CC5 and Photo Impact. The newly collected specimens were 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**).

Abbreviations used

A	pleonal (abdominal) segment or pleomere;	G2	male first gonopod; male second gonopod;
	sulci between adjacent pleomeres;	P2–5	pereiopods 2-5 or ambulatory
a.s.l.	above sea level;		legs 1–4;
CW	carapace width measured at wid-	SS	subterminal segment of G1 or G2;
	est point;	S4/E4	(S4/E4, S5/E5, S6/E6, S7/E7)
CL	carapace length measured along		episternal sulci between adjacent
	medial line from anterior to pos-		thoracic sternites and episternites;
	terior margin;	S	thoracic sternite;
CH	carapace height measured at maxi-	S1/S2	(or \$2/\$3, \$4/\$5, \$5/\$6, \$6/\$7)
	mum height of cephalothorax;		sternal sulci between adjacent tho-
Ε	episternite;		racic sternites;
FW	front width measured along ante-	TA	terminal article of G1 or G2;
	rior frontal margin between inner	TS	terminal segment of mandibu-
	angles of orbits;		lar palp.

Details for DNA extraction, DNA sequencing, PCR, and molecular phylogenetic analyses are given by Mvogo Ndongo et al. (2019, 2020). All sequences used in this study are given in Table 1.

Systematic account

Infraorder Brachyura Latreille, 1802 Superfamily Potamoidea Ortmann, 1896 Family Potamonautidae Bott, 1970 Subfamily Potamonautinae Bott, 1970

Potamonemus man sp. nov.

http://zoobank.org/58FD0C15-4CB9-4561-8453-B98255BBEE25 Figures 1d, 2d, 3d, 4d, 5g, h, l, 6j–l, 7d, h Common name: Man Lake freshwater crab

Holotype. Adult & (CW 24.51 mm, CL 17.09 mm, CH 9.62 mm, FW 7.62 mm), CAMEROON, Southwest Region, Mount Manengouba Ecological Reserve, Man Lake, Mount Manengouba (5.02414, 9.82142), 1,958 m a.s.l., 14 March 2017, coll. P.A. Mvogo Ndongo (ZMB Crust. 30320).

Paratypes. 1 adult 3 (CW 21.37 mm, CL 15.61 mm, CH 8.45 mm, FW 6.69 mm), 1 adult ♀ (CW 23.55 mm, CL 16.87 mm, CH 10.09 mm, FW 7.60 mm), CAMEROON, Southwest Region, Mount Manengouba Ecological Reserve, Man Lake, Mount Manengouba (5.03604, 9.82906), 1,958 m a.s.l., 14 March 2017, coll. P.A. Mvogo Ndongo (ZMB Crust. 30324). 2 adult 👌 (CW 20.12 mm, CL 14.64 mm, CH 7.92 mm, FW 6.76 mm; CW 20.40 mm, CL 14.73 mm, CH 8.16 mm, FW 6.63 mm); 3 subadult 👌 (CW 18.46 mm, CL 13.89 mm, CH 7.40 mm, FW 6.16 mm; CW 19.38 mm, CL 13.78 mm, CH 7.78 mm, FW 6.63 mm; CW 14.05 mm, CL 10.67 mm, CH 5.84 mm, FW 5.30 mm), CAMEROON, Southwest Region, Mount Manengouba Ecological Reserve, Man Lake, Mount Manengouba (5.03604, 9.82906), 1,958 m a.s.l., 14 March 2017, coll. P.A. Mvogo Ndongo (IFAS-017); 4 adult 🌻 (CW 19.39 mm, CL 14.07 mm, CH 7.74 mm, FW 6.48 mm; CW 17.37 mm, CL 12.46 mm, CH 6.94 mm, FW 6.33 mm; CW 16.88 mm, CL 12.06 mm, CH 6.36 mm, FW 5.20 mm), CAMEROON, Mount Manengouba Ecological Reserve, Man Lake, Mount Manengouba (5.03604, 9.82906), 1,958 m a.s.l., 14 March 2017, coll. P.A. Mvogo Ndongo (IFAS-018).

Other material. Bakossi National Park (Figs 1c, 2c, 3c, 4c, 5e, f, j, 6g–i, 7c, g). 1 adult ♂ (CW 30.41 mm, CL 20.57 mm, CH 12.50 mm, FW 9.32 mm), 1 adult ♀ (CW 27.48 mm, CL 20.06 mm, CH 11.31 mm, FW 8.19 mm), CAMEROON, Southwest Region, Bakossi National Park (5.031083, 9.687528), 1,253 m a.s.l., 15 March 2017, coll. P. A. Mvogo Ndongo (ZMB Crust. 30328). 5 adult ♂ (CW 27.61 mm, CL 19.37mm, CH 11.39 mm, FW 8.65 mm; CW 26.54 mm, CL 19.09 mm, CH 10.80 mm, FW 9.13 mm; CW 25.55 mm, CL 18.68 mm, CH 10.58 mm, FW 8.41 mm; CW 25.3 mm,

Species	Locality	Museum number	Reference study	GenBank accession number			
				CO1	12S rRNA	16S rRNA	
Louisea nkongsamba	Mt. Nlonako	ZMB Crust. 31618	Mvogo Ndongo	MN188072	MN217386	MN217393	
(CW 20.0)			et al. 2019				
Louisea balssi (CW 14.8)	Manengouba	ZMB Crust.29628	Mvogo Ndongo et al. 2019	MN188070	MN217384	MN217391	
Louisea edeaensis (CW 17.2)	Lake Ossa	LZUY 15-3 (T351-30)	Mvogo Ndongo et al. 2017c	KY964474	KY964479	KY964472	
Buea mundemba. (CW 26.2)	Korup N. P.	ZMB Crust. 30321	Mvogo Ndongo et al. 2019	MN188069	MN217388	MN217396	
Buea bangem (CW 26.5)	Bakossi N.P.	IFAS-010	Mvogo Ndongo et al. 2020	MT019691	MT021447	-	
Buea asylos (CW 25.4)	Buea and Kumba	NHM 1994.588-591	Daniels et al. 2015	KP640489	KP640410	KP640453	
Potamonemus man sp. nov	Bakossi N. P.	ZMB Crust. 30328	Mvogo Ndongo et al. 2019	MN188067	MN217390	MN217398	
Potamonemus man sp. nov	Mt. Manengouba R.	ZMB Crust. 30320	Present study	GenBank (submitted by the first author)	-	-	
Potamonemus mambilorum	Southwest Cameroon	NHM 1991.183	Daniels et al. 2015	-	KP640409	KP640452	
Potamonemus sachsi	Southwest Cameroon	NMU09.04.1983	Daniels et al. 2015	-	AY803490	AY803530	
Potamonautes idjiwiensis	D. R. Congo	SAM A78437	Daniels et al. 2015	KP640481	KP640402	KP640446	
Potamonautes obesus	Tanzania	Unaccessioned	Daniels et al. 2015	AY803647	AY803497	AY803537	
Afrithelphusa monodosa	Guinea	NMU 25.IV.2005.C	Daniels et al. 2015	KP640469	KP640386	KP640430	
Globonautes macropus	Liberia	NMU VII. 1988	Daniels et al. 2015	-	KP640391	KP640435	
Sudanonautes aubryi	Cameroon	LZUY-06	Mvogo Ndongo et al. 2017c	KY069938	KY964475	KY069950	
Sudanonautes tiko	Cameroon	ZMB Crust.29628	Mvogo Ndongo et al. 2017c	KY069941	KY964476	KY069954	

Table 1. Species and specimens of *Louisea, Buea, Potamonemus, Sudanonautes*, and *Potamonautes* and the outgroup taxa included in the molecular analysis. All measurements in mm.

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; NP, National park; ZMB: Museum für Naturkunde, Berlin, Germany.

CL 18.05 mm, CH 10.70, FW 8.41 mm) (IFAS-014); 2 subadult ♂ (CW 22.17 mm, CL 15.76 mm, CH 9.04 mm, FW 7.35 mm; CW 22.02 mm, CL 15.67 mm, CH 9.06 mm, FW 7.49 mm; CW 22.04 mm, CL 16.00 mm, CH 9.22 mm, FW 7.65 mm; CW 21.35 mm, CL 15.19 mm, CH 8.76 mm, FW 6.81 mm), CAMEROON, Southwest Region, Bakossi National Park (5.031083, 9.687528), 1,248 m a.s.l., 15 March 2017, coll. P.A. Mvogo Ndongo (IFAS-015). 2 subadult ♀ (CW 23.76 mm, CL 17.57 mm, CH 9.88 mm, FW 7.68 mm; CW 23.31 mm, CL 16.63 mm, CH 9.08 mm, FW 7.59 mm), CAMEROON, Southwest Region, Bakossi National Park (05.031083, 9.687528), 1,248 m a.s.l., 15 March 2017, coll. P.A. Mvogo Ndongo (IFAS-016).

Diagnosis. Carapace anterior surface smooth except for faint urogastric groove (Fig. 1d). Broad epimeral (longitudinal) suture on carapace sidewall (branchiostegite) dividing carapace sidewall into 2 regions, vertical (pleural) groove lacking (Fig. 1d). Outer lower margin of cheliped merus lined by small, blunt teeth, inner lower margin smooth, distal meral tooth distinct, pointed (Fig. 3d). Major chela dactylus straight (not arched) (Fig. 5g). Sternal sulcus S2/S3 completely traversing sternum; S3/S4 incomplete, reduced to 2 short, distinct notches on each side of sternum (Fig. 3d). G1



Figure 1. Species of *Potamonemus* from southwestern Cameroon, whole animal, dorsal view **a** largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **b** adult male (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **c** adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **d** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Scale bars: 15 mm (**a**), 16 mm (**b**), 17 mm (**c**), 16 mm (**d**).

with long TA (TA/SS 0.66), slim, curving outward, lacking marginal setae, tapering to pointed tip; G2 TA remarkably short (TA/SS 0.13) (Fig. 6j, l). A small species, mature between CWs 20–25 mm.

Description. Carapace ovoid, medium height (CH/FW 1.17), wide (CW/FW 3.19); carapace surface smooth; postfrontal crest distinct, completely traversing carapace, lateral ends meeting anterolateral margins (Fig. 1d); exorbital tooth low, distinct; intermediate, epibranchial teeth each reduced to small granule (Fig. 4d); anterolateral margin behind epibranchial tooth smooth (Fig. 4d). Carapace branchiostegite with prominent epimeral suture dividing wall into subhepatic/suborbital, pterygostomial regions; vertical (pleural) suture faint (Fig. 3d). Sternal sulcus S2/S3 deep, completely traversing sternum; S3/S4 incomplete, reduced to 2 short, distinct notches on each side of sternum (Fig. 3d); margins of S3, S4 raised, broad (Fig. 3d); episternal sulci S4/E4, S5/E5, S6/E6 faint or missing, S7/E7 complete (Fig. 3d). Mandibular palp 2-segmented; medium-sized anterior lobe at junction between segment (0.25 × TS length; Fig. 7h). Third maxillipeds filling entire buccal cavern, except for transversely oval, efferent respiratory openings in superior lateral corners; ischium smooth, lacking vertical groove; exopod lacking flagellum (Fig. 7d).

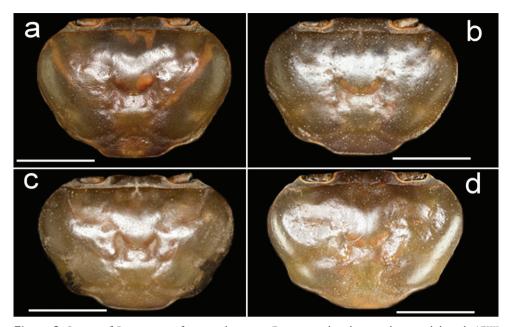


Figure 2. Species of *Potamonemus* from southwestern Cameroon, dorsal view **a** largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **b** adult male (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **c** adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **d** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Scale bars: 13 mm (**a**), 13 mm (**b**), 14 mm (**c**), 11 mm (**d**).

Male chelipeds greatly unequal, right cheliped larger than left (Figs 1d, 5g, h). Movable finger (dactylus), fixed finger (pollex of propodus) of right (major) chela both slim, elongated; fixed finger with 3 large pointed teeth (2 proximal, 1 distal); movable finger relatively stout, straight (not highly arched), with 4 small but distinct teeth (2 proximal, 2 distal; Fig. 5g). Left (minor) chela dactylus, propodus smaller than right chela, with small teeth on occluding margins (Fig. 5h). Inner inferior margin of cheliped merus lined by small teeth, outer inferior margin smooth; distal meral tooth large, pointed (Fig. 5d). Cheliped carpus inner margin with large pointed distal tooth; proximal tooth much smaller, followed by granule (Fig. 5l). Ambulatory legs (P2–5) slender, P4 longest, P5 shortest; dactyli P2–5 tapering to point, each bearing rows of downward-pointing sharp bristles, P5 dactylus shortest (Fig. 1d).

Male pleon triangular, margins not indented and lacking setae (Fig. 3d). G1 with long TA (TA/SS 0.66), slim, curving outward, lacking marginal setae, tapering to pointed tip; G2 TA remarkably short (TA/SS 0.13) (Fig. 6j, l); G1 SS, broad in basal, midsection, distal quarter tapering sharply, narrow at junction with G1 TA (Fig. 6j, l). G2 TA extremely short (G2 TA/SS 0.3; Fig. 6k).

Adult female. Right and left chelipeds subequal. Fixed, movable fingers of chela interspersed with series of smaller acute teeth along their length. Pleon wide, covering

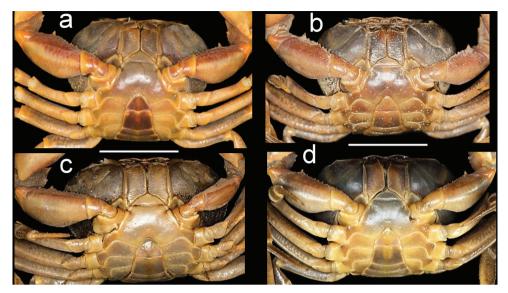


Figure 3. Species of *Potamonemus* from southwestern Cameroon, ventral view **a** largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **b** adult male, holotype (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **c** adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **d** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Scale bars: 16 mm (**a**), 17 mm (**b**), 16 mm (**c**), 17 mm (**d**).

entire sternum, reaching bases of coxae of P2–5; pleon with 6 free pleomeres (A1–6) becoming gradually wider proximally, telson wide, forming near semicircle.

Size. Small species, CW in mature individuals ranging from 20.0-30.4 mm.

Colour in life. Dorsal carapace and all ambulatory legs dark brown, chelipeds red.

Type locality. Stream flowing into Man Lake, Mount Manengouba, in Manengouba Ecological Reserve, Southwest Region of Cameroon.

Etymology. The species is named for Man Lake, one of a pair of small lakes in the caldera at the summit of Mount Manengouba (the other lake being Woman Lake). The species epithet is used as a noun in apposition.

Habitat. At the Man Lake locality at the summit of Mount Manengouba the species is found in a small stream flowing into the lake, and it was also collected from a small stream in the Bakossi National Park. Both of these localities are located in rainforest habitat found along the Cameroon Volcanic Line, a 1,600 km long chain of volcanoes that stretches from the islands in the Gulf of Guinea to the mountains of eastern Nigeria and western Cameroon, including Mount Cameroon.

Remarks. The new species is assigned to *Potamonemus* because it conforms to the genus diagnosis (Cumberlidge and Clark 1992; Cumberlidge 1993c; Cumberlidge et al. 2019). *Potamonemus man* sp. nov. most closely resembles *P. sachsi* in that the dactylus of the major cheliped of both species is straight rather than highly arched. These two species can be distinguished from each other by the smooth carapace and bran-



Figure 4. Species of *Potamonemus* from southwestern Cameroon, frontal view **a** largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **b** adult male, holotype (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **c** adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **d** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Scale bars: 13 mm (**a**), 12 mm (**b**), 14 mm (**c**), 11 mm (**d**).

chiostegal sidewalls in *P. man* sp. nov. (Figs 1D, 2D) (vs. patches of short setae along the anterolateral and posterolateral margins of the carapace that continue around to the sidewalls in the subhepatic and pterygostomial regions of the branchiostegite in *P. sachsi* (Cumberlidge et al. 2019: fig. 4c)). The highly arched dactylus of the major cheliped of *P. mambilorum* distinguishes it from both *P. man* sp. nov. and *P. sachsi*. Finally, *P. man* sp. nov. can be distinguished from *P. mambilorum* and *P. sachsi* by the body size of adult specimens: the new species and *P. sachsi* are adult between CW 20–30 mm and CW 23–28 mm, respectively, while *P. mambilorum* is the largest species (adult at CW 29–38 mm).

A phylogenetic tree (Fig. 8), based on 1,848 base pairs representing the combined partial sequences of three mtDNA markers (COI, 16S RNA, and 12S RNA), recovered three species of *Potamonemus* as a single clade with strong BI and ML confidence values (1/100 at this node). The three species of *Potamonemus* (*P. mambilorum*, *P. man* sp. nov., and *P. sachsi*) form an independent lineage within the subfamily Potamonautinae, and all are found in the same geographical area of Cameroon. The uncorrected *p*-distance between *Potamonemus man* sp. nov. and *P. mambilorum* is 2.2% for 12S RNA and 0.6% for 16S RNA, and between *P. man* sp. nov. and *P. sachsi* it is 4.5% for 12S RNA and 4.6% for 16S RNA.

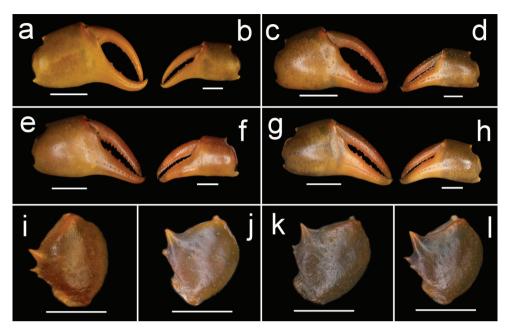


Figure 5. Frontal view of the right and left chelae of adult males of species of *Potamonemus* from southwestern Cameroon **a, b** largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **c, d** adult male (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **e, f** largest adult male, holotype (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **g, h** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Dorsal view of the right cheliped carpus of adult males of species of *Potamonemus* **i**, largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **j** adult male (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **k** adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **l** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30320). Scale bars: 5 mm (**a, c, g**), 2.5 mm (**b, d, f, h**), 5 mm (**i**, **j**, **k**, **l**).

Conservation. *Potamonemus man* sp. nov. is found in an area of great conservation interest. The aquatic habitats of this species in the Mount Manengouba Ecological Reserve and in the Bakossi National Park are both in montane tropical rainforest in the Cameroon highlands, an area with a high biodiversity and a high rate of endemism, including freshwater crabs (Cumberlidge et al. 2019; Mvogo Ndongo et al. 2017a, d, c, 2018, 2019, 2020). In Mount Manengouba Ecological Reserve the small, forested stream near Man Lake where *P. man* sp. nov. lives also supports a sympatric population of the endangered freshwater crab *Louisea balssi* (Bott, 1959). Significantly, there are no reports of any species of invertebrates (molluscs, insects, crustaceans) or vertebrates (fish, amphibians, snakes, and birds) from Man Lake itself. This inhospitality to life may be related to the unusual green colour of its waters which may be due to the accumulation of lethal compounds, which may also be a potential danger to humans (see Mvogo Ndongo et al. 2018). Both the Mount Manengouba Ecological Reserve and the Bakossi National Park are under increasing pressure from growing nearby human populations

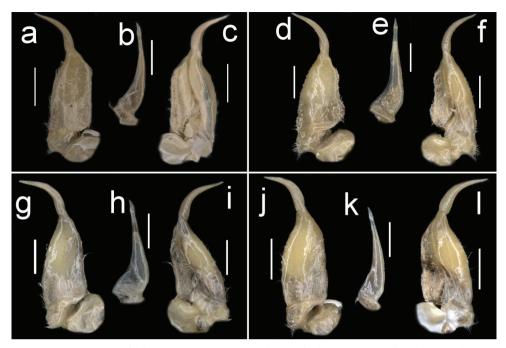


Figure 6. Dorsal view of left G1 (**a**, **d**, **g**, **j**) and ventral view of left G1 (**c**, **f**, **i**, **l**) of adult males of species of *Potamonemus* from southwestern Cameroon. **a**, **c** largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **d**, **f** adult male (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **g**, **i** adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **j**, **l** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Ventral view of G2 of adult males of species of *Potamonemus* from southwestern Cameroon **b** largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) **e** adult male (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) **h** adult male (CW 28 mm) of *P. man* sp. nov. from Bakossi (ZMB Crust. 30326) **k** adult male (CW 28 mm) of *P. man* sp. nov. from Mount Nlonako (ZMB Crust. 30326) **k** adult male (CW 28 mm) of *P. man* sp. nov. from Mount Nlonako (ZMB Crust. 30326) **k** adult male (CW 28 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30326) **k** adult male (CW 25 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) **k** adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Scale bars: 2 mm (**a–k**).

and from the associated clearance of land for agriculture. As a result, despite being found in protected areas, the habitat of *P. man* sp. nov. is increasingly threatened by nearby intensive agricultural practices and forest destruction for firewood collection. In addition, the farmers encroaching on these habitats use agrochemicals and pesticides on their crops, and these pollutants eventually drain into the aquatic systems, potentially poisoning the freshwater communities (Mvogo Ndongo et al. 2018).

Potamonemus mambilorum Cumberlidge & Clark, 1992

Figures 1b, 2b, 3b, 4b, 5c, d, i, 6d–f, 7b, f

Material examined. 6 adult ♂ (CW 29.05 mm, CL 21.17 mm, CH 12.10 mm, FW 8.85 mm; CW 29.56 mm, CL 21.35 mm, CH 12.34 mm, FW 9.12 mm;

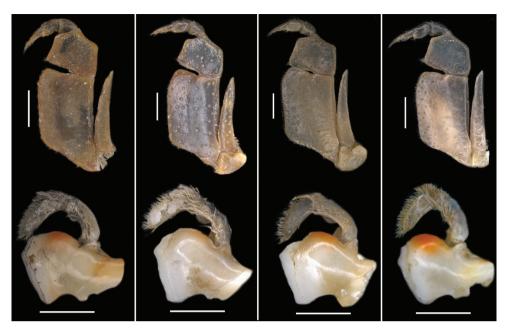


Figure 7. Frontal view of the left third maxilliped of adult males of species of *Potamonemus* from south-western Cameroon a largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 32428) b adult male (CW 28 mm) of *P. mambilorum* from small stream on Mount Manengouba (ZMB Crust. 30326) c adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) d adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30320). Frontal view of the left mandible of adult males of species of *Potamonemus* from southwestern Cameroon e largest adult male (CW 29 mm) of *P. man* sp. nov. from Man Lake, Mount Nlonako (ZMB Crust. 30320). Frontal view of the left mandible of adult males of species of *Potamonemus* from southwestern Cameroon e largest adult male (CW 29 mm) of *P. mambilorum* from Mount Nlonako (ZMB Crust. 30326) g adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30326) g adult male (CW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) h adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) h adult male, cCW 31 mm) of *P. man* sp. nov. from Bakossi National Park (ZMB Crust. 30328) h adult male, holotype (CW 25 mm) of *P. man* sp. nov. from Man Lake, Mount Manengouba (ZMB Crust. 30326). Scale bars: 5 mm (a–d), 2 mm (e–h).

CW 29.16 mm, CL 20.70 mm, CH 12.00 mm, FW 9.12 mm; CW 28.93 mm, CL 20.69 mm, CH 11.85 mm, FW 9.94 mm; CW 26.74 mm, CL 19.62 mm, CH 11.32 mm, FW 9.63 mm; CW 26.74 mm, CL 19.62 mm, CH 11.32 mm, FW 9.63 mm). 2 adult \bigcirc (CW 27.06 mm, CL 19.76 mm, CH 12.45 mm, FW 8.34 mm; CW 26.68 mm, CL 19.06 mm, CH 11.03 mm, FW 7.72 mm); Cameroon, Littoral region, Mount Nlonako Ecological Reserve (4.891820, 9.984830), 900 m a.s.l., 26 May 2018, coll. P.A. Mvogo Ndongo (ZMB Crust. 32428). 1 adult \bigcirc (CW 28.00 mm, CL 19.10 mm, CH 11.37 mm, FW 8.56 mm); 1 adult \bigcirc (CW 28.36 mm, CL 20.00 mm, CH 10.27 mm, FW 7.79 mm; Southwest Region, Mount Manengouba Ecological Reserve, small stream around the mountain (ZMB Crust. 30326) (5.034920, 9.836150), 1,958 m asl, 14 March 2017, coll. P.A. Mvogo Ndongo.

Remarks. The distributional range of *P. mambilorum* is extended in this work by the discovery of populations in Mount Nlonako Ecological Reserve in the littoral region of Cameroon. *Potamonemus mambilorum* was previously known from seven localities in

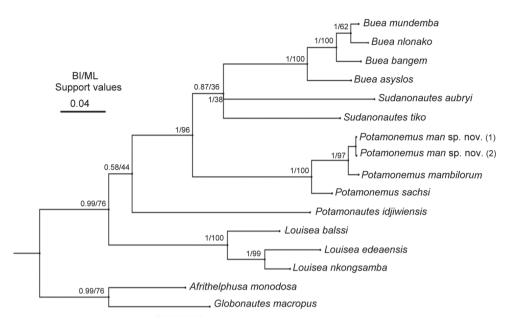


Figure 8. Bayesian Inference (BI) tree topology for the freshwater crab taxa from Cameroon included in this study derived from mtDNA sequences corresponding to three loci (partial 16S rRNA, COI, and 12S rRNA genes). Bayesian Inference (BI) and ML statistical values (%) on the nodes indicate bootstrap support and posterior probabilities, respectively.

the forested highlands and lowlands of southwestern Cameroon (extent of occurrence (EOO) 43,291 km²). The conservation status of this species was assessed as Least Concern (Cumberlidge 2008a), but this was before the threats to the freshwater ecosystems of this part of Africa were brought to light. The conservation status of *P. sachsi* was assessed as Vulnerable, B1ab(iii)+2ab(iii), based on its distributional range that includes the Bamenda highlands in southwest Cameroon and the neighboring Obudu plateau in southeast Nigeria, which is continuous with the Bamenda highlands (EOO 24,219 km²) and perceived threats (Cumberlidge 2008b). The areas where *P. mambilorum* and *P. sachsi* occur are now known to be at risk from a number of anthropogenic threats, including deforestation, together with intensive and encroaching agricultural practices and firewood collection, as well as release of pollutants such as agrochemicals potentially affecting the eggs, hatchling-carrying female crabs, and other aquatic organisms.

Key to the species of Potamonemus Cumberlidge & Clark, 1992

1	Dactylus of major cheliped highly arched (Fig. 5a)
_	Dactylus of major cheliped either straight or only slightly concave2
2	Carapace sidewalls in subhepatic and pterygostomial regions smooth
	<i>P. man</i> sp. nov.
_	Carapace sidewalls in subhepatic and pterygostomial regions with fields of
	short setae (Cumberlidge 1994: figs 3a, 4c)

Acknowledgements

We thank the Rufford Small Grant Foundation for funding the fieldwork in the South and Southwestern Regions of Cameroon, and the Museum für Naturkunde, Berlin (ZMB) for funding the first author during a research visit to Germany. We thank Dr. Paul F. Clark (Natural History Museum, London, UK) and Prof. Dr Christoph D. Schubart (University of Regensburg, Germany) for constructive comments in the earlier manuscript. Thanks are also due to the two anonymous reviewers and the Subject Editor for their important comments to improve the manuscript.

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