

Four new species of Aspidiotini (Hemiptera, Diaspididae, Aspidiotinae) from Panama, with a key to Panamanian species

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Abstract

Four new species of armored scale insect, *Clavaspis selvatica* **sp. nov.**, *Clavaspis virolae* **sp. nov.**, *Davidsonaspis tovomitae* **sp. nov.**, and *Rungaspis neotropicalis* **sp. nov.**, are described and illustrated from Panama. We also transfer two previously described species of Panamanian Aspidiotini to new genera, *Hemiberlesia crescentiae* (Ferris) **comb. nov.** and *Rungaspis rigida* (Ferris) **comb. nov.**, and report the first record of *Selenaspisopsis browni* Nakahara in Panama. A key to the species of Aspidiotini occurring in Panama is provided.

Keywords

Armored scale insect, biogeography, Coccoidea, Cocomorpha, Neotropics, taxonomy

Introduction

Armored scales are the most species-rich family of scale insects, comprising over 2600 species in 418 genera (García Morales et al. 2016). The family is characterized by the complete loss of legs and reduction of antennae in adult females, fusion of the posterior

abdominal segments into a pygidium, and the formation of a waxy test (Takagi 1990). Like all members of the suborder Sternorrhyncha, armored scales are strictly phytophagous. Many species of armored scales are pests of agricultural commodities (Miller and Davidson 2005). Heavy infestations inhibit photosynthesis through chlorophyll depletion and crowding of leaf surfaces, reducing plant vigor. Additionally, visible infestations and damage reduce the value of produce and nursery stock (Kosztarab 1990; Miller and Davidson 2005). However, unlike most scale insects, armored scales do not contribute to the growth of sooty molds on hosts because they do not produce honeydew (Henderson 2011).

Some armored scale insect species are extremely polyphagous, with host ranges among the widest known for any herbivorous insect, comprising in some cases over 100 families of plants (Normark and Johnson 2011; Ross et al. 2013; García Morales et al. 2016). The most highly polyphagous species have a strong tendency to be economic pests (Normark and Johnson 2011; Ross et al. 2013; Normark et al. 2014). Because armored scale insects appear to have essentially random dispersal via windblown larvae, Hardy et al. (2015) hypothesized that their host ranges are likely to reflect the plant diversity of their habitats, and that extreme polyphagy may have evolved in habitats with extreme plant diversity, such as tropical rainforests. Because of the economic importance of armored scale insects, they have been extensively sampled on cultivated plants, especially orchard crops and ornamentals (Rosen 1990; Miller and Davidson 2005). But their diversity, abundance, and host associations in natural environments are poorly known, and this is particularly true for tropical rainforests. Since 2010, one of us (BBN) has been systematically sampling armored scale insects in tropical forests. Two of the goals of this effort are to test for cryptic diversity within apparently polyphagous species and to test whether a species' local abundance is correlated with its host range. Results of tests of these hypotheses using samples from Panama and Borneo are reported in Peterson et al. (2020). Briefly, cryptic diversity is found within some apparently polyphagous species within their native ranges, but some invasive species are truly polyphagous. And local abundance is positively correlated with host range. Another goal of the rainforest sampling effort is to discover and describe new species of armored scale insects, which is the purpose of this article. Specifically, here we describe four new species within the tribe Aspidiotini collected from Panama.

Armored scales are currently classified into four subfamilies: Ancepspidinae, Aspidiotinae, Diaspidinae, and Furcaspinae (Normark et al. 2019). Aspidiotini is a large tribe within subfamily Aspidiotinae that includes many pest species that are globally invasive and economically damaging (Schneider et al. 2018). To date, 54 species of Aspidiotini in 16 genera have been recorded from Panama (García Morales et al. 2016; last accessed 31.iii.2021). In addition to the descriptions of four new species, this article includes the first report of *Selenaspidopsis browni* Nakahara from Panama. Additionally, this article assigns two Neotropical species to the genus *Rungaspis*, whose species are otherwise restricted to Africa and the southwestern Palearctic. With these records included, 58 species from 18 genera in Aspidiotini are known to occur in Panama, comprising roughly half of the total armored scale fauna for this country (58 out of 118 species reported in ScaleNet) (García Morales et al. 2016). The majority

of these species are likely native to the Neotropics (Ferris 1941, 1942; Deitz and Davidson 1986), but many are broadly distributed and are considered major, minor, or potential pests (Miller and Davidson 1990; Schneider et al. 2019). Species that are non-native to this region include members of *Aspidiella*, *Aspidiotus*, *Chrysomphalus*, and *Selenaspis*, which are widespread pests likely originating from the Australasian, Oriental, and Afrotropical regions (Schneider et al. 2018). An identification key to the species of Aspidiotini found in Panama is provided.

Material and methods

The sampling locality for new species described in this paper was the canopy crane in San Lorenzo National Park, Colón (9.2802°N, 79.9754°W). The locality was chosen because it offered access to the canopy via the crane and because every tree was reliably identified to species. The first survey was conducted in June 2012 by Geoffrey E. Morse and BBN, and the second in January 2015 by G. E. Morse, Daniel A. Peterson, Hannah Shapiro, and Shannon Trujillo. A full description of the sampling protocol is given in Peterson et al. (2020). Briefly, in each survey, investigators sampled all the tree species accessible from the canopy crane, and sampled multiple individuals of the more abundant species. Foliage of each sampled tree was searched visually for 20 person-minutes. Leaves that appeared to be infested with armored scale insects were collected into plastic bags, along with a 20 cm twig sample and 20 cm² bark sample. Collected material was refrigerated and examined under a dissecting microscope within 5 days; live armored scale insects were transferred to 100% ethanol. Subsequently, sampled scale insects were subjected to a joint morphological / molecular sample preparation that resulted in a sample of purified genomic DNA and a permanent microscope slide mount of the specimen's cuticle, following the method described in Normark et al. (2019).

In this paper, morphological terminology conforms to descriptions and illustrations provided by Schneider et al. (2019) and Miller and Davidson (2005). Vouchering of specimens was completed following the protocols described by Normark et al. (2019). Measurements were made on a Zeiss Axio Imager.M2 (Carl Zeiss Microscopy, LLC, White Plains, NY, USA) microscope with the aid of an AxioCam and AxioVision software. Illustrations were made using a Nikon Optiphot compound microscope (Nikon USA, Melville, NY, USA) with the aid of a camera lucida. Slide-mounted specimens were examined by the authors under phase contrast and DIC microscopy. The abbreviations L1, L2 and L3 refer to the median, second, and third pygidial lobes, respectively.

Depositories are abbreviated as follows:

- MIUP** Museo de Invertebrados G. B. Fairchild, Panama City, Panama;
- UMEC** University of Massachusetts Entomology Collection, Amherst, Massachusetts, USA;
- USNM** United States National Museum, scale insect collection at USDA Agricultural Research Service, Beltsville, Maryland, USA.

Taxonomy

Clavaspis selvatica Wei, Schneider, Normark & Normark sp. nov.

<http://zoobank.org/CDB99B24-3013-45F7-AA42-FE31CA298219>

Figure 1

Material examined. *Holotype*: PANAMA • 1 adult female; Parque Nacional San Lorenzo Canopy Crane, Colón; 9.2802°N, 79.9754°W; 15.i.2015; DA Peterson, GE Morse, H Shapiro, S Trujillo leg.; on *Embothrium coccineum*; MIUP (D6581C). *Paratypes*: • 1 adult female with second-instar exuviae; same data as holotype; USNM (D6581A); • 2 adult females; same data as holotype; UMEC (D6581B, D6581E).

Description ($N = 4$). **Adult female** not pupillarial. Appearance in life not recorded. Slide-mounted adult female 670–1450 μm long (holotype 670), 560–1100 μm wide (holotype 560), broadest at mesothorax or metathorax. Body outline turbinate to nearly oval. Derm membranous throughout at maturity except for pygidium. Antennae simple, each with one long seta. Distance between antennae 100–180 μm . Without disc pores associated with anterior or posterior spiracles. **Lobes**. L1 well developed, slightly wider than long, inner margins near parallel, with 1 notch on each side or without notches, rounded apically; space between lobes approximately 0.25 times width of L1. L2 and L3 absent. **Plates** cylindrical, narrow, pointed at apex, simple or with a few fine tines, about as long as L1; 2 plates present in first space, often with 1 or 2 tines near apex giving bifurcate or trifurcate appearance; 1 or 2 plates present in second space, simple or with minute tines; plates absent between L1. **Ducts**. Dorsal macroducts of 1-barred type, with 2–3 macroducts arising from first space, 8–10 arising from second space, and 7–8 arising from third space in singular rows. Series of marginal macroducts with wide orifices extending from mesothorax to abdominal segment II; at least two present per segment. Groups of ventral submarginal microducts occurring on head, thorax, and abdominal segments I–V. **Paraphyses**. With 1 pair of paraphysis-like basal scleroses near mesal margins of L1; 1 pair of paraphyses in first space, paraphysis arising from lateral margin of L1 slightly longer than paraphysis arising from medial margin of L2, both mushroom-like in shape with distinctive dome or cap at anterior end; 1 pair of small clavate paraphyses in second space. **Anal opening** longer than wide, 11–14 μm long, 5–7 μm wide, positioned 17–25 μm (1.5–2 anal lengths) from the base of L1, located within posterior third of pygidium. **Perivulvar pores** few, 2–6 pores in total, divided into 2–4 groups, with 1–4 in each group.

Remarks. This new species is most similar in appearance to *C. coursetiae* (Marlatt) with subtle differences distinguishing the two. Submarginal groups of microducts form a semicircle around the head, thorax, and pre-pygidial abdominal segments of *C. selvatica* but are more diffusely scattered in *C. coursetiae*, not organized in an obvious semicircular ring. In *C. selvatica*, at least two large macroducts are present on the mesothorax, while in *C. coursetiae* only one at most is present, falling near the posterior margin of the mesothorax. The plates are nearly as long as L1 and fringed in *C. selvatica*

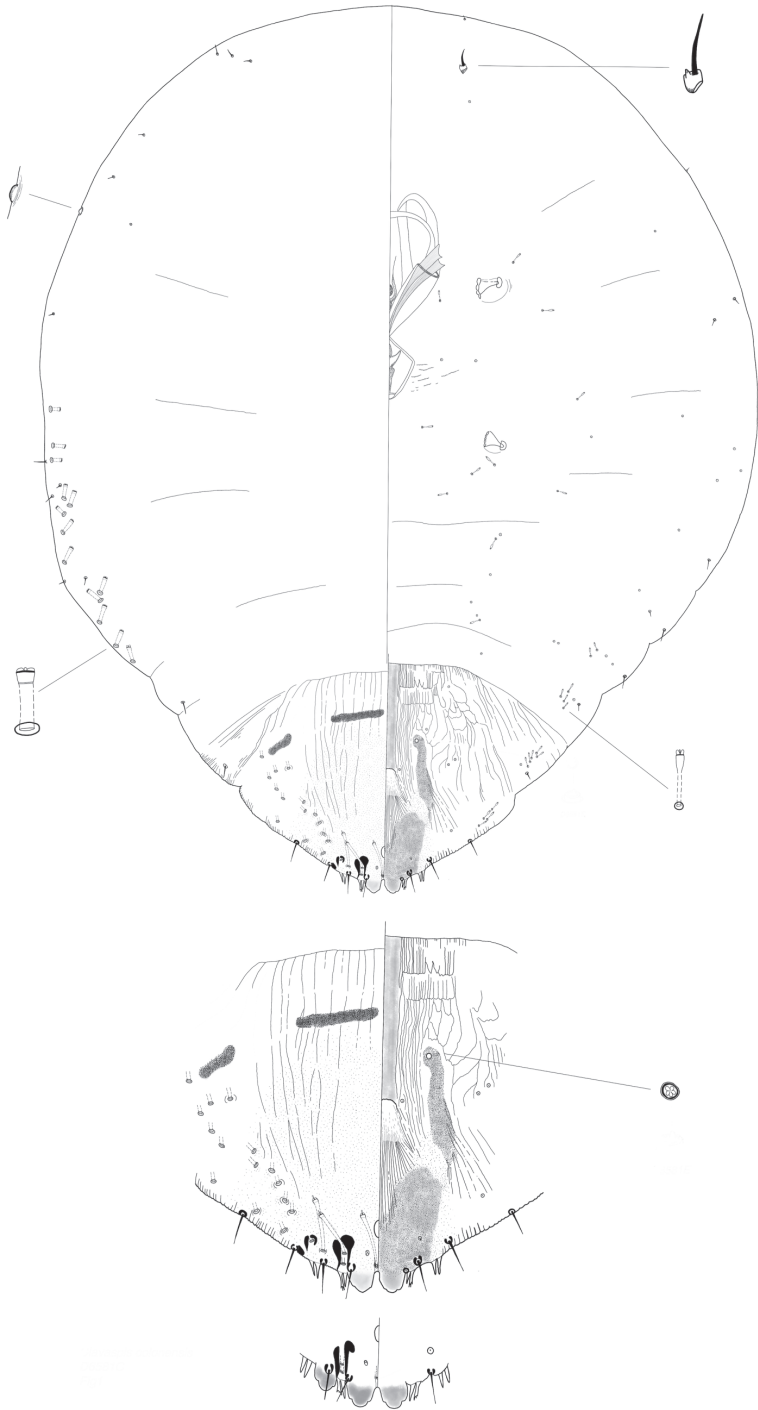


Figure 1. *Clavaspis selvatica* Wei, Schneider, Normark & Normark sp. nov. Adult female, full body view, illustrated from the holotype (D6581C); expanded views of pygidium showing variation, illustrated from the holotype (D6581C) and a paratype (D6581E).

but are short and simple in *C. coursetiae*. This species is also similar to *C. subsimilis* (Cockerell) in body shape and the shape of L1 but can be distinguished by possessing perivulvar pores on the pygidium (absent in *C. subsimilis*).

Host plant. *Apeiba aspera* Aubl. (family Malvaceae).

Etymology. The epithet *selvatica* is the Latin adjective meaning wild, literally “of the forest” (*selva*). Our choice of this name is influenced by the fact that in modern Spanish, the word *selva* is identical to its Latin ancestor in form, but now refers specifically to tropical rainforest.

Distribution. Panama (Colón).

***Clavaspis virolae* Wei, Schneider, Normark & Normark sp. nov.**

<http://zoobank.org/EEB45109-341D-44C4-98B8-E1BEC59F0CB1>

Figure 2

Material examined. Holotype: PANAMA • 1 adult female; Parque Nacional San Lorenzo Canopy Crane, Colón; 9.2802°N, 79.9754°W; 17.i.2015; DA Peterson, GE Morse, H Shapiro, S Trujillo leg.; on *Virola multiflora*; MIUP (D6676B). **Paratype:** • 3 adult females; same data as holotype; USNM (D6676A, D6676D, D6677A); • 3 adult females; same data as holotype; UMEC (D6674G, D6676C, D6677C).

Description (N = 7). Adult female not pupillarial. Appearance in life not recorded. Slide-mounted adult female 475–900 µm long (holotype 860, median 565), 410–630 µm wide (holotype 620, median 460), broadest near mesothorax and metathorax. Body outline oval, nearly circular in smaller individuals (< 600 µm long), becoming elongate-oval in larger individuals. Derm membranous throughout at maturity except for pygidium. Antennae simple, each with one long seta. Distance between antennae 40–100 µm. Without disc pores associated with anterior or posterior spiracles. **Lobes.** Pygidium with 2 pairs of lobes; L1 well developed, separated by space about one-fifth width of L1, lobes slightly wider than long, inner margins near parallel, with 1 medial and 1 lateral notch, rounded apically; L2 forming sclerotized point, about one-quarter to one-third size of L1, with 1 lateral notch; L3 absent, indicated at most by small, lightly sclerotized projection of pygidial margin. **Plates.** All plates simple; with or without fine plates in slight space between L1; with 2 pointed plates in first space; plates absent in second space; five simple microduct-bearing plates present laterad of L3, nearly as long as L1. **Ducts.** Dorsal macroducts of 1-barred type, slender, with orifices narrower in diameter than ventral microducts, restricted primarily to margin with one submarginal duct anterior to seta marking segment VI; 1 between L1, with 3–4 marginal ducts in first space, 2 marginal ducts in second space; with few short macroducts occurring on submarginal areas of pre-pygidial segments. Ventral microducts slightly wider in diameter than dorsal macroducts and present in small submarginal groups on pre-pygidial abdominal segments and segment V. **Paraphyses.** L1 each with a paraphysis-like basal sclerosis toward medial margin, slightly smaller than lobe; in first space, 1 clavate paraphysis arising from lateral angle of L1, 1 arising from me-

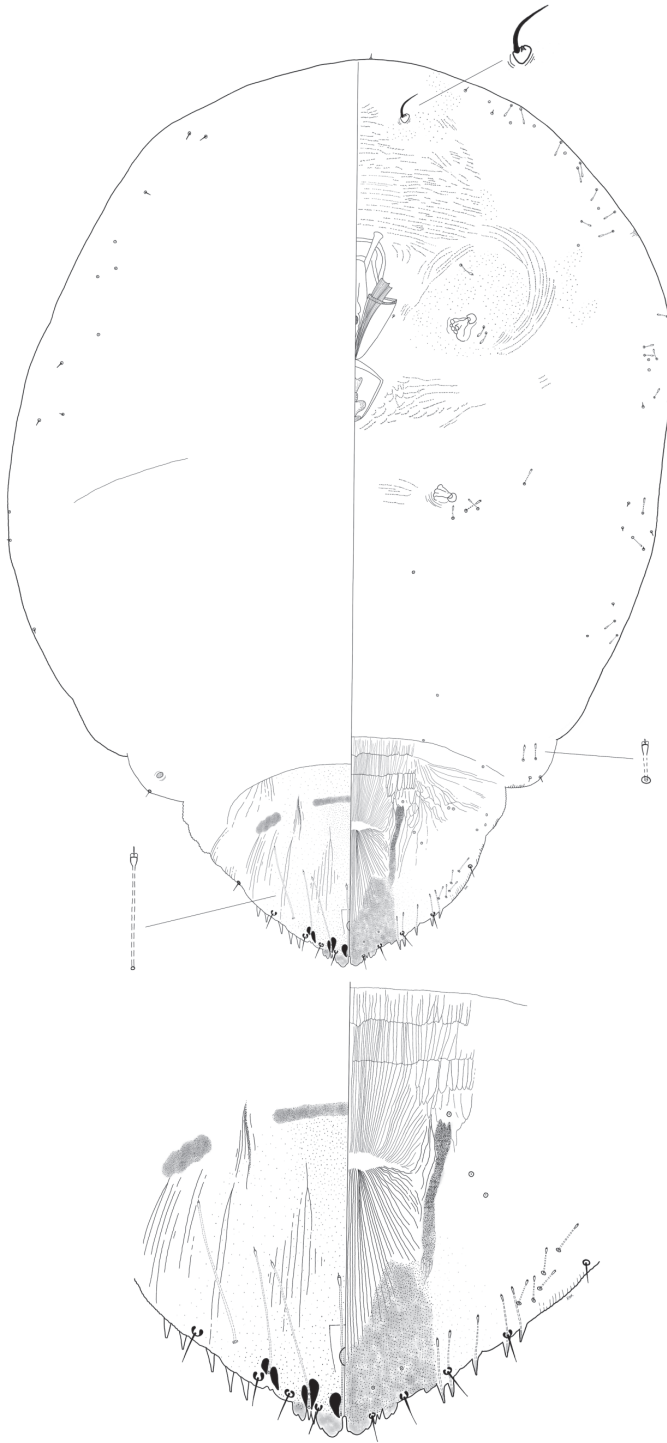


Figure 2. *Clavaspis virolae* Wei, Schneider, Normark & Normark sp. nov. Adult female, full body view, illustrated from the holotype (D6676B); expanded view of pygidium, illustrated from the holotype (D6676B).

sal angle of L2, posterior-most paraphysis slightly longer than L1; 2 smaller clavate paraphyses arising from mesal margin of L3. **Anal opening** oval, 8–13 μm in length, 4–6 μm in width, positioned 20–23 μm from base of L1, located within posterior third of pygidium. **Perivulvar pores** absent.

Remarks. This species is placed in the genus *Clavaspis* MacGillivray on the basis of the robust clavate paraphyses, small anal opening, and basal sclerosis of L1, resembling that of *Clavaspis ulmi* (Johnson). The paraphyses are not as elaborately developed as those of most *Clavaspis* species, but they are more developed than some species that have recently been recognized as members of *Clavaspis* on the basis of molecular phylogenetics – *C. perseae* (Davidson) and *C. patagonensis* Schneider, Claps, Wei, Normark & Normark (Normark et al. 2014; Schneider et al. 2020). *Clavaspis virolae* is similar to *Clavaspis ulmi*, but differs in having L2 present, plates fewer, dorsal macroducts fewer, medial paraphysis of first space less developed, and ventral macroduct orifices larger than those of dorsal macroducts. *Clavaspis virolae* also resembles species of *Hemiberlesia* Cockerell, especially *H. ignobilis* Ferris and *H. ocellata* Takagi & Yamamoto, but differs in having a smaller anal opening and fewer plates. It further differs from *H. ignobilis* in having L2 present and ventral macroduct orifices larger than those of dorsal macroducts, and from *H. ocellata* in having 2 pairs of conspicuous paraphyses present, L3 absent, and notching of L1 and L2 less deep. Yet another genus that *C. virolae* resembles is *Diaspidiotus* Berlese: the axes of L1 and L2 seem to converge slightly, causing the species to key out as *Quadraspidotus* MacGillivray, now a synonym of *Diaspidiotus*, in Ferris's (1942) key. But this is not as good a fit, as *Diaspidiotus* species lack basal scleroses of L1. It is also biogeographically less plausible, as *Diaspidiotus* is overwhelmingly a temperate Holarctic group. There exist Neotropical species assigned to *Diaspidiotus*, but these may be misplaced. The only such species reported from Panama, *D. crescentiae* Ferris, has a large anal opening and basal scleroses of L1, and is best regarded as *Hemiberlesia crescentiae* (Ferris), new combination.

Host plant. *Virola multiflora* (Standl.) A.C.Sm. (family Myristicaceae)

Etymology. The specific epithet is the Latin genitive of the host plant genus, *Virola*.

Distribution. Panama (Colón).

***Davidsonaspis tovomitae* Wei, Schneider, Normark & Normark sp. nov.**

<http://zoobank.org/EE712529-F0A9-4EAA-AB50-20F29BB27DF7>

Figure 3

Material examined. Holotype: PANAMA • 1 adult female; Parque Nacional San Lorenzo Canopy Crane, Colón; 9.2802°N, 79.9754°W; 12.vi.2012, GE Morse & BB Normark leg.; on *Tovomita longifolia*; MIUP (D3919A). **Paratype:** PANAMA • 1 adult female; Parque Nacional San Lorenzo Canopy Crane, Colón; 9.2802°N, 79.9754°W; 15.i.2015; DA Peterson, GE Morse, H Shapiro, S Trujillo leg.; on *Tovomita longifolia*; UMEC (D6433A).

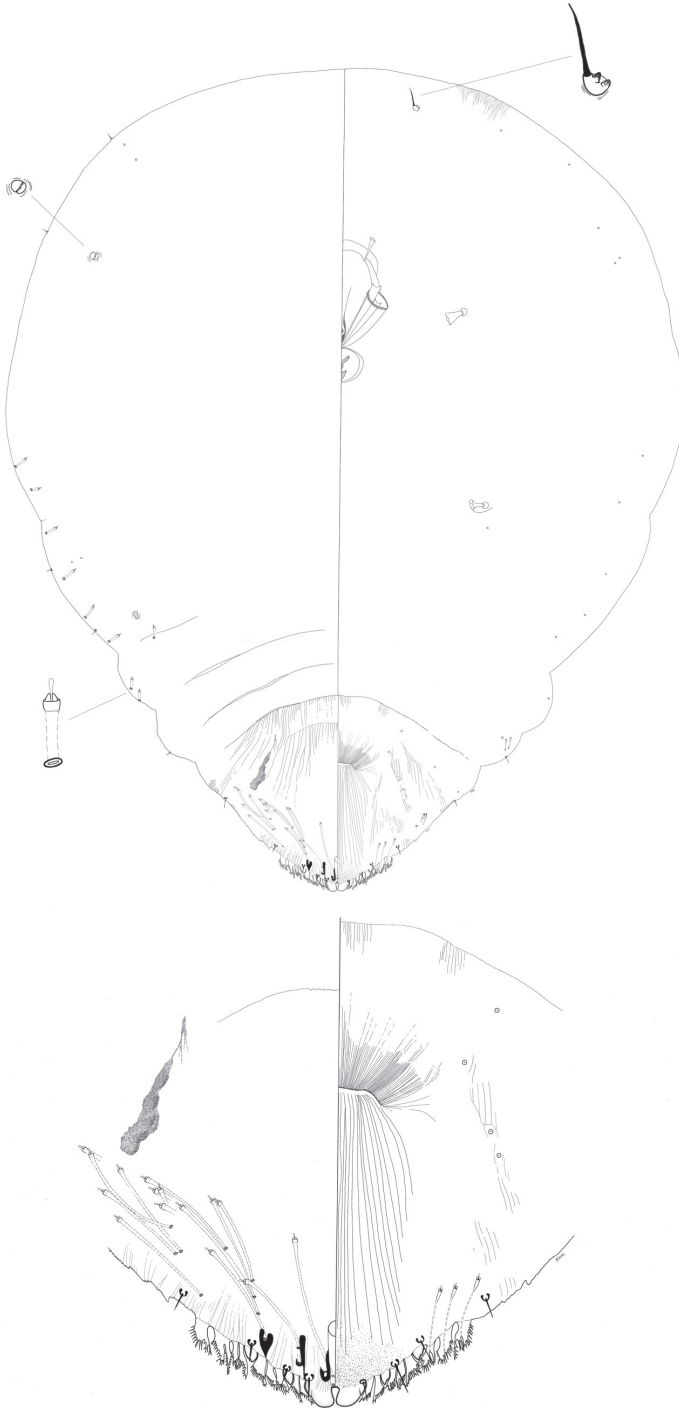


Figure 3. *Davidsonaspis tovomitae* Wei, Schneider, Normark & Normark sp. nov. Adult female, full body view, illustrated from the holotype (D3919A); expanded view of pygidium, illustrated from the holotype (D3919A).

Description ($N = 2$). **Adult female** not pupillarial. Appearance in life not recorded. Slide-mounted adult female 870–1060 μm long, 670–790 μm wide, broadest at mesothorax. Body outline broadly obovate. Antennae simple, each with one conspicuous long seta. Distance between antennae 160 μm . Without any disc pores associated with anterior or posterior spiracles. **Lobes.** Pygidium with 3 pairs of lobes extending out from posterior margin, well sclerotized. L1 large, apically convergent, each lobe oval in shape, with minute notch near midpoint of outer margin; L2 distinctly smaller than L1, with 1 or 2 small notches on outer margin; L3 similar in size and shape to L2, with 1 or 2 notches on outer margin and 0 or 1 notch on inner margin. **Plates.** Without plates between L1; with 2 plates between L1 and L2, flabellate, apically fringed, each slightly longer than L1, much longer than L2; three between L2 and L3, flabellate, apically fringed, each plate longer than L3; three anterior to L3, branched and elaborately fringed on apical and lateral margins, much longer than L3, each with internal microduct. **Ducts.** Dorsal macroducts of 1-barred type, dorsal submarginal macroducts about same size as marginal macroducts, long (120–140 μm) and narrowly ribbonlike, with minute orifices, few, only 10–15 on each side of pygidium. Also, with faux duct orifice on dorsum immediately anterior of L1 – circular structure slightly larger than duct orifices, but without duct. Pre-pygidial dorsal macroducts few, shorter than those on pygidium, confined to margin and submargin, absent on segments III and IV, two present on each side of segments II, I, metathorax, and mesothorax. Ventral microducts shorter and thinner than dorsal macroducts, with a few present on submargin of each segment from abdominal segment V to prothorax. **Paraphyses.** Three pairs of paraphyses present on each side of pygidium, variable, with lateral member of each pair often minute or absent. Medial pair of paraphyses anterior of L1, medial member of pair arising from near inner angle of L1, extending nearly to anus and terminating in rounded knob, lateral member of pair minute, forming part of sclerotized rim of faux duct orifice; pair of paraphyses between L1 and L2 also with medial paraphysis much larger than lateral paraphysis; pair between L2 and L3 usually about equal to each other in length, lateral member of pair sometimes obsolete. **Anal opening** nearly circular, maximum diameter 8 μm , located 23–25 μm (about 3 times diameter) from base of L1. **Perivulvar pores** absent.

DNA sequences. DNA sequences from 3 loci of the holotype of *Davidsonaspis tovoimitae* have been published: the large ribosomal subunit (28S; GenBank accession number KY219920), elongation factor 1-alpha (EF-1 α ; KY221745), and carbamoylphosphate synthetase (CAD; MH916177). The small ribosomal subunit (16S) sequences of the primary bacterial endosymbiont, *Uzinura diaspidicola*, of the holotype has also been published (KY220578).

Informal synonyms. The holotype of *D. tovoimitae* has appeared in published phylogenetic trees, where it was labeled “Davidsonaspis ud3919” (Schneider et al. 2018) or “Davidsonaspis undesr” (Normark et al. 2019).

Remarks. The only other known species in this genus is *Davidsonaspis aguacatae* (Evans, Watson, and Miller), found on avocados in Mexico. *D. aguacatae* had originally been assigned to *Abgrallaspis* Balachowsky (Evans et al. 2009), but was later reassigned

to a new genus *Davidsonaspis* Normark (Normark et al. 2014). The new species can be distinguished from *D. aguacatae* in having a series of 3 plates anterior to L3, each as broad as L3 and elaborately fringed on apical and lateral margins; in *D. aguacatae*, plates anterior to L3 are narrower than L3 and only slightly fringed. *D. tovomitae* otherwise closely resembles *D. aguacatae*, and the two species form a clade in published molecular phylogenetic trees (Schneider et al. 2018; Normark et al. 2019). The structure we refer to as a faux duct orifice anterior of L1 is illustrated by Evans et al. (2009) but not mentioned in their description. In one of their 2 illustrations of the pygidium of *D. aguacatae* the structure is shown with a central dot, as if it were the circular base of a seta, but in *D. tovomitae* no seta is present there.

Host plant. *Tovomita longifolia* (Rich.) Hochr. (family Clusiaceae)

Etymology. The specific epithet is the Latin genitive of the host plant genus, *Tovomita*.

Distribution. Panama (Colón).

***Rungaspis neotropicalis* Wei, Schneider, Normark & Normark sp. nov.**

<http://zoobank.org/02D416A8-3589-4AC4-877A-3F3A88E7C59B>

Figures 4, 5

Material examined. Holotype: PANAMA • 1 adult female; Parque Nacional San Lorenzo Canopy Crane, Colón; 9.2802°N, 79.9754°W; 20.vi.2012; GE Morse & BB Normark leg.; on *Marila laxiflora* Rusby; MIUP (D4168I). **Paratypes:** • 4 adult females; same data as holotype; USNM (D3953K, D4168B, D6550C, D6552B); • 5 adult females; same data as holotype; UMEC (D3953J, D3953P, D3995B, D4168E, D6703C).

Description. Adult female ($N = 10$) in some cases pupillarial, enclosed within sclerotized cuticle of 2nd instar; some individuals non-pupillarial. Appearance in life not recorded. Slide-mounted adult female 350–610 µm long (holotype 540 µm, median 540 µm), 280–500 µm wide (holotype 410 µm, median 420 µm), broadest at mesothorax. Body outline broadly oval, with slight indentation between prothorax and mesothorax. Derm membranous throughout at maturity in pupillarial individuals; cephalothorax and pygidium becoming sclerotized at maturity in some non-pupillarial individuals. Antennae simple, each with one long seta. Distance between antennae 51–73 µm. Eye a submarginal dorsal tubercle on prothorax. Without disc pores associated with anterior or posterior spiracles. Venter of mesothorax with about 6 transverse, irregular rows of sclerotized spicules in submedial area, posterolaterad of mouthparts.

Lobes. Pygidium with 1 or 2 pairs of lobes; L1 well developed, subquadrate, with parallel inner margins separated by exceedingly narrow space, lobes slightly longer than wide, rounded apically, with 1 large notch near apex on lateral margin and 0–1 notch near apex on medial margin; L1 each with well-developed basal sclerosis, slightly narrower and longer than lobe; L2, when fully developed, forming a small, sclerotized projection, about one-third length of L1 and much narrower, without notches; L2 often absent or represented by a membranous projection or low, sclerotized point; L3 absent.

Plates. With 2 narrow, elongate plates in first space, slightly fringed, with a few tines,

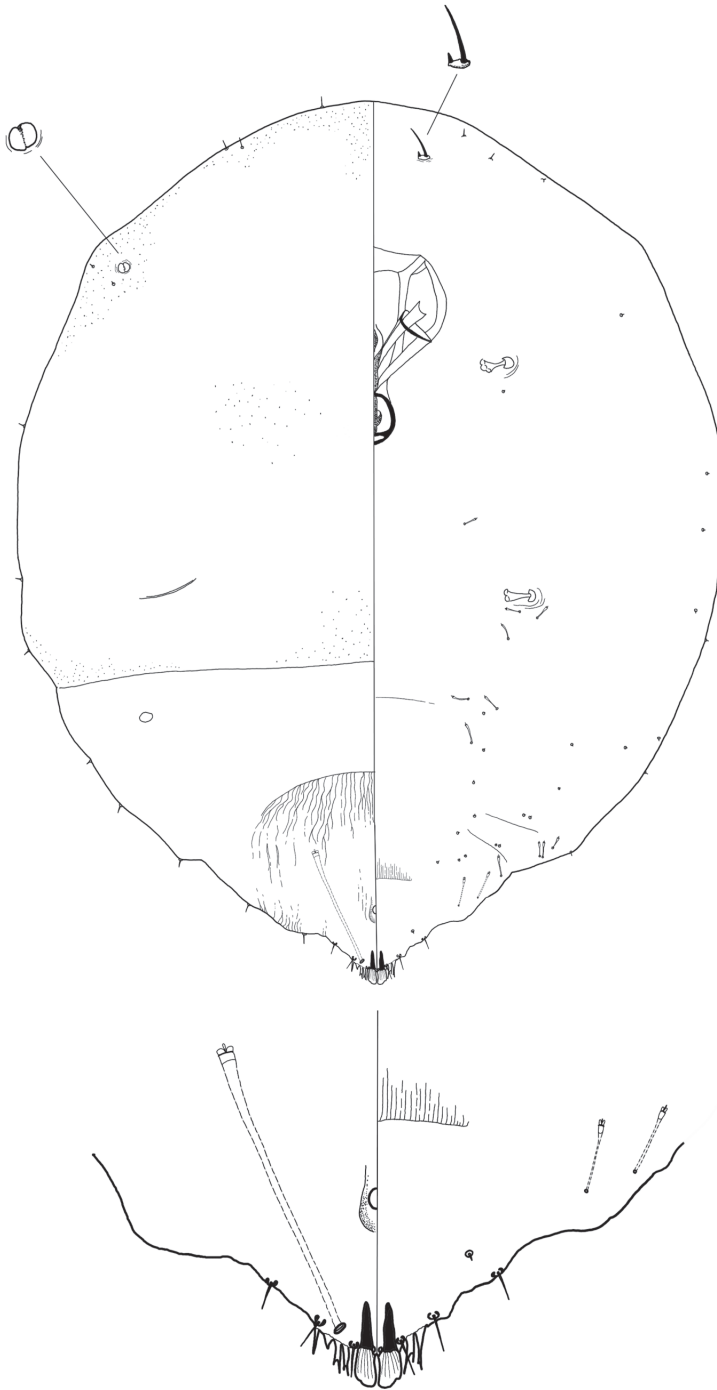


Figure 4. *Rungaspis neotropicalis* Wei, Schneider, Normark & Normark sp. nov. Adult female, full body view, illustrated from the holotype (D4168I); expanded view of pygidium, illustrated from the holotype (D4168I).

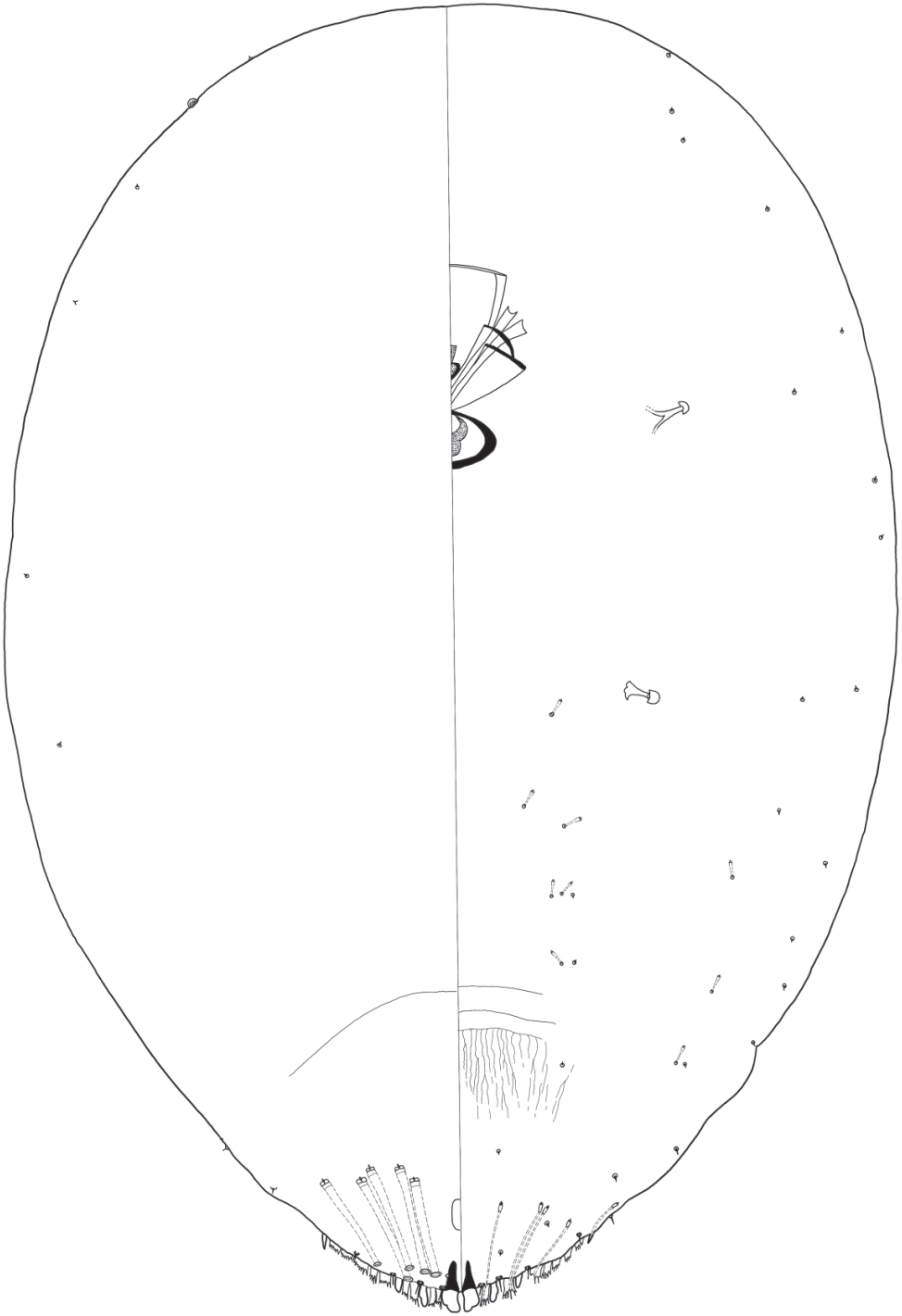


Figure 5. *Rungaspis neotropicalis* Wei, Schneider, Normark & Normark sp. nov. Second-instar female, full body view.

and 1 or 2 simple plates laterad of position of L2; no other plates present. **Ducts.** Dorsal macroducts of 1-barred type, slender, much broader than ventral microducts, few in number, restricted to margin of pygidium; with 1–3 (usually 2) ducts in first space, 0–2 (usually 1) immediately laterad of L2, and 0–1 (usually 0) laterad of seta marking segment VI, making a total of only 1–4 ducts (usually 4) on each side of pygidium. Ventral microducts exceedingly narrow, present along pygidial margin and scattered in submedial areas of other segments. **Paraphyses** absent. **Anal opening** subcircular, 8–11 μm in length and width, positioned 17–37 μm from base of L1, located within posterior half of pygidium. **Perivulvar pores** absent.

Second-instar female ($N = 8$) Appearance in life not recorded. Slide-mounted second-instar female 340–620 μm long (median 460 μm), 270–400 μm wide (median 340 μm), broadest at mesothorax. Body outline oval. Antennae simple, each with one long seta. Distance between antennae 54–96 μm . Without disc pores associated with anterior or posterior spiracles. **Lobes.** Pygidium with 3 pairs of well-developed lobes; L1 subquadrate, with parallel inner margins separated by exceedingly narrow space, lobes slightly longer than wide, rounded apically, with 1 large notch near apex on lateral margin and 0–1 notch near apex on medial margin; L1 each with well-developed basal sclerosis, slightly narrower and longer than lobe; L2 nearly as long as L1 but much narrower, rounded at apex, without notches or with slight notch on lateral margin; L3 subtriangular, slightly narrower and shorter than L2, without notches. **Plates.** Without plates between L1. With 2 narrow plates in first space, 2 broader plates in second space, and a series of 5 or 6 plates laterad of L3. All plates similar in length to adjacent lobes and fringed at apex, with plates anterior to L3 becoming progressively lower and less fringed anteriorly. Plates of the first and second spaces subtended by conspicuous ducts, about a third as wide as dorsal macroducts and nearly as long, much wider and longer than ventral microducts. **Ducts.** Dorsal macroducts of 1-barred type, broad, all submarginal; with 2 ducts in a short row arising from first space, 2 in the second space, and 1 laterad of L3, making a total of 5 on each side of pygidium. Ventral microducts exceedingly narrow, short, present along pygidial margin and scattered in submedial areas of other segments. **Paraphyses** absent. **Anal opening** oval to subcircular, 8–14 μm in length, 7–8 μm in width, positioned 23–40 μm from base of L1, located within posterior half of pygidium.

DNA sequences. Several DNA sequences of *Rungaspis neotropicalis* have been published, including fragments of 3 gene regions: the large ribosomal subunit (28S; D3953H, D3953J, D3953R, D3953V, D4168B, D4168E, D4168I, D4168J, D4249H, D4249L; Genbank accession numbers MT677181–MTT677184, MTT677266–MTT677296, MT677294), elongation factor 1- α (EF-1 α ; D3953J, D3953V, D3953W, D3995B; D4168A, D4168B, D4168E, D4168J, D4249H, D4249L; KY221749, MH915953, MH915954, MT64783, MT642022, MT642025–MT642029, MT642031, MT642032), and cytochrome oxidase I and II (COI–II; D3953H, D3953J, D3953R, D3953V, D3995B, D4168A, D4168B, D4168E, D4168I, D4168J, D4249G, D4249H, D4249L; KY221137, MH916549, MT676875–MT676878, MT676946–MT676950, MT676971, MT676972, MT676974).

Informal synonyms. Specimens of *R. neotropicalis* have appeared in published analyses and phylogenetic trees, where they were labeled “UG3995 ud3995” (Schneider et al. 2018; Normark et al. 2019), “UG3953 ud3953” (Schneider et al. 2018), or “Rungaspis ud3995” (Peterson et al. 2020).

Remarks. This is an unusual species both in its life history, showing intraspecific variation in the pupillarial habit, and in its biogeography, having affinities to African species. Some slide-mounted specimens are unequivocally pupillarial, having well-developed 1st instars inside of adult females that are themselves inside of 2nd-instar cuticles. More often than not, these adult females are flipped inside their puparia, with their head at the posterior end of the puparium. Other specimens are apparently non-pupillarial, and some of these have a sclerotized cephalothorax, a feature not seen, to our knowledge, in adult females of any pupillarial species. We had originally intended to describe the pupillarial and non-pupillarial forms as two different species, but the three sequenced gene regions show no differences between them and there are no consistent morphological differences either; therefore, we consider them to comprise a single species that includes both pupillarial and non-pupillarial developmental phenotypes. The second instar has a more completely developed secretory system than the adult, with more ducts, plates, and lobes – a pattern typical of pupillarial species and opposite to what is typical of non-pupillarial species. This may imply that this species is derived from a pupillarial ancestor and that the non-pupillarial form represents a secondary loss of the pupillarial habit.

Molecular phylogenetic studies have shown that *R. neotropicalis* has affinities with African species. Probably the best analysis is a recent study of Aspidiotini (Schneider et al. 2018), which shows *R. neotropicalis* nested within a clade of African *Aspidiotus* species (*A. fularum* Balachowsky, *A. elaeidis* Marchal, and an undescribed species from Uganda), with *R. neotropicalis* sister to *A. fularum*. *R. neotropicalis* was also included in a broader study of Diaspididae (Normark et al. 2019), where it appears in a clade that consists mostly of African species (*A. elaeidis*, *Selenaspis kamerunicus* Lindinger, *S. articulatus* Morgan, *Dynaspidotus rhodesiensis* (Hall), and *Entaspidotus lounsburyi* (Marlatt)) but that also includes one other New World species (*Rugaspidotus arizonicus* (Cockerell)). It is possible that *R. neotropicalis* is an African species that is invasive in the Neotropics, similar to *Selenaspis articulatus*, which is the single most abundant diaspidid species at the site where *R. neotropicalis* was collected (Peterson et al. 2020). But if this species is from Africa, it does not seem to have ever been found there. Based on the available evidence we regard it as a native Neotropical species, perhaps one resulting from an ancient trans-Atlantic dispersal event.

We tentatively place this species in the genus *Rungaspis* Balachowsky. *Rungaspis* presently comprises four species distributed in Africa and the southwestern Palearctic. *Rungaspis neotropicalis* resembles the other species of *Rungaspis* in having large basal scleroses of L1, reduced L2 and L3, cephalothoracic sclerotization at maturity (in non-pupillarial specimens), dorsal ducts with sclerotized orifices, and simplified plates located only in the first and second interlobular spaces. African *Rungaspis* species differ from *R. neotropicalis* in having conical plates without fringes (vs. slightly fringed)

and numerous narrow dorsal ducts (vs. few broad ducts). We considered three other possible placements for the species. One was the genus *Aspidiotus* Bouché. *Rungaspis neotropicalis* resembles *Aspidiotus* species in having basal scleroses of L1 and fringed plates, and molecular evidence indicates that its closest known relative is an African species of *Aspidiotus*. But we concluded that *R. neotropicalis* shares a greater number of characters with *Rungaspis*. Furthermore, *Aspidiotus* is radically non-monophyletic, and the mostly African clade to which *R. neotropicalis* belongs should probably be recognized as a distinct genus anyway (Schneider et al. 2018). Another possible placement we considered was the genus *Helaspis* McKenzie. *Helaspis* is a New World genus that “appears to suggest *Aspidiotus* more strongly than any known genus” (McKenzie 1963). With *R. neotropicalis* it shares basal scleroses of L1 and a sclerotized cephalothorax. But *Helaspis* has other extraordinary features – conical plates and bilobed L3 – that seem to indicate an affinity with the tribe Gymnaspidini rather than Aspidiotini (Normark et al. 2019). *Rungaspis neotropicalis* lacks these characters and is clearly a member of Aspidiotini. We also considered erecting a new genus for *R. neotropicalis* – this is the course taken by many diaspidid systematists faced with such an unusual species – but we concluded that that was not appropriate in this case given the evidence for affinity with *Rungaspis*.

Morphologically, *R. neotropicalis* also closely resembles *Aspidiotus rhusae* (Brain), a pupillarial species known from South Africa. The two species share a similar overall body shape, L1 with basal scleroses, absence of L3, absence of perivulvar pores, and presence of just a few slightly fringed plates and just a few broad, one-barred dorsal ducts near the pygidial margin. Characters that distinguish *R. neotropicalis* from *A. rhusae* are as follows (character of *A. rhusae* given in parentheses): L2 much narrower than L1 or absent (L2 nearly as broad as L1); space between L1 exceedingly narrow, without plates (space between L1 with pair of apically fringed plates); 4 or fewer dorsal ducts present on each side of pygidium (5 or more ducts present); 1–3 microducts present near each posterior spiracle (cluster of 5 or more ducts in this position); transverse rows of minute spicules present on mesothorax posterolaterad of mouthparts (absent); body margin slightly indented between prothorax and mesothorax (entire); eye a submarginal dorsal tubercle (eye marginal). The Neotropical species that *R. neotropicalis* most closely resembles is *Aspidiella rigida* Ferris. The two species both have L1 with basal scleroses and closely approximated medial margins, other lobes reduced or absent, cephalothorax becoming sclerotized at full maturity, and perivulvar pores absent. Characters that distinguish *Rungaspis neotropicalis* from *Aspidiella rigida* are as follows (character of *A. rigida* given in parentheses): plates present (absent); dorsal ducts of pygidium broad, much broader than ventral microducts, confined to margin and submargin (narrow, similar to ventral microducts, widely scattered); anus in posterior half of pygidium (anterior half).

Our study of Neotropical and African species that resemble *Rungaspis neotropicalis* has further led us to conclude that *Aspidiella rigida* belongs in the genus *Rungaspis*, and we transfer it accordingly: *Rungaspis rigida* (Ferris) comb. nov. Ferris (1941) remarked, “It is with much doubt that this species is here referred to the genus *Aspidiella*. In its pygidial characters it resembles the type genus closely enough except for the entire

absence of plates and the absence of the perivulvar pores... In the heavy sclerotization of the entire body it is peculiar and distinctive.” In each of these characters it resembles *Rungaspis* species more than *Aspidiella* species. Ferris further expressed puzzlement that an Oriental and Australian genus such as *Aspidiella* would include a species that was apparently native to the Neotropics. A biogeographic connection between the Neotropics and Afrotropics is better documented (by *Rungaspis neotropicalis* and in groups such as *Diaspis* Bouché) and less of a surprise.

Host plant. *Marila laxiflora* Rusby (family Calophyllaceae)

Etymology. The specific epithet is a Latin adjective; here it alludes to this species' unusual biogeography as a Neotropical member of a mostly African clade.

Distribution. Panama (Colón).

Selenaspidopsis browni Nakahara, 1984: 936. New country record

Material examined. PANAMA • 1 adult female; Parque Metropolitano Canopy Crane; 8.9944°N, 79.5431°W; 22.i.2015; DA Peterson, GE Morse, H Shapiro, S Trujillo leg.; on *Antirhea trichantha*; MIUP (D6765D); • 1 adult female; same data as previous; UMEC (D6765G).

Host plant. *Antirhea trichantha* (Griseb.) Hemsl. (Rubiaceae)

Distribution. Panama (Parque Metropolitano).

Key to species of Aspidiotini from Panama based on adult females

The key incorporates some modified excerpts drawn from Ferris (1942), Deitz and Davidson (1986), Smith-Pardo et al. (2012) and Normark et al. (2014). The key excludes *Hemiberlesia paucitatis* (McKenzie) due to insufficient information.

- 1 With deep thoracic constriction between prothorax and mesothorax or mesothorax and metathorax **2**
- Without deep thoracic constriction on thorax..... **3**
- 2 With deep thoracic constriction between mesothorax and metathorax; L3 spur-shaped, distinctly different from L2; perivulvar pores in 2 groups on pygidium (*Selenaspis*) ***Selenaspis articulatus* (Morgan)**
- With deep thoracic constriction between prothorax and mesothorax; L3 similar in shape to L2; perivulvar pores in 4 groups on pygidium (*Selenaspidopsis*)..... ***Selenaspidopsis browni* Nakahara**
- 3 Paraphyses absent on pygidium..... **4**
- Paraphyses present on pygidium..... **11**
- 4 Perivulvar pores absent..... **5**
- Perivulvar pores present..... **8**
- 5 With L3 definitely developed and easily distinguishable from pygidial margin.... **6**
- L3 lacking, at most represented by crenulations of pygidial margin (*Rungaspis*) **7**

- 6 L4 absent, plates beyond L3 simple (*Chortinaspis*)..... *Chortinaspis subchortina* (Laing)
- L4 present, plates beyond L3 apically fringed (*Nigridiapis*)..... *Nigridiapis armigera* Ferris
- 7 Dorsal ducts present in submedian areas of pygidium; entire body strongly sclerotized at maturity *Rungaspis rigida* (Ferris) **comb. nov.**
- Dorsal ducts absent from submedian areas of pygidium; cephalothorax slightly sclerotized at maturity or body membranous *Rungaspis neotropicalis* **sp. nov.**
- 8 L3 well developed and similar in shape to L2; pygidial macroduct orifices distinctly wider than ventral microducts; any plates present anterior to L3 deeply fringed (*Aspidiotus*) **9**
- L3 poorly developed and dissimilar in shape to L2; pygidial macroduct orifices not much wider than ventral microducts; any plates present anterior to L3 simple or minimally fringed (*Aspidiella*) **10**
- 9 Pre-pygidial marginal macroducts absent; with total of 15–29 dorsal macroducts on each side of body..... *Aspidiotus destructor* Signoret
- Pre-pygidial marginal macroducts present; with total of 22–38 dorsal macroducts on each side of body..... *Aspidiotus excisus* Green
- 10 Plates present anterior to L3; plates between L1 equal to or slightly longer than lobes, clearly visible; L3 represented by unsclerotized point..... *Aspidiella hartii* (Cockerell)
- Plates absent anterior to L3; plates between L1 about same length as lobes, somewhat obscured by lobes; L3 represented by swelling of margin only slightly larger than protrusions along remainder of pygidium.... *Aspidiella sacchari* (Cockerell)
- 11 Prosoma of mature female reniform; with 3 long fleshy plates laterad of L3; paraphyses short and indistinct; abdominal segments I–III with submarginal groups of macroducts (*Aonidiella*)..... *Aonidiella orientalis* (Newstead)
- Prosoma of mature female elongate, round, oval or turbinate, not reniform; combination of plates laterad of L3, paraphyses, and pre-pygidial macroducts not as described above **12**
- 12 Body elongate and more or less parallel-sided, 3× or 4× as long as wide (*Pseudischnaspis*) **13**
- Body round, turbinate, or oval, less than 3× as long as wide..... **14**
- 13 Body long and quite slender, cephalic margin almost straight; apical angle of pygidium more than 90 degrees; perivulvar pores in 5 groups..... *Pseudischnaspis acephala* Ferris
- Body elongate but broad, cephalic margin broadly rounded; apical angle of pygidium less than 90 degrees; perivulvar pores in 4 groups..... *Pseudischnaspis bowreyi* (Cockerell)
- 14 Most paraphyses shorter than or similar in length to L1, generally less than 2× its length; sometimes with 1 pair of paraphyses longer than L1 arising from first

- interlobular space and terminating in an abruptly swollen knob; never bearing paraphyses anterior to position of L3 **15**
- Paraphyses typically longer than L1, often exceeding 2× its length; with more than 1 pair of paraphyses exceeding length of L1, which either remain thin throughout or gradually expand apically; sometimes bearing paraphyses anterior to position of L3 **28**
- 15 Anal opening relatively large, distance between posterior edge of opening and base of L1 usually not more than 2× diameter of anal opening; plates usually with fringed apices (except 1 species bearing simple plates) (*Hemiberlesia*) **16**
- Anal opening small, distance between posterior edge of opening and base of L1 usually greater than 2× diameter of anal opening; plates simple or minimally fringed (*Clavaspis*) **24**
- 16 Perivulvar pores absent **17**
- Perivulvar pores present **21**
- 17 Having the following combination of characters, plates in the first and second interlobular spaces all simple, L2 and L3 entirely absent
 ***Hemiberlesia crescentiae* (Ferris) comb. nov.**
- Without this combination, at least some fringed plates present in first and second interlobular spaces, L2 and L3 at least indicated by a hyaline plate-like lobe **18**
- 18 Plates anterior to position of L3 absent, simple, or fringed, but without protruding central microduct **19**
- Plates anterior to position of L3 trifurcate, consisting of central protruding marginal microduct and 2 lateral processes that may be simple or fringed **20**
- 19 L1 with short, broad basal sclerosis, projecting anteriorly; L2 and L3 sclerotized and distinct from plates ***Hemiberlesia musae* Takagi & Yamamoto**
- L1 without broad basal sclerosis (with paraphysis-like sclerotization at base of medial or lateral margin in some specimens); L2 and L3 hyaline and plate-like...
 ***Hemiberlesia ignobilis* Ferris**
- 20 L2 and L3 hyaline; L1 subsemicircular, divergent; each plate between L1 and L2 with 1 associated microduct ***Hemiberlesia andradae* Okusu & Normark**
- L2 and L3 sclerotized; L1 with 1 lateral notch, closely appressed and parallel; each plate between L1 and L2 with 2 or 3 associated microducts
 ***Hemiberlesia diffinis* (Newstead)**
- 21 L2 definitely developed, sclerotized, distinctly dissimilar to a pygidial plate **22**
- L2 represented by unsclerotized point or lobe, similar in appearance to a pygidial plate **23**
- 22 L3 represented by sclerotized point without notches; plates beyond L3 variously fringed; eyes represented by distinct spurs
 ***Hemiberlesia cyanophylli* (Signoret)**
- L3 pointed but with at least 1 lateral notch and 0-1 medial notches; plates beyond L3 minimally fringed; eyes indistinct, not represented by spurs
 ***Hemiberlesia mendax* McKenzie**

- 23 Plates deeply fringed, definitely exceeding L1 in length, all similar in size and shape including plates beyond L3; L3 sclerotized ***Hemiberlesia palmae* (Cockerell)**
- Plates shallowly fringed, only slightly exceeding L1 in length, varying in size and shape, plates beyond L3 simple; L3 unsclerotized ***Hemiberlesia lataniae* (Signoret)**
- 24 Paraphyses arising from lateral angle of L1 typically elongate, slender and terminating in a sclerotized swollen knob **25**
- Paraphyses arising from lateral angle of L1 clavate but not terminating in a sclerotized swollen knob ***Clavaspis virolae* sp. nov.**
- 25 Perivulvar pores present, at least 1 pore per side **26**
- Perivulvar pores entirely absent **27**
- 26 With 2 plates between L1 and L2; submarginal groups of microducts form semi-circle around head, thorax and pre-pygidial abdominal segments; with at least 2 large macroducts on mesothorax ***Clavaspis selvatica* sp. nov.**
- With 1 plate between L1 and L2; submarginal groups of microducts not organized in obvious semicircular ring; with only 1 large macroduct on mesothorax.... ***Clavaspis coursetiae* Marlatt**
- 27 With L1 alone being well developed; plates fringed and equal in length to L1 ***Clavaspis herculeana* (Cockerell & Hadden)**
- With 4 pairs of well-developed lobes; plates simple and much shorter than L1 ... ***Clavaspis dentata* Ferris**
- 28 Paraphyses arising only from basal angles of lobes or position of obsolete lobes, never from within interlobular spaces; paraphyses in first interlobular space typically about 2× longer than those in second interlobular space (although nearly identical in length for 1 species); perivulvar pores absent (*Palinaspis*) **29**
- With at least 1 paraphysis arising from an interlobular space; paraphyses in first and second interlobular spaces not following this pattern; perivulvar pores present or absent **31**
- 29 Plates reduced to short membranous lobes, rounded apically ***Palinaspis lobulata* Ferris**
- Plates present or absent, if present, elongate **30**
- 30 Plates entirely lacking; with 1 notch on each side of each lobule L1; L2 entirely lacking or at most represented by very slight irregularity of margin ***Palinaspis sordidata* Ferris**
- Plates well-developed; with 1 notch on outer margin of each lobule of L1; L2 represented only by low, slightly sclerotized swelling of margin ***Palinaspis barbata* Ferris**
- 31 Having combination of 3 pairs of pygidial lobes, 1 paraphysis arising from first interlobular space, and lacking paraphyses beyond L3 ***Davidsonaspis tovoimitae* sp. nov.**
- Without above combination; paired paraphyses in first space arise from outer angles of lobes, with 3–4 well-developed lobes and with or without paraphyses present beyond L3..... **32**

- 32 Pygidial margin anterior to L4 not heavily sclerotized, without series of short paraphyses; plates anterior to L3 conspicuous, branched, fringed or clubbed, usually exceeding length of lobes; anus usually located closer to posterior margin than to vulva (*Chrysomphalus*) **33**
- Pygidial margin anterior to L4 heavily sclerotized, often with series of short paraphyses; plates anterior to L3 not as long and conspicuous, may be simple, branched, fringed or spine-like, usually not exceeding length of lobes; anus usually in center of pygidium or closer to vulva than posterior margin **34**
- 33 First 2 plates anterior to L3 with clavate apices; pre-pygidial segments lacking dorsal cluster of 4 or more ducts ***Chrysomphalus dictyospermi* Morgan**
- First 2 plates anterior to L3 with fringed apices; abdominal segment II with dorsal cluster of 5 or more ducts along lateral margin ***Chrysomphalus aonidum* (Linnaeus)**
- 34 Pygidium long and narrow, sharply tapering to acute apical point, lateral margins slightly concave; with 3 pairs of pygidial lobes, L4 reduced to point or absent; margin anterior to L4 heavily sclerotized; most paraphyses between L1 to L4 elongate (*Acutaspis*) **35**
- Pygidium short and broad, not tapering apically to acute point, lateral margins convex; usually with 4 or 5 pairs of pygidial lobes, L4 usually well developed; margin anterior to L4 lightly to heavily sclerotized; paraphyses between L1 to L4 variable in shape **38**
- 35 Lateral thoracic margin produced into distinct point or rounded umbo near posterior spiracles **36**
- Lateral thoracic margin without such point or umbo, at most with small, sclerotized spot in this position **37**
- 36 Lateral thoracic margins produced into very pronounced umbos; sclerotization of derm developed in sharply defined marginal zone extending from umbos, along sides, and across pygidium ***Acutaspis umbonifera* (Newstead)**
- Umbos quite small, sclerotization forming similar pattern as above, but rather weakly developed ***Acutaspis perseae* (Comstock)**
- 37 1 very long paraphysis arising from outer angle of L3, 1 or 2 small paraphyses arising from base of L4; pre-pygidial dorsal ducts present; derm membranous except for pygidium ***Acutaspis reniformis* (Cockerell)**
- 1 long paraphysis arising from outer angle of L3, small paraphyses absent from base of L4; pre-pygidial dorsal ducts absent; derm strongly sclerotized at full maturity ***Acutaspis albopicta* (Cockerell)**
- 38 Pygidium with longest paraphyses arising from lateral angles of lobes; large V-shaped reticulate sclerotized area on abdominal segment VI always present (*Crenulaspidiotus*) **39**
- Pygidium with longest paraphyses arising from interlobular spaces; without large V-shaped reticulate sclerotized area on abdominal segment VI **40**
- 39 With 5 pairs of lobes; with 2 plates anterior to L4; ventral microducts present between L3 and L4; paraphysis formula normally 1-1-1 ***Crenulaspidiotus maurellae* (Laing)**

- With 4 pairs of lobes, without plates anterior to L4; ventral microducts absent between L3 and L4; paraphysis formula normally 1-2-1.....
.....*Crenulaspidiotus sinuatus* (Ferris)
- 40 Anterior head margin of mature female forming distinctly sclerotized prominence, differentiated from lateral margin, resembling a “cap” (*Mycetaspis*) 41
- Anterior head margin of mature female not distinctly sclerotized or differentiated from lateral margin, not resembling a “cap” (*Melanaspis*)..... 44
- 41 Perivulvar pores present in 5 small groups; cephalic area very heavily sclerotized and bearing series of conspicuous setae..... *Mycetaspis sphaerioides* (Cockerell)
- Perivulvar pores absent; cephalic area without series of conspicuous setae..... 42
- 42 L1 each with elongate, tapering basal sclerosis with base about as wide as base of L1 *Mycetaspis personata* (Comstock)
- L1 with basal sclerosis narrow and arising from mesal angle, with base less than half as wide as base of L1 43
- 43 With sclerotized spur on head; longest paraphysis in third interlobular space arising from center of interlobular space, posterior to L4.....
.....*Mycetaspis apicata* (Newstead)
- Without sclerotized spur on head; longest paraphysis in third interlobular space arising near mesal angle of L4*Mycetaspis defectopalus* Ferris
- 44 Perivulvar pores present..... 45
- Perivulvar pores absent..... 47
- 45 Perivulvar pores present in 5 small groups; pygidium moderately acute at apex, lateral margins almost straight; first interlobular space with moderately long, apically swollen paraphysis followed by small process from mesal angle of L2.....
..... *Melanaspis nigropunctata* (Cockerell)
- Perivulvar pores present in 4 small groups; pygidium with lateral margins convergent; first interlobular space with quite long paraphysis followed by very small process from mesal angle of L2..... 46
- 46 L1 with very small basal process, without long paraphysis between them; without long paraphysis arising from outer angle of L3, with short and broad paraphysis arising from inner angle of L4.....*Melanaspis ponderosa* Ferris
- With long paraphysis between L1; with long paraphysis arising from outer angle of L3; without paraphysis arising from inner angle of L4
.....*Melanaspis bondari* Lepage & Giannotti
- 47 Pygidial lobes each with dorsal seta sunk to at least 0.25× length in distinct socket *Melanaspis sulcata* Ferris
- Pygidial lobes with dorsal setae not in distinct sunken sockets..... 48
- 48 With median band of dermal reticulations, squamations or transverse striations (median squamations may be indistinct) 49
- Without median band of dermal reticulations, squamations or transverse striations (median striations, if present, longitudinal) 50
- 49 Third interlobular space with longest paraphysis about equal in length to longest paraphysis in first and second interlobular spaces; venter without long microducts.....*Melanaspis coccolobae* Ferris

- Third interlobular space with longest paraphysis much shorter than longest paraphysis in first space or second interlobular spaces; venter with long microducts along pygidial margin anteriorly and in 2 irregular submarginal rows posterior to ventral pygidial scar..... *Melanaspis squamea* Ferris
- 50 With only 1 paraphysis in third interlobular space; with long microducts in 3 distinct longitudinal bands on each side of vulva on venter *Melanaspis longula* Ferris
- With various numbers of paraphyses in third interlobular space; with long microducts not arranged in distinct longitudinal bands on each side of vulva, or with 2 or fewer such bands..... 51
- 51 Usually with reduced paraphyses in third interlobular space; orifices of macroducts large and conspicuous in dorsal sclerotized areas 52
- Usually with 1 or more well-developed paraphyses in third interlobular space; orifices of macroducts smaller and less conspicuous in dorsal sclerotized areas. 54
- 52 With 3 paraphyses in second interlobular space; ventral seta in middle of or anterior to base of lobe on each L1 *Melanaspis smilacis* (Comstock)
- With 2 paraphyses in second interlobular space; ventral seta laterad of base of lobe on each L1 53
- 53 Orifices of macroducts absent on lateral margin of dorsal sclerotized area 2; paraphyses in third interlobular space nearly equal in length..... *Melanaspis odontoglossi* (Cockerell)
- Orifices of macroducts present on lateral margin of dorsal sclerotized area 2; paraphysis arising from outer angle of L3 slightly longer than others in third interlobular space *Melanaspis eglandulosa* (Lindinger) (in part)
- 54 Anal opening between or only slightly anterior to apices of paraphyses; without definite paraphyses beyond L4 *Melanaspis tenebricosa* (Comstock)
- Anal opening decidedly anterior to apices of all paraphyses; with paraphyses beyond L4 55
- 55 Pygidial margin with 2 or 3 conspicuous, spur-like processes anterior to L4..... *Melanaspis leivasi* (Costa Lima)
- Pygidial margin without spur-like processes anterior to L4..... 56
- 56 Anal opening located within posterior third of pygidium from base of median lobe; with 5 paraphyses in third interlobular space *Melanaspis tenax* (McKenzie)
- Anal opening located near center of pygidium; with 3–4 paraphyses in third interlobular space 57
- 57 Without macroduct orifices on membranous area in third interlobular space; all macroduct orifices with equal diameters..... *Melanaspis latipyga* Ferris
- With macroduct orifices on membranous area in third interlobular space; macroduct orifices in third interlobular space usually smaller in diameter than any macroduct orifices located more mesally (on abdominal segments VI–VIII)..... *Melanaspis eglandulosa* (Lindinger) (in part)

Additional online resources aiding in the identification of Aspidiotini are provided by Schneider et al. (2019) and Dooley (2006).

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A species of the genus *Panophrys* (Anura, Megophryidae) from southeastern Guizhou Province, China

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Abstract

Herein, we describe *Panophrys congjiangensis* **sp. nov.** obtained from the Yueliangshan Nature Reserve, Congjiang County, Guizhou Province, China. Phylogenetic analyses based on the mitochondrial genes 16S rRNA and COI indicated that this new species represented an independent lineage, closely related to *P. leishanensis*. The uncorrected genetic distances between the new species and its closest congener, *P. leishanensis*, were 3.0% for 16S rRNA and 8.4% for COI. The new species is distinguished from its congeners by a combination of the following morphological characteristics (1) medium body size (SVL 28.6–33.4 mm in males and 38.4–40.2 mm in females); (2) a small horn-like tubercle at the edge of each upper eyelid; (3) the tympanum distinctly visible (TD/ED ratio 0.47–0.66); (4) vomerine teeth absent; (5) the tongue not notched behind; (6) a narrow and unobvious lateral fringe on toes; (7) relative finger lengths $II < I < V < III$; (8) rudimentary webs on toes; (9) hindlimbs slender, heels overlapping when thighs are positioned at right angles to the body; (10) two metacarpal tubercles on the palm, with the inner metatarsal tubercle long and oval-shaped; (11) the tibiotarsal articulation reaching the nostril when the leg is adpressed and stretched forward; (12) dorsal skin rough with numerous orange–red granules, ventral surface smooth; (13) a single internal subgular vocal sac present in males; and (14) in breeding males, weak gray–black nuptial pads with black nuptial spines present on the dorsal surface of the bases of the first and second fingers. To date, the new species is only known from the type locality.

* Contributed equally as the first authors.

Keywords

Morphology, new species, *Panophrys*, southwest China, taxonomy

Introduction

The Asian horned toad subfamily Megophryinae (Bonaparte, 1850) is widely distributed in southern China, the southern and eastern Himalayas, and across Indochina to the islands of the Sunda Shelf and the Philippines (Fei and Ye 2016; Mahony et al. 2017; Liu et al. 2018; Frost 2021). The widespread distributions and morphological similarities of subfamily Megophryinae species have long made the discrimination of species in this subfamily controversial (Dubois 1987 “1986”; Rao and Yang 1997; Dubois and Ohler 1998; Jiang et al. 2003; Zheng et al. 2004; Frost et al. 2006; Li and Wang 2008; Fei et al. 2009; Fei and Ye 2016; Chen et al. 2017; Mahony et al. 2017). In recent phylogenetic analyses, subfamily Megophryinae has been recognized as a broadly monophyletic genus (i.e., *Megophrys* sensu lato; Chen et al. 2017; Mahony et al. 2017; Liu et al. 2018). These revisions have recommended that subfamily Megophryinae be divided into seven subgenera under the genus *Megophrys* sensu lato: *Atympanophrys* Tian & Hu, 1983; *Brachytarsophrys* Tian & Hu, 1983; *Megophrys* s.s. Kuhl & Van Hasselt, 1822; *Ophryophryne* Boulenger, 1903; *Pelobatrachus* Beddard, 1908; *Panophrys* Rao & Yang, 1997; and *Xenophrys* Günther, 1864. To resolve these classification conflicts, Li et al. (2020a) suggested to elevate the seven monophyletic subgenera by Mahony et al. (2017) to the level of genera, namely: *Atympanophrys*, *Brachytarsophrys*, *Megophrys*, *Ophryophryne*, *Panophrys*, *Pelobatrachus*, and *Xenophrys*. In this study, we have followed this recommendation (Li et al. 2020a; Frost 2021).

In the most recent revision, 59 species were assigned to the genus *Panophrys* (Frost 2021), 40 of these species were described in the past decade. In total, 55 *Panophrys* species have been described from China (see *Panophrys* species list in Frost 2021), and 11 are known specifically from Guizhou Province, i.e., *Panophrys anlongensis* (Li, Lu, Liu & Wang, 2020), *Panophrys binlingensis* (Ye & Fei, 1995), *Panophrys chishuiensis* (Xu, Li, Liu, Wei & Wang, 2020), *Panophrys jiangi* (Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020), *Panophrys leishanensis* (Li, Xu, Liu, Jiang, Wei & Wang, 2018), *Panophrys liboensis* (Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017), *Panophrys omeimontis* (Liu, 1950), *Panophrys shuichengensis* (Tian & Sun, 1995), *Panophrys spinata* (Liu & Hu, 1973), *Panophrys platyparietus* (Rao & Yang, 1997), and *Panophrys qianbeiensis* (Su, Shi, Wu, Li, Yao, Wang & Li, 2020). All of these species inhabit isolated mountain streams in evergreen broadleaf forests in Guizhou Province. Such isolated conditions may be favorable for species formation. For example, *P. spinata* has historically been recorded from several counties in Guizhou Province (Dafang, Jinsha, Suiyang, Jiangkou, Yinjiang, and Leishan; Wu et al. 1986; Fei et al. 2009). A recent phylogenetic analysis showed that the Suiyang population, originally recorded as *P. spinata*, was genetically closer to *P. spinata* and *Panophrys sangzhiensis* (Jiang, Ye & Fei, 2008). Thus, the Suiyang population was described as a new species, *P. qianbeiensis* (Su et al.

2020). Therefore, the diversity of *Panophrys* may be greater in Guizhou Province than is currently assumed.

During herpetological surveys conducted between 2019 and 2020 in Yueliangshan Nature Reserve, Congjiang County, Guizhou Province, China (Fig. 1), we captured several specimens of an unknown anuran species. Based on morphological characteristics, including body size (i.e., body length < 45 mm) and a small horn-like tubercle at the middle edge of each upper eyelid, these specimens were identified as a species of *Panophrys*, initially *P. minor* (Fei et al. 2009; Fei and Ye 2016). However, subsequent observation indicated that these newly collected specimens differed from any currently described *Panophrys* species. Indeed, molecular phylogenetics, comparative morphology, and bioacoustics data suggest that these specimens represent a previously unknown species. This new species is described herein.

Material and methods

Sampling

A total of 25 specimens were collected in this study: 22 were collected in Congjiang County, Guizhou Province, China, and were identified as an unknown species. The remaining 3 specimens, collected in Kuankuoshui National Nature Reserve, Suiyang County, Guizhou Province, China, were identified as *P. jiangi*. All specimens were fixed in 10% buffered formalin and later transferred to 75% ethanol for preservation. The muscle samples used for molecular analysis were preserved in 95% alcohol and stored at -20 °C. All specimens are housed at Guizhou Normal University (GZNU), Guiyang City, Guizhou Province, China.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from the muscle tissue samples using DNA extraction kits (Tiangen Biotech (Beijing) Co., Ltd.). We amplified and sequenced two mitochondrial genes from each DNA sample: partial 16S ribosomal RNA (16S rRNA), using primers L3975 (5'-CGCCTGTTTACCAAAAACAT-3') and H4551 (5'-CCGGTCT-GAACTCAGATCACGT-3') following Simon et al. (1994); and cytochrome C oxidase I (COI), using primers Chmf4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and Chmr4 (5'-ACYTCRGGRTGRCCRAARAATAATCA-3') following Che et al. (2012). PCR amplifications were performed in 25 µL reaction volumes with the following cycling conditions: an initial denaturing step at 95 °C for five min; 36 cycles of denaturation at 95 °C for 40 s, annealing at 52 °C (for 16S rRNA) or 47 °C (for COI) for 40 s, then extension at 72 °C for 1 min; and a final extension step at 72 °C for 10 min. The purified PCR products were sequenced with both forward and reverse primers using BigDye Terminator Cycle Sequencing Kits, following the manufacturer's instructions, on an ABI Prism 3730 automated DNA sequencer by Tsing KE Biological Technology Co. Ltd. (Chengdu, China). All sequences have been deposited in GenBank (Table 1).

Table 1. Localities, voucher information, and GenBank numbers for all samples used in this study.

ID	Species	Locality	Voucher number	Genbank accession No.	
				16S rRNA	COI
1	<i>Panophrys obesa</i>	Heishiding Nature Reserve, Guangdong, China	SYS a002271	KJ579121	MH406123
2	<i>Panophrys ombrophila</i>	Wuyi Shan, Fujian, China	WUYI2015101	KX856397	–
3	<i>Panophrys cheni</i>	Taoyuandong Nature Reserve, Hunan, China	SYS a002123	KJ560396	MF667904
4	<i>Panophrys dongguanensis</i>	Yinping Shan, Guangdong, China	SYS a002007	MH406654	MH406090
5	<i>Panophrys nankunensis</i>	Nankun Shan, Guangdong, China	SYS a004498	MK524108	MK524139
6	<i>Panophrys wugongensis</i>	Wugongshan Scenic Area, Jiangxi, China	SYS a002610	MK524114	MK524145
7	<i>Panophrys insularis</i>	Nan'ao Island, Guangdong, China	SYS a002169	MF667887	MF667924
8	<i>Panophrys lini</i>	Nanfengmian Nature Reserve, Jiangxi, China	SYS a002128	KJ560416	MF667907
9	<i>Panophrys nanlingensis</i>	Nanling Nature Reserve, Guangdong, China	SYS a001959	MK524111	MK524142
10	<i>Panophrys xiangnanensis</i>	Yangming Shan, Hunan, China	SYS a002875	MH406714	MH406166
11	<i>Panophrys baishanzuensis</i>	Baishanzu National Park, Qingyuan, Zhejiang, China	CIBQY20200719001	MW001150	MT998291
12	<i>Panophrys brachykolos</i>	Hongkong, China	SYS a005563	MK524122	MK524153
13	<i>Panophrys kuatunensis</i>	Wuyi Shan, Jiangxi, China	SYS a003449	MF667881	MF667916
14	<i>Panophrys lishuiensis</i>	Lishui City, Zhejiang, China	CIBWYF00169	KY021418	–
15	<i>Panophrys xianjuensis</i>	Xianju, County, Zhejiang, China	CIBXJ20190801	MN563754	MN563770
16	<i>Panophrys jinggangensis</i>	Jinggang Shan, Jiangxi, China	SYS a004028	MH406780	MH406239
17	<i>Panophrys liboensis</i>	Libo Country, Guizhou, China	GZNU20150813001	MF285253	MW959767
18		Libo Country, Guizhou, China	GZNU20160408007	MF285258	MW959768
19		Libo Country, Guizhou, China	GZNU20160408006	MF285257	MW959769
20		Libo Country, Guizhou, China	GZNU20160408004	MF285256	MW959770
21	<i>Panophrys boettgeri</i>	Longhu Forest Station, Fujian, China	SYS a004126	MH406785	MH406245
22	<i>Panophrys huangshanensis</i>	Huang Shan, Anhui, China	SYS a002702	MF667882	MF667919
23	<i>Panophrys congjiangensis</i> sp. nov.	Yueliangshan Nature Reserve, Congjiang, Guizhou, China	GZNU20200706003	MW959773	MW959761
24		Yueliangshan Nature Reserve, Congjiang, Guizhou, China	GZNU20200706004	MW959774	MW959762
25		Yueliangshan Nature Reserve, Congjiang, Guizhou, China	GZNU20200706005	MW959775	MW959763
26		Yueliangshan Nature Reserve, Congjiang, Guizhou, China	GZNU20200706000	MW959776	MW959764
27	<i>Panophrys leishanensis</i>	Leigong Shan, Guizhou, China	CIBLS20141004003	MK005308	MK005304
28		Leigong Shan, Guizhou, China	SYSa002213	MH406673	MH406113
29		Leigong Shan, Guizhou, China	CIBLS20160610005	MK005309	MK005305
30	<i>Panophrys baolongensis</i>	Baolong, Chongqing, China	KIZ019216	KX811813	KX812093
31	<i>Panophrys wushanensis</i>	Shennongjia Nature Reserve, Hubei, China	SYS a003008	MH406732	MH406184
32	<i>Panophrys tuberogranulata</i>	Badagong Shan, Hunan, China	SYS a004310	MH406801	MH406263
33	<i>Panophrys shimentaina</i>	Shimentai Nature Reserve, Guangdong, China	SYS a002078	MH406656	MH406093
34	<i>Panophrys yangmingensis</i>	Yangming Shan, Hunan, China	SYS a002889	MH406720	MH406172
35	<i>Panophrys jiulianensis</i>	Nankun Shan, Guangdong, China	SYS a003623	MK524103	MK524134
36	<i>Panophrys mirabilis</i>	Huaping Nature Reserve, Guangxi, China	SYS a002193	MH406670	MH406110
37	<i>Panophrys shunhuangensis</i>	Nanshan National Forest Park, Hunan, China	HNNU18NS01	MK836023	MK977594
38	<i>Panophrys acuta</i>	Heishiding Nature Reserve, Guangdong, China	SYS a002159	MF667869	MF667899
39	<i>Panophrys mufumontana</i>	Mufu Shan, Hunan, China	SYS a006390/CIB110012	MK524104	MK524135
40	<i>Panophrys caudoprocta</i>	Badagong Shan, Hunan, China	SYS a004281	MH406795	MH406257
41	<i>Panophrys sangzhiensis</i>	Badagong Shan, Hunan, China	SYS a004307	MH406798	MH406260
42	<i>Panophrys spinata</i>	Leigong Shan, Guizhou, China	SYS a002226	MH406675	MH406115
43	<i>Panophrys qianbeiensis</i>	Huanglian Nature Reserve, Guizhou, China	CIBTZ20190608015	MT651553	MT654520
44	<i>Panophrys binlingensis</i>	Wawu Shan, Sichuan, China	SYS a005313	MH406892	MH406354
45	<i>Panophrys binchuanensis</i>	Jizu Shan, Yunnan, China	KIZ019441	KX811849	KX812112
46	<i>Panophrys angka</i>	Kiew Mae Pan nature trail, Chiang Mai, Thailand	KIZ040591	MN508052	–
47	<i>Panophrys anlongensis</i>	Anlong County, Guizhou, China	CIBAL20190531018	MT823184	MT823261
48	<i>Panophrys omeimontis</i>	Laojun Shan, Sichuan, China	SYS a002741	MH406710	MH406162

ID	Species	Locality	Voucher number	Genbank accession No.	
				16S rRNA	COI
49	<i>Panophrys palpebralespinosa</i>	Pu Hu Nature Reserve, Thanh Hoa, Vietnam	KIZ011603	KX811888	KX812137
50	<i>Panophrys caobangensis</i>	Nguyen Binh, Cao Bang, Vietnam	IEBR 4385	LC483945	—
51	<i>Panophrys dauwimontis</i>	Dawei Shan, Yunnan, China	KIZ048997	KX811867	KX812125
52	<i>Panophrys jingdongensis</i>	Wuliang Shan, Yunnan, China	SYS a003928	MH406773	MH406232
53	<i>Panophrys rubrimera</i>	Lao Cai, Sa Pa, Vietnam	AMSR177676	MF536419	—
54	<i>Panophrys wuliangshanensis</i>	Wuliang Shan, Yunnan, China	SYS a003924	MH406771	MH406230
55	<i>Panophrys fansipanensis</i>	Lao Cai, Sa Pa, Vietnam	VNMN 2018.01	MH514886	—
56	<i>Panophrys hoanglienensis</i>	Lao Cai, Sa Pa, Vietnam	VNMN 2018.02	MH514889	—
57	<i>Panophrys jiangi</i>	Huoqiuba Nature Reserve, Guizhou, China	GZNU20180606020	MW959777	MW959765
58		Kuankuosui Nature Reserve, Guizhou, China	GZNU20070712001	MW959778	MW959766
59		Kuankuosui Nature Reserve, Guizhou, China	CIBKKS20180722006	MN107743	MN107748
60	<i>Panophrys minor</i>	Qingcheng Shan, Sichuan, China	SYS a003209	MF667862	MF667891
61	<i>Panophrys chishuiensis</i>	Chishui, County, Guizhou, China	CIBCS20190518031	MN954707	MN928958
62	<i>Ophryophryne pachyproctus</i>	Beibeng, Xizang, China	KIZ010978	KX811908	KX812153
63	<i>Panophrys yeae</i>	Didong, Medog, Tibet, China	CIB201706MT01	MN963217	MN964312
64	<i>Panophrys zhoui</i>	Rengqinbeng, Medog, Tibet, China	CIBMT171053	MN963207	MN964322
65	<i>Xenophrys vegrandis</i>	West Kameng district, Arunachal Pradesh, India	ZSI A 11605	KY022305	MH647530
66	<i>Ophryophryne elfina</i>	Bidoup Mountain, Lam Dong, Vietnam	ZMMU ABV000454	KY425379	—
67	<i>Ophryophryne gerti</i>	Nui Chua National Park, Ninh Thuan, Vietnam	ITBCZ 1108	KX811917	KX812161
68	<i>Ophryophryne synoria</i>	O'Reang, Mondolkiri, Cambodia	FMNH 262779	MN629394	—
69	<i>Ophryophryne hansi</i>	Phong Dien Nature Reserve, Thua Thien Hue, Vietnam	KIZ010360	KX811913	KX812155
70	<i>Ophryophryne microstoma</i>	Wuhuang Shan,, Guangxi, China	SYS a003492	MK524125	MK524156
71	<i>Megophrys montana</i>	Sukabumi, Java, Indonesia	LSUMZ 81916	KX811927	KX812163
72	<i>Megophrys parallela</i>	—	RMAS 021	KY679897	—
73	<i>Megophrys lancip</i>	Ulubelu, Ngrip, Indonesia	MZB:Amp:22233	KY679891	—
74	<i>Xenophrys medogensis</i>	Medog County, Tibet, China	SYS a002932	MH406725	MH406177
75	<i>Xenophrys robusta</i>	Darjeeling dist, West Bengal, India	SDBDU 2011.1057	KY022314	MH647535
76	<i>Xenophrys glandulosa</i>	Gaoligong Shan,, Yunnan, China	SYS a003758	MH406755	MH406214
77	<i>Xenophrys himalayana</i>	East Siang dist, Arunachal Pradesh, India	SDBDU2009.75	KY022311	—
78	<i>Xenophrys periosa</i>	East Siang dist, Arunachal Pradesh, India	BNHS 6061	KY022309	MH647528
79	<i>Xenophrys monticola</i>	Darjeeling dist, West Bengal, India	SDBDU 2011.1047	KX894679	—
80	<i>Xenophrys zhang</i>	Zhangmu, Xizang, China	KIZ014278	KX811765	KX812084
81	<i>Xenophrys flavipunctata</i>	Hills dist, East Khasi, Meghalaya	SDBDU2009.297	KY022307	MH647536
82	<i>Xenophrys mangshanensis</i>	Longtou glandulosa, Guangdong, China	SYS a002750	MF667866	MF667895
83	<i>Xenophrys maosonensis</i>	Xiaoqiaogou Nature Reserve, Yunnan, China	KIZ016045	KX811780	KX812080
84	<i>Xenophrys oreocrypta</i>	West Garo Hills dist, Meghalaya	BNHS 6046	KY022306	—
85	<i>Xenophrys major</i>	Zhushihe, Yunnan, China	SYSa002961	MH406728	MH406180
86	<i>Xenophrys awub</i>	—	SDBDU2007.161	KY022319	—
87	<i>Xenophrys serchhipii</i>	North dist, Tripura, India	SDBDU 2009.612	KY022323	MH647532
88	<i>Xenophrys zunhebotoensis</i>	—	SDBDU 2009.110	KY022321	—
89	<i>Xenophrys anrae</i>	Changlang dist, Arunachal Pradesh, India	SDBDU 2009.727	KY022318	MH647531
90	<i>Xenophrys numbumaeng</i>	—	SDBDU 2007.041	KY022316	—
91	<i>Xenophrys oropedion</i>	Mawphlang Sacred Forest, Meghalaya, India	SDBDU 2009.299	KY022317	MH647534
92	<i>Xenophrys megacephala</i>	—	ZSI A 11213	KY022315	MH647533
93	<i>Xenophrys dzukou</i>	—	SDBDU2007.106	KY022324	—
94	<i>Xenophrys lekaguli</i>	Pang Si Da National Park, Sa Kaeo, Thailand	FMNH 265955	KY022214	—
95	<i>Xenophrys takensis</i>	—	FMNH 261711	KY022215	—
96	<i>Xenophrys auralensis</i>	Aural, Kampong Speu, Cambodia	NCSM 79599	KX811807	—
97	<i>Xenophrys parva</i>	Zhushihe, Yunnan, China	SYSa003042	MH406737	MH406189
98	<i>Xenophrys aceras</i>	Khao Nan National Park, Nakhon Si Thammarat, Thailand	KIZ025467	KX811925	KX812159
99	<i>Xenophryslongipes</i>	Genting highland, Malaysia	IABHU 21101	AB530656	—
100	<i>Atympanophrys gigantea</i>	Ailao Shan, Yunnan, China	SYS a003883	MH406766	MH406225
101	<i>Atympanophrys shapingsensis</i>	Wawu Shan, Sichuan, China	SYS a005310	MH406890	MH406352
102	<i>Atympanophrys nankiangensis</i>	Nanjiang, Sichuan, China	CIB ZYC517	KX811900	—
103	<i>Atympanophrys wawuensis</i>	Wawu Shan, Sichuan, China	KIZ025799	KX811902	KX812062

ID	Species	Locality	Voucher number	Genbank accession No.	
				16S rRNA	COI
104	<i>Brachytarsophrys feae</i>	Huangcaoling, Yunnan, China	KIZ046706	KX811810	KX812056
105	<i>Brachytarsophrys platyparietus</i>	—	W01395	AY526206	—
106	<i>Brachytarsophrys chuannanensis</i>	Hejiang County, Sichuan, China	SYS a004926	MH406901	MH406364
107	<i>Brachytarsophrys carinense</i>	Dayao Shan, Guangxi, China	Tissue ID: YPX20455	KX811811	KX812057
108	<i>Brachytarsophrys popei</i>	Jinggang Shan, Jiangxi, China	SYS a004209	MK524124	MK524155
109	<i>Brachytarsophrys intermedia</i>	Phong Nha0Ke Bang NP, U Bo, Vietnam	ZFMK 87596	HQ588950	—
110	<i>“Megophrys” dringi</i>	Mulu National Park, Sarawak Gunung, Malaysia	UNIMAS 8943	KJ831317	—
111	<i>Pelobatrachus baluensis</i>	Gunung Kinabalu National Park, Kogopan Trail, Malaysia	ZMH A13125	KJ831310	—
112	<i>Pelobatrachus stejnegeri</i>	Pasonanca Natural Park, Zamboanga, Philippines	KU 314303	KX811922	KX812052
113	<i>Pelobatrachus kobayashii</i>	Gunung, Sabah, Meghalaya	UNIMAS 8148	KJ831313	—
114	<i>Pelobatrachus ligayae</i>	Palawan, Philippines	ZMMU NAP005015	KX811919	KX812051
115	<i>Pelobatrachus kalimantanensis</i>	Kalimantan Selatan, Borneo, Indonesia	MZB. Amph 21482	MG993554	—
116	<i>Pelobatrachus nasuta</i>	Sabah, Lahad Datu District, Malaysia	FMNH 231281	KY022186	—
117	<i>Pelobatrachus edwardinae</i>	Bintulu, Sarawak, Malaysia	FMNH 273694	KX811918	KX812050
118	<i>Leptobranchium boringii</i>	Emei Shan, Sichuan, China	Tissue ID: YPX37539	KX811930	KX812164
119	<i>Leptobranchella oshanensis</i>	Emei Shan, Sichuan, China	KIZ025778	KX811928	KX812166

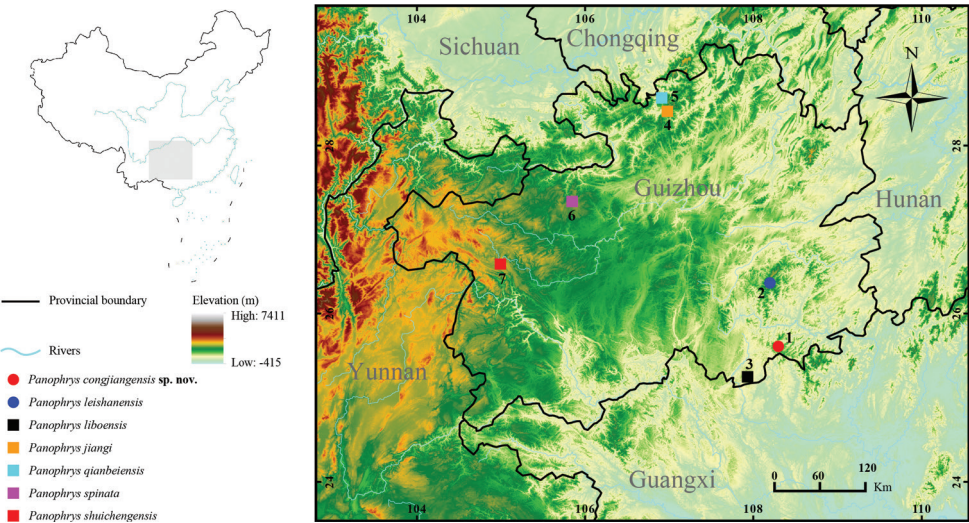


Figure 1. Sampling collection localities and distribution of the *Panophrys congiangensis* sp. nov., *P. leishanensis*, *P. liboensis*, and *P. jiangi* in Guizhou Province, China **1** Yueliangshan Nature Reserve, Congjiang County, Guizhou Province **2** Leigongshan National Nature Reserve, Leishan County, Guizhou Province **3** Maolan National Nature Reserve, Libo County, Guizhou Province **4, 5** Huoqiuba Nature Reserve, Suiyang County, Guizhou Province **6** Xingxiu Township, Dafang County, Guizhou Province **7** Fenghuang Township, Shuicheng County, Guizhou Province. The base maps are from Standard Map Service website (<http://bzdt.ch.mnr.gov.cn/index.html>).

Phylogenetic analyses

We used a total of 194 gene sequences (112 16S rRNA sequences and 82 COI sequences) for the molecular analyses, representing 102 species of subfamily Megophryinae. Two mitochondrial genes were sequenced in 10 muscle tissue samples from the

specimens collected in this study, and 178 sequences were downloaded from GenBank. Samples included those from the undescribed species collected and named in this study (Fig. 1). Following Mahony et al. (2017), we selected *Leptobrachium boringii* (Liu, 1945) and *Leptobrachella oshanensis* (Liu, 1950) as outgroups. The two outgroup sequences were obtained from GenBank. Details of the sequences used for phylogenetic analysis are given in Table 1.

All sequences were assembled and aligned using the MUSCLE (Edgar 2004) module in MEGA 7.0 (Kumar et al. 2016) with default settings. Alignments were checked by eye and revised manually if necessary. Trimming, with gaps partially deleted, was performed using GBLOCKS 0.91b (Castresana 2000). The best-fit partitioning schemes and corresponding substitution models for the concatenated-sequence supermatrix were selected in PartitionFinder 2.1.1 using the Bayesian information criterion (Lanfear et al. 2016). As a result, the analysis suggested that the best partition scheme 16S rRNA gene/each codon position of COI gene, and selected GTR+I+G model as the best model for 16S rRNA gene, and TRNEF+G, HKY+I+G, and TIM+G model as the best model for first, second and third codons position of COI gene, respectively. Phylogenetic analysis of the concatenated-sequence matrix was performed using maximum likelihood (ML) and Bayesian inference (BI). ML and BI phylogenies based on the concatenated-sequence matrix were constructed using both IQ-tree 2.0.4 (Nguyen et al. 2015) and MrBayes 3.2.1 (Ronquist et al. 2012), according to the best-fit partitioning schemes and the corresponding substitution models. The ML analysis was performed using the best-fit model for each partition with 2000 ultrafast bootstrap (UFB) replicates (Minh et al. 2013); the analysis was continued until a correlation coefficient of at least 0.99 was reached (Hoang et al. 2018). We performed two independent BI runs using four Markov chains (three heated chains and a single cold chain). The best-fit partitioning schemes and corresponding substitution models were selected. The BI analysis started with a random tree; each run consisted of 2×10^7 generations, sampled every 1000 generations. Runs were considered to have converged when the average standard deviation of split frequencies (ASDSF) was less than 0.01, and the effective sample sizes (ESS) in Tracer 1.7.1 (Rambaut et al. 2018) was greater than 200. Nodes in the trees were considered well-supported when Bayesian posterior probabilities (BPP) were ≥ 0.95 and ML ultrafast bootstrap values (UFB) were $\geq 95\%$. The phylogenetic trees were visualized using FigTree 1.4.3 (Rambaut 2016). The uncorrected *p*-distance model in MEGA 7.0 (Kumar et al. 2016) was used to calculate average genetic distances among species based on 16S rRNA and COI.

Species delimitation

To assess whether new species represent a valid species, two different methods were executed. We chose to include new species in the phylogenetic tree as well as several closely related species for species delimitation analysis. First, a Bayesian hypothesis-testing approach (Bayes Factor Delimitation, BFD) was implemented to statistically test alternate hypotheses of species delimitation (Gummer et al. 2014). Two species models were tested: 11 species (contains new species) and 10 species (lump new species with *P. leishanensis*). All analyses were performed in *BEAST using BEAST 1.8.2 (Drummond et al. 2012) under an uncorrelated lognormal relaxed molecular clock. A Yule process was used for

the species tree prior, and the piecewise linear and constant root was used for the population size model. Two independent runs for each model were performed in BEAST 1.8.2 to assess convergence of the MCMC runs. *BEAST was run each time for 1×10^7 generations of the MCMC algorithm sampling every 1000 generations and discarding the first 25% of the iterations as “burn-in”. After *BEAST analyses, two methods of marginal-likelihood estimation, including path-sampling (PS; Baele et al. 2012) and stepping-stone analysis (SS; Xie et al. 2011), were performed. PS and SS analyses were each run for a chain length of 1×10^6 generations for 100 path steps. We followed the suggestions provided by Gummer et al. (2014) to assess the strength of support for a particular species delimitation hypothesis.

In addition to the Bayesian methods tested, we also applied three tree-based species-delimitation methods, i.e., Bayesian implementation of the Poisson Tree Processes model (bPTP; Zhang et al. 2013). The parameters of these three analyses were set as follows: 1×10^5 generations, a thinning of 100 and burn-in of 10%. Convergence of models were assessed by visualizing plots of the MCMC iteration vs. the Log likelihood results. The bPTP analysis was conducted on the bPTP web server (<http://species.h-its.org/ptp/>) using mtDNA-based BI gene tree as input.

Morphological comparisons

Morphometric data were collected from 19 well-preserved adult specimens (voucher information given in Table 2). Measurements were recorded to the nearest 0.1 mm with digital calipers by Tao Luo following Fei et al. (2009). A total of 27 morphological features were measured in each well-preserved specimen. These following measurements were taken:

ED	eye diameter (diameter of exposed portion of eyeball);
FIL	first finger length;
FIIL	second finger length;
FIIL	third finger length;
FIVL	fourth finger length;
FL	foot length (distance from distal end of tibia to the tip of the distal phalanx of toe IV);
HDL	head length (from tip of snout to the articulation of the jaw);
HDW	head width (head width at the commissure of the jaws);
HLL	hindlimb length (distance from tip of fourth toe to vent);
HND	hand length (from the proximal border of the outer palmar tubercle to the tip of digit III);
IMTL	inner metatarsal tubercle length (taken as maximal length of inner metatarsal tubercle);
IND	internasal distance (distance between nares);
IOD	interorbital distance (minimum distance between upper eyelids);
IPTL	inner palmar tubercle length (measured as maximal distance from proximal to distal ends of the inner palmar tubercle);

LAHL	length of lower arm and hand (distance from the elbow to the distal end of finger IV);
LW	lower arm width (maximum width of the lower arm);
NED	nasal to eye distance (distance between the nasal and the anterior corner of the eye);
OPTL	outer metacarpal tubercle length (measured as maximal diameter of outer metacarpal tubercle);
SNT	snout length (from tip of snout to the anterior corner of the eye);
SVL	snout-vent length (from tip of snout to posterior margin of vent);
TD	tympanum diameter (horizontal diameter of tympanum);
TED	tympanum to eye distance (distance from anterior edge of tympanum to posterior corner of eye);
TFL	length of foot and tarsus (distance from the tibiotarsal articulation to the distal end of toe IV);
THL	thigh length (distance from vent to knee);
TL	tibia length (distance from knee to heel);
TW	tibia width (maximum width of the tibia);
UEW	upper eyelid width (greatest width of the upper eyelid margins measured perpendicular to the anterior-posterior axis).

To reduce allometric effects, all measurements were size-corrected with respect to SVL prior to morphometric analysis. Principal component analyses (PCAs) of size-corrected measurements and simple bivariate scatterplots were used to explore and characterize the morphometric differences between the new species and *P. leishanensis*. Mann–Whitney *U* tests were conducted to determine the significance of differences in morphometric characters between the new species and *P. leishanensis*. Mann–Whitney *U* tests also were used to test the significance of morphometric differences between males and females of the new species. All statistical analyses were performed using SPSS 21.0 (SPSS, Inc., Chicago, IL, USA), and differences were considered statistically significant at $P < 0.05$. Sex was determined based on male secondary sexual characters: the presence of a vocal sac and nuptial pads/spines (Fei and Ye 2016).

We compared the morphological characters of the new species with literature data for 59 other species in the *Panophrys* (Table 3). We also examined the type and/or topotype materials for *P. jiangi*, *P. liboensis*, *P. shuichengensis*, and *P. spinata* (see Appendix 1).

Bioacoustics analyses

The advertisement calls of the new species were recorded from the holotype specimen (voucher number GZNU20200706010) in the field on 5 July 2020 at the Yueliangshan Nature Reserve, Congjiang County, Guizhou Province, China. The advertisement calls were recorded in a stream, using a digital sound recorder (TASCAM DR-40) at an ambient air temperature of 25 °C and 92% humidity. Sounds were recorded within 5 cm of the calling individual. The wave-format sound files were sampled at 44 kHz, with sampling depth 24 bits. Praat 6.1.16 (Boersma 2001) was used to obtain

Table 2. Measurements of the adult specimens of *Panophrys congjiangensis* sp. nov. All units in mm. See abbreviations for the morphological characters in the Materials and Methods section. (M = male, F = female, other abbreviations defined in text), * for the holotype.

Voucher number	Sex	SVL	HDL	HDW	SNT	ED	IOD	IND	TD	UEW	NED	TED	HND	LAHL	LW	FIL	FIIL	FIHL	FIVL	TL	THL	FL	TFL	HLL	TW	IPTL	OPTL	IMTL
GZNU20200706010*	M	33.4	11.3	11.1	4.2	3.8	3.1	3.7	2.4	2.9	2.3	2.0	8.6	14.7	2.3	4.5	4.1	6.3	5.5	17.5	17.8	15.7	24.9	60.2	3.5	1.8	1.6	2.2
GZNU20200706001	M	33.1	11.2	11.4	4.2	3.5	3.2	3.7	2.3	2.8	2.5	1.9	8.3	14.3	2.1	4.7	4.2	6.1	5.2	16.7	16.5	15.6	23.6	56.8	3.2	2.1	1.5	2.3
GZNU20200706002	M	30.1	11.2	10.8	3.9	3.5	3.5	3.4	2.1	2.4	1.9	1.9	7.9	13.6	2	4.6	4.2	5.8	5.2	14.9	14.6	14.7	20.8	50.3	3.1	1.9	1.3	2.3
GZNU20200706003	M	30.6	10.8	10.3	4.0	3.6	3.7	3.1	2.3	2.7	2.4	1.8	7.7	13.9	2.1	3.9	3.3	6.2	4.9	15.3	14.6	13.4	20.8	50.7	2.6	2.0	1.2	1.8
GZNU20200706004	M	32.3	11.2	11.1	4.3	3.3	4.3	3.4	2.0	2.8	2.2	2.0	8.3	14.5	2.3	4.3	4.5	6.1	4.7	16.5	15.4	15.1	22.5	54.4	3.3	2.1	1.2	2.0
GZNU20200706005	M	29.8	11.0	11.2	4.4	3.7	3.7	3.3	2.0	2.6	2.5	2.2	8.2	14.3	2	3.7	5.7	7.7	5.9	15.9	15.3	14.6	22.8	54.0	3.1	1.6	1.2	1.7
GZNU20200706006	M	30.4	11.2	10.6	4.2	3.3	3.7	3.3	1.7	2.5	2.7	1.7	7.3	13.2	1.9	4.3	4.0	5.6	4.4	15.6	15.1	13.7	21.1	51.8	3.2	2.1	1.7	1.9
GZNU20200706007	M	28.6	10.2	9.1	3.9	3.4	3.9	2.8	1.7	2.7	2.3	2.1	7.5	13.9	1.9	3.9	3.6	5.8	4.1	14.6	13.8	13.5	20.9	49.3	2.8	1.8	1.6	1.9
GZNU20200706008	M	31.4	10.8	10.4	4.9	3.6	3.2	3.3	2.1	2.9	2.2	2.0	8.23	14.7	1.7	4.4	4.3	6.3	4.5	16.2	16.3	13.8	22.1	54.6	2.9	2.3	1.4	1.8
GZNU20200706009	M	31.1	10.7	11.2	3.4	3.9	3.2	3.5	2.4	2.9	1.9	1.9	7.9	14.8	2.4	4.4	3.9	6.3	5.1	15.6	15.2	14.5	22.9	53.7	3.1	1.7	1.4	1.9
GZNU20200706012	M	33.3	11.5	10.9	4.3	3.6	3.8	3.3	2.2	2.6	1.9	2.1	8.1	14.6	2.1	4.3	4.9	6.1	4.8	16.2	16.1	15.3	23.5	55.8	3.4	1.8	1.2	2.1
GZNU20200706013	M	30.2	10.6	9.8	4.1	3.6	3.5	3.1	1.7	3.0	1.9	1.9	7.9	13.8	2.1	4.5	3.9	5.7	4.9	14.8	13.4	13.5	22.1	50.3	3.1	2.3	1.4	2.2
GZNU20200707001	M	31.2	11.4	10.4	4.1	3.9	3.9	3.4	2.3	2.9	2.4	1.9	8.5	15.2	2.0	4.5	4.5	6.9	4.9	16.9	15.5	15.1	23.1	55.5	3.4	1.7	1.4	2.0
GZNU20200707002	M	30.1	11.2	10.8	3.9	3.5	3.5	3.4	2.1	2.4	1.9	1.9	7.9	13.6	2	4.1	3.6	5.7	4.9	14.9	14.6	14.7	20.8	50.3	3.1	2.2	1.6	1.9
GZNU20200707003	M	31.8	11.4	11.1	4.5	3.5	3.7	3.3	2.3	2.9	2.3	1.9	8.5	14.9	2.4	4.7	3.8	6.5	4.8	16.6	15.6	15.1	22.4	54.6	3.2	2.5	1.5	2.6
GZNU20200706011	F	38.4	12.1	11.8	4.5	4.8	3.8	3.5	2.5	3.7	2.5	1.8	8.9	16.2	2.0	5.1	4.3	6.6	5.7	19.1	18.6	16.6	25.7	63.4	3.9	2.8	2.1	2.2
GZNU20200706004	F	39.2	13.2	11.7	4.3	4.3	3.7	3.9	2.5	3.9	2.3	1.9	9.5	16.3	1.9	4.8	5.9	7.5	6.4	19.2	19.5	17.8	26.3	65	3.6	2.9	2.3	2.4
GZNU20200706005	F	39.5	13.3	11.5	4.4	4.2	3.8	3.5	2.4	3.8	2.6	1.9	9.1	16.2	2.1	4.8	5.9	7.7	6.4	19.3	19.2	18.2	26.2	64.7	3.5	2.8	2.4	2.6
GZNU20200706006	F	40.2	14.5	13.2	5.1	5.2	4.5	4.8	3.4	3.9	2.5	2.2	9.8	17.4	2.0	4.8	6.0	7.7	6.8	20	19.9	18.9	26.8	66.7	3.8	2.9	2.9	2.9

Table 3. References for morphological characters for congeners of the genus *Panophrys*.

ID	Species	Literature consulted
1	<i>Panophrys acuta</i> (Wang, Li & Jin, 2014)	Li et al. 2014
2	<i>Panophrys angka</i> (Wu, Suwannapoom, Poyarkov, Chen, Pawangkhanant, Xu, Jin, Murphy & Che, 2019)	Wu et al. 2019
3	<i>Panophrys anlongensis</i> (Li, Lu, Liu & Wang, 2020)	Li et al. 2020b
4	<i>Panophrys baishanzuensis</i> (Wu, Li, Liu, Wang & Wu, 2020)	Wu et al. 2020
5	<i>Panophrys baolongensis</i> (Ye, Fei & Xie, 2007)	Ye et al. 2007
6	<i>Panophrys binchuanensis</i> (Ye & Fei, 1995)	Ye and Fei 1995
7	<i>Panophrys binlingensis</i> (Jiang, Fei & Ye, 2009)	Fei et al. 2009
8	<i>Panophrys boettgeri</i> (Boulenger, 1899)	Fei et al. 2012
9	<i>Panophrys brachykolos</i> (Inger & Romer, 1961)	Inger and Romer 1961
10	<i>Panophrys caobangensis</i> (Nguyen, Pham, Nguyen, Luong & Ziegler, 2020)	Nguyen et al. 2020
11	<i>Panophrys caudoprocta</i> (Shen, 1994)	Fei et al. 2012
12	<i>Panophrys cheni</i> (Wang & Liu, 2014)	Wang et al. 2014
13	<i>Panophrys chishuiensis</i> (Xu, Li, Liu, Wei & Wang, 2020)	Xu et al. 2020
14	<i>Panophrys daiyunensis</i> Lyu, Wang & Wang, 2021	Lyu et al. 2021
15	<i>Panophrys daoji</i> Lyu, Zeng, Wang & Wang, 2021	Lyu et al. 2021
16	<i>Panophrys daweimontis</i> (Rao & Yang, 1997)	Fei et al. 2012
17	<i>Panophrys dongguanensis</i> (Wang & Wang, 2019)	Wang et al. 2019a
18	<i>Panophrys fansipanensis</i> (Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018)	Tapley et al. 2018
19	<i>Panophrys frigida</i> (Tapley, Cutajar, Nguyen, Portway, Mahony, Nguyen, Harding, Luong & Rowley, 2021)	Tapley et al. 2021
20	<i>Panophrys hoanglienensis</i> (Tapley, Cutajar, Mahony, Nguyen, Dau, Luong, Le, Nguyen, Nguyen, Portway, Luong & Rowley, 2018)	Tapley et al. 2018
21	<i>Panophrys huangshanensis</i> (Fei & Ye, 2005)	Fei et al. 2012
22	<i>Panophrys insularis</i> (Wang, Liu, Lyu, Zeng & Wang, 2017)	Wang et al. 2017a
23	<i>Panophrys jiangi</i> (Liu, Li, Wei, Xu, Cheng, Wang & Wu, 2020)	Liu et al. 2020
24	<i>Panophrys jingdongensis</i> (Fei & Ye, 1983)	Fei et al. 2012
25	<i>Panophrys jinggangensis</i> (Wang, 2012)	Wang et al. 2012
26	<i>Panophrys julianensis</i> (Wang, Zeng, Lyu & Wang, 2019)	Wang et al. 2019a
27	<i>Panophrys kuatunensis</i> (Pope, 1929)	Fei et al. 2012
28	<i>Panophrys leishanensis</i> (Li, Xu, Liu, Jiang, Wei & Wang, 2019 «2018»)	Li et al. 2018b
29	<i>Panophrys liboensis</i> (Zhang, Li, Xiao, Li, Pan, Wang, Zhang & Zhou, 2017)	Zhang et al. 2017
30	<i>Panophrys lini</i> (Wang & Yang, 2014)	Wang et al. 2014
31	<i>Panophrys lishuiensis</i> (Wang, Liu & Jiang, 2017)	Wang et al. 2017b
32	<i>Panophrys lushuiensis</i> (Shi, Li, Zhu, Jiang, Jiang & Wang, 2021)	Shi et al. 2021
33	<i>Panophrys minor</i> (Stejneger, 1926)	Wang et al. 2017a
34	<i>Panophrys mirabilis</i> (Lyu, Wang & Zhao, 2020)	Lyu et al. 2020
35	<i>Panophrys mufumontana</i> (J. Wang, Lyu & Y.Y. Wang, 2019)	Wang et al. 2019a
36	<i>Panophrys nankunensis</i> (Wang, Zeng & Wang, 2019)	Wang et al. 2019a
37	<i>Panophrys nanlingensis</i> (Lyu, J. Wang, Liu & Y.Y. Wang, 2019)	Wang et al. 2019a
38	<i>Panophrys obesa</i> (Wang, Li & Zhao, 2014)	Li et al. 2014
39	<i>Panophrys ombrophila</i> (Messenger & Dahn, 2019)	Messenger et al. 2019
40	<i>Panophrys omeimontis</i> (Liu, 1950)	Fei et al. 2009
41	<i>Panophrys palpebralespinosa</i> (Bourret, 1937)	Fei et al. 2012
42	<i>Panophrys qianbeiensis</i> (Su, Shi, Wu, Li, Yao, Wang & Li, 2020)	Su et al. 2020
43	<i>Panophrys rubrimera</i> (Tapley, Cutajar, Mahony, Chung, Dau, Nguyen, Luong & Rowley, 2017)	Tapley et al. 2017
44	<i>Panophrys sangzhiensis</i> (Jiang, Ye & Fei, 2008)	Jiang et al. 2008
45	<i>Panophrys sanmingensis</i> Lyu & Wang, 2021	Lyu et al. 2021
46	<i>Panophrys shimentaina</i> (Lyu, Liu & Wang, 2020)	Lyu et al. 2021
47	<i>Panophrys shuichengensis</i> (Tian & Sun, 1995)	Tian et al. 2000
48	<i>Panophrys shunhuangensis</i> (Wang, Deng, Liu, Wu & Liu, 2019)	Wang et al. 2019b
49	<i>Panophrys spinata</i> (Liu & Hu, 1973)	Fei et al. 2012
50	<i>Panophrys tongboensis</i> Wang & Lyu, 2021	Lyu et al. 2021
51	<i>Panophrys tuberganulata</i> (Shen, Mo & Li, 2010)	Mo et al. 2010
52	<i>Panophrys wugongensis</i> (J. Wang, Lyu & Y.Y. Wang, 2019)	Wang et al. 2019a
53	<i>Panophrys wuliangshanensis</i> (Ye & Fei, 1995)	Fei et al. 2012
54	<i>Panophrys wushanensis</i> (Ye & Fei, 1995)	Fei et al. 2012
55	<i>Panophrys xianjuensis</i> (Wang, Wu, Peng, Shi, Lu & Wu, 2020)	Wang et al. 2020
56	<i>Panophrys xiangnanensis</i> (Lyu, Zeng & Wang, 2020)	Lyu et al. 2021
57	<i>Panophrys yangmingensis</i> (Lyu, Zeng & Wang, 2020)	Lyu et al. 2020
58	<i>Panophrys yuae</i> (Shi, Zhang, Xie, Jiang, Liu, Ding, Luan & Wang, 2020)	Shi et al. 2020
59	<i>Panophrys zhoui</i> (Shi, Zhang, Xie, Jiang, Liu, Ding, Luan & Wang, 2020)	Shi et al. 2020

oscillograms, sonograms, and power spectra at a window length of 0.005 s. The ambient temperature at the type locality was measured using a digital hygrothermograph (ECOFIVE MS6508).

Results

Phylogenetic analyses and genetic divergence

ML and BI phylogenies were constructed based on two concatenated mitochondrial gene sequences: 16S rRNA (548 bp) and COI (672 bp). The ML and BI topologies were largely identical (Fig. 2). *Panophrys* (except for *P. yeae* and *P. zhoui*) was strongly supported as monophyletic by both phylogenetic analyses. The phylogenetic trees also supported the monophyly of four of the seven genera of subfamily Megophryinae proposed in the revision of Li et al. (2020a): *Ophyrophryne*, *Atympanophrys*, *Brachytarsophrys*, *Panophrys* (except for *P. yeae* and *P. zhoui*), and *Pelobatrachus*; the monophyly of *Xenophrys* and *Ophyrophryne* was not supported. In both analyses, the new species formed a lower supported clade (0.59 in BI and 56% in ML) with *P. leishanensis*, *P. baolongensis*, *P. wushanensis*, *P. tuberogranulata*, *P. shimentaina*, *P. yangmingensis*, *P. jilianensis*, *P. mirabilis*, *P. shunhuangensis*, and *P. acuta*. However, relationships among species in this clade were not well resolved except for the following well-supported sister relationships: *P. baolongensis* and *P. wushanensis*; *P. shimentaina* and *P. yangmingensis*; and *P. mirabilis* and *P. shunhuangensis*. The new species was recovered in a relatively poorly-supported sister relationship with *P. leishanensis* (0.60 in BI and 79% in ML; Fig. 2).

The smallest *p*-distance between this lineage and any other species of *Panophrys* was 1.2% in 16S rRNA (with *P. huangshanensis*) and 6.5% in COI (with *P. wushanensis*). These levels of divergence were similar to those between other pairs of recognized congeners. For example, the 16S rRNA *p*-distance was 1.2% between *P. leishanensis* and *P. huangshanensis*, 1.2% between *P. jingdongensis* and *P. binchuanensis*, while the COI *p*-distance was 5.9% between *P. lini* and *P. nanlingensis*, 3.6% between *P. spinata* and *P. sangzhiensis*, and 4.5% between *Brachytarsophrys carinense* and *B. popei* (Suppl. material 1: Table S1; Suppl. material 2: Table S2). These results suggested that this population, from the Yueliangshan Nature Reserve, Congjiang County, Guizhou Province, China, represented an independent evolutionary lineage.

Species delimitation

The results of the *BEAST analysis for the alternative species model are provided in Table 4. Both SS and PS estimations based on 16S rRNA+COI datasets had the largest values for the 11 species taxonomy, indicating that it was supported in favor of the currently accepted 11 species model. In addition, the results of the maximum likelihood

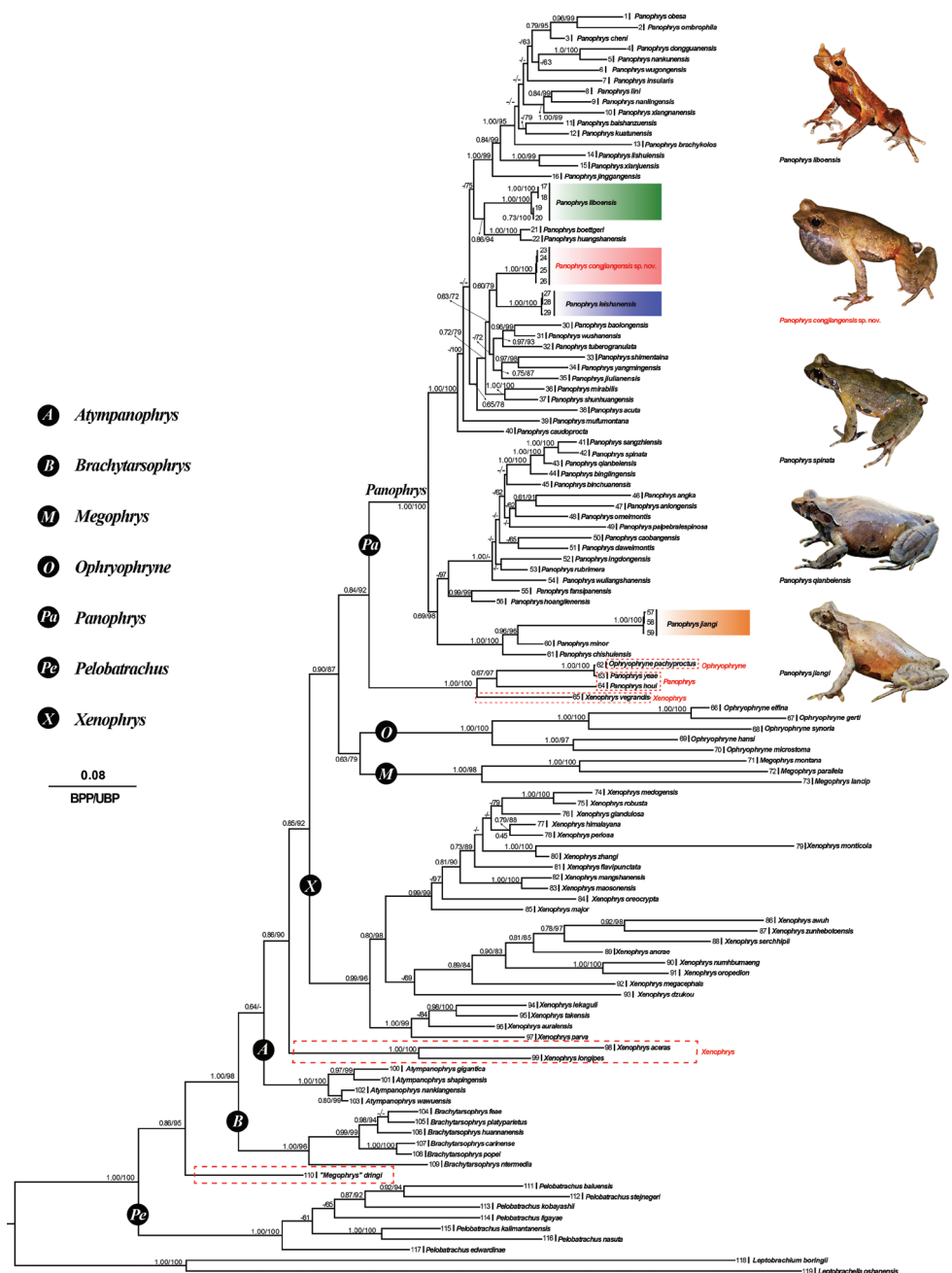


Figure 2. Phylogenetic tree based on mitochondrial 16S+COI genes. In this phylogenetic tree, ultrafast bootstrap supports (UFB) from ML analyses/Bayesian posterior probabilities (BPP) from BI analyses were noted beside nodes. The symbol “-” represents value below 0.60/60. Photos of new collections and 11 of 4 *Panophrys* species in Guizhou Province. The scale bar represents 0.08 nucleotide substitutions per site. The numbers at the tip of branches corresponds to the ID numbers in Table 1.

Table 4. The species delimitation results of new species and several closely related species in BF method.

Model	Species delimitation	MLE Path Sampling (PS)	MLE Stepping Stone (SS)	Rank	BF (PS)	BF (SS)
M1	11 species: AC+BA+JU+MI+SI+SU+TU+WU+YA+LE+CO	-4011.49	-4011.48	1	14.14	14.02
M2	10 species: AC+BA+JU+MI+SI+SU+TU+WU+YA+[LE+CO]	-4018.56	-4018.49	2	–	–

Each model represents a possible relationship of the new species to 10 closely related species. Abbreviation as: *P. acuta*: AC, *P. baolongensis*: BA, *P. julianensis*: JU, *P. mirabilis*: MI, *P. shimentaina*: SI, *P. shunhuangensis*: SU, *P. tuberogranulata*: TU, *P. wushanensis*: WU, *P. yangmingensis*: YA, *P. leishanensis*: LE, *P. congjiangensis* sp. nov.: CO.

solution of the bPTP analysis supported 11 species taxonomy model (Appendix 1). Thus, the results of the BFD and bPTP analyses suggest support for treating the new species as a single valid species.

Morphological analyses

The results of the Mann–Whitney *U* tests indicated that males of the new species differed significantly from *P. leishanensis* males based on several morphometric characters (all *p*-values < 0.05; Table 5). Using PCA, we extracted two and three principal component factors with Eigenvalues greater than two for males and females, respectively (Suppl. material 3: Table S3). The first two principal components explained 67.23% and 80.68% of the total variation in males and females, respectively. The variances in the data were mainly associated with limb and head characters, including TW, THL, HDL, LW, HDW, LAHL, HLL, FIIL, FIL, FIIL, TFL, TL, IND, and IOD (Table 5). The characters of the new species were distinct from those of *P. leishanensis* on two-dimensional plots of PC1 and PC2 for both males and females (Fig. 3).

Taxonomic account

Panophrys congjiangensis sp. nov.

<http://zoobank.org/B433A7B8-2C23-4EC7-8C94-BC7FECF8B584>
Table 2, Figs 4–6

Type material. Holotype. GZNU20200706010 (Figs 4, 5), adult male, collected by Tao Luo on 6 May 2020 in the Yueliangshan Nature Reserve, Congjiang County, Guizhou Province, China (25.614417°N, 108.410076°E; ca. 730 m a.s.l.).

Paratypes. Nineteen adult specimens (15 males and 4 females) from the same locality. Eleven males (GZNU20200706001–06009, GZNU20200706012–06013) collected with the holotype on 6 July 2020 by Tao Luo, Xueli Lu, and Weifeng Wang. One female (GZNU20200706011) collected with the holotype by Tao Luo. Three males (GZNU200707001–07003) collected on 7 July 2020 by Tao Luo. Three females (GZNU20200706004, GZNU20200706005, and GZNU20200706006) collected on 7 July 2020 by Tao Luo.

Table 5. Morphological comparison of *Panophrys congjiangensis* sp. nov. (PC) and *P. leishanensis* (PL). All units in mm. *P*-values are at 95% significance. Morphometric characters are explained in the methods section. CM and CF are the abbreviations of male and female from *Panophrys congjiangensis* sp. nov.

Measurements	<i>Panophrys congjiangensis</i> sp. nov.				<i>Panophrys leishanensis</i> ^a				<i>P</i> -value from Mann-Whitney U test		
	Male (N=15)		Female (N=4)		Male (N=10)		Female (N=2)		Male	Female	CM vs. CF
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range		PC vs. PL	PC vs. PL	
SVL	28.6–33.4	31.2 ± 1.4	38.4–40.2	39.3 ± 0.7	30.4–38.7	34.3 ± 2.7	42.3–42.3		0.000	0.133	0.003
HDL	10.2–11.5	11.0 ± 0.4	12.1–14.5	13.3 ± 1.0	9.1–11.0	10.1 ± 0.7	11.3–11.7		0.000	0.267	0.002
HDW	9.1–11.4	10.7 ± 0.6	11.5–13.2	12.1 ± 0.8	10.5–12.0	11.4 ± 0.5	12.1–12.4		0.014	0.133	0.003
SNT	3.4–4.9	4.2 ± 0.3	4.3–5.1	4.6 ± 0.4	3.6–4.5	4.2 ± 0.3	4.5–5.0		0.007	0.267	0.027
ED	3.3–3.9	3.6 ± 0.2	4.2–5.2	4.6 ± 0.5	3.3–4.3	3.9 ± 0.3	4.1–4.8		0.643	1.000	0.002
IOD	3.1–4.3	3.6 ± 0.3	3.7–4.5	4.0 ± 0.4	3.3–4.3	3.7 ± 0.3	3.9–4.2		0.062	0.267	0.084
IND	2.8–3.7	3.3 ± 0.2	3.5–4.8	3.9 ± 0.6	3.5–4.7	4.0 ± 0.4	4.1–4.3		0.392	0.267	0.011
TD	1.7–2.4	2.1 ± 0.2	2.4–3.4	2.7 ± 0.5	2.0–2.6	2.3 ± 0.2	2.5–2.8		0.461	1.000	0.003
UEW	2.4–3.0	2.7 ± 0.2	3.7–3.9	3.8 ± 0.1	/	/	/		/	/	0.002
NED	1.9–2.7	2.2 ± 0.3	2.3–2.6	2.5 ± 0.1	/	/	/		/	/	0.059
TED	1.7–2.2	1.9 ± 0.1	1.8–2.2	2.0 ± 0.2	/	/	/		/	/	0.750
HND	7.3–8.6	8.1 ± 0.4	8.9–9.8	9.3 ± 0.4	/	/	/		/	/	0.003
LAHL	13.2–15.2	14.3 ± 0.6	16.2–17.4	16.5 ± 0.6	14.4–16.3	15.3 ± 0.6	18.1–18.4		0.036	0.133	0.003
LW	1.7–2.4	2.1 ± 0.2	1.9–2.1	2.0 ± 0.1	2.7–3.9	3.2 ± 0.5	2.8–2.9		0.001	1.000	0.355
TL	14.6–17.5	15.9 ± 0.9	19.1–20	19.4 ± 0.4	16.2–18.6	17.5 ± 0.9	19.2–19.2		0.129	0.800	0.003
THL	13.4–17.8	15.3 ± 1.1	18.6–19.9	19.3 ± 0.5	14.4–16.8	15.4 ± 0.8	17.6–17.7		0.000	1.000	0.003
FL	13.4–15.7	14.6 ± 0.8	16.6–18.9	17.9 ± 1.0	14.9–17.3	15.9 ± 1.0	18.1–19.0		0.129	0.800	0.003
TFL	20.8–24.9	22.3 ± 1.2	25.7–26.8	26.3 ± 0.5	21.1–25.9	23.5 ± 0.5	27.5–27.9		0.004	1.000	0.003
HLL	49.3–60.2	53.5 ± 3.0	63.4–66.7	65.0 ± 1.4	50.3–60.2	54.2 ± 3.0	49.3–50.3		0.023	0.533	0.003
TW	2.6–3.5	3.1 ± 0.2	3.5–3.9	3.7 ± 0.2	3.6–4.7	4.2 ± 0.3	4.8–5.1		0.000	0.133	0.003
IPTL	1.6–2.5	2.0 ± 0.3	2.8–2.9	2.9 ± 0.1	/	/	/		/	/	0.003
OPTL	1.2–1.7	1.4 ± 0.2	2.1–2.9	2.4 ± 0.3	/	/	/		/	/	0.002
IMTL	1.7–2.6	2.0 ± 0.2	2.2–2.9	2.5 ± 0.3	/	/	/		/	/	0.014
FIL	3.7–4.7	4.3 ± 0.3	4.8–5.1	4.9 ± 0.2	3.2–3.9	3.5 ± 0.2	4.0–4.3		0.000	0.133	0.003
FIIL	3.3–5.7	4.2 ± 0.6	4.3–6	5.5 ± 0.8	2.8–3.5	3.2 ± 0.3	3.8–4.1		0.000	0.133	0.011
FIIL	5.6–7.7	6.2 ± 0.5	6.6–7.7	7.4 ± 0.5	4.2–5.4	4.8 ± 0.4	5.4–5.8		0.000	0.133	0.009
FIVL	4.1–5.9	4.9 ± 0.4	5.7–6.8	6.3 ± 0.5	3.4–4.1	3.7 ± 0.2	4.2–4.3		0.000	0.133	0.004

Note : ^a Morphological data from Li et al .(2018b).

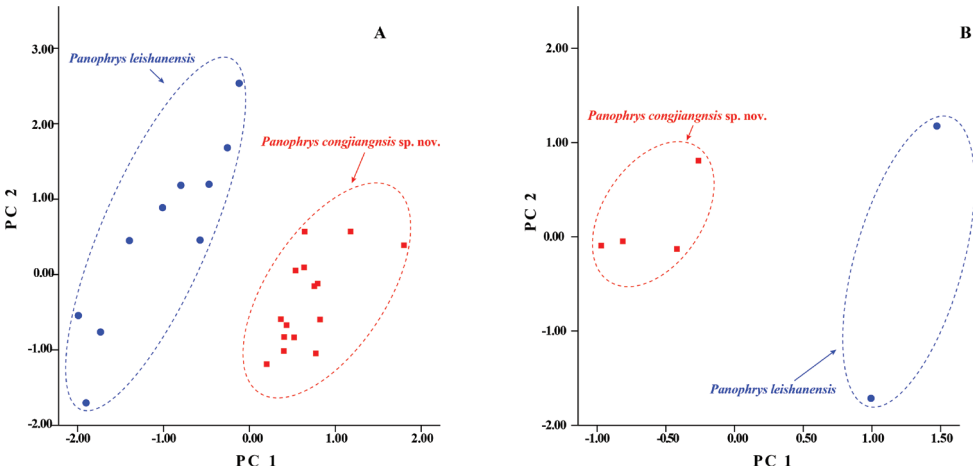


Figure 3. Plots of the first principal component (PC1) versus the second (PC2) for *Panophrys congjiangensis* sp. nov. and *P. leishanensis* from a principal component analysis **A** male **B** female.



Figure 4. Morphological features of the live adult male holotype GZNU20200706010 of *Panophrys congjiangensis* sp. nov. **A** single subgular vocal sac **B** dorsal view **C** dorsolateral view **D** ventral view **E** ventral view of hand **F** ventral view of foot **G** dorsal view of hand (1 indicates villiform gray-black nuptial spines) **H** iris. **A** was photographed at about 9 p.m., and **B** to **H** during the day, respectively.

Etymology. The specific epithet “congiangensis” refers to the holotype locality, which is Yueliangshan Nature Reserve, located in Congjiang County, Guizhou Province, China. We propose the English common name “Congjiang Horned Toad” and the Chinese common name “Cong Jiang Jiao Chan (从江角蟾)”.

Differential diagnosis. *Panophrys congiangensis* sp. nov. is assigned to the genus *Panophrys* based on molecular phylogenetic analyses and the following characteristics, which are diagnostic for this genus: (1) snout shield-like; (2) snout projecting beyond the lower jaw; (3) tympanum distinct (4) canthus rostralis distinct; (5) chest gland small and round, closer to axilla than to midventral line; (6) femoral gland on rear of thigh; (7) vertical pupils (Fei et al. 2006; Fei and Ye 2016; Su et al. 2020).

Panophrys congiangensis sp. nov. is distinguished from its congeners by a combination of the following characters: (1) medium body size (SVL: 28.6–33.4 mm in males and 38.4–40.2 mm in females); (2) single small horn-like tubercle at edge of each upper eyelid; (3) tympanum distinctly visible (TD/ED ratio 0.47–0.66); (4) vomerine teeth absent; (5) tongue not notched behind; (6) a narrow and unobvious lateral fringe on toes; (7) relative finger lengths $II < I < V < III$; (8) rudimentary webs on toes; (9) hindlimbs slender, heels overlapping when thighs are positioned at right angles to body; (10) two metacarpal tubercles on palm, with inner metatarsal tubercle long and oval-shaped; (11) tibiotarsal articulation reaching nostril when leg is stretched forward; (12) dorsal skin rough, with numerous orange-red granules, ventral surface smooth; (13) single internal subgular vocal sac present in males; (14) in breeding males, weak gray-black nuptial pads with black nuptial spines present on dorsal surfaces of bases of first and second fingers.

Description of holotype. GZNU20200706010 (Figs 4, 5), adult male. Medium body size, SVL 33.4 mm; head length slightly larger than head width (HDL/HDW ratio 1.02); snout short, rounded and projecting beyond the lower jaw in dorsal view, longer than eye diameter (SNT/ED ratio 1.11); nostril rounded, distinct, and closer to the tip of the snout than to the eye (SNT/NED ratio 1.83); internasal distance greater than interorbital distance (IND/IOD ratio 1.19); internasal distance greater than upper eyelid width (IND/UEW ratio 1.28); region vertical and concave; canthus rostralis well-developed; top of head slightly concave in dorsal view; a small horn-like tubercle at the edge of the upper eyelid; eyes large, slightly protuberant in dorsal view, eye diameter 34% of head length, pupils vertical (Fig. 4H); tympanum distinct, tympanum diameter less than eye diameter (TD/ED ratio 0.63); vomerine ridges and vomerine teeth absent; tongue is melon seed-shaped and not notched behind (Fig. 5E).

Forelimbs slender and comparatively short, the length of lower arm and hand 44.01% of SVL; fingers slender, relative finger lengths: $II < I < IV < III$; tips of fingers slightly dilated, round, without lateral fringes; one distinct subarticular tubercle at the base of each finger; two metacarpal tubercles on the palm; prominent, the outer one long and thin, the inner one oval-shaped, inner metacarpal tubercles longer than outer metacarpal tubercles (IPTL/OPT ratio 1.13).

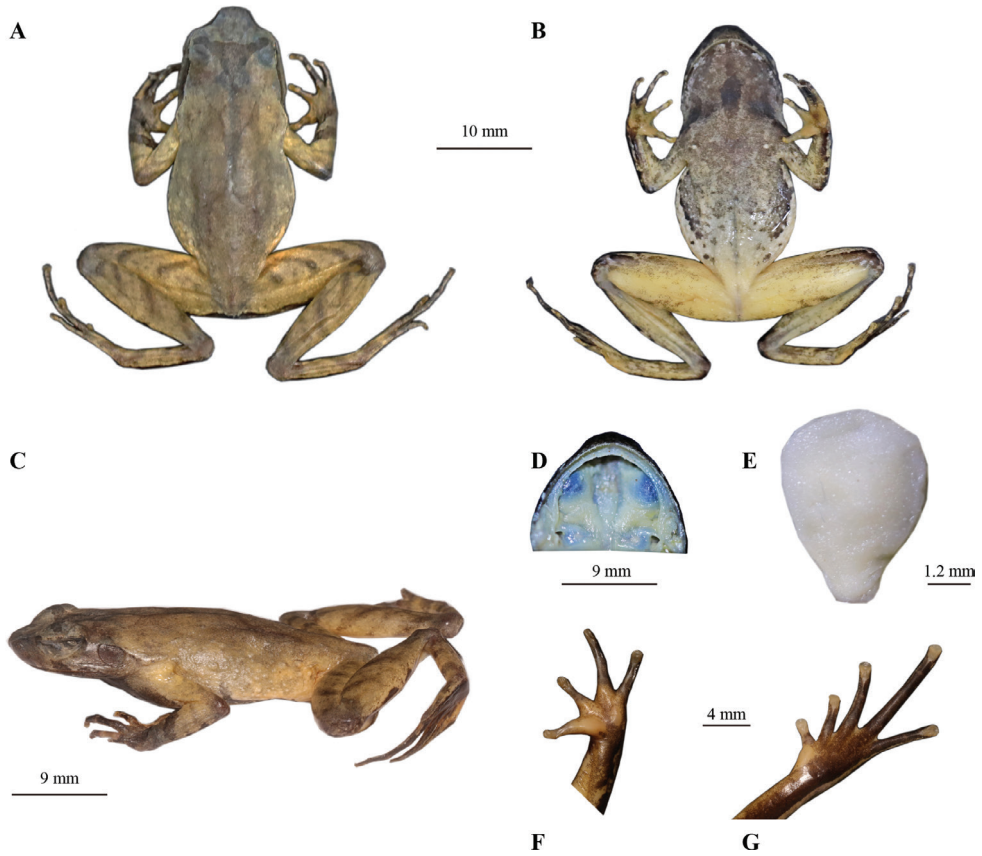


Figure 5. Morphological features of the preserved adult male holotype GZNU20200706010 of *Pano-phrys congjiangensis* sp. nov. **A** dorsal view **B** ventral view **C** lateral view **D** view of oral cavity **E** tongue **F** ventral view of hand **G** ventral view of foot.

Hindlimbs slender (HLL/SVL ratio 1.80); heels slightly overlapping when thighs are positioned at right angles to the body; tibiotarsal articulation reaching the nostril when leg stretched forward; foot length less than tibia length (FL/TL ratio 0.90); relative toe lengths $I < II < V < III < IV$; tips of toes round and slightly dilated; toes with narrow and unobvious lateral fringes and rudiment webs; one subarticular tubercle at the base of each toe; inner metatarsal tubercle long oval-shaped and the outer one absent.

Dorsal skin rough with numerous orange-red granules; several large warts scattered on flanks and dorsal limbs; several tubercles on upper eyelid, including a small horn-like prominent tubercle on the edge (Fig. 4H); supratympanic fold distinct; tubercles on the dorsum forming a discontinuous X-shaped ridge, the V-shaped ridges disconnected; two discontinuous dorsolateral parallel ridges on either side of the X-shaped ridges; an inverted triangular brown speckle between two upper eyelids; four transverse skin ridges on the dorsal shank and thigh; ventral surface smooth; chest with small, round glands, closer to the axilla than to midventral line; femoral glands on rear of

thigh; numerous white granules on ventral surface of thigh; posterior end of body distinctly protruding, forming an arc-shaped swelling above anal region.

Coloration of holotype in life (Fig. 4). Dorsal surfaces of head and trunk brownish gray; triangular marking with light edge between eyes; dark X-shaped marking with light edge on central dorsum; supratympanic fold light brown; four dark brown transverse bands on dorsal surfaces of thigh and shank; 2–4 dark brown and white vertical bars on lower and upper lip; dark vertical band below eye; iris copper-brown; throat and anterior chest light purple-brown; belly light orange-red with large white blotch and small grey blotch in belly center, and small white blotches and large black patches on belly sides, forming a discontinuous line; ventral surfaces of forelimbs purplish brown; some white spots on the ventral surfaces of hindlimbs; palms orange-red with a small black-brown blotch; ventral surfaces of first and second toes orange-red, ventral surfaces of remaining three toes black-brown; soles black-brown; tips of digits grey-white; pectoral and femoral glands white.

Preserved holotype coloration (Fig. 5). After preservation in ethanol, dorsal surfaces light brownish grey; dorsal surface of head dark gray; X-shaped ridges on dorsum indistinct and transverse bands on limbs and digits distinct, coloration lighter; throat dark black-brown; chest light black-brown; belly light gray-white with large black-brown blotches on sides and a small gray-brown blotch in center; posterior ventral body surface, inner thigh, and upper part of tibia milky yellow; palms and metatarsal tubercle milky yellow with a small gray-brown blotch; ventral surfaces of soles and toes dark black-brown; inner metatarsal tubercle milky yellow.

Variations. Measurements of the type series are shown in Tables 2, 4. Females (SVL 39.3 ± 0.7 mm, $N = 4$) had larger bodies than males (SVL 31.2 ± 1.4 mm, $N = 15$). In life, the diagnostic morphological characters of all paratypes were identical to those of the holotype. However, coloration and stripe patterns differed among individuals (Fig. 6). For example, male GZNU20200706007 (Fig. 6A) had a brown-black back and a black-brown belly with some large white patches, as well as two V-shaped markings that were virtually connected. This specimen also had warts on both sides of the body, forming a transverse skin ridge that almost connected to the second V-shaped marking. In contrast, male GZNU20200706008 (Fig. 6B) had a large black spot between the upper eyelids. The throat and anterior belly of this specimen were purple-brownish, while the belly was light milky yellow, with two large black blotches and a small white blotch on the body sides. In specimens GZNU20200706009 and GZNU20200706012 (Fig. 6C, E), the warts on both sides of the body formed transverse skin ridges connected to the second V-shaped marking and extending behind the tympanum; three white small blotches were present on the body sides. In specimens GZNU20200706013 and GZNU20200706012 (Fig. 6D, E), the back was light reddish brown.

Advertisement call. The call description is based on recordings of the holotype GZNU20200706010 (Fig. 7) from the bamboo forest near the streamlet. The ambient air temperature during the recording was 25.3°C . Each call contains 9–14 syllables (mean 11.60 ± 2.07 , $N = 5$). The call consists of a few strophes, each 2.41–3.43 s in duration (mean 2.75 ± 0.46 , $N = 4$). Each syllable has a duration of 0.05–0.09 s (mean 0.07 ± 0.06 , $N = 58$). The interval between syllables has a duration of 0.10–0.31 s (mean 0.167 ± 0.042 , $N = 53$).



Figure 6. Paratypes of *Panophrys congjiangensis* sp. nov. in life **A** GZNU20200706007, adult male **B** GZNU20200706008, adult male **C** GZNU20200706009, adult male **D** GZNU20200706013, adult male **E** GZNU20200706012, adult female. So, the images were all taken at 8 am.

Sexual dimorphism. Adult males (SVL 28.6–33.4 mm) smaller than adult females (SVL 38.4–40.2 mm). Adult males with single internal subgular vocal sac (Fig. 4A). Breeding males with grey-black nuptial pads with obvious black nuptial spines on dorsal surfaces of bases of first and second fingers.

Comparisons. Comparative data of *Panophrys congjiangensis* sp. nov. with 59 recognized congeners of *Panophrys* are given in Suppl. material 4: Table S4.

By having small body size, SVL 30.4–34.1 mm in males, *Panophrys congjiangensis* sp. nov. differs from *P. baolongensis* (42.0–45.0 in males), *P. binlingensis* (45.1–51.0 in

males), *P. boettgeri* (34.5–37.8 in males), *P. caobangensis* (34.9–38.9 in males), *P. caudo-procta* (81.3 in single male), *P. hoanglienensis* (37.4–47.6 in males), *P. huangshanensis* (36.0–41.6 in males), *P. insularis* (36.8–41.2 in males), *P. jingdongensis* (53.0–56.5 in males), *P. mirabilis* (55.8–61.4 in males), *P. obesa* (35.6 in single male), *P. palpebralespinosa* (36.2–38.0 in males), *P. sangzhiensis* (54.7 in single male), and *P. xiangnanensis* (38.6–42.0 in males). By having larger body size, SVL 30.4–34.1 mm in males, *Panophrys congjiangensis* sp. nov. differs from *P. cheni* (26.2–29.5 in males), *P. daiyunensis* (27.6–28.7 in males), *P. kuatunensis* (26.2–29.6 in males), *P. sanmingensis* (27.0–29.5 in males), *P. yae* (23 in single male), and *P. zhoui* (23.8–29.1 in males). By having small body size, SVL 38.9–40.2 mm in females, *Panophrys congjiangensis* sp. nov. differs from *P. fansipanensis* (41.7–42.5 in females), *P. minor* (42.0–48.2 in females), *P. tuberogranulata* (50.5 in single female), *P. wuliangshanensis* (41.3 in single female), *P. xianjuensis* (41.6 in single female), and *P. yangmingensis* (45.2 in single female).

Nine *Panophrys* species were previously recorded from the Guizhou Province, namely *P. anlongensis*, *P. chishuiensis*, *P. jiangi*, *P. leishanensis*, *P. liboensis*, *P. omeimontis*, *P. shuichengensis*, *P. spinata*, and *P. qianbeiensis*. *Panophrys congjiangensis* sp. nov. differs from *P. anlongensis* by having small body size, SVL 30.4–34.1 mm in males and 38.9–40.2 mm in females (vs. 40.0–45.5 mm in males and 48.9–51.2 in females), tibiotarsal articulation reaching the nostril when leg stretched forward (vs. reaching to the level of mid-eye). *Panophrys congjiangensis* sp. nov. differs from *P. chishuiensis* by having small body size, SVL 30.4–34.1 mm in males and 38.9–40.2 mm in females (vs. 43.4–44.1 mm in males and 44.8–49.8 in females), rudimentary webs on toes (vs. lacking webs), subarticular tubercles present on each toes (vs. absent), tibiotarsal articulation reaching the nostril when leg stretched forward (vs. reaching the level between tympanum and eye). *Panophrys congjiangensis* sp. nov. differs from *P. jiangi* by having slightly small body size, SVL 30.4–34.1 mm in males (vs. 34.4–39.2 mm in males), relative finger lengths are $II < I < V < III$ (vs. $I < II < V < III$), tibiotarsal articulation reaching the nostril when leg stretched forward (vs. reaching forward to the region between tympanum and eye). *Panophrys congjiangensis* sp. nov. differs from *P. liboensis*, *P. omeimontis*, *P. qianbeiensis*, *P. shuichengensis*, and *P. spinata* by having small body size, SVL 30.4–34.1 mm in males and 38.9–40.2 mm in females (vs. $SVL > 40$ mm in males in *P. liboensis*, *P. omeimontis*, *P. qianbeiensis*, *P. shuichengensis*, and *P. spinata*; vs. $SVL > 50$ mm in females in *P. liboensis*, *P. omeimontis*, *P. shuichengensis*, and *P. spinata*), small horn-like tubercle at the edge of each upper eyelid (vs. slightly large in *P. liboensis* and *P. shuichengensis*; absence in *P. qianbeiensis*), absence of vomerine teeth (vs. present in *P. liboensis*, *P. omeimontis*, and *P. qianbeiensis*), tongue not notched behind (vs. notched in *P. liboensis*, *P. omeimontis*, *P. qianbeiensis*, *P. shuichengensis*, and *P. spinata*), lateral fringes on toes narrow and unobvious (vs. wide in *P. liboensis*, *P. qianbeiensis*, *P. shuichengensis*, and *P. spinata*), rudimentary webs on toes (vs. more than one-fourth webs in *P. qianbeiensis*, *P. shuichengensis*, and *P. spinata*), subarticular tubercles present on each toes (vs. absent in *P. liboensis* and *P. shuichengensis*), tibiotarsal articulation reaching the nostril when leg stretched forward (vs. reaching to ocular region in *P. liboensis*, *P. omeimontis*, *P. shuichengensis*, and *P. spinata*; reaching to the level between tympanum and eye in *P. qianbeiensis*). *Panophrys congjiangensis* sp. nov. differs from *P. leishanensis* by having slightly small body size, SVL 38.9–40.2 mm in females (vs. 42.3 in single female), having

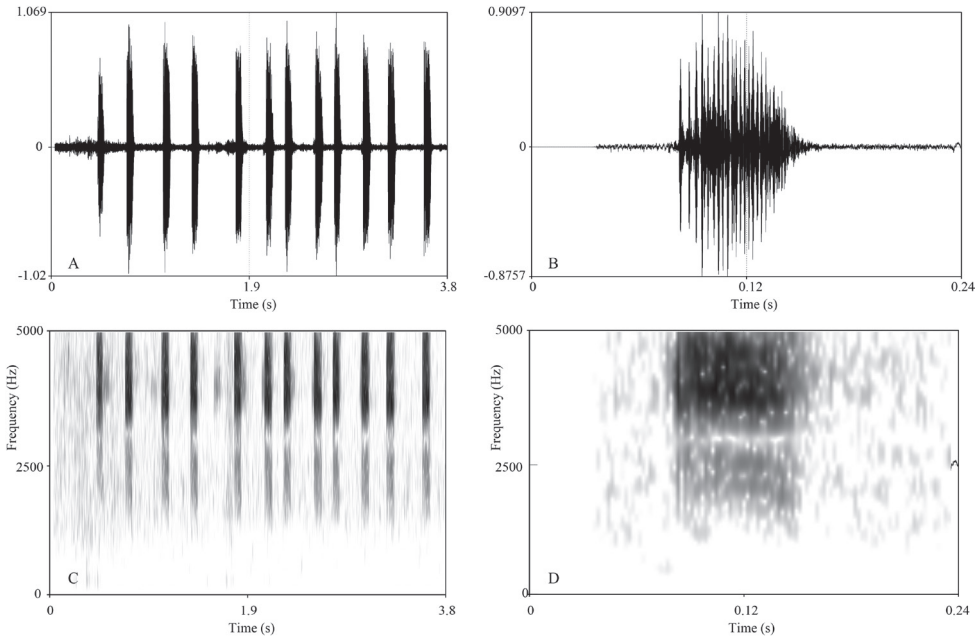


Figure 7. Visualization of advertisement calls of *Panophrys congjiangensis* sp. nov. **A** waveform showing 12 notes of one call **B** waveform showing one note **C** sonogram showing 12 notes of one call **D** sonogram showing one note.

narrow and unobvious lateral fringes on toes (vs. lacking), tibiotarsal articulation reaching the nostril when leg stretched forward (vs. reaching between tympanum to eye). The mean SVL of male *Panophrys congjiangensis* sp. nov. was significantly greater than that of *P. leishanensis*. In addition, the ratios of HDL, HDW, SNT, LAHL, LW, THL, TFL, HLL, and TW to SVL were all significantly greater in male *Panophrys congjiangensis* than in male *P. leishanensis* (all p -values < 0.05; Table 4). *Panophrys congjiangensis* sp. nov. also differs from *P. leishanensis* by having one call 9–14 syllables (vs. calls of *P. leishanensis*, which are 12–14 syllables long), shorter call intervals between syllables (0.167 ± 0.042 s, $N = 53$ in the new species vs. 0.409 ± 0.075 s, $N = 36$ in *P. leishanensis*), and shorter call syllables (0.07 ± 0.06 s, $N = 58$ in the new species vs. 0.105 ± 0.003 s, $N = 37$ in *P. leishanensis*).

From the remaining 24 species occurring in *Panophrys*, *Panophrys congjiangensis* sp. nov. can be distinguished by the absence of vomerine teeth (vs. present in *P. daweimontis*, *P. dongguanensis*, *P. frigida*, *P. jinggangensis*, *P. jiulianensis*, *P. nankunensis*, *P. nanlingensis*, *P. rubrimera*, *P. shimentaina*, and *P. tongboensis*), by the unnotched tongue (vs. tongue notched in *P. binchuanensis*, *P. cheni*, *P. kuatunensis*, and *P. lushuiensis*), by the small horn-like tubercle at edge of upper eyelid (vs. slightly large in *P. acuta*), by the absence of lateral fringes on toes (vs. lacking in *P. angka*, *P. brachykolos*, *P. lishuiensis*, *P. ombrophila*, *P. shunhuangensis*, and *P. wugongensis*; vs. wide in *P. lini*; vs. lacking in males in *P. wushanensis*, wide in females in *P. wushanensis*), by the subarticular tubercles present (vs. absent in *P. baishanzuensis* and *P. mufumontana*), tibiotarsal articulation

reaching the nostril when leg stretched forward (vs. reaching to ocular region in *P. acuta*, *P. baishanzuensis*, *P. binchuanensis*, *P. jiulianensis*, *P. lini*, *P. nanlingensis*, *P. ombrophila*, and *P. wushanensis*; vs. reaching to the level between tympanum and eye in *P. angka*, *P. dongguanensis*, *P. kuatunensis*, *P. lishuiensis*, and *P. nankunensis*; vs. reaching to the level between eye and snout in *P. cheni*, *P. daweimontis*, and *P. shunhuangensis*; vs. reaching to the level behind the eye in *P. brachykolos*, *P. mufumontana*, *P. shimentaina*, and *P. wugongensis*; vs. reaching to the level at center of tympanum *P. daoji*).

Distribution and ecology. *Panophrys congjiangensis* sp. nov. is only known from the type locality, Yueliangshan Nature Reserve, Congjiang County, Guizhou Province, China at elevations of 1142–1206 m. Individuals of the new species were frequently found in bamboo forests, grasses, and shrubberies near streams. Plants in the type locality predominantly fall into the families Urticaceae, Gramineae, Cyperaceae, Rosaceae, Dryopteridaceae, Polygonaceae, Aquifoliaceae, and Fagaceae. In the Yueliang Mountains, *Panophrys congjiangensis* sp. nov. is sympatric with *Pachytriton inexpectatus* Nishikawa, Jiang, Matsui & Mo, 2010; *Amolops sinensis* Lyu, Wang & Wang, 2019; *Nidirana leishanensis* Li, Wei, Xu, Cui, Fei, Jiang, Liu & Wang, 2019; *Hylarana latouchii* (Boulenger, 1899); *Quasipaa boulengeri* (Günther, 1889); *Hyla annectans* (Jerdon, 1870); *Opisthotropis zhaoermii* Ren, Wang, Jiang, Guo & Li, 2017; *Trimeresurus stejnegeri* (Schmidt, 1925); and *Rhabdophis tigrinus* (Boie, 1826). These species were often found in the same streams as *Panophrys congjiangensis* sp. nov.

Discussion

Phylogenetic analyses based on two mitochondrial genes suggested that the specimens collected in this study fell into the *Panophrys*, but were distinct from all previously described species in this genus. Genetic distances between *Panophrys congjiangensis* sp. nov. and its sister species *P. leishanensis* were 3.0% for 16S rRNA and 8.4% for COI, within the ranges expected for interspecific divergences in amphibians (Fouquet et al. 2007; Che et al. 2012). Indeed, other species have been distinguished and recognized based on much lower genetic distances. For example, the *p*-distance is 1.2% between *P. angka* and *P. anlongensis* for 16S rRNA, and 3.6% between *P. sangzhiensis* and *P. spinata* for COI (Suppl. material 1: Table S1; Suppl. material 1: Table S2). *Panophrys congjiangensis* sp. nov. is morphologically similar to *P. leishanensis*, but *Panophrys congjiangensis* sp. nov. is smaller, has a narrow and unobvious lateral fringe on the toes, and the tibiotarsal articulation of the hindlimb reaches the nostril when the leg is adpressed and stretched forward. The two species can also be distinguished based on bioacoustics characters: the call of *Panophrys congjiangensis* sp. nov. had fewer syllables than that of *P. leishanensis*, and the call intervals were shorter. Without phylogenetic, morphological, and bioacoustics data, it is difficult to determine the taxonomic status of new species. In this study, these multiple pieces of evidence supported the validity of *Panophrys congjiangensis* sp. nov. The new species described in this study increases the number of species assigned to *Panophrys* to 60, with 56 recorded from China (Fei and Ye 2016; AmphibiaChina 2020; Frost 2021).

Climatic fluctuations, habitat heterogeneity, habitat diversity, and the dynamics of montane forests may play important roles in driving diversification in the *Panophrys* (Chen et al. 2017; Liu et al. 2018). These factors may have led to the development of complex phenotypes in this genus. Recent studies have revealed high levels of species diversity in the *Panophrys* (Frost 2021). However, *Panophrys congjiangensis* sp. nov. does not belong to any of the clades identified by Chen et al. (2017) and Liu et al. (2018), suggesting that *Panophrys* diversity may remain severely underestimated, even where *Panophrys* species are sympatric ally distributed (Li et al. 2018; Lyu et al. 2020; Su et al. 2020). Until recently, it was difficult to perform taxonomic and phylogenetic studies of the *Panophrys* because many species in this genus are morphologically similar and have sympatric distributions; the many possible cryptic species in the *Panophrys* may have hindered our understanding of diversity in this genus (Chen et al. 2017; Liu et al. 2018; Li et al. 2018; Wang et al. 2019a, b; Mahony et al. 2020; Lyu et al. 2020; Liu et al. 2020; Xu et al. 2020). The high species diversity, strong forest dependence, and sympatric distributions in the *Panophrys* indicate that speciation patterns, niche differentiation, and coexistence mechanisms in this genus require further study.

Biodiversity conservation in southwestern China is a priority of the Chinese government (Ministry of Environmental Protection 2015). Biodiversity conservation programs in this region play an important role in maintaining the stability of mountain ecosystems as well as protecting biodiversity (Körner and Spehn 2002; Tang et al. 2006). Mountain ecosystems are characterized by high biodiversity, with species tending to exhibit a wide range of evolutionary adaptations (McCain and Colwell 2011; Elsen and Tingley 2015). Mountain ecosystems also serve as sanctuaries for many endemic and threatened species, and thus play a major role in the maintenance of biodiversity (Favre et al. 2016). Mountains ecosystems provide key ecological service functions and provide important natural resources that are utilized by local human populations (Körner and Spehn 2002; Grêt-Regamey et al. 2012). Thus, mountain species face a higher risk of extinction due to their limited range, unique environmental adaptations, and geographic isolation, rendering mountain taxa among the most likely to be threatened by climate change.

In the past three years alone, 11 new amphibian species have been described from Guizhou Province, China (Zhang et al. 2017; Li et al. 2018a, b; Li et al. 2019a, b; Lyu et al. 2019b; Wang et al. 2019c; Wei et al. 2020; Luo et al. 2020; Liu et al. 2020; Su et al. 2020). The discovery of these new species suggests that amphibian species diversity in this region is severely underestimated. In the context of global warming, there is an urgent need for a comprehensive, systematic, and in-depth survey of the impacts of climate change on terrestrial vertebrates to provide a basis for scientific decisions regarding amphibian conservation (IPCC 2014).

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Appendix I

Specimens examined

Panophrys jiangi (N=3): China: Guizhou Province: Suiyang County: Kuankuosui National Nature Reserve (type locality): GZNU20070712001. China: Guizhou: Suiyang: Huoqiuba Nature Reserve (topotype locality): GZNU20180606020–606022.

Panophrys liboensis (N=5): China: Guizhou Province: Libo County (type locality): GNUG20150813001, 408002, 408004, 408006–408008.

Panophrys shuichengensis (N=7): China: Guizhou Province: Shuicheng County (type locality): 944001, 98001, 98002, 945005, 2007030, 2007031, 2007032.

Panophrys spinata (N=6): China: Guizhou Province: Dafang County (topotype locality): GZNU201707015011–606016.

Supplementary material 1

Table S1. Uncorrected *p*-distance between Megophryinae species based on 16S rRNA sequences

Authors: Tao Luo, Yali Wang, Siwei Wang, Xueli Lu, Weifeng Wang, Huaiqing Deng, Jiang Zhou

Data type: molecular data

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Link: <https://doi.org/10.3897/zookeys.1047.61097.suppl1>

Supplementary material 2

Table S2. Uncorrected *p*-distance between Megophryinae species based on COI gene sequences

Authors: Tao Luo, Yali Wang, Siwei Wang, Xueli Lu, Weifeng Wang, Huaiqing Deng, Jiang Zhou

Data type: molecular data

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Link: <https://doi.org/10.3897/zookeys.1047.61097.suppl2>

Supplementary material 3

Table S3. Variable loadings for principal components with Eigenvalues greater than 2, from morphometric characters corrected by SVL

Authors: Tao Luo, Yali Wang, Siwei Wang, Xueli Lu, Weifeng Wang, Huaiqing Deng, Jiang Zhou

Data type: statistical data

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Link: <https://doi.org/10.3897/zookeys.1047.61097.suppl3>

Supplementary material 4

Table S4. Diagnostic characters separating all 59 species of the *Panophrys*

Authors: Tao Luo, Yali Wang, Siwei Wang, Xueli Lu, Weifeng Wang, Huaiqing Deng, Jiang Zhou

Data type: species data

Explanation note: Data modified from Wang et al. 2020; Li et al. 2020; Lyu et al. 2020; Nguyen et al. 2020.

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Link: <https://doi.org/10.3897/zookeys.1047.61097.suppl4>

Crenobiont, stygophile and stygobiont molluscs in the hydrographic area of the Trebišnjica River Basin

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Abstract

In the paper the crenobiont, stygophile and stygobiont malacofauna of the karst area of Popovo Polje around Trebinje (Eastern Herzegovina, BiH) is presented. The materials were collected from springs, caves and interstitial habitats (with a Bou-Rouch pump) at 23 localities. The following species were found: *Pisidium* cf. *personatum* A.W. Malm, 1855, *Theodoxus callosus* (Deshayes, 1833), *Sadleriana fluminensis* (Küster, 1852), *Radomaniola curta* (Küster, 1852), *Radomaniola* cf. *bosniaca* (Radoman, 1973), *Kerkia briani* Rysiewska & Osikowski, 2020, *Montenegrospeum bogici* (Pešić & Glöer, 2012), *Litthabittella chilodia* (Westerlund, 1886), *Travunijana vruljakensis* Grego & Glöer, 2019, a new genus and species of the Sadlerianinae, *Emmericia ventricosa* Brusina, 1870, *Iglica* cf. *absoloni* (A.J. Wagner, 1914), *Plagigeyeria tribuniciae* Schütt, 1963, *Paladilhops arion* Rysiewska & Osikowski, 2021, *Valvata montenegrina* Glöer & Pešić, 2008, *Radix labiata* (Rossmässler, 1835), *Galba truncatula* (O. F. Müller, 1774), *Ancylus recurvus* Martens, 1783, *Ancylus* sp. and the amphibiotic *Succinea* cf. *putris* (Linnaeus, 1758). The redescription of the genus *Travunijana* Grego & Glöer, 2019, applying the characteristics of shell, female reproductive organs and penis, is also presented. The new genus and species are described, based on the shell, penis, radula and fragmentary data on the female reproductive organs. For all species, the mitochondrial cytochrome oxidase subunit I (COI) is applied to confirm the determination; in the case of *Travunijana* and the new genus, the nuclear histone H3 locus is also used, in order to infer both their distinctiveness and phylogenetic relationships.

Keywords

Balkans, Bosnia and Herzegovina, cave, COI, H3, karst area, meiofauna, molecular systematics, new genus, new species, spring

Introduction

The Dinaric Karst is a global hotspot for subterranean biodiversity. This is particularly true in the case of its stygobiont, stygophilic and crenobiont communities. The present paper focusses on providing further evidence of one generally under-reported aspect of freshwater aquatic biodiversity – namely the malacofauna of the Trebišnjica River Basin, predominantly in the hydrographically complex karst area of Eastern Herzegovina in Bosnia and Herzegovina.

The study reported below, was undertaken under the remit of the RS-Bosnia and Herzegovina Official Government Licence, which is granted annually to the “Proteus Project in Bosnia and Herzegovina” to undertake its objective of protecting and conserving endangered cave fauna and by extension, to protect and conservation-manage the natural karst conduit-aquifer hypogean ecosystems containing the endangered cave faunal species. One of the objectives of the Project is to fully characterise these ecosystems and in doing so, to provide an inventory of their biodiversity.

In this context, the contribution made by the visiting team of malacologists from the Department of Malacology of the Jagiellonian University’s Institute of Zoology and Biomedical Research and from Department of Animal Reproduction, Anatomy and Genomics of University of Agriculture in Krakow, both in Poland, has provided the “Proteus Project” with vital information on the biological characteristics and geographic distribution of a range of genera and species of malacofauna collected at 23 locations connected to 11 separate karst conduit-aquifer ecosystems across a wide area of the Trebišnjica River Basin. The 23 sampling locations were purposely selected by the Director of the “Proteus Project” to represent a typical range of karst hydrological features, such as cave resurgence springs (vrela), ponors and estavelles, either underground or at surface outlets or inlets.

Speleomalacological research on this scale and in such an integrated form, has never been undertaken before now in Bosnia and Herzegovina. Not surprisingly, therefore, the Polish team has identified a new genus and species of meiofaunal gastropod (Mollusca). As a standalone account, these first results, containing verifiable genomic data are of great scientific importance in their own right, but when combined with the associated variety of environmental data being amassed by the “Proteus Project”, they assume a much greater value.

In regard to both ecosystem services and as a nutrient-rich food supply, the importance of the position of malacofauna near the bottom of the “foodchain” of the subterranean aquatic ecosystem, cannot be overstated. Without them being present in all their wonderful variety and population numbers, the diversity of many of the higher cave animals would certainly not be as great.



Figure 1. Selected studied localities from Trebinje area, part 1 **A** locality 1, Vrelo „Vrijeka” (Bijeljani), Dabarsko Polje **B** locality 5, Vrelo „Pokrivenik” (Muhareva Ljut), Popovo Polje **C** locality 6, Vrelo „Lukavac” (Zavala) **D** locality 9, Izvor „Knez” (Trklja) **E** pumping of interstitial fauna at locality 11, Vrelo „Tučevac” (Mostači) **F** locality 13, Vrelo „Polički Studenac” (Crkvina). See also Table 1.

Material and methods

In June and September 2019, we collected aquatic gastropods from springs, interstitial habitats and caves at 23 localities (Table 1, Figs 1–3). They were either collected by hand and sieve in caves and springs, or with a pump applying the Bou-Rouch technique (Bou and Rouch 1967), to sample interstitial fauna below the sedimented floor



Figure 2. Selected studied localities from Trebinje area, part 2 **A** locality 14, Vrelo “Oko” (Zasad) **B** locality 16, Igorovo Jezero (lake) (Gorica) **C** locality 17, Vrelo „Vruljak 2” (Gorica), Trebinjsko Polje **D** locality 20, confluence of Sušica River and Jazina River (Jazina). See also Table 1.

of streams, at a depth of about 50 cm. The tube was inserted in the sediment five times, and 20 litres were pumped each time. Samples were sieved through a 500 μm sieve and fixed in 80% analytically pure ethanol, replaced twice, and later sorted. Next, the snails were put in fresh 80% analytically pure ethanol and kept at $-20\text{ }^{\circ}\text{C}$ temperature in a refrigerator. Percentages of each identified taxon in each locality are presented in Table 1, with division into samples collected on the surface and with a pump.

The shells were photographed with a Canon EOS 50D digital camera, under a Nikon SMZ18 microscope. The dissections were done under a Nikon SMZ18 microscope with dark field, equipped with Nikon DS-5 digital camera, whose captured images were used to draw anatomical structures with a graphic tablet. Morphometric parameters of the shell were measured by one person using a Nikon DS-5 digital camera and ImageJ image analysis software (Rueden et al. 2017). The radulae were extracted with Clorox, applying the techniques described by Falniowski (1990), and examined and photographed using a HITACHI S-4700 scanning electron microscope.

DNA was extracted from whole specimens; tissues were hydrated in TE buffer ($3 \times 10\text{ min}$); then total genomic DNA was extracted with the SHERLOCK extraction kit (A&A Biotechnology), and the final product was dissolved in 20 μl of tris-EDTA (TE)

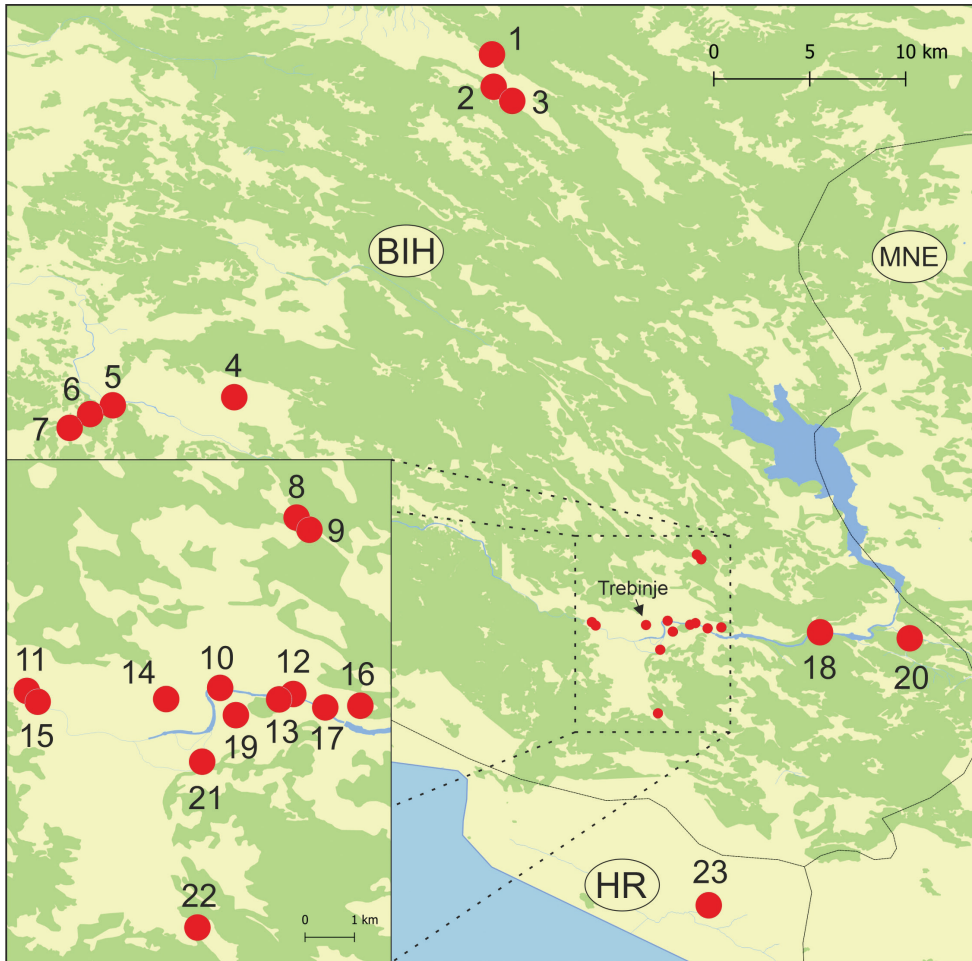


Figure 3. Studied localities.

buffer. The extracted DNA was stored at -80°C at the Department of Malacology, Institute of Zoology and Biomedical Research, Jagiellonian University in Kraków (Poland).

Mitochondrial cytochrome oxidase subunit I (COI) and nuclear histone 3 (H3) loci were sequenced. Details of PCR conditions, primers used and sequencing technique were as given in Szarowska et al. (2016a). Sequences were initially aligned in the MUSCLE (Edgar 2004) programme in MEGA 7 (Kumar et al. 2016) and then checked in BIOEDIT 7.1.3.0 (Hall 1999). Uncorrected p-distances were calculated in MEGA 7. Estimation of the proportion of invariant sites and the saturation test (Xia 2000; Xia et al. 2003) were performed using DAMBE (Xia 2018). In the phylogenetic analysis, additional sequences from GenBank were used (Table 2). The phylogenetic analysis was performed applying two approaches: Bayesian Inference (BI) and Maximum Likelihood (ML). The Bayesian analyses were run using MrBayes v. 3.2.3 (Ronquist et al. 2012) with defaults for most priors. Two simultaneous analyses were performed, each with 10,000,000 generations,

Table 1. The list of studied localities, with a short description of their characteristics, geographical coordinates and taxa identified.

Id	Site names, characteristics and codes	Coordinates	Taxa confirmed	% of taxa in site (surface/pump)
1	Vrelo „Vrijeka” (Bijeljani), Dabarsko Polje; at the outlet (BiH19_08) A permanent cave resurgence spring whose water originates from ponors located in Lukavačko Polje.	43.07417, 18.23899	<i>Emmericia ventricosa</i> <i>Montenegrospeum bogici</i> <i>Radomaniola</i> cf. <i>bosniaca</i>	0/12.6 100/0 0/87.4
2	Estavela „Ljelješnica” (Bijeljani); inside the cave (BiH19_14) When checked, this location was hydrologically inactive.	43.05400, 18.24069	–	–
3	Rijeka (river) „Vrijeka” (Dabarsko Polje); on the surface near entrance of Ponor „Ponikva” (BiH19_15) Samples taken under low-flow conditions.	43.04535, 18.25217	<i>Radomaniola</i> cf. <i>bosniaca</i>	100/0
4	Estavela „Kapuša” (Dračevo); inside the entrance (BiH19_24) Checked when the estavelle was hydrologically inactive.	42.85692, 18.07665	–	–
5	Vrelo „Pokrivenik” (Muhareva Ljut), Popovo Polje; spring at the cave entrance; high water level variation (BiH19_05) Samples taken when the location was hydrologically inactive.	42.85166, 17.99838	<i>Emmericia ventricosa</i>	0/100
6	Vrelo „Lukavac” (Zavala); outlet for Vjetrenica Pećina. Spring below the cave entrance; high water level variation (BiH19_06) Samples taken when the location was hydrologically inactive.	42.84643, 17.9846	<i>Radomaniola</i> cf. <i>bosniaca</i>	0/100
7	Vrelo „Bitomišlje” (Golubinać); in valley above Zavala, with Austro-Hungarian infrastructure (BiH19_07) Samples taken under extremely low-flow conditions.	42.83799, 17.97161	<i>Litthabitella chilodia</i> <i>Montenegrospeum bogici</i>	40.3/0 59.7/0
8	Izvor „Kneginja” (Trklja); a low-flow groundwater spring in Dolomite coming from a limestone blockhouse (BiH19_20)	42.75729, 18.3693	<i>Ancylus</i> sp. <i>Litthabitella chilodia</i>	0/2.7 0/97.3
9	Izvor „Knez” (Trklja); a low-flow groundwater spring in Dolomite coming from a limestone blockhouse (BiH19_21)	42.75463, 18.37218	<i>Ancylus</i> sp. <i>Litthabitella chilodia</i>	0/2.3 0/97.7
10	Confluence of Trebišnjica River with the Potok (stream) Blace (Blace); surface stream from a cave spring-group on the right bank of Trebišnjica River (BiH19_17)	42.71536, 18.35077	<i>Radomaniola curta</i> <i>Sadleriana fluminensis</i> <i>Succinea</i> cf. <i>putris</i>	100/32.1 0/64.3 0/2.6
11	Vrelo „Tučevac” (Mostači); the spring inside the cave (BiH19_13) A high-level overflow spring from a locally complex estavelle cave system. When active, its water originates from ponors in Ljubomirsko Polje 14 km away. This was hydrologically inactive when sampled.	42.71445, 18.30278	<i>Radomaniola</i> cf. <i>bosniaca</i>	100/0
12	Vrelo „Vruljak 1” (Gorica), Trebinjsko Polje. This was sampled in the resurgence pool before which 2 cave rivers Rijeka “Goričica” and Rijeka “Vrulje” have joined inside & emerge (BiH19_03) The cave resurgence spring is just one outlet from a locally very complex cave system, containing a very rich biodiversity. The water originates from ponors in Ljubomirsko Polje about 12 km away.	42.71393, 18.36833	<i>Emmericia ventricosa</i> <i>Pisidium</i> cf. <i>personatum</i> <i>Radomaniola</i> cf. <i>bosniaca</i> <i>Travunijana</i> <i>vruljakensis</i>	0/7.8 50/0 0/92.2 50/0
13	Vrelo „Polički Studenac” (Crkvina); a cave spring in the left bank of Trebišnjica River (BiH19_11)	42.71288, 18.36514	<i>Ancylus recurvus</i> <i>Emmericia ventricosa</i> <i>Iglicopsis butoti</i> sp. nov. <i>Kerkia briani</i> <i>Radomaniola curta</i> <i>Radomaniola</i> cf. <i>bosniaca</i> <i>Travunijana</i> <i>vruljakensis</i>	3.7/0 0/44.3 27.8/0 38.9/0 10.2/7.6 0/48.1 19.4/0
14	Vrelo “Okno” (Zasad); a spring in the entrance to the cave system; surrounded by ancient limestone-block housing; at the centre of Trebinje (BiH19_23) This location is permanently hydrologically active and its water originates from ponors in Ljubomirsko Polje 14 km away. Although it is locally regarded as a vrelo, it is actually an estavelle. This was once used as a public water supply.	42.71274, 18.33697	<i>Radomaniola</i> cf. <i>bosniaca</i> <i>Travunijana</i> <i>vruljakensis</i>	0/5.9 0/94.1
15	Estavela „Pećine” (Mostači) (BiH19_12) This is a major estavelle-type outlet for the karst conduit-aquifer originating at the ponors in Ljubomirsko Polje. It was hydrologically inactive when sampled.	42.71244, 18.30497	<i>Ancylus recurvus</i> <i>Galba truncatula</i>	100/0 0/100
16	Igorovo Jezero (lake) (Gorica); small lake in a collapsed cave passage with cave springs and containing many ponors; muddy bottom (BiH19_19) The water originates from ponors in both Ljubomirsko Polje and Cibrijansko Polje. The ponors in and around the lake feed water underground downstream to Vrelo “Vruljak 2” (Gorica).	42.71111, 18.38495	<i>Ancylus</i> sp. <i>Galba truncatula</i> <i>Radix labiata</i> <i>Sadleriana fluminensis</i>	0/9.1 0/36.4 0/9.1 0/45.4

Id	Site names, characteristics and codes	Coordinates	Taxa confirmed	% of taxa in site (surface/pump)
17	Vrelo „Vruljak 2” (Gorica) , Trebinjsko Polje; this location was sampled at the resurgence spring outlet before which 2 cave rivers have joined inside: Rijeka “Pešterčica” and Rijeka “Venator” (BiH19_02) This is a permanently hydrologically active outlet from a locally very complex cave system containing a very rich biodiversity.	42.71062, 18.37618	<i>Kerkia briani</i> <i>Plagigygeria tribuniciae</i> <i>Radomaniola curta</i> <i>Sadleriana fluminensis</i> <i>Travunijana vruljakensis</i>	15.9/0 2.3/0 0/96.5 0/3.5 81.8/0
18	The intermittently active cave spring, Vrelo „Vražiji Mlin” (D. Grančarevo) ; Trebišnjica Canyon (BiH19_04) This is fed by ponors in Jasen Polje. The location is set in dolomitic limestone.	42.70847, 18.44801	<i>Radomaniola cf. bosniaca</i>	0/100
19	“Slomljen pecina” (Mokri Dolovi) ; (BiH19_22) Since being sampled, this location has now been buried and made inaccessible by urban development.	42.70844, 18.35419	–	–
20	Confluence of Sušica River and Jazina River (Jazina) (BiH19_16) This was sampled under low-flow conditions. The source of the water is a giant estavelle situated in karstified dolomite with dolomitic limestone.	42.70429, 18.50491	<i>Iglica cf. absoloni</i> <i>Litthabitella chilodia</i> <i>Radix labiata</i> <i>Valvata montenegrina</i>	16.7/0 83.3/0 0/72.2 0/27.8
21	Vrelo „Lušac” (Gučina) ; at the surface outlet (BiH19_10) A permanently hydrologically active outlet from a complex karst conduit-aquifer, whose principal source is unproven. This was once a public water supply.	42.70111, 18.3575	<i>Litthabitella chilodia</i> <i>Montenegrospeum bogici</i> <i>Psidium cf. personatum</i> <i>Paladilbiopsis arion</i> <i>Travunijana vruljakensis</i>	14.6/0 22.0/0 4.9/0 58.5/0 0/100
22	Estavela „Mali Šumer” (Bugovina) , Mokro Polje; in the entrance shaft (BiH19_01) The entrance comprises a neo-circular stone wall leading down into the interior by more than 20 stone steps set into the natural stone floor of the karst conduit. The construction is of Austro-Hungarian origin and designed to give easy access to the potable water supply for local people. The location was hydrologically inactive when sampled.	42.65665, 18.34458	<i>Emmericia ventricosa</i>	0/100
23	River Konavoska Ljuta (Ljuta) , Croatia; samples from the surface (Stones, plants) (BiH19_18) This karst river originates from Vrelo “Konavoska Ljuta” a few metres upstream from the sampling location. However, the water itself originates from a ponor 10 km away in Zubačko Polje near Trebinje in Eastern Herzegovina. This cave resurgence spring is used as a public water supply. The samples were collected under low-flow conditions.	42.53408, 18.37647	<i>Psidium cf. personatum</i> <i>Radomaniola curta</i>	15.6/0 84.4/100

with one cold chain and three heated chains, starting from random trees and sampling the trees every 1000 generations. The first 25% of the trees were discarded as burn-in. The analyses were summarised as a 50% majority-rule tree. The Maximum Likelihood analysis was conducted in RAxML v. 8.2.12 (Stamatakis 2014) using the RAxML-HPC v.8 on XSEDE (8.2.12) tool via the CIPRES Science Gateway (Miller et al. 2010). We applied the GTR model whose parameters were estimated by RAxML (Stamatakis 2014).

Systematic part

Bivalvia

Pisidiidae

Pisidium cf. personatum A.W. Malm, 1855

Remarks. Specimens of this common, widely distributed, Holarctic and eurybiotic species were found in many springs. It was also collected from interstitial habitats (with a Bou-Rouch pump) at the localities 12, 21 and 23 (Fig. 4).

Table 2. Taxa used for phylogenetic analyses with their GenBank accession numbers and references.

Species	COI/H3 GB numbers	References
<i>Agrafia wiktoria</i> Szarowska & Falniowski, 2011	JF906762/MG543158	Szarowska and Falniowski 2011/Grego et al. 2017
<i>Alzoniella finalina</i> Giusti & Bodon, 1984	AF367650/-	Wilke et al. 2001
<i>Anagastina zetavalis</i> (Radoman, 1973)	EF070616/-	Szarowska 2006
<i>Ancylus</i> sp. B	DQ301830 DQ301838/-	Albrecht et al. 2006
<i>Ancylus</i> sp. C4	KY012232 KY012163/-	Macher et al. 2016
<i>Ancylus</i> sp. – clade 3	AY350516 AY350519/-	Pfenninger et al. 2003
<i>Ancylus</i> sp. – clade 4	AY350520 AY350521/-	Pfenninger et al. 2003
<i>Avenionia brevis berenguieri</i> (Bourguignat, 1882)	AF367638/-	Wilke et al. 2001
<i>Belgrandia thermalis</i> (Linnaeus, 1767)	AF367648/-	Wilke et al. 2001
<i>Belgrandiella kuesteri</i> (Boeters, 1970)	MG551325/-	Osikowski et al. 2018
<i>Belgrandiella kusceri</i> (A. J. Wagner, 1914)	-/MG551366	Osikowski et al. 2018
<i>Bithynia tentaculata</i> (Linnaeus, 1758)	AF367643/-	Wilke et al. 2001
<i>Bracenicia gloeri</i> Grego, Fehér & Eröss, 2020	MT396209/-	Hofman et al. 2020a
<i>Bythinella cretensis</i> Schütt, 1980	KT353689/-	Szarowska et al. 2016b
<i>Bythiospeum acicula</i> (Hartmann, 1821)	KU341350/MK609536	Richling et al. 2016/Falniowski et al. 2019
<i>Daphniola lousi</i> Falniowski & Szarowska, 2000	KM887915/-	Szarowska et al. 2014a
<i>Dalmatinella fluviatilis</i> Radoman, 1973	KC344541/-	Falniowski and Szarowska 2013
<i>Dalmatinella simonae</i> Beran & Rysiewska, 2021	MT773271/-	Beran et al. 2021
<i>Ecrobia maritima</i> (Milaschewitsch, 1916)	KX355835/MG551322	Osikowski et al. 2016/Grego et al. 2017
<i>Emmericia expansilabris</i> Bourguignat, 1880	KC810060/-	Szarowska and Falniowski 2013a
<i>Fissuria boui</i> Boeters, 1981	AF367654/-	Wilke et al. 2001
<i>Gracocarganiella parnassiana</i> Falniowski & Szarowska, 2011	JN202352/-	Falniowski and Szarowska 2011
<i>Gracocarganiella</i> sp.	JN202353/MN03140	Falniowski and Szarowska 2011/Hofman et al. 2019
<i>Graziana alpestris</i> (Frauenfeld, 1863)	AF367641/-	Wilke et al. 2001
<i>Grossuana hohenackeri</i> (Küster, 1853)	KC011749/-	Falniowski et al. 2012
<i>Hauffenia michleri</i> (Kuščer, 1932)	KT236156/KY087878	Falniowski and Szarowska 2015 /Rysiewska et al. 2017
<i>Heleobia maltzani</i> (Westerlund, 1886)	KM213723/MK609534	Szarowska et al. 2014b/ Falniowski et al. 2019
<i>Horatia klecakiana</i> Bourguignat, 1887	KJ159128/-	Szarowska and Falniowski 2014
<i>Iglica gracilis</i> (Clessin, 1882)	MH720985/MH721003	Hofman et al. 2018
<i>Islamia zermanica</i> (Radoman, 1973)	KU662362/MG551320	Beran et al. 2016/Grego et al. 2017
<i>Littorina littorea</i> (Linnaeus, 1758)	KF644330/KP113574	Layton et al. 2014/unpub.
<i>Lithoglyphus prasinus</i> (Küster, 1852)	JX073651/-	Falniowski and Szarowska 2012
<i>Marstoniopsis insubrica</i> (Küster, 1853)	AF322408/-	Falniowski and Wilke 2001
<i>Moitessieria</i> cf. <i>puteana</i> Coutagne, 1883	AF367635/MH721012	Wilke et al. 2001/Hofman et al. 2018
<i>Montenegrospeum bogici</i> (Pešić & Glöer, 2012)	KM875510/MG880218	Falniowski et al. 2014/Grego et al. 2018
<i>Montenegrospeum sketi</i> Grego & Glöer, 2018	MG880216/-	Grego et al. 2018
<i>Paladilhopsis grobbeni</i> Kuščer, 1928	MH720991/MH721014	Hofman et al. 2018
<i>Pontobelgrandiella</i> sp. Radoman, 1978	KU497024/MG551321	Rysiewska et al. 2016/Grego et al. 2017
<i>Pseudamnicola pieperi</i> (Schütt, 1980)	KT710670/-	Szarowska et al. 2016a
<i>Pseudorientalia</i> sp.	KJ920477/-	Szarowska et al. 2014c
<i>Radomaniola curta</i> (Küster, 1853)	KC011814/-	Falniowski et al. 2012
<i>Radomaniola curta curta</i> (Küster, 1853)	KC011781 KC011784 KC011787 KC011788 KC011791 KC011792 KC011810/-	Falniowski et al. 2012
<i>Radomaniola</i> sp.	KC011727 KC011745 KC011747 KC011763 KC011764 KC011766/-	Falniowski et al. 2012
<i>Sadleriana fluminensis</i> (Küster, 1853)	KF193067/-	Szarowska and Falniowski 2013b
<i>Sarajana apfelbecki</i> (Brancsik, 1888)	MN031432/MN031438	Hofman et al. 2019
<i>Sarajana</i> cf. <i>apfelbecki</i>	MN031431/-	Hofman et al. 2019
<i>Tanousia zermaniae</i> (Brusina, 1866)	KU041812/-	Beran et al. 2015

Gastropoda
Neritopsina: Neritidae

Theodoxus callosus (Deshayes, 1833)

Remarks. This species, described from Greece and reported from Greece and Albania, was found at some larger springs, but never in subterranean waters.

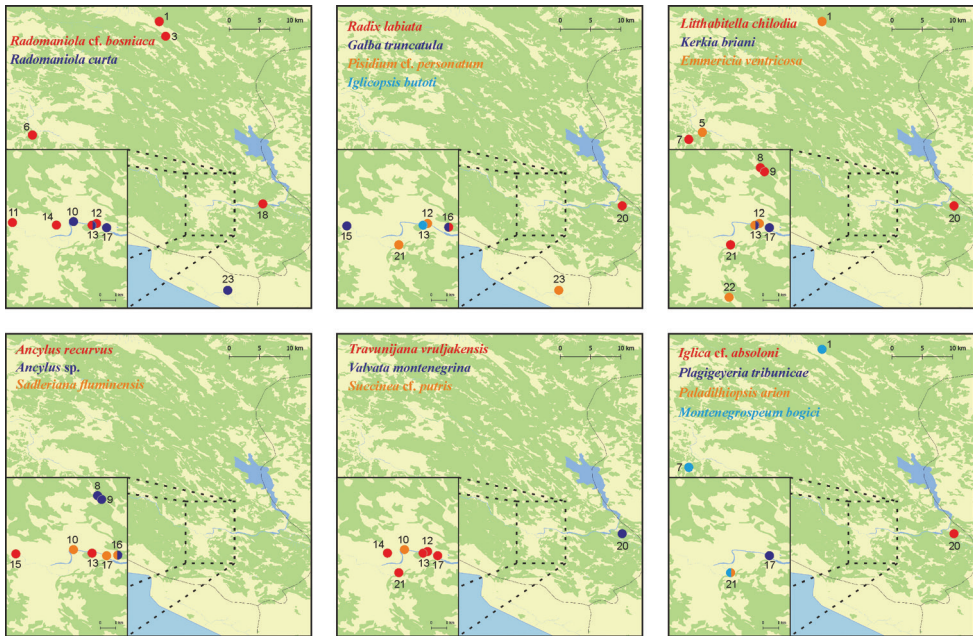


Figure 4. Distribution of the studied taxa. Localities' numbers correspond to Table 1.

Caenogastropoda

Hydrobiidae: Sadlerianinae

Sadleriana fluminensis (Küster, 1852)

Fig. 5A

GenBank no. COI: MZ027620–MZ027622

Remarks. The most widely distributed species of *Sadleriana*. Found at the localities 10, 16 and 17 (Fig. 4).

Radomaniola Szarowska, 2006

Remarks. Replacement name for *Orientalina* Radoman, 1978. The genus is widely spread in the former Yugoslavia, but recorded also from Italy. Radoman (1983) distinguished six species of *Radomaniola*, and in one of them – *R. curta* – eight subspecies. It has to be noted that in modern phylogenetics, the only acceptable meaning of a subspecies is a geographic race, which was hardly the case in Radoman's classification; also, far from being acceptable is that all his species-level taxonomy was based on the shell alone, strikingly variable in this genus (e.g., Falniowski et al. 2012; see also Fig. 5B–M). Molecular and anatomical data (Falniowski et al. 2012) did not confirm the classification of Radoman (1983), but demonstrated high genetic diversity, suggesting a flock of distinct species. The phylogeography as well as molecularly-based

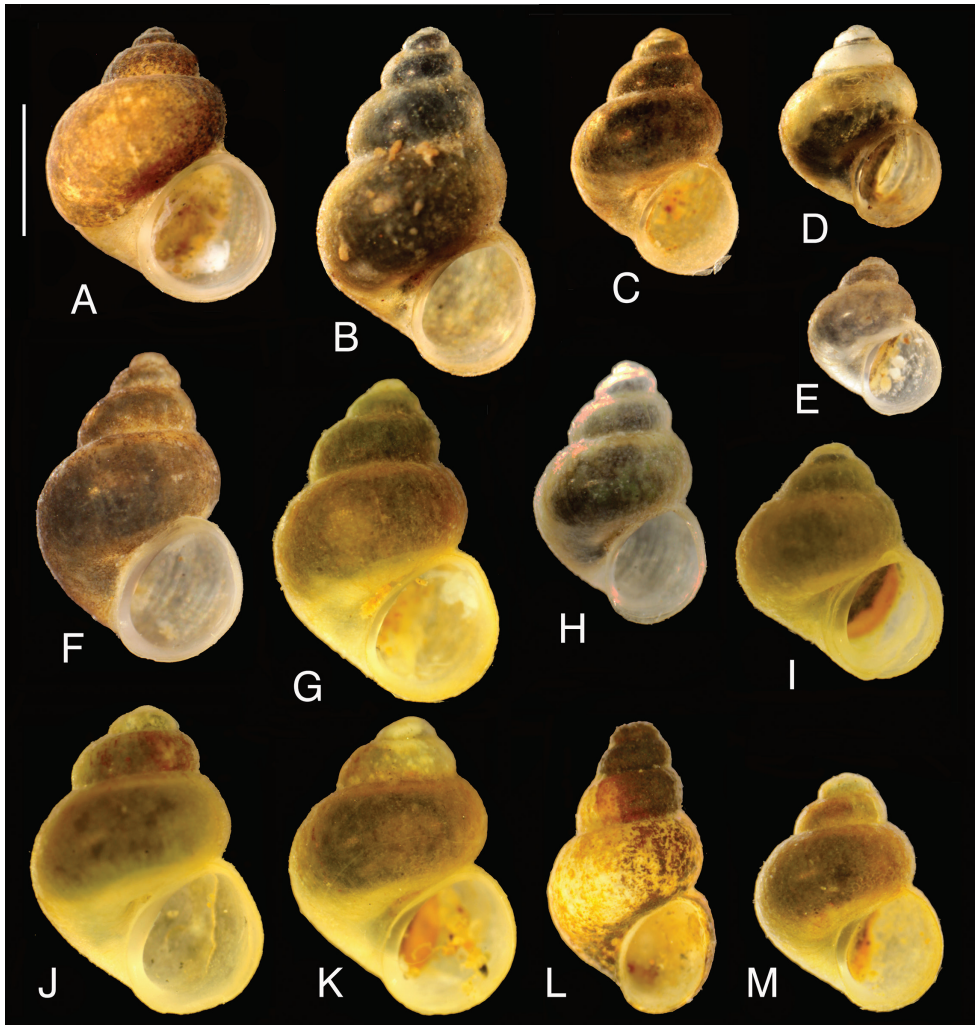


Figure 5. Shells of the studied gastropods: **A** *Sadleriana fluminensis*, locality 10 **B–M** *Radomaniola* **B–H** *R. curta* (localities: **B–D** – 10, **E, F** – 13, **G** – 17, **H** – 23) **I–M** *R. cf. bosniaca* (localities: **I–K** – 1, **L, M** – 12). Scale bar: 1 mm.

species discrimination in *Radomaniola* should be studied with more extensive material, which we are proposing to do. At the moment, considering only *Radomaniola* from the area sampled in this study, one can distinguish two main clades (Fig. 6), representing at least two distinct species. For the one including the sequences of the snails from the spring at Vranjicko Njive, type locality of *Radomaniola curta curta* (sequences KC011781 and KC011784), we used a provisional assignment to this species; for the second clade we provisionally used the name *R. cf. bosniaca*. In general, the representatives of *Radomaniola* were the most common snails at the studied localities, and were found at the surface, as well as in the pumped interstitial samples and could also be found in caves. *Radomaniola*, pigmented and with eyes, is a stygophile gastropod.

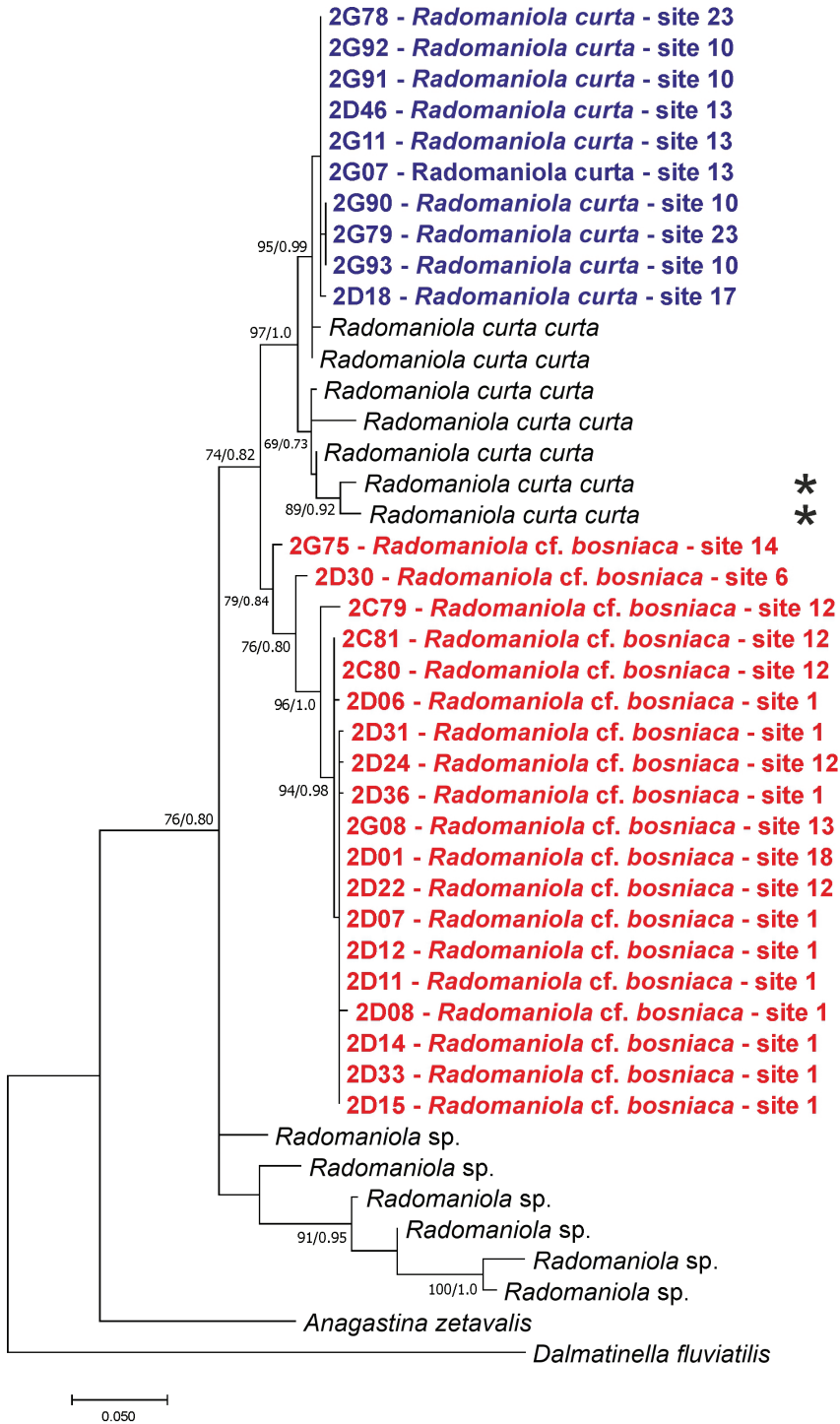


Figure 6. Maximum likelihood (ML) phylogram of the studied *Radomaniola*, based on the partial cytochrome oxidase subunit I (COI) sequences, bootstrap supports given if >60%, together with Bayesian probabilities; topotypes of *R. curta curta* marked with asterisks.

***Radomaniola curta* (Küster, 1852)**

Fig. 5B–H

GenBank no. COI: MW879241–MW879250

Remarks. Found at the localities 10, 13 and 23 (Fig. 4) on the surface and also interstitially and at the locality 17 only on the surface. At the locality 13 in the spring Polički Studenac, in sympatry with *R. cf. bosniaca*.

***Radomaniola cf. bosniaca* (Radoman, 1973)**

Fig. 5I–M

GenBank no. COI: MW879222–MW879240

Remarks. Collected at the localities 1, 6, 12, 13, 14 and 18 (Fig. 4) on the surface, but only at the localities 3 and 11 interstitially. At the locality 13 in sympatry with *R. curta*.

***Kerkia briani* Rysiewska & Osikowski, 2020**

Fig. 7A–C

GenBank no. COI: MT780191–MT780196; H3: MT786730–MT786735; Hofman et al. 2020b

Remarks. Found at the locality 13 (Fig. 4), its type locality, and at locality 17 (about 1 km away), where it is an element of the meiofauna; pumped with a Bou-Rouch pump (Hofman et al. 2020b).

***Montenegrospeum bogici* (Pešić & Glöer, 2012)**

Fig. 7D–K

GenBank no. COI: MZ266648–MZ266650

Remarks. Pešić and Glöer (2012) described a new species of *Bythiospeum* Bourguignat, 1882: *B. bogici* Pešić & Glöer, 2012 from underground waters of Vrelo “Taban”, in central Montenegro. Their description was based on empty shells. Later they (Pešić and Glöer 2013) collected live specimens, and described the lack of eyes and pigment and the penis with a lobe at its medial part. They considered *B. bogici* as belonging to a new genus: *Montenegrospeum* Pešić & Glöer, 2013. Later, Falniowski et al. (2014) demonstrated with molecular data that *Montenegrospeum* belongs to the Hydrobiidae, not Moitessieriidae, despite striking similarity of the shell between this snail and e.g., *Iglica* Wagner, 1927. Numerous live specimens of this species were pumped from interstitial habitats at the localities 1, 7 and 21 (Fig. 4).

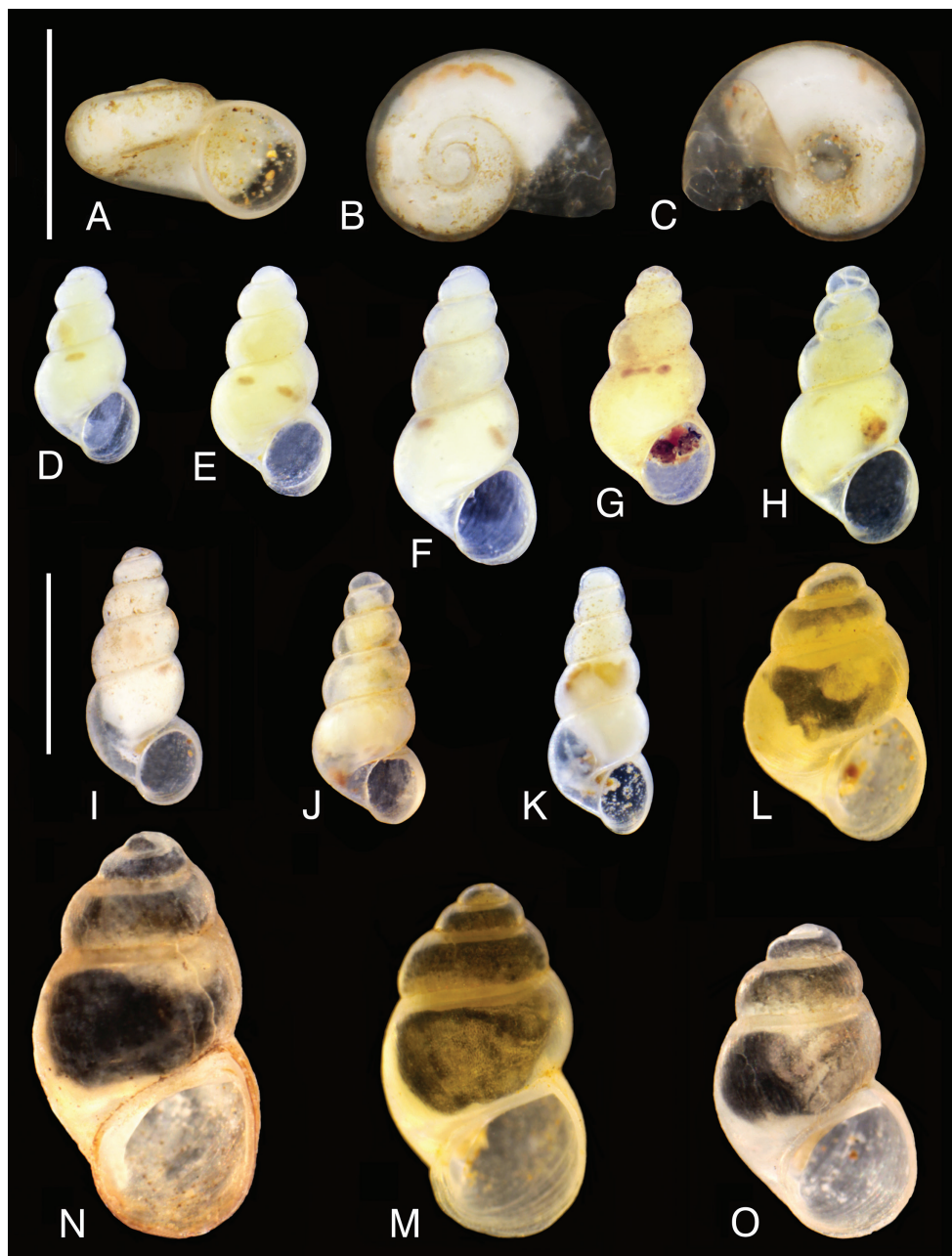


Figure 7. Shells of the studied gastropods: **A–C** *Kerkia briani* **D–K** *Montenegrospeum bogici* (localities: **D–F** – 1, **G, H** – 7, **I** – 13, **J** – 14, **K** – 21) **L–O** *Litthabittella chilodia* (localities: **L, M** – 17, **N–O** – 8). Scale bars: 1 mm.

***Litthabitella chilodia* (Westerlund, 1886)**

Fig. 7L–O

GenBank no. H3: MZ285059–MZ285063

Remarks. This species was found at the localities 7, 8, 9, 20 and 21 (Fig. 4). It was numerous and was also found in a cave and sometimes interstitially; pumped.

***Travunijana vruljakensis* Grego & Glöer, 2019**

Fig. 9

GenBank no. COI: MW879256–MW879272; H3: MW865737–MW865748

Remarks. Grego and Glöer (2019) described a new monotypic genus *Travunijana* from Vrelo “Goricki Studenac” (Gorica), a spring at the right bank of the Trebišnjica River, this being its type locality. They found it also in two other springs: Vrelo Vruljak 1 (Gorica; our locality 12), and Vrelo Vruljak 3 (Gorica). Their diagnosis of the genus was based on a single “unique” character – the strange morphology of the penis – which was based on artefactual appearance, caused by fixation: a nonglandular outgrowth on the left side, located distally (Grego and Glöer 2019). The penis photographed by them presents a bulbous, drastically contracted distal section, making copulation impossible.

Our molecular data (Fig. 8) confirmed the distinctiveness of the genus *Travunijana* Grego & Glöer, 2019. The phylograms based on H3, as well as on both concatenated loci placed *Travunijana* as the sister species with *Graecoarganiella* Falniowski & Szarowska, 2011, and *Sarajana* Radoman, 1975 (bootstrap 85%). The shell habitus is different (conic in *Travunijana*, ovate-conic in *Sarajana*), and the penial morphology differs (Hofman et al. 2019): the outgrowth on the left side is simple and filamentous in *Sarajana*, and short and bi-lobed in *Travunijana*. The phylogram based on COI showed a more complicated pattern, but bootstrap supports were too low for any more certain placement in the phylogeny.

Redescription of the genus *Travunijana* Grego & Glöer, 2019

Diagnosis. Shell conic with moderately convex whorls, big sphaerical bursa copulatrix and two nearly vestigial receptacula seminis, penis long and slender, distally forming a slightly bent filament, at the base of the filament an outgrowth on the left side of the penis, formed of two finger-like lobes.

Description. The shell (Fig. 9) as described by Grego and Glöer (2019). The female reproductive organs (Fig. 10) with bulbous loop of (renal) oviduct, big and spherical bursa copulatrix and two nearly vestigial receptacula seminis: proximal (rs_2 of Radoman 1973) and distal (rs_1 of Radoman 1973) one. The penis

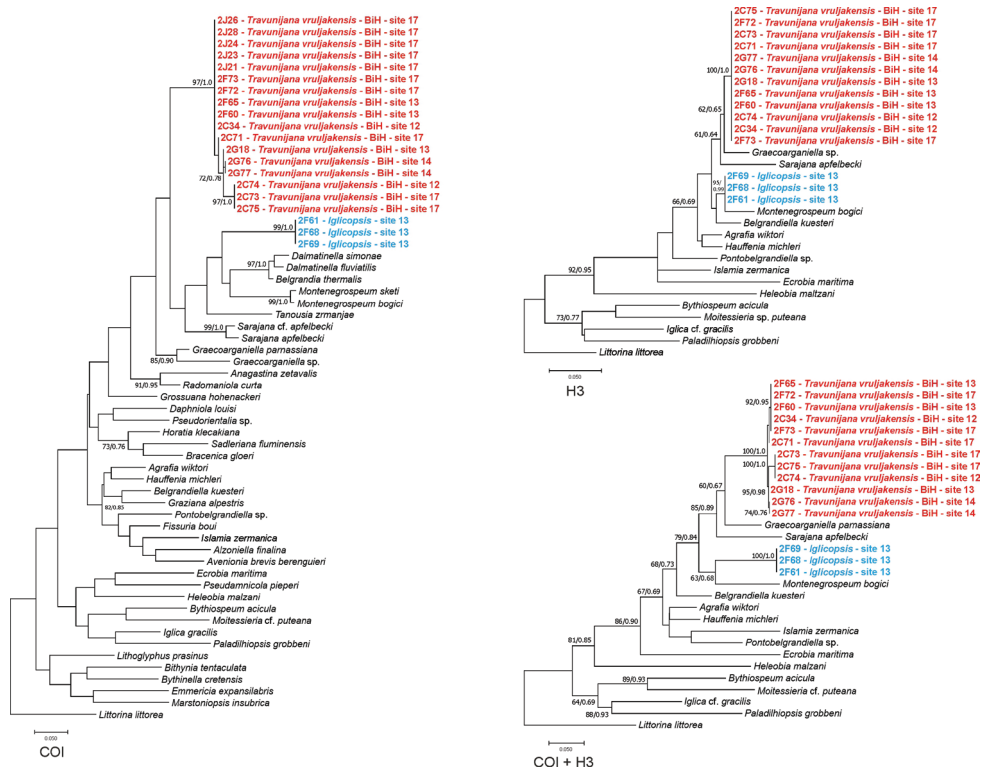


Figure 8. Phylogenetic relationships of *Travunijana* and *Iglicopsis* based on COI, H3 and concatenated loci; bootstrap supports given if over 60%, their values together with Bayesian probabilities.

(Fig. 11) long and slender, slightly bent at its medial section, at the base of the long filamentous distal section and an outgrowth on the left side, consisted of two finger-like lobes.

Travunijana vruljakensis was common at the studied territory, found at the localities 12, 13, 14, 17 and 21. At 12, 13 and 17 (Fig. 4) interstitially pumped.

Iglicopsis Falniowski & Hofman, gen. nov.

<http://zoobank.org/77758877-EEF4-448E-B727-D5632F9E5F51>

Type species. *Iglicopsis butoti* by original designation

Diagnosis. Shell ovate-conic with broad and flat apex, transparent, operculum smooth, no pigment, eyes absent, ctenidium present, penis long, tapering, with bilobed outgrowth on the left side and flat outgrowth at the right side, unpigmented renal oviduct, bursa copulatrix and two small receptacula seminis.

Remarks. *Iglicopsis* shell resembles that of *Montenegropeum*, but is more oval, with broader spire and broader flat apex, sometimes showing scalarity at the body

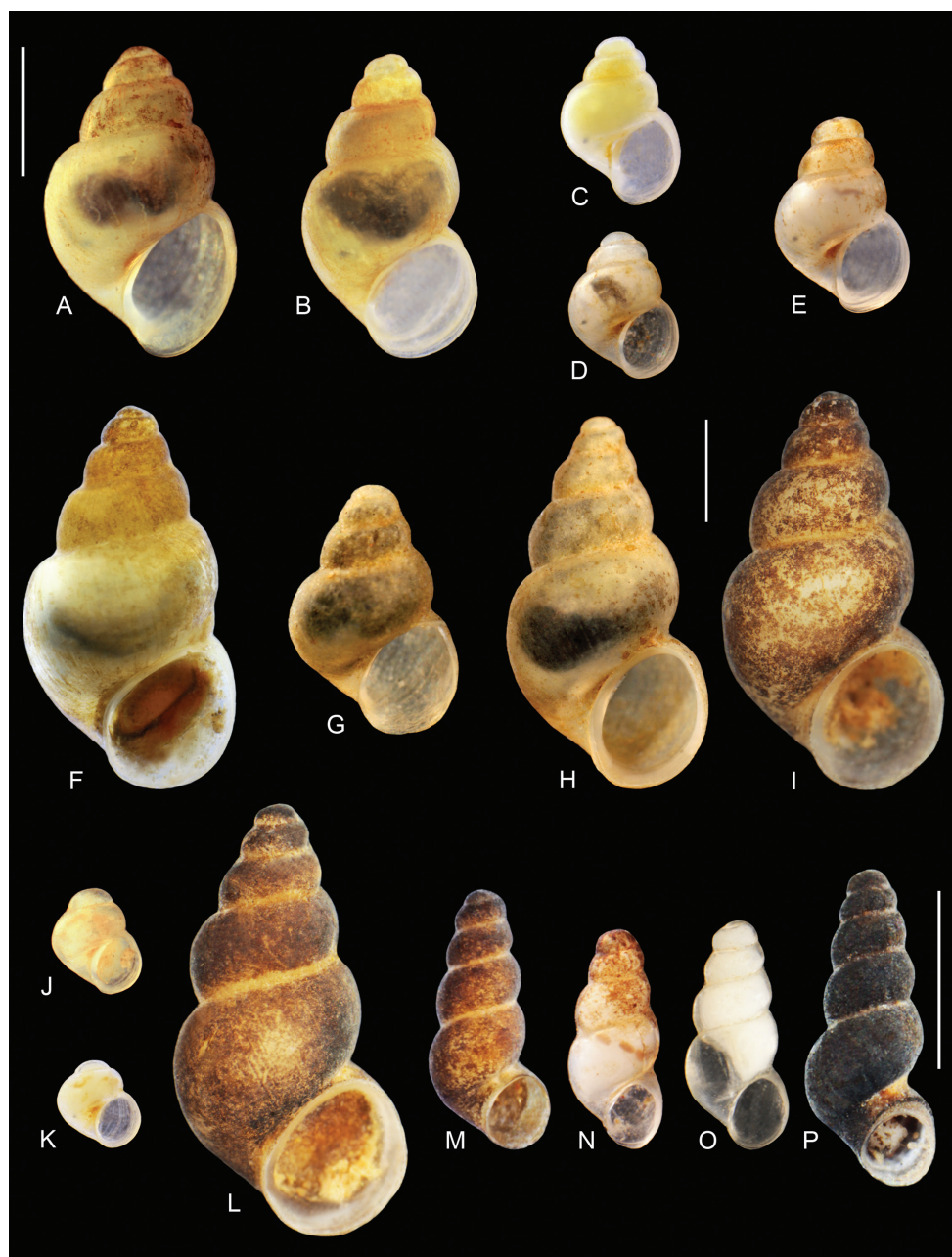


Figure 9. Shells: **A–L** *Travunijana vruljakensis* **M–P** *Iglicopsis butoti* **M** holotype **N** 2F61 **O** 2F68 **P** 2F69 (extraction numbers, see Table 3). Scale bars: 1 mm.

whorl; the penis with the left-side outgrowth located more proximally and bi-lobed and additional flat outgrowth on the right side; the molecular divergence ($p = 0.186$ for mitochondrial COI and $p = 0.114$ for nuclear H3) at the genus-level.

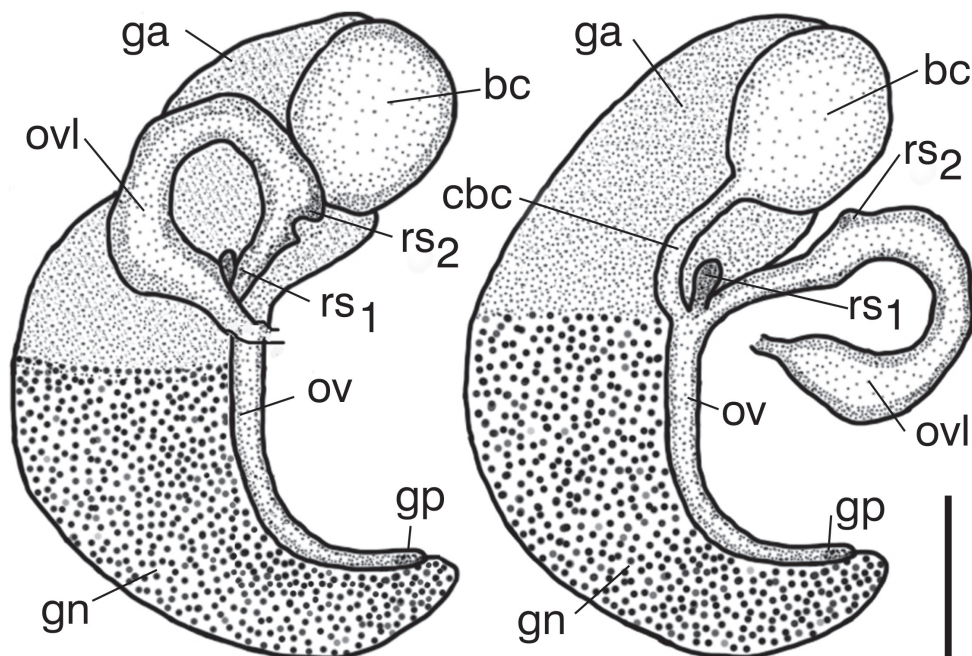


Figure 10. Female reproductive organs of *Travunijana vruljakensis* (bc – bursa copulatrix, cbc – duct of bursa, ga – albuminoid gland, gn – nidamental gland, gp – gonoporus, ov – oviduct, ovl – loop of (renal) oviduct, rs₁ – distal seminal receptacle, rs₂ – proximal seminal receptacle). Scale bar: 0.25 mm.

***Iglicopsis butoti* Falniowski & Hofman, sp. nov.**

<http://zoobank.org/C1A9D0B0-4B10-4977-B69B-7C4C42BB19D3>

Fig. 9M–P

GenBank no. COI: MW879273–MW879275; H3: MW865749–MW865751

Type materials. Holotype. Ethanol-fixed specimen (Fig. 9M), Vrelo „Polički Studenac” (Crkvina); a cave spring in the left bank of and adjacent to the Trebišnjica River (N 42.71288, E 18.36514) (our locality 13, Fig. 4) close to Trebinje (Bosnia and Herzegovina), interstitially, 50 cm below the gravel floor of the spring; in the collection of the Department of Malacology of Jagiellonian University, voucher number ZMUJ-M.2651.

Paratypes. Three paratypes destroyed to extract DNA, one specimen ethanol-fixed, in the collection of the Department of Malacology of Jagiellonian University, ZMUJ-M.2652.

Diagnosis. Shell minute, ovate-conic, distinguishable from *Montenegrospeum* by a more oval habitus, broader spire and broader flat apex, sometimes showing scalarity at the body whorl; the penis with the left-side outgrowth located more proximally and bi-lobed, and additional flat outgrowth on the right side.

Table 3. Shell measurements (in mm) of holotype and sequenced and illustrated specimens of *Iglicopsis butoti* sp. nov. For explanation of the symbols α - β , see Fig. 13B.

	Holotype	2F61	2F68	2F69
<i>a</i>	1.49	1.29	1.35	1.87
<i>b</i>	0.55	0.54	0.54	0.70
<i>c</i>	0.43	0.39	0.43	0.44
<i>d</i>	0.80	0.62	0.67	0.93
<i>e</i>	0.37	0.34	0.35	0.44
α	90	89	90	90
β	20	18	20	18

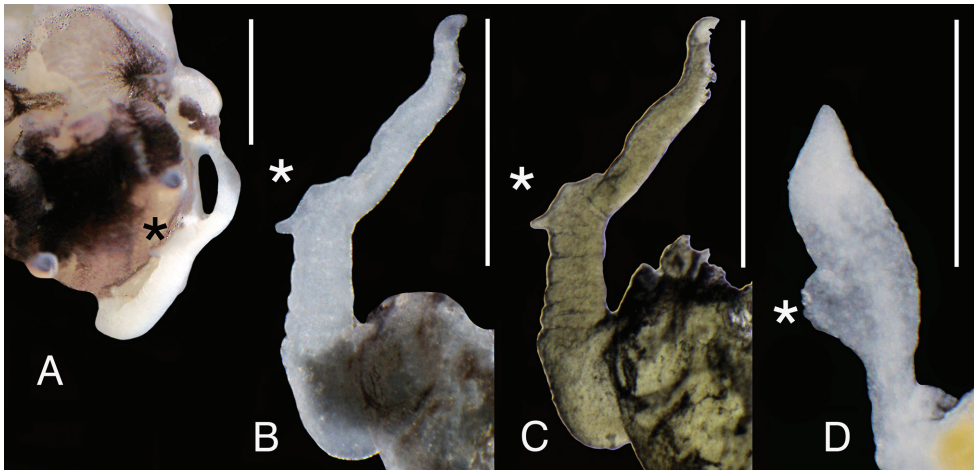


Figure 11. Penis of *Travunijana vruljakensis*. Scale bars: 0.5 mm.

Description. *Shell* (Fig. 9M–P) up to 1.49 mm high and 0.55 mm broad, ovate-conic, whitish, translucent, thin-walled, and consisting of about five whorls, growing regularly and separated by moderately deep suture. Spire high and broad, apex broad and flat, body whorl less than 0.5 of the shell height, Aperture small, prosocline, oval in shape, peristome complete and thin, somewhat swollen, in contact with the wall of the body whorl, in some specimens showing scalarity close to the aperture, umbilicus slit-like. Shell surface smooth, with growth lines hardly visible.

Measurements of holotype and sequenced and illustrated shells: Table 3. Shell variability slight; scalarity and much bigger dimensions of one specimen (Fig. 9P) most probably caused by the larval Trematoda (parasite gigantism).

Soft parts morphology and anatomy. Body white, pigmentless, with no eyes. Ctenidium with nine short lamellae, osphradium elongated. Tectum forming a characteristic broad loop (Fig. 9N). Female reproductive organs with unpigmented renal oviduct, bursa copulatrix and two small receptacula seminis; details unknown.

The radula (Fig. 12) with the central tooth cusp formula:

$$\frac{(4)3-1-3(4)}{1-1} \text{ or } \frac{(5)4-1-4(5)}{1-1}$$

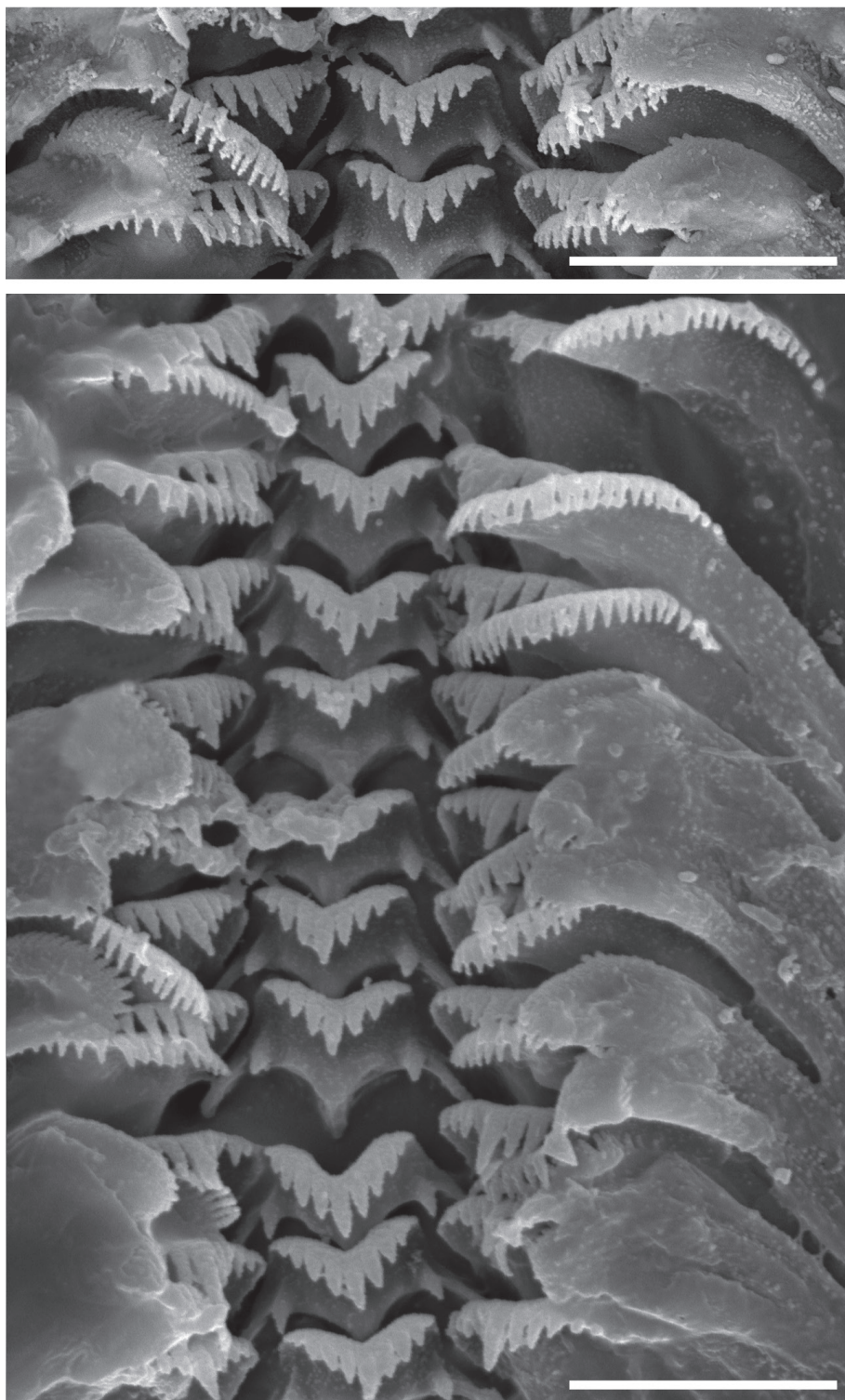


Figure 12. Radula of *Iglucopsis butoti*, scale bars: 10 μ m.

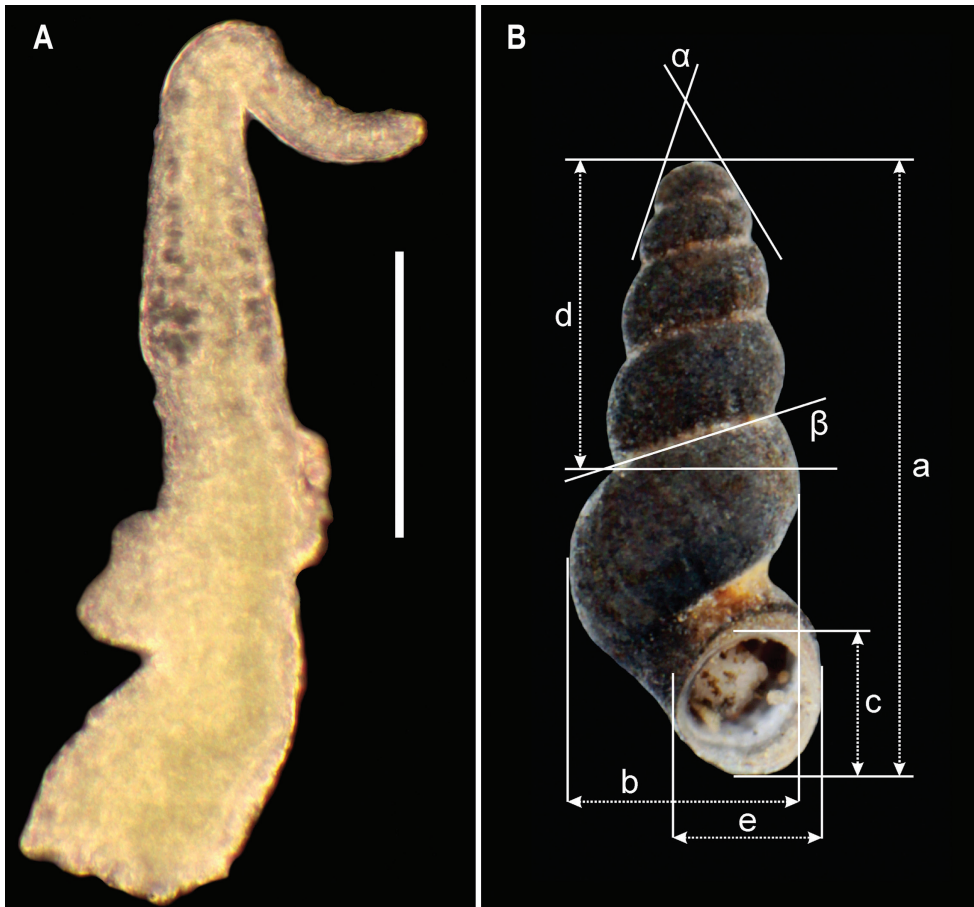


Figure 13. **A** Penis of *Igllicopsis butoti*, scale bar: 0.1 mm **B** shell measurements: a – shell height, b – body whorl breadth, c – aperture height, d – spire height, e – aperture breadth, α – apex angle, β – angle between body whorl suture and horizontal surface.

Rather long and slender cusps grow regularly to central one. Lateral cusp with 5 – 1 – 5(6) long and massive cusps. Inner marginal tooth with ca 23 slender cusps of nearly invariable length along the tooth edge, outer marginal tooth with 26 broadly triangular cusps.

Penis (Fig. 13A) long, tapering, below the half of its length, proximally, bi-lobed outgrowth on the left side and flat outgrowth at the right side, at the distal part and the vas deferens well visible inside, running in zigzags.

Derivatio nominis. The genus name refers to the similarity of the shell to the moitessieriid genus *Iglica* Wagner, 1927. The specific epithet *butoti* refers to the memory of Dr Louis J. M. Butot, a Dutch malacologist devoted mostly to the Greek malacofauna, good friend and the mentor of AF.

Distribution and habitat. Known from the type locality only.

Molecular relationships. despite its shell morphology, *Igllicopsis* clearly belongs to the Hydrobiidae Stimpson, 1865, Sadlerianinae Szarowska, 2006, and not to the

Moitessieriidae Bourguignat, 1863 (Fig. 8). Its sister species is *Montenegrospeum bogici* in the H3 tree (Fig. 8, bootstrap 95%), and on the tree based on both concatenated loci (but with bootstrap 63% only); in the COI tree the bootstrap does not support its phylogenetic position.

Emmericiidae

Emmericia ventricosa Brusina, 1870

Fig. 14A–C

GenBank no. COI: MZ027623–MZ027627

Remarks. The species was found at the localities 1, 5, 12, 13, 22 (estavelle) (Fig. 4), at the surface. Molecular data rather confirms its distinctiveness ($p = 0.038$) from *E. expansilabris* (Bourguignat, 1870), described from Vrelo “Ombla” on the Dalmatian coast in nearby Croatia.

Moitessieriidae

Iglica cf. *absoloni* (A.J. Wagner, 1914)

Remark. Empty shell was found interstitially at the locality 20 (Fig. 4).

Plagigeyeria tribunicae Schütt, 1963

Remark. Empty and incomplete shell was found interstitially at the locality 17 (Fig. 4).

Paladilhiopsis arion Rysiewska & Osikowski, 2021

Fig. 14D, E

GenBank no. COI: MW741739–MW741740; H3: MW776424–MW776425

Remarks. Live specimens were pumped from an interstitial habitat at the locality 21 (Fig. 4). They were recently described as new to science (Hofman et al. 2021). Morphologically and molecularly, they were distinct from the moitessieriid species discussed in Hofman et al. (2018). Rysiewska et al. (2021) demonstrated that at least some of the species assigned to the genus *Plagigeyeria* Tomlin, 1930 belong to the genus *Paladilhiopsis* Pavlović, 1913. Our specimens from Gučina in Trebinje molecularly formed the sister clade with *Plagigeyeria montenegrina* Bole, 1961 from Obodska Pečina in Montenegro. Also, the outline and orientation of the long axis of the aperture was characteristic of *Plagigeyeria*. The similarly shaped shell and geographic range may suggest assignment to *P. nitida* Schütt, 1963, but the number of whorls of our specimens is much higher than presented by Schütt (1972).

Heterobranchia**Heterostropho: Valvatidae*****Valvata montenegrina* Glöer & Pešić, 2008**

Fig. 14F

GenBank no. COI: MZ027632–MZ027633**Remark.** Some specimens found at the locality 20 (Fig. 4); in the surface waters.**Pulmonata****Lymnaeidae*****Radix labiata* (Rossmässler, 1835)**

Fig. 14G

GenBank no. COI: MZ027630**Remarks.** This common Central-European and Mediterranean species was found at the localities 16 and 20 (Fig. 4). Inhabits slowly running or stagnant small water bodies (e.g., Glöer 2019), preferably close to ground waters, but not found in subterranean habitats.***Galba truncatula* (O. F. Müller, 1774)**

Fig. 14H, I

GenBank no. COI: MZ027628–MZ027629**Remarks.** Common Palaearctic gastropod, inhabiting nearly all of Europe. This amphibious and calcifilous (e.g., Glöer 2019) species inhabits small water bodies, rich in vegetation, such as at our locality 16 – a small lake in a collapsed cave, rather than subterranean habitats, but at the locality 15 it was found in an estavelle, a kind of vast subterranean tunnel transporting water either down, as outlet of surface waters, or up, forming temporary active springs. Shells of our specimens (Fig. 14H, I) were somewhat untypical, with low and broad spire, but the variation of the shell in the Lymnaeidae has been long known (e.g., Roszkowski 1914; Falniowski 1980, 1981), as being wider than in any other gastropod group.**Ancylidae*****Ancylus recurvus* Martens, 1783**

Fig. 14J, K

GenBank no. COI: MW879251–MW879253**Remarks.** *Ancylus* is known as a stygophile gastropod (e.g., Culver and Pipan 2009; Macher et al. 2016; personal observations); also inhabiting caves. *Ancylus recurvus* at

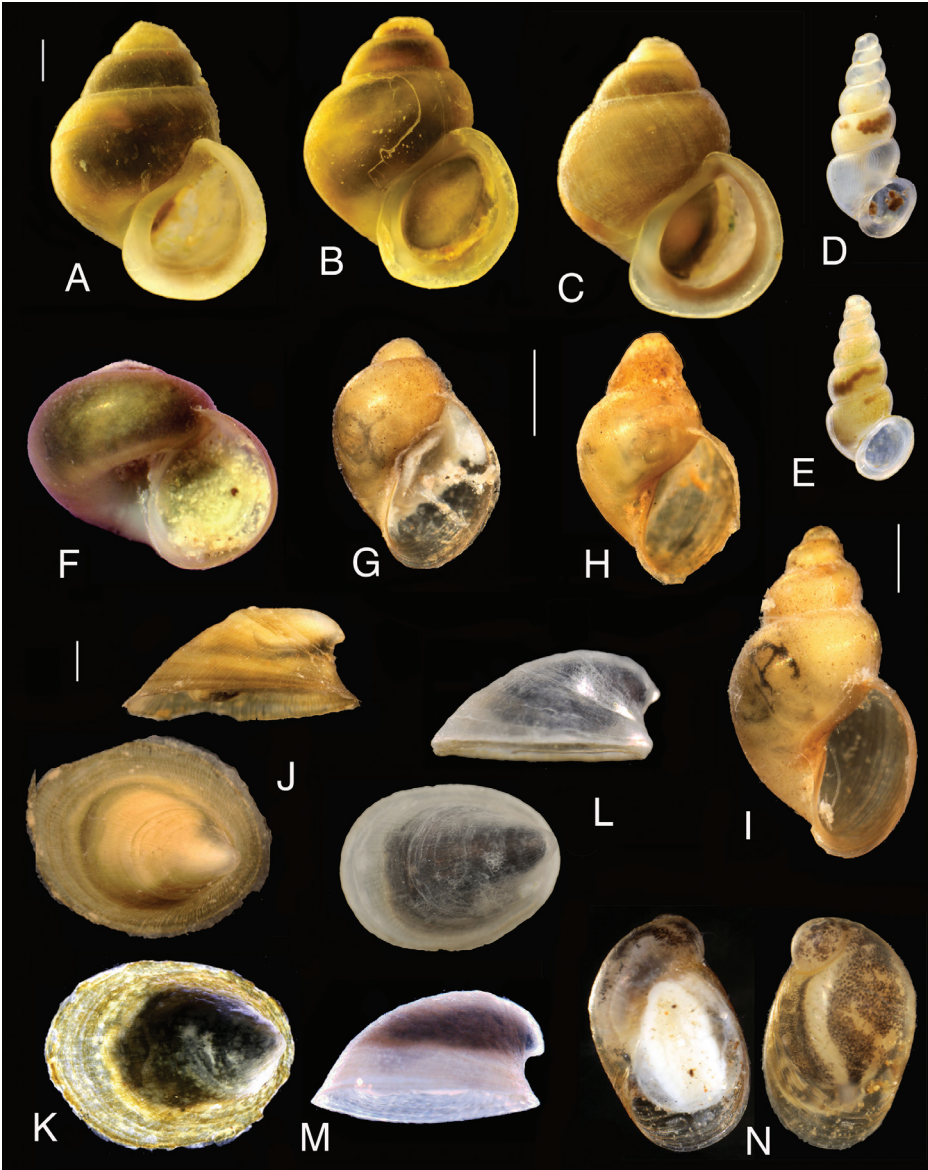


Figure 14. Shells of the studied gastropods: **A–C** *Emmericia ventricosa* (localities: **A** – 1, **B** – 5, **C** – 12) **D, E** *Paladilhiopsis arion* (locality 21) **F** *Valvata montenegrina* (locality 20) **G** *Radix labiata* (locality 16) **H, I** *Galba truncatula* (localities: **H** – 15, **I** – 16) **J, K** *Ancylus recurvus* (localities: **J** – 15, **K** – 13) **L, M** *Ancylus* sp. C4 (localities: **L** – 9, **M** – 16) **N** *Succinea* cf. *putris* (locality 10). Scale bars: 1 mm.

the locality 13 was also found interstitially, pumped, and at the locality 15 (Fig. 4) it inhabited an estavelle. Our *A. recurvus* molecularly belonged to the clade “*Ancylus* sp. B” of Albrecht et al. (2006), Clade 3 of Pfenninger et al. (2003) (Fig. 15). It is molecularly different from *A. fluviatilis* by 9%.

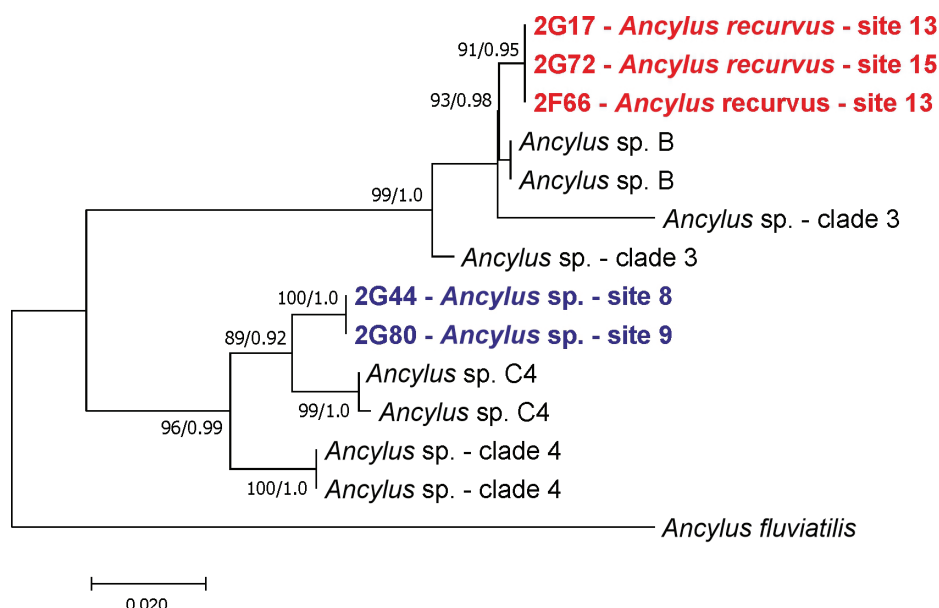


Figure 15. Molecular relationships of the studied *Ancyclus* based on COI; our sequences in red and orange, the other from GenBank; bootstrap supports given if over 60%, their values together with Bayesian probabilities.

Ancyclus sp.

Fig. 14L, M

GenBank no. COI: MW879254–MW879255

Remarks. Considering the shell morphology, it should be determined as *A. fluviatilis* O. F. Müller, 1774, a species reported from this region. However, Pfenninger et al. (2003) demonstrated that *A. fluviatilis* inhabits a wide range throughout Europe, but in the southern regions there are a few cryptic, molecularly defined species of *Ancyclus*. Our *Ancyclus* sp. molecularly belonged to the Clade 4 of Pfenninger et al. (2003) and “*Ancyclus* sp. C4” of Albrecht et al. (2006) (Fig. 15). It was found as a crenobiont in the cave springs at the localities 8, 9 and 16 (Fig. 4). Molecular divergence between this *Ancyclus* sp. and *Ancyclus recurvus* is 7%, and similar value (7.5%) is observed between this *Ancyclus* sp. and *A. fluviatilis*.

Stylommatophora: Succineidae

Succinea cf. *putris* (Linnaeus, 1758)

Fig. 14N

GenBank no. COI: MZ027631

Remarks. Our specimen differed by 12 substitutions (97.55% of identity) from *Succinea* sp. GenBank number KF412772 from “Egypt: Fayoum Governorate”. For the closest European *Succinea*, *S. putris* the identity was only 86.73%. In fact, this

value is close to the threshold one to distinguish species in the Pulmonata, thus our specimen may represent some still unsequenced species of *Succinea*. This amphibious snail was found at locality 10 (Fig. 4).

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A new species of *Dactylolabis* subgenus *Dactylolabis* Osten Sacken, 1860 from China (Diptera, Limoniidae)

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Abstract

Only two species of *Dactylolabis* subgenus *Dactylolabis* Osten Sacken, 1860 were previously known from China. Here, a new species, *Dactylolabis* (*Dactylolabis*) *wudangensis* **sp. nov.**, is reported from China. *Dactylolabis* (*D.*) *gracilistylus* Alexander, 1926 is re-described and illustrated. A key to males of species of the subgenus *Dactylolabis* from China is presented.

Keywords

Biodiversity, crane flies, Dactylolabinae, key, taxonomy

Introduction

Dactylolabis subgenus *Dactylolabis* Osten Sacken, 1860 (Diptera, Limoniidae) is a large subgenus in the subfamily Dactylolabinae. It is distributed worldwide with 50 known species, of which 32 taxa are from the Palaearctic Region, including 16 from Europe, and 18 from the Nearctic Region (Oosterbroek 2021). The subgenus is characterized by the following features: antennae 16-segmented; vein *MA* missing; crossvein *m-cu* near base of cell *dm*; outer gonostylus fleshy with many setae; cerci of ovipositor with wide apex (Osten Sacken 1860; Savchenko 1978; Alexander and Byers 1981; Starý 1992; Podenas et al. 2006; Ribeiro 2008).

* These authors contributed equally to this work.

Materials and methods

The specimens were studied and illustrated with a ZEISS Stemi 2000-c stereomicroscope. Details of the coloration were checked in specimens immersed in 75% ethyl alcohol (C_2H_5OH). Genitalic preparations of males were made by macerating the apical portion of the abdomen in cold 10% NaOH for 12–15 hours. After examination, the genitalia were transferred to fresh glycerine ($C_3H_8O_3$) and stored in a microvial pinned below the specimen. Type specimens of the new species are deposited in the Entomological Museum of China Agricultural University, Beijing, China (CAU). The holotype of *D. (D.) mohanica* Alexander, 1940 was borrowed from the Institute of Zoology, China Academy of Sciences, Beijing, China (IZCAS).

The morphological terminology mainly follows McAlpine (1981), Alexander and Byers (1981), and Savchenko (1978). The terminology applied to the wing veins follows the interpretations of Savchenko (1978) and de Jong (2017). Terminology of the male hypopygium follows Savchenko (1978) and Alexander and Byers (1981). The following abbreviations are used: og = outer gonostylus, ig = inner gonostylus, aed = aedeagus, gx = gonocoxite, 9t = ninth tergite, 9s = ninth sternite.

Taxonomy

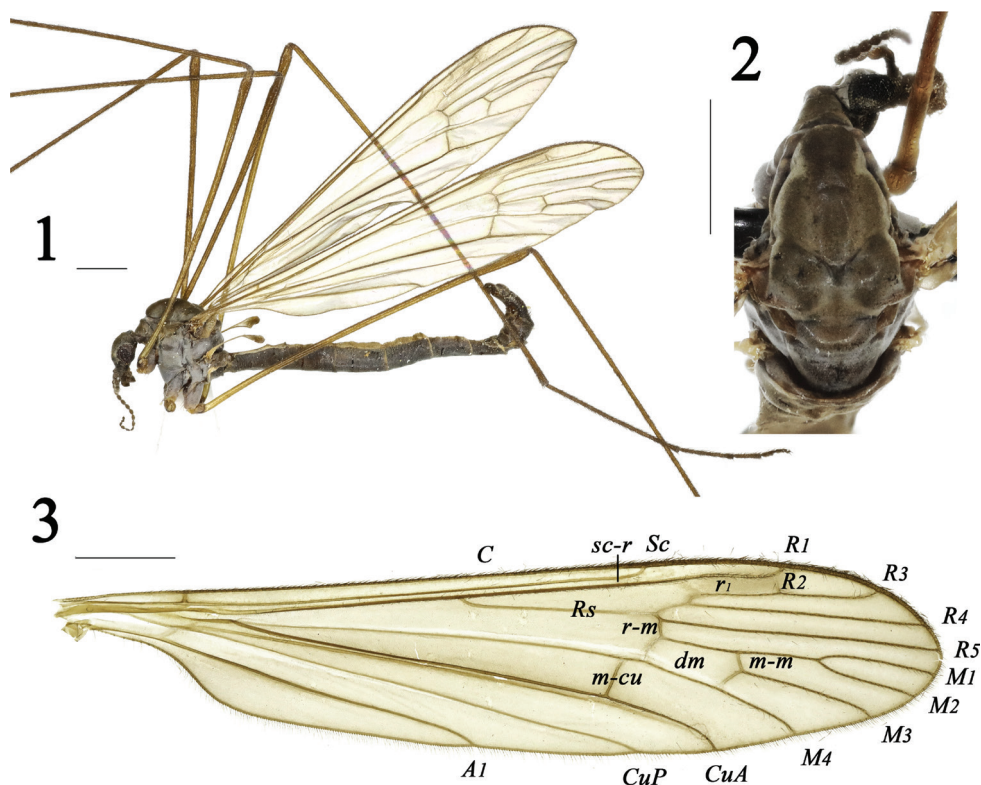
A key to adult males of the subgenus *Dactylolabis* from China

- 1 Wing yellowish hyaline throughout, except pterostigma (Figs 1, 3); vein R_4 relatively straight at tip (Figs 1, 3; Alexander 1926: pl. 1, fig. 8)
..... ***Dactylolabis (Dactylolabis) gracilistylus* Alexander, 1926**
- Wing yellowish hyaline with brownish markings, except pterostigma (Figs 10, 11, 17); vein R_4 relatively curved at tip (Figs 10, 11, 17; Alexander 1940: p. 22, fig. 12) **2**
- 2 Cell r_1 not broad at pterostigma; crossvein *sc-r* shorter than vein R_1 ; crossvein *m-cu* near 1/3 of cell *dm* (Fig. 10; Alexander 1940: p. 22, fig. 12); tips of veins A_1 and *CuP* with brownish markings (Fig. 10)
..... ***Dactylolabis (Dactylolabis) mohanica* Alexander, 1940**
- Cell r_1 rather broad at pterostigma; crossvein *sc-r* longer than vein R_1 ; crossvein *m-cu* near 1/5 of cell *dm*; tips of veins A_1 and *CuP* without brownish markings (Figs 11, 17) ***Dactylolabis (Dactylolabis) wudangensis* sp. nov.**

***Dactylolabis (Dactylolabis) gracilistylus* Alexander, 1926**

Figs 1–7

Dactylolabis gracilistylus Alexander, 1926: 372. Type locality: China: Zhejiang.



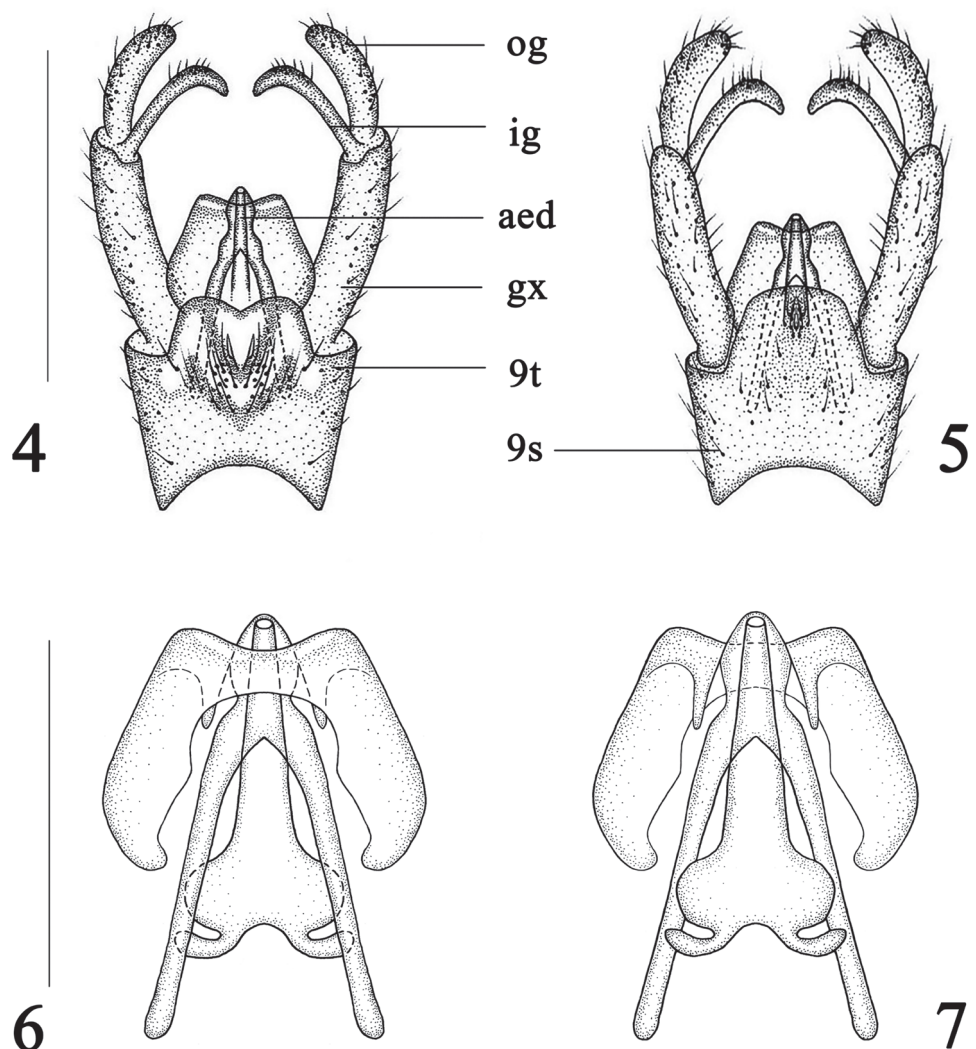
Figures 1–3. *Dactylolabis (Dactylolabis) gracilistylus* Alexander, 1926, male **1** habitus, lateral view **2** head and thorax, dorsal view **3** right wing. Scale bars: 1 mm.

Diagnosis. Wing yellowish hyaline, pterostigma brownish. Vein R_3 as long as vein R_{2+3} . Veins R_4 and R_5 relatively straight. Vein M_1 as long as vein M_{1+2} . Crossvein $m-cu$ located before or near base of cell dm . Posterior margin of 9t with an M-shaped process and a shallow V-shaped notch at middle. Inner gonostylus slender, curved; gonocoxite very elongate and slender, more than twice as long as outer gonostylus. Aedeagus very big, with a shallow V-shaped notch at posterior margin.

Redescription. Male ($n = 3$). Body length 8.2–8.5 mm, wing length 8.4–8.8 mm, antenna length 1.6–1.7 mm.

Head (Figs 1, 2) dark brown with pale gray pollen. Vertex with long setae. Rostrum and palpus brown. Antenna brown.

Thorax (Figs 1, 2) mostly dark brown with gray pollen. Pronotum rather long; mesonotum brownish, prescutum dark brown with pale gray pollen. Thoracic pleuron mostly dark brown with dense gray pollen. Legs: coxae brown with gray pollen; trochanters brownish-yellow; femora more yellow at base, brownish-yellow at tip; tibiae brownish-yellow; tarsi brown. Wing (Figs 1, 3) yellowish hyaline, pterostigma more brownish; veins brownish. Venation: R_s long; R_2 relatively oblique; R_3 as long as R_{2+3} ; R_4



Figures 4–7. *Dactylolabis (Dactylolabis) gracilistylus* Alexander, 1926, male **4** hypopygium, dorsal view **5** hypopygium, ventral view **6** aedeagal complex, dorsal view **7** aedeagal complex, ventral view. Scale bars: 1 mm (**4, 5**); 0.5 mm (**6, 7**).

and R_5 relatively straight; M_1 as long as M_{1+2} ; $m-cu$ located before or near base of cell dm . Halter (Fig. 1) length approximately 1.3 mm, halter stem yellowish; halter brownish.

Abdomen (Fig. 1) elongated, tergites brownish-yellow, sternites dark brown.

Hypopygium (Figs 1, 4–7) dark brown with brownish setae. Surface of 9t with plenty of long setae, posterior margin with an M-shaped process, medially with a shallow V-shaped notch; posterior margin of 9s with plenty of long setae; outer gonostylus cylindrical; inner gonostylus slender, curved; gonocoxite very elongate and slender, more than twice as long as outer gonostylus; aedeagus hyaline, very big, posterior margin with a shallow V-shaped notch.

Female. Similar to male (Alexander 1926: 372).

Material examined. 1 male (CAU), China: Zhejiang, Yuyao, Siming Mountain, 1980.IV.27, Jikun Yang. 1 male (CAU), China: Zhejiang, Qingyuan, Baishanzu, 1984.IV.19, Hong Wu. 1 male (CAU), China: Zhejiang, Deqing, Mogan Mountain, 1991.IV.20.

Distribution. China (Zhejiang).

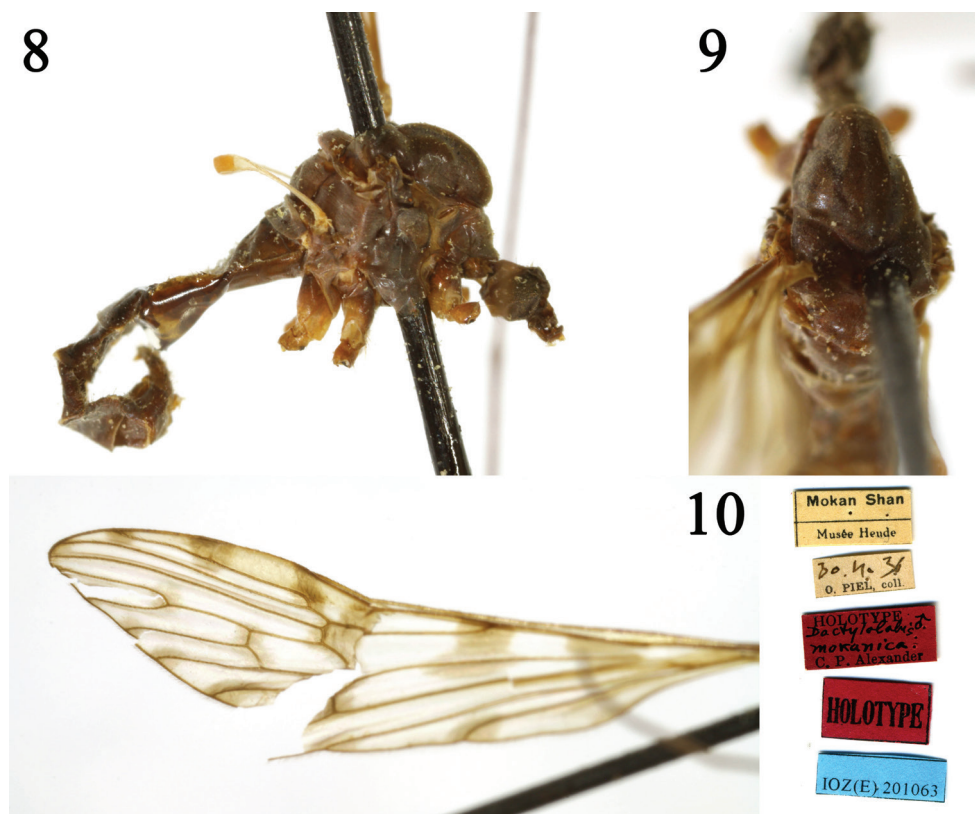
***Dactylolabis (Dactylolabis) moganica* Alexander, 1940**

Figs 8–10

Dactylolabis moganica Alexander, 1940: 22. Type locality: China: Zhejiang: Mogan Mountain.

Diagnosis. Tips of veins A_1 and Cup with brownish markings. Vein R_3 as long as vein R_{2+3} . Vein R_4 relatively curved at tip. Vein M_1 about twice as long as vein M_{1+2} . Cross-vein $m-cu$ located at basal 1/3 of cell dm .

Distribution. China (Zhejiang).



Figures 8–10. *Dactylolabis (Dactylolabis) moganica* Alexander, 1940, male **8** habitus, lateral view **9** head and thorax, dorsal view **10** left wing.

Material examined. *Holotype*, male, China: “Chekiang: Mogan Shan” (= Zhejiang: Mogan Mountain), April 30, 1936, Institute of Zoology, China Academy of Sciences, accession no. IOZ(E) 201063 (IZCAS).

***Dactylolabis (Dactylolabis) wudangensis* sp. nov.**

<http://zoobank.org/8882A009-75B2-4B7D-B5F6-B0A935B1CA4C>

Figs 11–21

Diagnosis. Cell r_1 relatively broad at pterostigma. Vein R_3 shorter than vein R_{2+3} . Vein R_4 relatively curved at tip. Vein M_1 about twice as long as vein M_{1+2} . Crossvein $m-cu$ located at 1/5 of cell dm . Posterior margin of 9t with an M-shaped process and a deep V-shaped notch at middle. Inner gonostylus stubbier than outer gonostylus, curved. Gonocoxite rather short, as long as outer gonostylus. Aedeagus very big, with an elongated tip at posterior margin.

Description. **Male** ($n = 3$). Body length 7.2–10.1 mm, wing length 14.2–18.8 mm, antenna length 2.2–2.4 mm.

Head (Figs 11, 12) dark brown with gray pollen. Rostrum and palpus brown. Antenna brown.

Thorax (Figs 11, 12) mostly dark brown with gray pollen. Pronotum rather long; mesonotum brownish, prescutum brown with four dark brown stripes. Thoracic pleuron mostly dark brown with pale gray pollen. Legs: base of coxae brown, tip of coxae and trochanters brownish-yellow; femora more yellow at base, brown at tip; tibiae and tarsi brown. Wing (Figs 11, 17) yellowish hyaline, pterostigma more brownish, and with brownish markings near base of wing, origin of R_s , around crossvein $sc-r$ and vein R_2 , base of vein R_4 , crossveins $r-m$ and $m-m$, crossvein $m-cu$, and vein CuA ; veins brown. Venation: cell r_1 relatively broad at pterostigma; R_s long; R_2 relatively straight; R_3 shorter than R_{2+3} ; R_4 relatively curved at tip; R_5 relatively straight; M_1 about twice as long as M_{1+2} ; $m-cu$ located at 1/5 of cell dm . Halter (Figs 11, 12) approximately 2.2 mm long, stem yellowish, rest gray.

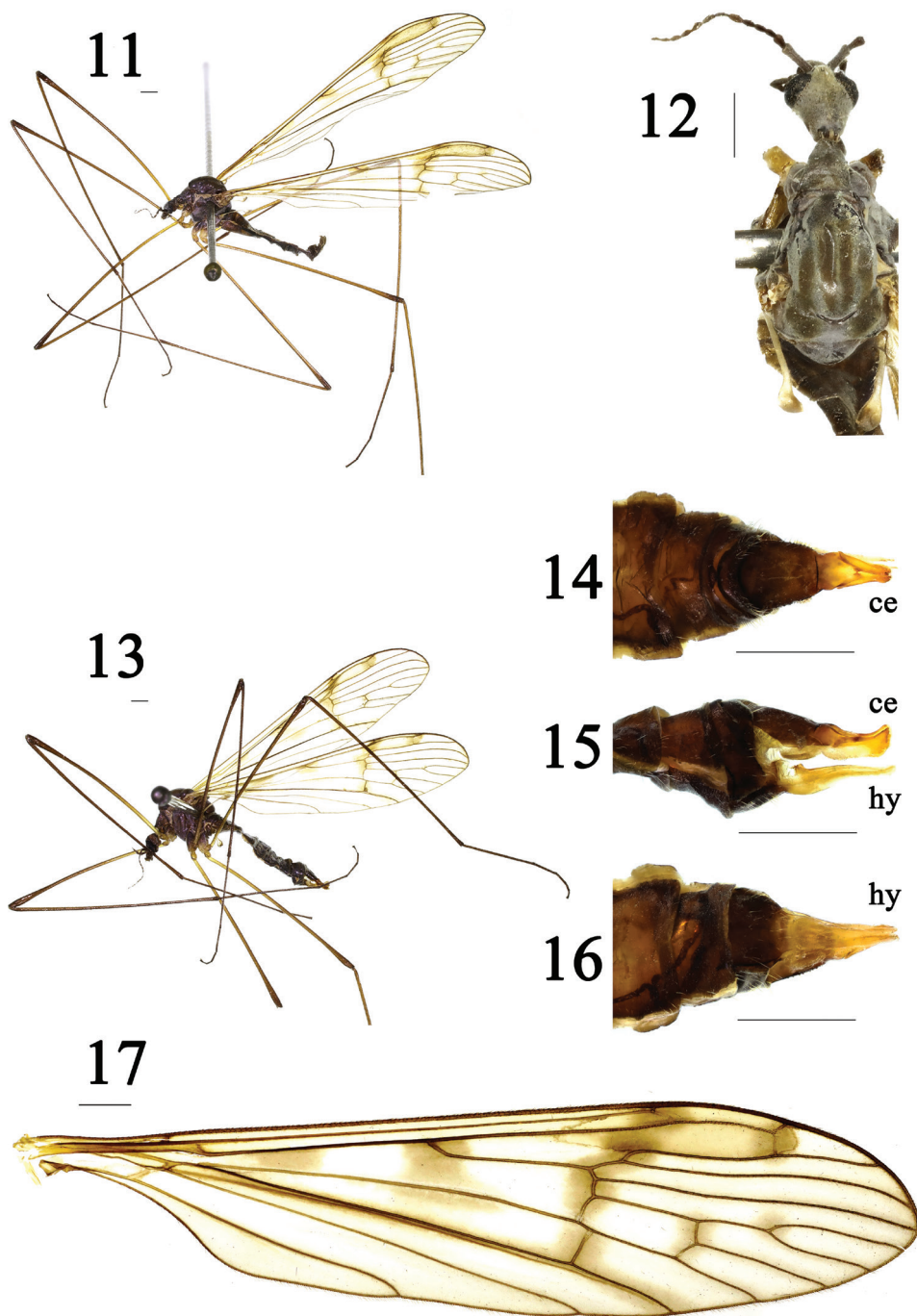
Abdomen (Fig. 11) mostly dark brown with brownish-yellow setae.

Hypopygium (Figs 11, 18–21) brown with brownish setae. Surface of 9t with numerous long setae, posterior margin with an M-shaped process, with a deep V-shaped notch at middle; outer gonostylus cylindrical; inner gonostylus stubby, curved; gonocoxite rather short, as long as outer gonostylus; aedeagus hyaline, very big, with an elongated tip at posterior margin.

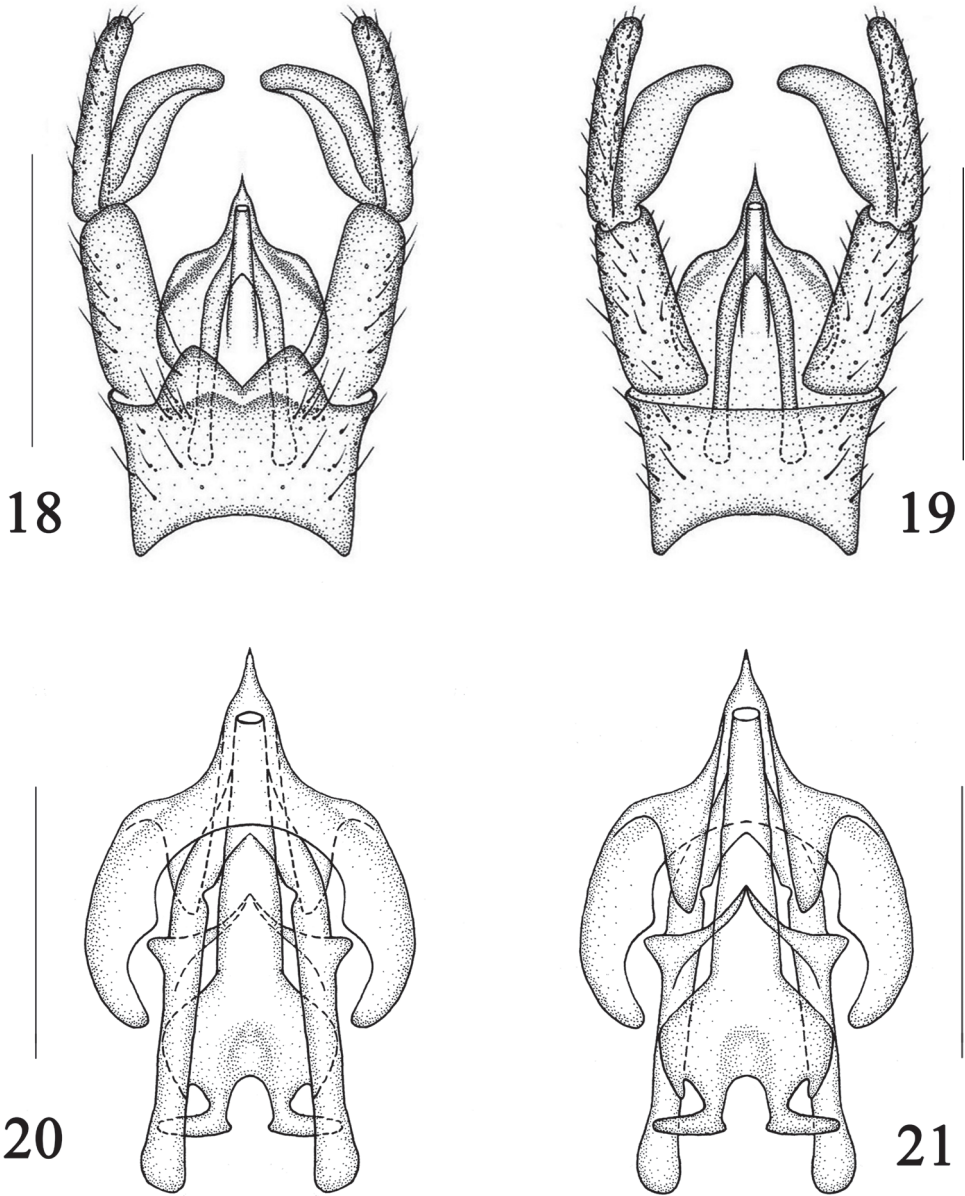
Female ($n = 1$). Similar to male. Body length 8.6 mm, wing length 13.5 mm, antenna length 2.3 mm.

Ovipositor (Figs 13–16) brown with yellow setae. Cercus reddish-brown, broadened at base. Hypogynial valve yellow, narrowed toward tip, longer than cercus.

Type material. *Holotype*: male (CAU), China: Hubei, Danjiangkou, Wudang Mountain, 1600 m, 1984.VI.31, Jikun Yang. *Paratypes*: 2 males, 1 female (CAU), China: Hubei, Danjiangkou, Wudang Mountain, 1600 m, 1984.VI.31, Jikun Yang.



Figures 11–17. *Dactylolabis (Dactylolabis) wudangensis* sp. nov. **11** male habitus, lateral view **12** male head and thorax, dorsal view **13** female habitus, lateral view **14** ovipositor, dorsal view **15** ovipositor, lateral view **16** ovipositor, ventral view **17** male right wing. Scale bars: 1 mm.



Figures 18–21. *Dactylolabis* (*Dactylolabis*) *wudangensis* sp. nov., male **18** hypopygium, dorsal view **19** hypopygium, ventral view **20** aedeagal complex, dorsal view **21** aedeagal complex, ventral view. Scale bars: 1 mm (**18, 19**); 0.5 mm (**20, 21**).

Distribution. China (Hubei).

Etymology. The species is named after the type locality, Wudang Mountain.

Remarks. The new species is somewhat similar to *D. (D.) mohanica* Alexander, 1940 from China (Zhejiang), but can be separated from the latter by crossvein *sc-r*

slightly longer than crossvein R_1 , $m-cu$ located at $1/5$ of cell dm , and tips of veins A_1 and CuA without brownish markings (Figs 11, 17). In *D. (D.) mokanica*, crossvein $sc-r$ is shorter than vein R_1 , crossvein $m-cu$ is located at $1/3$ of cell dm , and the tips of veins A_1 and CuA have brownish markings (Fig. 10; Alexander 1940: p. 22, fig. 12). The new species is somewhat similar to *D. (D.) dilatata* (Loew, 1856) from the West Palearctic and *D. (D.) subdilatata* Starý, 1969 from Czechia in having similar wing markings and venation, but can be separated from the latter two species by posterior margin of 9t with an M-shaped process and cercus shorter than hypogynial valve (Figs 13–16, 18, 19). In *D. (D.) dilatata* and *D. (D.) subdilatata*, the posterior margin of 9t lacks an M-shaped process and the cercus is longer than the hypogynial valve (Starý 1969: p. 125, figs 1, 4, 5, 8). The new species is somewhat similar to *D. (D.) dilatatooides* Savchenko, 1978 from Kazakhstan in having similar wing markings, but can be separated from the latter by vein R_{2+3+4} as long as vein R_2 and posterior margin of 9t with an M-shaped process (Figs 11, 17–19). In *D. (D.) dilatatooides*, vein R_{2+3+4} is almost absent and the posterior margin of 9t has a deep V-shaped notch (Savchenko 1978: p. 1176, fig. 1; p. 1177, fig. 3). The new species is somewhat similar to *D. (D.) laticellula* Savchenko, 1978 from Russia in having similar wing venation, but can be separated from the latter by wing with brownish markings and posterior margin of 9t with an M-shaped process (Figs 11, 17–19). In *D. (D.) laticellula*, the wing has no markings and the posterior margin of 9t lacks an M-shaped process (Savchenko 1978: p. 1176, fig. 2; p. 1177, fig. 4).

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Systematic review of the dextral *Hemiplecta* Albers, 1850 (Eupulmonata, Ariophantidae) from Thailand with description of a new species and list of all the Indochinese species

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Abstract

The genus *Hemiplecta* is a group of large-sized land snails which have long been used as a food resource by Indochinese people. There are five dextral and four sinistral species currently recognized from Thailand. The dextral group is comprised of two previously recorded species (*H. humphreysiana* and *H. distincta*), two newly recorded species (*H. funerea* and *H. esculenta*), and one new species (*H. nemorosa* **sp. nov.**) from northern Thailand is being proposed. We reassessed the diagnostic characters of the genitalia, mantle edge, and radula. Specimens were classified into the genus *Hemiplecta* on the basis of the penial verge and shell lobe, and on the characters of a bulbous gametolytic sac without a gametolytic duct. A complete species list, together with photographs of the name-bearing types or authenticated specimens and the taxonomic status of *Hemiplecta* s.l. that are known from Indochina including Peninsular Malaysia and Myanmar, is provided for the first time. In total, this species list contains 39 available nominal species names described from this area. Type or authentic specimens can be located for 37 nominal species names, of which 25 are illustrated herein and the other 12 were recently illustrated. However, two available species-level names could not be traced to any type specimens. In addition, lectotypes of *H. funerea* and *H. pluto* are designated herein to stabilize the names.

Keywords

Conservation, edible snails, lectotype, Southeast Asia, taxonomy type specimen

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Introduction

As currently understood, the diverse ariophantid snail genus *Hemiplecta* Albers, 1850 consists of around 50 dextral species as well as five sinistral species (MolluscaBase 2020; Sutcharit et al. 2021). They are distributed from Indochina through the Sunda Islands to New Guinea (Zilch 1959; Schileyko 2002), with vague records from the Maldives and Kerala, India (Schileyko 2002; Ramakrishna et al. 2010). The genus contains large ariophantid species (shell width up to 70 mm), and at least two species, *H. distincta* (Pfeiffer, 1850) and *H. esculenta* Maassen, 2006, have been known as food for local people in northeastern Thailand, Laos, Cambodia, and Vietnam. It is also an intermediate host of the rat lungworm (Panha 1987b, 1988b, 1994; Maassen 2006; Tesana et al. 2009; Mienis et al. 2010).

Taxonomically, *Hemiplecta* was first established as a distinct section [? subgenus] of *Nanina* Gray, 1834 with a brief definition to contain large helicoid species mainly from the Philippines and the Malay Archipelago (Albers 1850). It was then reassessed with an extended generic description and the addition of several species from India and Southeast Asia (Albers 1860; Godwin-Austen 1888b). Later, it was formally treated as a subgenus of *Nanina* (Adams and Adams 1858; Nevill 1878), but this was not widely accepted. The generic demarcation of *Hemiplecta* was systematically revised based on anatomical characters of its type species by Godwin-Austen (1897). In addition to their shell morphology, species attributed to this genus have a well-developed dart apparatus and a bulbous gametolytic sac without a duct (Godwin-Austen 1897). These characters were accepted as being more reliable than the shell morphology, and were

followed until recently (Blanford and Godwin-Austen 1908; Thiele 1931; Zilch 1959; Schileyko 2002). The recent phylogeny of some Indochinese *Hemiplecta* (including the type species) confirm that they are monophyletic and are comprised not only of dextral species but also several sinistral species that were previously included in the *Dyakia* Godwin-Austen, 1891 (see Sutcharit et al. 2021). In addition, the modern systematic revision of various helicarioid groups has illustrated the taxonomic importance of reproductive characters for distinguishing taxa at both the generic and specific levels (i.e. Hyman and Ponder 2010; Hyman and Köhler 2018, 2019a, b). Until now, the taxonomic treatment of many *Hemiplecta* species has been confusing and remained contentious due to the paucity of crucial reproductive characters.

In Thailand, thirteen nominal (dextral and sinistral) species have previously been attributed to *Hemiplecta*, many of which were uncritically listed in compilations derived from earlier literature (see Panha 1996; Hemmen and Hemmen 2001) and all are based only on shell morphology. Among these, four sinistral species previously attributed to *Dyakia* have been revised and transferred to *Hemiplecta* (see Sutcharit et al. 2021). The other nine dextral species have been re-classified based on analyses of molecular phylogeny and genitalia morphology. Three nominal species, *Helix crossei* Pfeiffer, 1862, *Helix danae* Pfeiffer, 1863a and *Helix weinkauffiana* Crosse & Fischer, 1863, were synonymized and relocated either to *Quantula* Baker, 1941 or *Phuphania* Tumpeesuwan et al., 2007 (Godwin-Austen 1891; Laidlaw 1931, 1933; Hausdorf 1995; Schileyko 2002; Jirapatrasilp et al. 2020). Two other nominal species, *Helix siamensis* Pfeiffer, 1856a and *Hemiplecta dichromatica* Morlet, 1889, were found to possess genitalia with a long gametolytic duct (Maneevong 2000; Boonmachai and Nantararat 2020; Sutcharit et al. 2020; Pholyotha et al. 2021), suggesting that they should be reclassified into the genus *Cryptozona* Mörch, 1872. Therefore, for Thai species, only four dextral species, *H. humphreysiana* (Lea, 1840), *H. distincta*, *H. neptunus* (Pfeiffer, 1861) and *H. zimmermanni* Godwin-Austen, 1888, and four sinistral species are retained in this genus.

In the present study, we aimed to establish a stable and objective taxonomy by incorporating data from the reproductive organs, pallial system and radula morphology. All recognized and undescribed dextral *Hemiplecta* species occurring in Thailand were critically examined, and their morphological variation and distribution ranges are presented. Previously, most of the *Hemiplecta* species have been described based solely on their shells. However, where anatomical data for additional *Hemiplecta* species was available in the literature, this was summarized and compared with the results of the present study. Furthermore, all the nominal taxa currently attributed to the genus *Hemiplecta* s.l. that have the type locality in Indochina, Peninsular Malaysia and Myanmar are alphabetically listed. In addition, the primary type specimens or authentic specimens (when possible) are figured for further comparisons and precise identification.

Materials and methods

Snails were sampled throughout Thailand. Living snails were euthanized by the two-step method (AVMA 2020), then transferred to 70% (v/v) ethanol for fixation, preservation,

and subsequent anatomical study. Genital systems of up to five specimens of each species were examined. Radulae were extracted, and examined under scanning electron microscopy (SEM; JEOL, JSM-5410 LV). Radula shape and teeth formula were analyzed.

Anatomical abbreviations. Descriptive terms are oriented with reference to the genital orifice. Abbreviations follow Godwin-Austen (1900, 1919), Schileyko (2002), Pholyotha et al. (2020) and Sutcharit et al. (2021): **ag**, albumin gland; **aldl**, anterior left dorsal lobe; **an**, anus; **at**, atrium; **da**, dart apparatus; **dp**, dart papilla; **e**, epiphallus; **ec**, epiphallic caecum; **fl**, flagellum; **fo**, free oviduct; **gs**, gametolytic sac; **h**, heart; **hd**, hermaphroditic duct; **hg**, hermaphroditic gland; **k**, kidney; **lsl**, left shell lobe; **ov**, oviduct; **p**, penis; **pg**, prostate gland; **pdl**, posterior left dorsal lobe; **pr**, penial retractor muscle; **ps**, penial sculpture; **psh**, penial sheath; **puv**, pulmonary vein; **pv**, penial verge; **r**, rectum; **rdl**, right dorsal lobe; **rsl**, right shell lobe; **ur**, ureter; **v**, vagina; **vd**, vas deferens.

Institutional abbreviations

CUMZ	Chulalongkorn University, Museum of Zoology, Bangkok
FMNH	Field Museum of Natural History, Chicago
MNHN	Muséum National d'Histoire Naturelle, Paris
NHM	Natural History Museum, London
NHMUK	when citing specimens deposited in the NHM
NMNH	National Museum of Natural History, Smithsonian Institute, Washington D.C.
RMBR	Raffles Museum of Biodiversity Research, Singapore
RMNH	Naturalis Biodiversity Center, Rijksmuseum van Natuurlijke Historie, Leiden
SMF	Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main
UMZC	University Museum of Zoology Cambridge, Cambridge

Photo credits

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Systematic account

Family Ariophantidae Godwin-Austen, 1888a

Genus *Hemiplecta* Albers, 1850

Hemiplecta Albers, 1850: 60, 61. Albers 1860: 52, 53. Godwin-Austen 1888b: 155–157. Godwin-Austen 1898: 70, 71. Zilch 1959: 317. Schileyko 2002: 1282, 1283.

Nanina (*Hemiplecta*) – Adams and Adams 1858: 222. Nevill 1878: 46–48.

Koratia Godwin-Austen, 1919: 202. Type species: *Helix distincta* Pfeiffer, 1850, by monotypy. Schileyko 2002: 1281, 1282.

Hemiplecta (*Koratia*) – Zilch 1959: 317. Vaught 1989: 97.

Ariophanta (*Semperia*) Godwin-Austen, 1898: 82 [non Crosse 1867: 74, 75]. Type species. *Helix retrorsa* Gould, 1843; by original designation.

Type species. *Helix humphreysiana* Lea, 1840; subsequent designation by Martens in Albers (1860).

Diagnosis. Shell dextral or sinistral, medium to large in size (width about 25 to 75 mm) and monochrome to with stripes, or banding patterns. Apertural lip simple to slightly thickened in adult snails; umbilicus open. Genitalia include penial sheath, straight or coiled epiphallid caecum and short flagellum; penial verge may be present or absent. Dart apparatus well developed; gametolytic sac bulbous to elliptical-shaped (without distinct duct). Mantle edge well developed with or without shell lobes. Jaw smooth (without vertical ribs) and crescentic. Radula with unicuspid central teeth, and bicuspid lateral and marginal teeth.

Remarks. Due to the high degree of similarity in shell morphology, the specific and generic classification within Ariophantidae is usually problematic. There are at least three nominal genera that are often confused, *Nanina* Gray, 1834, *Ariophanta* Des Moulins, 1829 and *Cryptozona* Mörch, 1872. The genitalia have proved to be the distinguishing characters for specific or generic recognition among the Ariophantidae (Laidlaw 1932a; Solem 1966; Sutcharit and Panha 2008). However, only a few species of each genus have been anatomically examined. Based on this limited anatomical information, the unique characters taken from the type species are summarized in Table 1. The genus *Hemiplecta* can be differentiated from these three genera by lacking a gametolytic duct, while the others possess a short to long gametolytic duct. *Hemiplecta* also differs from *Ariophanta* and *Cryptozona* by having unicuspid central teeth, very short or absent flagellum, and a mantle edge with shell lobes; while the latter two genera have tricuspid central teeth, long flagellum, and a mantle edge without shell lobes. In addition, *Hemiplecta* can be distinguished from *Nanina* by its short flagellum (Table 1).

Dextral species in Thailand

Hemiplecta humphreysiana (Lea, 1840)

Figures 1A, 2A, B, 3, 10A–C

Helix humphreysiana Lea, 1840: 175. Type locality: Pondicherry and Singapore. Lea 1841: 463, 464, pl. 12, fig. 16. Reeve 1854: *Helix* pl. 74, species 387.

Hemiplecta humphreysiana: Morgan, 1885a: 378. Godwin-Austen 1898: 74, pl. 80. figs 6, 6b; pl. 61, figs 1, 1e. Collinge 1902: 78, pl. 4, figs 16–23. Laidlaw 1932a: 78. Laidlaw 1932b: 40. Laidlaw 1933: 217. Benthem Jutting 1949: 69. Benthem Jutting 1950: 444, fig. 64. Laidlaw 1957: 134. Benthem Jutting 1959: 148–150. Ho 1995: 104, 105.

Table 1. Comparison of shell and genitalia characters among four genera of Ariophantidae, mainly based on the characteristics of the type species. The superscript numbers are the references: 1 = Schileyko (2002), Thiele (1931), Zilch (1959), 2 = Blanford and Godwin-Austen (1908), 3 = Wiegmann (1898), Sarasin and Sarasin (1899), 4 = Godwin-Austen (1899), and 5 = Sutcharit et al. (2021), present study.

Characters	<i>Ariophanta</i> Des Moulins, 1829 ^{1,2}	<i>Nanina</i> Gray, 1834 ^{1,3}	<i>Hemiplecta</i> Albers, 1850 ^{1,2,5}	<i>Cryptozonia</i> Mörch, 1872 ^{1,4}
Gametolytic duct	Short	long	absent	short / absent
Gametolytic sac	Bulbous	bulbous	bulbous	bulbous
Epiphallus	short, nearly absent	long	long	long
Epiphallic caecum	long and straight	short and straight	long and straight or coiled	long and straight
Flagellum	very short or absent	long	short	short
Shell lobe	Absent	present	present / absent	absent
Shell coiling	dextral / sinistral	dextral	dextral / sinistral	dextral
Central teeth	Tricuspid	unicuspid	unicuspid / tricuspid	tricuspid
Distribution	South Asia	Indonesia, New Guinea	Southeast Asia, New Guinea	South Asia, Indochina
Type species	<i>Helix laevipes</i> Müller, 1774	<i>Helix citrina</i> Linnaeus, 1758	<i>Helix humphreysiana</i> Lea, 1840	<i>Helix ligulata</i> Férussac, 1819

Nanina humphreysiana: Martens, 1867: 233, pl. 10, figs 2, 2b, 4. Tryon, 1886: 36, pl. 11, figs 52, 53, pl. 12, fig. 54.

Nanina (Hemiplecta) humphreysiana: Tryon, 1886: 36, pl. 11, figs 52, 53; pl. 12, fig. 54.

Type specimen. See the species list of the Indochinese species (Fig. 12D).

Material examined. SINGAPORE: Bukit Timah: RMBR 1990.1711 (1 specimen in ethanol), 1990.15781–2 (2 specimens in ethanol); CUMZ 4573 (1 shell; Figs 2A, 3). Botanic Garden: RMBR 1975.2.10.89 (1 shell), RMBR 1990.1710 (1 specimen in ethanol); CUMZ 4571/1 (1 shell), CUMZ 4572 (4 specimens in ethanol). Nee Soon: RMBR 1990.15945 (1 shell), RMBR 1990.16996 (1 specimen in ethanol), RMBR 1990.15103–4 (2 specimens in ethanol), RMBR 1992.3159 (1 specimen in ethanol), RMBR 1992.3160–1 (2 shells), RMBR 1992.3162 (1 specimen in ethanol), RMBR 1994.4116 (1 specimen in ethanol). Singapore: RMBR 1989.509–513 (5 shells), RMBR 1990.15105 (1 specimen in ethanol). THAILAND: Sirindhorn Waterfall, Halabala National Park, Narathivat Province: CUMZ 4647 (2 shells), CUMZ 4648 (1 shell; Fig. 2B).

Shell. Shell large (height up to 40 mm, width up to 55 mm), dextral and conic to depressed conic (Fig. 2A, B). Whorls 6 to 8, slightly convex; suture wide and shallow. Shell yellowish to brownish, usually with narrow dark brown band on periphery. Upper shell surface darker than lower surface. Apex obtuse; embryonic shell large and smooth; following whorls with thin growth lines. Last whorl rounded to slightly angulate; aperture ovate; lip simple but slightly thickened in adult snail. Columella slightly dilated; parietal callus thin and translucent. Umbilicus open and deep.

Genitalia. Atrium (at) very short (Fig. 3A). Penis (p) long, slender, cylindrical, and encircled by thin penial sheath (psh) extending about one-third of penis length. Epiphallic caecum (ec) short, straight; penial retractor muscle (pr) thin and attached to the tip. Epiphallus (e) short and about one-third of penis length. Flagellum (fl) short,

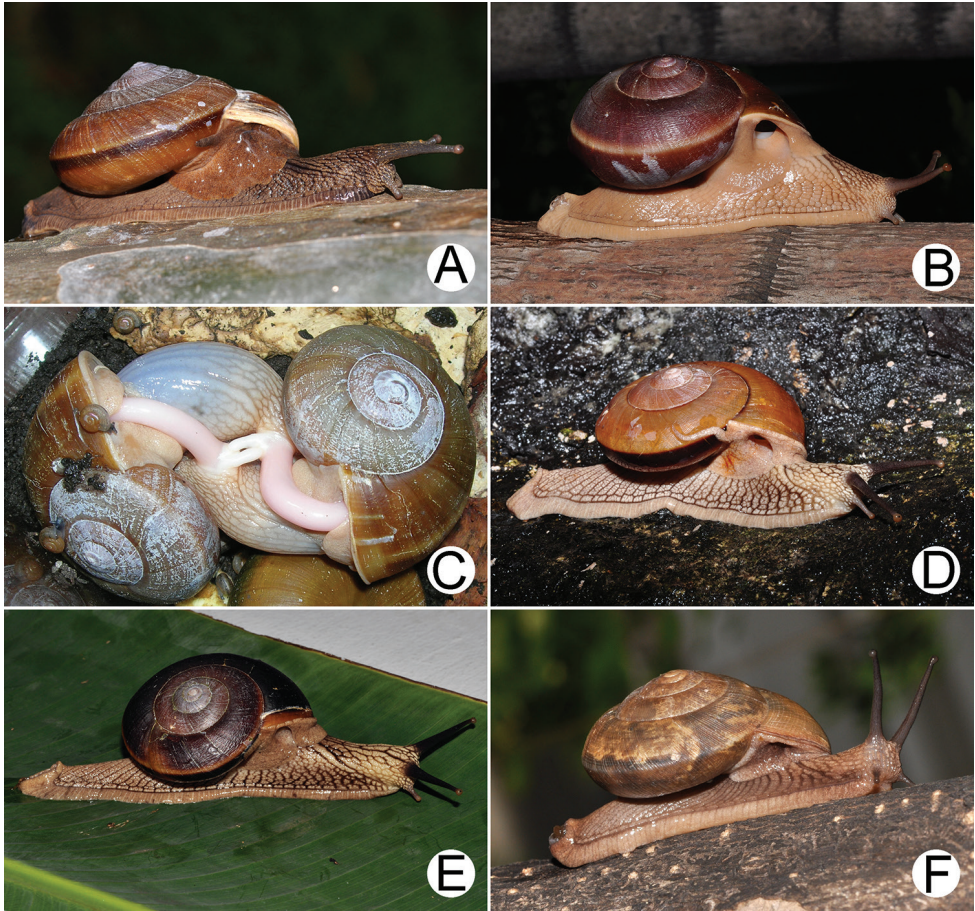


Figure 1. Living snail. **A** *Hemiplecta humphreysiana* from Singapore (width about 45 mm) **B, C** *Hemiplecta distincta* **B** from Saraburi, Thailand (width about 65 mm) and **C** mating pairs **D, E** *Hemiplecta funerea* from Nan, Thailand (width about 50 mm) **D** yellow shell form and **E** dark shell form (width about 50 mm) **F** *Hemiplecta esculenta* from Chiang Mai, Thailand (width about 30 mm).

stout, and with thin muscle bands connected to penial sheath. Vas deferens (vd) small tube. Internal wall of penis with sculpture encircling penial verge (Fig. 3B). Penial sculpture (ps) consists of scattering of small papillary knobs arranged randomly on penial wall. Penial verge (pv) long conic with smooth surface.

Vagina (v) long and cylindrical (Fig. 3A, B); internal wall with thin and smooth longitudinal vaginal pilasters (vp). Dart apparatus (da) long muscular cylinder, externally and internally smooth; dart papilla (dp) short, conic, and smooth. Gametolytic sac (gs) elongate or bulbous without distinct duct. Free oviduct (fo) long and encircled with thickened blackish muscular tissue (orange in fresh specimens). Oviduct (ov) long and with lobules; prostate gland bound to oviduct. Albumen gland (ag) small. Hermaphroditic duct (hd) small, convoluted, and connected to lobules of hermaphroditic gland (hg).

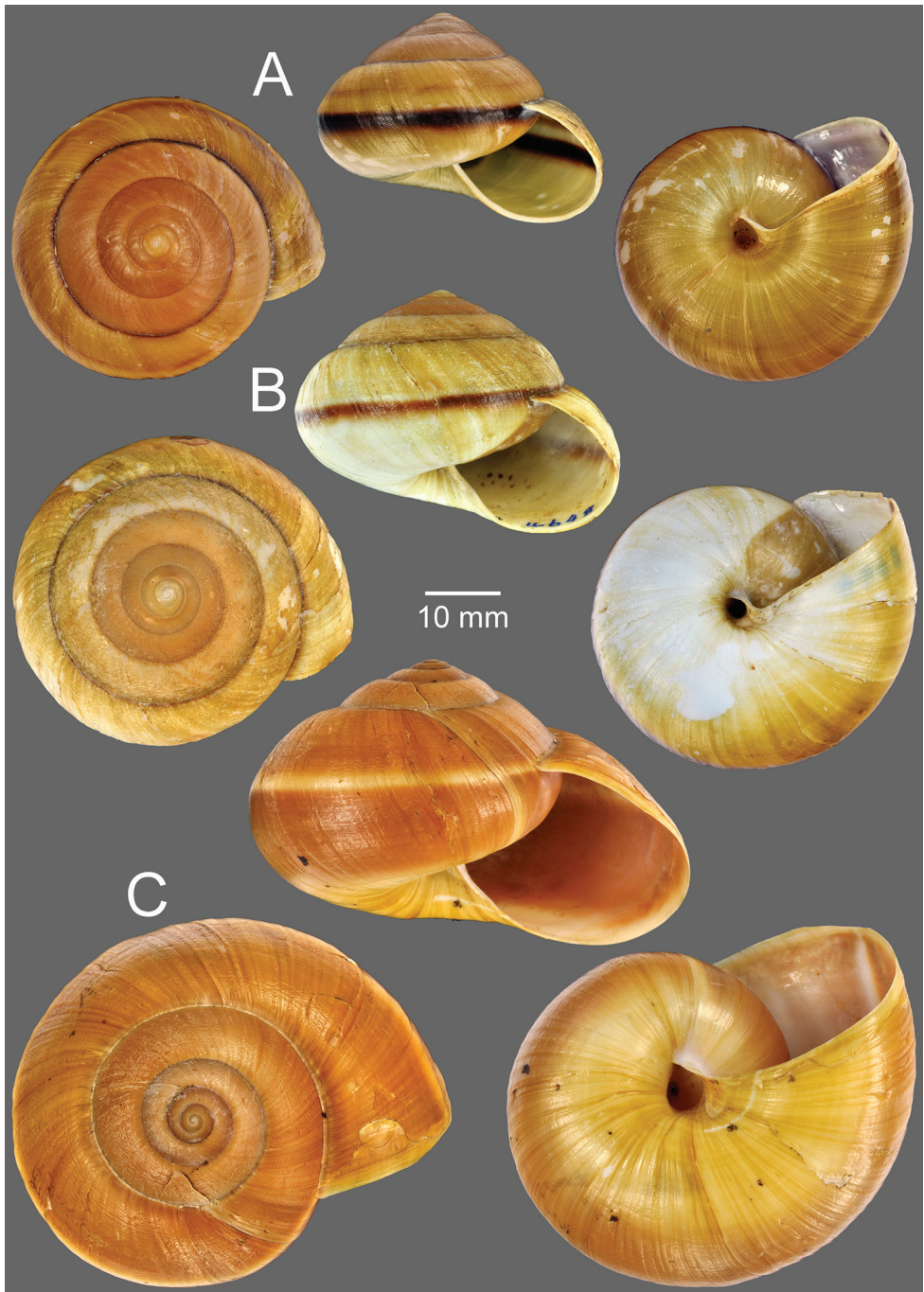


Figure 2. **A, B** *Hemiplecta humphreysiana* **A** specimen CUMZ 4573 from Singapore and **B** specimen CUMZ 4648 from Narathivat, Thailand **C** *Hemiplecta distincta*, specimen CUMZ 4531 from Chaeongsao, Thailand.

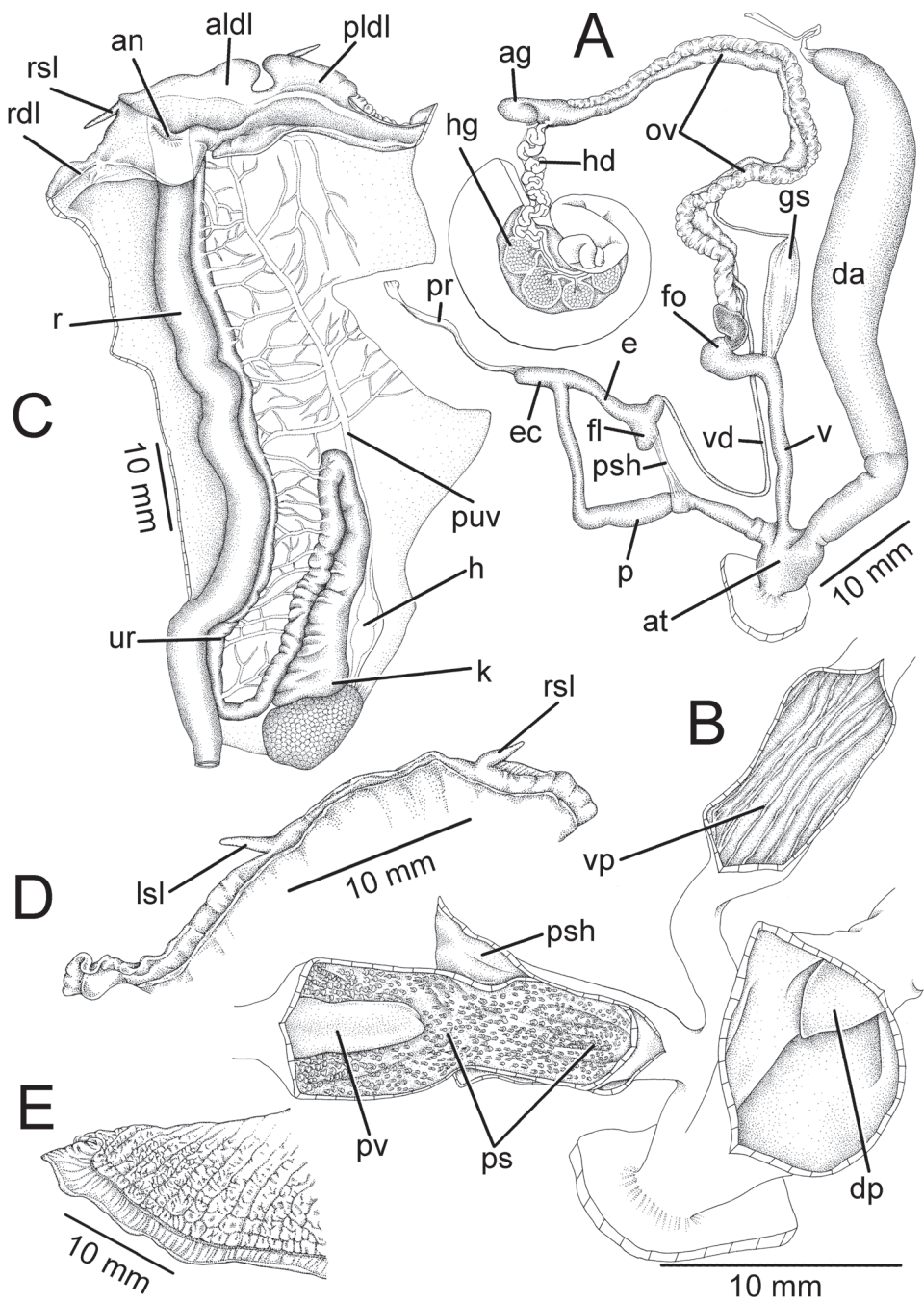


Figure 3. Genitalia, pallial system, mantle edge structure, and caudal region of *Hemiplecta humphreysiana*, specimen CUMZ 4573 from Singapore **A** whole genital organs **B** internal wall sculpture of penis, vagina and dart chamber **C** pallial system, lung cavity and ventral view of mantle edge **D** dorsal view of mantle edge showing shell lobes and **E** right view of caudal region.

Radula. Each row containing about 253 teeth (127–(18–32)–1–(29–32)–125). Central tooth unicuspid and triangular (Fig. 10A). Lateral teeth unicuspid, slanted, and with curved cusp. Outer lateral teeth with slightly curved cusps; latero-marginal transition from tooth numbers 28 to 32 (Fig. 10B). Marginal teeth bicuspid and curved; endocone and ectocone usually similar in shape and size (Fig. 10C).

External features. Mantle edge with large dorsal lobes. Right dorsal lobe (rdl) to right of anus (an; on the left in figure), large, and thick. Left dorsal lobe to left of anus (on the right in figure), composed of thin crescentic anterior left dorsal lobe (aldl), and thin elongated posterior left dorsal lobe (pldl). Right shell lobe (rsl) and left shell lobe (lsl) have short finger-shaped extensions located on mantle edge near tip of urinary groove and around junction of anterior and posterior left dorsal lobes, respectively, (Fig. 3C, D).

Pulmonary cavity typically sigmurethran, heart (h; auricle and ventricle) located left of kidney (k; on the right in figure). Pulmonary cavity approximately four times longer than wide. Pulmonary vein (puv) and venation on lung cavity well developed and distinct. Kidney (k) elongate, slender, and approximately one-third length of pulmonary cavity. Ureter (ur) sigmoid, closed tube arising from tip of kidney, extending along right side of kidney, and curved adjacent to rectum (r). Anus (an) adjacent to mantle edge (Fig. 3C).

Living snails possess long greyish-brown tentacles (Fig. 1A). Skin reticulated brownish with blackish reticulations around head. Foot sole relatively elongate, broad and unipartite. Sole of foot plain brownish; side of body brownish; upper part of tail dark greyish. Tail long, curved mid-dorsally, tall dome-shaped in cross section. Caudal horn not overhanging; caudal foss a long vertical slit arranged on tail above sole margin. Pedal groove typical aulacopod and well defined (Fig. 3E).

Distribution. The systematic studies of some *Hemiplecta* species have revealed incongruence between the traditional shell-based and molecular classifications (Sutcharit et al. 2021). Therefore, apart from Singapore (type locality) the historical record of *H. humphreysiana* from Sumatra, Borneo, and several localities in Peninsular Malaysia (Martens 1867; Tryon 1886; Godwin-Austen 1898; Collinge 1902; Laidlaw 1932a, 1933, 1957; Benthem Jutting 1950, 1959) needs to be confirmed by more convincing morphological and molecular evidences. In Thailand, this is the first and only record of this species from Narathivat, the southern-most province of Thailand.

Remarks. The specimens examined and described herein for the genitalia, pallial system, and radula were collected from Singapore (the correct type locality of this type species) to specify the characteristics for the genus. *Hemiplecta humphreysiana* clearly differs from all other species recorded both in Thailand and Peninsular Malaysia (compared with the type specimens in the list of the species). It can be distinguished from *H. floweri* Smith, 1899 (see Godwin-Austen 1900) from Peninsular Malaysia, by having a narrow umbilicus, without a brownish spiral band on the umbilical area, and more elevated spire (Smith 1898; Godwin-Austen 1900). In addition, the straight epiphallic caecum of this species is distinct from the coiled epiphallic caecum of *H. floweri* (Table 2). Unfortunately, none of the penial sculptures have been prepared for further comparison.

Table 2. Shell coiling, shell lobe, and genitalia variation among species that have been classified into the genus *Hemiplecta* s.l.: +, present; -, absent; ? not shown in the literature. References: 1 = Benthem Jutting (1950), 2 = Blanford and Godwin-Austen (1908), 3 = Collinge (1902), 4 = Godwin-Austen (1898), 5 = Godwin-Austen (1900), 6 = Godwin-Austen (1919), 7 = Maassen (2009), 8 = Panha (1987a), 9 = Schileyko (2002), 10 = Schileyko (2015), 11 = Stoliczka (1873), 12 = Sutcharit et al. (2012), and 13 = Sutcharit et al. (2021).

Taxa	Shell coiling	Right/left shell lobes	Epiphallic caecum	Penial verge	Dart apparatus	Gametolytic duct	References
<i>H. abbasi</i> Maassen, 2009	dextral	?	straight	+	+	-	7
<i>H. ceylanica</i> (Pfeiffer, 1850)	dextral	?	straight	+	+	-	9
<i>H. cymatium</i> Pfeiffer, 1856)	dextral	+/+	straight	+	+	-	11
<i>H. distincta</i> (Pfeiffer, 1850)	dextral	-/-	straight	-	+	-	5, 6, 8, 9 and present study
<i>H. esculenta</i> Maassen, 2006	dextral	-/-	straight	+	+	-	present study
<i>H. floweri</i> (Smith, 1899)	dextral	+/+	coiled	+	+	-	5
<i>H. finerea</i> (Smith, 1896)	dextral	+/-	coiled	-	+	-	present study
<i>H. humphreysiana</i> (Lea, 1840)	dextral	+/+	straight	+	+	-	1, 2, 3, 4 and present study
<i>H. ligorica</i> Sutcharit & Panha, 2021	sinistral	-/-	straight	-	+	-	13
<i>H. nemorosa</i> sp. nov.	dextral	-/-	straight	+	+	-	present study
<i>H. pernobilis</i> (Férussac, 1821)	dextral	?	coiled	-	+	-	10
<i>H. retrorsa</i> (Gould, 1843)	sinistral	-/-	straight	-	+	-	13
<i>H. salangana</i> (Martens, 1883)	sinistral	-/-	straight	-	+	-	12
<i>H. thailandica</i> Sutcharit & Panha, 2021	sinistral	-/-	straight	-	+	-	13
Doubtful generic status							
<i>H. malaouyi</i> (Morgan, 1885)	dextral	+/+	coiled	?	+	long cylindrical	Laidlaw (1932a)
<i>H. densa</i> (Adams & Reeve, 1850)	dextral	+/+	straight	?	+	long cylindrical	Wiegmann (1898)
<i>H. werberi</i> (Sarasin & Sarasin, 1899)	dextral	?	straight	?	+	long cylindrical	Niethammer (1937)
<i>H. foersteri</i> Kobelt, 1914	dextral	?	straight	?	-	long cylindrical	Wiktor (2003)
<i>H. belenang</i> Cilia & Abbas, 2012	dextral	?	straight	+	+	long cylindrical	Cilia and Abbas (2012)

Hemiplecta distincta (Pfeiffer, 1850)

Figures 1B, C, 2C, 4, 10D, E

Helix distincta Pfeiffer, 1850: 69, 70. Type locality: Insulis Moluccis [possibly error or mislabeling]. Pfeiffer 1853: 346, pl. 134, figs 1, 2. Reeve 1854: *Helix* pl. 86, species 465.

Nanina distincta: Martens, 1860: 7.

Helix neptunus Pfeiffer, 1861a: 190. Type locality: Siam [Thailand]. Pfeiffer 1861b: 176, 177. pl. 48, figs 1, 2. New synonym

Nanina (*Rhyssota*) *distincta*: Martens, 1867: 69, 70, pl. 6, fig. 8.

Nanina (*Hemiplecta*) *distincta*: Tryon, 1886: 30, pl. 8, fig. 26.

Nanina (*Hemiplecta*) *neptunus*: Tryon, 1886: 34, pl. 8, fig. 27.

Hemiplecta zimmayensis Godwin-Austen, 1888c: 241, 242. Type locality: Zimme, Siam territory [Chiang Mai Province, Thailand]. New synonym

Ariophanta (*Hemiplecta*) *distincta*: Morelet, 1891: 231.

Hemiplecta distincta: Morelet, 1889: 124. Blanford 1903: 277, 278. Panha 1987a: 108–115, figs 1–3. Panha 1987b: 25–34, fig. 9. Panha 1988a: 197–206, figs 6, 7. Panha 1988b 233–239. Panha 1994: 4–15. Inkhavilay et al. 2019: 76, figs 35b, c, 56c.

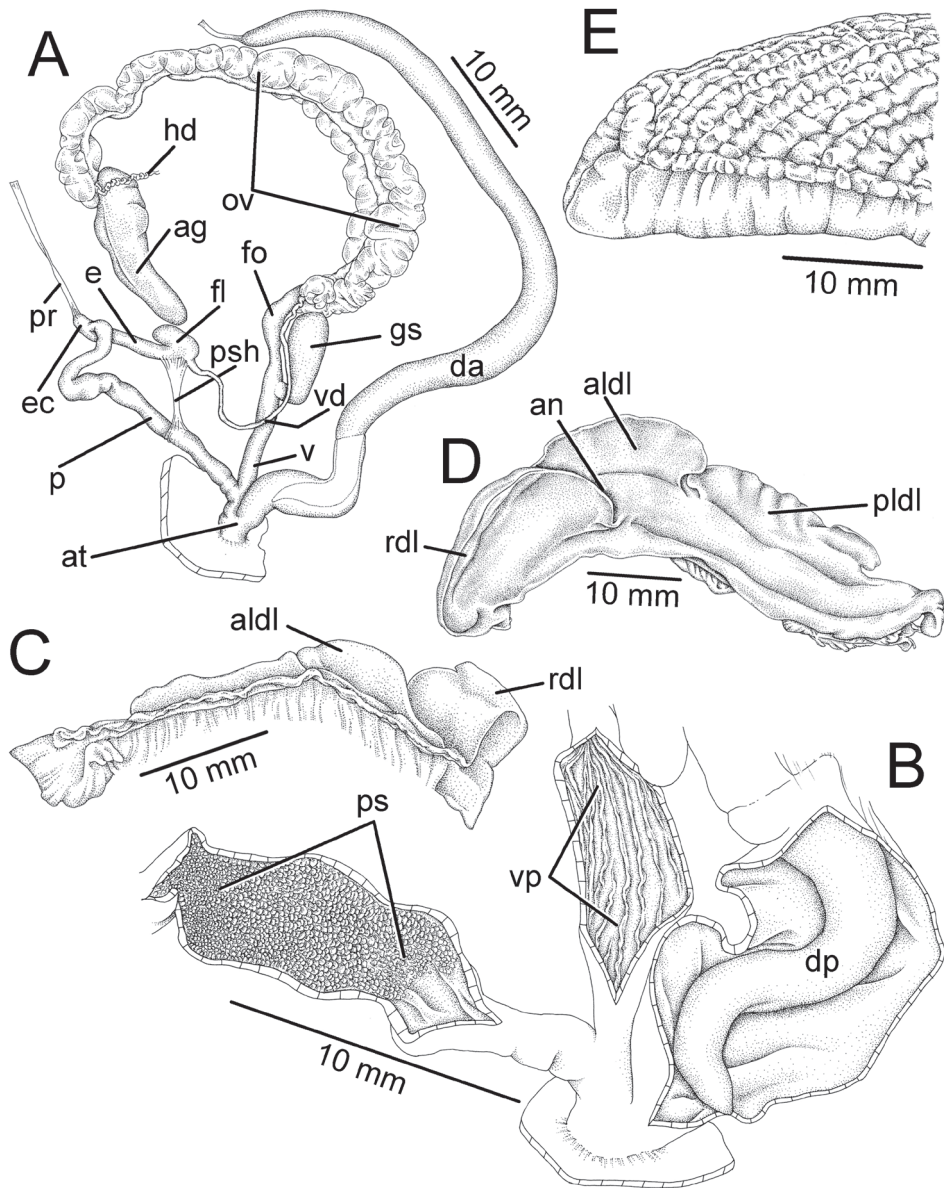


Figure 4. Genitalia, mantle edge structure, and caudal region of *Hemiplecta distincta*, specimen CUMZ 4560 from Chanthaburi, Thailand **A** whole genital organ **B** internal wall sculpture of penis, vagina, and dart chamber **C** dorsal view of mantle edge **D** ventral view of mantle edge and **E** right view of caudal region.

Nanina (Rhysota) distincta: Fischer and Dautzenberg 1904: 393. Dautzenberg and Fischer 1906: 346, 347.

Nanina (Rhysota) distincta var. *neptunus*: Dautzenberg and Fischer 1908: 171.

Koratia distincta: Godwin-Austen 1919: 199–202, figs 1, 2. Schileyko 2002: 1282, 1283, fig. 1685.

Nanina (Rhysota) distincta neptunus: Dautzenberg and Fischer, 1906: 347, 348.

Hemiplecta (Koratia) distincta: Solem, 1966: 27.

Hemiplecta (Hemiplecta) distincta: Hemmen and Hemmen, 2001: 44, fig. 12.

Hemiplecta (Hemiplecta) neptunus: Hemmen and Hemmen, 2001: 44.

Hemiplecta (Hemiplecta) zimmayensis: Hemmen and Hemmen, 2001: 44.

Type specimen. See the species list of Indochinese species (Fig. 11C).

Material examined. **THAILAND:** Tam Chiang Dao, Chiang Mai: CUMZ 4550 (1 shell), CUMZ 4558 (5 shells). Wang Chao Waterfall, Kamphangphet: CUMZ 4641 (4 shells). Klong Lann National Park, Kamphangphet: CUMZ 4579 (2 specimens in ethanol). Kaeng Jed Kwae, Watbot, Phitsanuloke: CUMZ 4638 (7 shells). Tam Wang Daeng, Nern Maprang, Phitsanuloke: CUMZ 4632 (1 shell). Khao Nang Rum, Huay Kla Klang National Park, Uthaitani: CUMZ 4502 (6 shells), CUMZ 4510 (3 shells), CUMZ 4538 (3 shells), CUMZ 4541 (6 shells), CUMZ 4607 (6 shells), CUMZ 4610 (9 shells), CUMZ 4611 (3 shells). Jed Sow Noi Waterfall, Muaklek, Saraburi: CUMZ 4548 (3 shells). Pu Kare Botanic Garden, Saraburi: CUMZ 4505 (4 shells), CUMZ 4506 (1 shell), CUMZ 4534 (5 shells). Tam Dao Khao Kaew, Muaklek, Saraburi: CUMZ 4624 (1 shell). Wat Tharawat, Saraburi: CUMZ 4508 (1 shell), 4530 (1 shell). Sam Larn National Park, Saraburi: CUMZ 4578 (2 specimens in ethanol). Bang Sri-thong, Bang Kruay, Nonthaburi: CUMZ 4555 (5 shells). Khao Look Chang, Pakchong, Nakhonratchasima: CUMZ 4501 (8 shells), CUMZ 4606 (9 shells), CUMZ 4612 (9 shells), CUMZ 4535 (3 shells). Tub Lann National Park, Nakhonratchasima: CUMZ 4617 (1 shell). Nawang, Nongbualumphu: CUMZ 4529 (1 shell). Tam Suwankuha, Nongbualumphu: CUMZ 4633 (3 shells), 4637 (3 shells). Thung Kra-Mang, Phu Kiew Wildlife Sanctuary, Chaiyaphum: CUMZ 4608 (5 shells). Pang Khone, Sakon-nakhon: CUMZ 4619 (4 shells). Phuphan Mountains, Sakonnakhon: CUMZ 4504 (6 shells), CUMZ 4507 (5 shells). Phu Kum Khao, Sahatsakhan, Kalasin: CUMZ 4557 (8 shells). Phu Sri Tharn Wildlife Sanctuary, Kalasin: CUMZ 4621 (1 shell). Huay Lao Waterfall, Phuluang Wildlife Sanctuary, Loei: CUMZ 4634 (1 shell). Tam Pha Bend, Chiang Karn, Loei: CUMZ 4532 (1 shell). Tam Pha Bing, Wangsapung, Loei: CUMZ 4636 (1 shell). Tam Piya, Loei: CUMZ 4639 (3 shells). Tam Mahasombat, Lomsak, Phetchabun: CUMZ 4567 (1 shell). Tam Yai Namnao, Namnao National Park, Phetchabun: CUMZ 4566 (1 shell), CUMZ 4622 (1 shell). Tam Phraya Nakarat, Phuphaman National Park, Khonkaen: CUMZ 4635 (1 shell). Pha Tam National Park, Ubonratchathani: CUMZ 4604 (3 shells), CUMZ 4616 (3 shells). Yod Dome National Park, Buriram: CUMZ 4629 (2 shells). Wang Ta Krai Waterfall, Nakhonnayok: CUMZ 4540 (2 shells), CUMZ 4549 (2 shells), CUMZ 4605 (1 shell), CUMZ 4640 (5 shells), CUMZ 4577 (1 specimen in ethanol). Khao Ang Rue Nai Wildlife Sanctuary, Chachoengsao: CUMZ 4531 (1 shell; Fig. 2B), CUMZ 4546 (7 shells), CUMZ 4609 (3 shells), CUMZ 4613 (4 shells), CUMZ 4620 (2 shells), CUMZ 4627 (1 shell), CUMZ 4630 (1 shell). Pang Srida National Park, Prachinburi: CUMZ 4631 (4 shells). Ra-Ru, Taphraya, Srakeow: CUMZ 4628 (1 shell). Tam Leum, Klonghad, Srakeow: CUMZ 4625 (1 shell). Khao Cha Ang-Oan, Borthong, Chonburi: CUMZ 4542 (4 shells), CUMZ 4618 (1 shell), CUMZ 4626 (4 shells). Khao Cha Mao Waterfall,

Rayong: CUMZ 4543 (1 shell). Tam Suwanphupha, Khao Chamao, Rayong: CUMZ 4545 (1 shell). Wat Ma-deau (Tam Khao Loi), Khao Chamao, Rayong: CUMZ 4544 (4 shells). Plieu National Park, Chanthaburi: CUMZ 4509 (1 shell), CUMZ 4536 (1 shell), CUMZ 4539 (9 shells), CUMZ 4560 (1 specimen in ethanol; Fig. 4), CUMZ 4601 (1 shell), CUMZ 4615 (4 shells). Sibha Shan Waterfall, Chanthaburi: CUMZ 4547 (6 shells). Tha Mai District, Chanthaburi: CUMZ 4603 (1 shell). Koh Kud, Trat: CUMZ 4559 (9 shells), CUMZ 4614 (7 shells). Kaeng Kracharn National Park, Phetchaburi: CUMZ 4527 (1 shell). Tam Nam Pud, Pangnga: CUMZ 4623 (1 shell).

Shell. Shell large (height up to 55 mm, width up to 75 mm), yellowish with white narrow peripheral band, and paler color below on lower shell surface. Upper shell surface with thin growth lines interrupted with spiral wrinkles. Last whorl large and rounded; aperture large ovate; lip simple but slightly thickened in adult snails (Fig. 2C).

Genitalia. The external genital organs were described in Godwin-Austen (1900, 1919). Gametolytic sac (gs) bulbous with undifferentiated duct. Internal wall of penis exhibits closely packed papilla knobs that abruptly cease near atrium; penial verge absent. Internal sculpture of vagina with thin and smooth longitudinal vaginal pilasters (vp). Internal surface of dart apparatus smooth; dart papilla (dp) conic, and with a smooth surface (Fig. 4A, B).

Radula. Each row with about 543 teeth (276–(15–20)–1–(15–20)–276). Central tooth unicuspid triangular with rounded head (Fig. 10D). Lateral teeth unicuspid, oblique and triangular. Outer lateral teeth unicuspid, sickle-shaped, with transition to curved and narrow sickle form; latero-marginal transition starts from tooth numbers 15 to 20. Marginal teeth narrowly curved, bicuspid; endocone and ectocone small and pointed (Fig. 10E).

External features. Living snails have a similar soft body morphology and pulmonary cavity to that of *H. humphreysiana*. The distinct characters are pale brown to brownish body (Fig. 1B, C). Sole of foot brownish; caudal horn not overhanging; caudal foss a long vertical slit arranged on tail above sole margin. Pedal groove typical aulacopod and well defined (Fig. 4E). Mantle edge narrow with large dorsal lobes. Right dorsal lobe (rdl) to right of pneumostome, large and thick; left dorsal lobe to left of pneumostome, composed of anterior left dorsal lobe (aldl) and posterior left dorsal lobe (pldl); shell lobe absent (Fig. 4C, D).

Distribution. Ranges from Cambodia to Laos, Thailand and southern Vietnam (Smith 1896; Laidlaw 1932a; Panha 1988a, 1994; Schileyko 2011; Inkhavilay et al. 2019). In Thailand, *H. distincta* is fairly abundant and occurs throughout the country, except for southern Thailand (Panha 1988b, 1994). The southern limit of the species appears to be near the Isthmus of Kra (10°N). We have a single and old shell from Pangnga Province, southern Thailand that needs to be confirmed.

Remarks. The type specimens of *Helix neptunus* Pfeiffer, 1861 and *Hemiplecta zimmayensis* Godwin-Austen, 1888 exhibit a shell morphology and color patterns identical to that of *H. distincta*. The absence of a whitish peripheral band in *Helix neptunus* Pfeiffer, 1861 and the strong growth lines of *Hemiplecta zimmayensis* are the only observed differences from *H. distincta*. Therefore, we recognize these two nominal species as junior subjective synonyms of *H. distincta*.

Hemiplecta distincta has long been considered a food item for local people in north-eastern Thailand (Panha 1987b), as well as in Cambodia and Laos (personal observation). It has also been found to be an intermediate host of the rat lungworm, a human pathogen (Panha 1987b, 1988b). The life cycle and breeding biology of this species have been extensively studied (Panha 1987a, b, 1988a, b, 1994).

***Hemiplecta funerea* (Smith, 1896)**

Figures 1D, E, 5A, B, 6, 10F, G

Nanina distincta var. *funerea* Smith, 1896: 128. Type Locality: Vanbu, Tonkin [Van Ban District, Lao Cai Province, Vietnam]. Fischer and Dautzenberg 1904: 393.

Nanina distincta var. *pallidior* Smith, 1896: 128. Type Locality: Vanbu, Tonkin [Van Ban District, Lao Cai Province, Vietnam]. Fischer and Dautzenberg 1904: 393.

Hemiplecta funerea: Inkhavilay et al. 2019: 76, 77, figs 35f, 36a.

Type specimen. See the species list of Indochinese species (Fig. 12B).

Material examined. THAILAND: Bor Klue District, Nan: CUMZ 4649 (5 shells). Ton Tong Waterfall, Doi Phu Ka National Park, Nan: CUMZ 4575 (8 specimens in ethanol; Figs 1D, E, 5A, B), CUMZ 4576 (8 shells).

Shell. Shell large (height up to 35 mm, width up to 55 mm), depressed conic, dextral, with 6–7 whorls; spire slightly elevated with wide and shallow suture. Shell almost black to dark brown with thin yellowish peripheral band. Apex obtuse; embryonic shell large with smooth surface; subsequent whorls with thin growth lines and thin radial wrinkles. Last whorl keeled; aperture large and ovate; lip simple, yellowish to dark yellow, and slightly thickened in adult snail. Columella slightly dilated; parietal callus thin and transparent. Umbilicus wide and deep (Fig. 5A, B).

Genitalia. Both male and female genital characters similar to that of *H. humphreysiana*. Gametolytic sac (gs) elongate with undifferentiated duct. The unique characters are a coiled epiphallic caecum (ec) and curved flagellum (fl), which are not found in the other species (Fig. 6A). Internally, penial sculpture (ps) consists of scattered papillary knobs lining penial wall; penial verge absent (Fig. 6B). Internal wall of vagina and internal structure of dart apparatus are similar to that in *H. humphreysiana* (Fig. 6C, D).

Radula. Each row contains about 286 teeth (140–(65–75)–1–(65–75)–135). Central tooth unicuspid conic-shaped, and dull cusp (Fig. 10F). Lateral teeth unicuspid, elongate, and slanted with pointed tip. Outer lateral teeth unicuspid, elongate; latero-marginal transition starts from tooth numbers 65 to 75. Marginal teeth slightly curved, bicuspid; endocone and ectocones small and of similar size (Fig. 10G).

External features. Living snails with long, black eye tentacles (Fig. 1D, E). Skin reticulated, pale brownish to brownish with dark reticulation across the entire head and foot above the lateral margin. Foot sole, caudal foss (Fig. 6G), caudal horn, and pedal groove similar to those in *H. humphreysiana*. Mantle edge, dorsal lobe, and shell lobe similar to those in *H. humphreysiana*, but only long and finger-shaped right shell lobe (rsl) present (Fig. 6E, F).

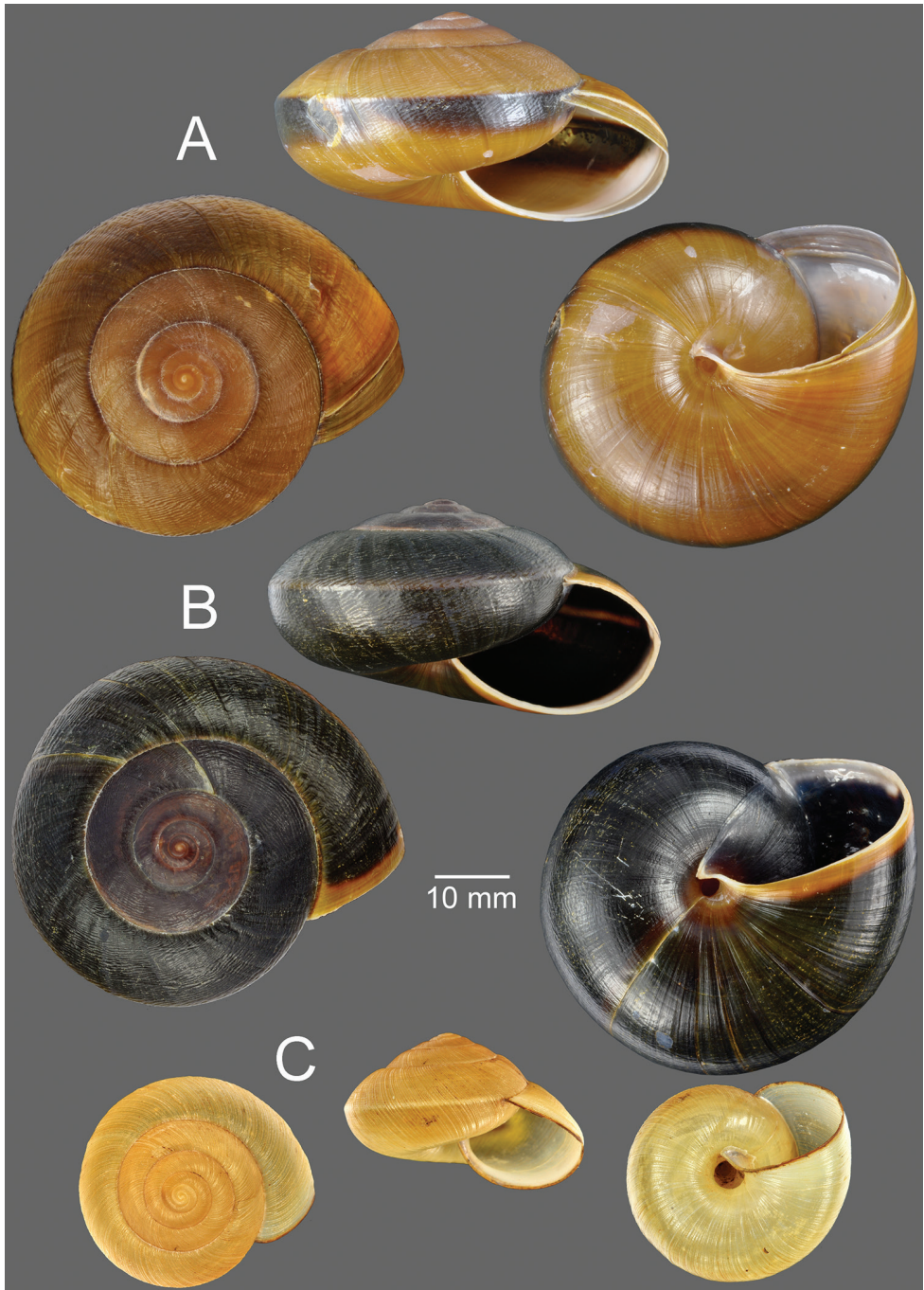


Figure 5. **A, B** *Hemiplecta funerea*, specimens CUMZ 4575 from Nan, Thailand **A** yellow shell form and **B** dark shell form **C** *Hemiplecta esculenta*, specimen CUMZ 4553 from Chiang Mai, Thailand.

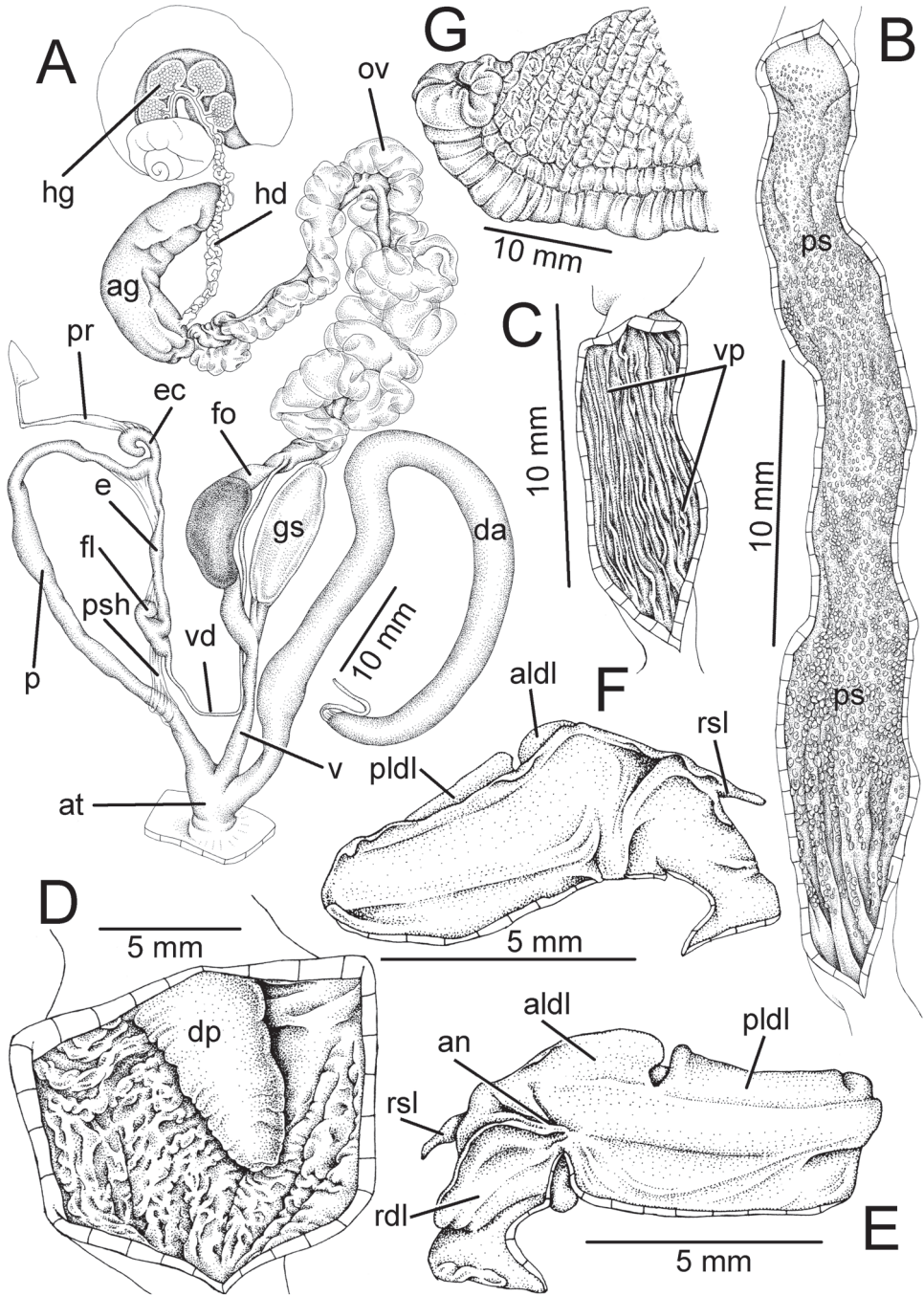


Figure 6. Genitalia, mantle edge structure and caudal region of *Hemiplecta funerea*, specimen CUMZ 4575 from Nan, Thailand **A** whole genital organs **B** internal wall sculpture of penis **C** internal wall sculpture of vagina **D** internal wall sculpture of dart chamber **E** ventral view of mantle edge **F** dorsal view of mantle edge showing right shell lobe and **G** right view of caudal region.

Distribution. Previously recorded from the type locality in northern Vietnam, and several localities in northern and central Laos (Smith 1896; Fischer and Dautzenberg 1904; Inkhavilay et al. 2019). Recently, we recorded this species from two localities in Nan Province, northern Thailand.

Remarks. Smith (1896: 128) introduced two nominal subspecies of *H. distincta* from northern Vietnam, which were distinguished on the basis of shell color. The “var. *funerea*” has a purplish-black tinted shell (Fig. 5B), while the “var. *pallidior*” possesses a yellowish or olive-yellow shell (Fig. 5A). Since then, no new specimens of these two color forms have been critically examined or their status verified. We have collected both living snails and empty shells of these two forms in recent surveys in Laos and Thailand. The two forms are anatomically and genetically identical, and occur syntopically, and thus, in our judgement, are examples of different shell colors of the same species. We recognize *H. funerea* as a distinct and valid species, and treat *Nanina distincta* var. *pallidior* Smith, 1896 as its junior synonym (ICZN 1999: Art. 24, 74).

Hemiplecta funerea can be distinguished from *H. distincta* by the angulated, dark brown or yellowish shell, distinct penial sculpture, and a long and distinctively coiled epiphallic caecum (Fig. 6A) compared to the short and straight epiphallic caecum in *H. distincta* (Fig. 4A). The blackish reticulated skin on a yellowish background and blackish eye tentacles contrast with the greyish body in *H. distincta* (Fig. 1B–E).

***Hemiplecta esculenta* Maassen, 2006**

Figures 1F, 5C, 7, 10H, I

Hemiplecta esculenta Maassen, 2006: 17, 18, figs 10–12. Type locality: limestone area near village Hang, Pu Luong National Park, Thanh Hoa, Vietnam. Inkhavilay et al. 2019: 76, fig. 35d, e. Páll-Gergely 2019: figs 11–13.

Type specimen. See the species list of Indochinese species.

Material examined. THAILAND: Tam Chiang Dao, Chiang Mai: CUMZ 4553 (5 specimens in ethanol; Fig. 1F), CUMZ 4564 (10 specimens in ethanol), CUMZ 4565 (9 specimens in ethanol), CUMZ 4574 (8 specimens in ethanol; Fig. 5C); Tam Tab-Tao, Chaiprakarn, Chiang Mai: CUMZ 4580 (10 specimens in ethanol).

Shell. Shell relatively small (height up to 25 mm, width up to 35 mm), elevated to slightly depressed, upper surface with distinct nodules arranged on growth line, and lower shell surface nearly smooth. Last whorl keeled; aperture large and ovate; lip simple to slightly expanded and dark brown. Umbilicus widely opened and deep (Fig. 5C).

Genitalia. Genital tracts similar to those of *H. humphreysiana* (Fig. 7A). Internal wall of penis with sculpture encircling penial verge. Penial sculpture (ps) consists of small to large papillary knobs arranged in oblique lines on penial wall; relatively smaller knobs surrounding penial verge. Penial verge (pv) small, short, conic, and with smooth surface (Fig. 7B). Gametolytic sac (gs) bulbous with undifferentiated duct. Internal wall of vagina with series of thin longitudinal vaginal

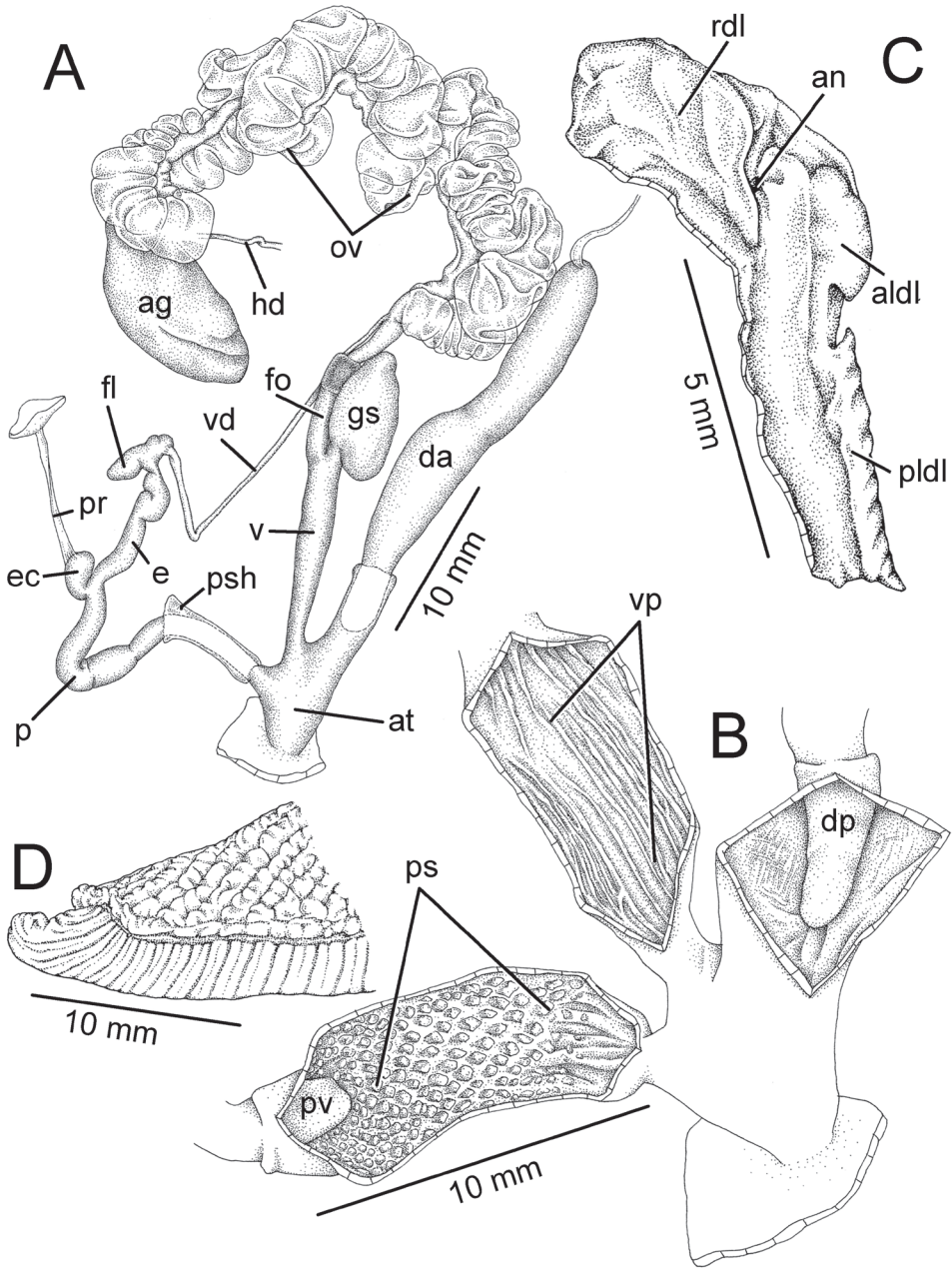


Figure 7. Genitalia, mantle edge structure, and caudal region of *Hemiplecta esculenta*, specimen CUMZ 4553 from Chiang Mai, Thailand **A** whole genital organ **B** internal wall sculpture of penis, vagina and dart chamber **C** ventral view of mantle edge and **D** right view of caudal region.

pilasters (vp). Dart apparatus (da) relatively short; internal wall of chamber with smooth wall, and papilla of dart apparatus (dp) slightly elongate, conic, and with smooth surface (Fig. 7B).

Radula. Each row possesses about 161 teeth (80–(18–21)–1–(19–21)–80). Central tooth triangular, tricuspid; ectocones small; mesocone large (Fig. 10H). Lateral teeth tricuspid; endocone small; mesocone large with pointed tip; ectocone large, basal, and with pointed tip. Outer lateral teeth arranged slightly obliquely, bicuspid; endocone very small to absent; mesocone large trapezoid; ectocone basal, relatively small, and pointed tip. Latero-marginal transition starts from tooth no. 18 to 21. Marginal teeth with curved teeth, bicuspid; endocone usually larger than ectocone (Fig. 10I).

External features. Living snail exhibits similar soft body morphology, pulmonary cavity and caudal structure (Fig. 7D) to that of *H. humphreysiana*. The distinct characters are the brownish to greyish body and mantle edge; right and left shell lobes absent (Fig. 1F).

Distribution. Previously known only from the type locality in northern Vietnam (Maassen 2006) and Xieng Khaung, northeastern Laos (Inkhavilay et al. 2019). Recently, we have located populations from northern Thailand in Chiang Mai Province.

Remarks. The shell features were carefully described in Maassen (2006). The original description of *H. esculenta* was based on seven shells and placement within *Hemiplecta* was provisional (Maassen 2006), and none of the topotypic specimens have subsequently been examined. The samples from Thailand show only minor variations from the type series, in the presence of a narrow brownish spiral band and slightly elevated spire, which we attribute to intraspecific variation. It is important to examine the genitalia of the topotypic material.

Hemiplecta nemorosa sp. nov.

<http://zoobank.org/5B542C60-C447-40B9-BC1D-AD24774C4AFD>

Figures 8–10J

Etymology. The species name is derived from the Latin word “*nemoris*” meaning “full of woods or shady,” which refers to the type locality of this new species in the dense deciduous forest.

Type specimen. **Holotype** CUMZ 5251 (height 24.6 mm, width 42.1 mm; Fig. 8A), **paratypes** CUMZ 5252 (2 shells; Fig. 8B), CUMZ 5253 (1 adult + 1 juvenile in ethanol; Fig. 8C) all from the type locality.

Type locality. Limestone outcrops with deciduous forest near road no. 1226, Pang Mapha Sub-district, Pang Mapha District, Mae Hongson Province, Thailand (19°34'10.2"N, 98°12'02.3"E).

Description. Shell medium sized (height up to 15 mm, width up to 45 mm), depressed conic, thin and dextral. Whorls 5 to 6, increasing regularly, slightly convex, with very wide and shallow suture. Spire convex; apex acute; embryonic shell smooth; following whorls with thin growth lines and radial wrinkles or undulating surfaces. Periostracum thin and transparent. Shell pale brownish to yellowish. Last whorl angular with strong peripheral keel which is much reduced near aperture. Aperture not descending, widely ovate and moderately oblique; lip simple to slightly thickened in adult specimen. Columella slightly dilated; parietal callus slightly thick and translu-

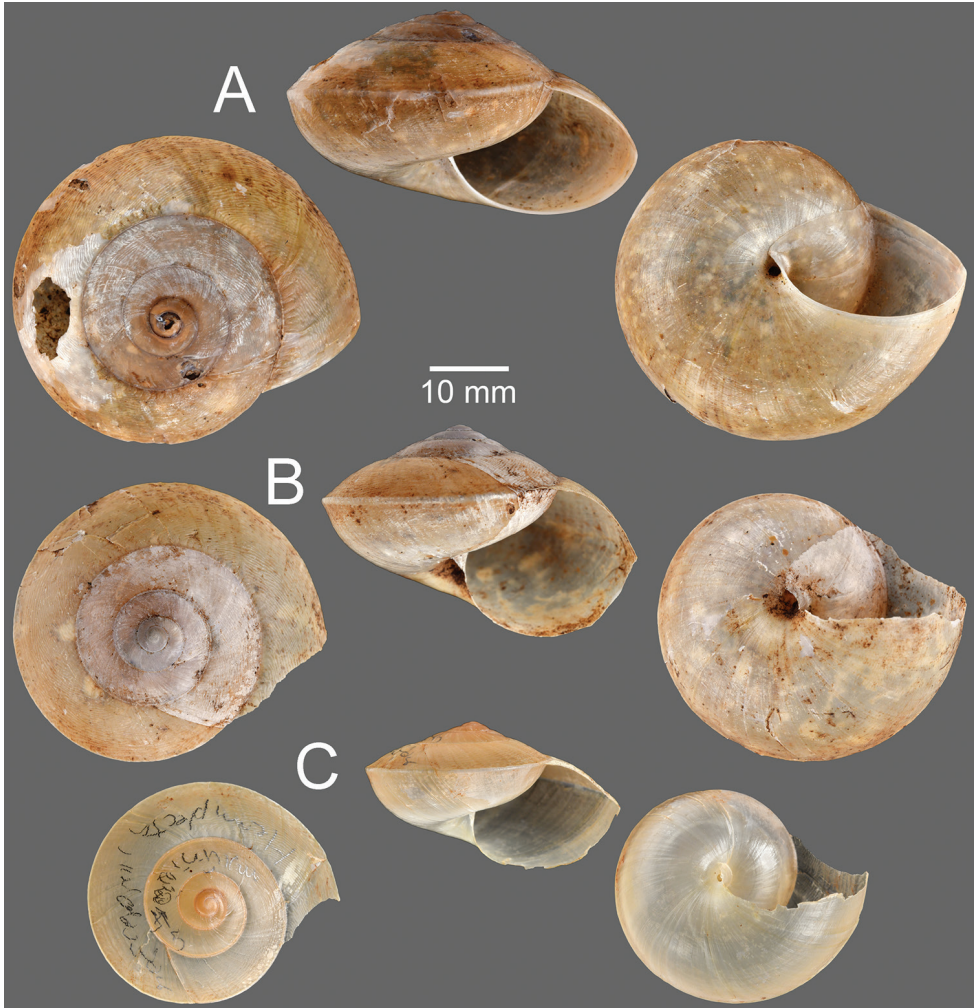


Figure 8. *Hemiplecta nemorosa* sp. nov. from Maehongsorn, Thailand **A** holotype CUMZ 5251 **B** paratypes CUMZ 5252, and **C** paratype CUMZ 5253 from the type locality.

cent. Umbilicus narrowly opened, deep, and partly covered by reflected columellar lip (Fig. 8).

Genitalia. Atrium (at) long. Penis (p) long slender, cylindrical, and encircled by thick penial sheath (psh) extending to about half of penis length. Epiphallic caecum (ec) short, straight; penial retractor muscle (pr) thin and attached to the tip. Epiphallus (e) short, about half of penis length. Flagellum (fl) short, stout, and with thin muscle bands connected to penial sheath. Vas deferens (vd) small tube (Fig. 9A). Internal wall of penis with sculpture over entire chamber with uniform scale-like or triangular linguulate pilasters varying in size from small to large and pilasters encircling penial verge smaller than in the middle of chamber. Penial verge (pv) small, conic, and with smooth surface (Fig. 9B).

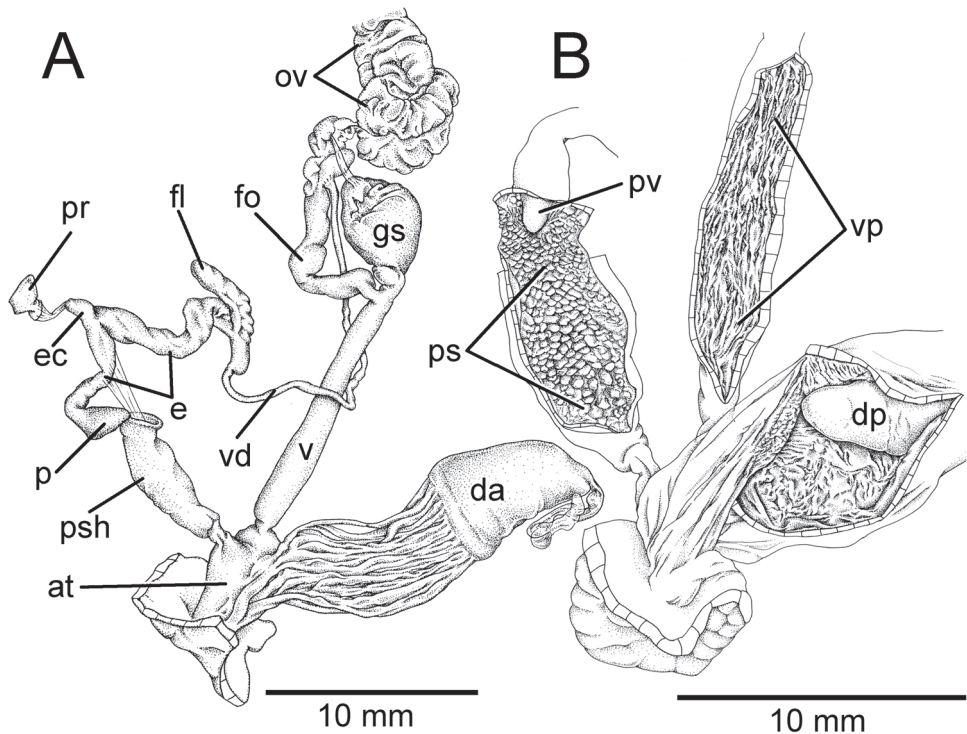


Figure 9. Genitalia, mantle edge structure, and caudal region of *Hemiplecta nemorosa* sp. nov., paratype CUMZ 5253 from Maehongsorn, Thailand **A** whole genital organ and **B** internal wall sculpture of penis, vagina and dart chamber.

Vagina (v) long, cylindrical, about same length as penis; internal wall with thin and smooth longitudinal vaginal pilasters (vp). Dart apparatus (da) short and enlarged muscular cylinder; externally covered with thin longitudinal muscular bands around half of dart apparatus length. Internally with irregular wall, dart papilla (dp) conic and smooth. Gametolytic sac (gs) bulbous without distinct duct. Free oviduct (fo) long and encircled with thin blackish muscular tissue. Oviduct (ov) long and with lobules; prostate gland bound to oviduct. Albumen gland, hermaphroditic duct, and hermaphroditic gland missing from the examined specimen (Fig. 9A, B).

Radula. Teeth arranged in wide angled U-shape. Each row containing more than 135 teeth (+58–(16–19)–1–(16–19)–75). Central tooth symmetrical tricuspid and triangular; mesocone conic shaped and with pointed cusp; ectocones short with dull cusps located at middle of tooth height. Lateral teeth asymmetrical tricuspid; endocone nearly absent; mesocone triangular with pointed cusp; ectocone with pointed cusps and located below endocone. Marginal teeth start around tooth numbers 16 to 19, elongate and obliquely bicuspid; endocone larger than ectocone and with pointed cusp; ectocone very small. Outer marginal teeth bicuspid and shorter than inner teeth (Fig. 10J).

Distribution. This new species is currently known only from the type locality in northern Thailand.

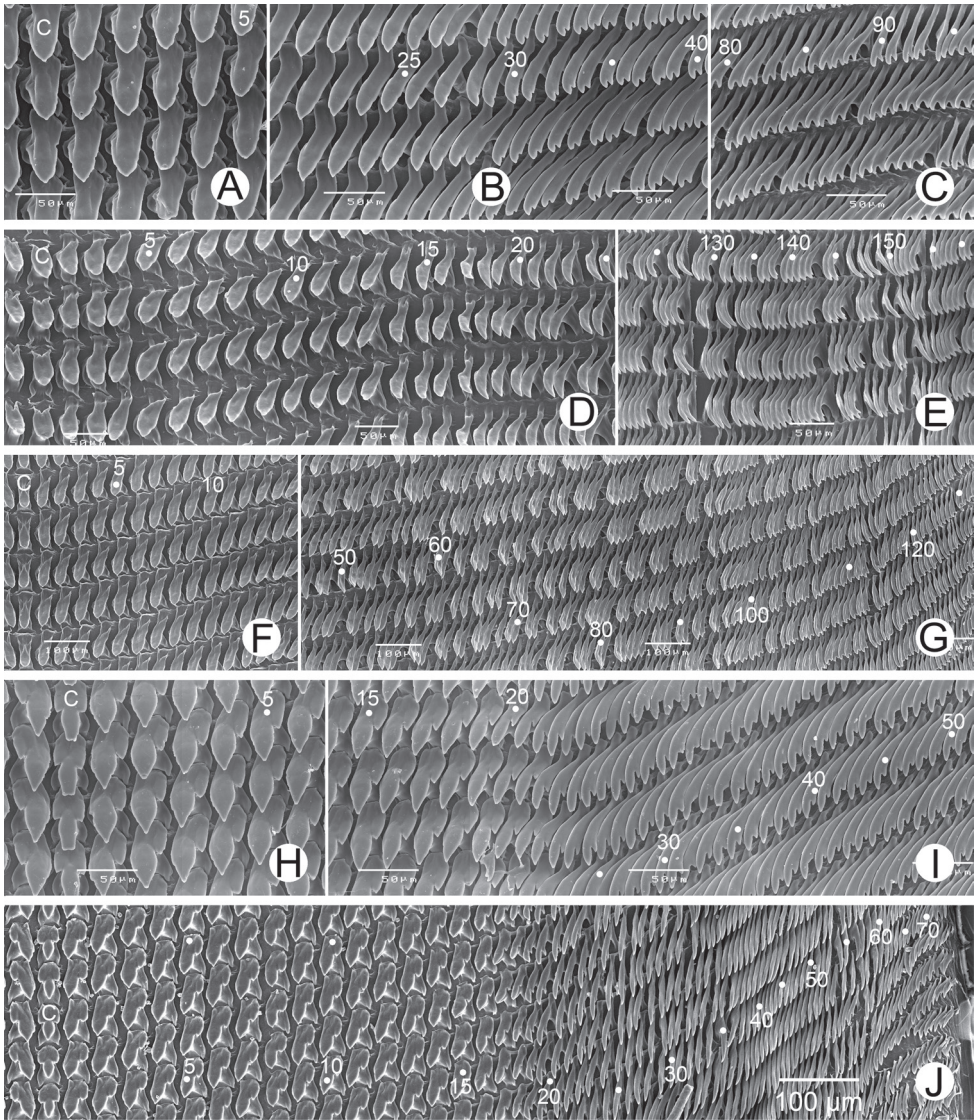


Figure 10. Representative SEM images of radula **A–C** *Hemiplecta humphreysiana*, specimen CUMZ 4573 from Singapore **A** central and lateral teeth **B** transition from lateral teeth to marginal teeth and **C** outermost marginal teeth **D, E** *Hemiplecta distincta*, specimen CUMZ 4560 from Chanthaburi, Thailand **D** central and lateral teeth and **E** outermost marginal teeth **F, G** *Hemiplecta funerea*, specimen CUMZ 4575 from Nan, Thailand **F** central and lateral teeth and **G** outermost marginal teeth **H, I** *Hemiplecta esculenta*, specimen CUMZ 4553 from Chiang Mai, Thailand **H** central and lateral teeth **I** transition from lateral teeth to marginal teeth **J** *Hemiplecta nemorosa* sp. nov., paratype CUMZ 5253 from Maehongsorn, Thailand. Central tooth indicated by 'C'. Numbers indicate the tooth order from lateral to marginal end.

Remarks. The shell morphology of this new species is similar to *H. uter* (Theobald, 1859) from Myanmar and *Falsiplecta integripedia* Schileyko & Semenyuk, 2018 from southern Vietnam. This new species, however, differs by having a shell width almost two-

times larger than *H. uter*, but further comparison of anatomical characters is necessary to confirm their distinction. *Hemiplecta nemorosa* sp. nov. clearly differs from *F. integripedia* in having a well-developed dart apparatus, globular gametolytic sac, and long epiphallus and flagellum. In contrast, *F. integripedia* has no dart apparatus, a long gametolytic duct, a very short epiphallus and the vas deferens attached near the tip of the epiphallus (flagellum lacking). *Hemiplecta nemorosa* sp. nov. also differs from *H. undosa* (Blanford, 1865) by having a relatively smaller shell size, an angular last whorl with strong peripheral keel, and a narrow umbilicus. In contrast, *H. undosa* has a rounded to slightly shouldered last whorl, and a wide and deep umbilicus.

Species list from Indochina including Peninsular Malaysia and Myanmar

This synoptic list includes all the nominal species-group names that have been attributed to *Hemiplecta* s.l. and have the type locality within the geographic area covered by mainland Indochina, Peninsular Malaysia or the southeastern part of Myanmar. All the nominal species group names are listed alphabetically where their original combinations and original publication were provided. In nearly all instances, the original literature was checked for authorship and date, page numbers of the original description and illustrations, and type locality to ensure accuracy of the entries. The usage of the nominal name, necessary references that provided descriptions or images of possible type specimens, and recent taxonomic treatment articles that placed species into the genus *Hemiplecta* are also listed. The current taxonomic status (validity or synonymy) of each taxon is provided, mainly following recent literature and this study. The depository information of the name-bearing types (holotype, lectotype, or syntype) is provided. The name-bearing types are illustrated when possible; exceptions are those recently published in Inkhavilay et al. (2019), Páll-Gergely (2019), and Sutcharit et al. (2020). However, in the cases where the name-bearing types could not be traced, topotypic or authentic reference specimens are illustrated instead for further comparison. In some instances, information about the authorship, type series, and type locality is discussed under the remarks section. The type specimens were located (preserved) in several museums, as follows:

Group I: Dextral species

1 *auriettae* (Tapparone Canefri, 1889)

Nanina (*Macrochlamys*) *auriettae* Tapparone Canefri, 1889: 318, 319, pl. 8, figs 4–6.

Type locality: Sul monte Mooleyit [Mulayit Hill, Hpa-An District, Kayin State, Myanmar].

Hemiplecta ? *auriettae*: Blanford and Godwin-Austen 1908: 293.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. The type specimens could not be traced.

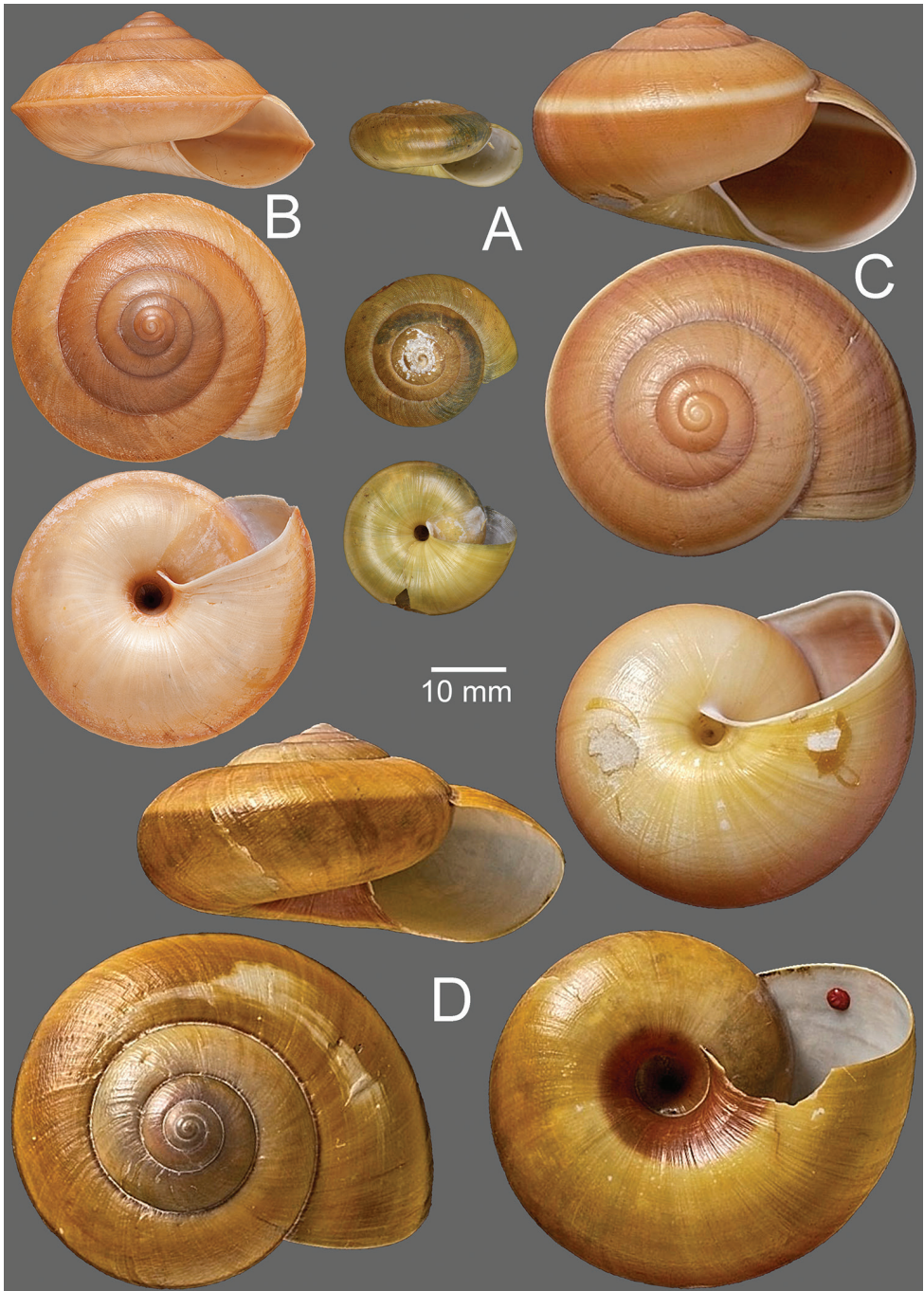


Figure 11. **A** *Hemiplecta auriettae*, specimen NHMUK 1912.4.16.497 **B** *Hemiplecta cymatium*, specimen UMZC I.104350 ex. Benson collection **C** *Hemiplecta distincta*, possible syntype NHMUK 20200199 **D** *Hemiplecta floweri*, syntype NHMUK 1899.3.16.1–2.

Remarks. The topotype specimen NHMUK 1912.4.16.497 (3 shells; Fig. 11A) from Mooley-it, Tenasserim is figured herein.

2 *chevalierii* (Souleyet, 1842)

Helix chevalierii Souleyet, 1842: 101. Type locality: Malacca [Malacca State, Malaysia].

Helix chevalierii: Souleyet, 1852: 503, 504, pl. 28, figs 24–26.

Hemiplecta chevalierii: Maassen 2001: 101.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. The type specimens could not be traced.

3 *cymatium* (Pfeiffer, 1856)

Nanina cymatium Bens. Pfeiffer 1855: 121. [unavailable name].

Helix cymatium Benson. Pfeiffer, 1856b: 58, pl. 17, figs 1, 2. Type locality: Pulo Lancavi, peninsulae Malaccanae [Langkawi Islands, Kedah State, Malaysia].

Hemiplecta cymatium: Maassen 2001: 101, 102.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. The type specimens could not be traced.

Remarks. The manuscript name “*cymatium* Bens.” was never published by Benson. It was first mentioned in the species list published by Pfeiffer (1855: 121), but without any indication to make the name available (ICZN 1999: Art. 12). Later, Pfeiffer (1856b) published this name with a description and illustration and attributed it to Benson. However, since Benson did not write the description, the authorship of this taxon should be attributed to Pfeiffer, who formally described it and made the name available.

The original description includes an illustration and one set of shell measurements. The type series of the taxa could not be traced in the UMZC and NHM collections. There are three specimens from UMZC I.104350 ex. Benson collection accompanied by a label with the taxon name but without collection locality. A specimen that closely matched the original description is figured herein (Fig. 11B).

4 *denserugata* (Möllerndorff, 1901)

Xestina denserugata Möllerndorff, 1901: 45, 46. Type locality: Berg Dran und Honggong, Süd Annam.

Hemiplecta denserugata: Schileyko 2011: 30.

Current taxonomic status. *Hemiplecta*. Valid species.

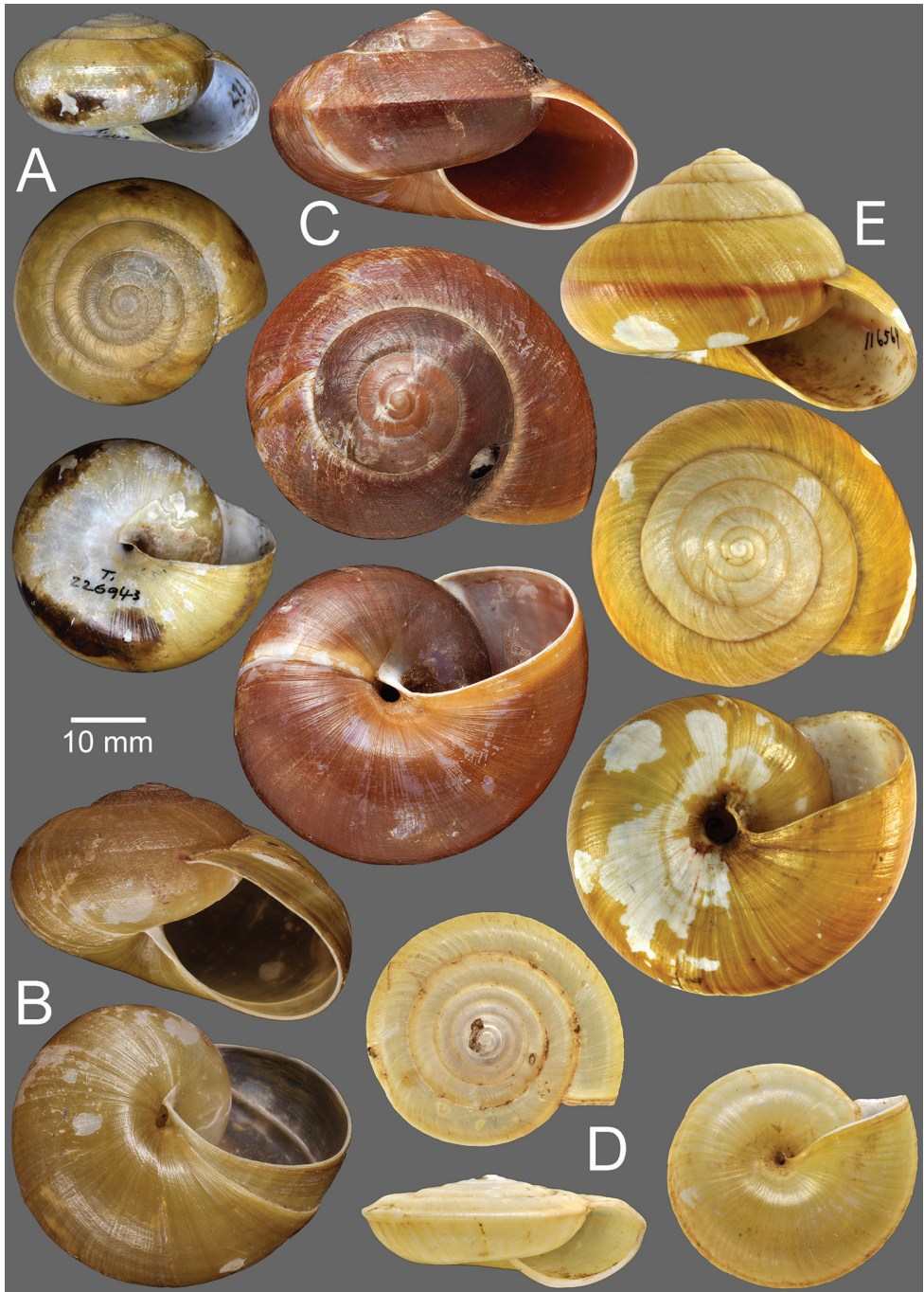


Figure 12. **A** *Hemiplecta denserugata*, syntype SMF 226943/1 **B** *Hemiplecta distincta*, holotype MNHN-IM-2000-35535 of *Hemiplecta franzhuberi* Thach, 2020 **C** *Hemiplecta funerea*, lectotype NHMUK 1896.1.25.4 **D** *Hemiplecta gordoniae*, specimen NHMUK 1903.7.1.309 **E** *Hemiplecta humphreysiana*, syntype NMNH 116569.

Type specimens. Syntype SMF 226943/1 (1 shell, height 20.1 mm, width 33.2 mm; Fig. 12A) from Süd Annam, Berg Dran, 3000 ft.

Remarks. The number of specimens was not clearly stated and only one set of shell measurements was given in the original description. The single specimen from the type lot is illustrated herein for the first time.

5 *distincta* (Pfeiffer, 1850)

Helix distincta Pfeiffer, 1850: 69, 70. Type locality: Insulis Moluccis [possibly error or mislabeling]. Pfeiffer 1853: 346, pl. 134, figs 1, 2. Reeve 1854: *Helix* pl. 86, species 465.

Hemiplecta (Hemiplecta) distincta: Hemmen and Hemmen 2001: 44, fig. 12.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Possible syntype NHMUK 20200199 (3 shells; Fig. 11C) from Moluccas [possibly error or mislabeling].

Remarks. This species was described based on specimens from the Cuming collection. The original description did not include an illustration and only one set of shell measurements was given. Later, Pfeiffer (1853) re-published the description and figured this species based on material from the Cuming collection. The NHM collections contain a lot of three shells from the Cuming collection. The original label, not in Pfeiffer's handwriting, states the taxon name and gives the collection locality as "Hab. Moluccas (Pfr. Zeitschr. 1850. p. 69)", and a small printed label stating "Type?". Additionally, the collection localities "Siam & Camboja" and "Siam & Cochin Chine (Martens)" were probably added at a later date. Therefore, we consider this lot to be possible syntypes. The specimen that closely matched the measurements in the original description and illustration in Pfeiffer (1853) is figured herein.

The museum collection and current published record with detailed geographical data of *H. distincta* are only from Indochina. Therefore, the type locality "Insulis Moluccis [Molucca Islands in eastern Indonesia]" possibly error or mislabeling.

6 *esculenta* Maassen, 2006

Hemiplecta esculenta Maassen, 2006: 17, 18, figs 10–12. Type locality: limestone area near village Hang, Pu Luong National Park, Thanh Hoa, Vietnam. Inkhavilay et al. 2019: 76, fig. 35d, e. Páll-Gergely 2019: figs 11–13.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype RMNH 99424 (see Inkhavilay et al. 2019: fig. 35d), paratype RMNH 99425 (1 shell).

Remarks. The type specimen was recently illustrated in Inkhavilay et al. (2019) and Páll-Gergely (2019).

7 *floweri* Smith, 1899

Hemiplecta floweri Smith, 1899: 284, 285, text figures. Type locality: Maxwell's Hill, Larut, Perak [Bukit Larut, Taiping, Perak State, Malaysia]. Maassen 2001: 103.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Syntype NHMUK 1899.3.16.1–2 (2 shells; Fig. 11D) from Malay Peninsula.

Remarks. The original description included illustrations and one set of shell measurements. However, the species description was not explicitly based on one specimen. There are two shells in the NHM type lot with an original label stating “Types”, subsequently changed to read “holotype red spot”. The shell that matched the measurements given in the original description and that has a red spot in the aperture is figured herein.

8 *franzhuberi* Thach, 2020

Hemiplecta franzhuberi Thach, 2020: 38, figs 442–444. Type locality: Thakhek, Laos.

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta distincta*.

Type specimens. Holotype MNHN-IM-2000-35535 (Fig. 12B) from Thakhek, Savannakhet, Laos.

Remarks. This species seems to be described based on a single specimen and the author refers to the diagnosed character “two broad shallow spiral grooves situated near the periphery of body whorl at dorsal side”. However, using just one character without any further independent diagnostic characters could raise doubt about the taxonomic status. The single shell may reflect an abnormality during the growth stage and, apart from this trait, all the other shell characters all are within the range of morphological variations seen within *H. distincta*. Therefore, we consider *H. franzhuberi* as a junior synonym of the more common and widespread *H. distincta*.

It would be very useful if this new species were compared with sympatric or geographically proximate species (i.e. *H. distincta* or *H. pluto*) instead of the distant species *H. abbasi* Maassen, 2009 from Sumatra (Maassen 2009).

9 *funerea* (Smith, 1896)

Nanina distincta var. *funerea* Smith, 1896: 128. Type Locality: Vanbu, Tonkin [Van Ban District, Lao Cai Province, Vietnam]. Fischer and Dautzenberg 1904: 393.
Hemiplecta funerea: Inkhavilay et al. 2019: 76, 77 [not figs 35f, 36a].

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Lectotype (design. n.) NHMUK 1896.1.25.4 (1 shell; Fig. 12C) designated from Vanbu, Tonkin.

Remarks. The species description is clearly based on more than one specimen. The original description does not include an illustration, and measurements of the largest specimen are given. The NHM collection contains a lot of a single specimen with a label stating “var. *funerea*”. This specimen matched well with the original description and is here designated as the lectotype to stabilize the name.

Inkhavilay et al. (2019: fig. 35f) state this specimen is the syntype of “var. *funerea*” but wrongly apply the images of “var. *pallidior*” instead.

10 *gordoniae* (Benson, 1863)

Helix gordoniae Benson, 1863: 87. Type locality: Birmanica prope Moulmein [Mawlamyine Township, Mawlamyine District, Mon State, Myanmar]. Hanley and Theobald 1870: 13, pl. 27, figs 1, 2.

Hemiplecta ? *gordoniae*: Blanford and Godwin-Austen 1908: 293.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. The type specimens could not be traced from the Benson collection.

Remarks. The topotype specimen from Godwin-Austen collection NHMUK 1903.7.1.309 (1 shell; Fig. 12D) with collection locality from Needoung Thoung, Ataran valley, Tenasserim is figured herein.

11 *huberi* Thach, 2017

Hemiplecta huberi Thach, 2017: 33, figs 389–391. Type locality: Thakhek, Khammouane Province, Central Laos. Inkhavilay et al. 2019: 77, fig. 36b. Páll-Gergely et al. 2020: 46.

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta pluto*.

Type specimens. Holotype MNHN-IM-2000-33196 (see Inkhavilay et al. 2019: fig. 36b).

Remarks. Páll-Gergely et al. (2020) attributed the diagnostic character of this name as a morphological variation of the widely distributed *H. pluto*, so this is treated as a junior synonym.

12 *huberi* Thach, 2017

Helminthoglypta huberi Thach, 2017: 54, figs 747–749 [non *Hemiplecta huberi* Thach, 2017: 33, figs 389–391]. Type locality: Thakhek, Khammouane Province, Central Laos.

Remarks. See under *Hemiplecta lanxangnica* Inkhavilay & Panha, 2019

13 *humphreysiana* (Lea, 1840)

Helix humphreysiana Lea, 1840: 175. Type locality: Pondicherry and Singapore. Lea 1841: 463, 464, pl. 12, fig. 16.

Hemiplecta humphreysiana: Godwin-Austen 1898: 74–76, pl. 80, fig. 6, 6b; pl. 81, fig. 1, 1e. Benthem Jutting 1959: 148–150.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Syntype NMNH 116569 (1 shell; Fig. 12E) from Pondicherry.

Remarks. This species was clearly described based on more than one specimen. The author's description clearly indicates that the type series was from two collection localities: "Pondicherry" received from Mr. Humphreys and "Singapore" received from Mr. Balastire. Later, Lea (1841) re-described the species and illustrated a single specimen. The Smithsonian collections contain a lot of a single shell from the Lea collection as from "Pondicherry". This specimen matched well with the illustration and the measurements given in the original description.

The records of this species from "Pondicherry" [the historical name probably referred to the cities on the east coast of India] have never been verified. Currently, the genus *Hemiplecta* are distributed from Southeast Asia to Southeast Asia and New Guinea, except one species recorded from the Maldives (Schileyko 2002). Therefore, "Pondicherry" is probably an erroneous record (see also Godwin-Austen 1898: 74), and "Singapore" is possibly the correct type locality of this species.

14 *jensi* Páll-Gergely, 2019

Hemiplecta jensi Páll-Gergely, 2019: 86–88, figs 1–6. Type locality: Vietnam, Thanh Hoa Province, Pu Luong N.R., surroundings of Village Am.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype SMF 353501 (see Páll-Gergely 2019: figs 1–4), from Vietnam, Thanh Hoa Province, Pu Luong N.R., surroundings of Village Am.

15 *khamducensis* (Thach & Huber, 2018)

Camaena khamducensis Thach & Huber in Thach, 2018: 67, figs 886–888. Type locality: Kham Duc area, Phuoc Son, District, Quang Nam Province, Central Vietnam.

Hemiplecta khamducensis: Páll-Gergely et al. 2020: 46.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype FMNH 386292.

Remarks. This nominal species was transferred to the genus *Hemiplecta* by Páll-Gergely et al. (2020); we agree with their decision. An image of a living specimen in the

original description (Thach 2018: fig. 888) shows an aulacopod type of pedal groove, whereas Camaenidae has a holopod type of pedal groove (see Solem 1959: fig. 2). The assignment of this species to the genus *Hemiplecta* is most likely, due to the helicarioid snails having a relatively large shell size, simple apertural lip (slightly thickened), and narrow umbilicus (Schileyko 2002). Anatomical examination will help elucidate the appropriate generic position of this species.

Thach (2018) mentioned depositing the holotype at the Field Museum of Natural History, Chicago. However, the holotype has not arrived at the FMNH collection (Jochen Gerber, personal communication on October 2020).

16 *khamducensis* Thach & Huber, 2000

Hemiplecta khamducensis Thach & Huber in Thach, 2020: 38, 39, figs 434–437. Type locality: Kham Duc, Phuoc Son District, Quang Nam Province, Central Vietnam.

Current taxonomic status. *Hemiplecta*. Junior homonym and junior synonym of *Hemiplecta khamducensis* (Thach & Huber, 2018).

Type specimens. Holotype NHMUK 20200208.

Remarks. This species was originally proposed as a junior secondary homonym from the same locality as the senior homonym. Basically, this junior homonym agrees well in all diagnostic shell characters of a red-brown shell, strong peripheral keel, and shell shape that all lie within the range of variation of the present species. This species is synonymized with *H. khamducensis* (Thach & Huber, 2018) herein, and, therefore, the replacement name is not necessary at present.

Thach (2020) stated that the holotype was deposited at the Natural History Museum in London. However, the holotype has not arrived in the NHM collection (Jonathan Ablett, personal communication on May 2021).

17 *lanxangnica* Inkhavilay & Panha, 2019

Helminthoglypta huberi Thach, 2017: 54, figs 747–749 [non *Hemiplecta huberi* Thach 2017: 33, figs 389–391]. Type locality: Thakhek, Khammouane Province, Central Laos.

Hemiplecta lanxangnica Inkhavilay & Panha in Inkhavilay et al., 2019: 77, 78, fig. 36c, d. [new replacement name]. Páll-Gergely et al. 2020: 46.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype RMNH 5006710 from Thakhek, Khammouane Province, Central Laos. Paratype MNHN-IM-2000-33215 (1 shell; see Inkhavilay et al. 2019: fig. 36c).

18 *laotica* (Möllerndorff, 1899)

Bensonina (*Oxytes*) *laotica* Möllerndorff, 1899: 165. Type locality: Oberer Mekong im Lande der Laos [upper Mekong in Laos].

Ariophanta laotica: Inkhavilay et al. 2019: 75, fig. 34d, e.

Hemiplecta laotica: Páll-Gergely 2019: 87, figs 6–10. Páll-Gergely et al. 2019: 605, fig. 7k–n.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Syntype SMF 226681 (1 shell; see Inkhavilay et al. 2019: fig. 34d), SMF 226682 (3 shells) from Laos.

Remarks. The type specimen was recently illustrated in Inkhavilay et al. (2019: fig. 34d).

19 *malaouyi* (Morgan, 1885)

Xesta malaouyi Morgan, 1885a: 374, 375, pl. 5, fig. 4. Type locality: Mont Kerbou, à 1800 mètre environ d'altitude [1800 m altitude, Gunung Korbu, Hulu Kinta, Perak State, Malaysia].

Hemiplecta malaouyi: Maassen 2001: 103.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Syntype MNHN-IM-2000-34170 (1 shell; Fig. 13A) from Perak, Mont Kerbou.

Remarks. Only one specimen in the syntype lot and the spire was broken after the original description.

20 *nemorosa* sp. nov.

Remarks. The species is described herein (see systematic part).

21 *neptunus* Pfeiffer, 1861

Helix neptunus Pfeiffer, 1861a: 190. Type locality: Siam [Thailand]. Pfeiffer 1861b: 176, 177. pl. 48, figs 1, 2.

Hemiplecta (*Hemiplecta*) *neptunus*: Hemmen and Hemmen 2001: 44.

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta distincta*.

Type specimens. Syntype NHMUK 20150065 (2 shells; Fig. 13B) from Siam.

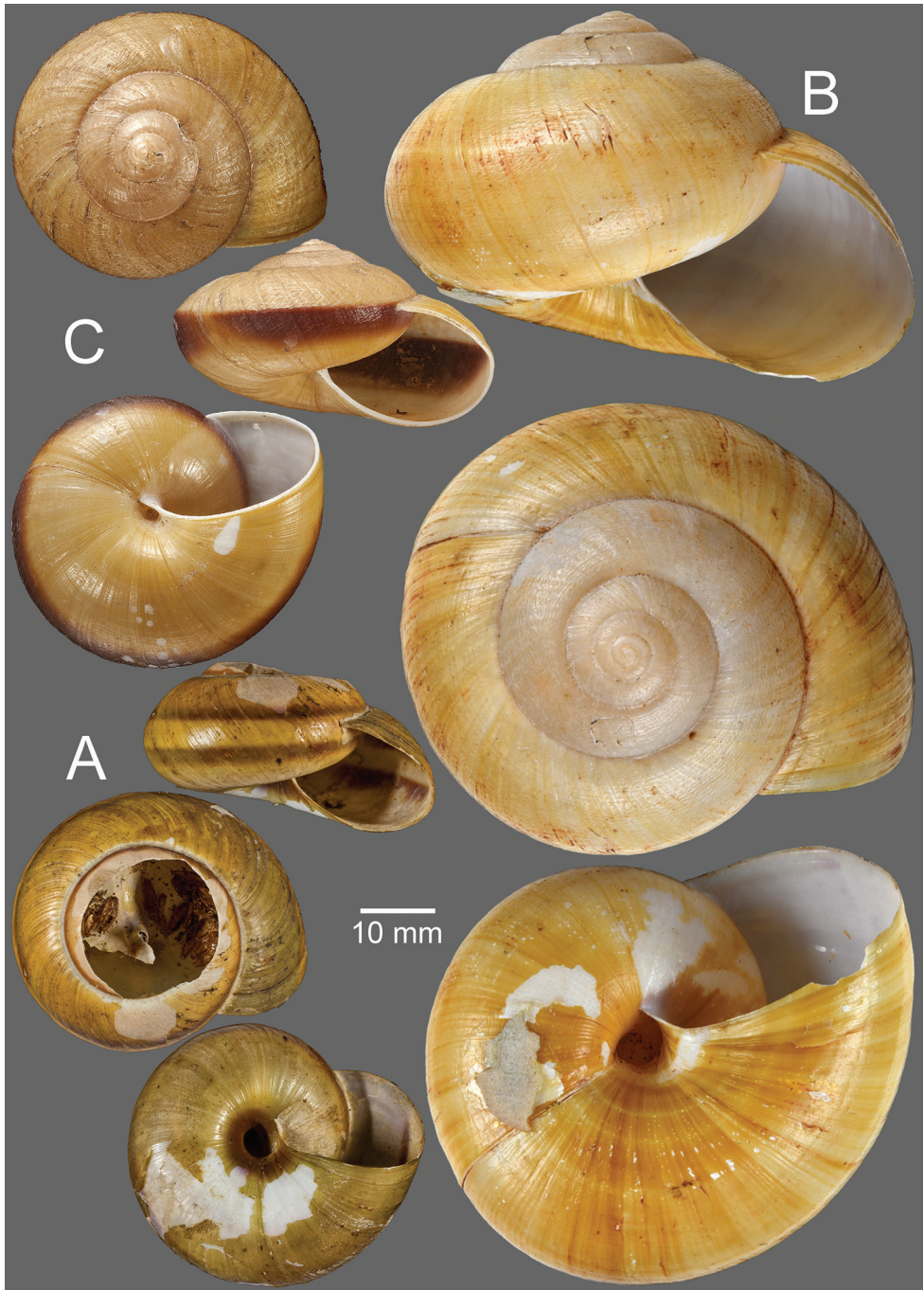


Figure 13. **A** *Hemiplecta malaouyi*, syntype MNHN-IM-2000-34170 **B** *Hemiplecta distincta*, syntype NHMUK 20150065 of *Helix neptunus* Pfeiffer, 1861 **C** *Hemiplecta funerea*, syntype NHMUK 1896.1.25.5 of *Nanina distincta* var. *pallidior* Smith, 1896.

Remarks. This name was described based on specimens from the Cuming ex. Mouhot collection. The original description did not include illustrations, and only one set of measurements was given. Later, Pfeiffer (1861b) re-described and illustrated a single specimen from the Cuming collection. There are two specimens from the Cuming collection in the NHM type lot with an original label in Pfeiffer's handwriting stating the species name, collection locality and "*pernobilis* Fer. Var.? and pl.74, f. 4.". The specimen that corresponded to the shell measurements in the original description and illustration in Pfeiffer (1861b) is figured herein.

22 *pallidior* (Smith, 1896)

Nanina distincta var. *pallidior* Smith, 1896: 128. Type locality: Vanbu, Tonkin [Van Ban District, Lao Cai Province, Vietnam]. Fischer and Dautzenberg 1904: 393. Inkhavilay et al. 2019: fig. 35f.

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta funerea*.

Type specimens. Syntype NHMUK 1896.1.25.5 (1 shell; Fig. 13C) from Vanbu, Tonkin.

Remarks. The image of Inkhavilay et al. (2019: fig. 35f) under the name *H. funerea* is the syntype of *Nanina distincta* var. *pallidior* Smith, 1896.

23 *pernobilis* (Férussac, 1821)

Helix pernobilis Férussac, 1821: 39, no. 182. Type locality: Poulo-Condor [Con Dao Island, South Vietnam].

Koratia distincta pernobilis: Schileyko 2011: 30

Koratia pernobilis: Schileyko 2015: 15–18, fig. 1.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. The type specimens could not be traced.

24 *pharangensis* (Möllerndorff, 1901)

Xestina pharangensis Möllerndorff, 1901: 46. Type locality: Pharang, Süd Annam [Phan Rang, Ninh Thuan Province, south Vietnam].

Hemiplecta pharangensis: Schileyko 2011: 30.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype SMF 226947/1 (height 15.1 mm, width 22.5 mm; Fig. 14A) from Pharang, Süd-Annam.

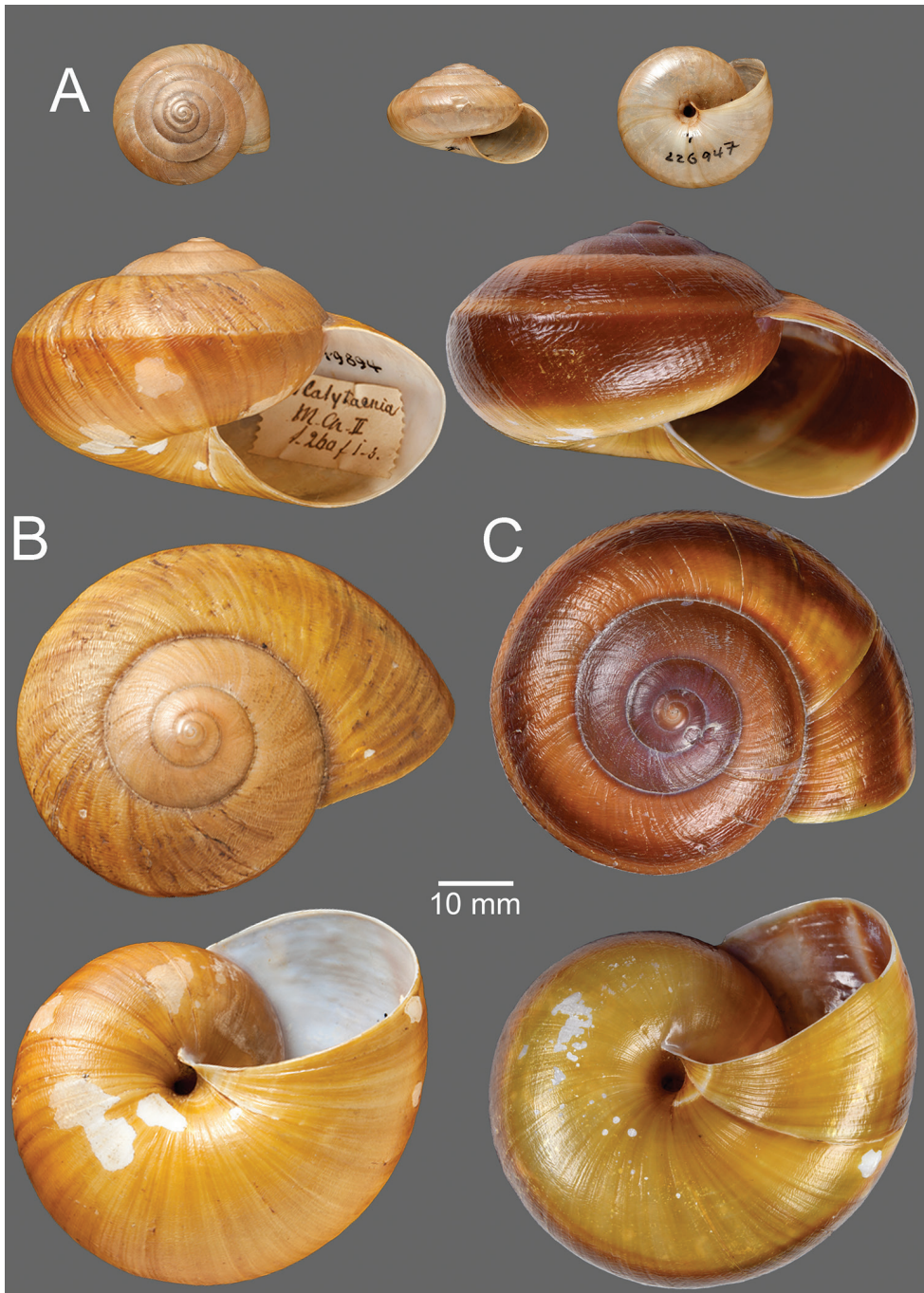


Figure 14. **A** *Hemiplecta pharangensis*, syntype SMF 226947/1 **B** *Hemiplecta platytaenia*, syntype SMF 149894 **C** *Hemiplecta pluto*, lectotype NHMUK 20200200.

Remarks. The number of specimens was not clearly stated, and only one set of shell measurements was given in the original description. The single specimen from the type lot is illustrated herein for the first time.

25 *platytaenia* Möllendorff, 1900

Hemiplecta platytaenia Möllendorff, 1900: 121. Type locality: Touranne [Da Nang, Vietnam]. Schileyko 2011: 30.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Syntype SMF 149894 (1 shell, height 41.2 mm, width 60.5 mm; Fig. 14B) from Annam: Touranne.

Remarks. The number of specimens was not clearly stated, and only one set of shell measurements was given in the original description. The single specimen from the type lot is illustrated herein for the first time. Möllendorff (1900: 121) also stated that this species differed from *H. neptunus* (= *H. distincta*) in having a flatter shape, with a weak keel, and a spiral band on periphery.

26 *pluto* (Pfeiffer, 1863)

Helix pluto Pfeiffer, 1863a [1862]: 268, 269. Type locality: Lao Mountains, Camboja [Luang Prabang, Laos]. Pfeiffer 1863b: 210, pl. 55, figs 8, 9.

Nanina (*Hemiplecta*) *pluto*: Kobelt 1900: 987, pl. 256, figs 1, 2.

Hemiplecta pluto: Schileyko 2011: 30. Inkhavilay et al. 2019:78, figs 36e, f, 56d. Páll-Gergely et al. 2020: 46.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Lectotype (design. n.) NHMUK 2020200 (1 shell; Fig. 14C) from Lao Mountains, Camboja.

Remarks. Pfeiffer (1863a) stated that this species was described based on specimens from the Cuming collection. The original description did not include an illustration, and only one set of shell measurements was given. There are two specimens in the mixed type-lot of different species. The specimen that had an original label in Pfeiffer's handwriting states "*H. pluto* Pfr." and the collection locality "Lao Mountains, Camboja". The specimen that matched the description and shell measurements given in the original description, and the illustration in Pfeiffer (1863b: pl. 55, figs 8, 9) is here designated as the lectotype to stabilize the name.

The other shell from the same collection lot with label stating "var. *neptunus* young" was identified as *H. distincta*. This specimen is not part of the type series and, therefore, excluded from this designation.

Pfeiffer (1863a) described this species based on the specimen collected by H. Mouhot in the Cuming collection, and “Lao Mountains, Camboja” is the type locality. We have seen specimens with more precise geographical location from the Khammouan Province to Luang Prabang Province, Laos (Inkhavilay et al. 2019).

27 *sakaya* (Morgan, 1885)

Oxytes sakaya Morgan, 1885a: 380, 381, pl. 6, fig. 1. Type locality: Mont Kerbou, à 1200 mètre environ d'altitude [1200 m altitude, Gunung Korbu, Hulu Kinta, Perak State, Malaysia].

Hemiplecta sakaya: Laidlaw, 1932a: 89. Maassen, 2001: 101.

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta cymatium*.

Type specimens. Syntype MNHN-IM-2000-34169 (2 shells; Fig. 15A) from Presquîle de Malacca, mont Kerbou.

Remarks. Laidlaw (1932a) seems to be the first to synonymize this species with *H. cymatium*, and this treatment has been followed by Maassen (2001) until recently. The type specimen ex. de Morgan collection is figured herein.

28 *textrina* (Benson, 1856)

Helix textrina Benson, 1856: 252. Type locality: ad Thyet Myo [Thayet District, Magway Region, Myanmar]. Pfeiffer 1860: 131, pl. 36, figs 5–7. Hanley and Theobald 1872: 24, pl. 52, figs 2, 5.

Hemiplecta ? *textrina*: Blanford and Godwin-Austen 1908: 292.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. The type specimens could not be traced.

Remarks. The specimen from the Blanford collection NHMUK 1906.1.1.389 from Bassein, Pegu is figured herein (Fig. 15B).

29 *theodori* (Philippi, 1846)

Helix theodori Philippi, 1846: 191, 192. Type locality: Prope Mergui Indiae orientalis [Myeik Township, Myeik District, Tanintharyi Region, Myanmar].

Hemiplecta theodori: Blanford and Godwin-Austen 1908: 292, 293.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. The type specimens could not be traced.

Remarks. The topotype specimen NHMUK 1888.12.4.1517 (1 shell; Fig. 15C) from Mergui is figured herein.

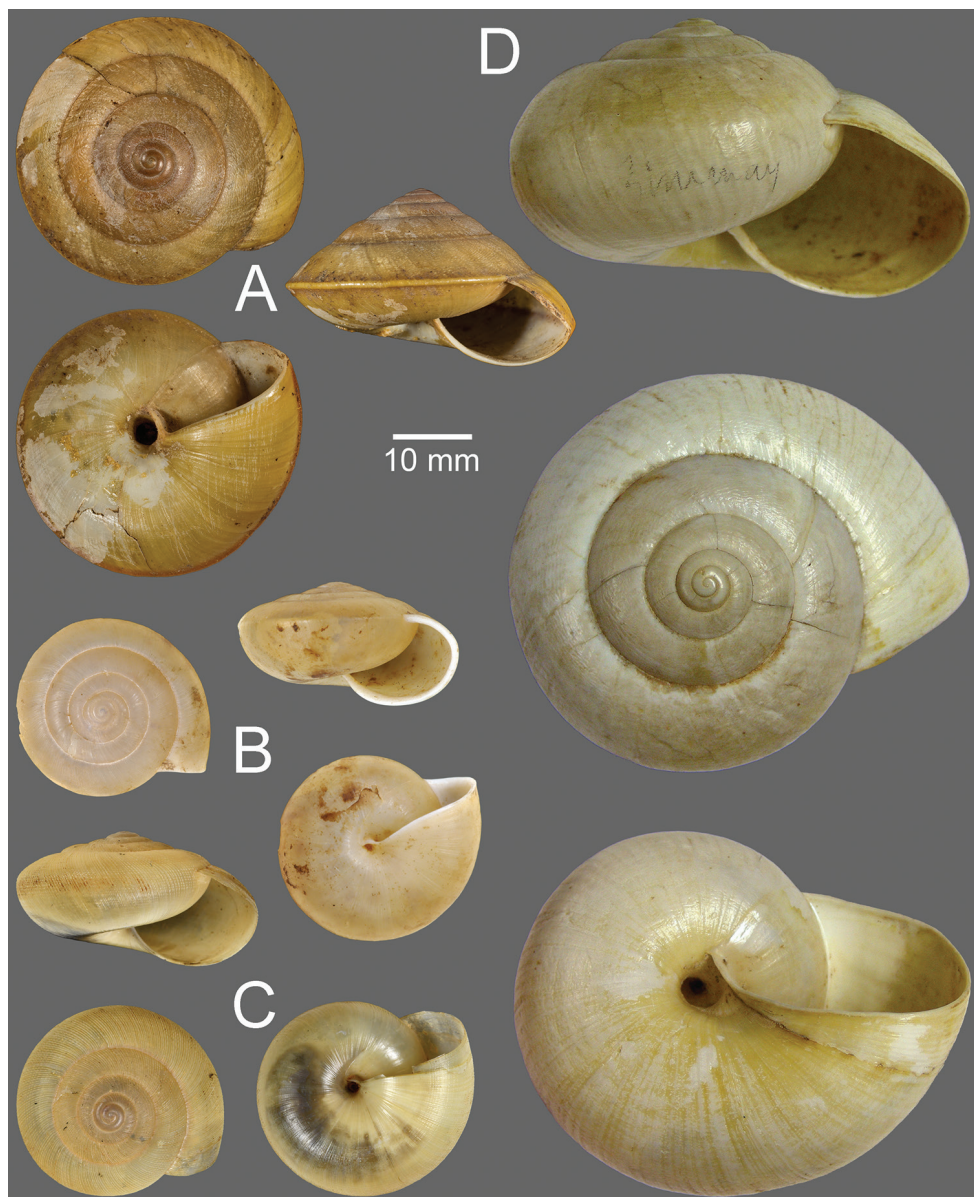


Figure 15. **A** *Hemiplecta sakaya*, syntype MNHN-IM-2000-34169 **B** *Hemiplecta textrina*, specimen NHMUK 1906.1.1.389 **C** *Hemiplecta theodori*, specimen NHMUK 1888.12.4.1517 **D** *Hemiplecta distincta*, syntype NHMUK 1888.12.4.2007 of *Hemiplecta zimmayensis* Godwin-Austen, 1888.

30 *undosa* (Blanford, 1865)

Nanina (*Hemiplecta*) *undosa* Blanford, 1865: 68. Type locality: Shan Hills, east of Ava [Shan Hills in Kyaukse District, Mandalay Region, Myanmar].

Helix undosa var. Hanley & Theobald, 1874: 45, pl. 111, figs 2, 3.

Hemiplecta undosa: Blanford and Godwin-Austen 1908: 291, 292.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Probable syntype NHMUK 20200201 (2 shells; Fig. 16A) from Ava, Hills east of Shan State.

Remarks. The original description did not include an illustration, and only one set of shell measurements was given. The author stated, “All the specimens”, implying that this description was based on more than one specimen. Blanford (1865) also mentioned “? horny when fresh” and “...dead and bleached” in the original description. The NHM collection contains a lot of two bleached shells from the Godwin-Austen ex. Blanford collection with an original label stating the species name; however, this is probably not in Blanford’s handwriting. Therefore, we consider this lot to be a probable syntype. However, the specimen that matched well with the original description and shell dimensions is figured herein.

31 *uter* (Theobald, 1859)

Helix uter Theobald, 1859: 305. Type locality: Maulmein [Mawlamyine Township, Mawlamyine District, Mon State, Myanmar]. Hanley and Theobald 1872: 27, pl. 58, figs 7, 8.

Hemiplecta uter: Blanford and Godwin-Austen 1908: 291.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype NMHUK 1888.12.4.1487 (height 16.3 mm, width 26.5 mm; Fig. 16B) from Moulmein.

Remarks. The original description clearly states that this taxon was described based on only one specimen collected by W.S. Atkinson (Theobald 1859). The NHM registration records show that a specimen was purchased from W. Theobald with the label stating “type” and locality given as “Moulmein”. Therefore, this single specimen is recognized as the holotype fixed by monotypy (ICZN 1999: Art. 73.1.2).

32 *zimmayensis* Godwin-Austen, 1888

Hemiplecta ? *zimmayensis* Godwin-Austen, 1888c: 241, 242. Type locality: Zimmay, Siam territory [Chiang Mai Province, Thailand].

Hemiplecta (*Hemiplecta*) *zimmayensis*: Hemmen and Hemmen 2001: 44.

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta distincta*.

Type specimens. Syntypes NHMUK 1888.12.4.2007 (1 shell; Fig. 15D) from Zimmay territory, Siam; NHMUK 1903.7.1.2108 (1 shell) from Siam.

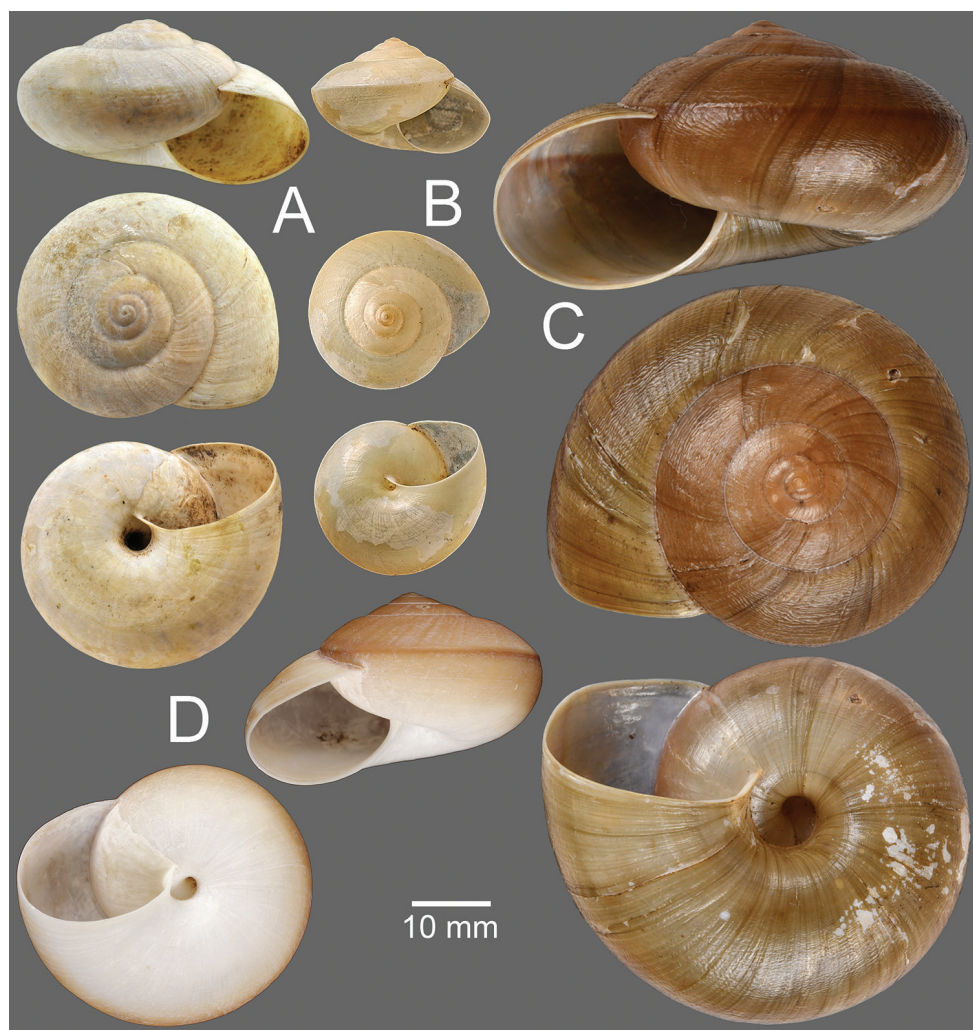


Figure 16. **A** *Hemiplecta undosa*, probable syntype NHMUK 20200201 **B** *Hemiplecta uter*, holotype NMHUK 1888.12.4.1487 **C** *Hemiplecta salangana*, syntype NHMUK 1904.5.26.18–19 of *Hemiplecta salangana* var. *martensi* Collinge, 1903 **D** *Hemiplecta salangana*, holotype MNHN-IM-2000-35533 of *Ariophanta trangensis* Thach & Huber, 2020.

Remarks. The original description did not contain any illustrations, and only one set of measurements was given. Godwin-Austen stated that the type series was from his own and Theobald’s collections. The NHM collection contains two lots that are considered to constitute the type series. Lot NHMUK 1903.7.1.2108 consists of a single specimen from the Godwin-Austen ex. Theobald collection and has original labels giving the species name “*zimmayensis*” and type collection locality “Siam”. The other lot consists of a single shell, NHMUK 1888.12.4.2007, and the NHM registration book

shows that this specimen lot was purchased from W. Theobald, and with an original label stating the species name “*H. Zimmayensis* G.A.” and type collection locality “Zim-may territory (Siam)”. This specimen (NHMUK 1903.7.1.2108) from the Godwin-Austen collection is figured herein.

Group II: Sinistral species

33 *lahatensis* (Morgan, 1885)

Helix lahatensis Morgan, 1885b: 69. Type locality: dans la forêt Lahat et Ipoli [Lahat, Ipoh, Perak State, Malaysia].

Ariophanta lahatensis: Morgan 1885a: 382, pl. 6, fig. 4.

Dyakia lahatensis: Laidlaw 1931: 193.

Hemiplecta lahatensis: Sutcharit et al. 2021: 206, 207, figs 3e, 4a.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Syntype MNHN-IM-2000-22834 (3 shells; see Sutcharit et al. 2021: fig. 3e) from Royaume de Pérak, vallée de Kinta.

Remarks. The type specimen of this nominal species seems to be based on the immature shell, and the genital organ was not examined. However, the molecular phylogeny based on the juvenile specimens from approximate type locality strongly suggests it is a member of the genus *Hemiplecta* (Sutcharit et al. 2021).

34 *ligorica* Sutcharit & Panha, 2021

Hemiplecta ligorica Sutcharit & Panha in Sutcharit et al. 2021: 208, 209, figs 4d, e, 5e, f, 6f. Type locality: The limestone hills at Tam Khao Lek, Nop Phitam District, Nakhon Si Thammarat Province, Thailand.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype CUMZ 5093/1.

Remarks. The species was recently described. Shell, genitalia, and DNA phylogeny confirm their generic status within the genus *Hemiplecta*.

35 *martensi* (Collinge, 1903)

Hemiplecta salangana var. *martensi* Collinge, 1903: 209. Type locality: Bukit Bersa [area in Khok Pho District, Pattani Province, Thailand].

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta salangana*.

Type specimens. Syntype NHMUK 1904.5.26.18–19 (2 shells; Fig. 16C) ex. Annandale and Robinson collection from Bukit Bersa.

Remarks. The original description does not include any illustration, and the author clearly stated that measurements given were based on two specimens. There is a specimen lot in the NHM ex. Annandale and Robinson collection consisting of two shells; one of these has a malformed shell form as stated in the original description. These two shells are considered as the syntypes and figured herein for the first time.

A recent phylogenetic study has recognized this taxon as a junior synonym of *H. salangana*, a widespread species in the southern peninsula of Thailand and northern Peninsular Malaysia (Sutcharit et al. 2021).

36 *retrorsa* (Gould, 1843)

Helix retrorsa Gould, 1843: 139. Type locality: Tavoy, British Burma [Dawei District, Tanintharyi Region, Myanmar]. Johnson 1964: 140, pl. 38, fig. 10.

Helix (Caracolla) retrorsa: Gould 1844: 455, pl. 24, fig. 5.

Dyakia retrorsa: Blanford and Godwin-Austen 1907: 300. Laidlaw, 1931: 191.

Hemiplecta retrorsa: Sutcharit et al. 2021: 200–205, figs 3a, b, 5a, b, 6a–c.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Lectotype (designated by Johnson (1964)) MCZ 169330 (1 shell, see Sutcharit et al. 2020, fig. 3a) from Tavoy, British Burma. Paralectotype MCZ 169331 (1 shell), USNM 611233 (1 shell), MCZ 220663 (1 shell).

Remarks. The type specimen was recently figured, and recent systematic revision based on both genitalia morphology and DNA phylogeny confirm their generic status within the genus *Hemiplecta* (Sutcharit et al. 2021).

37 *salangana* (Martens, 1883)

Nanina salangana Martens, 1883: 134–136, pl. 25, figs 8–12. Type locality: insulam Salanga (Junk Ceilon) ad oram occidentalem peninsulae Malaccanae [Phuket Province, Thailand and Peninsular Malaysia]. Sutcharit et al. 2012: 280, fig. 2.

Dyakia salangana: Laidlaw 1931: 191. Berry 1963: 14, pl. 9, fig. 61.

Hemiplecta salangana: Sutcharit et al. 2021: 205, 206, fig. 3c, d.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Syntypes ZMB/Moll 32578 (1 adult +1 juvenile, see Sutcharit et al. 2020: fig. 3c) from Salanga; ZMB/Moll 57522 (1 shell) from Salanga Hinterindien.

Remarks. The type specimen was recently published.

38 *thailandica* Sutcharit & Panha, 2021

Hemiplecta thailandica Sutcharit & Panha in Sutcharit et al. 2021: 207, 208, figs 4b, c, 5c, d, 6d, e. Type locality: Primary evergreen forest at Khao Soidao, Soidao District, Chanthaburi Province, Thailand.

Current taxonomic status. *Hemiplecta*. Valid species.

Type specimens. Holotype CUMZ 5095/1.

Remarks. The species was recently described. Shell, genitalia, and DNA phylogeny analyses confirm their generic status within the genus *Hemiplecta*.

39 *trangensis* Thach & Huber, 2020

Ariophanta trangensis Thach & Huber in Thach, 2020: 36, 37, figs 446–447. Type locality: Suburb of Trang City, Trang Province, Thailand.

Hemiplecta salangana: Sutcharit et al. 2021: 206.

Current taxonomic status. *Hemiplecta*. Synonym of *Hemiplecta salangana*.

Type specimens. Holotype MNHN-IM-2000-35533 (Fig. 16D).

Remarks. Thach (2020) stated that “...sinistral shell, elongate aperture extending leftward and very far from shell axis” are the diagnostic characters. However, these are synapomorphies of the sinistral-*Hemiplecta* clade. Recent systematic revision showed no evidence of the unique phylogenetic subdivision and, therefore, recognized this name as a junior synonym of *H. salangana* (see Sutcharit et al. 2021).

Results and conclusion

Nine valid species of the genus *Hemiplecta* occur in Thailand, five of these are the dextral shell coiling species, and the other four are sinistral shell coiling species. In order to broaden our comparison among species of *Hemiplecta* s.l., we gathered and compared anatomical data from the literature for nineteen species (Table 2). This comparison indicated that fourteen species are likely to have been correctly placed in the genus *Hemiplecta*, but not the other five species. The genital characters are relatively similar among species within this genus, except for the penial verge, penial sculpture, and terminal part of male genitalia (epiphallus, epiphallic caecum and flagellum), which are taxonomically informative at the species level (Table 2). Interestingly, these fourteen species all have a similar gametolytic organ structure: globular gametolytic sac with an undifferentiated duct. A recent systematic study has shown congruence between the traditional morphology-based species taxonomy and the molecular phylogeny (Sutcharit et al. 2021). This indicates that accurate generic recognition can be based on the genitalia character especially the globular shape of the gametolytic sac. However, the critical

role of the gametolytic organ other than extracellular digestion of excess reproductive products has never been reported in the stylommatophoran (Goméz 2001; Baur 2010).

This comparison further indicated that the other five species are likely to have been inappropriately placed in the *Hemiplecta* (Table 2). Four species: *H. densa* (Adams & Reeve, 1850) from the Philippines, *H. werberi* (Sarasin & Sarasin, 1899) from Sulawesi, *H. foersteri* Kobelt, 1914 from Papua New Guinea, and *H. belerang* Cilia & Abbas, 2012 from Sumatra exhibit a long gametolytic duct, with or without a dart apparatus (Table 2) and are clearly distinct from the typical characteristics of the *Hemiplecta* (Wiegmann 1898; Niethammer 1937; Wiktor 2003; Cilia and Abbas 2012). On the other hand, these four species have a dextral shell, and the presence of shell lobes suggest a close relationship to the genus *Nanina*. In addition, *H. malaouyi* (Morgan, 1885) from Peninsular Malaysia exhibits a coiled epiphallic caecum, long gametolytic organ, and presence of shell lobes (Table 2), which are the unique characteristics of the Macrochlamyidae (Blanford and Godwin-Austen 1908; Solem 1966; Schileyko 2003). However, the relatively large shell size (width about 40 to 60 mm) is distinct from other known genera within the Macrochlamyidae. Further anatomical information and molecular analyses will elucidate whether the generic placement is appropriate or whether these species form a distinct group.

In the species list, 39 available species-level names are recognized as part of the genus *Hemiplecta* and described from Indochina, including Peninsular Malaysia and Myanmar. There are six nominal species for which the name-bearing type could not be discovered, except the four nominal species: *H. aurietae*, *H. gordonae*, *H. textrina* and *H. theodori* where the topotypic specimen are figured as representative. However, generic placement of many species are still provisional because these species are known only from their shell descriptions without the genitalia characters. Like the other land snail group in Indochina, a systematic revision has never been studied, and species recognition is difficult. The species have long been described with only a brief description and without illustrations of unique characters of the species. This species list with illustrated type or authentic specimens provides a key species data and facilitates proper species identification.

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Multigene phylogeny and taxonomic revision of American shrimps of the genus *Cryphiops* Dana, 1852 (Decapoda, Palaemonidae) implies a proposal for reversal of precedence with *Macrobrachium* Spence Bate, 1868

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Abstract

The freshwater shrimp genus *Cryphiops* Dana, 1852 has a disjunct distribution in North (Mexico) and South (Brazil, Chile) America, and is composed of only six species. The current classification of genera in the Palaemonidae is controversial, based on variable morphological characters, and still far from a clear definition. *Cryphiops* differs from the speciose genus *Macrobrachium* Spence Bate, 1868 only by the absence of the hepatic spines on the carapace. Previous studies with a limited dataset suggested the necessity to link morphology and phylogeny to create an internal rearrangement in the genus to resolve the paraphyletic status. Through a molecular phylogenetic approach, the evolutionary relationships are inferred based on four (mitochondrial and nuclear) genes, among all recognized species of *Cryphiops* and, in combination with a taxonomic revision, a rearrangement in the systematics of the genus is suggested. The absence of hepatic spines on the carapace, the only character used to separate the genus *Cryphiops*, is subjective and should be considered as a homoplasy. This implies that *Cryphiops* and *Macrobrachium* are

subjective synonyms and, because the latter genus is much more diverse and widely known, with several economically important species, to avoid confusion and disturbance in nomenclatural stability and keep universality, a proposal for the priority of the older synonym (*Cryphiops*) to be partially suppressed in favor of maintaining the prevailing use of the younger synonym (*Macrobrachium*) is presented. As the species of *Cryphiops* should be accommodated in the genus *Macrobrachium*, new names to replace three preoccupied specific names that, by this action, resulted to be secondary homonyms are offered.

Keywords

Freshwater decapods, genetic variability, *Macrobrachium*, molecular phylogeny, Neotropical region, prawn

Introduction

During the 2010s, caridean shrimp systematics has undergone considerable changes at different levels (see De Grave et al. 2015a for review of the context and literature). The speciose Palaemonidae Rafinesque, 1815 is an example of this new tendency. The family consists of a large group of decapod crustaceans comprising 151 genera and approximately 780 species (WoRMS 2021), which reached a great evolutionary success, occupying marine, estuarine, and freshwater environments. Members of this group have a long taxonomic history and it can be considered a challenge to build a more natural classification since their morphology is highly conservative (Holthuis 1950, 1952a; Pereira 1997; Murphy and Austin 2005; Pileggi and Mantelatto 2010; De Grave and Ashelby 2013). Recently, considerable efforts have been taken to solve taxonomic incongruences and accommodate taxa in a more consistent classification (De Grave et al. 2009; De Grave and Fransen 2011). After the construction of this major guideline, some important specific and complementary taxonomic initiatives were developed focusing on different taxa (for a review see De Grave et al. 2015a). Despite this significant advance, the current knowledge is not sufficient to cover the tremendous diversity of palaemonids and the many questions that remain unanswered. One of these unsolved problems is that of *Cryphiops* Dana, 1852, a genus composed by six recognized species distributed in North (Mexico) and South (Brazil and Chile) America (Villalobos Hiriart et al. 1989; Baldari et al. 2010). Of the six species, only *Cryphiops* (C.) *caementarius* (Molina, 1782) needs estuarine water to complete its reproductive cycle while the other five [*Cryphiops* (*Bithynops*) *brasiliensis* Gomes Corrêa, 1973, *Cryphiops* (*Bithynops*) *luscus* (Holthuis, 1973), *Cryphiops* (*Bithynops*) *perspicax* (Holthuis, 1977), *Cryphiops* (*Bithynops*) *sbordonii* Baldari, Mejía-Ortiz & López-Mejía, 2010, and *Cryphiops* (*Bithynops*) *villalobosi* Villalobos Hiriart, Nates Rodríguez & Cantú Díaz Barriga, 1989] are restricted to inland waters with no apparent dependency of estuarine environments.

The taxonomic reappraisal of *Cryphiops* showed a close relationship with *Macrobrachium* Spence Bate, 1868, from which *Cryphiops* only differs by the absence of the hepatic spines on the carapace (Holthuis 1950, 1952a). The absence of one or both spines was also encountered by Short (2004) in some Australian species of

Macrobrachium [*M. hendersoni* (De Man, 1906), *M. hildebrandti* (Hilgendorf, 1893), *M. pilimanus* (De Man, 1879), and *M. koombooloomba* Short, 2004] who offered no explanation about the evolutionary importance of this character. Thus, considering the subjectivity of the character separating *Cryphiops* and *Macrobrachium*, it is imperative to conduct further in-depth studies, using different approaches, to resolve their relationship.

Molecular phylogenetic analysis including species of *Cryphiops* are scarce and appear only as part of broader studies with different objectives, for example Porter et al. (2005) and Page et al. (2008). Both studies presented *C. caementarius* nested within *Macrobrachium*. Further, in a molecular phylogenetic study of *Macrobrachium*, Pileggi and Mantelatto (2010) recovered the genus as a monophyletic clade if representatives of *Cryphiops* (*C. brasiliensis* and *C. caementarius*) were included. In addition, these authors pointed out two important aspects: first, the phylogenetic positioning regarding the type of larval development presented by both species of *Cryphiops* and, second, that the character used to separate both genera, the presence of the hepatic spine, is indeed subjective and should be reconsidered in a future revision.

Pereira (1997), using a cladistic analysis based on morphologic characters, stated that phylogenetic studies would be necessary to promote an internal rearrangement of the subgroups of Palaemonidae, because many of these proved to be paraphyletic (e.g., *Palaemonetes*, *Palaemon*, *Macrobrachium*, *Cryphiops*, *Bithynops*). Only recently some of these genera have been studied and undergone taxonomic changes, as was the case of *Palaemonetes* Heller, 1869 and *Palaemon* Weber, 1795 (see De Grave and Ashelby 2013 and Carvalho et al. 2017, 2020 for literature and details).

Thus, considering that the taxonomic status of this group is not yet fully resolved, and that no systematic rearrangement has been proposed, we used a multigene phylogenetic approach to assess the relationships among all species of *Cryphiops* in comparison with species of *Macrobrachium* from America, Africa, and the Indo-Pacific and, along with a taxonomic revision, we propose a rearrangement in the systematics of the group.

Materials and methods

Sample collection

Fresh specimens (*Cryphiops* and additional taxa) for molecular analysis were obtained from field collections in rivers and estuaries in Brazil, Chile, Venezuela, Costa Rica, and Mexico (Table 1). The new individuals were preserved in 75–90% ethanol. Additional material was obtained through donations, visits, or loans from various crustacean collections around the world (Table 1).

Repositories

Material examined is deposited in the Crustacean Collection of the Department of Biology (CCDB), Faculty of Philosophy, Sciences and Letters at Ribeirão Preto

Table 1. *Cryphiops* and *Macrobrachium* species used for molecular techniques. CCDB: Collection of Crustaceans, Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Brazil; CIB: Crustacean collection at Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mexico; CNCR: National Crustacean Collection, UNAM, México; GU: Griffith University, Australia; JC: Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah. USA; INPA: Instituto de Pesquisa da Amazônia, Brazil; MPEG: Museu Paraense Emilio Goeldi, Brazil; MZUCR: Museum of Zoology, University of Costa Rica, Costa Rica; UFRGS: Collection of Crustaceans, Federal University of Rio Grande do Sul, Brazil; OUMNHC-ZC: Zoological Collections, Oxford Museum of Natural History, UK; RMNH: Naturalis Biodiversity Center (former Rijksmuseum van Natuurlijke Historie), The Netherlands. Other abbreviations: AM, state of Amazonas; AP, state of Amapá; DF, Distrito Federal; PA, state of Pará; PR, state of Paraná; SP, state of São Paulo; USA, United States of America.

Species	Locality	Collection code and catalogue #	GenBank #			
			(16S)	(COI)	(18S)	(H3)
<i>Cryphiops</i> new status						
<i>M. alevillalobosi</i> nom. nov., comb. nov.	Ocosingo, Chiapas, Mexico	CNCR 3650b	–	–	MZ413044	–
	Ocosingo, Chiapas, Mexico	CNCR 5760	JF491348	–	–	–
<i>M. caementarius</i> (Molina, 1782) comb. nov.	Coquimbo, Chile	CCDB 1870	HM352453	HM352495	KM101490	–
	Chile	JC 1219	DQ079711	–	DQ079747	DQ079672
<i>M. candango</i> nom. nov., comb. nov.	Brasília-DF, Brazil	CCDB 2195	HM352434	–	–	–
	Brasília-DF, Brazil	CCDB 5894	MZ413047	–	MZ413038	MZ403772
	Brasília-DF, Brazil	CCDB 5897	MZ413048	–	MZ413039	MZ403773
<i>M. luscus</i> (Holthuis, 1973) comb. nov.	La Trinitaria, Chiapas, Mexico	CNCR 5759	JF491343	MZ423177	MZ413040	–
<i>M. persicax</i> (Holthuis, 1977) comb. nov.	La Trinitaria, Chiapas, Mexico	CNCR 7898	MZ413049	MZ423178	MZ413041	–
	La Trinitaria, Mexico	CNCR 25392	MZ413050	MZ423179	MZ413042	MZ403775
<i>M. valdonii</i> nom. nov., comb. nov.	La Trinitaria, Chiapas, Mexico	CNCR 25108	–	MZ423180	MZ413043	MZ403776
Comparative species						
<i>M. acanthurus</i>	Guaraqueçaba-PR, Brazil	CCDB 2546	HM352444	KM101538	KM101493	–
	Bocas del Toro, Panama	CCDB 3538	KM101467	KM101541	KM101496	–
<i>M. amazonicum</i>	Santana-AP, Brazil	CCDB 1965	HM352441	HM352486	KM101497	–
	Panama	CNCR 5151	KM101468	KM101542	KM101498	–
<i>M. americanum</i>	Puntarenas, Costa Rica	CCDB 2883	JQ805797	JQ805899	JQ805843	JQ805861
	Puntarenas, Costa Rica	MZUCR 3292-03	KM101473	KM101547	KM101504	–
	Isla Violines, Costa Rica	MZUCR 2970-01	KM101472	KM101546	KM101503	–
<i>M. australe</i>	Hualien, Taiwan	Not informed	DQ194904	AB235245	–	–
	Not informed	Not informed	–	–	GU204997	–
	Not informed	GU 363	–	–	–	FN995544
<i>M. borellii</i>	Buenos Aires, Argentina	UFRGS 3669	HM352426	HM352480	KM101505	–
<i>M. brasiliense</i>	Serra Azul-SP, Brazil	CCDB 2135	HM352429	HM352481	KM101506	–
<i>M. carcinus</i>	Santana-AP, Brazil	CCDB 2122	HM352448	HM352490	KM101507	–
	Isla Margarita, Venezuela	CCDB 2123	HM352450	HM352492	KM101508	–
	Cahuita, Costa Rica	CCDB 2145	HM352452	KM101548	KM101510	–
<i>M. crenulatum</i>	Isla Margarita, Venezuela	CCDB 2124	HM352463	HM352498	KM101512	JQ805865
	Parque Veragua, Costa Rica	CCDB 4874	KM101475	KM101550	KM101513	–
<i>M. digueti</i>	Puntarenas, Costa Rica	MZUCR 3292-01	KM101476	KM101551	KM101514	–
	Oaxaca, Mexico	CNCR 24811	JQ805808	JQ805905	JQ805849	JQ805870
<i>M. dux</i>	Limón, Costa Rica	CCDB 2882	JQ805806	JQ805903	JQ805847	JQ805868
	Warri, Nigeria	Not informed	KJ463388	KC688273	–	–
<i>M. equidens</i>	Pará, Brazil (introduced)	MPEG 0809	MZ413051	MZ423181	–	–
	Not informed	Not informed	–	–	GU205009	–
	Khatib Bongsu, Singapore	Not informed	–	–	–	FM958095
<i>M. faustinum</i>	Jamaica	RMNHD 17613	JQ805809	JQ805907	JQ805850	JQ805871
<i>M. ferreirai</i>	Manaus-AM, Brazil	CCDB 2125	HM352427	HM352483	–	–
<i>M. gracilirostre</i>	Hualien, Taiwan	Not informed	DQ194924	AB235258	–	–
	Not informed	Not informed	–	–	GU205013	–
<i>M. gracilirostre</i>	Manado, Indonesia	Not informed	–	–	–	FM958099
<i>M. hancocki</i>	Puntarenas, Costa Rica	CCDB3092	JQ805814	JQ805912	JQ805851	JQ805874
	Panama	RMNHD 8810	JQ805817	JQ805915	JQ805852	JQ805876

Species	Locality	Collection code and catalogue #	GenBank #			
			(16S)	(COI)	(18S)	(H3)
<i>M. heterochirus</i>	Ilha de São Sebastião-SP, Brazil	CCDB 2137	HM352454	HM352494	KM101515	–
	Cahuita, Costa Rica	CCDB 2899	KM101477	KM101552	KM101516	–
	Parque Veragua, Costa Rica	CCDB 4875	KM101478	KM101553	KM101517	–
<i>M. hobbsi</i>	Oaxaca, Mexico	CIB 1168.5	–	MH253251	–	–
	Huatabampo, Mexico	CNCR 2239	KF383306	–	–	–
<i>M. idae</i>	Khanom, Thailand	Not informed	DQ194930	AB235262	–	–
	Not informed	Not informed	–	–	GU205019	–
	Tioman, Malaysia	Not informed	–	–	–	FM958103
<i>M. iheringi</i>	Brasília-DF, Brazil	CCDB 5899	MZ413052	MZ423182	MZ413045	–
<i>M. inpa</i>	Manaus-AM, Brazil	CCDB 2127	HM352433	–	–	–
<i>M. jelskii</i>	Pereira Barreto-SP, Brazil	CCDB 2129	HM352437	HM352484	KM101519	–
<i>M. lar</i>	French Polynesian	GU 992	EF588316	–	–	EU249462
	Ryukyus, Japan	Not informed	–	AB235269	–	–
	Not informed	Not informed	–	–	KP215302	–
<i>M. latidactylus</i>	Tioman, Malaysia	Not informed	DQ194944	AB235272	–	–
	Not informed	Not informed	–	–	GU205024	–
	Tioman, Malaysia	Not informed	–	–	–	FM958109
<i>M. latimanus</i>	Cebu, Philippines	Not informed	DQ194937	AB235276	–	–
	Not informed	Not informed	–	–	GU205026	–
	Ciawi Tali, Indonesia	Not informed	–	–	–	FM958110
<i>M. nattereri</i>	Lago Tupé-AM, Brazil	CCDB 2130	HM352428	–	–	–
<i>M. occidentale</i>	Oaxaca, Mexico	CNCR 24838	KM101481	KM101556	KM101521	–
	Puntarenas, Costa Rica	MZUCR 3292-02	KM101482	KM101557	KM101522	–
<i>M. olfersii</i>	Ilha de São Sebastião-SP, Brazil	CCDB 2435	HM352459	HM352496	KM101523	–
	Isla Margarita, Venezuela	CCDB 2446	HM352460	KM101559	KM101525	JQ805886
	Parque Veragua, Costa Rica	CCDB 4873	KM101483	KM101560	KM101526	–
<i>M. obione</i>	Louisiana, USA	CCDB 4304	MZ413053	MZ423183	MZ413046	MZ403774
<i>M. panamense</i>	Guanacaste, Costa Rica	MZUCR 2971-01	KM101484	KM101561	KM101527	–
	Guanacaste, Costa Rica	MZUCR 3291-01	KM101486	KM101563	KM101529	–
<i>M. potiuna</i>	Eldorado-SP, Brazil	CCDB 2131	HM352438	KM101564	KM101530	–
	Cananéia-SP, Brazil	CCDB 3652	JX466936	–	KP179011	KP179067
<i>M. rosenbergii</i>	Jaboticabal-SP, Brazil (Culture)	CCDB 2139	HM352465	–	KM101531	–
	Kaohsiung Co., Taiwan	Not informed	–	AB235295	–	–
	Irian Jaya, Indonesia	Not informed	–	–	–	FM958123
<i>M. surinamicum</i>	Icangui-PA, Brazil	INPA CR 183	HM352446	KM101565	KM101532	–
<i>M. tenellum</i>	Oaxaca, México	CNCR 24831	KM101487	KM101566	KM101533	–
	Guanacaste, Costa Rica	MZUCR 3290-01	KM101489	KM101568	KM101535	–
<i>M. totonacum</i>	Oaxaca, Mexico	CNCR 19915	KF383311	–	–	–
<i>M. tuxtlense</i>	Veracruz, Mexico	CNCR 13174	KF383312	–	–	–
<i>M. vollenhoveni</i>	Badagry, Nigeria	Not informed	KJ463387	KC688272	–	–
<i>Palaemon argentinus</i>	Mar del Plata, Argentina	CCDB 3312	KP178997	–	KP179016	KP179115
	Mar del Plata, Argentina	CCDB 2011	HM352425	–	KM101536	–
<i>Palaemon modestus</i>	Kalkan, Kazakhstan	OUMNH-ZC 2012-01-0068	KP178986	–	KP179040	KP179099
	Jiangxi, China	Not informed	–	AB235307	–	–
<i>Palaemon orientis</i>	Kisarazu, Japan	OUMNH-ZC 2011-11-0028	KP178987	–	KP179044	KP179100
	Japan	Not informed	–	AB235306	–	–

(FFCLRP), University of São Paulo (USP), Brazil; National Crustacean Collection (CNCR), of the Institute of Biology, Universidad Nacional Autónoma de Mexico (UNAM), Mexico; and Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Brazil.

Molecular data

The molecular analysis was based on partial fragments of the 16S rDNA, COI mtDNA, 18S nDNA, and H3 nDNA genes, which have been effective in solving different levels of relationships among decapod species (Schubart et al. 2000; Porter et al. 2005; Pileggi and Mantelatto 2010; Mantelatto et al. 2011; Vergamini et al. 2011; Carvalho et al. 2013, 2017; Rossi and Mantelatto 2013; Álvarez et al. 2020; Robles et al. 2020).

DNA extraction, amplification and sequencing protocols followed Pileggi and Mantelatto (2010). Total genomic DNA was extracted from muscle tissue of the walking legs, chelipeds, or the abdomen. The amplification by polymerase chain reaction (PCR) was conducted with the following primers: 16Sar and 16Sbr (Palumbi et al. 1991) for the 16S mitochondrial gene; COI-a and COI-f (Palumbi and Benzie 1991) for the COI mitochondrial gene; 18Sai and 18Sb3.0 (Whiting et al. 1997) for the 18S nuclear gene; H3ar and H3af (Colgan et al. 1998) for the histone (H3) nuclear gene. PCR products were sequenced with the ABI Big Dye Terminator Mix (Applied Biosystems, Carlsbad, CA) in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems automated sequencer) following Applied Biosystems protocols. All sequences were confirmed by sequencing both strands. Genetic vouchers generated were deposited in the CCDB and CNCR under the catalogue numbers listed in Table 1.

Molecular analyses

Editing of sequences and *denovo* assembling were carried out with the computational program Geneious v2020.2.4 (Kearse et al. 2012). COI consensus sequences (protein-coding sequence) were checked for pseudogenes by translating them and checking for indels and stop codons (Song et al. 2008). We downloaded additional *Macrobrachium* and *Palaemon* (as outgroup) species sequences available from GenBank (*Cryphiops caementarius* – DQ079711, DQ079747, DQ079672; *Macrobrachium australe* Guérin-Meneville – DQ194904, AB235245, GU204997, FN995544; *M. dux* (Lenz) – KJ463388, KC688273; *M. equidens* (Dana) – GU205009, FM958095; *M. gracilirostre* (Miers) – DQ194924, AB235258, GU205013, FM958099; *M. hobbsi* (Villalobos Hiriart and Nates Rodriguez) – MH253251; *M. idae* (Heller) – DQ194930, AB235262, GU205019, FM958103; *M. lar* (Fabricius) – EF588316, AB235269, KP215302, EU249462; *M. latidactylus* (Thallwitz) – DQ194944, AB235272, GU205024, FM958109; *M. latimanus* (von Martens) – DQ194937, AB235276, GU205026, FM958110; *M. rosenbergii* (De Man) – AB235295, FM958123; *M. vollenhovenii* (Herklots) – KJ463387, KC688272; *Palaemon modestus* (Heller) – AB235307; *P. orientis* (Holthuis) – AB235306) (Table 1). Sequences were aligned using MAFFT v.7 (Katoh and Standley 2013) with default parameters, resulting in final alignments of ~540 base pairs (bp) for 16S rDNA, ~570 bp for COI mtDNA, ~550 bp for 18S nDNA and ~330 bp for H3 nDNA. The Maximum Likelihood (ML) approach was conducted in IQ-TREE (Nguyen et al. 2015) performed in the online platform Cyberinfrastructure for Phylogenetic Research (CIPRES) (Miller et al. 2010). The evolutionary model that best fitted the data (best fit model 16S: TPM3+F+I+G4; COI:

TN+F+I+G4; 18S: TIM2e+I+G4; H3: TNe+I+G4) was determined by IQ-TREE, using Bayesian Information Criterion (BIC) (Luo et al. 2010). Branch support was assessed by ultrafast bootstrap with 1,000 replicates. Genetic distances were computed for each gene using Kimura-2-parameter (K2P) in MEGA-X (Kumar et al. 2018).

In total, 88 specimens were used for the analyses, eleven belonging to *Cryphiops*, 71 to *Macrobrachium* and six to *Palaemon*, to obtain a robust representation of the ingroup and a consistent rooting of the phylogeny (Table 1). The selection of species composing both internal and external groups was based on the phylogenies proposed from morphological (Pereira 1997) and molecular characters (Murphy and Austin 2005; Liu et al. 2007; Pileggi and Mantelatto 2010). With this careful selection we covered all possible close and related species to *Cryphiops* reported previously, either by morphology and/or molecular affinities.

Taxonomic revision

The species identification was carried by us based on diagnostic morphological features in accordance with the literature (Holthuis 1952a, 1993; Gomes Corrêa 1973; Villalobos Hiriart et al. 1989; Baldari et al. 2010). We did not list all the synonyms for *Cryphiops* and *Macrobrachium* since a complete, detailed record can be found in Holthuis (1950, 1993) and De Grave and Fransen (2011). A non-exhaustive synonyms list containing post-1950 citations focused mainly on taxonomic and faunistic studies is provided for all species and it is partially based on the “Carideorum Catalogus L.B. Holthuis”, an extensive reference catalogue of scientific names of shrimps gathered by the late L.B. Holthuis during his 68 years of studying Crustacea (Fransen et al. 2010), which was digitized and kindly and unpretentiously made available by C.H.J.M. Fransen to the community of carcinologists in digital format on 7 April 2020. For pre-1952 citations regarding *C. caementarius*, see Holthuis (1952a, b).

The morphological data considered in this review for the comparative analysis of species were as follows. Measurements: total length (tl), from the anterior portion of the rostrum to the posterior portion of telson; and carapace length (cl), from the posterior margin of the orbit to the posterior margin of the carapace. Rostrum: shape, length in relation to scaphocerite, number of teeth and their distribution on the upper and lower margins. Orbit: shape of the lower margin. Scaphocerite: size and shape. Epistome: shape and arrangement. Carapace: presence of spinules, size and arrangement of hepatic and antennal spines. Pereiopods: size and shape of the first pereiopods (P1); size, shape, and proportion of the articles of the second pereiopods (P2); size and proportion of the articles of the third, fourth and fifth pereiopods (P3 to P5). Thoracic sternum: presence and shape of the median process (T4). Abdomen: surface roughness, shape of the pleura of the fifth somite. Pleopods: ratio appendix masculina/appendix interna of the second pair (PL2). Pre-anal keel: presence and shape in the inter-uropodal sclerite. Uropods: presence of external spines. Telson: general shape, shape of the posterior margin, presence, and distribution of dorsal spines, positioning of the posterior spines in relation to the posterior margin. Other aspects such as the size of males and ovigerous females, life cycle, color, distribution, systematic position, type locality and general considerations were also considered.

Results

Molecular approach

The concatenated phylogenetic analysis included 45 species of Palaemoninae: six belonging to *Cryphiops*, 36 to *Macrobrachium*, and three to *Palaemon*. A total of 35 new DNA sequences was generated in this study: seven 16S and seven COI mitochondrial sequences, ten 18S, and eleven H3 nuclear sequences. The final alignment of the four markers totaled 1,982 bp.

The topology obtained by ML with the four concatenated genes (Fig. 1) showed a clear positioning of the genus *Cryphiops* nested among the species of *Macrobrachium* as was also found in previous studies (Pereira 1997; Porter et al. 2005; Page et al. 2008; Pileggi and Mantelatto 2010). Genetic distances found among species of *Macrobrachium* and *Cryphiops* corroborate this inclusion (Suppl. material 1). The levels of divergence ranged from 2.8% to 18.8% (*Cryphiops* spp., *Macrobrachium* spp.) and from 0.23% to 15.1% among *Cryphiops*, for 16S; from 18.2% to 27.3% (*Cryphiops* spp., *Macrobrachium* spp.) and from 0.73% to 26.0% among *Cryphiops*, for COI; from 0.4% to 9.0% (*Cryphiops* spp., *Macrobrachium* spp.) and from 0.2% to 4.7% among *Cryphiops*, for 18S; and from 2.2% to 12.1% (*Cryphiops* spp., *Macrobrachium* spp.) and from 7% to 10.7% among *Cryphiops*, for H3.

The estuarine *C. caementarius*, which has extended larval development (ELD), is nested among the species of *Macrobrachium* that have the same type of larval development (Fig. 1). Similarly, the species of *Cryphiops* from inland waters (*C. brasiliensis*, *C. luscus*, *C. perspicax*, *C. sbordonii*, and *C. villalobosi*) with abbreviated larval development (ALD) are positioned in clades with species of *Macrobrachium* that have ALD (Fig. 1). Interestingly, the recovered phylogeny follows the previous subdivision proposed by Villalobos Hiriart et al. (1989) into two subgenera based on morphological and life-cycle characters, i.e., *Cryphiops* as ELD and *Bithynops* as ALD groups. The species of *Palaemon* showed a stable position in an external branch.

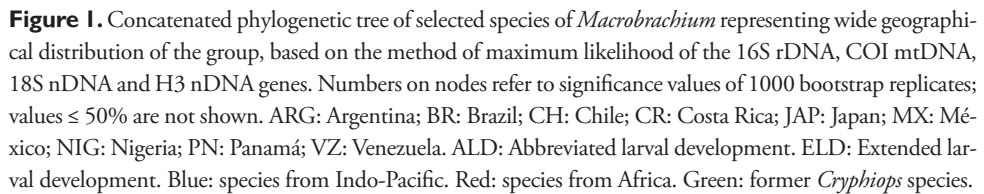
Systematic account

The following new taxonomic arrangement, including diagnoses of all six species, is provided.

Family Palaemonidae Rafinesque, 1815

Genus *Macrobrachium* Spence Bate, 1868

Cryphiops Dana, 1852: 18 [type species: *Cryphiops spinulosomanus* Dana, 1852] [based on Art. 23.9.3, ICZN 1999]. – Holthuis 1952a: 136. – Holthuis 1955: 51. – Maccagno and Cucchiari 1957: 206 (in list), 213. – Holthuis 1993: 105. – Jayachandran 2001: 14 (in key), 24. – Álvarez et al. 2011: 257.



Macrobrachium Spence Bate, 1868: 363 [type species: *Macrobrachium americanum* Spence Bate, 1868]. – Holthuis 1952a: 10. – Holthuis 1955: 53. – Maccagno and Cucchiari 1957: 206 (in list), 214. – Hobbs Jr. et al. 1977: 50. – Rodríguez 1980: 113. – Holthuis 1993: 109. – Chace Jr, Bruce 1993: 8. – Jayachandran 2001: 14 (in key), 49. – Valencia and Campos 2007: 3. – Álvarez et al. 2011: 260. – Dueñas et al. 2012: 69. – Campos 2014: 56.

Eupalaemon Ortmann, 1891: 696, 697 [type species: *Palaemon acanthurus* Wiegmann, 1836].

Parapalaemon Ortmann, 1891: 696, 731 [type species: *Palaemon dolichodactylus* Hilgendorf, 1879].

Macroterochair Stebbing, 1908: 39 [type species: *Palaemon lepidactylus* Hilgendorf, 1879].

Bithynops Holthuis, 1973: 135 [type species: *Bithynops luscus* Holthuis, 1973]. – Holthuis 1993: 102. – Hobbs Jr. et al. 1977: 46. – Jayachandran 2001: 14.

Cryphiops (*Cryphiops*). – Villalobos Hiriart et al. 1989: 161.

Cryphiops (*Bithynops*). – Villalobos Hiriart et al. 1989: 163. – Álvarez et al. 2011: 258.

Type species. *Macrobrachium americanum* Spence Bate, 1868, subsequent designation by Fowler (1912).

Diagnosis (modified from Holthuis 1950, 1952a). Body compressed, generally robust, sometimes slender. Rostrum well developed, compressed, toothed, size varying from shorter to longer than distal margin of scaphocerite. Carapace with anterolateral portion smooth or bearing numerous small spinules. Carapace armed with antennal spine; hepatic spines present in most species, branchiostegal groove present, distinct. Mandible with 3-segmented palp. All maxillipeds with well-developed exopods. Pleurobranchs on third maxilliped and all pereopods. P1 slender. P2 more robust than other pereopods, usually longer than entire body in adult males, left and right legs often equal in size and shape or markedly different in several species. P3–P5 with dactylus simple. P5 with propodus bearing numerous transverse rows of setae in distal part of posterior margin. PL1 with endopod much smaller than exopod, endopod of male without appendix interna. Pleon with pleurae smooth in most species or with small spinules. Uropods overreaching telson; exopod with distolateral spine, endopod unarmed. Telson elongate, subtriangular, narrowing posteriorly, with two pairs of dorsal spines, anterior pair placed in middle, posterior pair usually placed midway between anterior pair and posterior margin; posterior margin ending in sharp median point, flanked by two pairs of spines, outer pair usually shorter than inner one, inner pair overreaching apex of telson in most species. See detailed description in Holthuis (1950).

Remarks. See Discussion.

***Macrobrachium alevillalobosi* nom. nov., comb. nov.**

Cryphiops (*Bithynops*) *villalobosi* Villalobos Hiriart, Nates Rodríguez and Cantú Díaz Barriga, 1989: 166, figs 1–5, 7b, d, 8c. – Villalobos-Hiriart et al. 1993: 281, table 5 (list). – De Grave and Fransen 2011: 316 (catalog). – Álvarez et al. 2011: 258, fig. 2a. – Quiroz-Martínez et al. 2014: table S1 (list). – Alvarez and Villalobos 2016: 250, table 8.1 (list).

Cryphiops villalobosi. – Baldari et al. 2010: 48, fig. 1 (map), 52, table 1. – Botello and Álvarez 2013: 776, table 1 (list). – Mantelatto et al. 2020: 916 (key).

Material examined. Holotype: MEXICO – **Chiapas** • male, tl 51.8 mm; Municipality of Ocosingo, km 140 carretera Palenque-Ocosingo, ca. 5 km NW of the town of Ocosingo, Arroyo La Laja, 24 Jan. 1985, J.L. Villalobos, J.C. Nates, A. Cantú leg.; CNCR 3650. **Paratypes:** 1 female, tl 42.7 mm, allotype; same data as for holotype; CNCR 3650 • 16 males, tl 44.3–57.0 mm, 7 females, 26.2 to 46.0 mm, 2 ovigerous females, 38.1, 42.7 mm; same date as for holotype; CNCR 3650a.

Additional material examined. MEXICO – **Chiapas** • 9 males, tl 25.0–45.8 mm, 30 females, 36.8–39.2 mm; Municipality of Ocosingo, km 140 carretera Palenque-Ocosingo, ca. 5 km NW of the town of Ocosingo, Arroyo La Laja; 07 Aug 1983; J.L. Villalobos, J.C. Nates, A. Cantú leg.; CNCR 2940 • 16 males, tl 24.3–54.5 mm, 23 females, tl 17.1–27.4 mm; Municipality of Yajalón, carretera Palenque-Ocosingo, Arroyo Yajalón; 07 Aug. 1983; J.L. Villalobos, J.C. Nates, A. Cantú; CNCR 2941.

Description. Rostrum. Short, straight, reaching slightly beyond first third of third article of antennular peduncle; upper margin with 6–9 regularly spaced teeth, first one behind posterior edge of orbit; lower margin with 1–3 teeth.

Cephalon. Scaphocerite nearly $2.6 \times$ as long as wide, outer margin straight.

Carapace. Smooth; antennal spine small, slightly overreaching lower portion of orbit; hepatic spine absent. Lower orbital angle obtuse, moderately pronounced.

Pereiopods. P1: slender, reaching with distal third of carpus beyond scaphocerite; carpus slightly longer than merus; chelae 0.68 length of carpus. P2: moderately robust, with small spines, equal in form and size, reaching with distal third of merus beyond scaphocerite; ischium 0.75 length of merus; merus as long as carpus; carpus as long as palm, with slight basal constriction; propodus $2.5 \times$ as long as dactylus, and $1.6 \times$ as long as carpus; palm compressed, nearly $5 \times$ as long as high; fingers 0.62 length of palm, with numerous spinules, not gapping, tips crossing, cutting edge with 3–6 teeth on proximal third in both fingers. P3–P5 with all joints covered with rows of small spinules. P3 reaching with entire dactylus beyond scaphocerite, propodus $2.5 \times$ as long as dactylus, propodus nearly $2 \times$ as long as carpus, propodus slightly longer than merus. P4 reaching with entire dactylus beyond scaphocerite, propodus $3 \times$ as long as dactylus, nearly $2 \times$ as long as carpus, propodus slightly longer than merus. P5 reaching with half-length of dactylus end of scaphocerite, propodus $3 \times$ as long as dactylus, propodus nearly $2 \times$ as long as carpus, propodus slightly longer than merus.

Pleon. Smooth; somite 5 with posteroventral angle of pleuron acute; abdominal somite merely $2 \times$ as long as somite 5. Inter-uropodal sclerite without keel-shaped pre-anal carinae.

Pleopods. PL2 with appendix masculina less than $2 \times$ length of appendix interna.

Uropods. Exopodite with mobile spines as long as spiniform projection of outer margin.

Telson. Broad, smooth, slightly longer than abdominal somite 6, bearing two pairs of dorsal spinules close to posterior margin. Posterior margin ending in moderately acute triangular point; two pairs of posterior spinules with several plumose setae, inner pair overreaching distal margin of telson.

Etymology. Villalobos Hiriart et al. (1989) dedicated this species to Dr. Alejandro Villalobos Figueroa, eminent Mexican carcinologist and founder of the CNCR. We maintain this homage by just adding the first part of his name to the specific epithet.

Size. See in material examined.

Color. Body translucent with orange punctuations.

Type locality. México, Chiapas, Municipality of Ocosingo, Arroyo La Laja, km 140 carretera Palenque-Ocosingo, ca. 5 km NW of the town of Ocosingo.

Distribution. Mexico, Chiapas, in the Valle de Ocosingo, Río La Virgen, Arroyos La Laja, Maravilla, Pasilá, and Yajalón (Villalobos Hiriart et al. 1989).

Life cycle. Exclusive of inland waters, therefore independent of brackish waters to complete its life cycle. The eggs are few and large: 1.3–2.4 mm (Villalobos Hiriart et al. 1989). Its larval development is not known but given the characteristics of the eggs, it should be abbreviated, following the same pattern of congeners inhabiting continental waters (Magalhães and Walker 1988; Pereira and García 1995).

Remarks. The name *Macrobrachium villalobosi* was used by Hobbs Jr. (1973) for a new species from Mexico. Villalobos et al. (1989) used the same name for a new species of *Cryphiops* (*Bithynops*) also from Mexico. Since the synonymization of both genera makes these specific names secondary homonyms, *Macrobrachium alevillalobosi* is proposed as a replacement name for *Cryphiops* (*Bithynops*) *villalobosi* Villalobos Hiriart, Nates Rodríguez & Cantú Díaz Barriga, 1989.

Macrobrachium alevillalobosi nom. nov., comb. nov. differs from *M. candango* nom. nov. and *M. perspicax* comb. nov. mainly in the form, size, and proportion of the articles of the second pereopod (Table 2). The chelipeds are long and similar in size and shape, overreaching the scaphocerite with distal third of the merus; the ischium is shorter than merus; the palm is long and cylindrical, almost five times as long as high, and the dactylus is 0.62 times the length of the palm. Finally, *M. alevillalobosi* nom. nov., comb. nov. is the only species of this group in which the appendix masculina is almost as long as the endopod of the second pleopod.

***Macrobrachium caementarius* (Molina, 1782), comb. nov.**

Cancer caementarius Molina, 1782: 208.

Palaemon Gaudichaudii H. Milne Edwards, 1837 in H. Milne Edwards 1834–1840: 400.

Cryphiops spinuloso-manus Dana, 1852: 26.

Bithynis longimana Philippi, 1860: 164.

Macrobrachium africanum Spence Bate, 1868: 366, pl. 31, fig. 3.

Cryphiops caementarius. – Holthuis 1952a: 137, pls. 33–35. – Holthuis 1952b: 74, fig. 17. – Holthuis 1955: 52, fig. 28. – Bahamonde 1957: 7. – Hartmann 1957: 117. – Hartmann 1958: 15, figs 1–5. – Bahamonde 1962: 7. – Del Solar et al. 1970: 19 (catalog). – Chirichigno Fonseca 1970: 16 (list), fig. 28. – Boschi 1977: 7. – Manning and Hobbs Jr. 1977: 158 (list). – Retamal 1977: 4 (table), 5, fig. 4. – Viacava et al. 1978: 161. – Holthuis 1980: 81 (list). – Méndez 1981: 14

(list), 73 (list), 75 (key), pl. 33 figs 246, 247. – Retamal 1981: 14 (list), fig. 35. – Rodríguez 1981: 47 (list). – Pérez Farfante 1982: 375. – Pretzmann 1983: 316. – Wicksten and Hendrickx 1992: 7 (list). – Holthuis 1993: 106, fig. 93. – Pereira 1997: 21, fig. 18C, 47, table 6 (list). – Bahamonde et al. 1998: 93. – Kameya et al. 1998: 90 (list). – Meléndez and Maldonado 1999: 125, 130. – Jayachandran 2001: 24. – Retamal and Jara 2002: 195, 204 (list). – Zuñiga Romero 2002: 21, 1 fig. – Wicksten and Hendrickx 2003: 60 (list). – Jara et al. 2006: 42, table I, 43, table II, 46, table IV. – Meruane et al. 2006: 285, fig. 1. – Báez and López 2010: 244. – Retamal and Moyano 2010: 307, table 1. – Pileggi and Mantelatto 2010: 197, table 1. – Ríos-Escalante et al. 2013: 850, table 1. – Rossi and Mantelatto 2013: 3, table 1 (list). – Morales and Meruane 2013: 1441, figs 1, 3–5. – Moscoso 2014: 12 (list), 44. – De Grave et al. 2015a: 5, table 1. – Zacarías Ríos and Yépez Pinillos 2015: 398, fig. 1. – Wasiw G., Yépez P. 2015: 166, fig. 2D. – Mantelatto et al. 2020: 915 (key). – Velásquez et al. 2020: 1062.

Cryphiops spinolosomanus. – Maccagno and Cucchiari 1957: 213.

Cryphiops (Cryphiops) caementarius. – Villalobos Hiriart et al. 1989: 162. – De Grave and Fransen 2011: 316 (catalog), fig. 36. – Ashelby et al. 2012: 295, table 1 (list).

Material examined. CHILE – **Coquimbo** • 2 males, cl 28.2, cl 36.4 mm; río Limari, Jul. 2006; C. Gaymer leg.; CCDB 1870 • 2 males, cl 30.3, cl 86.5 mm; Limari, río Puente; 19 Oct. 2007; L.G. Pileggi, E.C. Mossolin leg.; CCDB 2146 • 2 males, cl 5.4, cl 5.7 mm, 4 females, cl 5.6 to 10.1 mm, 2 ovigerous females, cl 14.9, cl 16.8 mm, 8 juveniles, cl 3.4 to 4.8 mm; La Serena, Playa el Faro, Avenida de Mar; 18 Oct. 2007; F.L. Mantelatto, L.G. Pileggi, E.C. Mossolin; CCDB 2327.

Description. Rostrum. Straight, short, nearly reaching first article of antennular peduncle; upper margin with 6–8 teeth, regularly spaced, one and/or two behind posterior margin of orbit; lower margin with 0–4 teeth.

Cephalon. Scaphocerite $2 \times$ as long as wide; outer margin convex proximally.

Carapace. Smooth, with strong, acute antennal spine; hepatic spine absent. Lower orbital angle obtuse, moderately pronounced.

Pereiopods. P1 slender, reaching with most of carpus beyond scaphocerite; fingers slightly longer than palm; carpus slightly shorter than chelae; ischium and merus distinctly spinulated; carpus and chelae smooth. P2 strong, with many spines, strong heterochely; largest cheliped reaching with half-length of merus beyond scaphocerite; ischium larger than half-length of merus; merus longer than carpus; carpus short, slightly shorter than half length of palm, with strong basal constriction; propodus $2.1 \times$ as long as dactylus, $3.3 \times$ as long as carpus; palm slightly inflated, more than $2.3 \times$ as long as high; fingers shorter than palm, with numerous small spinules, cutting edges with 4–7 denticles of equal size. P3–P5 smooth, except for sparse setae and spinules along lower margin of propodus; propodus nearly $2 \times$ as long as carpus; propodus slightly shorter than merus; P3 reaching with half-length of dactylus beyond scaphocerite, propodus $2 \times$ as long as dactylus; P4 reaching with tip of dactylus end of scaphocerite, propodus $1.5 \times$ as long

Table 2. Morphological comparison of key characters for the species previously included in the genus *Cryphiops* Dana, 1852.

	<i>M. alenillalobos</i> nom. nov., comb. nov.	<i>M. caementarius</i> (Molina, 1782), comb. nov.	<i>M. candango</i> nom. nov., comb. nov.	<i>M. luscus</i> (Holthuis, 1973), comb. nov.	<i>M. perspicax</i> (Holthuis, 1977), comb. nov.	<i>M. valdonii</i> nom. nov., comb. nov.
Rostrum	Reaching slightly beyond first third of ultimate article of antennular peduncle, and at level of distal fourth of scaphocerite. Upper margin with 6–9 teeth regularly spaced, first one behind of posterior edge of orbit; lower margin with 1–3 teeth	Reaching or slightly beyond first article of antennular peduncle, and at level of proximal third of scaphocerite. Upper margin with 6–8 teeth regularly spaced, first one or two behind of posterior edge of orbit; lower margin with 0–4 teeth	Reaching end of antennular peduncle, and little before the distal margin of scaphocerite. Upper margin convex over orbit, with 7 teeth, first and sometimes the second, slightly behind posterior edge of orbit; lower margin with 1 tooth	Reaching or slightly overreaching joint between second and third article of antennular peduncle, and at level of distal third of scaphocerite. Upper margin convex over orbit, with 5–8 teeth regularly spaced, first over or slightly behind posterior edge of orbit; lower margin with 0–1 tooth	Reaching joint between second and third articles of antennular peduncle, and at level of distal third of scaphocerite. Upper margin with 5–8 teeth regularly spaced, first one at level of orbit behind posterior edge of orbit; lower margin with 1 or 2, rarely 3 teeth	Almost reaching the third article of antennular peduncle, and before the distal border of scaphocerite. Upper margin with 8 teeth, lack teeth in postorbital position and on ventral margin
Eyes	Cornea normal and larger than the peduncle	Cornea normal and larger than the peduncle	Cornea normal and larger than the peduncle	Cornea reduced, smaller than the peduncle	Cornea normal and larger than the peduncle	Cornea with a small apical black point, smaller than the peduncle
Scaphocerite	2.6 × as long as wide	2 × as long as wide	2.5 × as long as wide	2.5 × as long as wide (Holthuis, 1973)	2.6 × as long as wide	2.4 × as long as wide
Lower orbital angle	Rounded, moderately pronounced	Rounded, pronounced, as long as antennal spine	Subacute, strongly pronounced	Obtuse, moderately pronounced	Subacute, moderately pronounced	Subacute, moderately pronounced
Antennal spine	In the middle of the lower orbital angle	Little below the lower orbital angle	Little below the lower orbital angle	Little below the lower orbital angle	Little below the lower orbital angle	Below the lower orbital angle
First male periepod	Reaching with distal third of carpus beyond scaphocerite	Reaching with the larger part of the carpus beyond scaphocerite	Reaching with almost half length of carpus beyond scaphocerite	Reaching with nearly entire chelae beyond scaphocerite	Reaching with nearly entire chelae or small part of carpus beyond scaphocerite	Reaching with the palm beyond scaphocerite
Second male periepod	Equal in form and size, reaching with distal third of merus beyond scaphocerite; ischium 0.7× length of merus; merus as long as carpus; carpus as long as palm, with slight basal constriction; propodus 2.5× as long as dactylus, and 1.6× as long as carpus; palm compressed, nearly 5× as long as high; fingers 0.62× length of palm	Different in form and size. Largest reaching with half of merus beyond scaphocerite; ischium, merus and carpus are covered with spinules, smaller than those of the chela; ischium more than 0.5× length of merus; merus longer than carpus; carpus slightly less 0.5× length of palm, with strong basal constriction; propodus 2.5× as long as dactylus, and 3.3× as long as carpus; palm compressed, nearly 2.3× as long as high; fingers 0.75× length of palm, little gaping	Similar in shape, different in size. Largest, reaching with distal portion of merus beyond scaphocerite; ischium nearly as long as carpus; merus as long as carpus; carpus as long as carpus; merus slightly shorter than palm; propodus 2.5× the length of dactylus, 2× as long as carpus; dactylus, 2× as long as carpus; palm inflated, less than 3× as long as high; fingers 0.71× the length of palm	Equal in form and size, reaching with proximal third of carpus beyond scaphocerite; ischium evidently shorter than merus; merus as long as carpus; carpus as long as palm, with moderate basal constriction; propodus 2× as long as dactylus, 2× as long as carpus; palm inflated, less than 3× as long as high; fingers little longer or as long as palm	Equal in form and size, reaching with proximal third of carpus beyond scaphocerite; ischium slightly shorter than merus; merus as long as carpus; carpus as long as palm, with a moderate basal constriction; propodus 2.2× as long as dactylus, 2× as long as carpus; palm inflated, 3× as long as high; fingers slightly shorter (0.8) than palm	Subequal in size, reaching with half of carpus beyond scaphocerite; ischium 0.9× merus; carpus 0.8× as long as merus and 0.85× palm length; propodus 1.5× as long as dactylus, 2.5× as long as carpus; palm 3.3× as long as high and 0.8× of dactylus length
Appendix masculina	Almost as long than endopod of second pleopod. Setae thick and short	Little longer than half endopod length of second pleopod. Setae thick and short	Shorter than endopod of second pleopod.	Shorter than endopod of second pleopod. Setae thick and short	Shorter than endopod of second pleopod. Setae slender and long	Shorter than endopod of second pleopod
Interuropodial sclerite	Without keel-shaped pre-anal carinae	With strong, keel-shaped pre-anal carinae	Without keel-shaped pre-anal carinae	Without keel-shaped pre-anal carinae	Without keel-shaped pre-anal carinae	Without keel-shaped pre-anal carinae

as dactylus; P5 reaching with tip of dactylus half-length of scaphocerite, propodus $1.5 \times$ as long as dactylus.

Pleon. Smooth. Somite 5 with posteroventral angle of pleuron acute; somite 6 slightly longer than somite 5. Inter-uropodal sclerite with strong, keel-shaped pre-anal carinae.

Pleopods. PL2 with appendix masculina $2 \times$ as long as appendix interna.

Uropods. Exopodite with mobile spines slightly longer than spiniform projection of outer margin.

Telson. Broad, smooth; $1.5 \times$ as long as abdominal somite 6, bearing 2 pairs of dorsal spinules, first pair located in middle of telson, second pair located $\frac{3}{4}$ of length of telson. Posterior margin rounded, ending in truncated tip, with several plumose setae and two pairs of posterior spinules, inner pair not reaching end of telson.

Size. See in material examined.

Color. Yellowish green with light brown spots dorsally. P2 with reddish joints and greenish blue color.

Type locality. Chile.

Distribution. Pacific coastal river basins from Perú and Chile (Holthuis 1952a, b; Jara et al. 2006; Morales and Meruane 2013).

Life cycle. Exclusive of coastal waters, dependent of brackish waters to complete its life cycle. The eggs are numerous and small: 0.43–0.62 mm of major diameter (Norambuena 1977; Yávar and Dupré 2007; Bazán et al. 2009). The larval development is long, with many free-swimming larval stages (Morales et al. 2006), following the usual pattern of coastal palaemonid species.

Remarks. For the heterochelia, the robustness and strong shape, as well as the ornamentation of the second pereopod, *M. caementarius* comb. nov. is comparable with *M. hancocki* Holthuis, 1950, and *M. occidentale* Holthuis, 1950 from the Pacific slope. The species is morphologically similar to *M. heterochirus* (Wiegmann, 1836) from the Atlantic slope, particularly concerning the shape of the rostrum, carapace, and telson.

***Macrobrachium candango* nom. nov., comb. nov.**

Cryphiops brasiliensis Gomes Corrêa, 1973: 169, figs 1–26. – Rodríguez 1981: 47 (in list). – Coelho and Ramos-Porto 1985: 407, 409 (table II). – Ramos-Porto and Coelho 1990: 98. – Pereira 1997: 21, fig. 18B, 47, table 6 (in list). – Ramos-Porto and Coelho 1999: 330 (catal.). – Melo 2003: 332, figs 180, 181. – Pileggi and Mantelatto 2010: 197 (table 1). – Mantelatto et al. 2016: 261 (in list). – Mantelatto et al. 2020: 915 (in key), fig. 23.102C.

Cryphiops (Bithynops) brasiliensis. – Villalobos Hiriart et al. 1989: 164, fig. 6b, d. – De Grave and Fransen 2011: 316 (catal.).

Material examined. Holotype: BRAZIL – Distrito Federal • male, cl 18.2 mm; Brasília, riacho da Granja do Ipê; 13 Sep. 1966; Emílio Varolli (SUDEPE) leg.; M.M.G. Corrêa det.; MNRJ 903.

Additional material examined. BRAZIL – **Distrito Federal** • 1 ovigerous female, cl 15.6 mm, [allotype]: Brasília, riacho da Granja do Ipê; 13 Sep. 1966; Emilio Varolli (SUDEPE) leg.; M.M.G. Corrêa det.; MNRJ 6464 • 1 male, cl 17.93 mm, 2 females, cl 15.3, cl 15.3 mm; Brasília, riacho da Granja do Ipê; 23 Feb. 1972; M.M.G. Corrêa leg.; MNRJ 2668 • 1 male, cl 14 mm, 2 females, cl 12.6, cl 13.8 mm; Brasília, córrego Taquara, Reserva Ecológica do IBGE (Instituto Brasileiro de Geografia e Estatística); 05 Aug. 2014, F.L. Mantelatto, L.G. Pileggi, F.L. Carvalho leg.; CCDB 5894 • 2 males, cl 22.9, cl 23.9 mm; Brasília, córrego Onça, upper Paraná River basin; 18 Aug. 1988; E.C. Lopes leg.; CCDB 5895 • 2 males, cl 21.7, cl 24.4 mm; Brasília, córrego Taquara, Onça, upper Paraná River basin; 18 Aug. 1988; E.C. Lopes leg.; CCDB 5896 • 2 females, cl 13.8, cl 14 mm, 5 juveniles, cl 4.4 to 6.2 mm; Brasília, riacho da Granja do Ipê, rio Roncador, Reserva Ecológica do IBGE (Instituto Brasileiro de Geografia e Estatística); 05 Aug. 2014, F.L. Mantelatto, L.G. Pileggi, F.L. Carvalho leg.; CCDB 5897 • 2 males, cl 9.49, cl 10.23 mm, 2 females, cl 11.34, cl 11.70 mm, 4 juveniles, cl 4.55 to 6.30 mm; Brasília, riacho da Granja do Ipê, 22 Feb. 2008, F.L. Mantelatto, E.C. Mossolin leg.; CCDB 2195.

Description of the holotype. Rostrum. Moderately high, nearly straight, distal end slightly directed upwards, reaching end of antennular peduncle, and little before the distal margin of scaphocerite; upper margin convex over orbit, with seven teeth, first and sometimes the second, slightly behind posterior edge of orbit; lower margin with one tooth.

Cephalon. Scaphocerite $2.5 \times$ as long as wide; outer margin slightly concave. Epistome forming two lobes; lobes with laterally arranged carinae.

Carapace. Anterolateral region slightly roughened; hepatic spine absent. Lower orbital angle obtuse, strongly pronounced.

Pereiopods. P1 reaching with almost half length of carpus beyond scaphocerite; fingers as long as palm; carpus $1.5 \times$ as long as chelae, $1.5 \times$ as long as merus; articles with scattered setae, fingers with tufts of setae. P2 similar in shape, different in size; largest one reaching with distal portion of merus beyond scaphocerite; smallest one reaching with distal end of carpus beyond scaphocerite, with fingers as long as palm; all articles with sparse setae and spines. Larger cheliped with ischium nearly as long as merus, with spinulation as in palm; merus as long as carpus, swollen, with spinulation as in palm; carpus slightly shorter than palm, swollen, with strong basal constriction; spinulation as in palm; propodus $2.5 \times$ as long as dactylus, $2 \times$ as long as carpus; palm with upper surface slightly compressed, somewhat swollen, covered with spinules, nearly $3 \times$ as long as high; fingers $2/3$ as long as palm, with numerous spinules; cutting edge of dactylus with large tooth in proximal third, slightly lower tooth in between large tooth and proximal part; cutting edge of fixed finger with tooth opposing two teeth of dactylus, with row of three denticles between proximal part and this tooth. P3–P5 smooth, except for sparse setae and spinules along lower margin of propodus; propodus nearly $2 \times$ as long as carpus; propodus slightly shorter than merus; P3 reaching with half-length of dactylus beyond scaphocerite, propodus $2 \times$ as long as dactylus; P4 reaching with tip of dactylus end of scaphocerite, propodus $2.5 \times$ as long as dactylus; P5 reaching with tip of dactylus half-length of scaphocerite, propodus $2.5 \times$ as long as dactylus.

Pleon. Smooth. Somite 5 with posteroventral angle of pleuron rectangular, not spinose; somite 6 $1.5 \times$ as long as somite 5. Inter-uropodal sclerite with strong, keel-shaped pre-anal carinae.

Pleopods. PL2 with appendix masculina $2 \times$ as long as appendix interna.

Uropods. Exopodite with mobile spines slightly shorter than spiniform projection of outer margin.

Telson. Broad, smooth, $1.5 \times$ as long as abdominal somite 6, bearing two pairs of dorsal spinules, first pair slightly behind middle portion of telson, second pair located closer to first pair than to posterior margin. Posterior margin distinct, ending in acute point, with several plumose setae and two pairs of posterior spinules, inner pair reaching end of telson.

Etymology. The specific epithet *brasiliensis* was used by Gomes Corrêa (1973) to refer to the type locality of the species, Brasília, the capital of Brazil. To keep that intention, we rename the species using the word *candango*, a demonym referring to those who are native to Brasília.

Size. See in material examined.

Color. From colorless to light brown, with dark brown carapace, mimicking the color of the substrate where they inhabit.

Type locality. Brazil, Distrito Federal, Brasília, Riacho da Granja do Ipê.

Distribution. Endemic of inland waters from Central Brazil (Distrito Federal) (Gomes Corrêa 1973; present paper).

Life cycle. Exclusive of inland waters, therefore independent of brackish waters to complete its life cycle. The fecundity is low, 38–61 eggs, and the eggs are large, their volume ranged from 4.41 to 7.71 mm^3 (Nogueira et al. under revision). Its larval development is not known but given its fecundity and egg size, it should be abbreviated, following the same pattern of congeners inhabiting continental waters (Magalhães and Walker 1988; Pereira and García 1995).

Remarks. Gomes Corrêa (1973) named *Cryphiops brasiliensis* a species from the vicinities of Brasília, Brazil. This specific epithet, however, was already used by Heller (1868) for a species of *Macrobrachium* described from the state of Mato Grosso, Brazil. With the synonymization of both genera, these specific names become secondary homonyms. We, therefore, propose the name *Macrobrachium candango* nom. nov., comb. nov. as a replacement name for *Cryphiops brasiliensis* Gomes Corrêa, 1973.

We examined specimens from three lots used by Gomes Corrêa (1973) to describe *C. brasiliensis* and deposited at the MNRJ: the holotype (MNRJ 903: male, cl 18.2 mm) and two others labeled as allotype (MNRJ 6464: 1 ovigerous female, cl 15.6 mm) and paratypes (MNRJ 2668: 1 male, cl 17.9 mm, 2 females, cl 15.3 and cl 15.3 mm), although the author did not explicitly designate the latter two as type material. We had this material on loan, which was returned to MNRJ in July 2008. After the fire at the Museu Nacional do Rio de Janeiro in September 2018, the lot MNRJ 2668 is missing, but the other two, including the holotype, preserved in alcohol, are safe and in good condition (I.A. Cardoso, curator of Crustacea, pers. comm. to FLM, Nov 2020). When carrying out aquatic surveys in the region around the type locality, we (FLM, LGP) visited the

Reserva Ecológica do IBGE (Brasília, DF) and found a well-preserved collection of specimens (> 260, not listed herein) made during previous aquatic faunistic surveys in the area (Takahashi et al. 2019). The main area of occurrence of this species is in a protected reserve, which may avoid possible impacts. This species was classified in the IUCN's Data Deficient (DD) category (Mantelatto et al. 2016). However, due to anthropic pressures in the region, future monitoring is necessary to evaluate its conservation conditions.

***Macrobrachium luscus* (Holthuis, 1973), comb. nov.**

Bythinops luscus Sbordoni, Argano & Zullini, 1973: 24 (nomen nudum).

Bithynops luscus Holthuis, 1973: 136, figs 1, 2. – Holthuis 1977: 181. – Hobbs, Hobbs and Daniel 1977: 25 (key), 46, fig. 17. – Reddell 1981: 108, fig. 15 (map), 319 (in list), 323 (list). – Villalobos 1982: 217 (in list). – Fitzpatrick 1983: 217. – Holthuis 1986: 606 (list). – Holthuis 1993: 103, fig. 89. – Hobbs III 1993: 20 (list). – Álvarez et al. 1996: 110, chart 12.2 (list). – Pereira 1997: 47, table 6 (list). – Fransen et al. 1997: 15 (catalog). – Jayachandran 2001: 17, fig. 1. – Palacios-Vargas 2006: 7 (list). – Mejía-Ortiz et al. 2013: 32, table 1. – Lamoreux et al. 2015: 306, Appendix 2 (list). – De Grave et al. 2015b: 7, table 3.

Cryphiops (Bithynops) luscus. – Villalobos Hiriart et al. 1989: 163, figs 1, 6a, c, 8a. – Villalobos-Hiriart et al. 1993: 281, table 5 (list). – Hobbs III 1994: 98 (list). – Fransen et al. 2010: 30, Appendix III (list). – De Grave and Fransen 2011: 316 (catalog). – Álvarez et al. 2011: 258, fig. 4a. – Palacios-Vargas and Reddell 2013: 43 (list). – Palacios-Vargas et al. 2014–2015: 22. – Quiroz-Martínez et al. 2014: table S1 (list). – Alvarez and Villalobos 2016: 250, table 8.1 (list).

Cryphiops luscus. – Palacios-Vargas 2006: 7 (list). – Baldari et al. 2010: 48, fig. 1 (map), 52, table 1. – Botello and Alvarez 2013: table 1 (list). – Mantelatto et al. 2020: 915 (key).

Material examined. MEXICO – **Chiapas** • 8 males, tl 30.5–49.4 mm, 15 ovigerous females, tl 30.8–46.3 mm; Municipality of La Trinitaria, Rancho de San Rafael del Arco, Gruta del Arco; 07 Apr. 1986; J.L. Villalobos leg.; CNCR 5759.

Description. Rostrum. Short, directed slightly downwards, tip directed slightly upwards, reaching or slightly overreaching joint between second and third article of antennular peduncle, and at level of distal third of scaphocerite; upper margin convex over orbit, with 5–8 teeth regularly spaced, first over or slightly behind posterior edge of orbit; lower margin with none or one tooth.

Cephalon. Scaphocerite 2.5 × as long as wide; outer margin straight.

Carapace. Smooth, with minute punctuations; antennal spine small, slightly overreaching lower portion of orbit; hepatic spine absent. Lower orbital angle obtuse, moderately pronounced.

Pereiopods. P1 slender, reaching with nearly entire chelae beyond scaphocerite; fingers slightly longer than palm; chelae 2/3 length of carpus. P2 moderately robust,

with several spines, equal in form and size, reaching with proximal third of carpus beyond scaphocerite; ischium evidently shorter than merus; merus as long as carpus; carpus as long as palm, with basal constriction; propodus $2 \times$ as long as dactylus, $2 \times$ as long as carpus; palm inflated, nearly $3 \times$ as long as high; fingers as long as palm, with numerous small spinules; cutting edge with two denticles of same size in both teeth. P3–P5 with all joints covered with row of small spinules on the lower margin; P3 reaching with entire dactylus beyond scaphocerite, propodus $2 \times$ as long as dactylus, propodus slightly longer than merus; P4 reaching with tip of dactylus end of scaphocerite, propodus $3 \times$ as long as dactylus, propodus slightly longer than merus; P5 reaching with tip of dactylus half-length of scaphocerite, propodus $3 \times$ as long as dactylus, propodus slightly longer than merus.

Pleon. Smooth. Somite 5 with posteroventral angle of pleuron acute; somite 6 nearly $2 \times$ as long as somite 5. Inter-uropodal sclerite without, keel-shaped pre-anal carinae.

Pleopods. PL2 with appendix masculina $2 \times$ as long as appendix interna.

Uropods. Exopodite with mobile spines as long as spiniform projection of outer margin.

Telson. Broad, smooth, slightly longer than abdominal somite 6, bearing two pairs of dorsal spinules closer to posterior margin of telson. Posterior margin ending in moderately acute triangular point, with several plumose setae and two pairs of posterior spinules, inner pair overreaching end of telson.

Size. See in material examined.

Color. Whitish to transparent.

Type locality. México, Chiapas, Municipality of La Trinitaria, Gruta del Arco, El Rancho de San Rafael Del Arco, Lagunas de Montebello, altitude 1,470 m. Recent visits to the type locality showed an increasing contamination in the lakes that supply water to the underground stream of the Gruta del Arco, and the collections of specimens were not successful, at least in the closest access to the water pools. Possibly, *M. luscus* comb. nov. is seriously threatened.

Distribution. Only known from the type locality (Holthuis 1973; present paper).

Life cycle. This is a cave species exclusive of inland waters, therefore independent of brackish to complete its life cycle. The eggs are few and large: 1.8–2.4 mm (Villalobos Hiriart et al. 1989). The duration of the embryonic development is probably long and with few larval stages following the pattern of other inland species.

Remarks. This species is similar to *Macrobrachium valdonii* nom. nov., comb. nov., which is the other hypogean species with abbreviated development and without hepatic spine. The most remarkable differences between them concerns the length of the rostrum, and the proportion of the articles of second pereopod (Table 2). In *M. luscus* comb. nov., the rostrum is short, reaching or slightly overreaching joint between second and third article of antennular peduncle, and at level of distal third of scaphocerite. The ischium of the second pereopod is evidently shorter than the merus, and the dactyl is little longer or as long as palm. In *M. valdonii* nom. nov., comb. nov., the rostrum is longer, reaching the third article of antennular peduncle and the distal border of scaphocerite; the ischium of the second pereopod is slightly shorter than the merus, and the dactyl is slightly shorter than the palm.

***Macrobrachium perspicax* (Holthuis, 1977), comb. nov.**

- Bithynops perspicax* Holthuis, 1977: 182, figs 3, 4. – Reddell 1981: 108, fig. 15 (map).
 – Villalobos 1982: 217 (list). – Holthuis 1986: 606 (list). – Álvarez et al. 1996: 110, chart 12.2 (list). – Pereira 1997: 47, table 6 (list). – Fransen et al. 1997: 16 (catalog). – Jayachandran 2001: 17. – Mejía-Ortiz et al. 2013: 32, table 1 (list).
Bithynops perspicax. – Sbordoni et al. 1977: 52, pl. 3 [error].
Cryphiops (Bithynops) perspicax. – Villalobos Hiriart et al. 1989: 165, figs 1, 7a, c, 8b. – Villalobos-Hiriart et al. 1993: 281, table 5 (list). – Hobbs III 1994: 98 (list). – Fransen et al. 2010: 30, Appendix III (list). – De Grave and Fransen 2011: 316 (catalog). – Álvarez et al. 2011: 258, fig. 2c. – Palacios-Vargas and Reddell 2013: 43 (list). – Palacios-Vargas et al. 2014–2015: 22. – Quiroz-Martínez et al. 2014: table S1 (list). – Alvarez and Villalobos 2016: 250, table 8.1 (list).
Cryphiops perspicax. – Palacios-Vargas 2006: 7 (list). – Baldari et al. 2010: 48, fig. 1 (map), 52, table 1. – Mantelatto et al. 2020: 916 (key).

Material examined. MEXICO – **Chiapas** • 16 males, tl 31.1–43.3 mm, 16 ovigerous females, tl 21.6–35.5 mm; Municipality of La Trinitaria, Ruinas de Chincultik, Cenote La Cueva; 07 Apr. 1986; J.L. Villalobos-Hiriart, J.C. Nates-Rodríguez, A. Cantú-Díaz Barriga leg; CNCR 7898.

Description. Rostrum. Short, directed downwards, reaching joint between second and third articles of antennular peduncle; upper margin with 5–8 teeth regularly spaced, first one at level or slightly behind posterior edge of orbit; lower margin with 1–3 teeth.

Cephalon. Scaphocerite $2.6 \times$ as long as wide, outer margin straight.

Carapace: Smooth, with minute punctuations; antennal spine small, slightly overreaching lower portion of orbit; hepatic spine absent. Lower orbital angle subacute, moderately pronounced.

Pereiopods. P1 slender, reaching with entire chelae or small part of carpus beyond scaphocerite; fingers slightly longer than palm; chelae $2/3$ length of carpus. P2 moderately robust, with spines, equal in form and size, reaching with proximal third of carpus beyond scaphocerite; ischium evidently shorter than merus; merus as long as carpus; carpus as long as palm, with basal constriction; propodus $2.2 \times$ as long as dactylus, $2 \times$ as long as carpus; palm inflated, nearly $3 \times$ as long as high; fingers slightly shorter (0.8) than palm, with numerous small spinules, not gaping, tips crossing, cutting edges with two similar denticles closer to proximal portion. P3–P5 with all joints covered with row of small spinules on lower margin; P3 reaching with entire dactylus beyond scaphocerite, propodus $2 \times$ as long as dactylus, propodus nearly $2 \times$ as long as carpus, propodus slightly longer than merus; P4 reaching with tip of dactylus end of scaphocerite, propodus $3 \times$ as long as dactylus, propodus nearly $2 \times$ as long as carpus, propodus slightly longer than merus; P5 reaching with tip of dactylus half-length of scaphocerite, propodus $3 \times$ as long as dactylus, propodus nearly $2 \times$ as long as carpus, propodus slightly longer than merus.

Pleon. Smooth, somite 5 with posteroventral angle of pleuron acute; somite 6 nearly $2 \times$ as long as somite 5. Inter-uropodal sclerite without keel-shaped pre-anal carinae.

Pleopods. PL2 with appendix masculina nearly $2 \times$ as long as appendix interna.

Uropods. Exopodite with mobile spines as long as spiniform projection of outer margin.

Telson. Broad, smooth, slightly longer than abdominal somite 6, bearing two pairs of dorsal spinules close to posterior margin of telson. Posterior margin ending in moderately acute triangular point, with several plumose setae and two pairs of posterior spinules, inner pair overreaching end of telson.

Size. See in material examined.

Color. Body translucent with orange punctuations.

Type locality. México, Chiapas, Municipality of La Trinitaria, Cenote La Cueva, Ruinas de Chincultik, altitude 1,480 m.

Distribution. Only known from the type locality (Holthuis 1977; present paper).

Life cycle. Exclusive of inland waters, therefore independent of brackish waters to complete its life cycle. The eggs are few and large: 1.9–2.5 mm (Villalobos Hiriart et al. 1989). Its larval development is not known but given the characteristics of the eggs, it should be abbreviated, following the same pattern of congeners inhabiting continental waters (Magalhães and Walker 1988; Pereira and García 1995).

Remarks. Among the epigeal forms of this group of species with abbreviated development and without hepatic spine, *M. perspicax* comb. nov. can be distinguished from *M. candango* nom. nov., comb. nov. and *M. alevillalobosi* nom. nov., comb. nov. by the total length of the body, and by the similar form and size of the second pereopod and the proportion of its articles (Table 2). Specimens of *M. perspicax* comb. nov. are generally smaller (31.1–43.3 mm) than those of the other two species; the second pereopods are shorter, do not present heterochely like *M. candango* nom. nov., comb. nov. and the chelae are slender, the palm is $3 \times$ as long as high, and the dactylus is slightly shorter.

***Macrobrachium valdonii* nom. nov., comb. nov.**

Cryphiops sbordoni Baldari, Mejía-Ortíz & López-Mejía, 2010: 48, figs 2–4. – Mantelatto et al. 2020: 915 (key).

Cryphiops (Bithynops) sbordonii. – De Grave and Fransen 2011: 316 (catalog). – Palacios-Vargas and Reddell 2013: 43 (in list). – Palacios-Vargas et al. 2014–2015: 22. – Quiroz-Martínez et al. 2014: table S1 (list). – Alvarez and Villalobos 2016: 250, table 8.1 (list).

Material examined. Holotype: MEXICO – Chiapas • male, cl 25 mm; Las Margaritas, Cueva Chambarro; 01 Mar. 2001; V. Sbordoni leg.; CNCR 25106. **Paratypes:** 1 ovigerous female, cl 22.5 mm, allotype; same data as for holotype; CNCR 25107 • 1 female, cl 12.3 mm; same data as for holotype; CNCR 25108.

Description. Rostrum. Short, straight, tip not reaching distal border of scaphocerite, almost reaching third article of antennular peduncle; upper margin bearing eight teeth, lower margin smooth.

Cephalon. Eyes reduced, globular cornea with facets, pigmented area reduced to a black point. Scaphocerite $2.4 \times$ as long as wide.

Carapace. Smooth, maximum length 25 mm, with only antennal spine; branchiostegal groove shallow; hepatic spine absent.

Pereiopods. P1: slender, smooth, with few tufts of setae on both fingers; palm surpassing distal margin of scaphocerite; palm slightly compressed, as long as dactylus; carpus $1.75 \times$ as long as palm, $1.12 \times$ as long as merus. P2: subequal in size, subequal in size, reaching with half of carpus beyond scaphocerite, without spines; ischium $0.9 \times$ merus; carpus $0.8 \times$ as long as merus, $0.85 \times$ as long as palm; propodus $1.5 \times$ as long as dactylus, $2.5 \times$ as long as carpus; palm semi-cylindrical, $3.3 \times$ as long as high, with dispersed tufts of setae, $0.8 \times$ as long as dactylus; fingers elongated, not gaping, cutting edges covered with tufts of setae, dactylus without teeth. P3: propodus, dactylus with several short setae, row of seven spines on ventral margin, propodus $3 \times$ as long as dactylus, $2.05 \times$ as long as carpus. P4: sparsely pilose, propodus $3.4 \times$ as long as dactylus, $1.8 \times$ as long as carpus, propodus with row of nine movable spines on ventral margin, propodus-dactylus articulation with pair of setae. P5: longest, propodus, carpus pilose, with longitudinal row of 12 movable spines, distal four close together, propodus-dactylus articulation with one spine; propodus $4 \times$ as long as dactylus, $2.1 \times$ as long as carpus.

Pleon. Smooth; somites 1–3 with pleura broadly rounded; somites 4 and 5 with posteroventral margin of pleura rounded; pleura of all somites bearing setae on ventral border; somite 6 nearly $1.5 \times$ as long as somite 5. Inter-uropodal sclerite without keel-shaped pre-anal carinae.

Telson. Nearly $1.5 \times$ longer than abdominal somite 6, shorter than uropodal rami, bearing two pairs of dorsal spines, first pair on distal fifth, second pair on middle section, with a single spine in the middle on left side; posterior margin broadly triangular bearing two pairs of lateral spines, inner pair $5 \times$ longer than outer one, with plumose setae between inner spines, center ending in acute tip.

Etymology. Baldari et al. (2010) named this species in honor of Prof. Valerio Sbordoni, a studious of the cave fauna of Chiapas, Mexico, and collector of the specimens. We maintained this homage by forming the specific epithet with parts of his first and last name.

Size. See in material examined.

Color. Live specimens are white, without pigment in/on the body.

Type locality. Mexico Chiapas, Las Margaritas, Cueva Chamburro.

Distribution. Only known from the type locality (Baldari et al. 2010).

Life cycle. Stygobitic species exclusive of inland waters, therefore independent of brackish waters to complete its life cycle. Female allotype with eggs (not measured).

Remarks. Mejía-Ortíz et al. (2008) described *Macrobrachium sbordonii* from Mexico, naming it after Valerio Sbordoni. Shortly thereafter, Baldari et al. (2010) pay

homage to the very same person again by describing a new species of *Cryphiops* also from Mexico. Since the synonymization of both genera makes the names secondary homonyms, *Macrobrachium valdonii* nom. nov., comb. nov. is proposed as a replacement name for *Cryphiops sbordonii* Baldari, Mejía-Ortiz & López-Mejía, 2010.

Similar to *M. luscus* comb. nov. (see remarks of that species and Table 2).

Discussion

Taxonomic issues

The phylogenetic analysis presented here, including freshwater prawns of the genus *Cryphiops* and species of *Macrobrachium* from four different geographic regions revealed that they form an unnatural group inside the Palaemonidae. All the species of *Cryphiops*, however, were considered valid taxonomic entities and all of them were recovered in the proper group of *Macrobrachium* species in terms of distribution and type of larval development.

Macrobrachium caementarius comb. nov. was consistently recovered associated to species with an estuarine affinity, supporting the taxonomic similarity showed in the phylogenetic analysis. The endemic species from Mexico, *Macrobrachium luscus* comb. nov., *M. perspicax* comb. nov., *M. valdonii* nom. nov., comb. nov., and *M. alevillalobosi* nom. nov., comb. nov., appear to have a joint position, always close to the species of *Macrobrachium* from Mexican inland waters (Fig. 1), which confirms the phylogenetic relationships among the four species. Similarly, the endemic species from central Brazil, *M. candango* nom. nov., comb. nov. is related to species of *Macrobrachium* also endemic to Brazil, in particular *M. iheringi* (Fig. 1), with a high degree of morphological similarity between these species.

The results of the taxonomic analysis of the species of *Cryphiops* corroborated the findings reported by Holthuis (1950, 1952a), who listed a series of morphological and biological reasons to explain why the taxonomy of the genera within the family Palaemonidae is considered of difficult resolution and deserved more refined studies. Therefore, it is not surprising that the current systematics of the group used so far exhibited several inconsistencies at both the generic and specific levels, such as those already reported for other species when molecular analysis were contrasted with morphologically based classifications (Murphy and Austin 2002, 2003).

The morphological character used to define *Cryphiops* is clear and easily discernible: "This genus differs from *Macrobrachium*, with which it often is united, mainly by the absence of the hepatic spine on the carapace" (see Holthuis 1952a: 136). That is, the only synapomorphy separating the two genera is the absence of the hepatic spine in *Cryphiops*. In accordance with Short (2004), the presence or absence of a hepatic spine is a doubtful character in Palaemonidae because it sometimes can be absent from one or both sides in specimens of *Macrobrachium*. Therefore, this single character used to

separate *Cryphiops* is subjective, and its usefulness should be reconsidered. Clearly, the absence of the hepatic spine refers to a case of homoplasy, in which the independently acquired apomorphies do not represent phylogenetic proximity. In this case, two hypotheses can be considered: 1) parallelism, losing the hepatic spine independently in the two lineages from a plesiomorphic with-hepatic-spine state, or 2) reversal, when the apomorphic state (absence of hepatic spine) becomes similar to the previous plesiomorphic state (absence of hepatic spine) present in the ancestor of the group. From a parsimony point of view, however, we believe that the first hypothesis seems more plausible, i.e., an independent loss of the hepatic spine that was propagated from generation to generation in different populations.

Nomenclatural issues

The obtained concatenated topology (Fig. 1) shows that there is high genetic similarity among the species of *Macrobrachium* and *Cryphiops*, coinciding with several previous studies that suggested that the latter should be part of *Macrobrachium* (Pereira 1997; Porter et al. 2005; Page et al. 2008; Pileggi and Mantelatto 2010). Following these studies, the robust results obtained here, considering all species of *Cryphiops* and almost all of the Neotropical species of *Macrobrachium*, corroborate the paraphyletic nature of these groups and indicate that the current classification should be amended accordingly. In this way, as De Grave and Ashelby (2013: 341) pointed out, such amendment will induce a nomenclatural issue regarding the priority of the names *Cryphiops* / *Macrobrachium*, a situation that demands extra caution and that will require an evaluation by the International Commission on Zoological Nomenclature (ICZN). The name *Cryphiops* Dana, 1852 precedes *Macrobrachium* Spence Bate, 1868 and, if the Principle of Priority is strictly followed, the former should have priority over the latter (ICZN 1999, Art. 23). However, *Macrobrachium* is a much more speciose genus with many species of economic interest and importance, and extensively cited in the scientific literature. Therefore, a change in the generic name that at present is very well known would certainly cause taxonomic confusion and nomenclatural instability. The provisions of the Article 23.9.1 of the Code for a Reversal of Precedence cannot be applied in this case because the older synonym (*Cryphiops*) was used as a valid name after 1899 (see synonymic list under *Macrobrachium*). We, nevertheless, invoke the provision of Article 23.9.3 to propose herein that the younger synonym (*Macrobrachium*) keeps the priority over the older one. An application to the International Commission on Zoological Nomenclature to suppress the priority of *Cryphiops* and rule this proposal of Reversal of Precedence is being concurrently prepared. We also suggest that the prevailing use of the name *Macrobrachium* is maintained while the matter is under consideration by the Commission (ICZN 1999, Art. 82). Meanwhile, those who believe the taxa to be distinct could still use both names (L.B. Holthuis, pers. comm. to FLM on 27 Nov 2007). The arguments to support this proposal are detailed below.

In an essay on Chile's natural history, Molina (1782) introduced "*Cancer caementarius*" to name a freshwater shrimp abundant in the rivers of that country.

This species was later treated under different names or combinations [for instance: *Astacus caementarius* by Molina (1810), *Palaemon caementarius* by Poepig (1835), *Palaemon gaudichaudii* by H. Milne Edwards (1837, in H. Milne Edwards 1834–1840), *Cryphiops spinuloso-manus* by Dana (1852), *Bithynis longimana* by Philippi (1860), *Macrobrachium africanum* by Spence Bate (1868), and *Bithynis caementarius* by Rathbun (1910); see Holthuis (1952a, b) for a complete synonymy] until Holthuis (1950, 1952b) noted that the specimen described by Dana (1852) was actually a mutilated specimen of *Bithynis caementarius* (Molina, 1782) and pointed out that *Cryphiops* Dana, 1852 had priority over the name *Bithynis* Philippi, 1860. Therefore, Holthuis (1952a) established the type species of the genus as being *Cryphiops spinulosomanus* Dana, 1852 (= *Cryphiops caementarius* (Molina, 1782)).

The genus remained monotypic for more than 120 years until Gomes Corrêa (1973) described *Cryphiops brasiliensis*, endemic to central Brazil. In that same year, Holthuis (1973) erected *Bithynops* to include a new cave species from Mexico, *Bs. luscus*. Soon after, Holthuis (1977) included another new species from Mexico in this genus: *Bithynops perspicax*. Subsequently, in a review of the genera *Cryphiops* and *Bithynops*, Villalobos Hiriart et al. (1989) proposed the synonymization of both taxa based on the fragility of the characters used to separate them (e.g., eyes with reduced cornea in *Bithynops*), but kept both taxa with subgeneric status. They retained *C. caementarius* under *Cryphiops* s. s., moved *C. brasiliensis*, *Bs. luscus*, and *Bs. perspicax* into *Cryphiops* (*Bythynops*), in addition to describing a new species, *Cryphiops* (*Bithynops*) *villalobosi* Villalobos Hiriart, Nates Rodriguez & Diaz Cantú, 1989. Later, Baldari et al. (2010) described a new cave species from Chiapas, Mexico, named *Cryphiops sbordonii* Baldari, Mejía-Ortiz & López-Mejía, 2010. It is noteworthy that Holthuis (1993), in his robust review of the caridean genera, did not follow this subgeneric arrangement, which is widely accepted (De Grave and Fransen 2011; WoRMS 2021).

The genus *Macrobrachium* was erected by Spence Bate (1868) to accommodate four species with males presenting “immensely developed” second pair of pereopods without, however, designating a type species. This was subsequently done by Fowler (1912), who chose an American species, *Macrobrachium americanum* Spence Bate, 1868, as the type species.

Holthuis and Ng (2010) gave a historical overview of the nomenclatural situation of the name *Macrobrachium*, in particular regarding the confusing usage of the names *Macrobrachium* and *Palaemon* Weber, 1795, which led the matter to be ruled by the International Commission of Zoological Nomenclature in the Opinion 564 (ICZN 1959). Due to the very conservative nature of the morphological traits used to differentiate this group of palaemonid shrimps both to generic and specific ranks, the taxonomic status of *Macrobrachium* has undergone several changes, especially until the first half of the 20th century. Spence Bate (1868) confessed his hesitation in creating the new genus, since he did not perceive any structural differentiation separating the new species of *Macrobrachium* from those of *Palaemon* but considered that the extremely long P2 would be a strong evidence that both taxa formed a natural classification. Shortly thereafter, the author did not follow his own system and treated

Macrobrachium as a junior synonym of *Bithynis* (see Spence Bate 1888: 788). Ortmann (1891), based on characters of the P2 (shape of the palm and length ratio between carpus and merus), split up *Palaemon* into four subgenera: *Eupalaemon* Ortmann, 1891; *Brachycarpus* Spence Bate, 1888; *Parapalaemon* Ortmann, 1891; and *Macrobrachium*. His system was followed by Coutière (1901), but not by Stebbing (1908), who, in view of the inconsistency of such arrangement, argued that the retention of the name *Macrobrachium* was not justified and replaced it with *Macroteroceris*, a genus defined by one of the chelipeds of the second pair being exceedingly longer than the other. Henderson and Matthai (1910) found the subgeneric arrangement of doubtful utility, since those characters were age dependent, and kept all species under the genus *Palaemon*. Holthuis (1950, 1952a) presented a comprehensive discussion on the difficulties of studying the taxonomy of this group regarding the few useful differential characters and their large variability individually, ontogenetically or between the sexes. Holthuis (1950: 104) also considered the subgeneric division unfeasible, as it could lead to confusion, and treated *Macrobrachium* as a unity.

Since Holthuis' revision (1952a) of the American Palaemoninae and, particularly, after the Opinion 564 (ICZN, 1959), the taxonomic and nomenclatural status of the genus has remained stable. As a pantropical and subtropical genus occurring in a wide variety of habitats, the number of species from around the world added or described in it grew so rapidly that 41 years after his revision, Holthuis (1993) himself remarked that "it is now a quite respectable generic name". Today, the genus is one of the most speciose of the infraorder Caridea, with 243 valid species until 1 June 2011 (De Grave and Fransen 2011) and 259 until 5 Jan 2021 (WoRMS 2021), with this number varying either due to the frequent addition of new species (e.g., Mejía-Ortíz and López-Mejía 2011; Pillai and Unnikrishnan 2012, 2013; Pillai et al. 2014, 2015; Fujita et al. 2015; Cai and Vidhayanon 2016; Lan et al. 2017; Saengphan et al. 2018, 2019; Xuan 2019; Zheng et al. 2019; Rossi et al. 2020; Siriwtut et al. 2020; Zhu et al. 2020; Myo et al. 2021) or due to synonymization or revalidation of species (e.g., Pileggi and Mantelatto 2012; Castelin et al. 2017).

The high diversity and worldwide tropical-subtropical distribution of *Macrobrachium*, combined with the scarcity of morphologic characters for accurate generic and specific delimitation, has long been intriguing taxonomists regarding its systematics, phylogenetic affinities, and biogeographic patterns. Several studies have been published on these topics using both morphological and molecular data, and, more recently, applying integrative approaches (Pereira 1997; Bowles et al. 2000; Murphy and Austin 2003, 2005; Short 2004; Hernández et al. 2007; Liu et al. 2007; Valencia and Campos 2007; Wowor and Ng 2007; Parhi et al. 2008; Wowor et al. 2009; Pileggi and Mantelatto 2010; Acuña Gómez et al. 2013; Rossi and Mantelatto 2013; Pileggi et al. 2014; Jose et al. 2016; Jose and Harikrishnan 2019; Mokambu et al. 2019; Molina et al. 2020). Among other factors, the high number of species has been hampering a comprehensive study on the phylogeny of the genus, but several articles were published on this at a regional level, either based on American (e.g., Pileggi and Mantelatto 2010; Acuña Gómez et al. 2013; Rossi and Mantelatto 2013; Pileggi et al. 2014), African

(e.g., Mokambu et al. 2019), or Indo-West Pacific species (e.g., Murphy and Austin 2005; Liu et al. 2007; Parhi et al. 2008; Chen et al. 2009; Wowor et al. 2009; Jose and Harikrishnan 2019; Siriwt et al. 2020). As one of the most conspicuous constituents of the aquatic fauna in estuarine and continental aquatic environments, a multitude of studies on the biology, ecology, reproduction, development, and physiology of many of its species have already been published. Jayachandran (2001) and Anger (2013) made a comprehensive review on the biology, ecology, and biogeography of *Macrobrachium* (see also the references therein).

The large size, high fecundity, and abundance of some species of the genus have made them an economically valuable fisheries and aquaculture resource and, consequently, numerous scientific and technical publications on different aspects related to their culture and fisheries have been made around the world (see New and Valenti 2000; Jayachandran 2001; New et al. 2008, 2010). *Macrobrachium rosenbergii* (De Man, 1879) and *Macrobrachium nipponense* (De Haan, 1849 [in De Haan 1833–1850]) are the most commercially important species, but other species of *Macrobrachium* have also been used for aquaculture or studied as potentially cultivable species (New and Valenti 2000; Jayachandran 2001; New et al. 2008, 2010; Hongtuo et al. 2012; New and Mohanakumaran Nair 2012; FAO 2020).

Holthuis and Ng (2010), considering the circumtropical, disjunct geographic distribution of this highly diverse group, raised doubts as to whether the genus would form a monophyletic clade. To this regard, we included eight Asian and two African species of *Macrobrachium* (Table 1); however, they were recovered either nested within American species or well within what is considered the genus *Macrobrachium* (Fig. 1). Although our study is limited to the available sequences and species that we were able to analyze and sequence, it contributes to the assumption that the genus is monophyletic and is supported by a multigene analysis. Other studies using molecular approaches, but also including a limited number of representatives either with preponderance of Indo-Pacific species (Murphy and Austin 2005; Liu et al. 2007; Parhi et al. 2008; Wowor et al. 2009; Jose and Harikrishnan 2019) or focused on American species (Pileggi and Mantelatto 2010; Acuña Gómez et al. 2013; Rossi and Mantelatto 2013; Pileggi et al. 2014; García-Velazco et al. 2017, 2018), have also pointed to a monophyletic status of the genus. Anger (2013) assumed that all *Macrobrachium* species originated from the same ancestor in proposing a robust scenario for explaining the origin, evolutionary history, and modern biogeography of the genus. Assuming that it is indeed monophyletic and considering that the type species of *Macrobrachium* is an American species, then our proposal of Reversal of Precedence of *Macrobrachium* over *Cryphiops*, if so ruled by the ICZN, should not affect the status and situation of the African and Indo-West Pacific species of *Macrobrachium*. On the other hand, if future, more comprehensive studies including a large number of worldwide representatives of the genus will eventually not corroborate its monophyly, then the taxonomic and nomenclatural situation of the non-American species might become somewhat complicated. Among the other generic names available, *Eupalaemon* Ortmann, 1891 cannot be used because its type species, designated by Holthuis (1955), is *Macrobrachium acanthurus*

(Wiegmann, 1836), a well-established American species. If the African and Asian species constitute a separate clade, then *Parapaleomon* Ortmann, 1891 would be the name to be used, as Holthuis (1955) established its type species as being *Macrobrachium dolichodactylus* Hilgendorf, 1879, a species from the eastern coast of Africa (Mozambique). If, however, the results of such a study pose more atomized groups, the introduction of new generic names for those clades might be necessary, since the type species of *Macroterochair* Stebbing, 1908, the only other name available for this group, is *Macrobrachium lepidactylus* Hilgendorf, 1879 (designated by Holthuis 1955), also from Mozambique.

Conclusions

Our phylogenetic analysis of all species of *Cryphiops*, including species of *Macrobrachium* from America, Africa, and the Indo-Pacific, using morphological and multigene approaches in combination with a taxonomic revision, revealed that the morphological character used to separate the genus *Cryphiops* is subjective and homoplastic, and that all *Cryphiops* species are nested within *Macrobrachium*. Such results corroborate the assumption about the monophyly of the genus *Macrobrachium*, which implies that *Cryphiops* Dana, 1852 and *Macrobrachium* Spence Bate, 1868 are subjective synonyms and, as a consequence, three specific secondary homonyms are established: *M. brasiliense* (Heller, 1862) × *C. brasiliensis* Gomes Corrêa, 1973; *M. villalobosi* Hobbs Jr, 1973 × *C. (Bithynops) villalobosi* Villalobos Hiriart, Nates Rodríguez & Cantú Díaz Barriga, 1989; and *M. sbordonii* Mejía-Ortiz, Baldari & López-Mejía, 2008 × *C. sbordonii* Baldari, Mejía-Ortiz & López-Mejía, 2010. We therefore present a systematic rearrangement in which all species of *Cryphiops* are included in *Macrobrachium* and introduce replacement names for the three resulting specific secondary homonyms.

The available genetic data argues for the synonymy of *Macrobrachium* Spence Bate, 1868 under *Cryphiops* Dana, 1852. Considering the large number of species under both names and the fact that they have a pan-tropical distribution, it is likely this taxonomy may be challenged by new genetic techniques and finer morphological analyses. To change the generic names at this stage would be very disruptive, resulting in nomenclatural instability and causing confusion for other researchers, especially since there are several economically important species (notably *Macrobrachium rosenbergii*). Moreover, many species are also important in conservation efforts and used for a wide variety of biological studies in many parts of the world. Therefore, until a larger data set can be assembled, we recommend maintaining the status quo with regards to the generic names, i.e., use *Macrobrachium* sensu lato and restrict the use of *Cryphiops* for *C. caementarius* (Molina, 1782) and its immediately allied species. Under the current code (ICZN 1999: Arts. 23.9.3, 81.2.2), the senior synonym (*Cryphiops*) should be partially suppressed in favor of maintaining the prevailing use of the junior synonym (*Macrobrachium*) under the provision of the Article 82 of the Code (ICZN 1999). In

this sense, an application is concurrently being prepared to the ICZN for using their Plenary Powers to partially suppress the priority of the name *Cryphiops* over the name *Macrobrachium* and rule a case of Reversal of Precedence regarding these names.

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Supplementary material I

Table S1

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Data type: Pairwise genetic distance matrix (excel table)

Explanation note: Pairwise genetic distance matrix of 16S (below diagonal) and COI (above diagonal) sequences among species previously included in the genus "*Cryphiops*" Dana, 1852 and "selected species" of *Macrobrachium* Spence Bate, 1868 from America, Africa, and the Indo-Pacific.

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