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Uncovering local endemism from southeastern Myanmar: description of the new karst-associated terrestrial snail genus *Burmochlamys* (Eupulmonata, Helicarionidae)

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Abstract

Salween River basin's karst ecosystems in southeastern Myanmar remain largely unexplored and are likely to harbour a high terrestrial snail diversity that are often associated with high levels of snail endemism. Here, an outstanding group of new karst-associated terrestrial snails, *Burmochlamys* **gen. nov.,** are discovered. A study of the comparative morphological and anatomical data reveals that the reproductive tract and radula of this new genus are closely related to the helicarionid genus *Sophina* Benson, 1859 but shell morphology (shape, size, and sculpture) and mantle extensions are distinct from the latter genus. *Burmochlamys* **gen. nov.** now consists of four known nominal species, *B. cassidula* **comb. nov.,** *B. cauisa* **comb. nov.,** *B. perpaula* **comb. nov.,** and *B. poongee* **comb. nov.,** and five new species; *B. albida* **sp. nov.,** *B. fasciola* **sp. nov.,** *B. moulmeinica* **sp. nov.,** *B. versicolor* sp. nov., and *B. whitteni* **sp. nov.** The highlight is that the members of the new genus show site-specific endemism, being restricted to karstic habitat islands of the Salween River basin. In addition, the discovery supports that the unique and complex structure of Salween River basin's karst ecosystems are habitats in which the terrestrial malacofauna have speciated and become endemic.

Keywords

Diversity, endemic, Indochina, land snail, limestone, Salween River basin, taxonomy

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Introduction

Myanmar is globally recognised as a highly important biodiversity hotspot that supports a great number of several endemic species of animals (Myers et al. 2000; Grismer et al. 2018a, b; Sutcharit et al. 2020). Especially the Salween River basin located in the southeastern Myanmar has a wide diversity of limestone hills and outcrops surrounded by lowland areas that are temporarily flooded during the monsoon season (Figs 1, 2) that can form island-like habitats (Clements et al. 2006; Grismer et al. 2018a, b, 2021; Sutcharit et al. 2020). The Salween River basin's karst ecosystems also serve as foci for speciation and endemism of the terrestrial malacofauna (Sutcharit et al. 2020) as well as geckos (Grismer et al. 2018a, b). Under these conditions, it is no surprise that although there have been several documentations of land snail fauna over many years, including Benson (1859), Theobald (1859), Stoliczka (1871), Godwin-Austen (1882–1920), Blanford and Godwin-Austen (1908), Pholyotha et al. (2020b), and Sutcharit et al. (2020), a large proportion of land snail fauna still remains undescribed. On the other hand, many known taxa from the region also need to be re-investigated because the current classification does not include any distinguishing characteristics, especially the genitalia, to achieve a more reliable generic classification level.

One group of snails with a small, depressed to conical, and rounded body whorl constitutes a distinctive part of the southeastern Burmese fauna (Stoliczka 1871; Blanford and Godwin-Austen 1908; Sutcharit et al. 2020). However, shell-based taxonomy of the group is insufficient for generic classification where the generally conserved shell form has created long-lasting taxonomic confusion. There are many genera of the speciose limacoid families in the Helicarionidae Bourguignat, 1877 (i.e., *Sophina* Benson, 1859) and Ariophantidae Godwin-Austen, 1883 (i.e., *Macrochlamys* Gray, 1847 and *Microcystina* Mörch, 1872) that present these shell morphs (Blanford and Godwin-Austen 1908; van Benthem Jutting 1950; Solem 1966; Schileyko 2002, 2003; Vermeulen et al. 2019; Pholyotha et al. 2020b; Sutcharit et al. 2020). Fortunately, the morphological characters of the mantle extensions, radula, and genitalia can distinguish between all these genera. In addition, integrative systematic revision of several Southeast Asian helicarionoids (i.e., Páll-Gergely et al. 2016; Pholyotha et al. 2020a, b, c, 2021a, b; Sutcharit et al. 2020, 2021) and the Australian helicarionids (i.e., Hyman et al. 2017; Hyman and Köhler 2018) confirms the taxonomic importance of these characters.

After intensive sampling from karstic and non-karstic habitats in southeastern Myanmar, the two overlooked species, *Helix poongee* Theobald, 1859 and *Helix cassidula* Benson, 1859, were found and showed a surprising incongruence in the radular and genital morphology that prevented their classification into the current known genus *Macrochlamys* (Blanford and Godwin-Austen 1908; Pholyotha et al. 2018, 2020b). In this survey, additional undescribed species of small-sized snails were also found. Noticeably, all seven species are likely to be the intermediate form to several genera because they have a mantle morphology similar to those of *Macrochlamys*, a small-sized shell with a spiral striated shell sculpture like *Microcystina*, and spatulate radula teeth identical to those of *Sophina*. Despite the absence of a molecular framework, the morphology-based revision has been widely accepted and could greatly contribute to a more robust taxonomy of the Asian helicarionoids (Solem 1966; Páll-Gergely et al. 2016; Vermeulen et al. 2019; Pholyotha et al. 2020a, b, c, 2021a, b; Sutcharit et al. 2020, 2021). Therefore, in this study, we describe a new land snail genus *Burmochlamys* gen. nov. together with the re-description of the two known species: *Helix poongee* and *H. cassidula* and five newly described species based on the comparative morphological and anatomical dataset. This discovery has also highlighted the growing knowledge of the diverse and endemic terrestrial snail fauna in Myanmar.

Materials and methods

Sampling, material preserving, identification, and morphological examination

Land snails were collected by direct visual searching and hand collecting from several accessible localities, including limestone and non-limestone habitats, from Shan State,

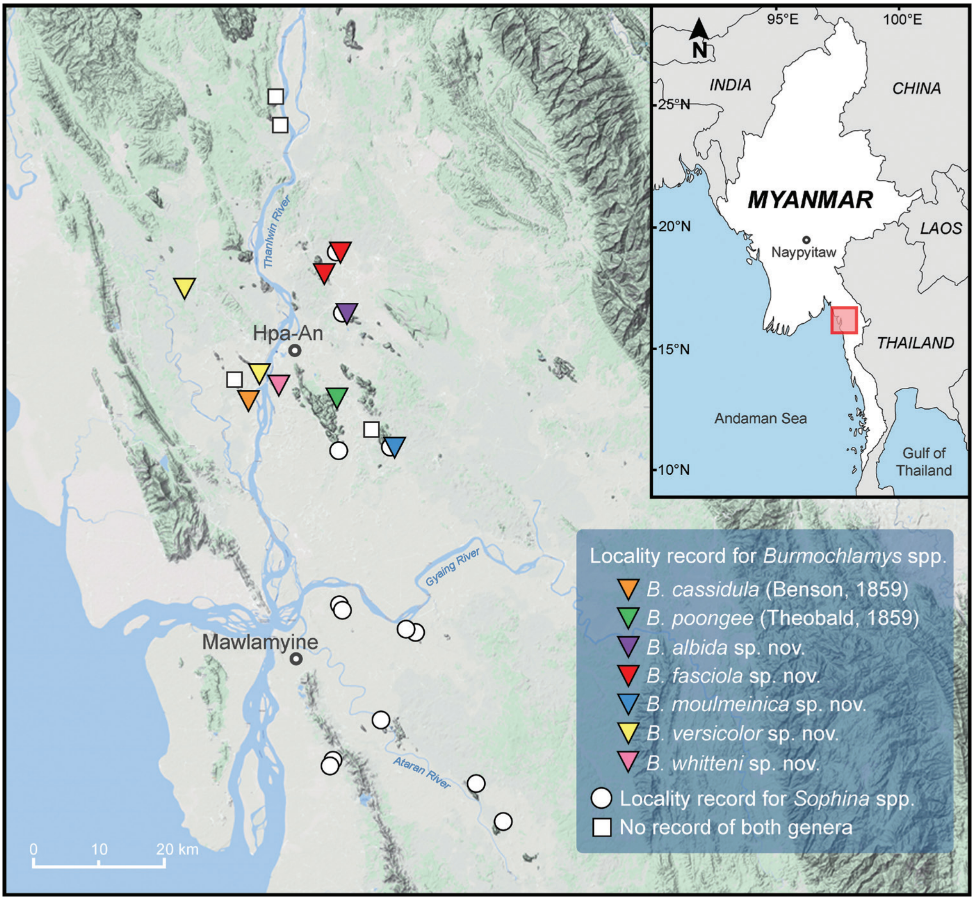

Figure 1. Map of the Salween River basin (known as Thanlwin River) in the southeastern Myanmar showing the sampling sites. Coloured triangles indicate the localities recorded for each *Burmochlamys* species. White circles indicate the localities recorded for *Sophina* species. White squares indicate the sampling localities with no records of both *Sophina* and *Burmochlamys* species.

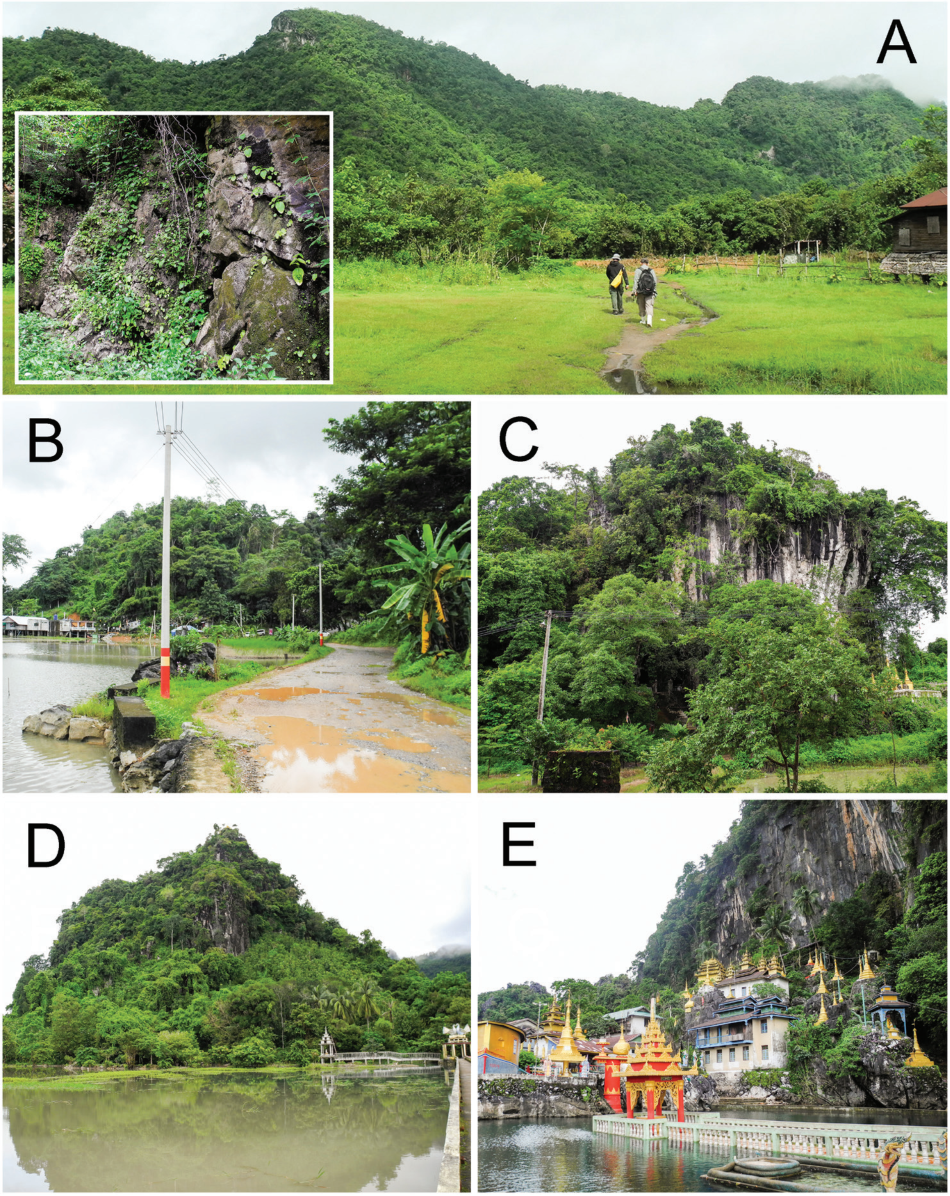

Figure 2. Habitat of some species of *Burmochlamys* gen. nov. in the karst basin of Hpa-An, Kayin State, Myanmar. **A** type locality of *B. fasciola* sp. nov. at Bardai Mountain and microhabitat structure of the karst wall **B** newly discovered locality of *B. poongee* at Kaw Ka Thaung Cave **C** type locality of *B. albida* sp. nov. at Waiponla Monastery **D** type locality of *B. moulmeinica* sp. nov. at Lun Nya Pagoda **E** type locality of *B. versicolor* sp. nov. at Bayin Nyi Cave. Photos by Ruttapon Srisonchai.

Mon State, Kayin State, and the Tanintharyi Region; however, only the limestone area of the Salween River basin in southeastern Myanmar was found to house populations of *Burmochlamys* species (Fig. 1). Field surveys were conducted during the years 2015 to

2016 under an MOU between the Forest Department, Ministry of Natural Resources and Environmental Conservation and Forestry, Myanmar, and Fauna & Flora International. Prior to preservation of the collected specimens in the field, we took pictures of each individual species in life. Snails were then euthanised following the standard two-step method protocols (American Veterinary Medical Association 2020), and then preserved in 95% (v/v) ethanol for further morphological and molecular works. The animal use protocol was approved by the Chulalongkorn University Animal Care and Use Committee (**CU-ACUC**) under the approval number 1723018. Type material and other voucher specimens are deposited in the Chulalongkorn University Museum of Zoology (**CUMZ**), Bangkok, Thailand and additional paratype specimens are deposited at the Natural History Museum, London, United Kingdom (**NHM** or **NHMUK** when citing specimen lots deposited in the NHM). Species identification was made based on the current literature [i.e., Benson (1859), Theobald (1859), Stoliczka (1871), Blanford and Godwin-Austen (1908), Pholyotha et al. (2020b), and Sutcharit et al. (2020)], and then compared with the available reference collection of the NHM and the University Museum of Zoology, Cambridge (**UMZC**). For the descriptive work, adult shells and genitalia were imaged using a Nikon camera (DSLR D850) with a Nikon 105 Macro lens (AF-S VR Micro-Nikkor 105 mm f/2.8G IF-ED). Adult shells were measured for size using a Vernier caliper and counting the number of whorls. Three to ten specimens of each species were dissected and examined under an Olympus SZX2-TR30 stereoscopic light microscope. Radulae were extracted, soaked in 10% (w/v) sodium hydroxide, cleaned with distilled water, and then imaged by scanning electron microscopy (SEM; JEOL, JSM-6610 LV).

List of abbreviations used in the figures: **ant-ldl** (anterior left dorsal lobe), **at** (atrium), **cf** (caudal foss), **ch** (caudal horn), **da** (dart apparatus), **e1** (portion of epiphallus nearer to penis), **e2** (portion of epiphallus nearer to retractor muscle), **ec** (epiphallic caecum), **fo** (free oviduct), **gd** (gametolytic duct), **gs** (gametolytic sac), **lsl** (left shell lobe), **p** (penis), **post-ldl** (posterior left dorsal lobe), **prm** (penial retractor muscle), **rdl** (right dorsal lobe), **rsl** (right shell lobe), **v** (vagina), **vd** (vas deferens).

Systematic descriptions

Family Helicarionidae Bourguignat, 1877
Subfamily Durgellinae Godwin-Austen, 1888

Genus *Burmochlamys* Pholyotha & Panha, gen. nov.

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Type species. *Burmochlamys fasciola* sp. nov., by original designation.

Etymology. The name combines *Burmo* in reference to Burma, the historical name of Myanmar, and the Greek word *chlamys* meaning mantle or cloak in reference to land snail with well-developed mantle extensions. Therefore, the generic name means the Burmese land snail with the well-developed mantle extensions. The gender of the new generic name is feminine.

Diagnosis. Shell subglobose to globose, small size, little high spire, and sculptured with spiral furrows and undulating radial lines. Snail with five well-developed mantle extensions; caudal horn raised. Genitalia with penial retractor muscle attached at tip of epiphallic caecum; gametolytic organ with rather short to moderate cylindrical duct and bulbous sac; well-developed dart apparatus; flagellum absent. Radula with large monocuspid central tooth and attached by two smaller teeth; laterals and marginals undifferentiated, large monocuspid, and at base of each tooth on outer side attached by a smaller tooth.

Description. ***Shell*** subglobose to globose, small-sized, thin, whitish to brownish, with or without yellowish brown band on the periphery. Shell surface with distinct to faintly spiral furrows, crossed by distinct to faintly undulating radial lines. Whorls 5½–7, regularly increasing; spire rather elevated; body whorl rounded. Aperture oblique and crescentic with simple lip. Umbilicus open, narrow to moderate, and deep.

Animal reticulated skin with pale to dark greyish or with a brown or yellow tinge. Mantle lobes or mantle extension well developed, divided into two shell lobes and three dorsal lobes, and somewhat thickened near their margins (Fig. 3A–C). Shell lobes short to moderate, slender, finger-like, and same colour as body. Right shell lobe (rsl) rather longer and larger than left shell lobe (lsl). Dorsal lobes large, broad, normally larger than shell lobes, crescent-shaped, and same colour as body. Right dorsal lobe (rdl) larger than left dorsal lobe. Left dorsal lobe divided into anterior (ant-ldl) and posterior lobes (post-ldl). Sole tripartite and lateral foot margin present. Caudal foss (cf) and caudal horn (ch) present (Fig. 3B).

Genitalia possess penis with thin penial sheath; penial retractor muscle varying in size and attached at tip of short and straight epiphallic caecum. Flagellum absent. Dart apparatus present. Gametolytic organ with rather short to moderate cylindrical duct and bulbous sac.

Radular teeth arranged in wide U-shaped rows. Central tooth monocuspid, large, narrow to broad spatulate shape, and with two smaller triangular-shaped teeth located at base (Fig. 3D). Lateral and marginal teeth not differentiated, monocuspid, large spatulate shape, and with only one smaller triangular-shaped tooth located at base on outer side. From inner to outer, lateromarginal teeth gradually narrower, smaller, and rather pointed cusp (Fig. 3D, E), and tiny inner cusp sometimes present on outermost teeth (yellow arrow in Fig. 3E).

Constituent species. *Burmochlamys* gen. nov. currently contains: *B. cassidula* (Benson, 1859), comb. nov., *B. cauisa* (Benson, 1859), comb. nov., *B. perpaula* (Benson, 1859), comb. nov., *B. poongee* (Theobald, 1859), comb. nov., *B. albida* sp. nov., *B. fasciola* sp. nov., *B. moulmeinica* sp. nov., *B. versicolor* sp. nov., and *B. whitteni* sp. nov.

Distribution. *Burmochlamys* gen. nov. shows a remarkable degree of endemism and localisation being restricted to the limestone karsts in the south of Salween River basin, Myanmar (Fig. 1). The limestone formations in the region are notable for their fragmented, island-like nature, with hills, caves, and towers forming archipelagos of habitat islands and some areas are temporarily flooded during the monsoon season (Fig. 2).

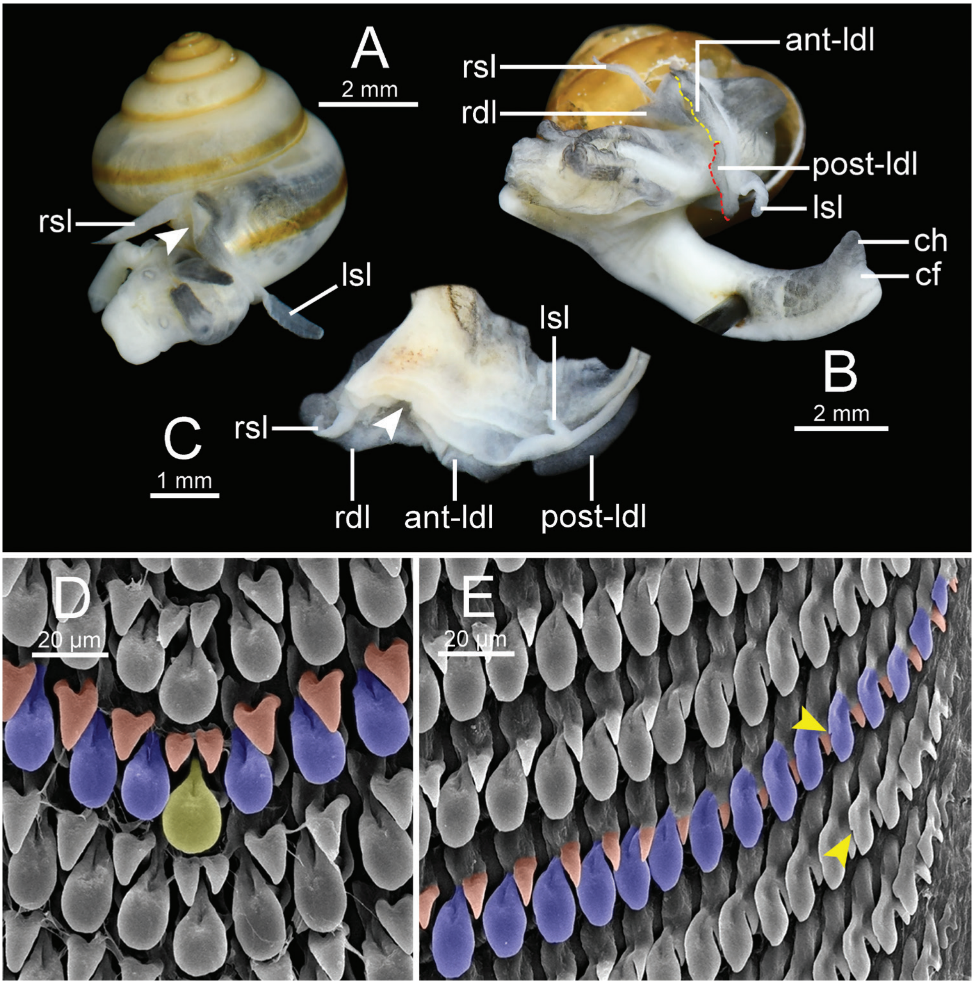

Figure 3. Synoptic illustration of mantle extensions, body terminology and radular morphology of *Burmochlamys* gen. nov. **A** right and left shell lobes of *B. fasciola* sp. nov. **B** mantle extensions (shell lobes and dorsal lobes) and posterior body of *B. poongee* **C** dorsal view of mantle extensions (shell lobes and dorsal lobes) of *B. cassidula* **D, E** representative SEM images of radula showing monocuspid central tooth (yellow) and lateromarginal teeth (blue) attached by the smaller triangular-shaped teeth (red). White arrow indicates pneumostome or breathing pore. Yellow arrow indicates a tiny cusp on outermost lateromarginal teeth.

Remarks. *Burmochlamys* gen. nov. possesses a similar radular morphology (monocuspid and spatulate shape) to those of *Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017, *Chalepotaxis* Ancey, 1887, and *Sophina*. However, the new genus is easy to distinguish from these three genera by having a microscopic shell sculpture, slender mantle extensions (left and right shell lobes) and genitalia with a well-developed dart apparatus and without a flagellum. In contrast, those three genera have a smooth shell surface and well-developed left and right shell lobes that can be enlarged and cover most of the shell. The genitalia of *Sophina* is more similar to that of *Burmochlamys* gen. nov., while *Aenigmatoconcha* has a small flagellum and no dart apparatus, and *Chalepotaxis* has neither flagellum nor dart apparatus (Páll-Gergely et al. 2016; Sutcharit et al. 2020; Pholyotha et al. 2021b).

In addition, *Burmochlamys* gen. nov. is clearly discriminated from other helicarionid and ariophantid genera with or without shell lobes by the presence of the monocuspid radular teeth (see Table 1). In comparison, the radular morphology of most genera in these families possess bicuspid, tricuspid, or multicuspid teeth (Stoliczka 1871; Blanford and Godwin-Austen 1908; van Benthem Jutting 1950; Solem 1966; Schileyko 2002, 2003; Páll-Gergely et al. 2016; Sutcharit et al. 2020; Pholyotha et al. 2021b).

As observed in the field, we searched after rain and found the snails climbing on the limestone walls or hiding under the slope of rocks (Fig. 4A). Regarding the simultaneous hermaphroditism, many copulating pairs were also discovered (Fig. 4B). Information on its natural predators and parasites remains scarce, but the carnivorous streptaxid snails were found sympatrically in some localities (Fig. 4C, D).

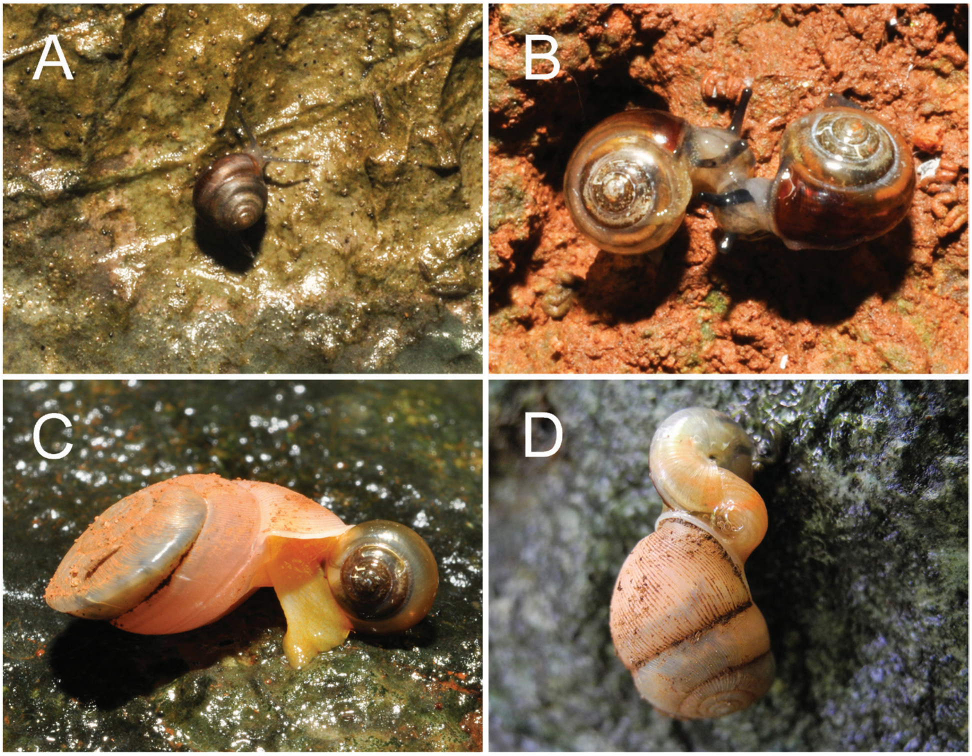

Figure 4. A adult of *B. moulmeinica* sp. nov. while climbing slowly on the karst wall after raining at Lun Nya Pagoda **B** mating pairs of *B. versicolor* sp. nov. on the karst wall at Bayin Nyi Cave **C** *B. versicolor* sp. nov. eaten by the carnivorous snail *Carinartemis* sp. on the karst wall at Bayin Nyi Cave **D** *B. cassidula* eaten by the carnivorous snail *Haploptychius* sp. on the karst wall at Kaw Gon Cave.

Table 1. Comparison of the morpho-anatomical characteristics of *Burmochlamys* gen. nov. and the possibly related genera of the Helicarionidae and Ariophantidae in mainland Southeast Asia. References: 1 = this study, 2 = Stoliczka (1871), 3 = Blanford and Godwin-Austen (1908), 4 = van Benthem Jutting (1950), 5 = Solem (1966), 6 = Schileyko (2002), 7 = Schileyko (2003), 8 = Páll-Gergely et al. (2016), 9 = Tanmuangpak et al. (2017), 10 = Pholyotha et al. (2018), 11 = Pholyotha et al. (2020b), 12 = Pholyotha et al. (2020c), 13 = Pholyotha et al. (2021a), 14 = Pholyotha et al. (2021b), and 15 = Sutcharit et al. (2020).

	Shell size (diameter)	Periphery	Shell lobe	Radula teeth	Epiphallic caecum	Flagellum	Dart apparatus	Ref.
Family Ariophantidae Godwin-Austen, 1883								
Khasiella* Godwin-Austen, 1899 (*Helix vidua* Hanley & Theobald, 1876)	small to medium	keeled or subangulate	short, slightly extended	central tricuspid, lateral tricuspid, marginal bicuspid	free bent	present	present	3,6
***Macrochlamys* Gray, 1847 (*Helix vitrinoides* Deshayes, 1831)**	small to large	round or anglulate	moderate to long, slender	central tricuspid, lateral tricuspid, marginal bicuspid	coiled	present	present	3, 7, 10, 11
Microcystina* Mörch, 1872 (*Nanina rinki* Mörch, 1872)	small	round	short to moderate, slender	central tricuspid, laterals tricuspid, marginals bicuspid	absent	absent	present[1]	3, 4, 7
Sakiella* Godwin-Austen, 1908 (*Helix honesta* Gould, 1846)	medium	subangulate	moderate, slender	central tricuspid, lateral tricuspid, marginal bicuspid	n.a.	n.a.	present	2, 3, 7
Sarika* Godwin-Austen, 1907 (*Helix resplendens* Philippi, 1847)	medium to large	round or angulate	moderate to long, slender	central tricuspid, lateral tricuspid, marginal bicuspid	straight	present	present	3, 11, 6, 12
Sesara* Albers, 1860 (*Helix infrendens* Gould, 1843)	small to medium	round, angulate, or carinate	absent	central tricuspid, lateral tricuspid, marginal bicuspid	straight[2]	present	absent	2, 3, 5, 6, 9
Taphrenalla* Pholyotha & Panha, 2020 (*Nanina diadema* Dall, 1897)	medium to large	round	long, slender	central tricuspid, lateral tricuspid, marginal bicuspid	straight to little bent	present	present	13
***Taphrospira* Blanford, 1904 (*Helix convallata* Benson, 1856)**	medium	round	long and fairly broad, covering partially	central tricuspid, laterals tricuspid, marginals bicuspid	straight	present	absent	3, 6
Family Helicarionidae Bourguignat, 1877								
Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017 (*Aenigmatoconcha clivicola* Tumpeesuwan & Tumpeesuwan, 2017)	medium	round	long, broad, enlarged and covering most of shell	central monocuspid, lateromarginal monocuspid	straight	present	absent	14
Chalepotaxis* Ancey, 1887 (*Helix similaris* var. *infantilis* Gredler, 1881)	small to medium	round	long, broad, enlarged and covering most of shell	central monocuspid, lateromarginal monocuspid	straight	absent	absent	8
Cryptaustenia* Cockerell, 1891 (*Vitrina planospira* Benson, 1859 (= *Vitrina succina* Reeve, 1862))	small to medium	round	long, broad, enlarged and covering most of shell	central tricuspid, lateral bi- or tricuspid, marginal bicuspid	absent	absent	present[3]	3, 5, 7
Durgella* Blanford, 1863 (*Helix levicula* Benson, 1859)	small to medium	round	long, broad, enlarged and covering most of shell	central unicuspid, lateromarginal teeth bicuspid with numerous cusps on the outer side	straight	absent	present[4]	3, 5, 6
Holkeion* Godwin-Austen, 1908 (*Helix anceps* Gould, 1843)	medium to large	sharply angulate	short to moderate, slender	central tricuspid, lateral tricuspid, marginal bicuspid	absent	present	present	3,7

	Shell size (diameter)	Periphery	Shell lobe	Radula teeth	Epiphallic caecum	Flagellum	Dart apparatus	Ref.
Sitala* Adams, 1865 (*Helix infula* Benson, 1848)	small to medium	subangulate or carinate	short, slightly extended	central tricuspid, lateromarginal teeth pointed with 2–5 cusps on the outer side	straight	absent	absent[5]	2,3
Sophina* Benson, 1859 (*Helix schistostelis* Benson, 1859)	small to medium	round	long, broad, enlarged and covering most of shell	central monocuspid, lateromarginal monocuspid	straight	absent	present	2,3,15
Burmochlamys* gen. nov. (*Burmochlamys fasciola* sp. nov.)	small	round	short to moderate, slender	central monocuspid, lateromarginal monocuspid	straight	absent	present	1

* Genitalia of the type species were examined.

[1] *Microcystina bintennensis* Godwin-Austen, 1899 does not have dart apparatus (see Blanford and Godwin-Austen 1908).

[2] *Sesara parva* Solem, 1966 has a spirally coiled epiphallic caecum (Solem 1966).

[3] *Cryptaustenia gadinodromica* Solem, 1966 does not have dart apparatus (Solem 1966).

[4] *Durgella assamica* Godwin-Austen, 1881 and *D. rogersi* Godwin-Austen, 1907 do not have dart apparatus (Blanford and Godwin-Austen 1908).

[5] *Sitala attegia* (Benson, 1859) has a well-developed dart apparatus (Stoliczka 1871; Blanford and Godwin-Austen 1908).

***Burmochlamys cassidula* (Benson, 1859), comb. nov.**

Figs 1, 3C, 4D, 5B, 6A, B, 8A–C, 9A, 11A

Helix cassidula Benson, 1859: 186. Type locality: ad Moulmein, nee raro [Mawlamyine, Mon State, Myanmar].

Hyalinia cassidula — Tryon, 1886: 177, pl. 53, fig. 68.

Material examined. Type material. Moulmein: probable syntype UMZC I.104235.

Other material. Kaw Gon Cave, Hpa-An, Kayin State, Myanmar (16°49'22.2"N, 97°35'08.9"E): CUMZ 14209 (Fig. 6A, B).

Diagnosis. Shell globose, milky white with yellow tinge, and with wide yellowish brown band. Animal greyish with five mantle extensions. Genitalia with very short epiphallic caecum attached by thin penial retractor muscle, very short and large vagina, and short and large gametolytic duct.

Description. ***Shell*** (Figs 6A, B, 8A–C). Shell globose, small (width up to 7.8 mm, height up to 6.3 mm), rather thin, semi-translucent. Colour milky-white with yellow tinge with wide yellowish brown band above periphery. Protoconch and teleoconch surface with similar sculpture. Surface of body whorl with distinct spiral furrows at regular intervals, cut by distinctly undulating radial lines (Fig. 8A–C). Whorls 6–6½, increasing regularly; suture impressed; spire much elevated; varix usually present; last whorl rounded on periphery, and little convex below. Aperture obliquely crescent-shaped; peristome simple. Columellar margin simple and slightly reflected near umbilicus. Umbilicus open, narrow, and deep.

Genital organs (Fig. 9A). Atrium (at) very short. Penis (p) rather short, cylindrical, and with a penial sheath. Epiphallus (e1+e2) ca. three-quarters of penis length; e1 cylindrical and its diameter smaller than penis and e2; e2 bulbous and ca. half of e1

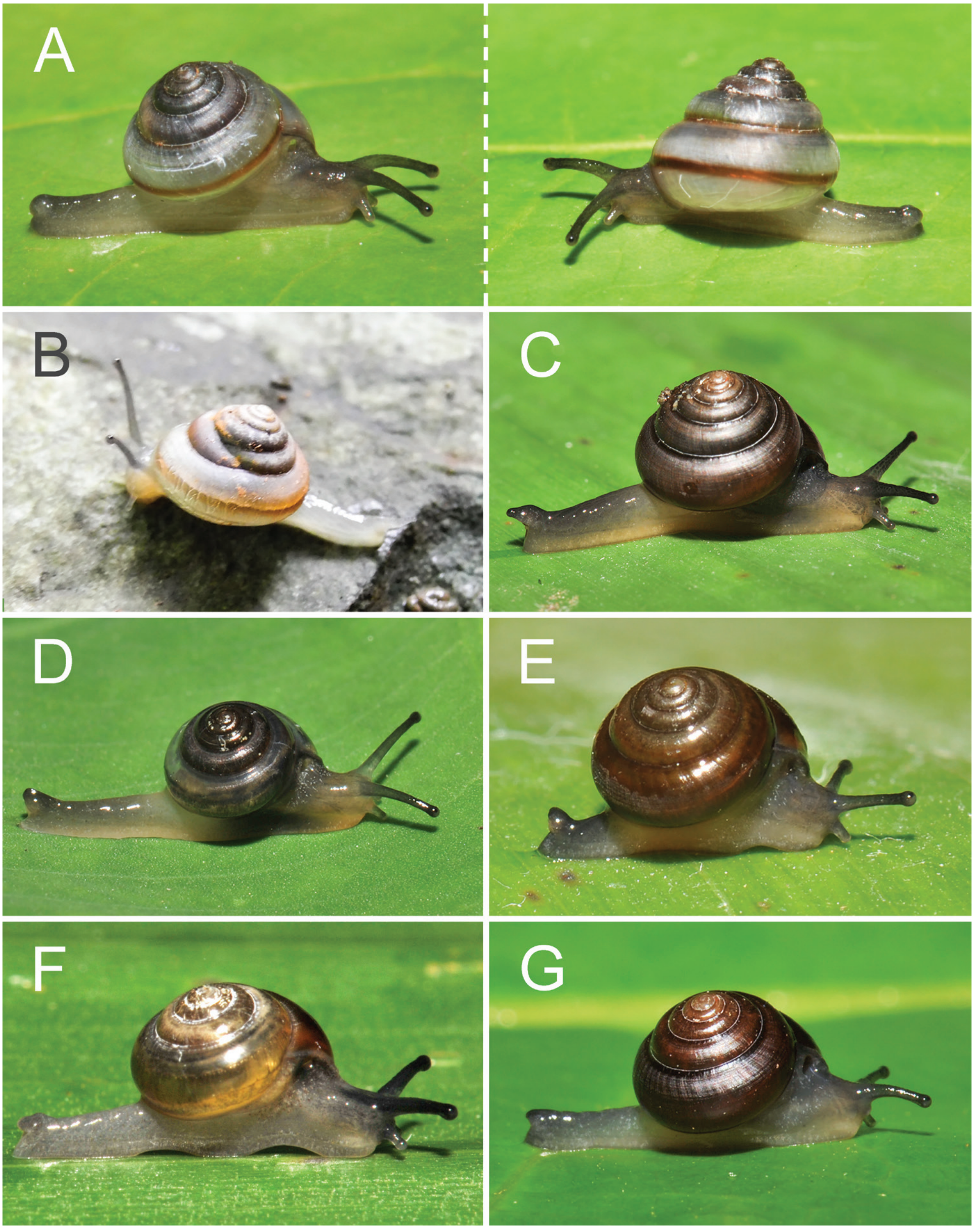

Figure 5. Living snails of *Burmochlamys* species **A** *B. fasciola* sp. nov. paratype CUMZ 14214 showing left and right lateral views **B** *B. cassidula* specimen CUMZ 14209 **C** *B. poongee* specimen CUMZ 14210 **D** *B. albida* sp. nov. paratype CUMZ 14212 **E** *B. moulmeinica* sp. nov. paratype CUMZ 14217 **F** *B. versicolor* sp. nov. paratype CUMZ 14219 **G** *B. whitteni* sp. nov. paratype CUMZ 14222.

length. Epiphallic caecum (ec) very short, cylindrical and with thin penial retractor muscle (prm) attached at tip. Vas deferens (vd) thin tube. Dart apparatus large, rather long cylindrical, and located on atrium at vagina and penis junction. Vagina (v) large, very short, and cylindrical. Gametolytic sac (gs) bulbous; gametolytic duct (gd) short, ca. one-quarter of penis length, cylindrical, and very enlarged near vagina. Free oviduct (fo) as long as penis, cylindrical, and encircled with thick tissue near vagina.

Radula (Fig. 11A). Teeth arranged in wide U-shaped rows with each row consisting of ~ 75 teeth. Central tooth monocuspid, large and ovate spatulate shape, with two smaller triangular-shaped teeth located at base of central tooth. Laterals and marginals not differentiated, monocuspid, large spatulate shape then gradually become narrower, elongated and rather pointed cusp. One smaller triangular-shaped tooth located at base on outer side of each tooth, and then gradually reduced in size outwards. Some outermost teeth with tiny inner cusp.

External appearance (Figs 3C, 5B). Animal with reticulated skin, pale creamy grey to greyish body. Five well-developed mantle extensions same colour as body; right shell lobe (rsl) larger and longer than left shell lobe (lsl); right dorsal lobe (rdl) larger than both anterior left dorsal lobe (ant-ldl) and posterior left dorsal lobe (post-ldl). Sole divided into three parts longitudinally. Caudal foss and caudal horn present.

Distribution. *Burmochlamys cassidula* is known only from Kaw Gon Cave in Myanmar (Fig. 1). The isolated limestone outcrop is surrounded by paddy fields and temporary wetlands (flooded in the monsoon season). We searched after rain and found them on the surface of limestone walls. The carnivorous snail *Haploptychius* sp. was also found sympatrically at this locality (Fig. 4D).

Remarks. *Burmochlamys cassidula* is easy to distinguish from all other recognised congeners by its unique shell colour pattern: milky-white with a yellow tinge and with one wide yellowish brown peripheral band.

Burmochlamys cauisa (Benson, 1859), comb. nov.

Fig. 6C

Helix cauisa Benson, 1859: 388. Type locality: Phie Than, vallis Tenasserim [Phie Than, Tenasserim Valley].

Helix causia [sic] — Pfeiffer, 1868: 118; Hanley and Theobald 1874: 37, pl. 90, figs 2, 3; Tryon 1887: 102, pl. 15, figs 67, 68.

Macrochlamys causia [sic] — Godwin-Austen, 1907: 163; Blanford and Godwin-Austen 1908: 117.

Macrochlamys cauisa — Pholyotha et al. 2020b: 186, 187, fig. 3a.

Material examined. Type material. Tenasserim: probable syntypes UMZC I.102465.

Other material. Kalryenmullay Hills, Tenasserim: NHMUK 1888.12.4.465–466 ex. Blanford Coll. (two shells; Fig. 6C; specimen figured in Pholyotha et al. 2020b: fig. 3a).

Remarks. Shell morphology of *B. cauisa* is matched well to this new genus rather than the depressed and lustrous shell of the *Macrochlamys* (see Pholyotha et al. 2020b). The unique shell morphology is subglobose, small size (width of ~ 7.0 mm, height of ~ 4.0 mm), obliquely striated and very minutely longitudinal lines, moderately elevated spire, enlarged and well-rounded last whorl, ovate lunate aperture, simple peristome,

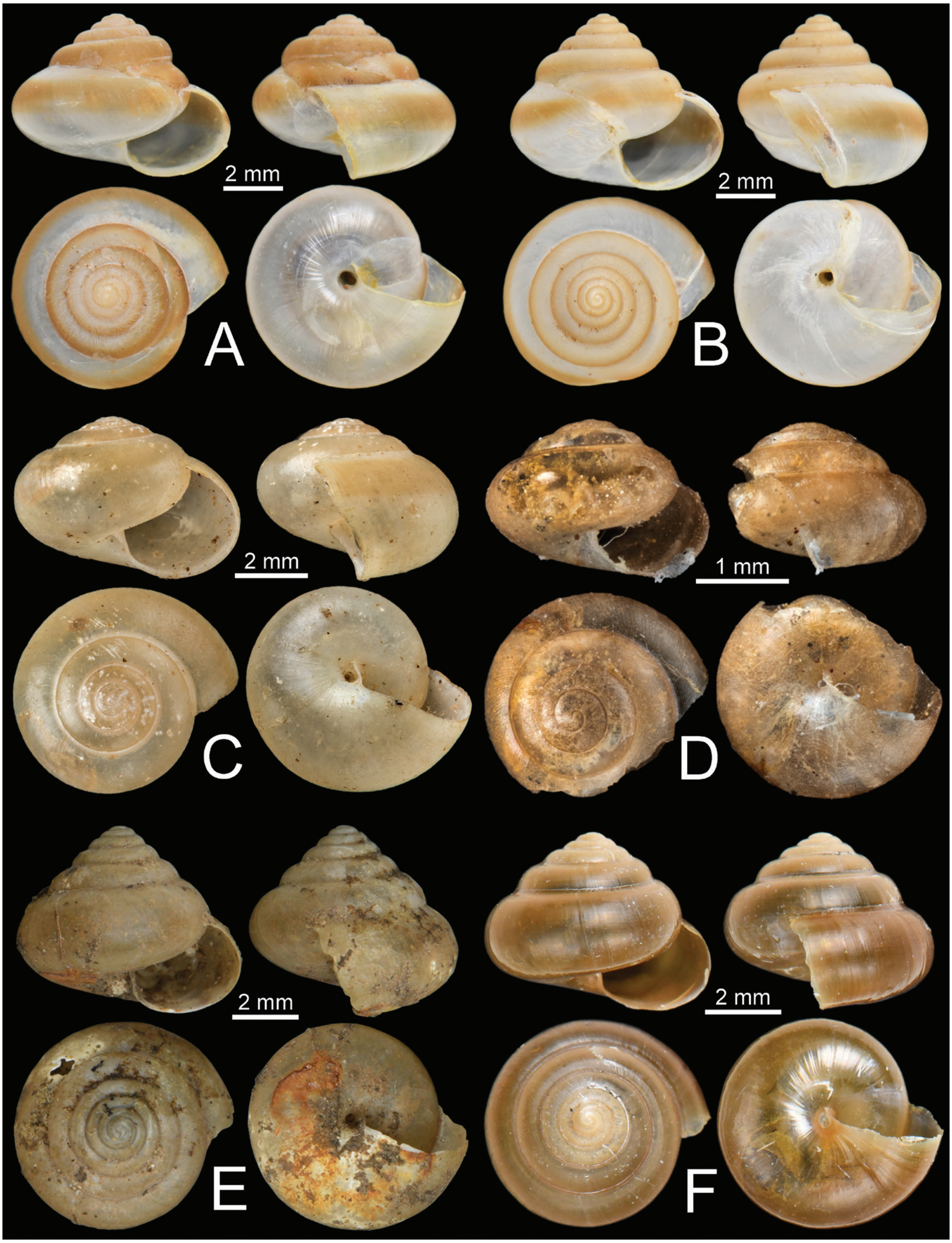

Figure 6. Shells of *Burmochlamys* species **A, B** *B. cassidula* specimen CUMZ 14209 **C** *B. cauisa* specimen NHMUK 1888.12.4.465-466 **D** *B. perpaula* specimen NHMUK 1903.7.1.533 **E, F** *B. poongee* **E** specimen NHMUK 1888.12.4.23-6 **F** specimen CUMZ 14210.

simple columellar margin with slightly reflected near umbilicus, and narrowly open umbilicus (Fig. 6C; Benson 1859; Blanford and Godwin-Austen 1908). Unfortunately, we could not find any specimens identifiable to this species during this survey.

Burmochlamys cauisa is currently known only from the type locality and vicinity of Salween River basin (Blanford and Godwin-Austen 1908). It is easy to distinguish from all other congeners by its moderately elevated spire and rather broad ovate lunate aperture. In comparison, most species of *Burmochlamys* gen. nov. have a higher shell spire and narrower aperture opening.

Burmochlamys perpaula **(Benson, 1859), comb. nov.**

Fig. 6D

Helix perpaula Benson, 1859: 390. Type locality: Phie Thán, raro [Phie Than, Tenasserim Valley].
Helix perpaula — Pfeiffer, 1868: 69.
Nanina (Macrochlamys) perpaula — Tryon, 1886: 89, pl. 29, fig. 37.
Macrochlamys perpaula — Godwin-Austen, 1883: 89, pl. 14, fig. 5; Blanford and Godwin-Austen 1908: 123; Pholyotha et al. 2020b: 187, 188, fig. 3b.

Material examined. Type material. The type series could not be located.

Other material. Moulmein: NHMUK 1903.7.1.533 ex. Godwin-Austen Coll. (one shell; Fig. 6D; specimen figured in Godwin-Austen 1883: pl. 14, fig. 5 and Pholyotha et al. 2020b: fig. 3b), NHMUK ex. MacAndrew Coll. Acc. No. 1563 (six shells), NHMUK 1912.4.16.400 (three shells).

Remarks. *Burmochlamys perpaula* is currently known only from the type locality in Tenasserim Valley (Benson 1859; Blanford and Godwin-Austen 1908). The original type series could not be located, and no specimens were found in this study. Therefore, the generic placement is still provisional and awaiting for further anatomical information. However, *Helix perpaula* is transferred to this new genus, which it distinct from the *Macrochlamys* by a numbers of shell morphology (see Pholyotha et al. 2020b).

Burmochlamys perpaula is characterised by subglobose, small size (width of ~ 2.0 mm, height of ~ 1.3 mm), obliquely striated and very minutely spirally ribbed throughout, moderately elevated spire, rather more convex body whorl, and narrowly crescent-shaped aperture, simple peristome, simple columellar margin with slightly reflected near umbilicus, and narrowly open umbilicus (Fig. 6D; Benson 1859; Blanford and Godwin-Austen 1908). In addition, this species can be distinguished from all other congeners by its moderately elevated spire, rather more convex body whorl, and narrower umbilicus. In comparison, most species of *Burmochlamys* gen. nov. have a higher shell spire, rather broad and well-rounded last whorl, and relatively wider umbilicus.

Burmochlamys poongee **(Theobald, 1859), comb. nov.**

Figs 1, 2B, 3B, 5C, 6E, F, 8D–F, 9B, 11B

Helix poongee Theobald, 1859: 307. Type locality: prope Moulmein [Mawlamyine, Mon State, Myanmar].

Helix poongee — Pfeiffer, 1868: 134; Hanley and Theobald 1876: 8, pl. 16, fig. 9.
Helix poongi Theobald, 1876: 19 [incorrect subsequent spelling].
Macrochlamys poongee — Godwin-Austen, 1882: 90, pl.14, fig. 1; Pholyotha et al. 2020b: 190, 191, figs 3e, 3f.
Nanina poongee — Tryon, 1886: 98, pl. 33, fig. 70.
Macrochlamys pungi — Blanford and Godwin-Austen, 1908: 122. [unjustified emendation; ICZN 1999: Art.19.1 and 33.2.3].

Material examined. Type material. The type series could not be located.

Other material. Moulmein: NHMUK 1869.06.1.1 ex. Blanford Coll. (one shell; specimen figured in Pholyotha et al. 2020b: fig. 3e), NHMUK 1888.12.4.23–26 ex. Blanford Coll. (four shells; Fig. 6E; specimen figured in Pholyotha et al. 2020b: fig. 3f), NHMUK ex. Blanford Coll. (two shells). Kaw Ka Thaung Cave, Hpa-An, Kayin State, Myanmar (16°49'42.0"N, 97°42'22.9"E): CUMZ 14210 (Fig. 6F).

Diagnosis. Shell globose and pale to dark brownish. Animal pale to dark greyish with a brown or yellow tinge and five mantle extensions. Genitalia with slender epiphallus, rather short epiphallic caecum attached by a thin penial retractor muscle, and very long and slender at the base of dart apparatus.

Description. ***Shell*** (Figs 6E, F, 8D–F). Shell globose, small (width up to 6.5 mm, height up to 5.2 mm), rather thin, semi-translucent. Colour pale to dark brownish. Protoconch and teleoconch surface with similar sculpture. Surface of body whorl with distinct spiral furrows at regular intervals, crossed by distinctly undulating radial lines (Fig. 8D–F). Whorls 6–7, increasing regularly; suture shallowly impressed; spire much elevated; last whorl well-rounded. Aperture obliquely crescent-shaped; peristome simple. Columellar margin simple, slightly reflected near umbilicus. Umbilicus open, narrow, and deep.

Genital organs (Fig. 9B). Atrium (at) rather short. Penis (p) moderate, cylindrical, and with a penial sheath. Epiphallus (e1+e2), slender, ca. four-fifths of penis length; e1 cylindrical and its diameter smaller than penis and e2; e2 bulbous and ca. one-third of e1 length. Epiphallic caecum (ec) rather short, cylindrical, and with a thin penial retractor muscle (prm) attached at tip. Vas deferens (vd) thin tube. Dart apparatus large, very long cylindrical with at the base very long, small, convoluted, and located on atrium near genital orifice. Vagina (v) rather short, and cylindrical. Gametolytic sac (gs) bulbous; gametolytic duct (gd) cylindrical, moderate, ca. three-fifths of penis length. Free oviduct (fo) ca. three-fifths of penis length, cylindrical, and encircled with thick tissue near vagina.

Radula (Fig. 11B). Resembles *B. cassidula*. Teeth arranged in wide U-shaped rows with each row consisting of ~ 60 teeth. Central tooth monocuspid, large and ovate spatulate shape; with two smaller triangular-shaped plates. Laterals and marginals not differentiated, monocuspid, large spatulate shape and then gradually become narrower, elongate, and rather more pointed cusps, and with one smaller triangular-shaped plate at base. Some outermost teeth with small and pointed cusp at inner side.

External appearance (Fig. 3B, 5C). Living animal with reticulated skin, pale to dark greyish with a brown or yellow tinge, slightly lighter on foot sole and darker colour on caudal horn. Mantle extension well developed with three dorsal lobes and two shell lobes, similar colour to body.

Distribution. *Burmochlamys poongee* can be found only from Kaw Ka Thaung Cave in Myanmar (Figs 1, 2B). This limestone is surrounded by paddy fields which are temporarily flooded during the monsoon season (Fig. 2B).

Burmochlamys albida Pholyotha & Panha, sp. nov.

https://zoobank.org/CF80F28E-FFF3-4C4E-A453-D383CA8FA1E2
Figs 1, 2C, 5D, 7A, B, 8G–I, 9C, 11C

Material examined. Type material. ***Holotype*****:** CUMZ 14211 (Fig. 7A; width 4.5 mm, height 3.7 mm). ***Paratypes*****:** Same locality as holotype: CUMZ 14212 (Fig. 7B; width 4.4 mm, height 3.2 mm), NHMUK (two shells).

Type locality. Limestone outcrop at Waiponla Monastery, Hpa-An, Kayin State, Myanmar (16°56'07.4"N, 97°42'56.8"E).

Diagnosis. Shell globose and milky-white with a yellow tinge, rarely with a faintly yellowish brown peripheral band. Animal pale fleshy grey with brown or yellow tinge and five mantle extensions. Genitalia with rather short epiphallic caecum attached by a very large and thick penial retractor muscle and rather short vagina.

Description. ***Shell*** (Figs 7A, B, 8G–I). Shell globose, small (width up to 4.5 mm, height up to 3.7 mm), thin, semi-translucent. Colour milky-white with a yellow tinge and without or rarely with faintly yellowish brown band above periphery. Protoconch and teleoconch surface with similar sculpture. Surface of body whorl with little distinct spiral furrows at regular intervals, crossed by very faintly undulating radial lines (Fig. 8G–I). Whorls 6–6½, increasing regularly; suture shallowly impressed; spire much elevated; last whorl well-rounded. Aperture obliquely crescent-shaped; peristome simple. Columellar margin simple, slightly reflected near umbilicus. Umbilicus open, narrow, and deep.

Genital organs (Fig. 9C). Atrium (at) rather short. Penis (p) moderate, cylindrical, and with penial sheath covering more than half of its length. Epiphallus (e1+e2) ca. three-sevenths of penis length; e1 cylindrical and small diameter smaller than penis and e2; e2 bulbous and ca. half of e1 length. Epiphallic caecum (ec) rather short, cylindrical, and with very large and thick penial retractor muscle (prm) attached at tip. Vas deferens (vd) thin tube. Dart apparatus large, long cylindrical, and located on atrium close to genital orifice. Vagina (v) rather short, cylindrical-shaped. Gametolytic sac (gs) bulbous; gametolytic duct (gd) cylindrical, moderate, ca. half of penis length. Free oviduct (fo) ca. one-quarter of penis length, cylindrical, and encircled with thick tissue near vagina.

Radula (Fig. 11C). Generally resembles *B. cassidula*. Teeth arranged in wide U-shaped rows, each row consisting of ~ 55 teeth. Central tooth monocuspid, large, and ovate spatulate shape; with two smaller triangular-shaped teeth. Laterals and marginals not differentiated, monocuspid, large spatulate shape and then gradually become narrower, elongate, rather more pointed cusp, and with one smaller triangular-shaped tooth. Some outermost teeth with a small, pointed cusp at the inner side.

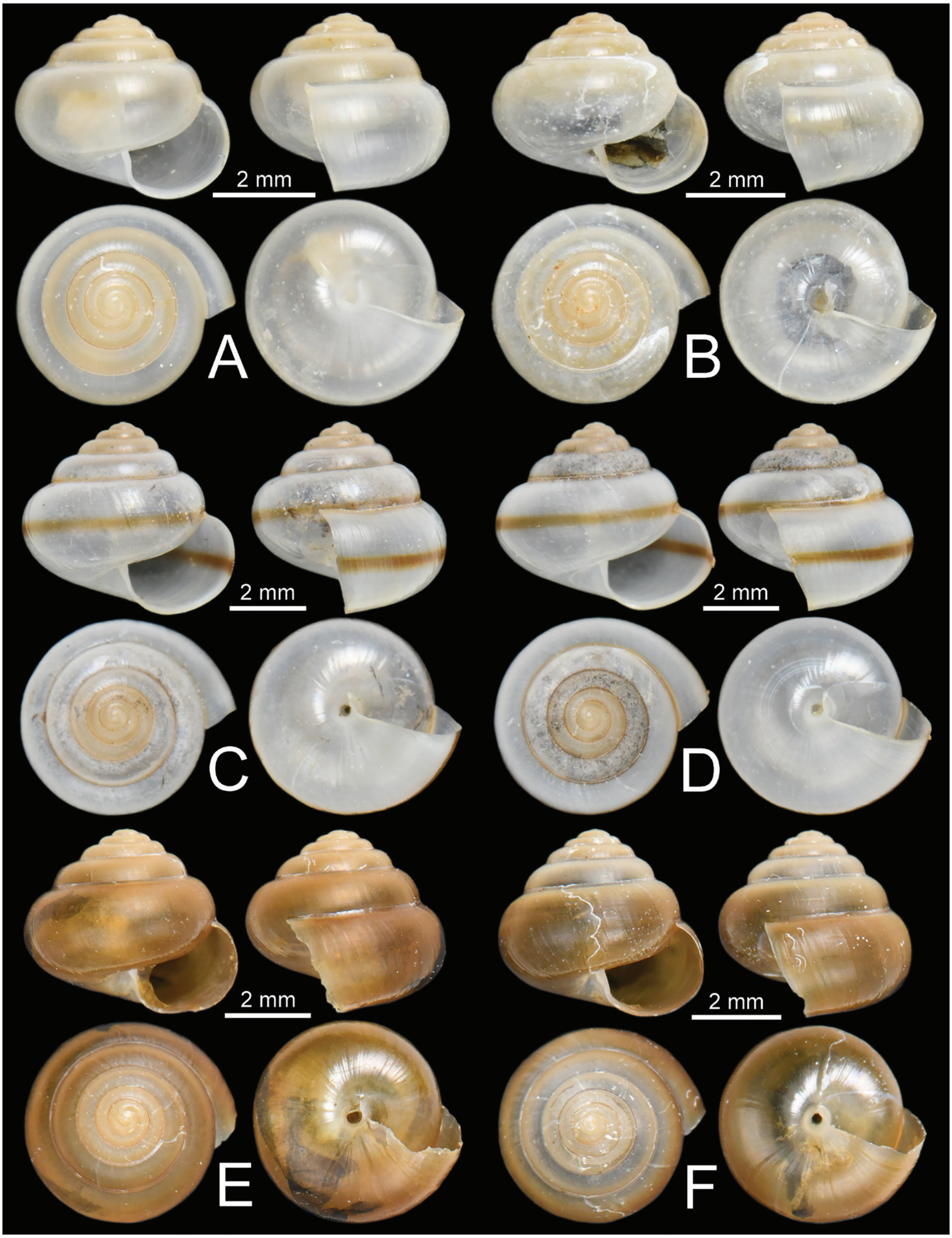

Figure 7. Shells of *Burmochlamys* species **A, B** *B. albida* sp. nov. **A** holotype CUMZ 14211, and **D** paratype CUMZ 14212 **C, D** *B. fasciola* sp. nov. **C** holotype CUMZ 14213 **D** paratype CUMZ 14214 **E, F** *B. moulmeinica* sp. nov. **E** holotype CUMZ 14216 **F** paratype CUMZ 14217.

External appearance (Fig. 5D). Living animal with reticulated skin, pale freshy-grey with a brown or yellow tinge. Five well-developed mantle extensions, with similar colour to body. Sole divided into three parts longitudinally; caudal foss and caudal horn well developed, similar colour to body.

Etymology. The specific epithet *albida* is from the Latin word *albidus* meaning white. It refers to the whitish shell, which characterises this species.

Distribution. *Burmochlamys albida* sp. nov. is endemic to a limestone outcrop at Waiponla Monastery. The surrounding paddy fields usually become flooded during the monsoon season (Figs 1, 2C). In addition, this new species is also sympatric with the limestone karst-restricted species, *Sophina salweenica* Sutcharit & Panha, 2020.

Remarks. Among the whitish-shelled species (see Table 2), *B. albida* sp. nov. has a very indistinctly narrow yellowish brown peripheral band, whereas *B. cassidula* has a wide yellowish brown peripheral band, *B. fasciola* sp. nov. has a narrow yellowish brown peripheral band, and *B. versicolor* sp. nov. has yellowish brown body whorl near the aperture and wider umbilicus than the other preceding species. Anatomically, *B. albida* sp. nov. has a large and thick penial retractor muscle, rather slender vagina, and simple at the tip of dart apparatus. In comparison, *B. cassidula* has a thin penial retractor muscle, large and short vagina, and soft at the tip of dart apparatus, while *B. fasciola* sp. nov. has a thin penial retractor muscle, very short vagina, and solid at the tip of dart apparatus. In addition, *B. versicolor* sp. nov. has a thin penial retractor muscle, rather slender vagina, and relatively smaller dart apparatus with soft at the tip.

Burmochlamys albida sp. nov. differs from the remaining *Burmochlamys* species by having a milky-white shell. While most other species have a pale to dark brownish shell without a peripheral band (see Table 2). Regardless of the shell colour and genitalia, this new species differs from *B. cauisa* and *B. perpaula* by spire, body whorl, and aperture. In comparison, *B. cauisa* has slightly elevated spire, well-rounded last whorl, and rather broad crescent-shaped aperture, while *B. perpaula* has slightly elevated spire, rather more convex body whorl, and narrower crescent-shaped aperture.

Burmochlamys fasciola Pholyotha & Panha, sp. nov.

https://zoobank.org/BDE9EEDE-1DAC-418E-9097-1EDCC579299B
Figs 1, 2A, 3A, 5A, 7C, D, 8J–L, 10A, 11D

Material examined. Type material. ***Holotype*:** CUMZ 14213 (Fig. 7C; width 5.6 mm, height 5.1 mm). ***Paratypes*:** Same locality as holotype: CUMZ 14214 (Fig. 7D; width 5.8 mm, height 4.9 mm), NHMUK (two shells).

Other material. Kyankaw Mountain, Hpa-An, Kayin State, Myanmar (17°00'59.5"N, 97°42'12.4"E): CUMZ 14215.

Type locality. Bardai Mountain, Hpa-An, Kayin State, Myanmar (17°00'00.5"N, 97°41'41.6"E).

Diagnosis. Shell globose and milky white with narrow yellowish brown band. Animal pale freshy-grey with five mantle extensions. Genitalia with rather short epiphallic caecum attached by thin penial retractor muscle, very short vagina, and solid at the tip of dart apparatus.

Description. ***Shell*** (Figs 7C, D, 8J–L). Shell globose, small (width up to 6.1 mm, height up to 6.0 mm), thin, semi-translucent. Colour milky-white with a narrow yellowish brown band above periphery. Protoconch and teleoconch surface with similar

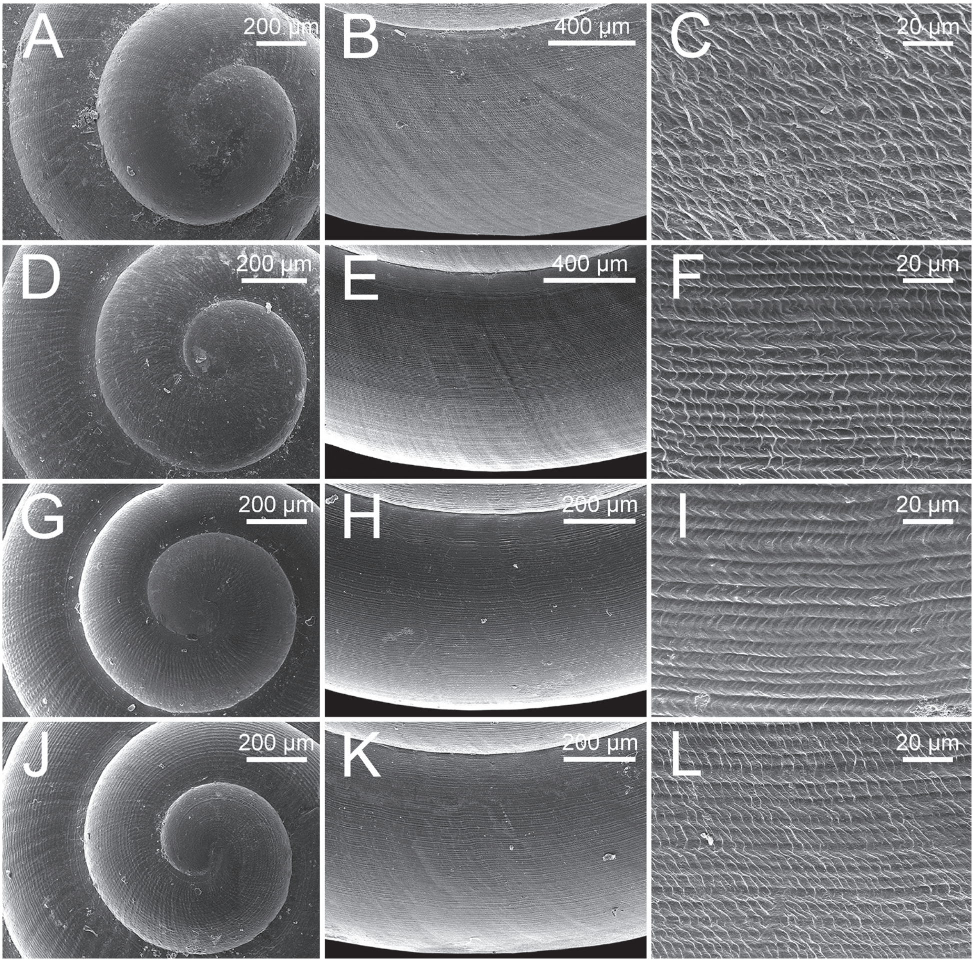

Figure 8. Representative SEM images of the shell of *Burmochlamys* species **A–C** *B. cassidula* specimen CUMZ 14209 **A** protoconch **B** body whorl **C** zoom-in view of body whorl **D–F** *B. poongee* specimen CUMZ 14210 **D** protoconch **E** body whorl **F** zoom-in view of body whorl **G–I** *B. albida* sp. nov. paratype CUMZ 14212 **G** protoconch **H** body whorl **I** zoom-in view of body whorl **J–L** *B. fasciola* sp. nov. paratype CUMZ 14214 **J** protoconch **K** body whorl **L** zoom-in view of body whorl.

sculpture. Surface of body whorl with distinct spiral furrows at regular intervals, cut by distinctly undulating radial lines (Fig. 8J–L). Whorls 6–6½, increasing regularly; suture shallowly impressed; spire much elevated; last whorl well-rounded. Aperture obliquely crescent-shaped; peristome simple. Columellar margin simple, slightly reflected near umbilicus. Umbilicus open, narrow, and deep.

Genital organs (Fig. 10A). Atrium (at) very short or indistinct. Penis (p) moderate, cylindrical and with a thin penial sheath. Epiphallus (e1+e2) ca. three-fifths of penis length; e1 cylindrical and smaller diameter than penis and e2; e2 bulbous and ca. half of e1 length. Epiphallic caecum (ec) rather short, cylindrical and with a thin penial retractor muscle (prm) attached at tip. Vas deferens (vd) thin tube. Dart apparatus large, long cylindrical, with solid at the tip (yellow arrow in Fig. 10A), and located on atrium at vagina and penis junction. Vagina (v) very short to indistinguishable. Gametolytic sac (gs) bulbous; gametolytic duct (gd) cylindrical, moderate, slightly shorter than penis. Free oviduct (fo) ca. half of penis length, cylindrical, and encircled with thick tissue near vagina.

Radula (Fig. 11D). Teeth arranged in wide U-shaped rows with each row consisting of ~ 50 teeth. Central tooth monocuspid, large and oblong spatulate plate, and attached by two smaller triangular-shaped teeth at its base. Laterals and marginals not differentiated and monocuspid; each tooth with large and oblong spatulate plate attached by only a smaller triangular-shaped tooth at base on outer side. From inner to outer, lateromarginal teeth gradually narrower, smaller, and rather more pointed cusp. Some outermost teeth with a small and pointed cusp at inner side.

External appearance (Figs 3A, 5A). Living animal with reticulated skin and pale freshy-grey body. Five mantle extensions present, same colour as body. Sole divided into three parts longitudinally. Caudal foss (cf) and caudal horn (ch) present with similar colour to body.

Etymology. The specific epithet is the Latin word *fasciola* meaning band or stripe. It refers to the presence of a brownish peripheral band, which characterises this species.

Distribution. *Burmochlamys fasciola* sp. nov. is known from two limestone areas in the south of the Salween River basin (Fig. 1). The Bardai Mountain (Fig. 2A) and Kyankaw Mountain are surrounded by paddy fields that are temporarily flooded during the monsoon season. In Kyankaw Mountain, this new species is sympatric with the limestone karst-restricted land snail species, *Sophina salweenica*.

Remarks. The distinguishing characters of *B. fasciola* sp. nov. is a milky-white shell with a narrow yellowish brown peripheral band and rather elongated radular teeth. Whereas the other *Burmochlamys* species have a pale to dark brownish shell without any band and the radular teeth have a broad spatulate shape (see Table 2).

Burmochlamys moulmeinica Pholyotha & Panha, sp. nov.

https://zoobank.org/E00C82AB-384E-408D-AEBC-F011AA9C2DD7
Figs 1, 2D, 4A, 5E, 7E, F, 10B, 13A–C, 15A

Material examined. Type material. *Holotype*: CUMZ 14216 (Fig. 7E; width 5.0 mm, height 4.2 mm). ***Paratypes*:** Same locality as holotype: CUMZ 14217 (Fig. 7F; width 4.5 mm, height 4.2 mm), NHMUK (two shells).

Type locality. Limestone outcrop at Lun Nya Pagoda, Hpa-An, Kayin State, Myanmar (16°44'53.8"N, 97°47'09.1"E).

Diagnosis. Shell globose and pale to dark brownish. Animal greyish with five mantle extensions. Genitalia with very short epiphallic caecum attached by a thin penial retractor muscle and rather short vagina.

Description. *Shell* (Figs 7E, F, 13A–C). Shell globose, small (width up to 5.0 mm, height up to 4.2 mm), rather thin, and semi-translucent. Colour pale to dark brownish. Protoconch and teleoconch surface with similar sculpture. Surface of body whorl with dis-

Table 2. Comparison of shell, radula, genital system, and distribution of all members of *Burmochlamys* gen. nov. in Myanmar.

	B. cassidula	***B. cauisa****	***B. perpaula****	***B. poongee***	***B. albida* sp. nov.**	***B. fasciola* sp. nov.**	***B. moulmeinica* sp. nov.**	***B. versicolor* sp. nov.**	***B. whitteni* sp. nov.**
shell colour	whitish with a yellow tinge	pale yellowish brown	dark brown	pale to dark brown	whitish with a yellow tinge	whitish	pale to dark brown	whitish yellow / yellowish brown	pale to dark brown
peripheral band	wide	absent	absent	absent	absent or rarely indistinct	narrow	absent	absent	absent
shell shape	globose	subglobose	subglobose	globose	globose	globose	globose	subglobose	globose
microscopic sculpture	present	present	present	present	present	present	present	present but indistinct	present
umbilicus	narrow	narrow	very narrow	narrow	narrow	narrow	narrow	moderate	narrow
central tooth shape	broad spatulate	–	–	broad spatulate	broad spatulate	narrow spatulate	broad spatulate	narrow spatulate	broad spatulate
penis	rather short			moderate	moderate	moderate	moderate	moderate	rather long
penial retractor muscle	thin	–	–	thin	thick	thin	thin	thin	thick
vagina	very short	–	–	short	short	very short	short	short	very short
the tissue surrounding gametolytic part and free oviduct	absent	–	–	absent	absent	absent	absent	absent	present
dart apparatus near atrium	rather long	–	–	very long	rather long	rather short	rather short	rather short	rather long
at the tip of dart apparatus	soft	–	–	soft	soft	solid	soft	soft	soft

*Information from Blanford and Godwin-Austen (1908).

tinct spiral furrows at regular intervals, cut by distinctly undulating radial lines (Fig. 13A–C). Whorls 6–6½, increasing regularly; suture shallowly impressed; spire much elevated; last whorl well-rounded. Aperture obliquely crescent-shaped; peristome simple. Columellar margin simple, slightly reflected near umbilicus. Umbilicus open, narrow, and deep.

Genital organs (Fig. 10B). Atrium (at) very short. Penis (p) moderate, cylindrical and with thin penial sheath. Epiphallus (e1+e2) ca. half of penis length; e1 cylindrical and smaller diameter smaller than penis and e2; e2 bulbous and ca. half of e1 length. Epiphallic caecum (ec) very short, bulbous, and with thin penial retractor muscle (prm) attached at tip. Vas deferens (vd) thin tube. Dart apparatus large, long cylindrical, and located on atrium at vagina and penis junction. Vagina (v) rather short, cylindrical. Gametolytic sac (gs) bulbous; gametolytic duct (gd) cylindrical, rather short, ca. half of penis length. Free oviduct (fo) ca. two-third of penis length, cylindrical, and encircled with thick tissue near vagina.

Radula (Fig. 15A). Resembles *B. cassidula*. Teeth arranged in wide U-shaped rows with each row consisting of ~ 50 teeth; central tooth monocuspid, large and ovate spatulate shape with both sides of its base attached by two triangular-shaped plates; laterals and marginals not differentiated, monocuspid, large spatulate shape then gradually become narrower, elongate, smaller, and rather more pointed cups outwards, and attached at base and on outer side by one smaller triangular-shaped plate.

External appearance (Figs 4A, 5E). Living animal with reticulated skin, grey body, slightly lighter on foot sole and darker colour on caudal horn. Mantle extensions with three dorsal lobes and two shell lobes; same colour as body.

Etymology. The specific epithet *moulmeinica* is a noun in reference to the historical name of Mawlamyine city, pertaining to the Salween River basin, where the type locality is situated.

Distribution. *Burmochlamys moulmeinica* sp. nov. is endemic to a small limestone area at Lun Nya Pagoda in Myanmar (Figs 1, 2D). The isolated limestone is surrounded by paddy fields that are temporarily flooded during the monsoon season (Fig. 2D). In addition, this new species is also sympatric with the limestone karst-restricted species, *Sophina pisinna* Sutcharit & Panha, 2020.

Remarks. *Burmochlamys moulmeinica* sp. nov. is similar to *B. poongee* in shell morphology but can be differentiated by genitalia. This new species has a relatively short epiphallus and a rather short at the base of dart apparatus, whereas *B. poongee* has a slender and longer epiphallus and a very long and small at the base of dart apparatus.

Burmochlamys versicolor **Pholyotha & Panha, sp. nov.**

https://zoobank.org/096635E4-809B-437B-BE16-197FD4D38D1B
Figs 1, 2E, 4B, C, 5F, 10C, 12A, B, 13D–F, 15B

Material examined. Type material. *Holotype*: CUMZ 14218 (Fig. 12A; width 6.0 mm, height 4.2 mm). ***Paratypes*:** Same locality as holotype: CUMZ 14219 (Fig. 12B; width 5.9 mm, height 4.5 mm), NHMUK (two shells).

Other material. Limestone outcrop at Golden valley, Hpa-An, Kayin State, Myanmar (16°51'02.3"N, 97°36'26.1"E): CUMZ 14220.

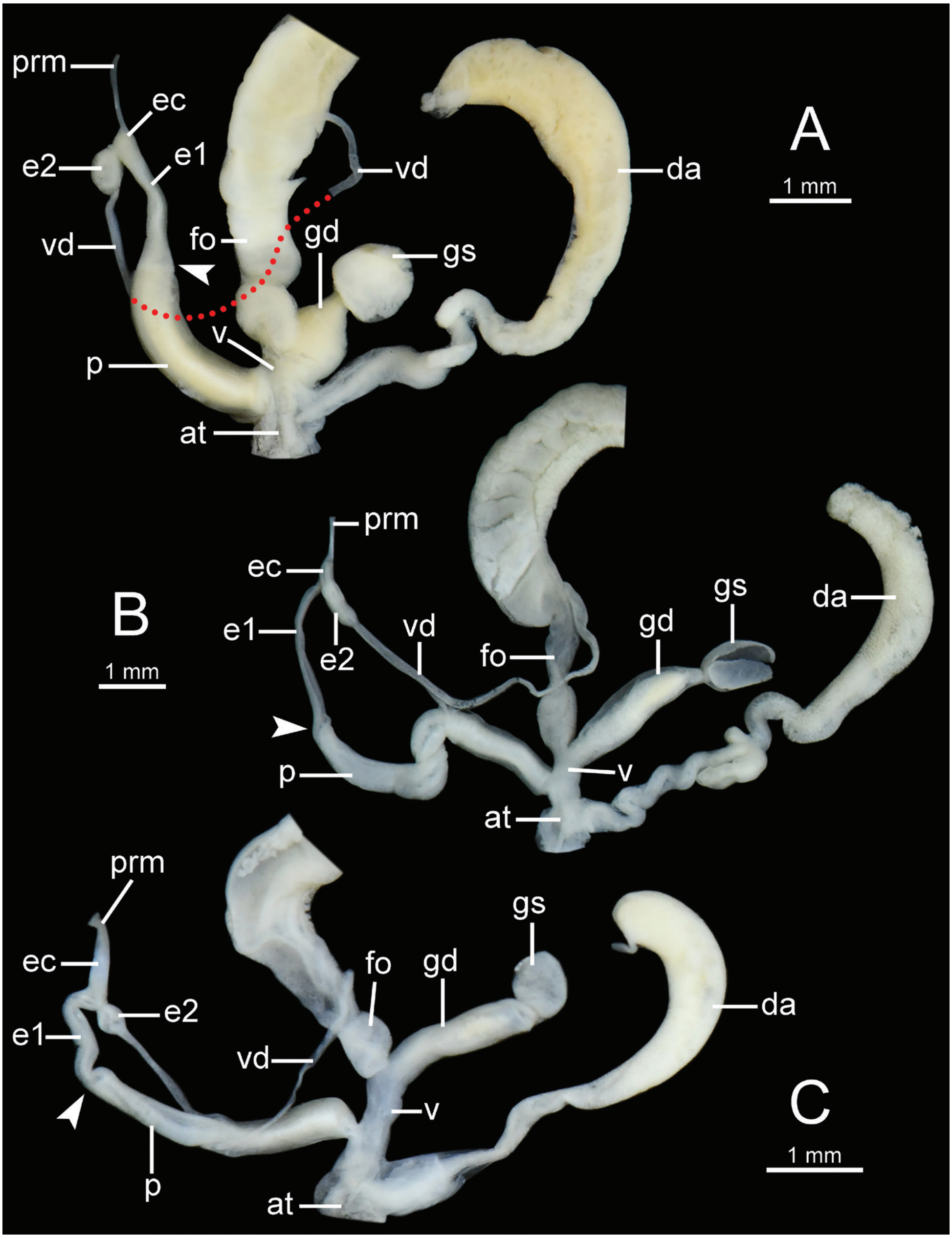

Figure 9. General view of the genital system of *Burmochlamys* species **A** *B. cassidula* specimen CUMZ 14209 **B** *B. poongee* specimen CUMZ 14210 **C** *B. albida* sp. nov. paratype CUMZ 14212. White arrow indicates the end of the penis. Red-dotted line indicates vas deferens missing.

Type locality. Limestone outcrop at Bayin Nyi Cave, Hpa-An, Kayin State, Myanmar (16°58'10.1"N, 97°29'30.6"E).

Diagnosis. Shell subglobose, whitish yellow and then gradually turned yellowish brown near aperture opening, and umbilicus somewhat narrow and very deep. Animal dark greyish with five mantle extensions. Genitalia with very short epiphallic caecum attached by thin penial retractor muscle, rather short vagina, and small, slender, rather short gametolytic duct.

Description. ***Shell*** (Figs 12A, B, 13D–F). Shell subglobose, small (width up to 6.3 mm, height up to 4.9 mm), thin, semi-translucent. Colour whitish yellow and then gradually turned yellowish brown on body whorl near aperture opening. Protoconch and teleoconch surface with similar sculpture. Surface of body whorl with indistinct spiral furrows without radial lines (Fig. 13D–F). Whorls 5½–6, increasing regularly; suture shallowly impressed; spire rather elevated; last whorl broad and well-rounded. Aperture obliquely crescent-shaped; peristome simple. Columellar margin simple, slightly reflected near umbilicus. Umbilicus open, moderate, and very deep that show preceding whorl.

Genital organs (Fig. 10C). Atrium (at) very short. Penis (p) moderate, cylindrical and with penial sheath. Epiphallus (e1+e2) ca. one-third of penis length; e1 cylindrical and its slightly smaller diameter than penis and e2; e2 bulbous and slightly longer than e1. Epiphallic caecum (ec) very short, small, bulbous with thin penial retractor muscle (prm) attached at tip. Vas deferens (vd) thin tube. Dart apparatus rather small, long cylindrical, and located on atrium at vagina and penis junction. Vagina (v) rather short and cylindrical. Gametolytic sac (gs) rather large, elongate bulbous; gametolytic duct (gd) small, slender, and rather short ca. half of penis length. Free oviduct (fo) cylindrical, very long ca. half of penis length, and encircled with thick tissue near vagina.

Radula (Fig. 15B). Teeth arranged in wide U-shaped rows, each row consisting of ~ 40 teeth. Central tooth monocuspid, large and oblong spatulate plate, and attached at both sides of its base by two smaller triangular-shaped teeth. Laterals and marginals not differentiated and monocuspid; each tooth with large and oblong spatulate plate attached by one smaller triangular-shaped tooth on outer side of its base. Outermost teeth shorter and smaller than inner teeth.

External appearance (Figs 4B, 5F). Living animal with reticulated skin and pale to dark greyish body. Mantle extensions well developed, divided to three dorsal lobes and two shell lobes; same colour as body. Foot margin and caudal horn well-developed, dark greyish.

Etymology. The specific epithet is the Latin word *versicolor* meaning of various colours. It refers to the two distinct shell colours which characterise this species.

Distribution. *Burmochlamys versicolor* sp. nov. is confirmed from two localities in the south of Salween River basin (Fig. 1). Bayin Nyi Cave is surrounded by paddy fields that are temporarily flooded during the monsoon season (Fig. 2E), while Golden valley is a small limestone outcrop located close to the Thanlwin River (= Salween River). Occurrence of the new species between the two limestone areas is expected. A living snail was found climbing up a limestone wall and many mating pairs were also discovered at Bayin Nyi Cave (Fig. 4B), where the carnivorous snail *Carinartemis* sp. was also found sympatrically (Fig. 4C).

Remarks. *Burmochlamys versicolor* sp. nov. is easy to distinguish from all known species by having (i) a whitish yellow shell with yellowish brown colour on ca. one-fourth of body whorl near the aperture, (ii) shell sculpture as only shallow spiral lines, and (iii) much wider and larger umbilicus that shows the preceding whorl. In com-

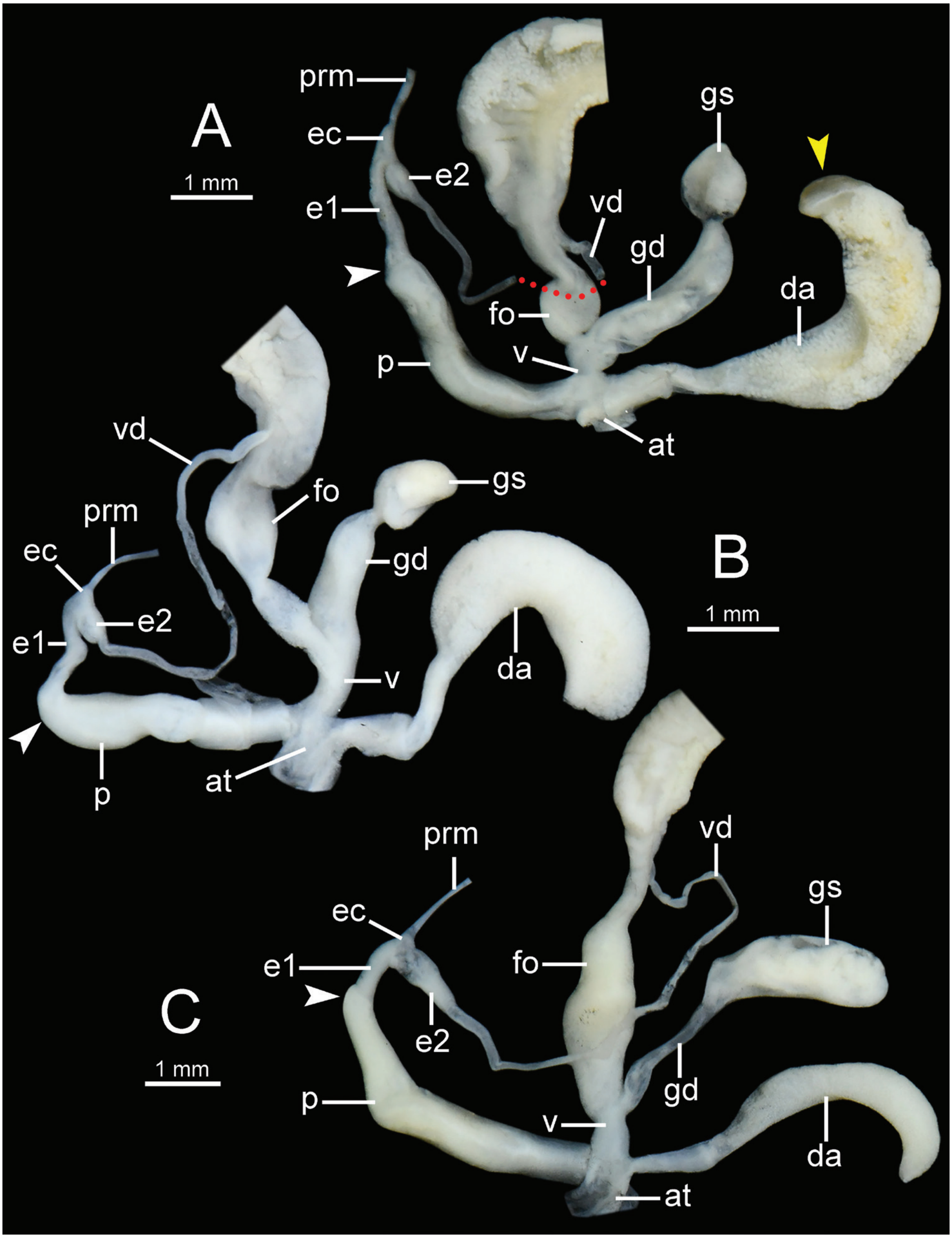

Figure 10. General view of the genital system of *Burmochlamys* species **A** *B. fasciola* sp. nov. paratype CUMZ 14214 **B** *B. moulmeinica* sp. nov. paratype CUMZ 14217 **C** *B. versicolor* sp. nov. paratype CUMZ 14219. White arrow indicates the end of the penis. Yellow arrow indicates the solid at the tip of dart apparatus. Red-dotted line indicates vas deferens missing.

parison, all other congeneric species have (i) a brownish or whitish shell colour, with or without peripheral band, (ii) a shell surface with both impressed spiral lines and undulating radial lines, and (iii) a small umbilicus (see Table 2).

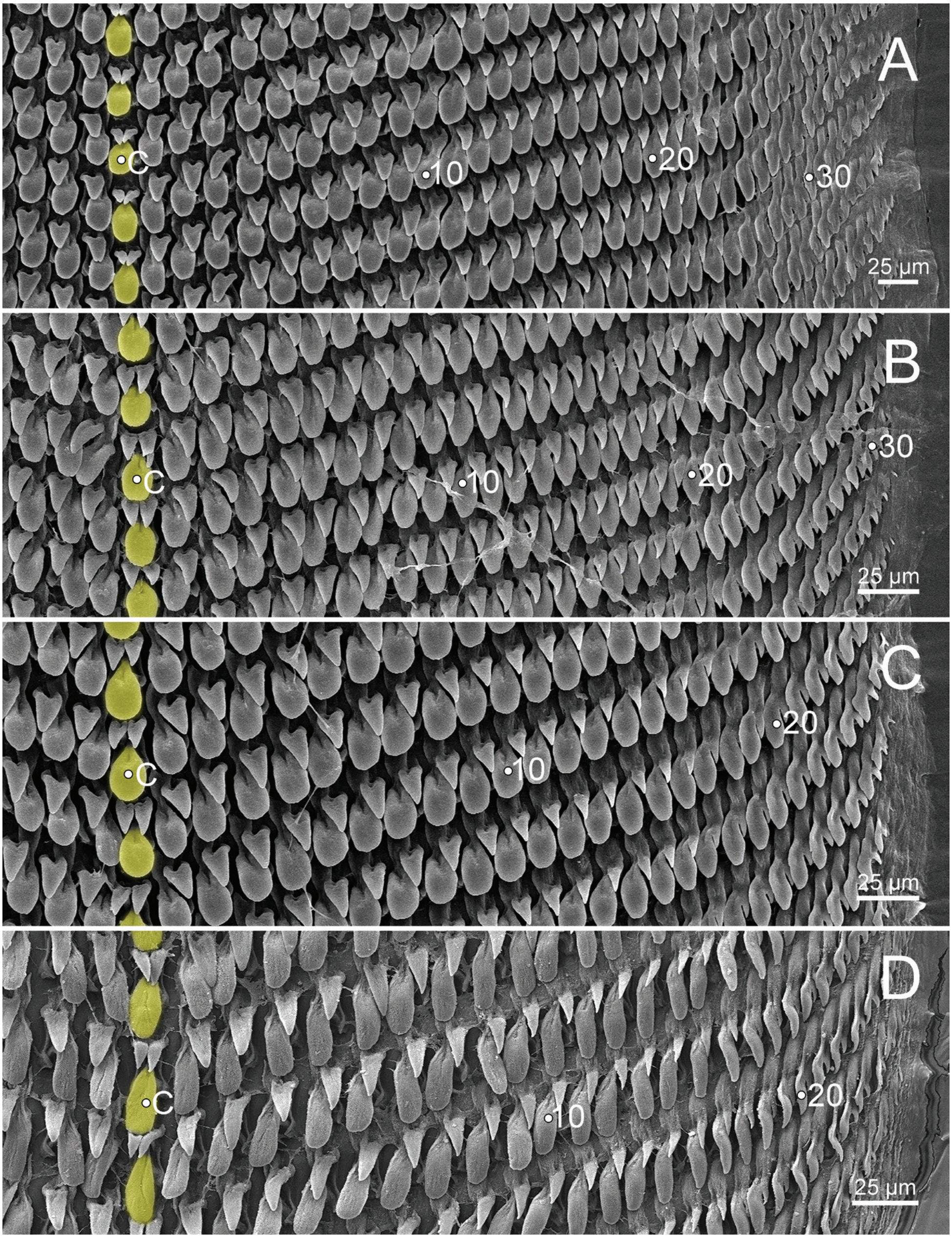

Figure 11. Representative SEM images of the radula of *Burmochlamys* species **A** *B. cassidula* specimen CUMZ 14209 **B** *B. poongee* specimen CUMZ 14210 **C** *B. albida* sp. nov. paratype CUMZ 14212 **D** *B. fasciola* sp. nov. paratype CUMZ 14214. Central tooth indicated by 'C'; yellow colour indicates central tooth row.

Burmochlamys whitteni Pholyotha & Panha, sp. nov.

https://zoobank.org/3F800E38-AB3F-4185-9871-CA7DA26456A2

Figs 1, 5G, 12C, D, 13G–I, 14, 15C

Material examined. Type material. *Holotype*: CUMZ 14221 (Fig. 12C; width 5.8 mm, height 5.0 mm). ***Paratypes*:** Same locality as holotype: CUMZ 14222 (Fig. 12D; width 5.6 mm, height 5.0 mm), NHMUK (two shells).

Type locality. Limestone outcrop at Htaung Wee Cave, Hpa-An, Kayin State, Myanmar (16°50'31.1"N, 97°37'18.4"E).

Diagnosis. Shell globose and pale to dark brownish. Animal pale grey with five mantle extensions. Genitalia with slender epiphallus, rather short epiphallic caecum attached by a thick and short penial retractor muscle, and gametolytic part and free oviduct entirely encircled by connective tissue.

Description. *Shell* (Figs 12C, D, 13G–I). Shell globose, small (width up to 5.8 mm, height up to 5.0 mm), rather thin, and semi-translucent. Colour pale to dark brownish. Protoconch and teleoconch surface with similar sculpture. Surface of body whorl with distinct spiral furrows at regular intervals, cut by undulating radial lines (Fig. 13G–I). Whorls 6–6½, increasing regularly; suture shallowly impressed; spire much elevated; last whorl well-rounded. Aperture obliquely crescent-shaped; peristome simple. Columellar margin simple, slightly reflected near umbilicus. Umbilicus open, narrow, and deep.

Genital organs (Fig. 14). Atrium (at) short. Penis (p) rather long, cylindrical and with penial sheath. Epiphallus (e1+e2) ca. five-eighths of penis length; e1 cylindrical and smaller diameter than penis and e2; e2 bulbous and ca. two-third of e1 length. Epiphallic caecum (ec) rather short, cylindrical, and with thick and short penial retractor muscle (prm) attached at tip. Vas deferens (vd) thin tube. Dart apparatus large, long cylindrical, and located on atrium at vagina and penis junction. Vagina (v) very short and cylindrical. Gametolytic sac (gs) bulbous; gametolytic duct (gd) cylindrical, moderate, ca. three-eighths of penis length; thin connective tissue encircled entire gametolytic organ and free oviduct (Fig. 14A, B). Free oviduct (fo) as long as gametolytic duct.

Radula (Fig. 15C). Resembles *B. cassidula*. Teeth arranged in wide U-shaped rows with each row consisting of ~ 55 teeth; central tooth monocuspid, large and ovate spatulate shape and attached at both sides of its base with two smaller triangular-shaped plates; lateromarginals monocuspid, large and ovate spatulate shape and then gradually smaller, narrower, and rather more pointed cusp; each lateromarginal tooth with one smaller triangular-shaped plate attached at base outwards. Some outermost teeth with a small and pointed cusp at inner side.

External appearance (Fig. 5G). Living animal with reticulated skin, pale grey body, slightly paler on foot sole and darker colour on caudal horn. Mantle extensions with two shell lobes and three dorsal lobes, similar colour to body.

Etymology. The specific epithet *whitteni* is named in honour of the late Dr. Tony Whitten (1957–2017) of Fauna & Flora International, who invited our team to explore the land snails in southern Myanmar during 2015 to 2016.

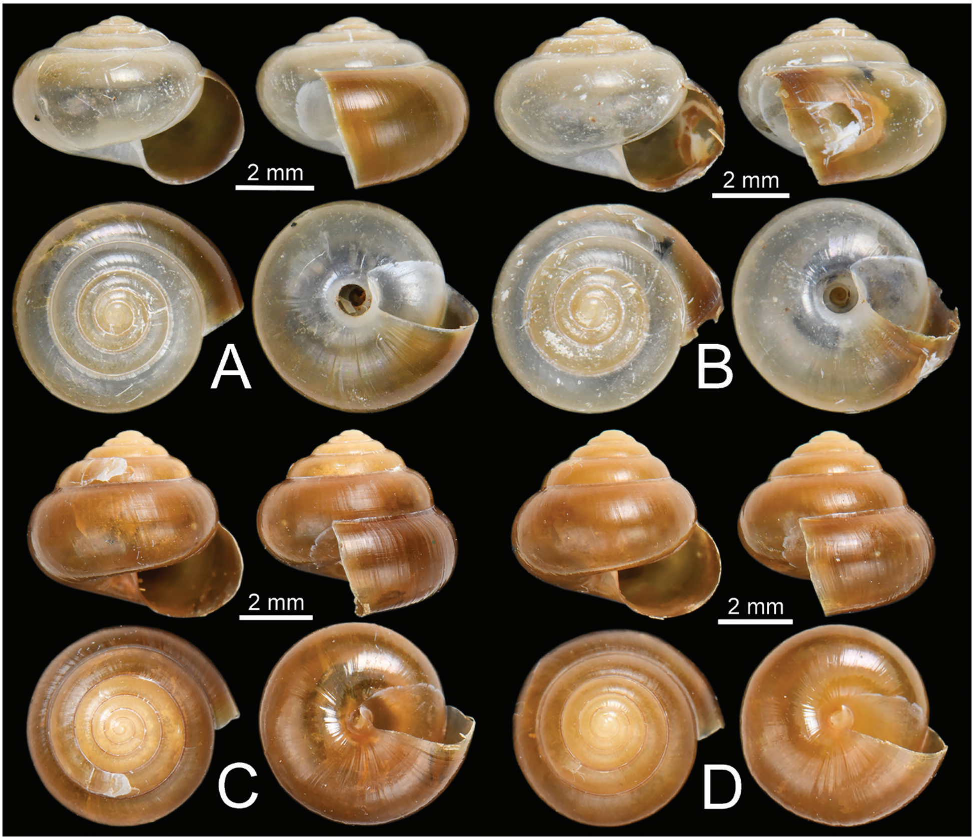

Figure 12. Shells of *Burmochlamys* species **A, B** *B. versicolor* sp. nov. **A** holotype CUMZ 14218, and **D** paratype CUMZ 14219 **C, D** *B. whitteni* sp. nov. **C** holotype CUMZ 14221, and **D** paratype CUMZ 14222.

Distribution. *Burmochlamys whitteni* sp. nov. is known only from the type locality (Fig. 1). This isolated limestone of Htaung Wee Cave is situated close to the east-bank of the Thanlwin River and is surrounded by paddy fields that are temporarily flooded during the monsoon season.

Remarks. Among the brownish-shelled species (see Table 2), *B. whitteni* sp. nov. can be distinguished from *B. poongee* and *B. moulmeinica* sp. nov. by the presence of the connective tissue encircled entirely at the gametolytic part and free oviduct, whereas in the latter two species this tissue is absent. In addition, *B. whitteni* sp. nov. has a thick penial retractor muscle, relatively long epiphallus, and rather short at the base of dart apparatus. In contrast, *B. poongee* has a thin penial retractor muscle, relatively long epiphallus, and very long at the base of dart apparatus, while *B. moulmeinica* sp. nov. has a thin penial retractor muscle, relatively short epiphallus, and rather short at the base of dart apparatus.

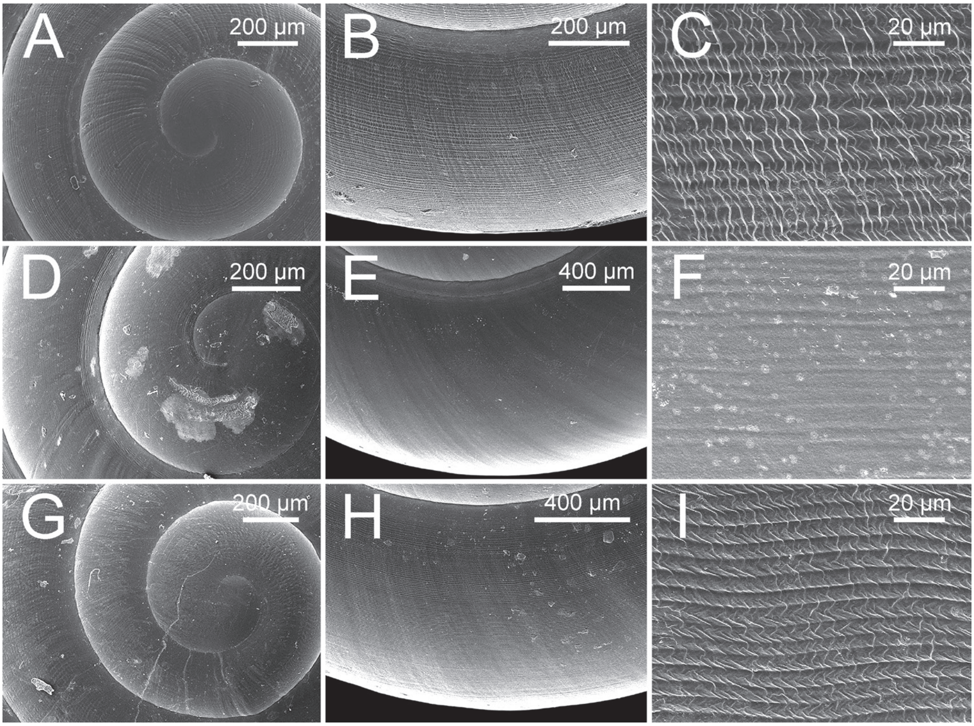

Figure 13. Representative SEM images of the shell of *Burmochlamys* species **A–C** *B. moulmeinica* sp. nov. paratype CUMZ 14217 **A** protoconch **B** body whorl **C** zoom-in view of body whorl. **D–F** *B. versicolor* sp. nov. paratype CUMZ 14219 **D** protoconch **E** body whorl **F** zoom-in view of body whorl **G–I** *B. whitteni* sp. nov. paratype CUMZ 14222 **G** protoconch **H** body whorl **I** zoom-in view of body whorl.

Discussion

Taxonomic implications from morphological and anatomical studies

With regard to the lack of synapomorphies, the delimitation of the Helicarionidae is vague and the relationship within this family is still far from resolved (Hausdorf 1998; Hyman and Ponder 2010). Hausdorf (1998) stated that only the Durgellinae Godwin-Austen, 1888 can be substantiated as a monophyly and are characterised by the reduced flagellum and large shell lobes (apparently secondarily reduced in *Sitala*). At present the only limacoid groups that possess monocuspid radula with spatulate shape are *Aenigmatoconcha* and *Sophina*, placed in the Durgellinae (Tumpeesuwan and Tumpeesuwan 2017; Sutcharit et al. 2020; Pholyotha et al. 2021b), and *Chalepotaxis*, placed in the Helicarionidae (Páll-Gergely et al. 2016). Therefore, we tentatively assign *Burmochlamys* gen. nov. under the Durgellinae of the Helicarionidae based on the morphological characters outlined above (flagellum reduced, shell lobes possibly secondarily reduced, and monocuspid radula present). Because the higher classification

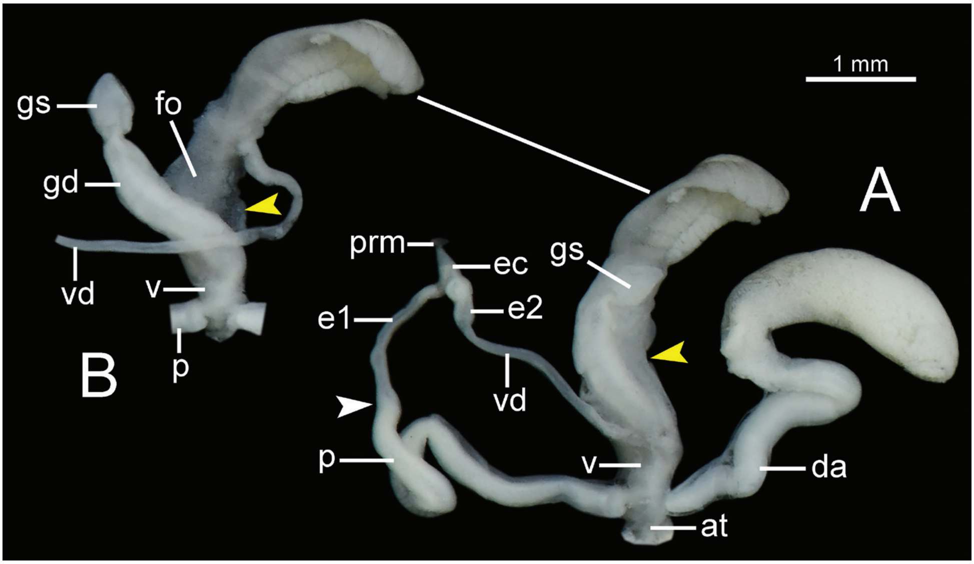

Figure 14. Genital system of *Burmochlamys whitteni* sp. nov. paratype CUMZ 14222 **A** general view of the genital system **B** a part of gametolytic organ (duct and sac) and free oviduct after the connective tissue is removed. White arrow indicates the end of the penis. Yellow arrow indicates the connective tissue surrounding the gametolytic organ and free oviduct.

is still provisional, future studies will combine molecular phylogenetic analyses to investigate its true evolutionary position.

Considering only the shell morphology, *Burmochlamys* gen. nov. is similar to some Southeast Asian genera of the Helicarionidae (i.e., *Aenigmatoconcha*, *Chalepotaxis*, *Sitala* and *Sophina*) or the Ariophantidae (i.e., *Macrochlamys* and *Microcystina*). Among these six genera, *Microcystina* has the most similar shell to this new genus in having a microscopic shell sculpture and rounded body whorl, but the usual shell shape of *Microcystina* species is depressed and rarely globosely depressed (Blanford and Godwin-Austen 1908; van Benthem Jutting 1950; Schileyko 2003; Vermeulen et al. 2019), whereas *Burmochlamys* gen. nov. has a subglobose to globose shell shape. However, most *Microcystina* species are known only from their shell information and need to be taxonomically revised. Compared with other genera, *Burmochlamys* gen. nov. can be easily distinguished from *Sitala* by the shell shape and the body whorl that *Sitala* has more or less trochiform and subangulated to carinate at the periphery. The presence of the microscopic shell sculpture can be used to separate this new genus from *Aenigmatoconcha*, *Chalepotaxis*, *Macrochlamys*, and *Sophina.* Shells of the speciose genus *Macrochlamys* are depressed to globose, smooth, and rounded to angulated at the periphery (Blanford and Godwin-Austen 1908; Pholyotha et al. 2018; Pholyotha et al. 2020b), while *Aenigmatoconcha*, *Chalepotaxis*, and *Sophina* have depressed to globose, smooth, and rounded at the periphery (Páll-Gergely et al. 2016; Sutcharit et al. 2020; Pholyotha et al. 2021b). Despite the similarity of many of the shell traits between *Burmochlamys* gen. nov. and other genera that has made a lot of

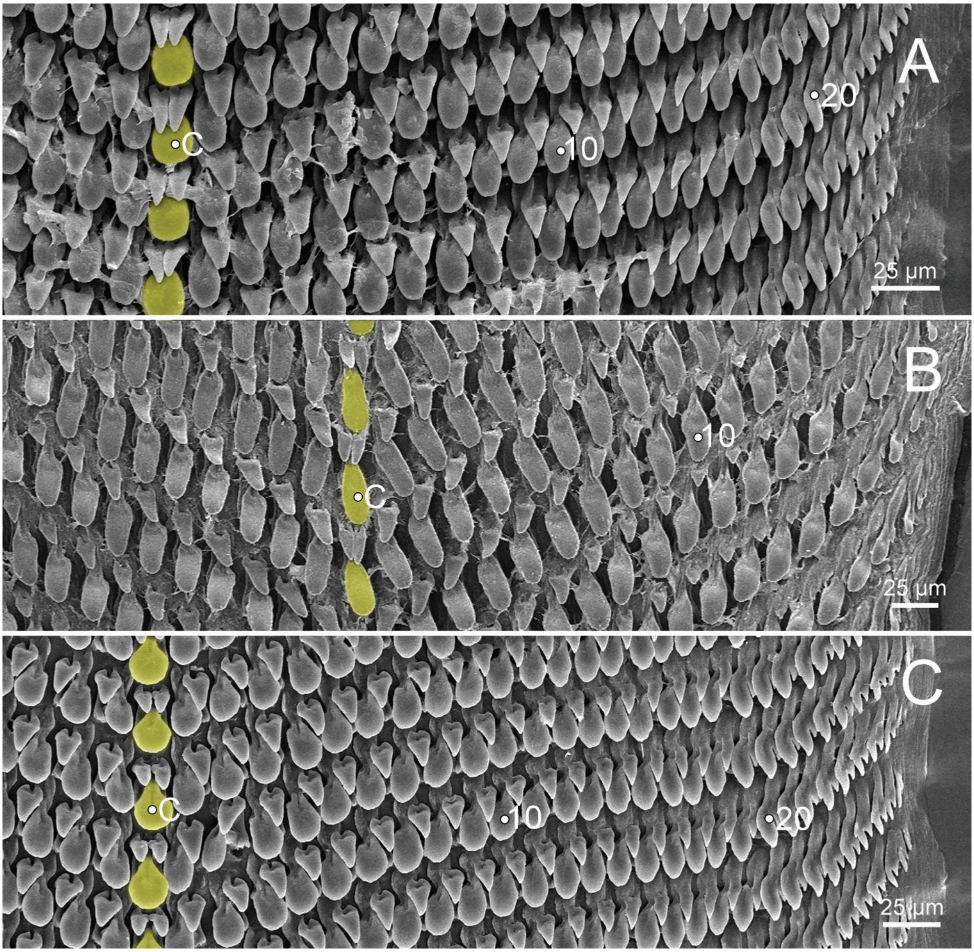

Figure 15. Representative SEM images of the radula of *Burmochlamys* species **A** *B. moulmeinica* sp. nov. paratype CUMZ 14217 **B** *B. versicolor* sp. nov. paratype CUMZ 14219 **C** *B. whitteni* sp. nov. paratype CUMZ 14222. Central tooth indicated by 'C'; yellow colour indicates central tooth row.

taxonomic confusion in the past, the peculiarities of the morphological characters of the mantle extension, radula, and genitalia deserve special consideration (see Table 1).

The noticeable characters of *Burmochlamys* gen. nov. are the finger-like shell lobe and the spatulate-shaped radula. Previous studies of the anatomy of the Southeast Asian helicarionids and ariophantids noted differences in the mantle morphology (especially the shell lobe), described as a slender or slightly extended shell lobe (i.e., *Macrochlamys*, *Microcystina*, and *Sitala*), or as a broad and enlarged shell lobe (i.e., *Aenigmatoconcha*, *Chalepotaxis*, and *Sophina*). So, the mantle morphology can be used as an informative character to distinguish members of these two families even though reduction of the mantle extension within the same genus has been documented (Blanford and Godwin-Austen 1908; Pholyotha et al. 2020c; Sutcharit and Panha 2021).

With regards to the radular morphology, most genera of helicarionids and ariophantids have a tricuspid central tooth, tricuspid laterals, bicuspid marginals, or undifferentiated lateromarginal teeth with several cusps (Stoliczka 1871; Blanford and Godwin-Austen 1908; van Benthem Jutting 1950; Solem 1966; Schileyko 2002, 2003). While only the *Aenigmatoconcha*, *Chalepotaxis*, *Sophina*, and *Burmochlamys* gen. nov. have radular teeth with a monocuspid and spatulate shape (Páll-Gergely et al. 2016; Sutcharit et al. 2020; Pholyotha et al. 2021b). The radular morphology of *Burmochlamys* gen. nov. consists of two types. The large one is spatulate in shape (highlight in yellow and blue colour Fig. 3D, E) and the smaller one is triangular in shape (highlight in red colour Fig. 3D, E). Similarly, *Chalepotaxis infantilis* (Gredler, 1881) and some species of *Sophina* (i.e., *S. tonywhitteni* Sutcharit & Panha, 2020) exhibit this smaller tooth but it is very small (Páll-Gergely et al. 2016; Sutcharit et al. 2020). In contrast, all *Aenigmatoconcha* species do not have this smaller tooth (Pholyotha et al. 2021b). The same modification of radula that present another smaller tooth located behind the larger tooth is recorded in the arboreal land snails, *Amimopina macleayi* (Brazier, 1876) and *Rhachistia histrio* (Pfeiffer, 1855), in the family Cerastidae Wenz, 1923 (Solem 1973). Solem (1973) hypothesised that the functional aspects in the two radular are identical, where the large spatulate tooth scrapes against the food source surface, then another smaller tooth catches and pulls any loose pieces.

Among the genera having the monocuspid radular teeth (*Aenigmatoconcha*, *Burmochlamys* gen. nov., *Chalepotaxis*, and *Sophina*), the genital organ of this new genus shows a strong similarity to those of *Sophina* because of the absence of a flagellum and the presence of a straight epiphallic caecum and dart apparatus (Páll-Gergely et al. 2016; Sutcharit et al. 2020; Pholyotha et al. 2021b). In contrast, *Aenigmatoconcha* has a straight epiphallic caecum and a small flagellum but lacks a dart apparatus (Pholyotha et al. 2021b), while *Chalepotaxis* has a straight epiphallic caecum but has neither a flagellum nor a dart apparatus (Páll-Gergely et al. 2016).

Regardless of the unique radular teeth of the new genus, the genitalia of *Burmochlamys* gen. nov. and *Microcystina* (at least the type species) differ by the epiphallic caecum, which in *Burmochlamys* gen. nov. is present but *Microcystina* is absent. Additionally, reduction of dart apparatus in *M. bintennensis* Godwin-Austen, 1899 (not the type species) is also reported and differs from this new genus (Blanford and Godwin-Austen 1908).

The dart apparatus has chiefly functions during the courtship of the limacoid snails to increase the male reproductive success (Lodi and Koene 2016). With a handful of anatomical information, the dart apparatus has long been used to distinguish genera among the limacoid snails, even though there are some arguments because it seems to have been gained or lost multiple times during snail evolution (Blanford and Godwin-Austen 1908; Hausdorf 1998; Schileyko 2002, 2003; Sutcharit et al. 2020). With regard to the type species, *Cryptaustenia* Cockerell, 1891, *Durgella* Blanford, 1863 and *Microcystina* deserve a special mention because they tend to have a well-developed dart apparatus, but some non-type species classified to these respective genera have no dart apparatus (see Table 1). Likewise, the reversed phenomenon occurs in *Sitala*, whose type species has no dart apparatus while *S. attegia* (Benson, 1859) (not the type species) has a well-developed dart apparatus (Table 1). A comprehensive phylogenetic study among these genera has not been published so far, but it would be interesting to know whether the gain or loss of the dart apparatus are facultative or indicate phylogenetic signal.

Geographic distribution of *Burmochlamys* gen. nov.

Although several localities in Shan State, Mon State, Kayin State, and the Tanintharyi Region were surveyed during 2015 and 2016, only some localities of the karst habitat islands in the Salween River basin of Kayin state were found to harbour *Burmochlamys* gen. nov. where two known and five new species of *Burmochlamys* gen. nov. were discovered. Thus, a narrow distribution range of the new genus is suggested; however, this is not ascertained because there are several limestone karsts yet to be surveyed in adjacent regions. Interestingly, all species recognised herein (except *B. cauisa* and *B. perpaula* which are known only from the type specimen) show high degrees of endemism and localisation (one species per location), which has possibly resulted from the great variety of ecological niches afforded by their complex karst formations and highly fragmented island-like habitat of the Salween River basin (Grismer et al. 2018a, b; Sutcharit et. al. 2020). Similarly, the karst formations of the Mekong Delta Limestones form archipelagos of habitat islands that host an exclusive concentration of endemic land snail taxa along the Vietnamese and Cambodian coast (Vermeulen et al. 2019; Pholyotha et al. 2020a).

Currently restricted to only the Salween River basin (Fig. 1), *Burmochlamys* gen. nov. and *Sophina* indeed show high levels of local endemism. Sutcharit et al. (2020) reported that *Sophina* is genetically divided into two principal groups that are distributed allopatrically on either side of the Gyaing River, and this similar pattern is also documented in geckos at the species-group level (Grismer et al. 2018a, b). Interestingly, all *Burmochlamys* species show a confined distribution in the north part of the Gyaing River, which possibly implies that the river acts as the geographical barrier and then allopatry and local endemicity play an important role in the diversification of the *Burmochlamys* species. However, at a few localities, sympatry of *Burmochlamys* and *Sophina* species was observed, i.e., Kyankaw Mountain (*B. fasciola* sp. nov. and *S. salweenica*), Waiponla Monastery (*B. albida* sp. nov. and *S. salweenica*), and Lun Nya Pagoda (*B. moulmeinica* sp. nov. and *S. pisinna*). In two sympatric species of *Sophina* which are distinct in shell size, divergence in body size may reduce interspecific competition and reflect niche partitioning between species (Goodfriend, 1986; Sutcharit et al. 2020). The phenomenon of reducing competition by different body size might possibly also hold for sympatric species of *Burmochlamys* (shell width about 6.0–8.0 mm) and *Sophina* (shell width about 9.0–13.0 mm; Sutcharit et al. 2020). However, no molecular phylogenetic analysis of *Burmochlamys* is available, and so further research on this issue has to reveal how the ecological and evolutionary processes have shaped its diversity patterns.

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RESEARCH ARTICLE

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Revision of the carnivorous land snail family Streptaxidae (Stylommatophora, Achatinina) in Myanmar, with description of four new species

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Abstract

The carnivorous terrestrial snail family Streptaxidae, recognized by having a regular to eccentric shell with complex apertural dentition, remains little-known and largely unexplored in Myanmar. This article presents historically recorded species and provides new data on this family. A total of eighteen species in five genera, namely *Carinartemis*, *Discartemon*, *Haploptychius*, *Oophana*, and *Perrottetia* from the southeastern and eastern parts of Myanmar, is examined herein. Among these, *Haploptychius* is the most diverse with eight species, while the remaining genera are comprised of fewer than five species each. *Streptaxis birmanica* and *Streptaxis blanfordianus* are herein synonymized with *Haploptychius blanfordi*, while *Streptaxis hanleyanus* is synonymized with *Carinartemis sankeyi*. Furthermore, the first genitalia and radula descriptions for three previously known species, *D. tonywhitteni*, *C. exacutus*, and *C. sankeyi*, are provided. Using comparative morphological and anatomical approaches, four new species are described: *D. paurodeviatus* **sp. nov.,** *H. heliakosus* **sp. nov.,** *H. tenasserimicus* **sp. nov.,** and *H. karenorum* **sp. nov.** This present study enhances the understanding of the land snail fauna in Myanmar, specifically the streptaxids, and highlights that limestone areas are important for biodiversity conservation.

Keywords

Biodiversity, endemic, Fauna & Flora international, genitalia, limestone, systematics, taxonomy

Introduction

Having the greatest land area in mainland Southeast Asia, Myanmar, formerly known as Burma, is recognized as having a high level of ecosystem diversity that can support a diverse variety of organisms and is part of the Indo-Burma biodiversity hotspot (Myers et al. 2000; FAO 2010; Asian Development Bank 2012). Intensive surveys of the malacofauna in Myanmar were performed in the mid-nineteenth to early twentieth centuries and were mainly conducted by American and European naturalists (see Pholyotha et al. 2020; Grego et al. 2021). However, the investigation on mollusks did not receive much attention after the independence of Myanmar in the 1940s. Fortunately, since Myanmar became more open in the 1990s, studies of several groups of Burmese land snails have gradually been published, i.e., Streptaxoidea Gray, 1860 (Páll-Gergely et al. 2020a; Sutcharit et al. 2020), Helicarionoidea Bourguignat, 1877 (Pholyotha et al. 2020; Sutcharit et al. 2020; Sutcharit and Panha 2021), Pupinidae Pfeiffer, 1853 (Páll-Gergely et al. 2015; Páll-Gergely and Grego 2019), Plectopylidae von Möllendorff, 1898 (Páll-Gergely 2018), Camaenidae Pilsbry, 1895 (Páll-Gergely et al. 2020b, 2022), and Clausiliidae Gray, 1855 (Nordsieck 2002a, b; Grego et al. 2021; Szekeres et al. 2021a, b).

The members of family Streptaxidae Gray, 1860 are known as carnivorous land snails and are characterized by an eccentric to cylindrical shell with complex apertural dentition, genitalia with hook-like structures, and living animals usually having bright yellow to red or greenish bodies (Blanford and Godwin-Austen 1908; van Benthem Jutting 1954; Berry 1963; Schileyko 2000; Verdcourt 2000; Rowson et al. 2009; Siriboon et al. 2013, 2014a, b). This group is distributed worldwide across the tropical and subtropical areas of South America, Africa, and Asia (van Bruggen 1967; Schileyko 2000; Sutcharit et al. 2010; Rowson et al. 2010). As in many predator-prey relationships, most streptaxid taxa are abundant in limestone microhabitats where their preferred food items are also abundant (Siriboon et al. 2013, 2014a, b; Inkhavilay et al. 2016). In Southeast Asia, karsts cover an area of approximately 400,000 square kilometers and are known to host high levels of animal diversity and endemism (Clements et al. 2006). Currently, ten genera and approximately 140 nominal species of streptaxid snails are recognized in the region (Blanford and Godwin-Austen 1908; van Bruggen 1972; Richardson 1988; Schileyko 2000; Siriboon et al. 2013, 2014a, b; Do and Do 2015; Inkhavilay et al. 2016; MolluscaBase 2021).

In Myanmar, the most recent reports on the superfamily Streptaxoidea (Streptaxidae and Diapheridae Panha & Naggs, 2010) were published more than one hundred years ago (see Blanford and Godwin-Austen 1908). While only a few land snail groups have been systematically revised in Myanmar (Páll-Gergely et al. 2020a; Sutcharit et al. 2020), the systematic revision of the streptaxid snails in several other countries of mainland Southeast Asia (i.e., Laos, Vietnam, and Thailand) has advanced during the past decade (Siriboon et al. 2013, 2014a, b; Inkhavilay et al. 2016; Do 2017; Bui et al. 2019; Siriboon et al. 2020; Sutcharit et al. 2020).

During land snail expeditions in Myanmar in 2015 and 2016, streptaxid species were discovered in several localities in southeastern (Mon State, Kayin State, and Tanintharyi Region) and eastern (Shan State and Mandalay Region) parts of Myanmar under the cooperation of the Forest Department of Myanmar (FDM), Fauna & Flora

International (FFI), and Chulalongkorn University. Herein, we discuss the taxonomy of the carnivorous terrestrial snail family Streptaxidae collected in Myanmar. The molecular phylogeny by Siriboon et al. (2020) suggests that some genera are non-monophyletic, especially *Oophana* Ancey, 1884 and *Haploptychius* von Möllendorff, 1906. However, in this study the traditional concept based on morphological characters is applied, without acknowledging their respective type species. Therefore, this research aims to 1) revise the systematics of the streptaxid species with eccentric shell shape based on the shell, radula morphology and genital anatomy (if available), especially the penial hooks; and 2) record all species of streptaxid species with an eccentric shaped shell that have been reported from Myanmar based on the literature and historical museum collections.

Materials and methods

Streptaxid specimens were collected during 2015 and 2016 by the Animal Systematics Research Unit (**ASRU**) members, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. These field surveys were conducted under a MOU between the Forest Department, Ministry of Natural Resources and Environmental Conservation and Forestry, Myanmar and Fauna & Flora International (**FFI**) with Letter No. 0092. The field surveys focused on non-limestone and limestone areas in Shan State, Mon State, Kayin State, Mandalay Region, and Tanintharyi Region. The coordinates were recorded using GPS. Approximate collection localities are presented in Table 1 and Figure 1.

For anatomical studies, living specimens were photographed, euthanized following the protocol by the American Veterinary Medical Association (2020), and then preserved in 70% ethanol (v/v). Taxonomic identifications were made based on Kobelt (1905–1906), Blanford and Godwin-Austen (1908), Schileyko 2000; Siriboon et al. (2013, 2014a, b), Inkhavilay et al. (2016), and Sutcharit et al. (2020). Shell height, shell width and whorl count were measured, and the height/width ratio was calculated following Siriboon et al. (2013). The shells were photographed using a Nikon camera with a Nikon 105 Macro lens. One to five adult specimens in ethanol were dissected, and the genitalia were examined under a stereomicroscope. A representative specimen was illustrated with the aid of a camera lucida. The buccal masses were removed, and the radulae were soaked in 10% (w/v) NaOH, and then cleaned in distilled water. Radula, penial hooks, and vaginal hooks were examined and photographed using scanning electron microscopy (**SEM**; JEOL, JSM-5410 LV).

All the nominal species names described as new to science in this work are attributed to the first and last authors (Man & Panha). Thus, a complete citation of the authors is Man & Panha in Man et al.

Anatomical abbreviations

ag, albumen gland; **at**, atrium; **fo**, free oviduct; **gd**, gametolytic duct; **gs**, gametolytic sac; **hd**, hermaphroditic duct; **ov**, ovary; **p**, penis; **pr**, penial retractor muscle; **ps**, penial sheath; **psr**, penial sheath retractor muscle; **sv**, seminal vesicle; **ta**, talon; **v**, vagina; **vd**, vas deferens (Siriboon et al. 2013, 2014a, b; Sutcharit et al. 2010).

Table 1. Shell measurements of streptaxid species from Myanmar recognized in this study. Numbers listed before collection locality correspond to those in the map in Fig. 1.

Species, localities and CUMZ no.	Number of specimens	Ranges, mean ± S.D. in mm.			Number of whorls
		Shell height	Shell width	H/W ratio	
Discartemon tonywhitteni					
20. Phra (Buddha) Cave, Tanintharyi: (13001)	7	3.4–5.1 4.5±0.64	9.5–12.7 11±1.05	0.27–0.5 0.4±0.08	6–6½
Discartemon paurodeviatus sp. nov.					
17. Phataw Phatet Island, Tanintharyi: (13002, 13003, 13004)	17	6.8–8.1 7.5±3.48	10.8–12 11.3±0.35	13.8–16.8 1.5±0.07	6–6½
Oophana mouhoti					
19. Phra (Buddha) Cave, Tanintharyi: (13005)	1	11.2	10	0.6	6½
Perrottetia theobaldi					
3. Aik Kham Cave, Shan: (13006)	4	3.6–4.2 3.8±0.31	5.6–5.8 5.7±0.10	1.4–1.6 1.5±0.10	5–5½
Haploptychius solidulus					
16. Pathen mountain, Hpa-an, Kayin: (13007)	15	10.8–12.4 11.6±0.61	11.3–12.6 11.8±0.46	0.9–1.1 1.02±0.03	6–6½
Haploptychius thebawi					
1. Pyinyaung Village, Meiktila, Mandalay Region (Apache Cement Co. Ltd): (13008)	4	7.5–9.0 7.8±0.69	7.5–8.7 8.1±0.51	0.8–1.1 1.03±0.13	6–6½
2. Lin Way Monastery, Ywangan, Shan: (13009)	7	6.7–7.3 7.1±0.24	6.5–7.9 7.2±0.42	0.9–1.2 1.02±0.08	5½–6
3. Aik Kham Cave, Taunggyi, Shan: (13010)	3	4.6–5.3 5.0±0.36	5.0–5.7 5.4±0.37	1.1–1.2 1.08±0.03	6–6½
Haploptychius tenasserimicus sp. nov.					
18. Lampane Cave, Tanintharyi: (13011, 13012)	5	4.1–5.0 4.5±0.36	8.1–9.7 8.7±0.59	1.7–2.0 1.9±0.08	6
Haploptychius heliakosus sp. nov.					
9. Bardai Mountain, Hpa-an, Kayin: (13013, 13014, 13015)	39	9.7–11.6 10.6±0.60	7.3–10.3 9.1±1.05	0.6–0.9 0.8±0.10	7–7½
6. Kyonknow Cave, Hpa-an, Kayin: (13016)	7	8.4–9.9 9.2±0.61	8.1–9.9 8.9±0.57	0.8–1.2 0.9±0.10	6½–7
Haploptychius karenorum sp. nov.					
10. Waiponla Mountain, Hpa-an, Kayin: (13017, 13018)	6	11.4–12.4 11.8±0.40	8.2–10.0 8.9±0.70	0.6–0.8 0.7±0.08	6–7½
5. Taung Lay Cave, Hpa-an, Kayin: (13019)	2	8.8–9.7 9.3±0.4	10.5–12 11.3±0.8	1.14–1.36 1.22±1.10	6½–7
Carinartemis exacutus					
11. Sadhdan Cave, Hpa-an, Kayin: (13020)	3	6.2–7.3 6.7±0.45	13.0–14.0 13.03±0.4	1.8–2.0 1.9±0.15	6–6½
4. Bayin Nyi Cave, Hpa-an, Kayin: (13021, 13022)	23	6.3–6.8 6.6±0.22	6.8–12.6 12.2±0.4	1.7–2.0 1.8±0.09	6–6½
12. Lun Nga Mountain, Hpa-an, Kayin: (13023, 13024)	64	5.5–6.3 5.9±0.33	13.2–13.9 13.5±0.28	2.1–2.5 2.2 ±0.16	5½–6
7. Taung Wine Cave, Hpa-an, Kayin: (13025, 13026)	9	7.4–7.5 7.5±0.05	12.0–13.1 12.5±0.56	1.6–1.7 1.7±0.89	6–6½
8. Kaw Ka Taung, Hpa-an, Kayin: (13027)	1	7.6	12	1.6	6½
Carinartemis sankeyi					
14. Saddan Cave, Mawlamyine, Mon: (13028)	7	6.9–8.3 7.6±0.51	9.0–10.5 9.7±0.55	1.2–1.4 1.3±0.11	6–7
13. Kayon Cave, Mawlamyine, Mon: (13029, 13030)	18	7.0–8.2 7.5±0.49	9.1–10.2 9.7±0.49	1.2–1.4 1.2±0.07	6½–7
15. Dhammatat Cave, Mawlamyine, Mon: (13031)	12	5.5–7.4 6.5±0.57	10.4–11.6 11.1±0.44	1.5–2.0 1.7±0.15	6–7

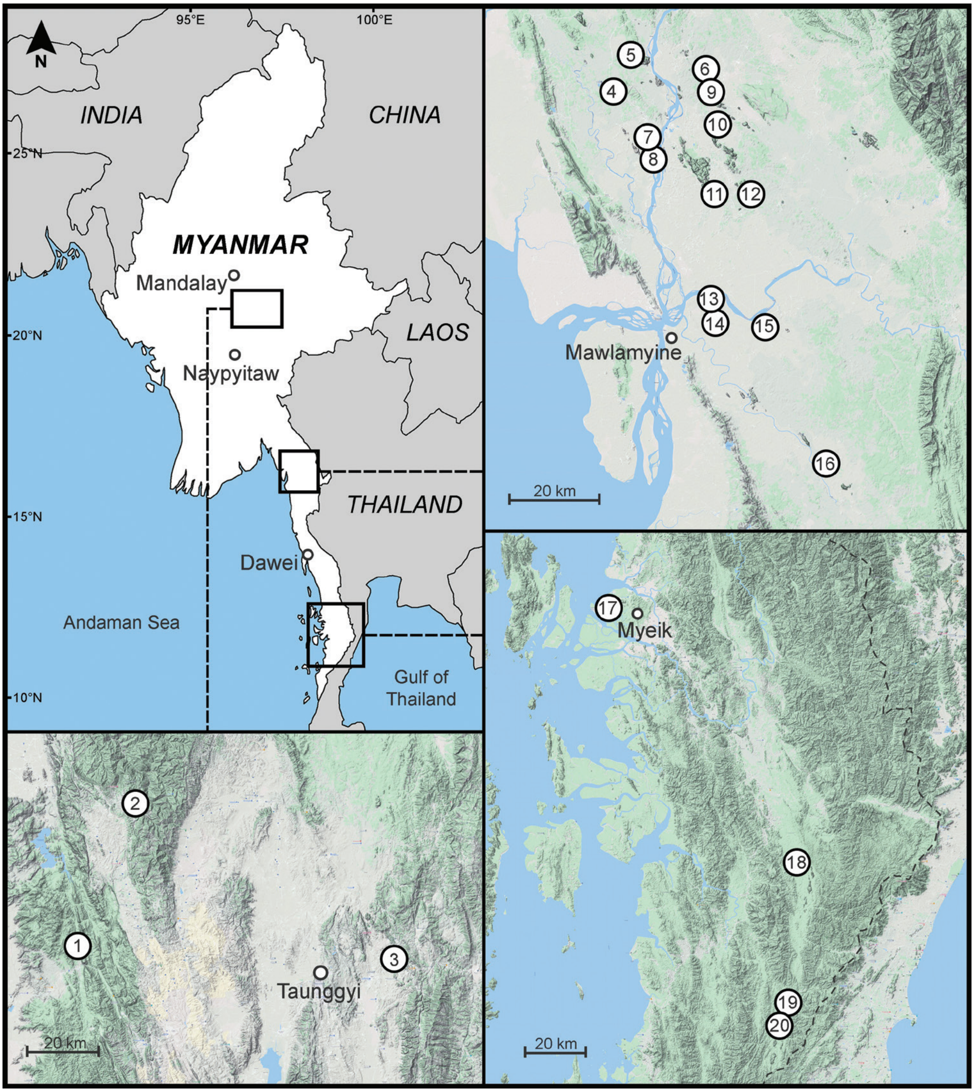

Figure 1. Approximate sampling locations used in this study. The location name and coordinates are listed in Table 1. Historical type localities of a broad scale, such as Tavoy, Burma, Moulmein, Mergui, and Tenasserim, are not indicated on this map.

Institutional abbreviations

Materials examined in this study were deposited in the following institutions:

CUMZ	Chulalongkorn University Museum of Zoology, Bangkok;
MCZ	Museum of Comparative Zoology, Harvard University, Massachusetts;
MNHN	Muséum National d'Histoire Naturelle, Paris;

NHM	The Natural History Museum, London;
NHMUK	when citing specimens deposited in the NHM;
SMF	Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main.

Systematics

Family Streptaxidae Gray, 1860

Genus *Discartemon* Pfeiffer, 1856

Discartemon Pfeiffer, 1856: 173. Ancey 1884: 399. Tryon 1885: 58. Gude 1903: 226. van Benthem Jutting: 1954: 71–94. Zilch 1960: 560. Richardson 1988: 182–185. Schileyko 2000: 784. Siriboon et al. 2014a: 48, 49. Inkhavilay et al. 2016: 25. Sutcharit et al. 2020: 151.

Odontartemon (*Discartemon*) – Kobelt 1905: 91, 96.

Type species. *Streptaxis discus* Pfeiffer, 1853, by subsequent designation by Ancey (1884: 399).

Diagnosis. Shell flattened to globose-heliciform. Last whorl rounded to angular, less distorted, and often with peripheral keel; whorls regularly to rapidly expanded. Apertural dentition varied, ranging from only one parietal lamella to additional dentition: upper palatal, palatal, basal, columellar and supracolumellar lamellae. Genitalia with short to long penis, sometimes with penial appendix and penial sheath covering entire penis length. Penial hooks present and vaginal hook absent.

Remarks. This genus was recently revised in both shell and genital diagnostic characters, and the intrageneric relationship was discussed (Siriboon et al. 2014a, 2020). At present, the genus *Discartemon* comprises thirty recognized species, including a new species described herein (Sutcharit et al. 2020).

Discartemon tonywhitteni Sutcharit & Panha, 2020

Figs 1, 2A, 3A, B, 4A, B, 5, 24A

Discartemon tonywhitteni Sutcharit & Panha in Sutcharit et al. 2020: 154, fig. 3a–c. Type locality: Phra (Buddha) Cave, Lenya National Park, Tanintharyi Township, Tanintharyi Region, Myanmar.

Material examined. ***Holotype*** CUMZ 5108; ***paratypes*** CUMZ 5107 (14 shells), CUMZ 5101 (30 shells), CUMZ 5102 (25 shells), CUMZ 5104 (18 shells), CUMZ 5105 (30 shells), CUMZ 5106 (20 juvenile shells). Limestone outcrop ~ 3.4 km south of the Phra (Buddha) Cave, Lenya National Park, Tanintharyi Township, Tanintharyi Region, Myanmar (11°11'56.2"N, 99°10'25.7"E): CUMZ 13001 (7 specimens in ethanol; Fig. 3A, B).

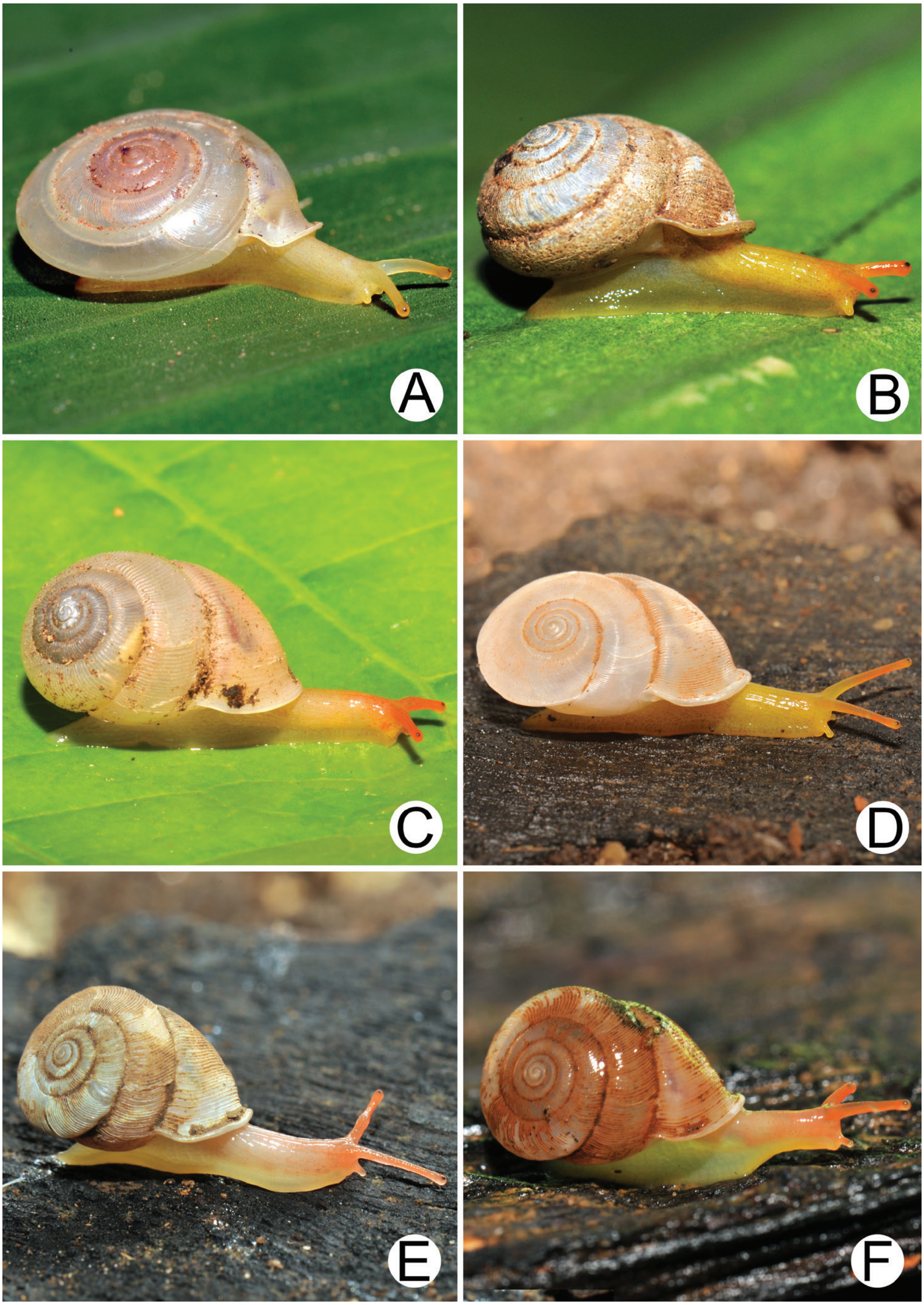

Figure 2. Living snails **A** *Discartemon tonywhitteni* from near Phra (Buddha) Cave, Tanintharyi Region (shell width ~ 10 mm) **B** *Discartemon paurodeviatus* sp. nov. from the type locality (shell width ~ 11 mm) **C** *Haploptychius heliakosus* sp. nov. from the type locality (shell height ~ 9 mm) **D** *Carinartemis exacutus* from Lun Nga Mountain, Kayin State (shell height ~ 14 mm) **E, F** *Carinartemis sankeyi* **(E)** from Kayon Cave, Mon State (shell height ~ 10 mm) and **(F)** from Saddan Cave, Mawlamyine, Mon State (shell height ~10 mm).

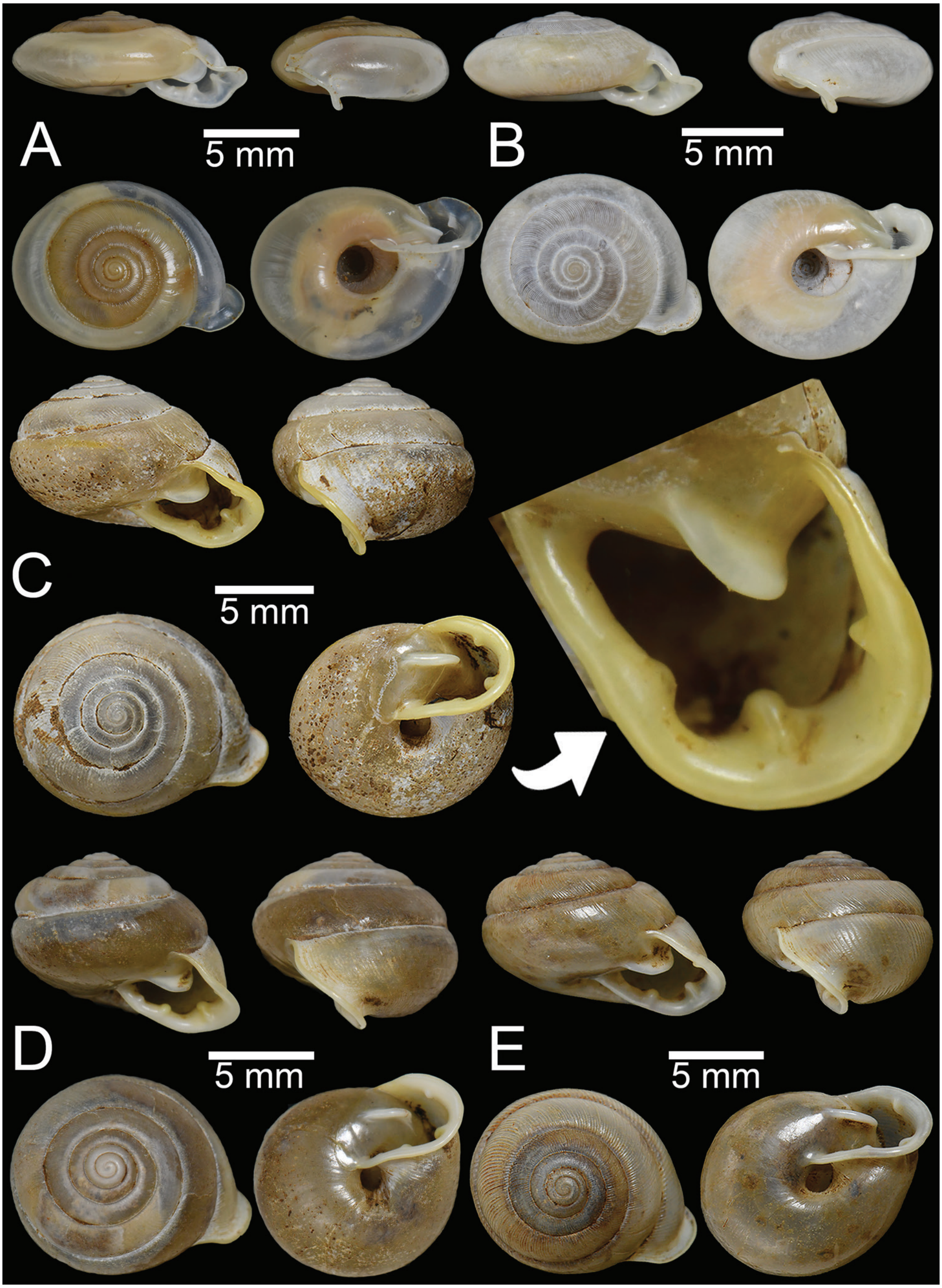

Figure 3. A, B *Discartemon tonywhitteni*, specimen CUMZ 6264 from Phra (Buddha) Cave, Tanintharyi Region **C–E** *Discartemon paurodeviatus* sp. nov. **C** holotype CUMZ 13002 with apertural dentition and **D, E** paratypes CUMZ 13003 from the type locality.

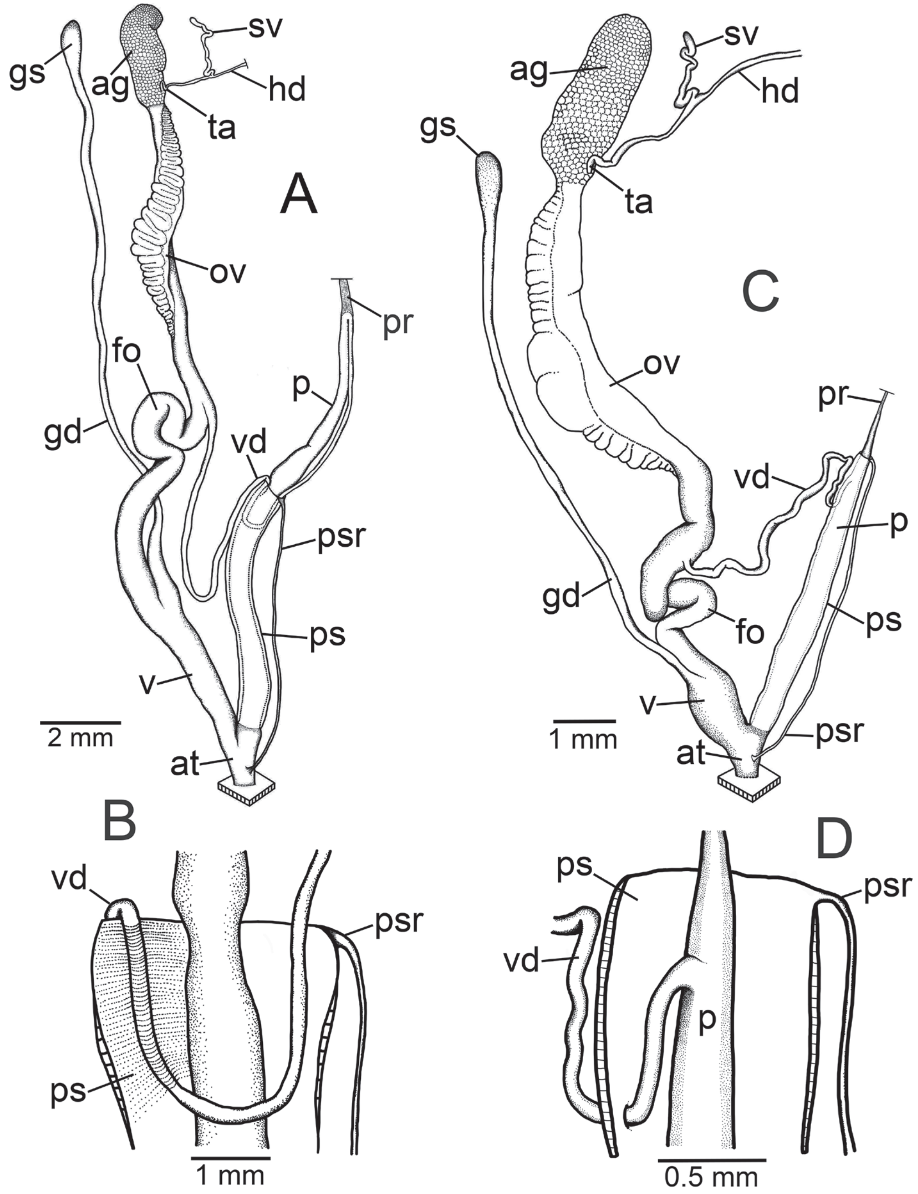

Figure 4. Genital anatomy of **A, B** *Discartemon tonywhitteni*, specimen CUMZ 13001 **A** reproductive system and **B** insertion of vas deferens into penial sheath **C, D** *Discartemon paurodeviatus* sp. nov., paratype CUMZ 13003 **C** reproductive system and **D** insertion of vas deferens into penial sheath.

Diagnosis. This species was clearly described in Sutcharit et al. (2020). Shell is depressed-heliciform, white and translucent, spire low conical to convex with wide and depressed suture. Shell surface glossy, with fine transverse ridges that diminish below

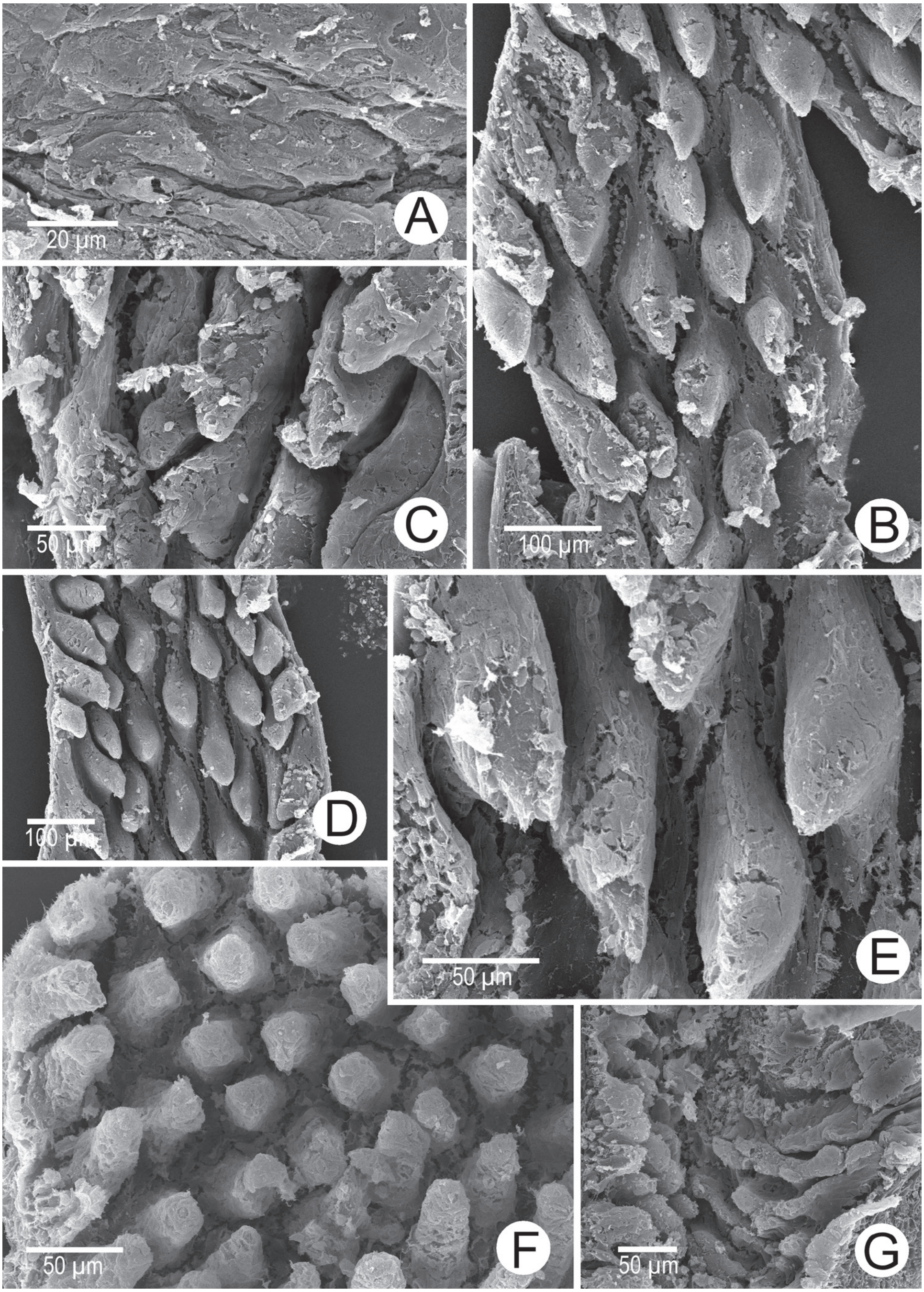

Figure 5. Internal sculpture of genitalia of *Discartemon tonywhitteni*, specimen CUMZ 13001 **A** atrium surface **B** arrangement of penial hooks on proximal part of penis **C** lateral view of proximal penial hooks **D** arrangement of penial hooks on middle part of penis **E** high magnification view of pointed penial hooks on middle part of penis **F** top view of distal penial hooks embedded in penial papillae **G** arrangement of transverse vaginal folds.

periphery; varices present. Embryonic shell large, ~ 2½ whorls with smooth surface; following whorls in regularly expanding coil. Last whorl usually angular; widely open umbilicus. Apertural dentition with one strong parietal, one palatal, one basal, one columellar and one supracolumellar lamella (Fig. 3A, B).

Genital organs. Atrium (at) short. Penis (p) long and slender. Penial sheath (ps) thin and extending ~1/2 to 3/4 of penis length; penial sheath retractor muscle (psr) very thin, originating at atrium and inserting distally on penial sheath (Fig. 4A). Vas deferens (vd) very short, ~ 1/6 of penial sheath length and passes through penial sheath before entering penis distally (Fig. 4B). Penial retractor muscle (pr) thin and very long, inserting at penis and vas deferens junction.

Internal wall of atrium generally smooth (Fig. 5A). Proximal and middle penial wall with dense and translucent penial hooks (~ 5 hooks/200 μm^2) and located on conical papillae (Fig. 5B–D). Penial hooks small (<0.04 mm in length), expanded at base, tips pointed (Fig. 5C, E). and curved towards genital orifice. Most penial hooks with blunt tips (Fig. 5E). Distal penial wall with dense and translucent conical penial papillae with embedded penial hooks, ~ 12 penial papillae/200 μm^2 (Fig. 5F).

Vagina (v) short, ~ 1/3 of penis length. Gametolytic duct (gd) a long tube extending as far as albumin gland; gametolytic sac (gs) ovate. Free oviduct (fo) long, ~ 2 times of vagina length. Oviduct (ov) slender and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) small, short and club shaped. Hermaphroditic duct (hd) bearing long seminal vesicle (sv) ca. twice as long as the length from talon to branching point of seminal vesicle (Fig. 4A).

Vaginal wall with transverse vaginal folds; vaginal hook absent (Fig. 5G).

Radula. Each row consists of ~ 35−49 teeth with formula (24−17) −1− (17−24). Central tooth very small with pointed cusp. Lateral and marginal teeth undifferentiated, unicuspid and lanceolate. Latero-marginal teeth gradually reducing in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 24A).

Distribution. *Discartemon tonywhitteni* is the southernmost distributed species and, currently, is known as the only form from the type locality (Fig. 1), which is an isolated limestone outcrop in Tanintharyi Region, southern Myanmar (Sutcharit et al. 2020).

Remarks. Sutcharit et al. (2020) described *D. tonywhitteni* based only on shells. Fortunately, seven alcohol-preserved specimens collected near the type locality were sent by the FFI staff after the species was published. These additional specimens allow us to describe the genitalia and radular morphology of this species herein.

Discartemon paurodeviatus **Man & Panha, sp. nov.**

https://zoobank.org/7F0B6632-7902-483B-A580-A3900EB06D6E
Figs 1, 2B, 3C–E, 4C, D, 6, 24B

Type material. ***Holotype*** CUMZ 13002 (Fig. 3C). Measurements: shell height 7.6 mm, shell width 11.0 mm, and 6 whorls. ***Paratypes*** CUMZ 13003 (14 shells;

Fig. 3D, E), CUMZ 13004 (3 specimens in ethanol), NHMUK (2 shells), all from the type locality.

Type locality. Small hill on Pahtaw Pahtet Island (~ 500 m west of Myeik Town), Myeik Township, Tanintharyi Region, Myanmar (12°26'3.5"N, 98°35'14.1"E).

Diagnosis. *Discartemon paurodeviatus* sp. nov. can be distinguished from *D. collingei* (Sykes, 1902) from Malaysia by having a larger shell, rounded last whorl, semi-ovate and slightly reflected aperture, and sometimes supracollumellar lamellae are present. Compared with *D. vandermeermohri* van Benthem Jutting, 1959 and *D. mekalostraka* Siriboon & Panha, 2014 from Thailand, this new species has a slightly extended last whorl from the penultimate whorl, last whorl slightly axially deflected, widely open umbilicus, and semi-ovate aperture. The genital organs of *D. paurodeviatus* sp. nov. differ from *D. mekalostraka* by having corrugated atrium without pore, short penis, dense brownish and long slender penial hooks located on penial papillae, and short vagina with thickened and reticulated vaginal folds.

Description. Shell globose-heliciform, white and translucent; whorls 6–6½; spire conical with distinct suture. Shell surface glossy with fine transverse ridges that diminish below periphery; varices present. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Last whorl rounded, little axially deflected and extruded from the penultimate whorl. Aperture semi-ovate; peristome yellowish to whitish, discontinuous, thickened, expanded, and slightly reflected. Apertural dentition with one strong parietal, one small palatal, one large basal, and one small columellar lamella; sometimes with small supracolumellar lamellae. Umbilicus open and deep (Fig. 3C–E).

Genital organs. Atrium (at) short. Penis (p) long and slender. Penial sheath (ps) very thin, extending entire penis length; penial sheath retractor muscle (psr) very thin, originating at atrium and inserting distally on penial sheath (Fig. 4C). Vas deferens (vd) passes through penial sheath without insertion before entering penis distally (Fig. 4D). Penial retractor muscle (pr) thin and long, inserting at penis and vas deferens junction.

Internal wall of atrium corrugated with sparse atrial pores (Fig. 6A). Penial wall with scattered, very long, slender, and pale brown penial hooks (~ 10 hooks/200 μm^2), hooks located on conical papillae that are separated by distinct reticulated folds (Fig. 6C, D). Penial hooks of small size (< 0.08 mm in length), slightly expanded and conical at base, tip sharp and directed towards genital orifice (Fig. 6B, E, F).

Vagina (v) very short and stout, ~ 1/7 of penis length. Gametolytic duct (gd) a long and slender tube extending as far as albumin gland; gametolytic sac (gs) ovate. Proximal free oviduct (fo) convoluted and distally long and thick, ~ 3 times of vagina length. Oviduct (ov) enlarged and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) small, short and club shaped. Hermaphroditic duct (hd) bearing short and thickened seminal vesicle (sv) and ca. same length as from talon to branching point of seminal vesicle (Fig. 4C).

Vaginal wall generally with thickened reticulated vaginal folds, smooth surface, and vaginal pores present (Fig. 6A, G).

Radula. Each row consists of ~ 21−33 teeth with formula (16−10)−1−(10−16). Central tooth small with pointed cusp. Lateral and marginal teeth largest and

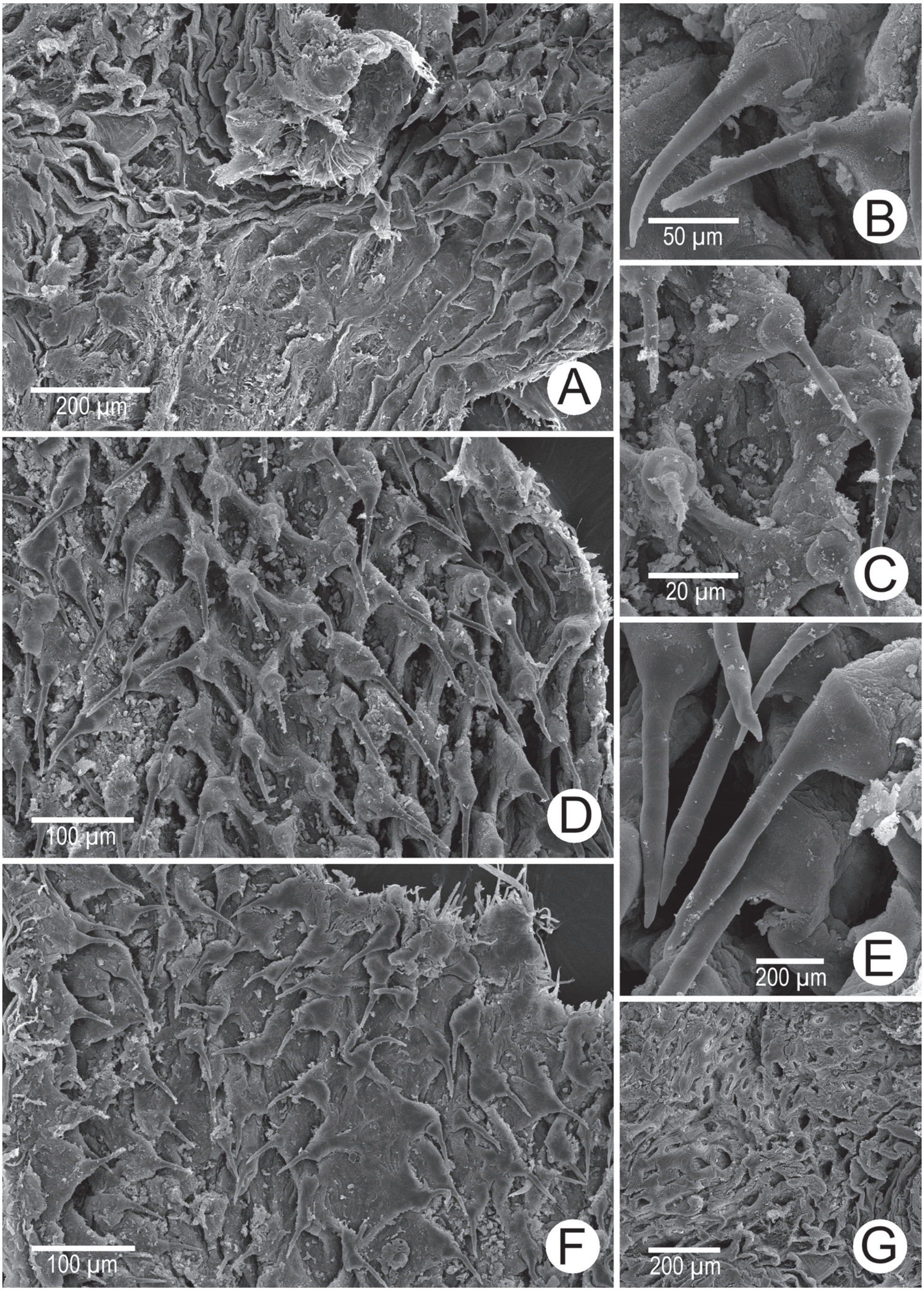

Figure 6. Internal sculpture of genitalia of *Discartemon paurodeviatus* sp. nov., paratype 13003 **A** junction of atrium, vagina, and proximal penis **B, C** lateral view of penial hooks on proximal part of penis **D** reticulated arrangement of penial hooks on middle part of penis **E** lateral view of penial hooks **F** arrangement of penial hooks on distal part of penis **G** arrangement of thickened reticulated vaginal folds.

undifferentiated, unicuspidal, and lanceolate. Latero-marginal teeth rapidly reducing in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 24B).

Etymology. The specific name *paurodeviatus* is derived from the Greek word *pauros* meaning little or few and the Latin word *devius* meaning out of the way. It refers to the last whorl of the new species as being slightly axially deflected and extruded from the penultimate whorl.

Distribution. The species is only known from the type locality in southern Myanmar (Fig. 1).

Remarks. Variation occurs in the possession of supracolumellar lamellae in some specimens. Currently, two species of *Discartemon* (*D. paurodeviatus* sp. nov. and *D. tonywhitteni*) have been recognized from Myanmar (Sutcharit et al. 2020). These two species are noticeably different in shell morphology and internal structure of genitalia and found in distant localities from each other.

Genus *Oophana* Ancey, 1884

Oophana Ancey, 1884: 508. Tryon 1885: 58. Richardson 1988: 233. Schileyko 2000: 796. Clements 2006: 125.

Odontartemon (*Oophana*) – Kobelt 1906: 91, 101. Thiele 1931: 730. van Benthem Jutting 1954: 95. Zilch 1960: 562.

Type species. *Ennea bulbulus* Morelet, 1862 by subsequent designation by Kobelt (1906: 101).

Diagnosis. Shell globosely ovate to sub-heliciform, translucent to opaque. Penultimate whorl slightly extended beyond last whorl. Last whorl rounded or flattened along the periphery, and axially deflected from penultimate whorl. Aperture oblique-ovate to squarish; apertural dentition with parietal (one or two), palatal, basal, and columellar lamellae. Genitalia with penial sheath covering ~ 1/2 of penis length, and penial hook present.

Remarks. The genus *Oophana* shares the ovate shell shape with the *Haploptychius*. However, *Oophana* can be distinguished by generally having a greater number of apertural dentitions (parietal, palatal, basal and columellar lamellae), while *Haploptychius* has only one parietal lamella (Schileyko 2000; Inkhavilay et al. 2016).

Generally, despite *Oophana* and *Indoartemon* Forcart, 1946 possessing an ovate shell with a blunt spire, the former can be distinguished by its parietal, palatal, basal, and columellar lamellae, while the latter possesses only one parietal and one palatal lamella (Schileyko 2000; Siriboon et al. 2014a). *Oophana* can be distinguished from *Discartemon* and *Perrottetia* Kobelt, 1905 by its globosely ovate shell, axially deflected last whorl, and penultimate whorl more or less extended from the last whorl. In comparison, *Discartemon* has a flattened to a globose-heliciform shell, regularly to rapidly growing last whorl that is less deflected, and penultimate whorl does not extend from the last whorl (Siriboon et al. 2014a). *Perrottetia* possesses a sub-heliciform and depressed shell, with longitudinal furrows behind the apertural lip, and a less deflected last whorl (Siriboon et al. 2013; Inkhavilay et al. 2016). Moreover, *Oophana* can easily be separated from the *Carinartemis* Siriboon & Panha, 2014 by their rounded penultimate whorl with more apertural dentition, whereas the latter genus has a sharply keeled penultimate whorl, and without or with only one parietal lamella (Siriboon et al. 2014b).

Generally, *Oophana* shows high variability in shell form, and apertural dentition with upper palatal and supracolumellar lamellae occurring in some species (Siriboon et al. 2020). However, examination of the genitalia in most species including the type species is still limited, making comparisons with other congeners difficult.

The phylogenetic relationships of the *Oophana* s.l. from Thailand were recently shown to be polyphyletic and comprised of three groups. These polyphyletic groups could possibly be recognized as distinct genera supported by their unique shell and genital characters (Siriboon et al. 2020). Furthermore, the oblique-heliciform shell and apertural dentition with four lamellae are shared among these three polyphyletic groups. Nevertheless, without the information on the type species, these characters are insufficient to restrict the true *Oophana* s.s., and so, in this revision we place the streptaxids that have an oblique-ovate shell and apertural dentition with four lamellae within the genus *Oophana* s.l.

***Oophana elisa* (Gould, 1856)**

Streptaxis elisa Gould, 1856: 13. Type locality: an island in the Mergui Archipelago. Pfeiffer 1868: 448. Blanford and Godwin-Austin 1908: 14.
Odontartemon elisa – Kobelt 1906: 126.
Perottetia elisa – Kobelt 1910: 151.
Oophana elisa – van Benthem Jutting 1954: 96. Richardson 1988: 234, 235. Clements 2006: 126.

Remarks. The shell is oblique-heliciform with convex spire and a distinct suture. Whorls 7 and shell surface with transverse ridges that diminish below the periphery. Shell periphery is keeled, and last whorl axially deflected. Umbilicus open and deep. Aperture is subquadrangular, peristome reflected. Apertural dentition with one parietal, one basal, two weak palatal, and one columellar lamella.

This species is known only from the type locality, in the southern part of Myanmar. No fresh materials were collected in this survey. Herein, the description of this species is based only on the original description. Gould (1856) received the specimen from Rev. J. Benjamin and described it without providing any figures. In the description, Gould compared *O. elisa* with two species: *Seychellaxis souleyetianus* (Petit, 1841) and *Haploptychius pyriformis* (Pfeiffer in Philippi, 1845). In differentiating the three, Gould noted that *O. elisa* has a larger and a more depressed shell than *S. souleyetianus*, and that *O. elisa* has a more elongated shell that is two-fold larger in size and posterior denticle (presumably parietal lamella) smaller than *H. pyriformis*. Based on this comparison, we assume that the shell of *O. elisa* differs from all other known congeners from Myanmar and Peninsular Malaysia by its largely deflected last whorl, discoid spire and keeled penultimate whorl. Besides probably having the same distribution range, *O. elisa* is similar to *D. paurodeviatus* sp. nov. in having a quite straight and parallel aperture lip, and four apertural dentitions. However, *O. elisa* seems to have an oblique-ovate shell with more deflected last whorl, keeled penultimate whorl, and discoid spire. Meanwhile, *D. paurodeviatus* sp. nov. possesses a globose shell with less deflected last whorl, and penultimate whorls being rounded. To date, no specimens or illustrations have been found after the first record.

Oophana mouhoti **(Pfeiffer, 1863)**

Figs 1, 7A, B

Streptaxis mouhoti Pfeiffer, 1863 [1862]: 273. Type locality: Siam [Thailand]. Martens 1867: 84, pl. 22, fig. 22. Pfeiffer 1868: 446. Nevill 1878: 4. Tryon 1885: 80, pl. 15, fig. 46. Bourguignat 1889: 133.

Streptaxis johswichi Martens, 1864: 528. Type locality: Siam, bei Petchaburi [Petchaburi Province, Thailand].

Streptaxis mouhoti var. *johswichi* – Martens 1864: 528. Pfeiffer 1868: 446.

Gonaxis mouhoti – Bourguignat 1889: 133.

Odontartemon (*Oophana*) *mouhoti* – Kobelt 1906: 104, 105, pl. 55, fig. 23.

Oophana mouhoti – Richardson 1988: 235, 236. Siriboon et al. 2020: 5, 14, 15.

Material examined. ***Possible syntype*** NHMUK ex. Cuming collection (3 shells; Fig. 7A) from Camboja [Cambodia]. NHMUK ex. Cuming collection (2 shells) from Siam. Phra (Buddha) Cave, Tanintharyi Region, Myanmar (11°14'01.5"N, 99°10'42.8"E): CUMZ 13005 (1 shell; Fig. 7B).

Diagnosis. *Oophana mouhoti* can be distinguished from *O. obtusus* (Stoliczka, 1871) by its higher spire, expanded aperture lip, deflected last whorl, and slightly angulate penultimate whorl. In contrast, *O. obtusus* exhibits a narrow aperture, spire convex, periphery of penultimate whorl usually equal to last whorl, strong columellar lamella, and small tubercle near the posterior angle of aperture.

Description. Shell oblique-ovate, white, and translucent; whorls 6½; spire elevated conical with distinct suture. Shell surface glossy with fine transverse ridges that diminish below the periphery; following whorls regularly coiled. Shell periphery rounded and last whorl axially deflected. Aperture subcircular; peristome expanded, thickened, discontinuous, and reflected. Apertural dentition with one strong parietal, one small upper palatal, one small palatal, and one basal lamella. Umbilicus open and deep (Fig. 7A, B).

Distribution. This species has been recorded from several localities in Peninsular Thailand (Siriboon et al. 2020) along the southeastern part of the Tenasserim Range. However, only one specimen was collected in Myanmar from the Tanintharyi Region.

Remarks. The only shell was collected from the limestone hills at Buddha Cave, Tanintharyi Region; it matches well with this species.

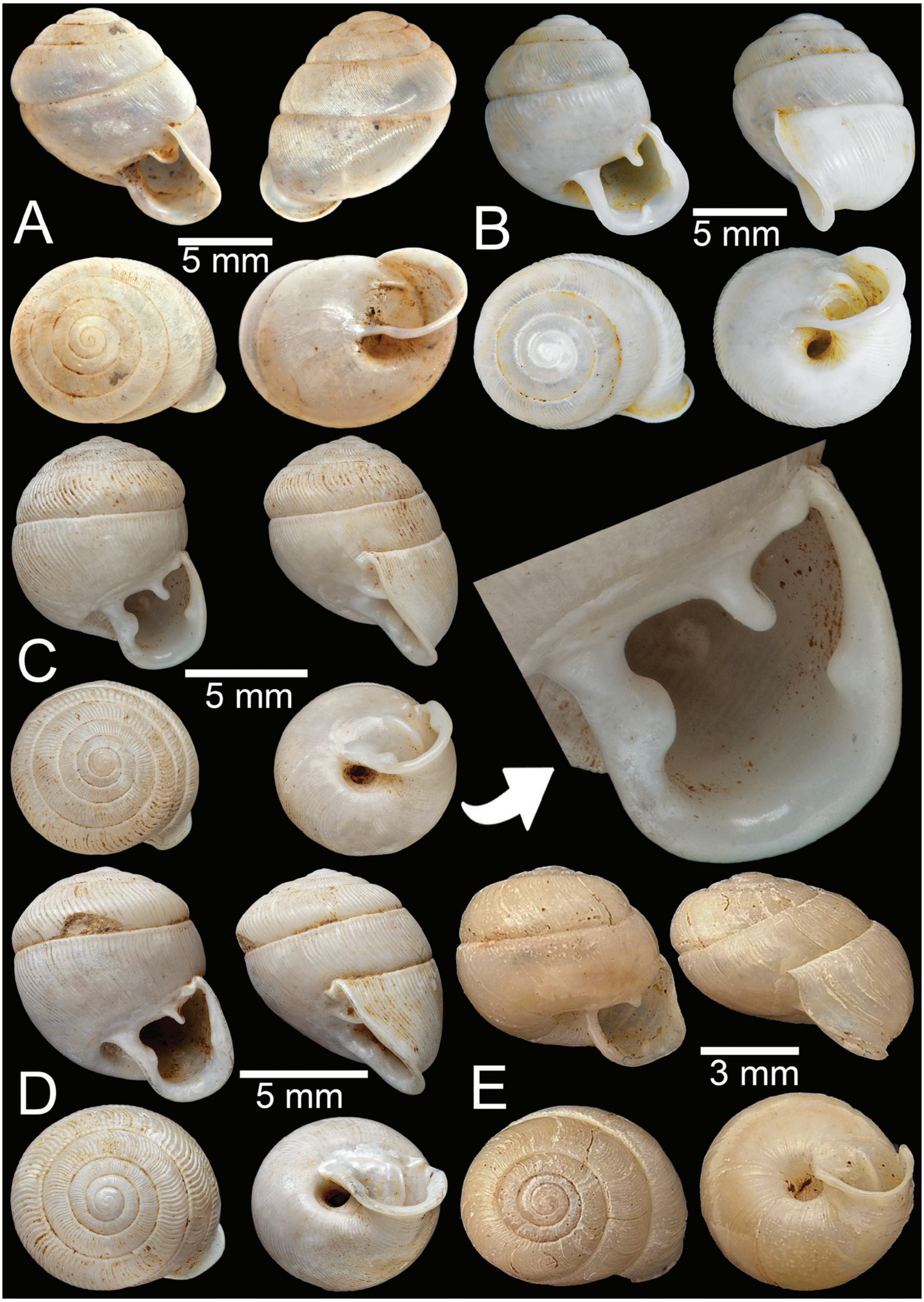

Figure 7. A, B *Oophana mouhoti* **A** possible syntype NHMUK ex. Cuming collection from Camboja and **B** specimen CUMZ 13005 from Phra (Buddha) Cave, Tanintharyi **C, D** *Oophana obtusus* **C** specimen NHMUK 1888.12.4.788–790 from Chauktalon, Moulmein with apertural dentition and **D** specimen NHMUK 1871.9.23.64 from Moulmein **E** *Oophana laevis*, possible syntype NHMUK 1899.7.4.11 from Burma.

Oophana mouhoti was originally described based on specimens in the collection of H. Cuming with the very brief collection locality given as Siam. The NHM, London holds a lot of three specimens in the Cuming collection collected by H. Mouhot but has Camboja as the collection locality. This specimen lot does not have Pfeiffer' handwriting on the label; therefore, they are considered possible syntypes. However, Mouhot's recorded localities were generally imprecise. No additional specimens for this species are available, nor are records or literature references from Cambodia, other than peninsular Thailand (Sutcharit et al. 2020). A similar situation of imprecise type locality records with Mouhot-collected specimens have been clarified in the case of helicarionid species (see Pholyotha et al. 2020). The recent works on land snails from Thailand confirmed that the specimens from the limestone hills along peninsular Thailand identified as *O. mouhoti* match well with the possible syntype. Therefore, peninsular Thailand (Petchaburi and Prachuap Khiri Khan provinces) are possibly the collecting locality for this species. Furthermore, H. Mouhot visited Petchaburi Province once from May to August in 1860 [see travel routes map in Mouhot (1864a, b)]. Additionally, *Streptaxis johswichi* Martens, 1864 was described based on specimens collected from Petchaburi Province, Thailand. Later, Martens (1867) examined the *O. mouhoti* specimens in the Cuming collection and agreed that they belonged to the same species.

***Oophana obtusus* (Stoliczka, 1871)**
Fig. 7C, D

Streptaxis obtusus Stoliczka, 1871: 166, 167, pl. 7, figs 11–13, pl. 8, figs 1–4. Type locality: Prope Moulmein, provincia Tenasserim [Mawlamyine District, Mon State, Myanmar]. Pfeiffer 1876: 495. Nevill 1878: 3. Tryon 1885: 76, pl. 15, fig. 45. Gude 1903: 323, pl. 12, figs 8–10. Blanford and Godwin-Austin 1908: 9.
Odontartemon (*Oophana*) *obtusus* – Kobelt 1906: 106, pl. 56, figs 1–3.
Oophana obtusa – Richardson 1988: 236.

Material examined. Moulmein: NHMUK 1903.7.1.4002 (2 shells) ex. Godwin-Austen collection. NHMUK 1871.9.23.64 (1 shell, Fig. 7D). NHMUK 1937.7.91–92. Chauktalon, Moulmein: NHMUK 1906.2.2.342 (3 shells + 1 juvenile) ex. Blanford collection. NHMUK 1888.12.4.788–790 (3 shells, Fig. 7C) ex. Blanford collection. Sacred Lakes, Moulmein: NHMUK 1889.3.15.1 (1 shell) ex. Godwin-Austen collection.

Description. Shell oblique-ovate, white, translucent; whorls 6–7; spire convex with distinct suture. Shell surface glossy with fine transverse ridges, nearly smooth with few transverse ridges near peristome. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl slightly axially deflected. Aperture subcircular; peristome continuous, expanded, slightly reflected, and very short sinulus. Apertural dentition with one strong parietal, one palatal, and one strong columella lamella. Umbilicus open and deep (Fig. 7C, D).

Distribution. This species is only known from the type locality, the southern part of Myanmar (Stoliczka 1871).

Remarks. No new specimens were collected in this survey; however, authenticated museum specimens were examined. Regarding genitalia, *Oophana obtusus* and *Haploptychius burmanicus* were the first two species anatomically examined and illustrated among several streptaxids in Myanmar by Stoliczka (1871), who noted that there was no noticeable difference in reproductive forms between these two species. Based on the genitalia drawing, *O. obtusus* has a thick penial sheath covering almost the entire penis, and vas deferens not inserted into the penial sheath, but the lengths of vagina and atrium are unclear. Thus, the description and drawing remain insufficient to confirm the distinguishing characters of this species.

Oophana laevis (Blanford, 1899)

Fig. 7E

Streptaxis laevis Blanford, 1899: 765, pl. 50, figs 11–12. Type locality: Tenasserim [probably Tanintharyi Region, Myanmar]. Gude 1903: 218. Blanford and Godwin-Austen 1908: 13.
Streptaxis (*Odontartemon*) *laevis* – Kobelt 1905: 93, 94, pl. 60, figs 11, 12.
Odontartemon (*Oophana*) *laevis* – Kobelt 1910: 150.
Indoartemon laevis – Richardson 1988: 225. Siriboon et al. 2014b: 163.

Material examined. ***Possible syntype*** NHMUK 1899.7.4.11 (1 shell; Fig. 7E) ex. Beddome collection from Burma [Myanmar].

Diagnosis. *Oophana laevis* differs from *O. mouhoti* and *O. obtusus* by having a rounded penultimate whorl, convex spire, and apertural dentition with parietal and basal lamellae. Although *O. mouhoti* and *O. obtusus* have a slightly angular penultimate whorl and elevated spire, *O. mouhoti* usually has only a parietal lamella (sometimes with basal lamella). In contrast, *O. obtusus* has parietal, palatal, and columellar lamellae.

Description. Shell oblique-heliciform, white, and translucent; whorls 5½; spire low convex with distinct suture. Shell surface glossy with transverse ridges that diminish below periphery. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl axially deflected. Aperture subquadrangular with sinulus; peristome discontinuous, thin, expanded and reflected. Apertural dentition with one parietal and one basal lamella, and sometimes columellar lamella is absent. Umbilicus open and shallow (Fig. 7E).

Distribution. This species is known only from the type locality, which was mentioned only as 'Tenasserim' (Blandford 1899). This locality possibly refers to the Tanintharyi Region, southeastern Myanmar.

Remarks. No new specimens were collected in this study. However, we compared the possible type specimen with the other congeners. Blanford (1899) clearly stated that the type series was collected by Beddome and consisted of three specimens. However, the NHM, London holds a lot of only a single specimen from the Beddome collection, with the word India, probably added at a later date, on the label. Therefore, we consider this lot to be a possible syntype.

Genus *Perrottetia* Kobelt, 1905

Odontartemon (*Perrottetia*) Kobelt, 1905: 91. Kobelt 1906: 108. Thiele 1931: 730. Forcart 1946: 215.
Oophana (*Perrottetia*) – van Benthem Jutting 1954: 95.
Perrottetia – Zilch 1960: 562, 563. Richardson 1988: 237. Schileyko 2000: 777, 778. Siriboon et al. 2013: 44, 45.

Type species. *Helix perrotteti* Petit, 1841 by subsequent designation by Forcart (1946: 215).

Diagnosis. Shell oblique-heliciform, and translucent. Longitudinal furrows present behind apertural lip. Apertural dentition usually consists of two parietal, one palatal, one basal and one columellar lamella, and upper palatal and supracolumellar lamellae may be present. Genitalia with long and slender penis, penial sheath thin and not extending the entire penis length. Penial hooks dense, pale brown, expanded at base, with pointed tips, located on penial papillae, and curved towards genital orifice. Vaginal hooks may be present.

Remarks. *Perrotettia* and *Discartemon* are generally similar in having complex apertural dentition; however, their shell morphology is obviously different. *Perrottetia* has a sub-heliciform shell, smaller size, last whorl rounded and more or less deflected, and mostly with two parietal lamellae. Although parietal, palatal, basal and columellar lamellae are always present in *Perrottetia*, possession of second parietal, upper palatal and supracolumellar lamellae is variable, as is the presence of bifid lamellae (Siriboon et al. 2013; Inkhavilay et al. 2016; Bui et al. 2019). In contrast, *Discartemon* exhibits flattened to globose-heliciform shells, last whorl rounded to angular and less deflected with, usually, one parietal lamella.

Nonetheless, some *Discartemon* species from Thailand have two parietal lamellae, for example, *D. afthonodontia* (see Siriboon et al. 2014a: fig. 7a, b) and *D. flavacandida* (see Siriboon et al. 2014a: fig. 7e, f). Likewise, some *Perrottetia* species are also described with only a single parietal lamella, for example, the Vietnamese species *P. aberrata* (Souleyet, 1852) and *P. namdongensis* Bui & Do, 2019 (see Bui et al. 2019: figs 2c–e, 3a–c), and the Laos species *P. unidentata* Inkhavilay & Panha, 2016 (see Inkhavilay et al. 2016: fig. 5f–i).

At present, the genus *Perrottetia* is comprised of about 30 nominal species distributed from India and Sri Lanka to Indochina and southern China (Kobelt 1906; Richardson 1988; Schileyko 2011; Siriboon et al. 2013; Inkhavilay et al. 2016). So far, *P. theobaldi* Benson, 1859 is the only species known from Myanmar.

Perrottetia theobaldi (Benson, 1859)

Fig. 8

Streptaxis theobaldi Benson, 1859b: 187. Type locality: Nauclai (lat. 25°15', long. 92°30') [probably in Meghalaya State, India]. Pfeiffer 1868: 449. Hanley and Theobald 1870: 4, pl. 8, fig. 9. Nevill 1878: 3. Tryon 1885: 77, pl. 16, figs 14, 86. Blanford and Godwin-Austen 1908: 9, 10. Ramakrishna et al. 2010: 189.

Odontartemon (*Perrottetia*) *theobaldi* – Kobelt 1906: 111, 112, pl. 57, fig. 25. Kobelt 1910: 151.

Perrottetia theobaldi – Richardson 1988: 242, 243. Gittenberger et al. 2021: 74, fig. 1.

Material examined. ***Syntype*** UMZC I.102535 (2 shells) from unknown locality. Khasi Hill: NHMUK 1888.12.4.784–786 (3 shells; Fig. 8A) ex. Theobald collection. Siam boundary: NHMUK 1903.7.1.4005 (7 shells + 1 juvenile; Fig. 8C) ex. Godwin-Austen collection. Burma: NHMUK 1871.9.23.143 (1 shell; Fig. 8B). Aik Kham Cave, Taunggyi Township, Shan State, Myanmar (20°49'07.0"N, 97°13'42.0"E): CUMZ 13006 (4 shells; Fig. 8D).

Diagnosis. *Perrottetia theobaldi* is similar to *P. dugasti* (Morlet, 1892) from Vietnam; however, the former species can be distinguished by having a second parietal lamella running to the main parietal lamella, thicker and less reflected peristome, subquadrangular aperture, and shallow suture. In contrast, *P. dugasti* has a weak second parietal lamella running to the sinulus, thinner and more reflected peristome, semi-ovate aperture, and a deeper suture with a clear bifid columellar lamella. Similarly, *P. mabillei* (Bavay & Dautzenberg, 1903) from Vietnam can be separated from *P. theobaldi* by its strong radial ridges, wide and short sinulus, basal lamella absent, bifid columellar lamellae, and deep umbilicus, while *P. theobaldi* has a smooth shell surface, long and narrow sinulus, strong basal lamella, columellar lamellae separated, and shallow umbilicus. Furthermore, *P. theobaldi* differs from the Thai species *P. aquilonaria* Siriboon & Panha, 2013 by having a shallow suture, narrow and long sinulus, rounded last whorl, peristome thicker and less reflected, aperture subquadrangular and columellar lamellae are separated. In contrast, *P. aquilonaria* possesses deep suture, shorter and wider sinulus, the last whorl shouldered, aperture subcircular, more expanded peristome, and bifid columellar lamella.

Description. Shell sub-oblique heliciform, white, and translucent; whorls 5–5½; spire convex with distinct suture. Shell surface glossy with transverse ridges that diminish below periphery. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl axially deflected; two deep and short longitudinal furrows present. Aperture triangular with sinulus; peristome continuous, thickened, expanded, and reflected. Apertural dentition with one large and strong parietal, nearly adjoined small second parietal lamella; adjoined at right angles, one small upper palatal, one strong palatal, one strong basal, and bifid columellar lamella. Columellar lamella is sometimes absent. Umbilicus narrow (Fig. 8).

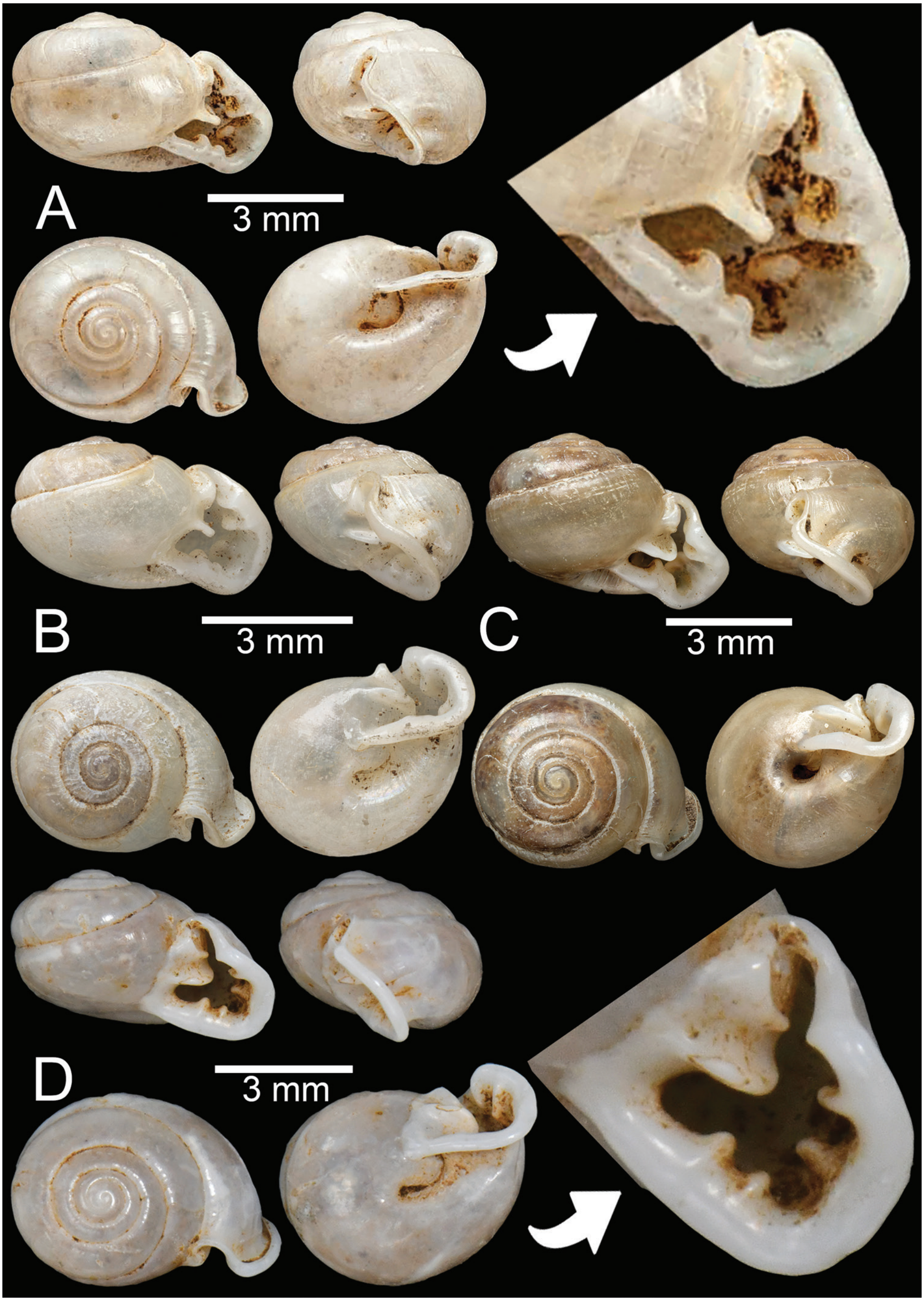

Figure 8. *Perrottetia theobaldi* **A** syntype UMZC I.102535 (no locality data) with apertural dentition **B** specimen NHMUK 1871.9.23.143 from Burma **C** specimen NHMUK 1903.7.1.4005 from Siam boundary **D** specimen CUMZ 13006 from Aik Kham Cave, Taunggyi, Shan State with apertural dentition.

Distribution. This species has been widely recorded from India to Bhutan and Myanmar (Blanford and Godwin-Austen 1908; Ramakrishna et al. 2010; Gittenberger et al. 2021). In Myanmar, the previous record was from Bhamo, Kachin State (Blanford and Godwin-Austin 1908) and the empty shells together with *H. thebawi* (Godwin-Austin, 1888) were found from Shan State in this sampling.

Remarks. All the specimens of *P. theobaldi* (Fig. 8) showed slight differences in the apertural dentition and peristome positions, particularly the columellar and supra-columellar lamellae that are closer or almost united compared to the specimens from the Aik Kham Cave, Shan State (Fig. 8D). However, all were collected from the same range; we place them as the same species.

Genus *Haploptychius* von Möllendorff, 1906

Haploptychius von Möllendorff in Kobelt, 1906: 127. Zilch 1960: 562. Richardson 1988: 211. Schileyko 2000: 796, 797. Inkhavilay et al. 2016: 26, 27. Do et al. 2015: 40. Bui et al. 2019: 90.
Odontartemon (*Haploptychius*) – Thiele 1931: 730. Forcart 1946: 215.
Oophana (*Haploptychius*) – van Benthem Jutting 1954: 76, 95.

Type species. *Streptaxis sinensis* Gould, 1859, by original designation.

Diagnosis. Shell oblique-heliciform to ovate and last whorl deflected. Penultimate whorl round to bluntly angular and extended beyond last whorl. Apertural dentition includes only one parietal lamella. Genitalia with penial sheath thin to thick and extending ~ 1/2 to entire penis length. Penial hooks dense, slightly expanded at base, tips pointed, located on low to high conical papillae and vaginal hooks absent.

Remarks. *Haploptychius* is almost identical with *Carinartemis* in having a deflected last whorl, extended penultimate whorl, and bearing a single parietal lamella. However, *Haploptychius* can be recognized by its oblique-ovate shell and rounded to angular penultimate whorl, while *Carinartemis* possesses an oblique-heliciform shell with sharply keeled penultimate whorl. In genitalia, *Haploptychius* has long and slender penial hooks, without vaginal hooks, while *Carinartemis* possesses shorter and blunt penial hooks, and sometimes transparent vaginal hooks may be present (Siriboon et al. 2014b; Inkhavilay et al. 2016).

A recent molecular phylogeny revealed that *Haploptychius* is polyphyletic, and the traditional genus concept using shell shape and apertural dentition seems unreliable (Siriboon et al. 2020). However, further intensive genital examination and molecular analysis of the type species are necessary to restrict the *Haploptychius* s.s., and generic revision absolutely requires both forms of evidence. Therefore, since the morphological and molecular evidence have never been evaluated, we follow the traditional genus concept in recognizing the streptaxids with oblique-heliciform shells and only one parietal lamella as members of the genus *Haploptychius* s.l.

Currently, the genus *Haploptychius* is comprised of about 40 nominal species distributed from India to Indochina, southern and central China, and Sulawesi of Indonesia (Kobelt 1906; Blanford and Godwin-Austen 1908; Zilch 1961; Richardson 1988; Schileyko 2000; Do and Do 2015; Inkhavilay et al. 2016). In addition, five species of *Haploptychius* s.l. have been documented mainly in south-eastern Myanmar (Blanford and Godwin-Austen 1908), and three new species are added in this study.

***Haploptychius bombax* (Benson, 1859)**

Fig. 9

Helix bombax Benson, 1859b: 186. Type locality: Moulmein, necnon and Phie Than in provincia Tenasserim [Mawlamyine, Mon State and Payathonzu, Kayin State, Myanmar]. Pfeiffer 1868: 151. Hanley and Theobald 1870: 15, pl. 31, figs 1, 4.

Streptaxis bombax – Stoliczka 1871: 167. Hanley and Theobald 1876: 63, pl. 156, fig. 9. Nevill 1878: 3. Blanford and Godwin-Austen 1908: 4, 5.

Haploptychius bombax – Kobelt 1906: 147, 148, pl. 63, figs 7–9. Richardson 1988: 212.

Material examined. ***Syntype*** UMZC I.102470 (3 shells; Fig. 9A) from Moulmein. India [error]: NHMUK ex. Cuming collection (1 juvenile). Moulmein: NHMUK 1903.7.1.3990 (4 shells; Fig. 9D) ex. Godwin-Austen collection. NHMUK 1906.2.2.159 (1 juvenile; Fig. 9B) ex. Blanford collection. NHMUK 1906.2.2.340 (2 shells) ex. Blanford collection. NHMUK ex Godwin-Austen collection (2 shells). NHMUK 1888.12.4.768–770 (3 shells; Fig. 9C).

Diagnosis. *Haploptychius bombax* can be differentiated from *H. burmanicus* by having a large oblique-ovate shell, without sinulus, and larger or wider last whorl. In contrast, *H. burmanicus* possesses a small sub-oblique heliciform shell, wide sinulus, and compressed last whorl. Compared with *H. pellucens* (Pfeiffer, 1863) from Laos, *H. bombax* differs by having strong radial ridges, convex spire, and narrow umbilicus, while *H. pellucens* exhibits a nearly smooth surface, more ovate shape, elevated spire, and umbilicus widely open.

Description. Shell oblique-ovate, white, and translucent; whorls 5½–7; spire convex with distinct suture. Shell surface glossy with fine transverse ridges that diminish below periphery of penultimate whorl. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl axially deflected. Aperture semi-ovate; peristome discontinuous, thickened, slightly expanded, and reflected. Apertural dentition with one strong parietal lamella. Umbilicus open and deep (Fig. 9).

Distribution. *Haploptychius bombax* is only known from the type locality in south-eastern Myanmar (Benson 1859b).

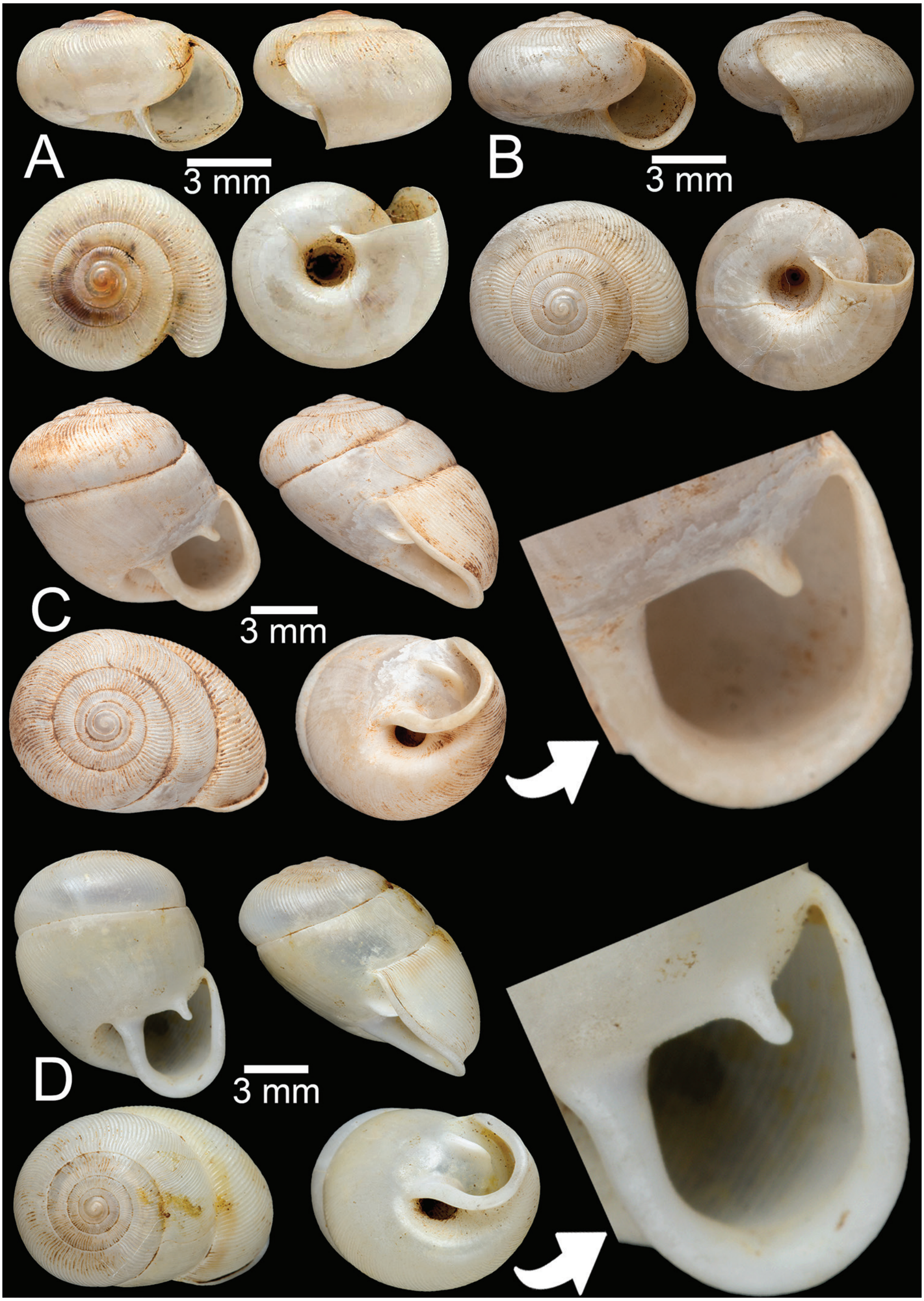

Figure 9. *Haploptychius bombax* **A** syntype UMZC I.102470 from Moulmein **B** juvenile specimen NHMUK 1906.2.2.159 from Moulmein **C** specimen NHMUK 1903.7.1.3990 from Moulmein with apertural dentition **D** specimen NHMUK 1888.12.4.768–770 from Moulmein with apertural dentition.

Remarks. The original description mentioned only 5½ whorls, but the museum specimen (Fig. 9C, D) had 7 whorls. This study collected no specimens, but authenticated museum specimens (juveniles and adults) were examined.

Haploptychius blanfordi (Theobald, 1864)

Fig. 10A–C

Streptaxis blanfordi Theobald, 1864: 245. Type locality: Montibus Arakanensibus provincia Pegu [Arakan Mountains in Bago Region, Myanmar]. Nevill 1878: 2.

Streptaxis birmanica Blanford, (in MSS.) Theobald, 1864: 245, 246 (in part). Type locality: Pegu [Bago Region, Myanmar]. Theobald 1865: 277.

Streptaxis blanfordianus Stoliczka 1871: 163, pl. 7, figs 8, 9 (unjustified emendation). Pfeiffer 1876: 494.

Streptaxis birmanica – Hanley and Theobald 1876: 4, pl. 8, fig. 5 (non Theobald 1864).

Streptaxis blanfordi var. – Godwin-Austen 1895: 443.

Haploptychius blanfordianus – Kobelt 1906: 143, 144, pl. 57, fig. 15, pl. 62, figs 4, 5.

Haploptychius blanfordi – Richardson 1988: 212.

Streptaxis blanfordi – Hanley and Theobald 1870: 4, pl. 8, fig. 10 (non Theobald 1864).

Streptaxis birmanicus [sic] – Richardson 1988: 251.

Material examined. ***Syntype*** NHMUK 1888.12.4.777–779 (3 shells; Fig. 10A) from Pegu.NHMUK 1909.3.15.71 (1 shell; Fig. 10C) ex. Godwin-Austen collection. Pegu, Arakan: NHMUK 1909.3.15.93 (3 shells) ex. H. Blanford collection. Akouktoung, Pegu: NHMUK 1906.2.2.338 (4 shells; Fig. 10B) ex. W.T. Blanford collection. Pegu, Arakan: NHMUK 1909.3.15.93 (3 shells) ex. H. Blanford collection.

Diagnosis. *Haploptychius fischeri* (Morlet, 1887) almost shares the same shell form as *H. blanfordi*, but the latter possesses a more depressed shell, penultimate whorl more bulging, aperture wider, longer and subquadrangular shape.

Description. Shell oblique-heliciform, white, and translucent; whorls 6; spire convex with distinct suture. Shell surface glossy with fine transverse ridges that diminish below periphery. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl axially deflected. Aperture semi-ovate; peristome discontinuous, thickened, expanded, and reflected. Apertural dentition with one strong parietal and small palatal lamellae. Umbilicus open and deep (Fig. 10A–C).

Distribution. This species has been recorded with a wide distribution range from Arakan [Rakhine State], Pegu [Bago Region], and Shan State in Myanmar. However, the record from Cocos Islands in the southern Indian Ocean by Blanford and Godwin-Austen (1908) needs to be verified with modern evidence rather than by shell morphology alone.

Remarks. The taxonomic status of this species is still ambiguous because it is identical in shell morphology to *Streptaxis birmanica*. Theobald (1864) consecutively de-

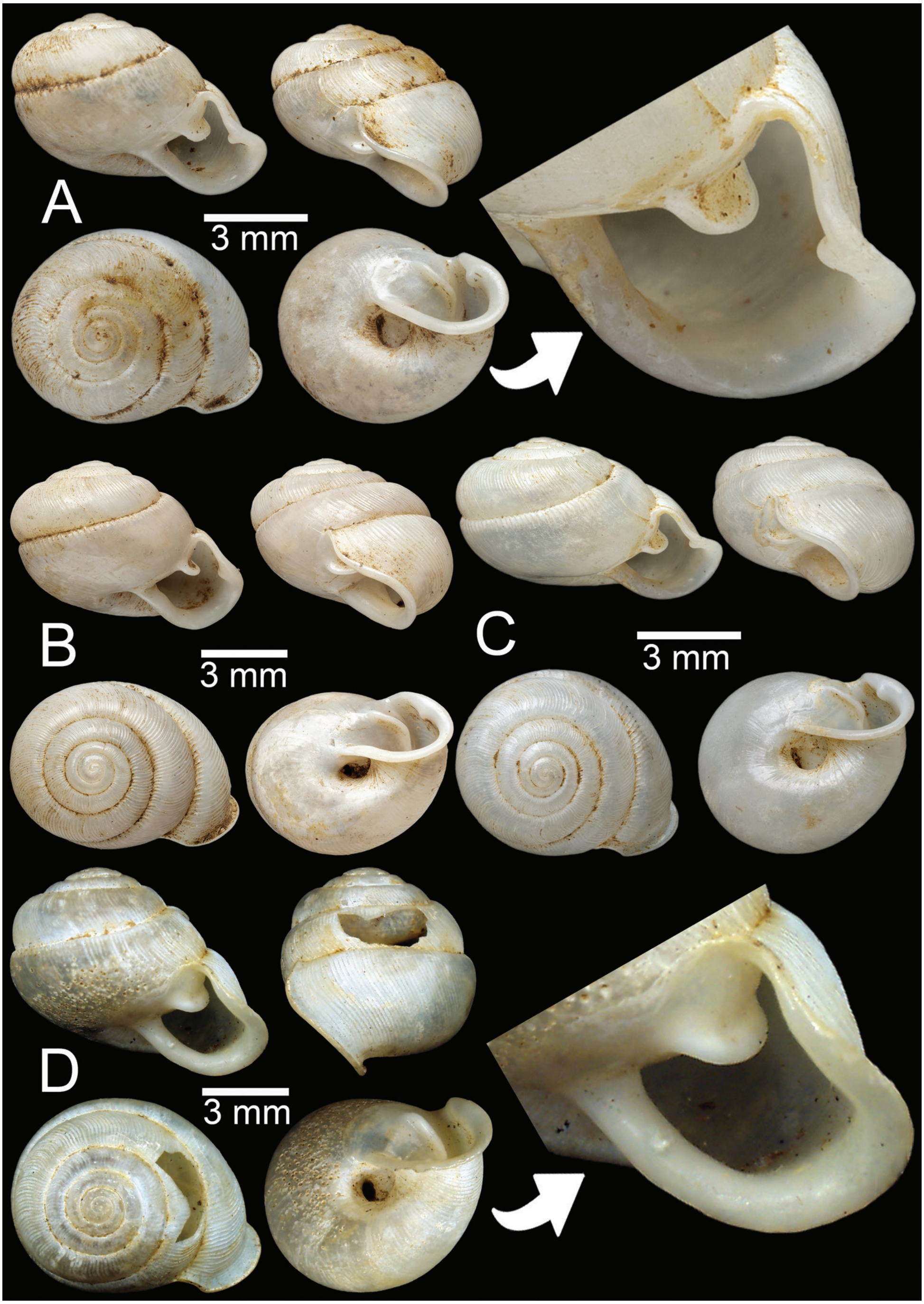

Figure 10. A–C *Haploptychius blanfordi* **A** syntype NHMUK 1888.12.4.777–779 from Pegu with apertural dentition **B** specimen NHMUK 1906.2.2.338 from Akouktoung, Pegu **C** specimen NHMUK 1909.3.15.71 from Pegu **D** *Haploptychius burmanicus*, syntype NHMUK 1906.2.2.199 from Tongoop, Arakan with apertural dentition.

scribed *Streptaxis blanfordi* and *Streptaxis birmanica* based on specimens from the same geographical area as Pegu. These two nominal species resemble each other in having one parietal and one palatal lamella, whereas they differ in only minor characters of shell shape and size. In this revision, we consider these two nominal species as synonyms. Only the type specimens of *H. blanfordi* could be located and examined; we therefore consider *Streptaxis birmanica* as the junior synonym.

The original spelling of the species was *blanfordi*, which was intentionally modified to *blanfordianus* by Stoliczka (1871) but for an unclear reason. This subsequent spelling of *blanfordianus* is an unjustified emendation made available with its own authorship and date, and so became a junior objective synonym (ICZN 1999: Arts 32.3, 33.2.3, 50.5).

Theobald (1864) clearly stated that *Streptaxis birmanica* W. Blanford, (in MSS.) was described based on a single specimen received from W.T. Blanford. However, we could not locate this single shell as the holotype fixed by monotypy. Theobald (1864) also stated that the other two shells lacked the palatal lamella and were of a relatively smaller size, which are recognized as distinct varietal entities. Therefore, these two specimens are excluded from the type series of *Streptaxis birmanica*. The authorship was originally attributed to W.T. Blanford as the manuscript name. However, since Blanford did not write the description or credit the description to W.T. Blanford, the taxon is attributed to Theobald only (ICZN 1999: Art. 50.1.1).

Haploptychius burmanicus **(Blanford, 1865)**

Fig. 10D

Streptaxis burmanica Blanford 1865: 81, 82. Type locality: Tongoop, Arakan [Toungup, east of Arakan Hills, Thandwe District, Rakhine State, Myanmar].

Streptaxis burmanicus [sic] – Pfeiffer 1868: 444. Stoliczka 1871: 163, pl. 7, figs 5–7. Nevill 1878: 2. Blanford and Godwin-Austen 1908: 6, fig. 5. Richardson 1988: 251.

Haploptychius burmanicus – Kobelt 1906: 145: pl. 57, figs 19, 20, pl. 62, figs 1–3. Richardson 1988: 213. Ramakrishna et al. 2010: 190.

Material examined. ***Possible syntype*** NHMUK 1906.2.2.199 (2 adults + 1 juvenile; Fig. 10D) ex. W.T. Blanford collection from Tongoop, Arakan [Taungup, Rakhine State].

Description. Shell oblique-heliciform, white, and translucent; whorls 6; spire convex with distinct suture. Shell surface glossy with fine transverse ridges that diminish below periphery. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl axially deflected. Aperture semi-ovate; peristome continuous; sometimes discontinuous, thin, expanded, and slightly reflected. Apertural dentition with one strong parietal lamella. Umbilicus open and deep (Fig. 10D).

Distribution. *Haploptychius burmanicus* is still known only from the type locality in southern Myanmar (Blanford 1865). Outside Myanmar, it is also reported from Mizoram in India (Ramakrishna et al. 2010); however, this record needs to be verified.

Remarks. This species highly resembles *H. blanfordi* in shell form, but *H. burmanicus* is more globosely heliciform, with a higher spire and only one parietal lamella. Without anatomical information, the generic placement of this species is still tentative, and we retain this as Blanford and Godwin-Austen (1908). In addition, *H. perlissus* Vermeulen et al. 2019 from Vietnam (Vermeulen et al. 2019: figs 49–51) can be distinguished from *H. burmanicus* by having an oblique-ovate shell, less extended penultimate whorl from the last whorl, lower to nearly flattened spire, the palatal side is almost straight, basal side rounded, and columellar side broadly rounded, whereas *H. burmanicus* has a higher spire, more compressed shell, and the last whorl is more axially deflected.

Haploptychius solidulus (Stoliczka, 1871)

Figs 1, 11

Streptaxis solidulus Stoliczka, 1871: 166, pl. 7, fig. 10. Type locality: Moulmein, Tenasserim [Mawlamyine District, Mon State, Myanmar]. Pfeiffer 1876: 494. Hanley and Theobald 1874: 40, pl. 98, fig. 7. Nevill 1878: 3. Tryon, 1885: 71, pl. 14, fig. 99. Gude 1903: 212. Blanford and Godwin-Austen 1908: 7. Kobelt 9010: 152.
Haploptychius solidulus – Richardson 1988: 221, 222.

Material examined. Moulmein: NHMUK 1937.7.9.10 (1 shell). NHMUK 1906.2.2.339 (1 shell) ex. Blanford collection. NHMUK 1888.12.4.774–776 (3 shells; Fig. 11B). NHMUK 1909.3.15.75 (1 shell; Fig. 11A) ex. Godwin-Austen collection. Pathen Mountain, east bank of Attaran River and ~ 45 km southeast of Kyaikmaraw Township, Mawlamyine District, Mon State, Myanmar (16°14'7.5"N, 97°56'48.1"E): CUMZ 13007 (15 shells; Fig. 11C, D).

Diagnosis. Among the *Haploptychius* species from Myanmar, this species is distinctly large with an oblique-ovate shell, and less deflected last whorl. *Haploptychius solidulus* has almost the same shell form as *Oophana mouhoti*. However, it differs from *O. mouhoti* by having somewhat enlarged last whorl, more deflected last whorl, and a conical spire with only one parietal lamella. In contrast, *O. mouhoti* shows a depressed and obtuse conical spire, with one parietal and small basal lamellae.

Description. Shell oblique-ovate, white, and opaque; whorls 6½–7; spire elevated conical with distinct suture. Shell surface glossy with fine transverse ridges that diminish below periphery. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl axially deflected. Aperture semi-ovate; peristome discontinuous, thickened, expanded, and slightly reflected. Apertural dentition with one strong parietal lamella. Umbilicus open and deep (Fig. 11).

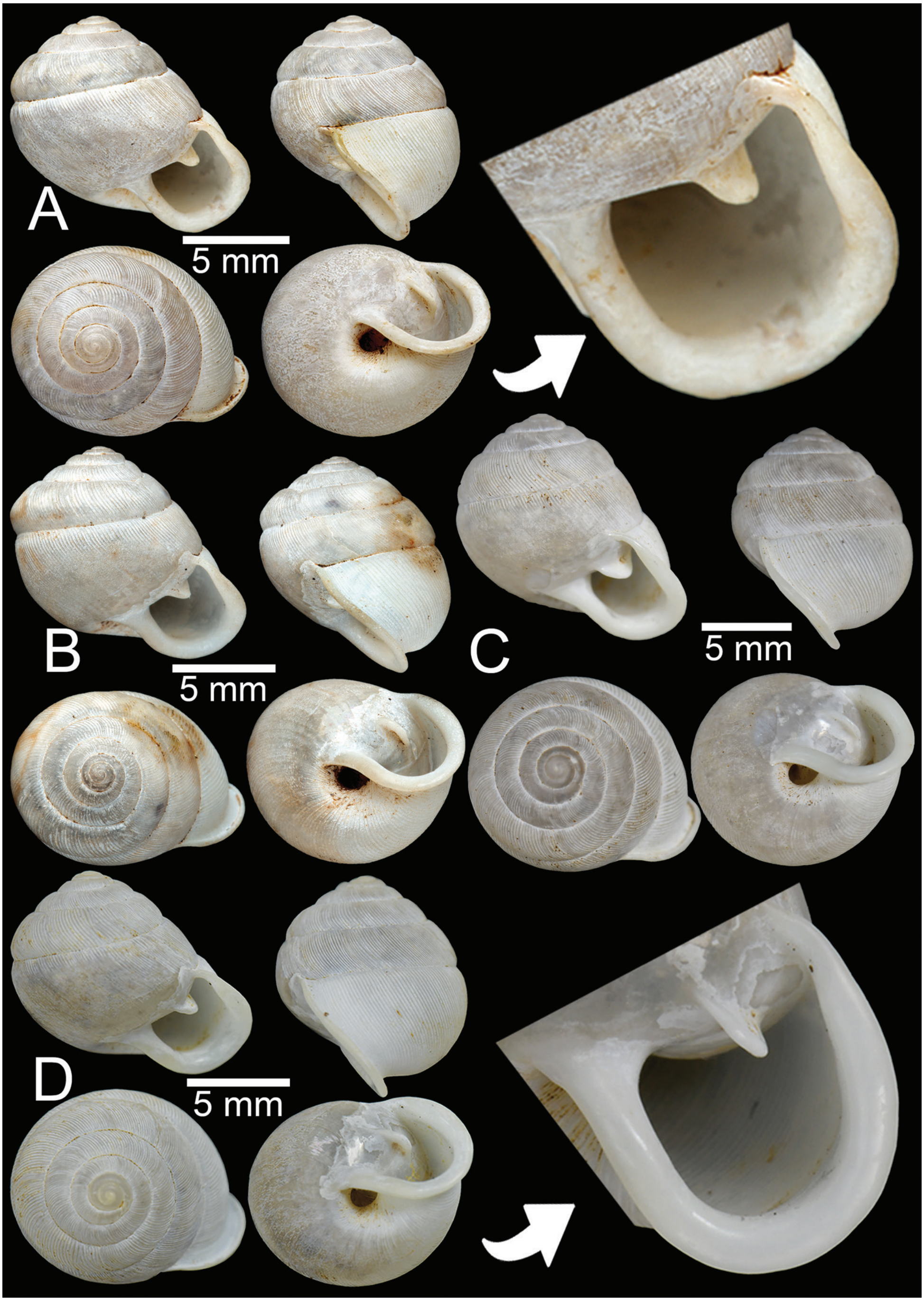

Figure 11. *Haploptychius solidulus* **A** specimen NHMUK 1909.3.15.75 from Moulmein with apertural dentition **B** specimen NHMUK 1888.12.4.774–776 from Molumein **C, D** specimen CUMZ 13007 from Pathen Mountain, Mawlamyine, Mon State with apertural dentition.

Distribution. This species appears to be restricted to limestone karsts and is only recorded from southern Myanmar.

Remarks. Stoliczka (1871) mentioned the specimens received from Theobald, who collected at Yethebiankoo [Rathe Pyan Cave] on the Attaran river, south-east of Moulmein [Mawlamyine]. Therefore, the exact type locality is assumed to be Rathe Pyan Cave, but it is located southwest of Hpa-an, Kayin State. Unfortunately, no living specimens could be examined.

Haploptychius thebawi **(Godwin-Austin, 1888)**

Figs 1, 12

Streptaxis thebawi Godwin-Austin, 1888: 243. Type locality: Pingoung, Shan Hills [Pinlaung Township, Taunggyi District, Shan State, Myanmar]. Gude 1903: 212, 322, 325, pl. 12, figs 11–13.

Haploptychius thebawi – Kobelt 1906: 145, 146: pl. 62, figs 11–13. Richardson 1988: 222.

Material examined. Limestone hills (Apache Cement Factory), Pyinyaung Village, Thazi Township, Meiktila District, Mandalay Region, Myanmar (20°49'39.1"N, 96°23'35.1"E): CUMZ 13008 (4 shells; Fig. 12A–C). Ywangan Village, near Lin Way Monastery, Kalaw Township, Taunggyi District, Shan State, Myanmar (21°13'43.3"N, 96°33'19.2"E): CUMZ 13009 (7 shells). Aik Kham Cave, Taunggyi Township, Taunggyi District, Shan State, Myanmar (20°49'07.0"N, 97°13'42.0"E): CUMZ 13010 (3 shells; Fig. 12D, E).

Diagnosis. *Haploptychius thebawi* is similar to *H. solidulus* in having a high conical spire and globosely ovate shell. The former species can be discriminated by its smoother shell surface and narrower aperture, more rounded and extended penultimate whorl, and less inflated last whorl. Additionally, *H. thebawi* resembles *H. porrectus* (Pfeiffer, 1863) from Laos in having strong radial ridges, conical spire, and an inflated and deflected last whorl, whereas *H. thebawi* exhibits a larger shell, less depressed and deflected last whorl, fine radial ridges, and higher spire.

Description. Shell oblique-heliciform, white and translucent; whorls 5½–6½; spire conical with distinct suture. Shell surface glossy with transverse ridges that diminish below periphery. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery rounded; last whorl axially deflected. Aperture subcircular; peristome discontinuous, thickened, expanded, and slightly reflected. Apertural dentition with one strong parietal lamella. Umbilicus open and deep (Fig. 12).

Distribution. This species occurs in central-eastern Myanmar, based on the type locality and two localities in Shan State, one locality in Mandalay Region, and it is relatively low in population density.

Remarks. Originally, *H. thebawi* was described based on specimens from Pingoung, Shan Hills, which is probably now referred to as Pinlaung Township, Taunggyi District,

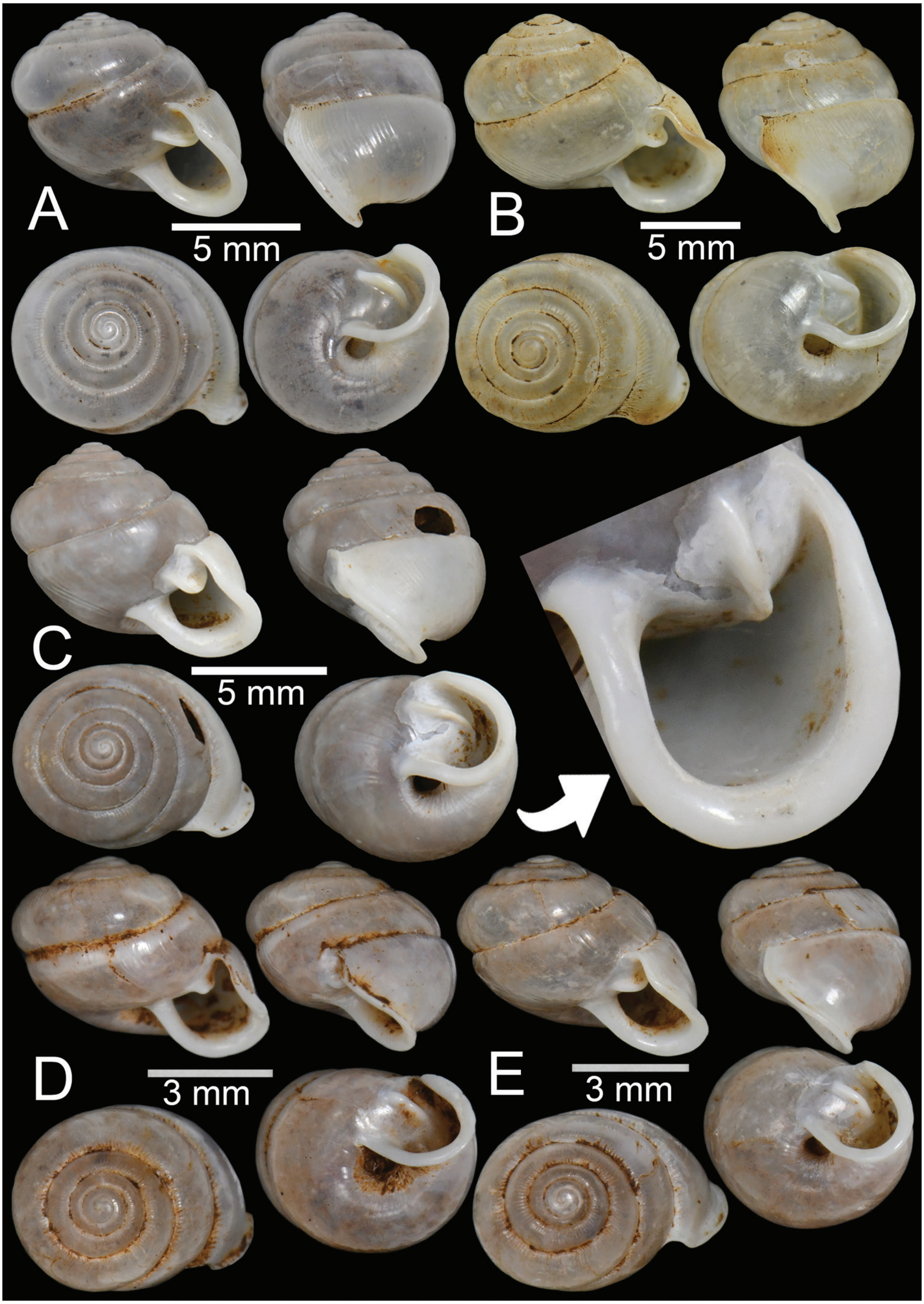

Figure 12. *Haploptychius thebawi* **A–C** specimens CUMZ13008 from Pyinyaung Village, Meiktila, Mandalay Region, with apertural dentition **D, E** specimens CUMZ 13010 from Aik Kham Cave, Taunggyi, Shan State.

Shan State. Unfortunately, the type specimens of *H. thebawi* could not be located in the NHM, London collection. However, the specimens examined herein match well with the original description and the syntype illustrated in Gude (1903: figs 11–13). Despite that, the specimens from Mandalay Region tend to have a more ovate shell and elongated last whorl, and their smooth shell surface, narrow umbilicus, and conical spire make them most similar to this nominal species. Therefore, we treat this population as an intraspecific variation. Examination of genitalia of specimens from this population is necessary to clarify their taxonomic status.

Blanford and Godwin-Austen (1908) treated this nominal species as a junior synonym of *H. burmanicus*. However, the recently collected specimens of *H. thebawi* can be distinguished from *H. burmanicus* and *H. blanfordi* by having an oblique-ovate to oblique-heliciform shell, elevated and conical spire, nearly smooth shell surface, penultimate whorl extended beyond last whorl, only one parietal lamella, and narrow umbilicus. Conversely, the two latter species have sub-oblique heliciform shells, depressed spire, prominent radial ridges on the shell surface, penultimate whorl slightly extended beyond the last whorl, with one parietal and one palatal lamella, and a wide umbilicus. Therefore, we consider *H. thebawi* to stand as a separate species.

Among three populations of *H. thebawi* (Table 1), Aik Kham Cave specimens have the smallest size with a more depressed shell, followed by Ywangan Village; Apache Cement Factory specimens have the largest shell with more conical spire and rounded last whorl.

***Haploptychius tenasserimicus* Man & Panha, sp. nov.**

https://zoobank.org/2391DEBA-7A84-4520-AF32-7BEB712C45EB
Fig. 13A–C

Type material. ***Holotype*** CUMZ 13011 (Fig. 13A). Measurements: shell height 4.5 mm, shell width 8.6 mm, and 6 whorls. ***Paratypes*** CUMZ 13012 (5 shells; Fig. 13B, C), NHMUK (2 shells).

Type locality. This new species was found from the limestone karsts near Lampane Village, Tanintharyi Region, Myanmar (11°40'18.1"N, 99°13'30.1"E).

Etymology. The specific name *tenasserimicus* refers to the type locality of this new species located on the Tenasserim Mountain Range, which forms the backbone of Indochina.

Diagnosis. This species is distinguishable by its small size, angular penultimate whorl, low convex spire, and aperture that elongates and grows almost horizontally. *Haploptychius tenasserimicus* sp. nov. differs from *H. burmanicus* and *H. blanfordi* species by having oblique-ovate shells, prominent transverse ridges, higher spire, rounded penultimate whorl, and less deflected last whorl. This new species also differs from *H. blaisei* (Dautzenberg & Fischer, 1905) from Laos and Vietnam by having a relatively smaller shell (width ~ 8 mm), nearly flattened spire, angular penultimate whorl and rounded last whorl. In contrast, *H. blaisei* possesses a relatively larger shell (width

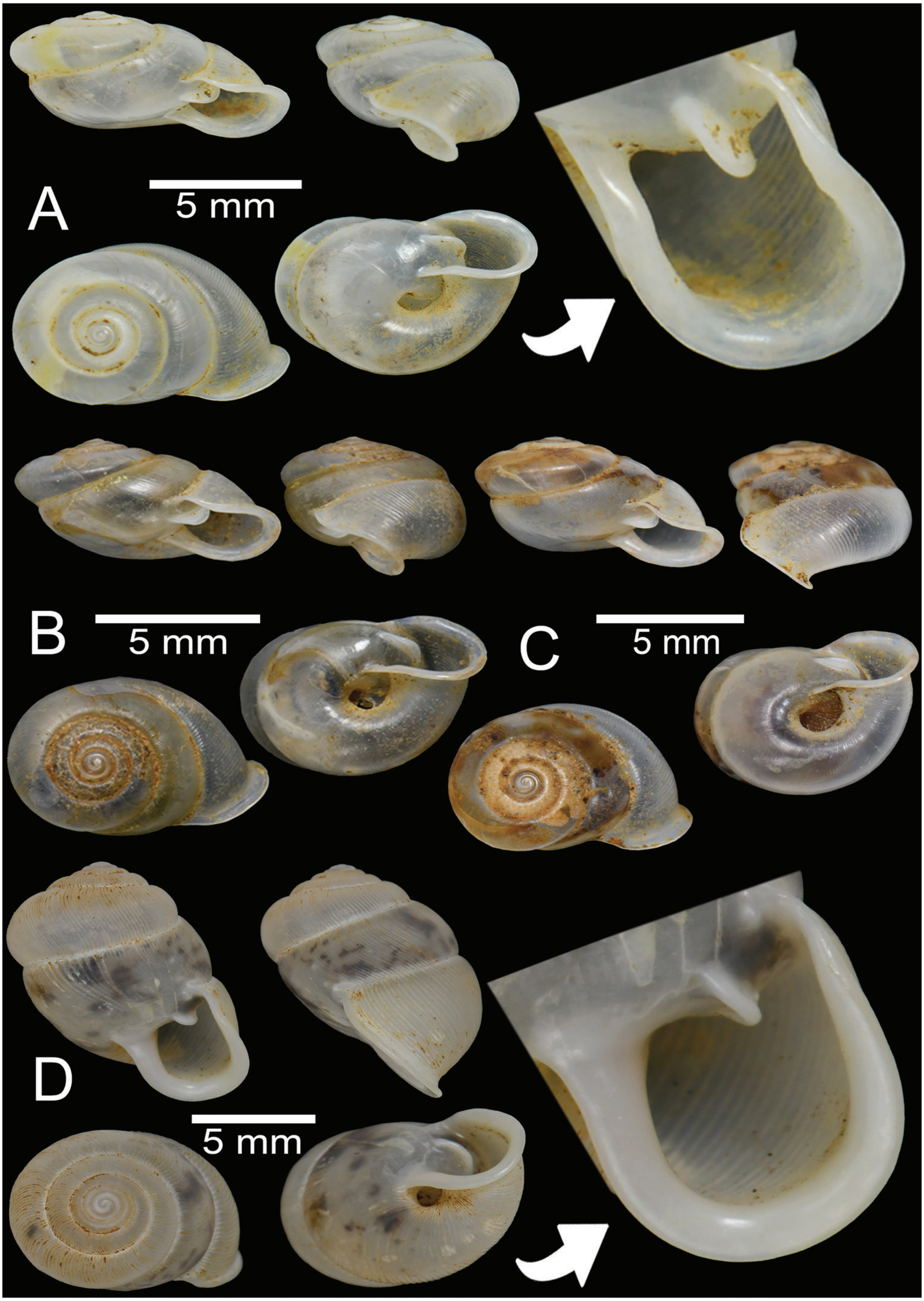

Figure 13. A–C *Haploptychius tenasserimicus* sp. nov. **A** holotype CUMZ 13011 with apertural dentition **B, C** paratypes CUMZ 13012 from the type locality **D** *Haploptychius heliakosus* sp. nov. holotype CUMZ 13013 with apertural dentition.

~ 10 mm), higher spire, rounded penultimate whorl, and compressed last whorl. In addition, *H. tenasserimicus* sp. nov. can be distinguished from *H. dorri* (Dautzenberg, 1894) from Vietnam (see Inkhavilay et al. 2016) by having a more depressed and larger shell (width ~ 8 mm), angular penultimate whorl, aperture longer and more axially deflected last whorl. *Haplotychius dorri* exhibits a depressed and smaller shell (width ~ 5 mm), smooth shell surface, rounded penultimate whorl, shorter aperture and last whorl less axially deflected from the vertical axis.

Description. Shell sub-oblique heliciform, white and translucent; whorls 6; spire low convex with distinct suture. Shell surface glossy with fine transverse ridges that diminish below periphery; varices present. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery angular; last whorl axially deflected. Aperture semi-ovate; peristome discontinuous, expanded and slightly reflected. Apertural dentition with one parietal lamella. Umbilicus open and shallow (Fig. 13A–C).

Distribution. This species was only collected from the type locality; limestone hills in primary forest in the Tanintharyi Region, Myanmar.

Remarks. The genitalia information is not known.

Haploptychius heliakosus **Man & Panha, sp. nov.**

https://zoobank.org/2ED29774-4354-40B7-AC84-8C74CE43AE3E

Figs 2C, 13D, 14A, B, 15, 16, 24C

Type material. ***Holotype*** CUMZ 13013 (Fig. 13D). Measurements: shell height 9.0 mm, shell width 10.2 mm, and 7 whorls. ***Paratypes*** CUMZ 13014 (24 shells; Fig. 14A), CUMZ 13015 (15 specimens in ethanol), NHMUK (2 shells).

Type locality. Bardai Mountain, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (16°59'50"N, 97°41'48"E).

Other material examined. Kyonknow Cave, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (17°01'00.1"N, 97°41'42.1"E): CUMZ 13016 (7 shells; Fig. 14B).

Diagnosis. *Haplotychius heliakosus* sp. nov. differs from *H. bombax* by having a deeper suture, higher spire, rounded penultimate whorl, and subquadrangular aperture. In contrast, *H. bombax* possesses a relatively shallower suture, lower spire, angular penultimate whorl, and semi-ovate aperture. *Haplotychius heliakosus* sp. nov. also differs from *H. burmanicus* and *H. blanfordi* by having an oblique ovate shell, higher spire, and less axially deflected last whorl. In contrast, the two latter species exhibit a depressed heliciform shell and lower spire, and more axially deflected last whorl. Although *H. heliakosus* sp. nov. has a shell similar to *H. pellucens* from Laos, this new species has a less axially reflected last whorl, subquadrangular aperture, and more ridges on the shell surface. Additionally, the genitalia of *H. heliakosus* sp. nov. has a thickened penial sheath covering almost the entire penis, and short and stout penial hooks on papillae, while *H. pellucens* has a thin penial sheath covering ~ 1/2 of the penis, and

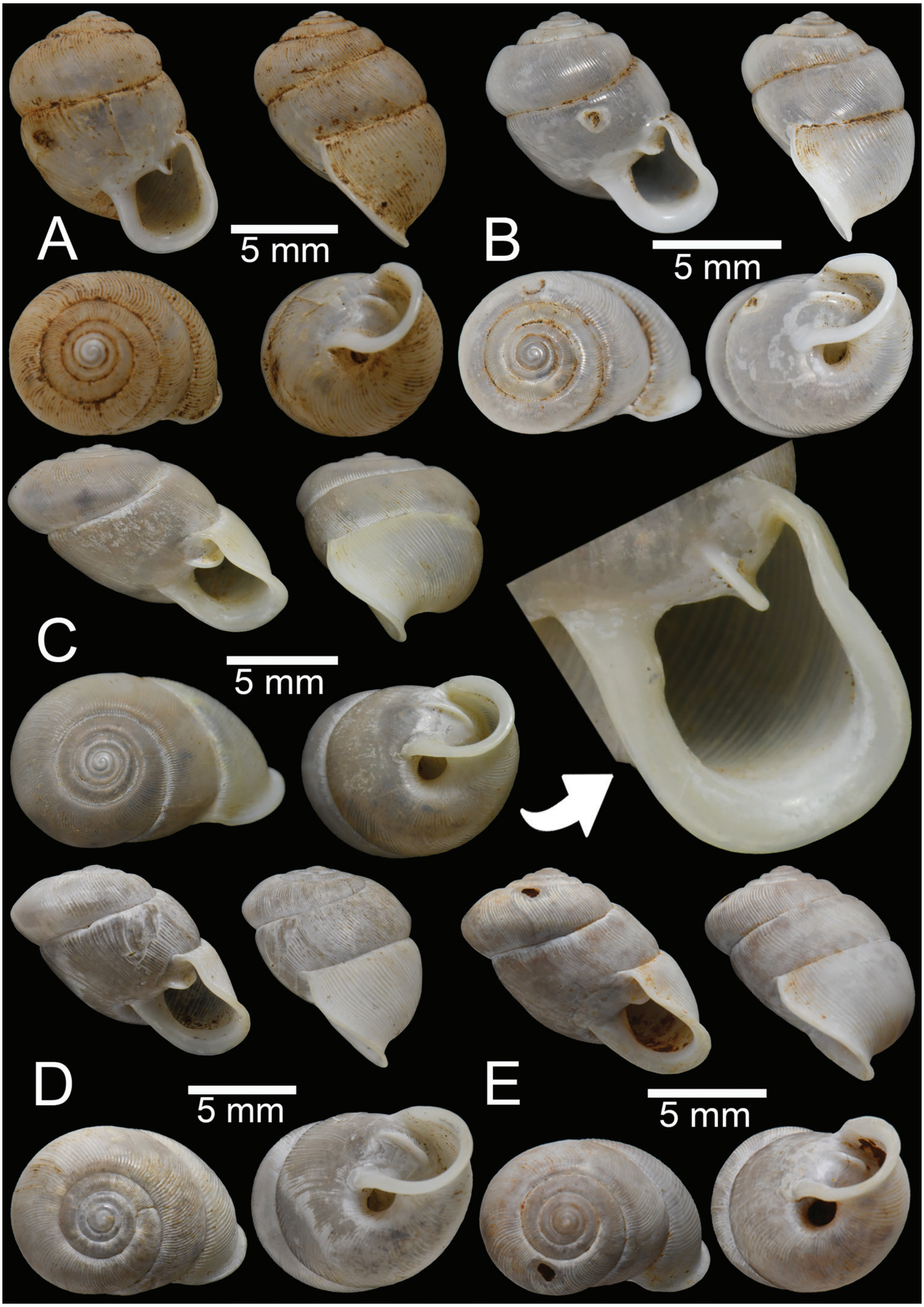

Figure 14. A, B *Haploptychius heliakosus* sp. nov. **A** paratype CUMZ 13014 from the type locality and **B** specimen CUMZ 13016 from Kyonknow Cave, Hpa-an, Kayin State **C–E** *Haploptychius karenorum* sp. nov. **C** holotype CUMZ 13017 with apertural dentition **D** paratype CUMZ 13018 from the type locality and **E** specimen CUMZ 13019 from Taung Lay Cave, Hpa-an, Kayin State.

long and slender penial hooks without papillae. This new species differs from *C. exacutus* (Gould, 1856) by having penial sheath retractor muscle originating at atrium, vas deferens passing through a short section of thin penial sheath before extending ~ 1/3 of the penial sheath length to the curved portion, shorter free oviduct, seminal vesicle ca. twice the length from talon to branching point of seminal vesicle, thickened atrial folds with sparse atrial pores, and stout distal penial hooks.

Description. Shell oblique-ovate, white, and translucent; whorls 7–7½; spire low conical with distinct suture. Shell surface glossy with transverse ridge, nearly smooth with few transverse ridges near peristome. Embryonic shell large, ~ 2½ whorls with smooth surface; following whorls regularly coiled. Penultimate whorl rounded; last whorl axially deflected. Aperture subquadrangular; peristome discontinuous, thickened, expanded, and slightly reflected. Apertural dentition with one strong parietal lamella and sometimes with small second parietal lamella adjoined at a right angle. Umbilicus open and deep (Fig. 13D, 14A, B).

Genital organs. Atrium (at) short. Penis (p) very thin, and long tube. Penial sheath (ps) muscularly enlarged, very thickened, and extending entire penis length; penial sheath retractor muscle (psr) thin, originating at atrium, and inserting distally on penial sheath (Fig. 15A). Vas deferens (vd) passes through a short section of thin penial sheath then extends ~ 1/3 of the penial sheath length to a curved portion before entering penis distally. Curved portion with very thin connective tissue attached between vas deferens and penial sheath wall (Fig. 15B). Penial retractor muscle (pr) thin, very long, inserting at junction of penis and vas deferens.

Internal wall of atrium generally smooth with transverse thickened atrial folds with sparse atrial pores (Fig. 16A). Proximal penial wall with scattered pale brownish penial hooks, ~ 26 hooks/200 μm^2; hooks located on irregular trapezoidal penial papillae and separated by longitudinal folds (Fig. 16B). Penial hooks small (< 0.04 mm in length), slightly expanded at base, tips pointed, and slightly curving away from genital orifice (Fig. 16C). Penial wall on middle to distal parts with scattered light brownish penial hooks, ~ 24 hooks/200 μm^2; hooks located on laterally-flattened penial papillae separated by longitudinal folds (Fig. 16D, E). Penial hooks short, stout, small (< 0.01 mm in length), expanded at base, tips obtuse and curved towards genital orifice (Fig. 16F–H).

Internal wall of atrium generally smooth with sparse atrial pores (Fig. 23A). Proximal penial wall covered with scattered and pale brownish penial hooks, ~ 12 hooks/200 μm^2. Proximal penial hooks located on laterally flattened penial papillae; hooks small and short (< 0.03 mm in length), slightly expanded at base, tips obtuse and curved towards genital orifice (Fig. 23B, C). Middle and distal penial walls densely covered with pale brownish hooks, ~ 20 hooks/200 μm^2. Middle and distal hooks located on laterally compressed penial papillae separated by reticulated folds; hooks small, short (< 0.01 mm in length), slightly expanded at base, tips pointed (Fig. 23D–G).

Vagina very short, stout, and ~ 1/10 of penis length. Gametolytic duct (gd) a long tube extending as far as albumin gland; gametolytic sac (gs) ovate. Proximal free oviduct (fo) enlarged, tapering to a smaller tube in the middle part, then enlarged

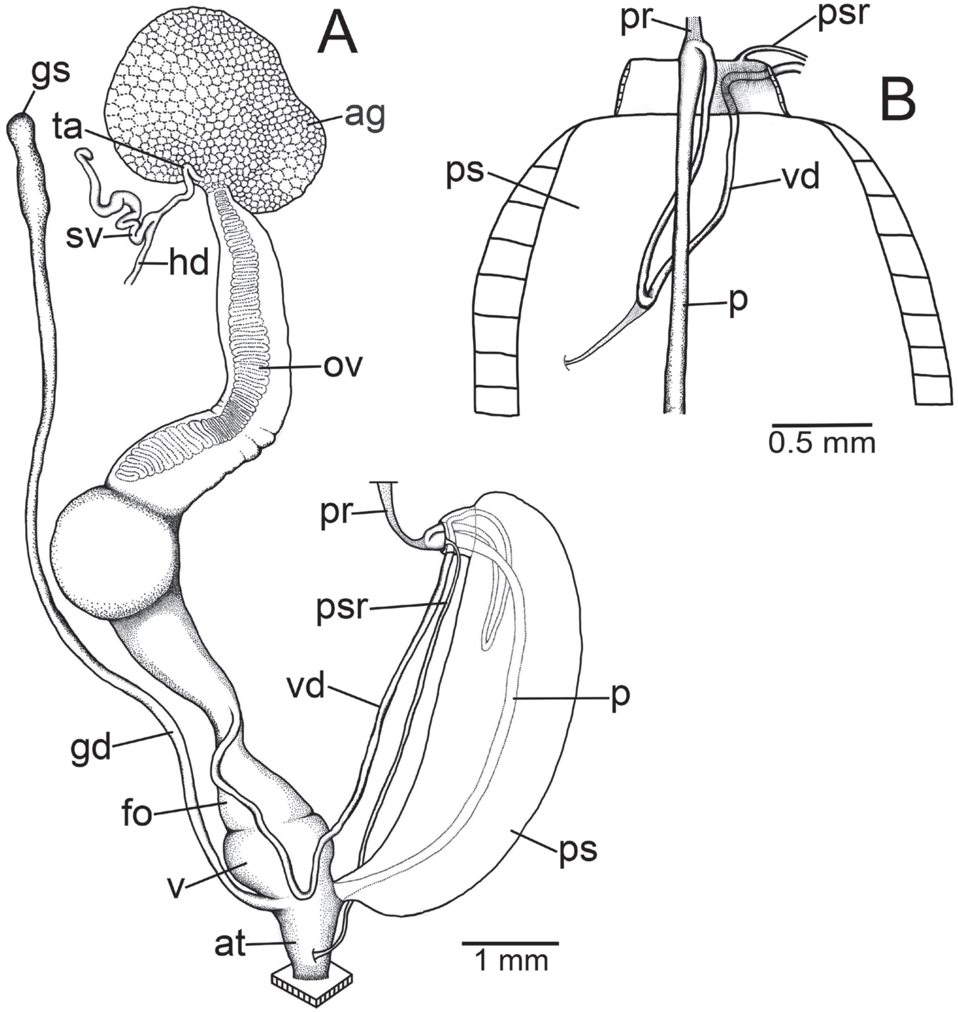

Figure 15. Genital anatomy of *Haploptychius heliakosus* sp. nov. paratype CUMZ 13014 **A** reproductive system **B** insertion of vas deferens into penial sheath.

distally. Oviduct (ov) enlarged and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) small, short, and club shaped. Hermaphroditic duct (hd) bearing long seminal vesicle (sv) of about twice the length from talon to branching point of seminal vesicle (Fig. 15A).

Vaginal wall with longitudinal vaginal folds (Fig. 16I), folds with nearly smooth surface, and vaginal hook absent.

Radula. Each row consists of ~ 35 teeth with formula (17)–1–(17). Central tooth very small with pointed cusp. Lateral and marginal teeth undifferentiated, lanceolate, unicuspid, and lanceolate. Latero-marginal teeth gradually reduced in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 24C).

Etymology. The specific name *heliakosus* is derived from the Greek word *heliakos* meaning of the sun. It honors our colleague, Dr. Arthit Pholyotha, who collected the specimens and took the photos of the living snails used in this study. His first name Arthit means the Sun.

Distribution. This new species is currently known from two localities in the limestone karsts near Salween (Thanlwin) Basin, Kayin State, southeastern Myanmar.

Remarks. Shell variations of *H. heliakosus* sp. nov. were found between two populations. Specimens from the Kyonknow population (Fig. 14B) have a straighter periphery or nearly cylindrical last whorl compared to those from the type locality, Bardai Mountain (Fig. 13D, 14A). As no living specimens from the Kyonknow Cave were collected, we considered the Kyonknow population as an intraspecific variation of *H. heliakosus* sp. nov. because this locality is very close to the type locality. Living specimens from the Kyonknow population and genital examination are necessary to resolve these systematic issues.

Haploptychius karenorum **Man & Panha, sp. nov.**

https://zoobank.org/556A1CE1-7E73-4466-9C69-F5454EB4E5CC
Fig. 14C–E

Type material. ***Holotype*** CUMZ 13017 (Fig. 14C). Measurements: shell height 9 mm, shell width 11.6 mm and 7 whorls. ***Paratypes*** CUMZ 13018 (6 shells; Fig. 14D), NHMUK (2 shells).

Type locality. Limestone outcrops at Waiponla Hill, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (16°56'7.4"N, 97°42'56.8"E).

Other material examined. Taung Lay Cave, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (17°11'40.3"N, 97°37'47.0"E): CUMZ 13019 (2 shells; Fig. 14E).

Diagnosis. *Haploptychius karenorum* sp. nov. can be differentiated from *H. heliakosus* sp. nov. by having a convex spire, penultimate whorl angular and extended well beyond the diameter of the last whorl, and more axially deflected last whorl. In contrast, *H. heliakosus* sp. nov. possesses an elevated spire, penultimate whorl rounded, less extended beyond the diameter of the last whorl, and less axially deflected last whorl. This new species differs from *H. bombax* by having an angular penultimate whorl, more axially deflected last whorl, subcircular aperture, and broadly expanded lip. In comparison, *H. bombax* has a rounded penultimate whorl, less axially deflected last whorl, semi-ovate aperture, with thickened and slightly expanded lip. For further comparison, *H. karenorum* sp. nov. differs from *H. burmanicus*, *H. blanfordi*, and *H. thebawi* in having a more axially deflected last whorl, lower spire, penultimate whorl angular and strongly extended beyond the diameter of the last whorl, and without sinulus. The three latter species have an elevated spire, penultimate whorl rounded and slightly extended beyond the diameter of the last whorl, and less axially deflected last whorl.

Description. Shell oblique-ovate, solid and translucent; whorls 6–7½; spire depressed convex and with distinct suture. Embryonic shell ~ 2½ whorls with smooth surface;

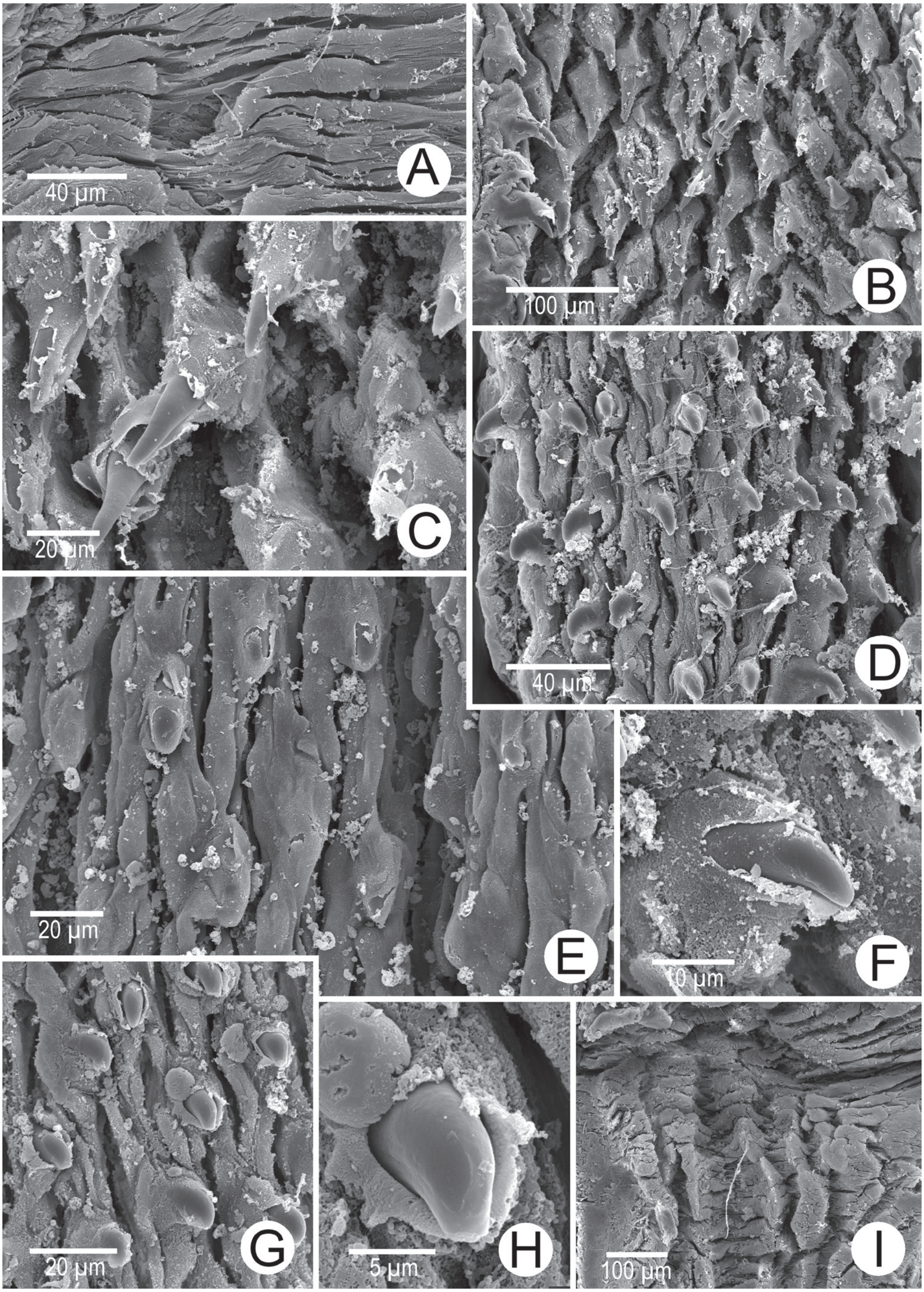

Figure 16. Internal sculpture of genitalia of *Haploptychius heliakosus* sp. nov. paratype CUMZ 13014 **A** atrium surface **B** arrangement of penial hooks on proximal part of penis **C** top view of penial hook **D, E** arrangement of penial hooks on middle part of penis **F** top view of penial hook with obtuse tip **G** arrangement of penial hooks on distal part of penis **H** high magnification of penial hook with obtuse tip **I** vaginal folds.

following whorls growing regularly and last whorl intermediately expanded. Shell surface has moderately strong radial ridges that diminish below periphery of last whorl and around umbilicus. Penultimate whorl bluntly angular and extended beyond last whorl. Last whorl compressed to flattened, axially deflected from columellar axis. Aperture subcircular; peristome thickened, expanded, and slightly reflected. Apertural dentition with one strong parietal lamella. Umbilicus widely open and deep (Fig. 14C–E).

Etymology. The specific name *karenorum* refers to the Karen people, the major ethnicity in Kayin State, Myanmar.

Distribution. This species is known from two localities in the limestone karts of Kayin State, southern Myanmar.

Remarks. Comparing populations from the two localities, shells of *H. karenorum* sp. nov. from the Taung Lay population (Fig. 14E) have a more elevated spire and weaker parietal lamella. However, the bluntly angular penultimate whorl and oblique ovate shells are identical to the type specimens, which we consider an intraspecific shell variation. However, these two localities are quite distant from each other, and on opposite sides of the Attaran River (Fig. 1). Thus further, genitalia information will help determine whether they are just geographical variations.

Genus *Carinartemis* Siriboon & Panha, 2014

Carinartemis Siriboon & Panha in Siriboon et al. 2014b: 166–168.

Type species. *Carinartemis vesperus* Siriboon & Panha, 2014 by original designation.

Diagnosis. Shell obliquely heliciform and with conical spire. Penultimate whorl keeled and extended beyond the diameter of the last whorl. Last whorl rounded to shouldered and strongly axially deflected. Aperture semi-ovate to subcircular; apertural dentition with or without one or two parietal lamellae. Genitalia with thin to thick penial sheath that covers entire penis length; penial hooks and vaginal hooks may be present.

Remarks. The genus is comprised of two endemic species occurring in limestone outcrops in western Thailand. A recent phylogenetic study revealed that the previously recognized *Haploptychius petitii* (Gould, 1844) and *Indoartemon medius* Siriboon & Panha, 2014 are clustered with members of the *Carinartemis* (see Siriboon et al. 2020) with strong support. Siriboon et al. (2020) suggested reassigning these two species into *Carinartemis*. Therefore, based on this phylogenetic result, the previously recognized *H. petitii* is relocated here under the genus *Carinartemis*.

Carinartemis petitii (Gould, 1844)

Fig. 17A, B

Streptaxis petitii Gould, 1844: 456, 457, pl. 24, fig. 7. Type locality: Tavoy [Dawei District, Tanintharyi Region, Myanmar].

Streptaxis petiti [sic] – Pfeiffer 1847: 8. Hanley and Theobald 1870: 4, pl. 8, fig. 4. Nevill 1878: 3. Tryon 1885: 74, pl. 14, figs 16–18. Gude 1903: 216. Kobelt 1910: 151, 152. Blanford and Godwin-Austin 1908: 4.
Haploptychius petiti [sic] – Kobelt 1906: 142, pl. 57, fig. 14. Richardson 1988: 218.
Haploptychius petitii – Siriboon et al. 2020: 7, 14, 16, fig. 3.

Material examined. ***Syntype*** MCZ 169290 (1 shell; Fig. 17A) from Burmah. Moulmein: NHMUK 1909.3.15.67 (2 shells, Fig. 17B) ex. Godwin-Austen collection. NHMUK 67.9.3.15 (1 shell) ex. Blanford collection.

Diagnosis. *Carinartemis petitii* can be distinguished from *C. sankeyi* (Benson, 1859) by having a fine transverse ridge on the upper periphery, penultimate whorl keeled and little extended beyond the diameter of last whorl, and a subcircular aperture. In addition, *C. petitii* differs from *I. medius* from Thailand by having a more axially deflected last whorl, and only one parietal lamella present, while *I. medius* has a less axially deflected last whorl, and with one additional small palatal lamella. *Carinartemis petitii* is superficially similar to *H. blaisei* but it has an elevated spire, keeled penultimate whorl, subcircular aperture, and thicker lip.

Description. Shell oblique-heliciform, white, and translucent; whorls 6½–7; spire conical with distinct suture. Shell surface glossy with fine transverse ridges, nearly smooth with a few transverse ridges near peristome. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery keeled nearly the entire penultimate whorl; last whorl axially deflected. Aperture subcircular; peristome discontinuous, thickened, expanded, and reflected. Apertural dentition with one strong parietal lamella. Umbilicus open and deep (Fig. 17A, B).

Distribution. This species is known from the type locality in Myanmar (Gould 1844) and Kanchanaburi Province in Thailand (Siriboon et al. 2020).

Remarks. No new specimens were collected in this survey. However, the syntype (Fig. 17A) and the museum specimens (Fig. 17B) show slightly different shell forms. *Carinartemis petitii* is similar in shell form to the genus *Haplotychius* in having one parietal lamella and a deflected last whorl. However, an initial molecular analysis placed this species within the *Carinartemis* clade (Siriboon et al. 2020). This species is clearly distinct from other recognized *Carinartemis* species by its oblique-heliciform shell, translucence and a high to low conical spire with a distinct suture. Moreover, the shell surface has fine transverse ridges that diminish below periphery, whorls regularly coiled, shell periphery is keeled; umbilicus open and deep. Aperture is semi-ovate, peristome thickened, expanded, reflected, and apertural dentition with one strong parietal lamella.

Carinartemis exacutus (Gould, 1856)

Figs 1, 2D, 17C, D, 18, 21A, B, 22, 24D

Streptaxis exacutus Gould, 1856: 13. Type locality: Burma [Myanmar].
Streptaxis exacuta [sic] – Pfeiffer 1859: 331.

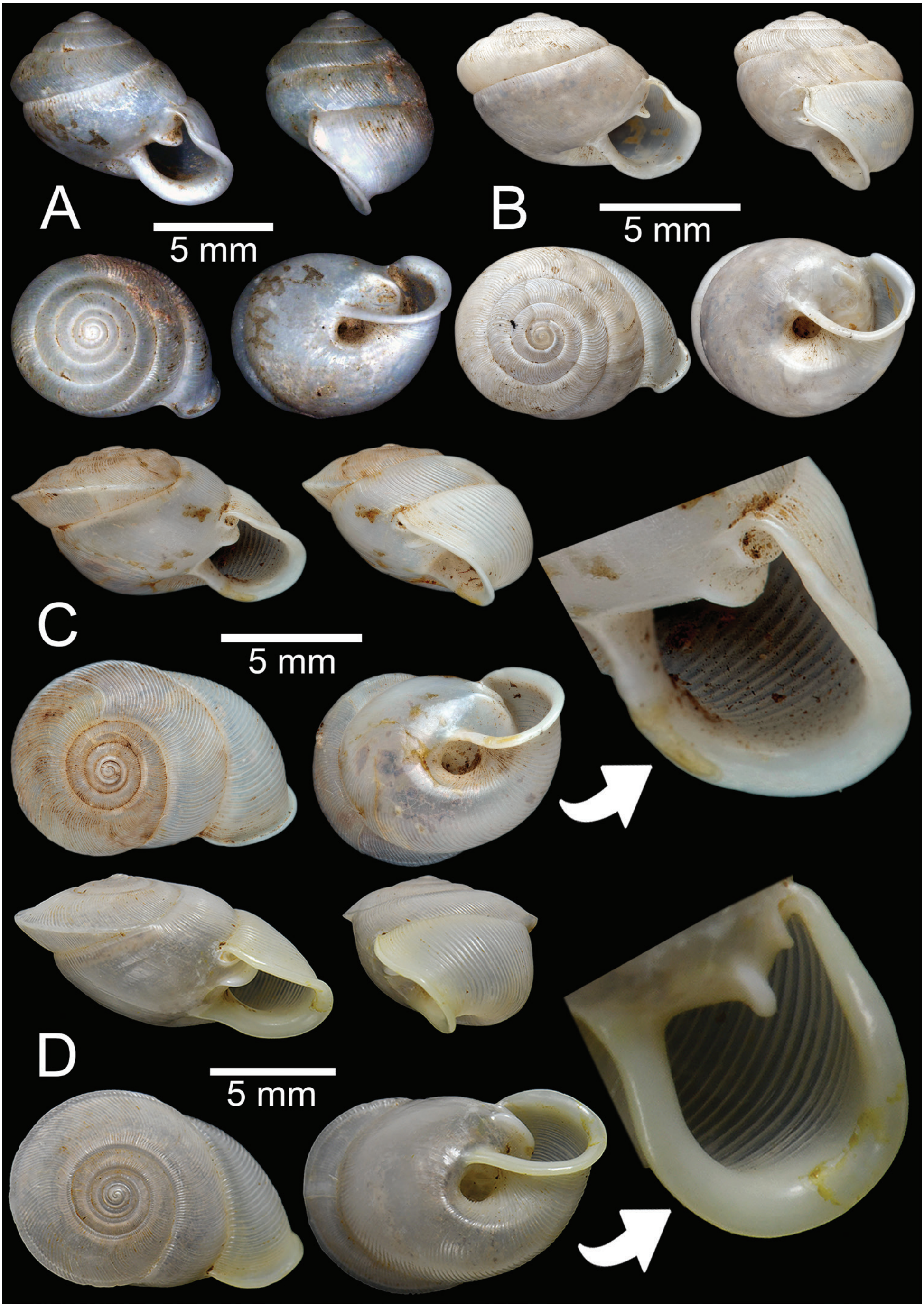

Figure 17. A, B *Carinartemis petitii* **A** syntype MCZ 169290 from Tavoy, British Burmah and **B** specimen NHMUK 1909.3.15.67 from Moulmein **C, D** *Carinartemis exacutus* **C** specimen NHMUK 1888.12.4.771–773 from Moulmein with apertural dentition and **D** specimen CUMZ 13025 from Taung Wine Cave, Hpa-an, Kayin State with apertural dentition.

Streptaxis exacutus – Pfeiffer 1871: 30, 31, pl. 115, figs 13, 14. Hanley and Theobald 1874: 40, pl. 98, figs 8–10. Nevill 1878: 3. Tryon 1885: 72, pl. 14, figs 11, 12. Blandford and Godwin-Austin 1908: 8.

Haploptychius exacutus – Kobelt 1906: 142, 143, pl. 57, figs 16–18. Richardson 1988: 215. Siriboon et al. 2014b: 169, 171.

Material examined. Moulmein: NHMUK 1874.9.3.15 (3 shells) ex. Godwin-Austen. NHMUK 1888.12.4.771–773 (3 shells; Fig. 17C). NHMUK 1906.2.2.198 (3 shells + 2 juveniles; Fig. 18A) ex. Blanford collection. NHMUK 1903.7.1.3999 (3 shells) ex. Godwin-Austen collection. Burma: NHMUK1950.12.9.170 (1 shell) ex. Laidlaw collection. Mergui: NHMUK ex. Cuming collection (2 shells). Sadhdan Cave, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (16°44'23.4"N, 97°43'04.2"E): CUMZ 13020 (3 shells). Bayin Nyi Cave, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (16°58'10.1"N, 97°29'30.6"E): CUMZ 13021 (8 shells; Fig. 18B), CUMZ 13022 (15 specimens in ethanol). Lun Nga Mountain, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (16°44'53.2"N, 97°47'09.5"E): CUMZ 13023 (14 shells; Fig. 18C), CUMZ 13024 (50 specimens in ethanol). Taung Wine Cave, near Thiri Hpa-an Hotel, Hpa-an Township, Hpa-an District, Kayin State, Myanmar (16°50'31.1"N, 97°37'18.4"E): CUMZ 13025 (3 shells; Fig. 17D), CUMZ13026 (6 specimens in ethanol). Kaw Ka Taung Cave (Golden valley), Hpa-an Township, Hpa-an District, Kayin State, Myanmar (16°50'32.4"N, 97°37'10.9"E): CUMZ 13027 (1 shell).

Diagnosis. *Carinartemis exacutus* is superficially similar to *C. sankeyi*, *C. vesperus* Siriboon & Panha, 2014, and *C. striatus* Siriboon & Panha, 2014 from western Thailand, but it has a larger shell, convex spire, immediately expanded penultimate whorl, semi-ovate aperture, two parietal lamellae, very thickened penial sheath, vas deferens passes through penial sheath, curved portion of vas deferens with very thin connective tissue, and hooks located on irregular trapezoidal penial papillae separated by longitudinal folds. In comparison, *C. sankeyi*, *C. vesperus*, and *C. striatus* have an elevated spire, regularly expanded penultimate whorl, but *C. sankeyi* has fine transverse ridges on the entire shell, subquadrangular aperture, one parietal lamella, slender atrium, thin penial sheath, vas deferens does not pass through a penial sheath, curved portion of vas deferens is without connective tissue, proximal penial hook located on laterally-flattened penial papillae, and distal penial hooks located on laterally compressed penial papillae separated by reticulated folds. In contrast, *C. vesperus* has a subcircular aperture, lacks parietal lamellae, has a less axially deflected last whorl, vas deferens passes through the penial sheath, curved portion of vas deferens is without connective tissue, and penial papillae absent. Meanwhile, *C. striatus* has strong transverse ridges over the entire shell, a semi-ovate aperture, one parietal lamella, vas deferens is attached to the distal end of the penial sheath with very thin connective tissue, and hooks are located on papillae without connected longitudinal folds.

Description. Shell oblique-heliciform, white, and translucent; whorls 6–6½; spire convex with distinct suture. Shell surface glossy with fine transverse ridges, nearly

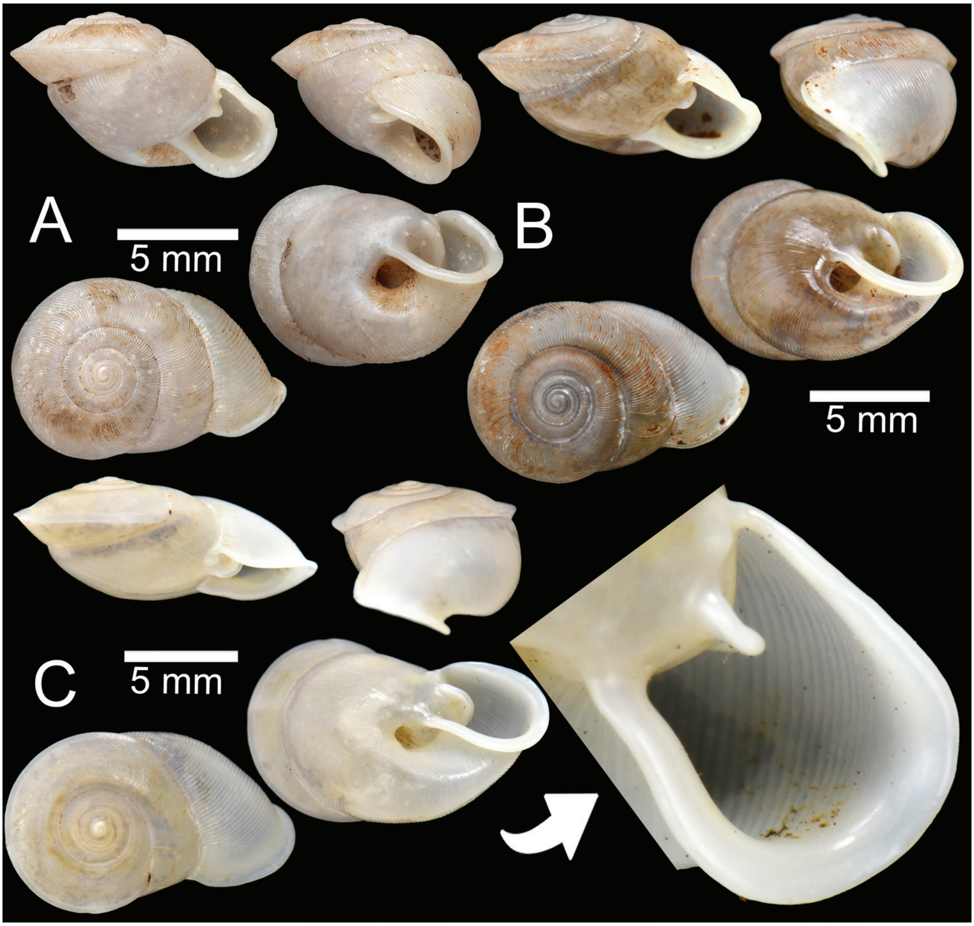

Figure 18. *Carinartemis exacutus* **A** specimen NHMUK 1906.2.2.198 from Moulmein **B** specimen CUMZ 13021 from Bayin Nyi Cave, Hpa-an, Kayin State **C** specimen CUMZ 13023 from Lun Nga Mountain, Hpa-an, Kayin State with apertural dentition.

smooth with few transverse ridges near peristome; varices present. Embryonic shell ~ 2½ whorls with smooth surface; following whorls intermediately coiled. Shell periphery wide and sharply keeled along nearly the entire penultimate whorl; last whorl axially deflected. Aperture semi-ovate; peristome discontinuous, thickened, expanded, and slightly reflected. Apertural dentition with one strong parietal lamella and sometimes with a second parietal lamella adjoined at a right angle. Umbilicus open and deep (Figs 17C, D, 18).

Genital organs. Atrium (at) short. Penis (p) a very thin, and long tube. Penial sheath (ps) muscularly enlarged, very thickened and extending entire length of penis; penial sheath retractor muscle (psr) thin, originating near genital orifice, attached to atrium with short and thin connective tissue, and inserting distally on penial sheath (Fig. 21A). Vas deferens (vd) passes through a short portion of penial sheath, then extends to curved portion at ~ 1/11 of the penial sheath length before entering penis distally. Curved portion of vas deferens with very thin connective tissue originating at penial sheath (Fig. 21B). Penial retractor muscle (pr) thin, very long, inserting at penis and vas deferens junction.

Internal wall of atrium generally smooth with sparse atrial pores (Fig. 22A). Penial wall with scattered pale brownish penial hooks, ~ 20 hooks/200 μm^2; hooks located on laterally flattened penial papillae and separated by longitudinal folds (Fig. 22B–D). Penial hooks small (<0.02 mm in length), expanded at base, tips pointed and curved towards genital orifice (Fig. 22E, F).

Vagina (v) very short and ~ 1/12 of penis length. Gametolytic duct (gd) a long tube extending as far as albumin gland; gametolytic sac (gs) ovate. Proximal free oviduct (fo) enlarged then tapering to smaller diameter distally. Oviduct (ov) enlarged and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) small, short and club shaped. Hermaphroditic duct (hd) bearing long seminal vesicle (sv) ~ 1/2 the length from talon to branching point of seminal vesicle (Fig. 21A).

Vaginal wall with longitudinal oblique vaginal folds, folds with nearly smooth surface and vaginal hook absent (Fig. 22G).

Radula. Each row consists of 41–45 teeth with formula (22–20)–1–(20–22). Central tooth is very small with pointed cusp. Lateral and marginal teeth undifferentiated, lanceolate, unicuspid, and lanceolate. Latero-marginal teeth gradually reduced in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 24D).

Distribution. This species was collected from five limestone hills in Kayin State, southern Myanmar, in this survey.

Remarks. This species appears at a high abundance among the limestone karsts in Hpa-an, Kayin State. All the specimens examined from the populations from Lun Nga Mountain and Bayin Nyi Cave have a very small to indistinct upper parietal lamella, and some specimens from Bayin Nyi Cave have strong transverse ridges on almost the entire whorl. Moreover, the Lun Nga Mountain population have a last whorl that is more extended anteriorly and an elongated semi-ovate aperture (Fig. 18C). However, the genitalia in all localities show no difference from this locality; thus, we consider them as intraspecific variations within *C. exacutus*. Some specimens from Bayin Nyi Cave have strong transverse ridges on almost the entire whorl.

Although the type locality was listed as 'Burma' [Myanmar], all the known records and specimens examined in this study were collected from Kayin State. Therefore, the precise type locality of this species is probably southwestern Myanmar in Mon State and Kayin State.

Carinartemis sankeyi (Benson, 1859)

Figs 1, 2E, F, 19, 20, 21C, D, 23, 24E, F

Streptaxis sankeyi Benson, 1859a: 472. Type locality: Moulmein [Mawlamyine Township, Mon State, Myanmar]. Pfeiffer 1868: 442. Hanley and Theobald 1870: 4, pl. 8, fig. 7. Tryon 1885: 72, pl. 14, figs 2, 3. Blanford and Godwin-Austen 1908: 8, fig. 7.

Streptaxis sankeyanus Stoliczka, 1871: 167, 168, pl. 7, fig. 14 (unjustified emendation). Hanley and Theobald 1870: 4, pl. 8, fig. 7. Nevill 1878: 3. Tryon 1885: 72, pl. 14, figs 9, 10.

Streptaxis hanleyanus Stoliczka, 1871: 168, 169, pl. 7, fig. 15. Type locality: Prope Moulmein, ad flumen Attaran [Attaran River, Mawlamyine, Mon State, Myanmar]. Blanford and Godwin-Austen 1908: 8, 9.

Haploptychius sankeyi – Kobelt 1906: 147, pl. 57, figs 11, 12, pl. 62, figs 6, 7. Richardson 1988: 220. Siriboon et al. 2014b: 169.

Oophana hanleyana – Richardson 1988: 235.

Material examined. ***Syntype*** UMZC I.102740 (6 shells; Fig. 19A) from Moulmein. Moulmein: NHMUK 1954.6.3.556 (1 shell) ex. Hawkins collection. NHMUK 1906.2.2.341 (4 shells; Fig. 19C) ex. Blanford collection. NHMUK 1872.9.3.15 (5 shells) ex. Godwin-Austen collection. NHMUK 1903.7.1.4001 (1 shell; Fig. 20A) ex. Godwin-Austen collection. NHMUK Acc. No. 1733 ex. Oldham collection (2 shells). NHMUK 1888.12.4.792–794 (3 shells; Fig. 19B). NHMUK 1871.9.23.61 (1 shell; Fig. 19D). Paboung Toung, Burma: NHMUK 1891.3.17.577–578 (2 shells) ex. Hungerford collection. Mergui: NHMUK 20210051 (1 shell; Fig. 19E) ex. Cuming collection. Kwengan Hill: NHMUK 1888.12.4.781–783 (3 shells; Fig. 20B) ex. Blanford collection. Saddan Cave situated on same karsts ~ 600 m south of Kayon Cave, Mawlamyine Township, Mawlamyine District, Mon State, Myanmar (16°31'42.8"N, 97°43'2.1"E): CUMZ 13028 (7 shells). Kayon Cave [previously known as Farm Caves] ~ 10 km from Mawlamyine Township, Mawlamyine District, Mon State, Myanmar (16°32'0.5"N, 97°42'53.5"E: CUMZ 13029 (13 shells; Fig. 20D), CUMZ 13030 (5 specimens in ethanol). Dhammatat Cave, Mawlamyine Township, Mon State, Myanmar (16°30'23.0"N, 97°48'36.3"E): CUMZ 13031 (12 shells; Fig. 20C).

Diagnosis. *Carinartemis sankeyi* is superficially similar to *C. vesperus* and *C. striatus* in having a subquadrangular aperture, and less expanded and continuous peristome, slender atrium, vas deferens does not pass through a penial sheath, proximal penial hooks located on laterally flattened penial papillae, and distal penial hooks located on laterally compressed penial papillae separated by reticulated folds. In contrast, the shell of *C. vesperus* has fine transverse ridges (nearly smooth) with few transverse ridges near peristome, varices absent, periphery more extended beyond the diameter of last whorl, lacking parietal lamella, and penial papillae absent. Meanwhile, *C. striatus* has vas deferens attached to the distal end of penial sheath with very thin connective tissue, and hooks located on papillae without connected longitudinal folds.

Description. Shell oblique-heliciform, white, translucent; whorls 6–7; spire conical with distinct suture. Shell surface glossy with fine transverse ridge across the entire shell; varices present. Embryonic shell ~ 2½ whorls with smooth surface; following whorls regularly coiled. Shell periphery wide and sharply keeled around nearly the entire penultimate whorl; last whorl axially deflected. Aperture subquadrangular;

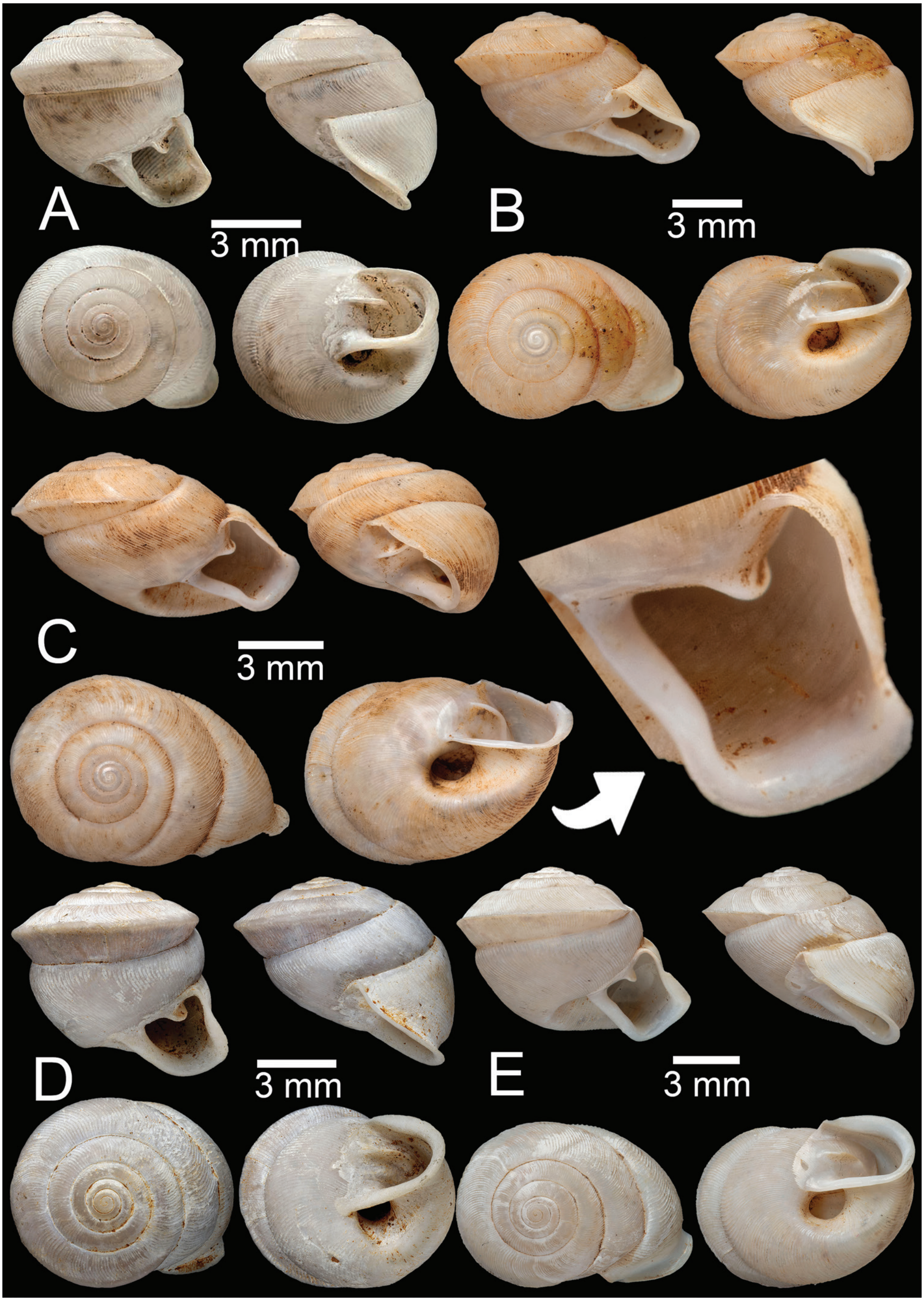

Figure 19. *Carinartemis sankeyi* **A** syntype UMZC I.102740 from Moulmein **B** specimen NHMUK 1888.12.4.792–794 from Moulmein **C** specimen NHMUK 1906.2.2.341 from Moulmein with apertural dentition **D** specimen NHMUK 1871.9.23.61 from Moulmein **E** specimen NHMUK ex. Cuming collection from Mergui.

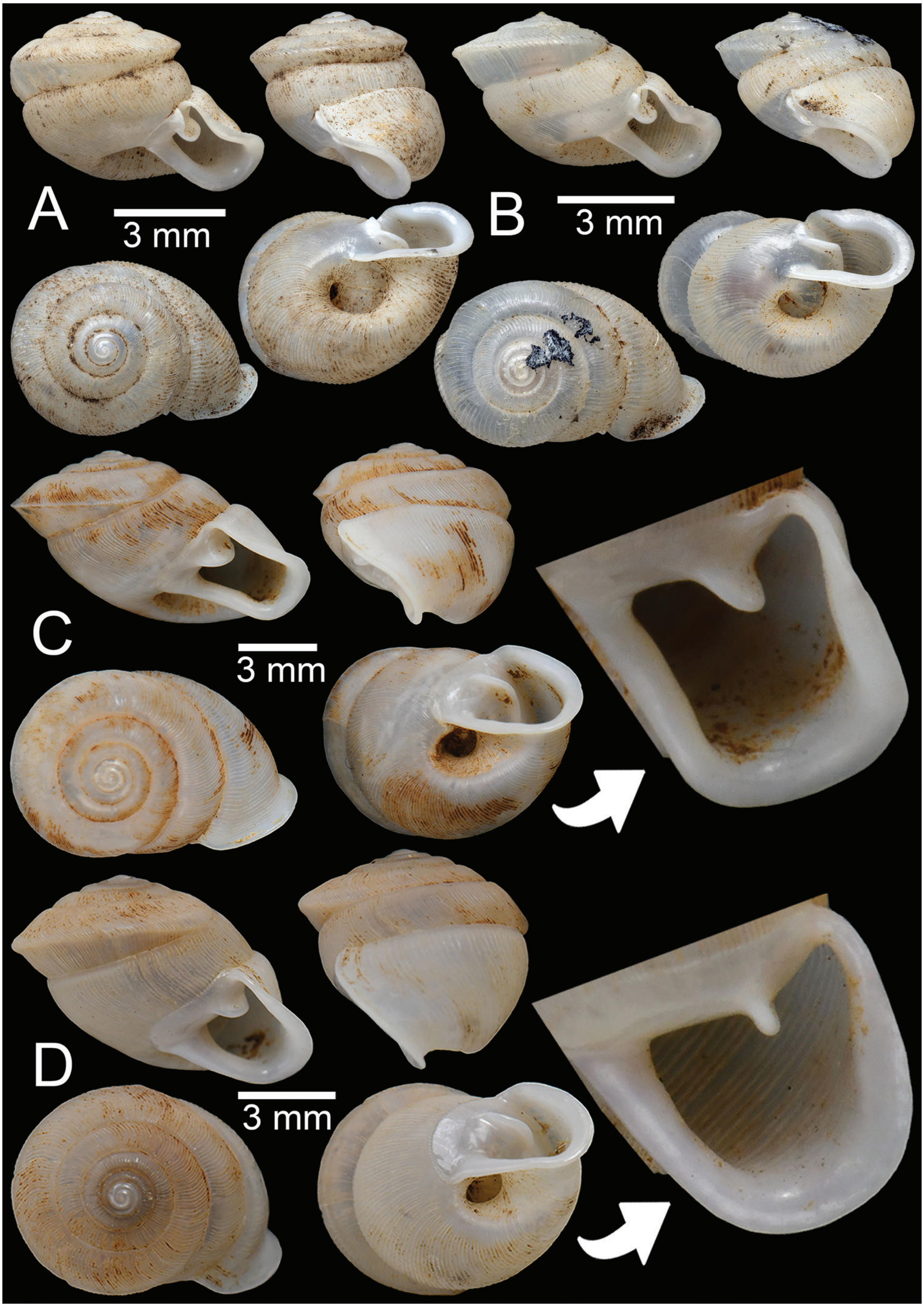

Figure 20. *Carinartemis sankeyi* **A** specimen NHMUK 1903.7.1.4001 from Moulmein **B** specimen NHMUK 1888.12.4.781–783 from Kwengan Hill **C** specimen CUMZ 13031 from Dhammatat Cave, Mawlamyine, Mon State with apertural dentition **D** specimen CUMZ 13029 from Kayon Cave, Mawlamyine, Mon State with apertural dentition.

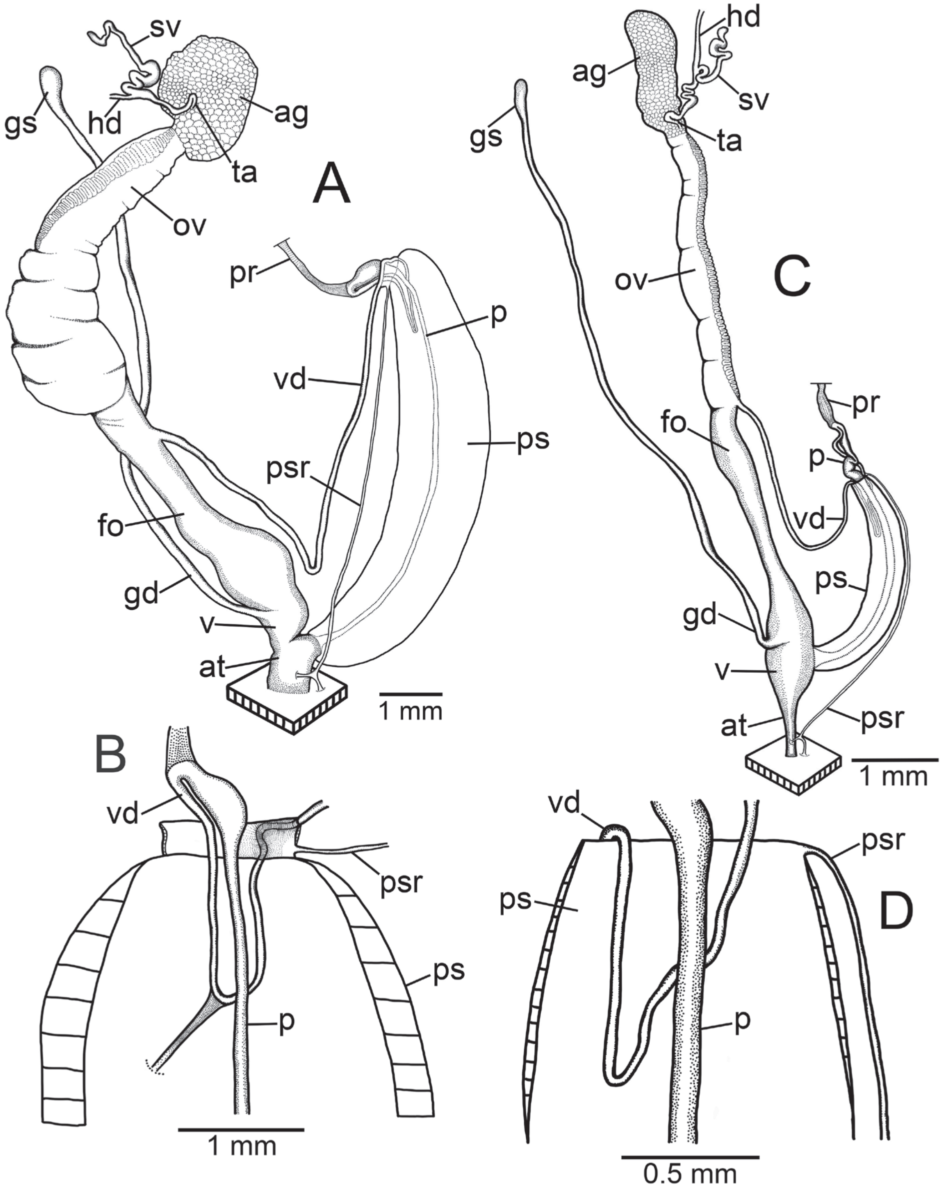

Figure 21. Genital anatomy of **A, B** *Carinartemis exacutus*, specimen CUMZ 13021 **A** reproductive system and **B** insertion of vas deferens into penial sheath **C, D** *Carinartemis sankeyi*, specimen CUMZ 13029 **C** reproductive system and **D** insertion of vas deferens into penial sheath.

peristome continuous, thickened, expanded, and reflected. Apertural dentition with one strong parietal lamella. Umbilicus open and deep (Figs 19, 20).

Genital organs. Atrium (at) short, thin and slender. Penis (p) very thin and long. Penial sheath (ps) thin and extending nearly entire length of penis; penial sheath

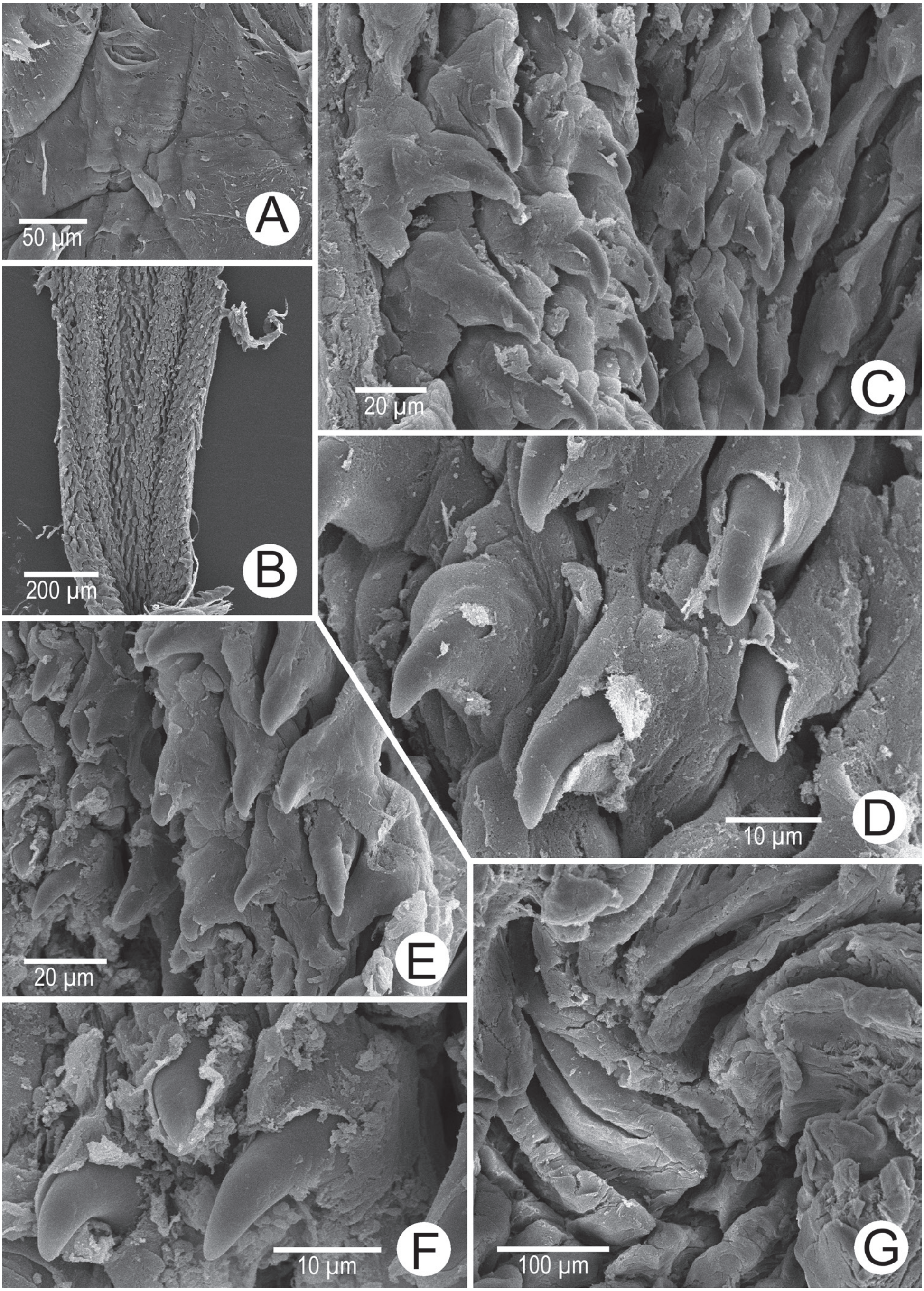

Figure 22. Internal sculpture of genitalia of *Carinartemis exacutus*, specimen CUMZ 13021 **A** atrium surface **B** overview of internal penial wall **C** arrangement of penial hooks on proximal part of penis **D** lateral view of penial hooks **E** arrangement of penial hooks on middle part of penis **F** lateral view penial hooks **G** arrangement of longitudinal oblique vaginal folds.

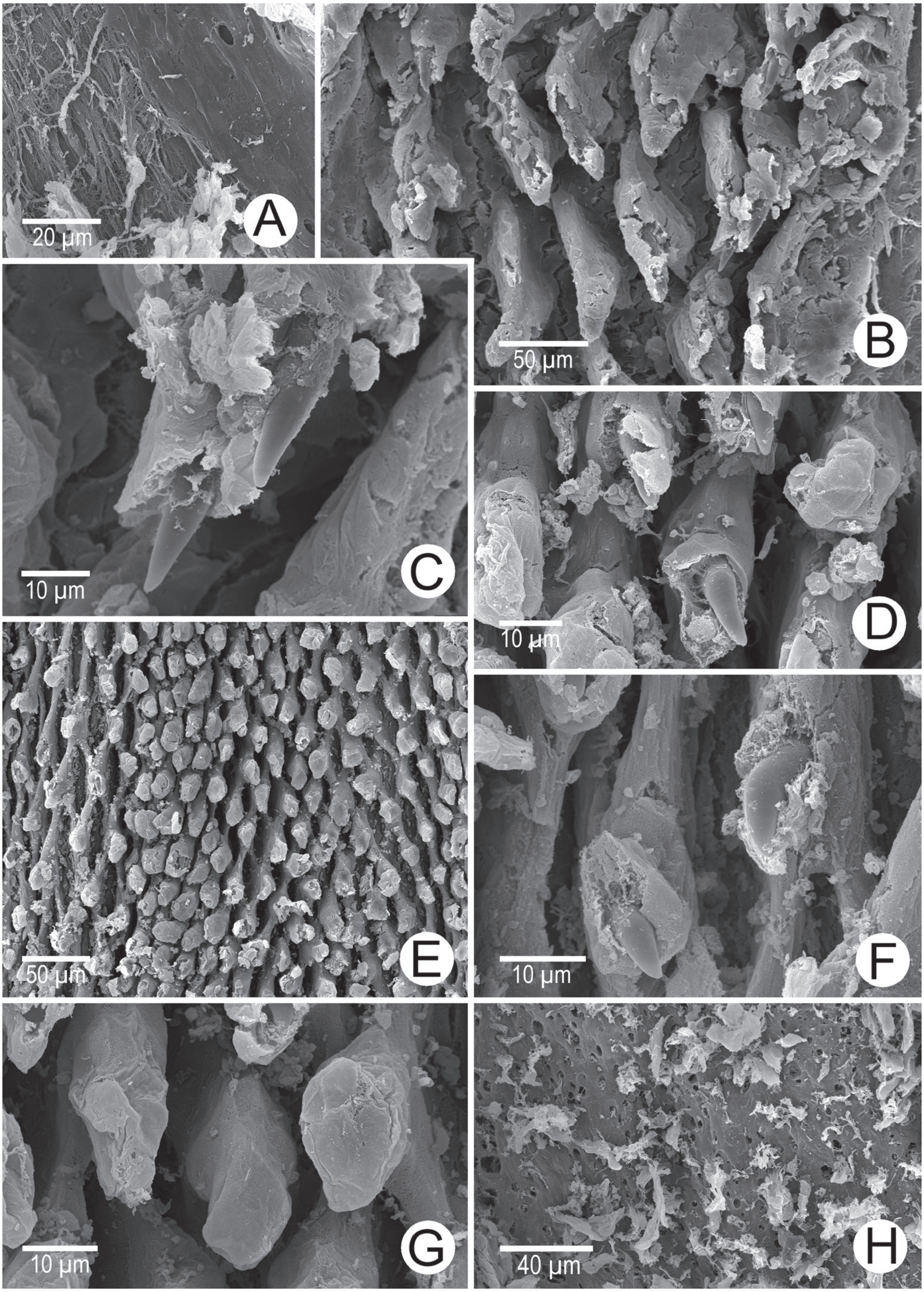

Figure 23. Internal sculpture of genitalia of *Carinartemis sankeyi*, specimen CUMZ 13029 **A** atrium surface **B** arrangement of penial hooks on proximal part of penis **C** lateral view of exposed proximal penial hooks **D** penial hooks on middle part of penis **E** arrangement of penial hooks on distal part of penis **F** uncovered distal penial hooks with pointed tip **G** distal penial hooks embedded in penial papillae **H** vaginal surface with vaginal pores.

retractor muscle (psr) thin, originating near genital orifice and attached to atrium with short and thin connective tissue, and inserting distally on penial sheath (Fig. 21C). Vas deferens (vd) runs downwards to curved portion, ~ 1/3 of the penial sheath length, without insertion before entering penis distally (Fig. 21D). Penial retractor muscle (pr) thin, very long, inserting at penis and vas deferens junction.

Internal wall of atrium generally smooth with sparse atrial pores (Fig. 23A). Proximal penial wall covered with scattered and pale brownish penial hooks, ~ 12 hooks/200 μm^2. Proximal penial hooks located on laterally flattened penial papillae; hooks small and short (< 0.03 mm in length), slightly expanded at base, tips obtuse and curved towards genital orifice (Fig. 23B, C). Middle and distal penial walls densely covered with pale brownish hooks, ~ 20 hooks/200 μm^2. Middle and distal hooks located on laterally compressed penial papillae separated by reticulated folds; hooks small, short (<0.01 mm in length), slightly expanded at base, and tips pointed (Fig. 23D–G).

Vagina (v) short, stout and ~ 1/5 of penis length. Gametolytic duct (gd) a long tube extending as far as albumin gland; gametolytic sac (gs) ovate. Proximal free oviduct (fo) enlarged then tapering to smaller diameter in middle section, and slightly enlarged distally. Oviduct (ov) enlarged and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) small, short and club shaped. Hermaphroditic duct (hd) bearing long seminal vesicle (sv) ca. the same length as from talon to branching point of seminal vesicle (Fig. 21C).

Vaginal wall generally smooth with vaginal pores, and vaginal hook absent (Fig. 23H).

Distribution. This species occurs from three localities in Mon State, southern Myanmar and is likely to be endemic to this area.

Radula. Each row consists of 43–49 teeth with formula (21–24)–1–(21–24). Central tooth small with pointed cusp. Lateral and marginal teeth undifferentiated, unicuspid and lanceolate. Latero-marginal teeth gradually reduced in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 24D).

Remarks. Benson (1859a) introduced *C. sankeyi* based on a single specimen from 'Moulmein'. Later, Stoliczka (1871) provided some diagnostic characters with a description of the soft body color and suggested that the Farm-Cave is possibly the correct type locality. This historical locality name refers to a group of caves located on a karst ridge, namely Kayon Hill, situated on the west bank of the Attaran River. At the same time, Stoliczka (1871) proposed *Streptaxis hanleyanus* based on a single specimen with a small and depressed shell, wide umbilicus, and almost rectangular aperture. However, this holotype specimen was collected from the same geographical area as *C. sankeyi*. Therefore, we consider this nominal species as a smaller shell form and so treat it as a junior synonym with *C. sankeyi*.

The original spelling of this nominal species was *sankeyi*, which was intentionally modified to *sankeyanus* by Stoliczka (1871) without a clear reason. Therefore, this unjustified emendation name was made available with its authorship and date and became a junior objective synonym (ICZN 1999: Arts 32.3, 33.2.3, 50.5).

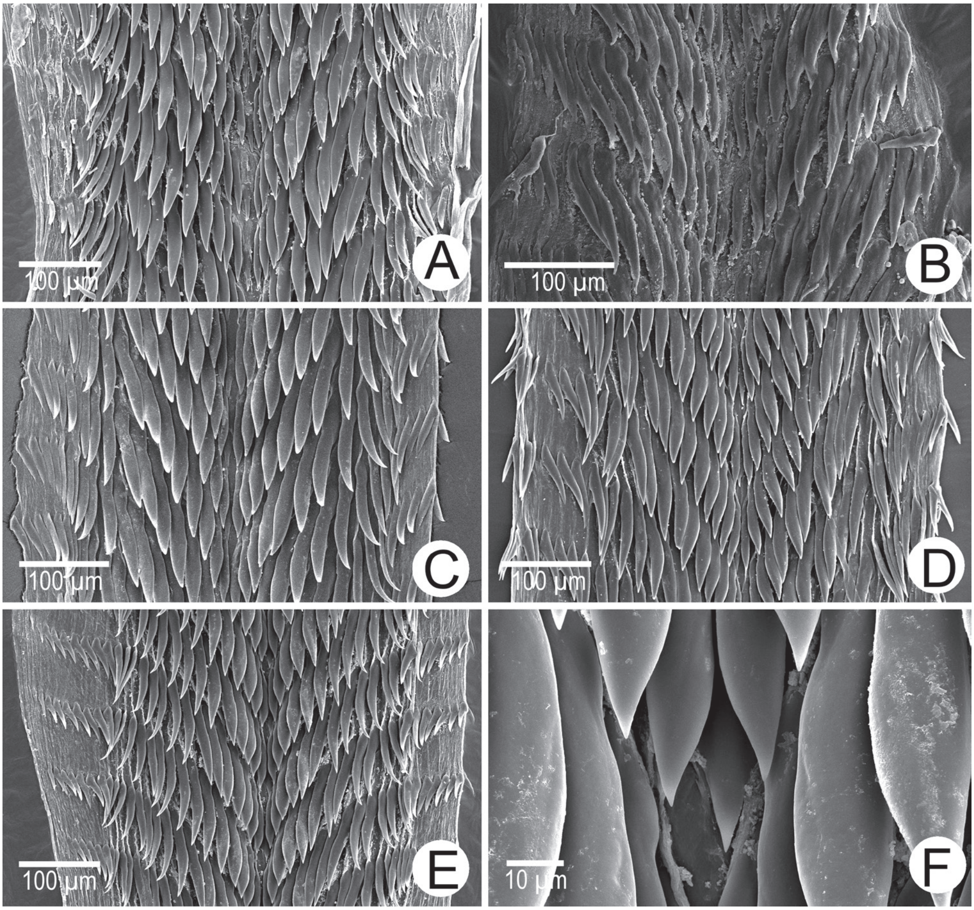

Figure 24. Radula of specimens **A** *Discartemon tonywhitteni*, paratype CUMZ 13001 **B** *Discartemon paurodeviatus* sp. nov., paratype CUMZ 13003 **C** *Haploptychius heliakosus* sp. nov., paratype CUMZ 13014 **D** *Carinartemis exacutus*, specimen CUMZ 13021 **E, F** *Carinartemis sankeyi*, specimen CUMZ 13029 **E** overview of radula and **F** high magnification of central teeth.

Recently, the population collected from Dhammatat Cave showed a more rectangular aperture, slightly compact penultimate whorl, and larger parietal lamella (Fig. 20C), but this species could only be examined from the empty shells. Therefore, we have to conclude that these differences are an intraspecific variation until more specimens are available, or at least the genitalia.

Discussion

This research presents all known Streptaxidae in Myanmar, comprising eighteen species belonging to five genera (*Carinartemis*, *Discartemon*, *Haploptychius*, *Oophana*, and

Perrottetia), including fourteen formerly known species and four new species (Table 2). In addition, this study has added the first anatomical information for three formerly known species (*D. tonywhitteni*, *C. exacutus*, and *C. sankeyi*) and one new species (*H. heliakosus* sp. nov.). The diversity yielded from the southern part of Myanmar (four genera with nine species) was higher than in the central-northeast part (two genera with two species). However, the number of streptaxid taxa found in the central-northeast part of Myanmar are believed to be low because sampling could only be conducted in a few areas. Further land snail surveys should also focus on areas we could not cover in this expedition, such as Rakhine State and Bago Region, areas that contain many of the formerly known species (Theobald 1864; Blanford 1865).

Most reported streptaxids in Myanmar occupy a narrow distribution, and some species were found in only a single area, such as one species of *Perrottetia* in Shan State, two species of *Discartemon* in the Tanintharyi Region, and two species of *Carinartemis* in Kayin and Mon States, which are adjacent regions. In contrast, *Haploptychius* has a wide distribution, ranging from central-northeastern to southern Myanmar, including previously recorded localities, such as Rakhine State and the Bago Region (Theobald 1864; Blanford 1865). Notably, the shell size of streptaxids from the northern part of Myanmar is smaller than those from the southern part, and the population density is also lower. In southern regions, geomorphological and climatic conditions seem to offer more favorable habitats for snails (Siriboon et al. 2020; Sutcharit et al. 2020). On the other hand, this may reflect very narrow distribution ranges, naturally low population levels, or difficulty in finding the northern species due to their smaller size. The two species from the genus *Discartemon* are clearly different in shell morphology, genital structure, and geographic distribution. *Discartemon tonywhitteni* is distributed on the mainland, while *D. paurodeviatus* sp. nov. occurs on an isolated island. *Discartemon* species are mainly localized in southern Thailand and Peninsular Malaysia (Siriboon et al. 2014a, 2020).

Herein, all previously documented species of *Oophana* have been redescribed, based on original descriptions compared with historical museum specimens except for *O. mouhoti*, a new record in Myanmar but based on only a single shell. Siriboon et al. (2020) reported this species from several localities in western Thailand, adjacent to Mon State and Kayin State, but it seems rare in Myanmar even though most the historical materials are recorded from Mawlamyine and Tanintharyi. A phylogenetic analysis revealed that *O. mouhoti* and *O. strangulatus* (von Möllendorff, 1894) were members of the same clade, and were distinct from other congeners in having a more cylindrical shell. In comparison to the Thailand species, most *Oophana* species from Myanmar show a more elongated globose-ovate shell form and weaker apertural dentition (see Siriboon et al. 2020), but *O. elisa* is still ambiguous. Genetically, *Oophana* from Myanmar appear to be closer to the *O. mouhoti* clade and have a similar shell shape and apertural dentition; however, the genital anatomy of the *Oophana* species remains largely unknown.

The most dominant genus, *Haploptychius*, are found mainly in Hpa-an, in the southern part of Myanmar. This genus also shows variability in its shell shape related to their geographic distribution. For example, snails from the (i) Kayin State have more

Table 2. Comparative shell morphology of all recognized streptaxid species in Myanmar.

Species	Shell shape	Spire	Shell surface	Penultimate whorl	Last whorl	Apertural dentition (lamellae)
D. tonywhitteni	depressed heliciform	low-conical to convex	glossy with fine transverse ridges	flattened	angular (sometimes narrowly rounded)	one parietal, one palatal, one basal, one columellar, and one supracolumellar (sometimes with small upper palatal)
***D. paurodeviatus* sp. nov.**	globose heliciform	conical	glossy with fine transverse ridges	rounded, not extended beyond last whorl	rounded, little axially deflected	one parietal, one palatal, one basal, and one columellar (sometime with supracolumellar)
O. elisa	oblique heliciform	convex	transverse ridges	angular, extended beyond last whorl	axially deflected	one parietal, one palatal, one basal, and one columellar (sometimes with supracolumellar)
O. mouhoti	oblique ovate	elevated conical	glossy with fine transverse ridges	slightly angular, not extended beyond last whorl	axially deflected	one parietal, one upper palatal, one palatal, and one basal
O. obtusus	oblique ovate	convex	glossy with fine transverse ridges	rounded to weakly angular and scarcely extended beyond last whorl	slightly axially deflected	one parietal, one palatal, and one columellar
O. laevis	oblique heliciform	low convex	glossy with transverse ridges	rounded, not extended beyond last whorl	axially deflected	one parietal, and one basal, (columellar lamella present or absent)
P. theobaldi	sub-oblique heliciform	convex	glossy with transverse ridges	rounded, not extended beyond last whorl	axially deflected	one parietal nearly adjoint with small second parietal, one upper palatal, one palatal, one basal, and one bifid columellar
H. bombax	oblique ovate	convex	glossy with fine transverse ridges	rounded, slightly extended beyond last whorl	axially deflected	only one parietal lamella
H. blanfordi	oblique heliciform	convex	glossy with fine transverse ridges	rounded, slightly extended beyond last whorl	axially deflected	one parietal (sometimes with small palatal)
H. burmanicus	oblique heliciform	convex	glossy with fine transverse ridges	rounded, slightly extended beyond last whorl	axially deflected	one parietal
H. solidulus	oblique ovate	elevated conical	glossy with fine transverse ridges	slightly angular, not extended beyond last whorl	axially deflected	one parietal
H. thebawi	oblique heliciform	conical	glossy with transverse ridges	rounded, less extended beyond last whorl	axially deflected	one parietal
***H. tenasserimicus* sp. nov.**	sub-oblique heliciform	low convex	glossy with fine transverse ridges	angular, extended beyond last whorl	axially deflected	one parietal
***H. heliakosus* sp. nov.**	oblique ovate	low conical	glossy with transverse ridges	rounded, extended beyond last whorl	axially deflected	one parietal
***H. karenorum* sp. nov.**	depressed ovate	depressed-convex	moderately strong transverse ridges	bluntly angular, extended beyond last whorl	axially deflected	one parietal
C. petitii	oblique heliciform	high to low conical	fine transverse ridges	subangular, slightly extended beyond last whorl	axially deflected	one parietal
C. exacutus	oblique heliciform	convex	glossy with fine transverse ridges	sharply keel, extended beyond last whorl	axially deflected	one parietal (sometimes with second parietal)
C. sankeyi	oblique heliciform	convex	glossy with fine transverse ridges	sharply keeled, extends beyond last whorl	axially deflected	only one parietal

globosely ovate shells and a larger size, while those from (ii) Tanintharyi Region have depressed shells, and those from (iii) Mandalay Region and Shan State have smaller, ovate shells and most bear only one parietal lamella. Exceptionally, *H. heliakosus* sp. nov. from the type population has an extra small tubercle next to the parietal lamella, and similarly, *H. blanfordi* has a small palatal lamella. While the male reproductive organ of the *Haploptychius* species in Laos shows a relatively thin penial sheath with slender, elongate penial hooks (Inkhavilay et al. 2016), surprisingly, *H. heliakosus* sp. nov. and *C. exacutus* superficially share a lack of differentiation in their external genitalia features by having a very thickened penial sheath that covers the entire penis, with broader basal penial hooks, and obvious penial papillae (Table 2). Further molecular analysis may suggest the possible cause of these shared characteristics among the streptaxids.

So far, five species in the genus *Carinartemis* have been recorded from Thailand and Myanmar (Siriboon et al. 2014b), of which four species have been investigated in terms of their genitalia. The distinctive traits of the male organs of *Carinartemis* from Myanmar are the possession of penial hooks with reticulated folds that are covered with penial papillae (Table 2). The internal sculpture of the genitalia of *C. exacutus* and *C. sankeyi* could be useful in discriminating the species, although their shell morphology is highly similar, but distinct from the Thai species (Siriboon et al. 2014b). In addition, the shell shape shared among *Carinartemis* species was observed in *H. karenorum* sp. nov. and *H. tenasserimicus* sp. nov., which tend to have an angular penultimate whorl that extends beyond the last whorl. This angular or slightly keeled penultimate whorl also occurs in some species of *Oophana* and *Indoartemon* (Siriboon et al. 2020). As Siriboon et al. (2020) suggested, to stabilize the traditional taxonomic boundary in these groups, more information on at least the genitalia is required. Furthermore, these records of new biological diversity in Myanmar indicate the uniqueness of its ecosystems and should promote more discussion concerning conservation efforts in Myanmar.

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RESEARCH ARTICLE

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Biodiversity of Nearctic inland water: discovery of the genus *Heterorotula* (Porifera, Spongillida, Spongillidae) in the Appalachian Mountains, with biogeographical implications and description of new species

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Abstract

This paper reports the discovery of a small population of sponges in the Pigeon River of eastern Tennessee, USA, which were morphologically distinct from Spongillida of North America. A morphological comparative analysis resulted in the first Nearctic record of the genus *Heterorotula* with the description of a new species *Heterorotula lucasi* **sp. nov.** diverging from all other known species by its unique combinations of diagnostic morphotraits of spicules and gemmules. The new record enlarges the geographic range of the genus which has been known until now only from Australia, New Zealand, New Caledonia, Japan (as an alien species), and from subequatorial Brazil (as subfossil remains). The discovery of a biogeographic enclave of *Heterorotula* in the southeastern United States contributes to the understanding of Porifera inland water biodiversity, biogeographic patterns, and adaptive morphotraits in the Nearctic and globally. Data confirm that the Appalachian region (Ordovician–Permian origin) of Tennessee and, in general, of North America have high levels of diversity and endemicity.

Keywords

Aquatic biodiversity, biogeography, evolutionary history, freshwater sponges, morphotraits, Tennessee, United States

Introduction

The aquatic biodiversity of the southeastern United States is recognized as the most biologically rich on the North American continent (Elkins et al. 2019). Assessments of aquatic biodiversity have found the southeastern United States to have levels of diversity and endemism rivalling those of tropical regions (Abell 2000; Collen et al. 2014).

The mountains and valleys of the Appalachian region of North America extend for 3,200 km from the Canadian province of Newfoundland to central Alabama of the United States. There was no one-time orogeny creating the Appalachian Mountains; instead, these mountains were formed by several major and minor orogenies. Erosion has reduced ancient Appalachian peaks of over 5,000 m to slightly over 2,000 m today (Pickering et al. 2003).

The central and southern Appalachians did not experience Pleistocene glaciation. Their alignment north to south provided organisms a migration corridor to the south thereby avoiding extinctions (Pickering et al. 2003). Due to topography, climate, and being spared Pleistocene glaciation, the southern Appalachian Mountains are one of the most biologically diverse regions in the temperate world. High aquatic species diversity has led to this region being recognized as a global center of aquatic biodiversity (McLarney 1999; Curtin et al. 2002).

Until recently, little was known concerning the sponge fauna of the Appalachian region of Tennessee. However, including the species described in this article, 13 species have been documented from this region (Table 1) (Copeland et al. 2015, 2019, 2020). During July 2013 in the Pigeon River, a small population of sponges was discovered which was morphologically distinct from all other known extant Spongillida of North America. We report here on this population as the first record of the genus *Heterorotula* Penney & Racek, 1968 from the Nearctic Region and describe it as a new species.

Table 1. Checklist of the freshwater sponges (Porifera: Spongillida) of the Appalachian region of Tennessee (southeastern Nearctic Region) with the new species record (bold) from Pigeon River.

Family	Species
Potamolepidae	*Cherokeesia armata* Copeland, Pronzato & Manconi, 2015
Spongillidae	*Corvospongilla becki* Poirrier, 1978
	Ephydatia fluviatilis (Linnaeus, 1759)
	Ephydatia muelleri (Lieberkuhn, 1855)
	Eunapius fragilis (Leidy, 1851)
	Heteromeyenia latitenta (Potts, 1881)
	Heteromeyenia tubisperma ((Potts, 1881)
	***Heterorotula lucasi* sp. nov.**
	Racekiela ryderi (Potts, 1882)
	Radiospongilla cerebellata (Bowerbank, 1863)
	Radiospongilla crateriformis (Potts, 1882)
	Radiospongilla crateriformis (Potts, 1882)
	Trochospongilla horrida (Weltner, 1893)

Study area

The Pigeon River arises in the Blue Ridge Mountains of western North Carolina and flows to the northwest until its confluence with the French Broad River in eastern Tennessee (Fig. 1A, B). The Pigeon and French Broad rivers are eastern head water tributaries of the Tennessee River. The Pigeon River traverses the Pisgah National Forest, the Cherokee National Forest, and drains much of the northeastern Great Smoky Mountains National Park. From Canton, North Carolina to its confluence with the French Broad River in Tennessee, anthropogenic impacts to the river have been substantial. The Pigeon River was once considered one of the most polluted rivers in North America (Etnier and Starnes 1993). In 1908 a paper mill began production in Canton, upstream of the Tennessee study area. Effluent from this mill contained a mixture of chemicals including dioxin, chloroform, organic halides, phenols, and catechols (Bartlett 1995). The toxicity of this mixture caused a significant loss of biodiversity. All gastropods (10 species), mussels (40 species), and many fish species disappeared from the river (Coombs 2010). Releases of high levels of tannins and lignin resulted in the

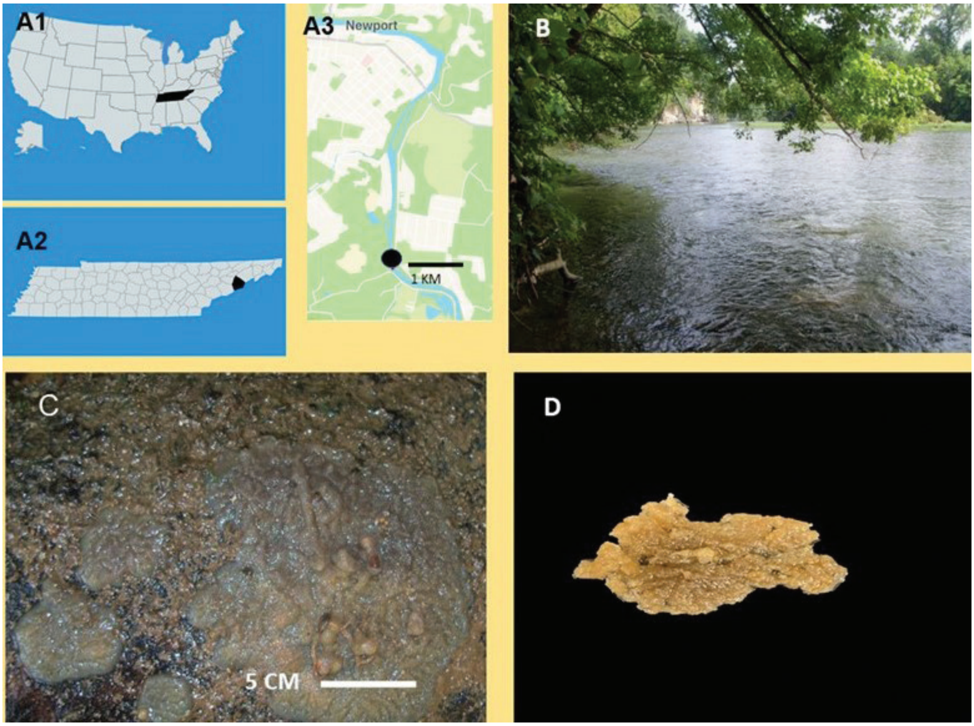

Figure 1. Study area **A1** Tennessee in the southeastern region of the United States **A2** location of Cocke County in Tennessee **A3** type locality of *Heterorotula lucasi* sp. nov. is indicated by a black circle in the Pigeon River (35.9396, –83.1786) **B** riparian habitat of the new sponge species at the type locality **C** macrophotograph *in vivo* of holotype (USNM 1662182) with tawny color and encrusting growth form on a rocky substrate **D** macrophotograph of holotype in alcohol.

river water becoming coffee brown in color and emitting a bad odor. Hot water releases from the mill raised water temperature.

For 52 years there was no treatment of the mill's effluent. The first effort at treatment occurred in 1960 with the removal of settleable solids (University of Tennessee 2021). In 1989 the mill began an effective dioxin control program. Dioxin has not been detected in tested water since 1989 (Coombs et al. 2010). In 1996 aquatic snails were re-introduced. The survival and recolonization of these snails led to the formation of the Pigeon River Recovery Project (PRRP) in 2001 (Coombs et al. 2010). The PRRP which is composed of volunteers from federal and state agencies, industry, and private organizations has the goal of increasing the aquatic biodiversity of the Pigeon River (Coombs 2010). Due to the reintroduction of aquatic organisms by the PRRP the Pigeon River has regained some of its biodiversity

Materials and methods

Two sites on the Pigeon River were surveyed by viewing appropriate hard substrates (i.e., rocks and logs) while wading. A total of 71 sponge specimens were collected, four of which were morphologically distinct from all other extant Spongillida of the Nearctic Region. A minimum of 1 human-hour of search time was spent at each collection site. Latitude and longitude were obtained using a Garmin GPSmap 76CSx receiver. Sponges were viewed using a 10× head-band magnifier for the presence of gemmules. If gemmules were found a section of the sponge was collected. Sponges were preserved in 70% ethanol until processed for light microscopy (LM) and scanning electron microscopy (SEM).

To characterize morphotraits and obtain clean spicule preparations for SEM observation and LM slides, excised sponge was dissolved in test tubes containing 65% nitric acid. Once dissolved, the remaining spicules were centrifuged to create a pellet. Pellets were rinsed and centrifuged three times in distilled water, followed by a final rinse and spin in 70% ethanol. Spicules were pipetted on to glass slides for LM analysis and onto stubs for SEM analysis. A glass substratum was placed under the spicules providing a black background in the SEM photomicrographs (Manconi and Pronzato 2000). Skeleton sections, disassociated spicules, entire gemmules, and gemmule cross-sections were sputter coated with gold-palladium using an Anatech Hummer IV (6.2) sputter coater and observed by Leo 982 and Hitachi TM 3000 SEM. Sponge identification was made using the keys of Manconi and Pronzato (2002, 2016) and descriptions of Reiswig et al. (2010), Penney and Racek (1968), and Racek (1969). To determine range, mean ± standard deviation for spicular lengths, widths, and rotule diameters 75 megascleres and gemmuloscleres were measured using the measurement program of the Hitachi TM 3000. Diameters of 10 gemmules were measured. A paired *t*-test analysis, using JMP software, was used to test the null hypothesis of no significant difference between diameters of gemmulosclere rotules.

Institutional acronyms

FW-POR	R. Manconi and R. Pronzato collection, Italy.
USNM	National Museum of Natural History, Smithsonian Institution, Washington DC, USA.
WAM	Western Australian Museum, Perth, Western Australia.

Systematic account

Phylum Porifera Grant, 1836
Class Demospongiae Sollas, 1885
Subclass Heteroscleromorpha Cárdenas, Perez & Boury-Esnault, 2012
Order Spongillida Manconi & Pronzato, 2002
Family Spongillidae Gray, 1867

Genus *Heterorotula* Penney & Racek, 1968

Type species. *Heterorotula capewelli* (Bowerbank, 1863).

Heterorotula lucasi Manconi & Copeland, sp. nov.

https://zoobank.org/5886FA19-94E5-43D8-8EF6-AB41E0D819F3
Figs 1–9; Tables 1, 2

Type locality. Pigeon River 35.9396; –83.1786, Cocke County, Tennessee, USA.

Type data. ***Holotype.*** USNM 1662182, entire specimen, 70% ethanol, coll. J. Copeland, 30 July 2013. ***Paratype.*** USNM 1662183, fragments, 70% ethanol. Pigeon River, Cocke County, Tennessee, USA, 35.9396; –83.1786, coll. J. Copeland, 30 July 2013.

Other material. BMNH 1890.1.9.339, holotype, *Heterorotula capewelli* type species of genus *Heterorotula*. WAM Z27997, FW-POR 881, *Heterorotula multiformis* (Weltner, 1910) (Australia), WAM Z98316, FWPOR 883, *Heterorotula* cf. *multidentata* (Weltner, 1895) (Australia).

Diagnosis. *Heterorotula lucasi* sp. nov. is characterized by (a) gemmuloscleres as spiny birotules with flat rotules (distal and proximal) of significantly different diameters with crenulated/notched to shallowly incised margins, (b) absence of skeletal microscleres, (c) skeletal acanthoxeas spiny, and (d) free (not sessile) gemmules.

Etymology. The specific epithet *lucasi* refers to *lux* meaning light in Latin and is dedicated to Lucas Edward Copeland whose enthusiasm and love for the natural history of the forests and streams of the Appalachian Mountains of Tennessee resulted in many wonderful discoveries.

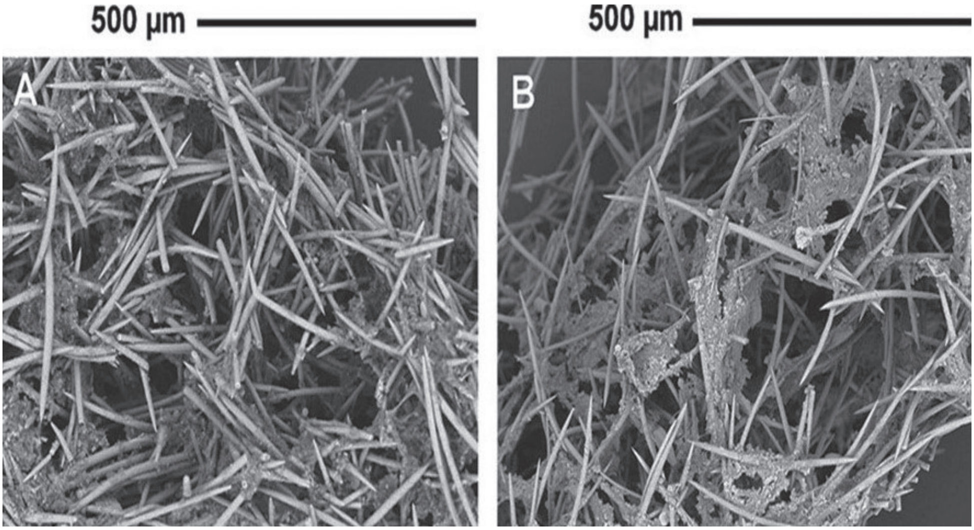

Figure 2. *Heterorotula lucasi* sp. nov. from the Pigeon River, Tennessee (type locality). Micrographs of skeletal network with scanty spongin by SEM **A** multispicular fibres of entosomal area **B** paucispicular fibers of ectosomal area. Scale bar: 500 µm.

Description. Adult sponges with gemmules. No brooded larvae were found. ***Growth form*** (Fig. 1C) encrusting 1–4 mm in thickness and to 16 cm in diameter. ***Surface*** with irregular ridges, hispid from emerging spicules, and with a network of subdermal canals covered by a hyaline dermal membrane. ***Color*** tawny *in vivo* (Fig. 1C), light yellow to light brown in alcohol (Fig. 1D). ***Consistency*** of live sponge soft and fragile. Spongin scanty in skeletal network, arranged as irregular polygonal meshes, to abundant in gemmular theca and basal spongin plate. ***Ectosomal skeleton*** of slender megascleres in paucispicular fibres, with no special architecture (Fig. 2B) supports the dermal membrane. ***Choanosomal skeleton*** (Fig. 2A) as a network of multi-spicular fibres, with scanty spongin. ***Basal spongin plate*** well developed.

Megascleres (Figs 2, 3) fusiform acanthoxeas 223.1–335.0 µm (276.8 ± 24.7) in length and 7.7–13.7 µm (10.9 ± 1.0) in width, slightly curved, with variably dense spines except towards the variably pointed tips, to less frequently nearly spineless. Acanthoxeas shaft from slender in ectosomal area to stouter in the endosome. ***Microscleres*** absent.

Gemmules (Fig. 4) scattered in skeletal network, subspherical, 448–613 µm (528 ± 55.9) in diameter. Foramen simple (Fig. 4E, F) with smooth undulate margins, slightly elevated above gemmule surface. ***Gemmular cage*** (Fig. 4C, D) of acanthoxeas with small spines.

Gemmular theca trilayered ~50 µm in thickness (Fig. 5). **Outer layer** fibrous to compact with distal rotules more or less embedded (Fig. 5A, B, D). **Pneumatic layer** well developed and thick, ranging in the same gemmule from mainly fibrous to chambered network of irregularly polygonal meshes of variable size (Fig. 5B, C). ***Inner layer*** (Fig. 5A, D) multilayered of 3–5 layers of compact spongin. ***Gemmuloscleres*** radially embedded (Fig. 5A, B) as a dense monolayer in pneumatic layers of theca, with distal

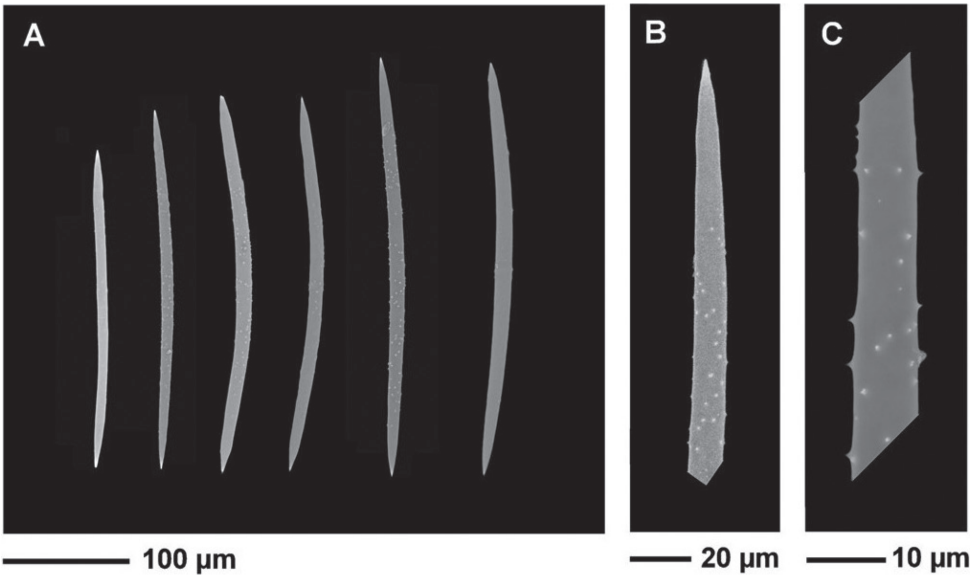

Figure 3. *Heterorotula lucasi* sp. nov. from the Pigeon River, Tennessee (type locality). Micrographs of skeletal megascleres by SEM **A** acanthoxeas fusiform from nearly spineless to variably spined excepts towards the tips **B** detail of an oxea smooth only at the tip **C** spiny shaft of an acanthoxea; scattered spines and microspines on shaft. Scale bars: 100 µm (**A**); 20 µm (**B**); 10 µm (**C**).

smaller rotules partly covered by outer layer and proximal larger rotules, partly overlapping one to each other, not embedded into inner layer (Fig. 5A, B).

Gemmuloscleres (Figs 6–8) slender birotules 19.8–48.6 µm (35.1 ± 5.5) in length, with narrow spiny shaft 2.7–4.4 µm (3.3 ± 0.3) in width. Shaft spines of three types (a) simple, short curved to straight, smooth spines, (b) spines with tips arranged in asterose clusters (microspines in rosettes), and (c) large, acute spines up to 3 µm long bearing secondary microspines (Fig. 7). ***Rotules*** flat with crenulated/notched to shallowly incised margins and both rotules inner and outer surfaces bearing numerous microspines sometimes in radial tows (Fig. 8A) of significantly different diameters $t(74) = 18.67$, $p < 0.00005$) (Fig. 6). Large proximal rotules 19.4–24.4 µm (21.6 ± 1.1) in diameter, small distal rotules 16.6–21.7 µm (18.9 ± 1.1) in diameter.

Habitat (Fig. 1B). All four specimens of *Heterorotula lucasi* sp. nov. were found bearing gemmules on the underside of shaded rocky substrate in shallow running water at an altitude of 325 m. Associated aquatic community was composed of diatoms, Spongillidae of three genera/species *Heteromeyenia latitenta* (Potts, 1881), *Radiospongilla cerebellata* (Bowerbank, 1863), and *Trochospongilla horrida* (Weltner, 1893); Mollusca of two families Pleuroceridae and Corbiculidae, Diptera larvae of the families Chironomidae and Simuliidae; Ephemeroptera larvae of two families Baetidae and Heptageniidae; Trichoptera larvae of family Hydropsychidae, and Odonata larvae of family Gomphidae. The climate of the study area is temperate having an average annual rainfall of 112 cm. In addition to rain events, flow rates of the Pigeon River are influenced by water releases from a hydroelectric dam in Haywood County, North

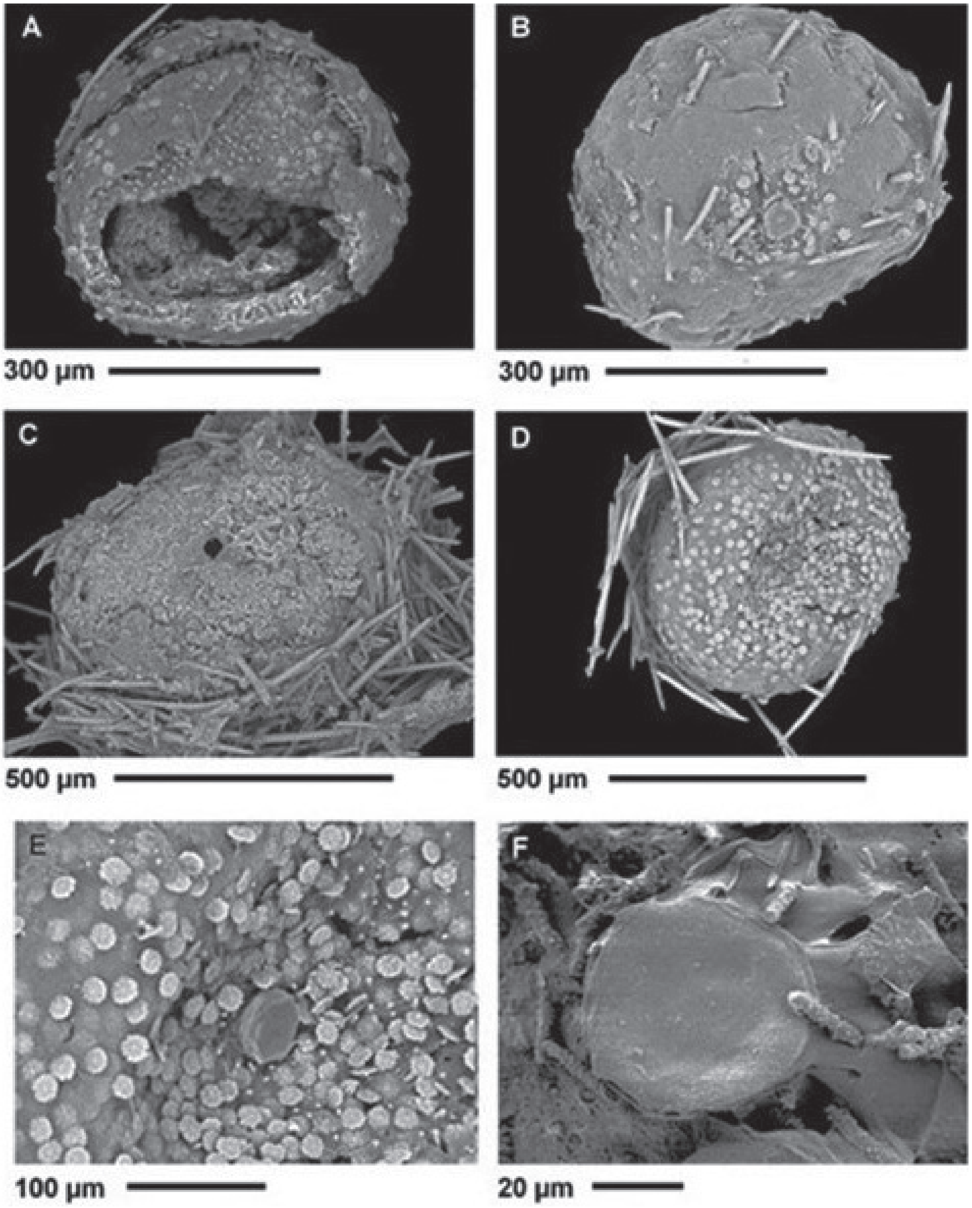

Figure 4. *Heterorotula lucasi* sp. nov. from the Pigeon River, Tennessee (type locality). Micrographs of gemmules by SEM **A** gemmule with multilayered theca armed by radial gemmuloscleres around the central cavity bearing a mass of totipotent cells **B** outer layer at theca surface with a few emerging rotules of gemmuloscleres, acanthoxeas of the cage and sligtly elevated foramen closed by a membrane **C** theca of an aged gemmule covered by diatoms frustules, surrounded by the spicular cage of acanthoxeas and outer layer with open foramen after hatching **D** theca with a fragment of spicular cage and outer layer armed by dense distal rotules of gemmuloscleres **E** fibrous structure of outer layer with sligtly elevated foramen closed by a membrane before hatching **F** fibrous structure of outer layer around foramen and sparse broken shafts of gemmuloscleres. Scale bars: 500 µm (**C, D**); 300 µm (**A, B**); 100 µm (**E**); 20 µm (**F**).

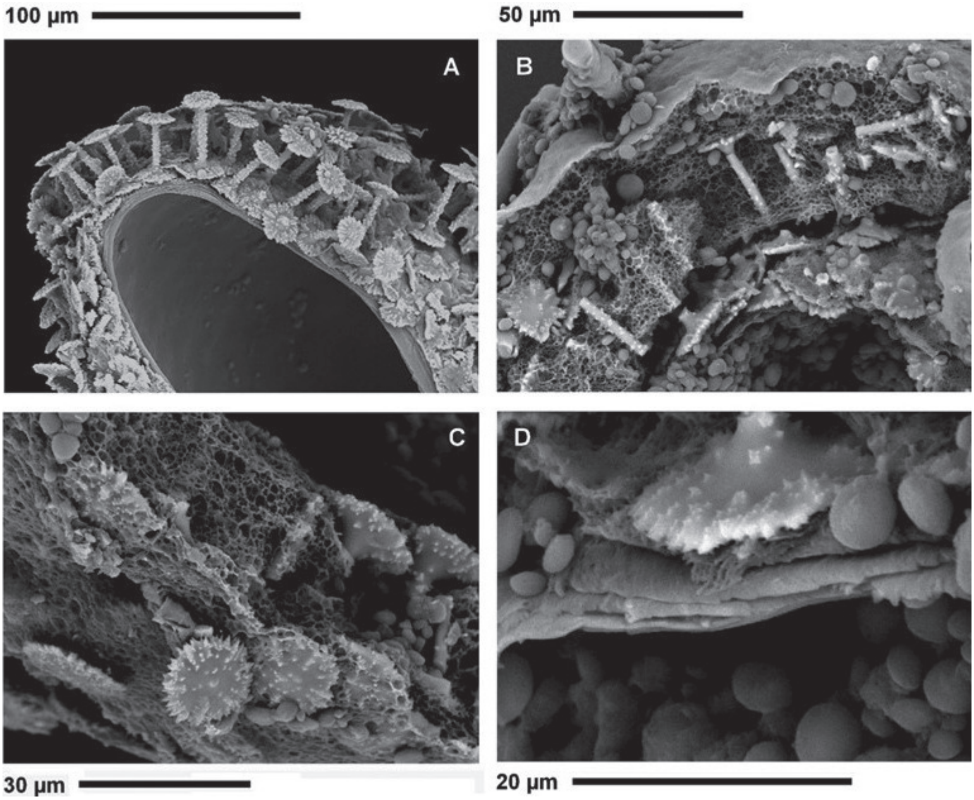

Figure 5. *Heterorotula lucasi* sp. nov. from the Pigeon River, Tennessee (type locality). Micrographs of gemmular theca cross sections by SEM **A** theca with the radial monolayer of short and long spiny birotules bearing crenulated/notched margins of rotules. proximal, partly overlapping, rotules adherent to the multilayered inner layer (cross section, outer layer, and pneumatic layer not evident) **B** trilayered theca with short and long spiny birotules embedded in the fibrous/chambered pneumatic layer in between the smooth outer layer and the multilayered inner layer, **C** radial birotules in the fibrous to chambered pneumatic layer **D** proximal rotule with crenulated/notched margins adherent to the multilayered inner layer surrounding the central gemmular cavity containing totipotent cells. Scale bars: 100 µm (**A**); 50 µm (**B**); 30 µm (**C**); 20 µm (**D**).

Table 2. Length and width ranges, in microns, for spicules and gemmules of living species of *Heterorotula*.

Species	ML	MW	LBL	SBL	BSW	PRD	DRD	GW
H. capewelli	195–330	13–18	38–52	34–45	3–4	24–45	20–23	510–560
H. nigra	224–360	7–13	56–73	35–48	2–4	13–16	10–14	230–360
H. multidentata	284–320	10–18	64–84	32–48	4–6	19–22	17–20	490–580
H. kakahunesis	185–260	9–12	30–42			17–22		380–540
H. multiformis	330–420	13–20	35–52	24–44	2–4	14–24	14–18	480–680
H. caldonensis	250–410	8–20	35–85		4	19–26	18–22	450–600
H. controversa	210–395	12–21	21–57		3–4	20–24	18–21	470–590
H. lucasi	223–335	7.7–13.7	19.8–48.6		2.7–4.4	19.4–24.4	16.6–21.7	448–613

Range values positioned between columns indicate no separation into two distinct size classes for that variable. Legend: ML megasclere length; MW megasclere width; LBL large birotule length; SBL small birotule length; BSW birotule shaft width; PRD proximal rotule diameter; DRD distal rotule diameter, GW gemmule width.

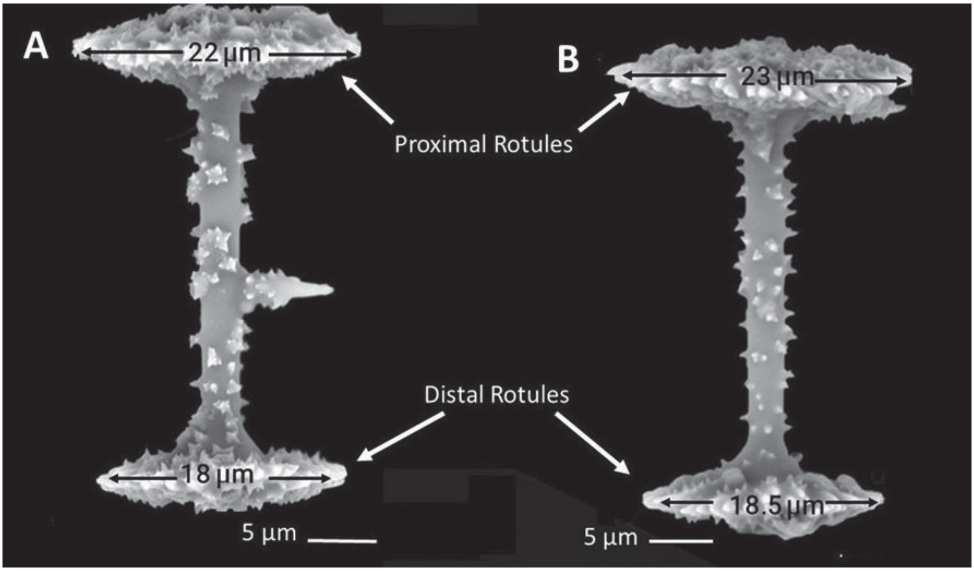

Figure 6. *Heterorotula lucasi* sp. nov. from the Pigeon River, Tennessee (type locality). Size differences in proximal and distal rotules. Scale bars: 5 µm (**A**); 5 µm (**B**); scale bars are slightly different in length.

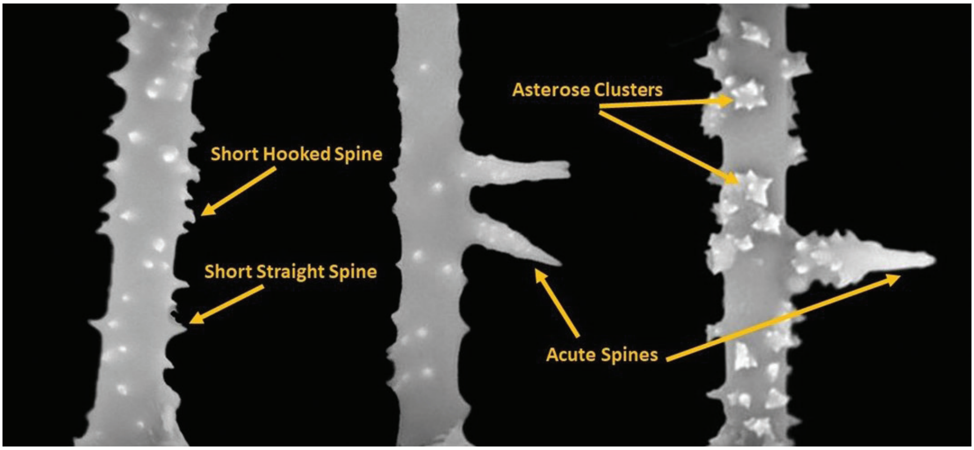

Figure 7. *Heterorotula lucasi* sp. nov. from the Pigeon River, Tennessee (type locality). Spine morphology of shafts of gemmuloscleres by SEM.

Carolina. Over its 113 km course the Pigeon River drops from an elevation of 800 m at Canton, North Carolina to 310 m at its confluence with the French Broad River near Newport, Tennessee.

Geographic range. *Heterorotula lucasi* sp. nov. is only known from Pigeon River (type locality, 35.9396, –83.1786) in Tennessee. The location of this single population lays far outside the previously known range of the genus.

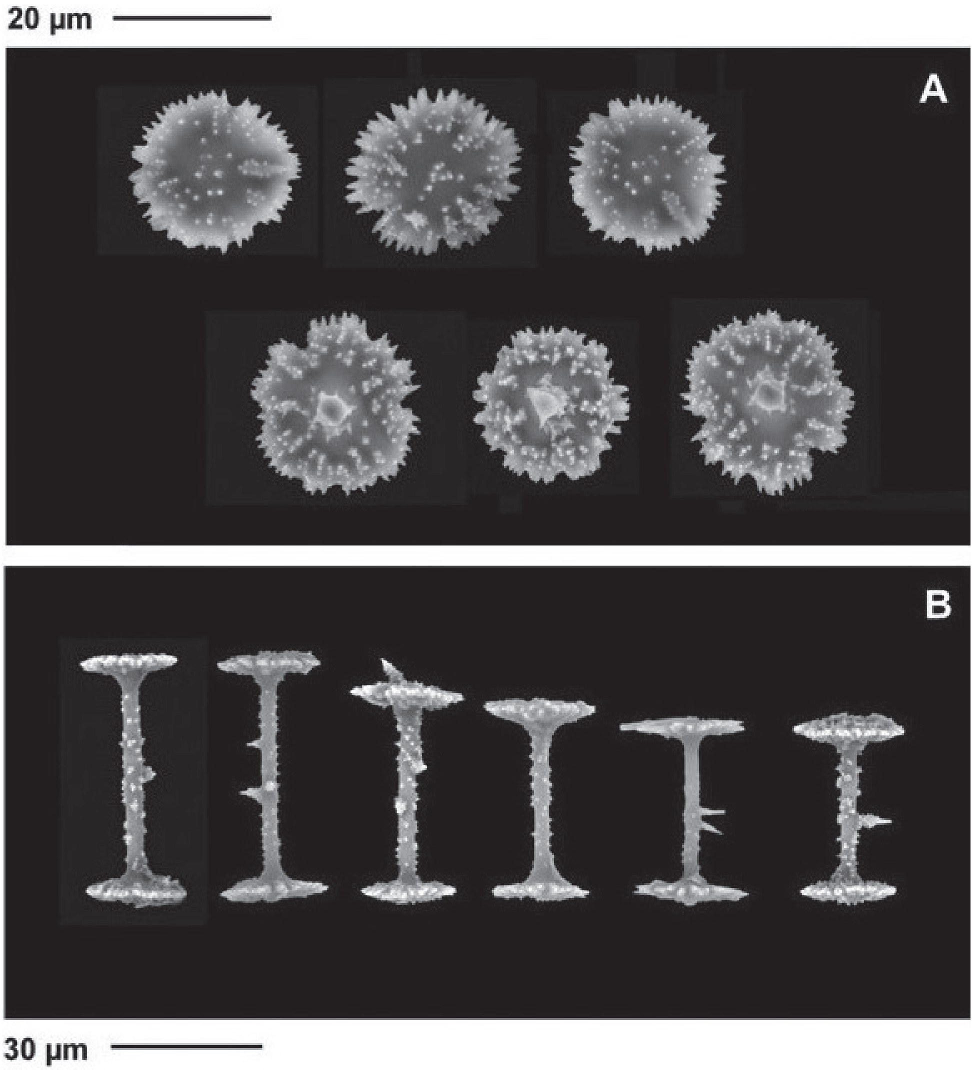

Figure 8. *Heterorotula lucasi* sp. nov. from the Pigeon River, Tennessee (type locality) **A** crenulated margins and miceospines of rotules and gemmulosclers **B** variation in size and spines of gemmuloscleres. Scale bars: 30 µm (**B**); 20 µm (**A**).

Discussion

The genus *Heterorotula* was erected by Penney and Racek (1968: 96–97) to allocate five species from the Australasian Region (Fig. 9), i.e., *Heterorotula capewelli* (Bowerbank, 1863) as type species, *Heterorotula kakahuensis* (Traxler, 1896), *Heterorotula multidentata* (Weltner, 1895), *Heterorotula multiformis* (Weltner, 1910), and *Heterorotula nigra* (Lendenfeld, 1887) for genus transfer (see Bowerbank 1863; von Lendenfeld

1887; Weltner 1895, 1910; Traxler 1896; Penney and Racek 1968; Rützler 1968) from genera *Ephydatia* Lamouroux, 1816, *Spongilla* Lamarck, 1816, *Tubella* Carter, 1881, and *Meyenia* Potts, 1882 with junior synonyms, e.g., *Spongilla capewelli* Bowerbank, 1863; *Spongilla sphaerica* Lendenfeld, 1887, *Tubella nigra* Lendenfeld, 1887, *Ephydatia multidentata* (Weltner, 1895), *Tubella multidentata* Weltner, 1895, *Ephydatia kakahuensis* Traxler, 1896, *Ephydatia lendenfeldi* Traxler, 1896, *Ephydatia multiformis* Weltner, 1910, and *Ephydatia nigra* Gee, 1931.

Figure 9. Disjunct biogeographic pattern of genus *Heterorotula* (Porifera, Spongillida, Spongillidae) with enclaves in Nearctic and Neotropical regions. The Nearctic *H. lucasi* sp. nov. from Tennessee is indicated in red. *Heterorotula multidentata* is considered an alien species in Japan.

A year later, *Heterorotula caledonensis* (Rützler, 1968) was described as *Ephydatia multidentate* f. *caledonensis* by Rützler (1968). In his synopsis on the Australian freshwater sponges, Racek erected *Heterorotula contraversa* (Racek, 1969). Later, Volkmer-Ribeiro and Motta (1995) described *Heterorotula fistula* from Brazil. Additionally, *H. multidentata* was reported in Japan (Palaearctic) by Masuda (1998, 2006) and Ishijima et al. (2008) as an alien species (Fig. 9).

The reason given by Penney and Racek (1968: 96–97) to justify the erection of the new genus was that "The peculiar structure and often greatly varying length of the gemmoscleres, so typical for this genus." They also stated that: "*Heterorotula* is more closely related to *Ephydatia* than to any other genus." Effectively not all the *Heterorotula* species share very close gemmuloscleres morphologies and, in some cases, a correct identification is not easy.

As for the morphotraits of *Heterorotula* species, Penney and Racek (1968), Racek (1969), and Manconi and Pronzato (2002) focused on length differentials of gemmuloscleres, morphologies of rotule margins, and diameter differences between proximal and distal rotules. An exhaustive and clear key together with images was provided by Racek (1969: 292–293) for morphological divergencies among the five Australasian *Heterorotula* species. As for the main diagnostic traits of the two genera *Heterorotula* and *Ephydatia*, see the following key.

Key to the genera *Ephydatia* and *Heterorotula*

1 Gemmuloscleres only birotules of one length (short shaft) radially arranged; rotule margins evidently indented and spiny with marginal teeth deep and straight; pneumatic layer chambered; microscleres absent; megascleres as microspiny and/or smooth oxeas .... ***Ephydatia***

– Gemmuloscleres only birotules of varying length with rotules (short to long shaft) radially arranged; rotules frequently of different diameter, margins crenulated/incised, with many small spines; pneumatic layer fibrous to chambered; microscleres absent; megascleres as microspiny and/or smooth oxeas .. .. ***Heterorotula***

As for the only record of the genus known until now from Americas, *Heterorotula fistula* is an intriguing species for its biogeographic location and palaeoecology. It is recorded as a fossil/subfossil taxon from spongolites remains in sedimentary deposits of permanent and temporary freshwater bodies of the subequatorial Brazilian areas of Rio Grande do Norte, Goiás Mato Grosso do Sul and Minas Gerais in the Neotropical Region (Volkmer-Ribeiro and Motta 1995; Machado et. al. 2014, 2016; Docio et. al. 2021). Although its subfossil condition cannot support its status as either a true living species or a recently extinct one, the Neotropical enclave of this species enlarged the disjunct biogeographic pattern of *Heterorotula* to the western hemisphere (Fig. 9).

In any case, the very different geological histories of the Gondwanian, Australasian, and Neotropical regions, after the Pangea breakup (~175 Mya), do not provide an explanation for this biogeographic pattern of *Heterorotula*. This task is made more difficult because very few ancient fossils of freshwater sponges have been found (Pronzato et al. 2017; Samant et al. 2021).

Results of a comparative analysis of *H. lucasi* sp. nov. with congeneric species, on the basis of museum collection and original descriptions, revealed that it significantly diverges from all other species of *Heterorotula* by a few robust characters.

The growth form, oscules, color, and skeletal architecture are not diagnostic morphotraits for the new species and for genus *Heterorotula*, as for most Spongillida. The absence of skeletal microscleres is shared by all species of the genus.

The trait "megascleres as fusiform oxeas prevalently spiny to nearly spineless" is partly and differently shared with some *Heterorotula* species. The comparsion between the type species *H. capewelli* and *H. lucasi* sp. nov. revealed that the megascleres of the former (195–300 × 13–18 μm) are stout oxeas predominantly smooth with few evident scattered spines, while those of the new species are slimmer oxeas predominantly and abundantly microspiny. The oxeas lengths range reported by Penney and Racek (1968) for other species is from *H. nigra* (224–360 × 7–13 μm), *H. multidentatae* (284–320

× 10–18 µm), *H. multiformis* (330–420 × 13–20 µm) to *H. kakahuensis* (185–260 × 9–12 µm). Length and width measurements for megascleres, gemmuloscleres, and gemmules of the known living species of *Heterorotula* are presented in Table 2.

The gemmule architecture and dimensions and its morphotraits, i.e., spicular cage, pneumatic layer, and foramen matches the genus range. The gemmuloscleres of *H. lucasi* sp. nov. are unique. Both rotules are finely spined and with crenulated/incised margins. The slim shaft of gemmuloscleres displays spines moderately long and variable in length.

Only the type species of the genus, *H. capewelli*, shares with *H. lucasi* sp. nov. the trait "crenulated rotules." The term "crenulated" refers to the New Latin term *crenulatus*, from *crenula*, diminutive of Medieval Latin *crena* (notch) was introduced by Racek (1969: 293) and meaning "Having a margin contour with shallow, usually rounded notches and projections; finely notched or scalloped." However, the two species diverge for gemmulosclere shaft morphologies, *H. capewelli* has smooth to finely granulated shafts whereas the shafts of the new species are variously spined. All other species of the genus show gemmuloscleres with margins of rotules variously indented/incised and with variably long to very long shafts.

Among freshwater sponges gemmuloscleres birotules are shared by several genera having a wide range of rotule morphotraits, e.g., flat, curved, umbonate, indented, hooked, smooth, and variably spiny/tubercled. In *Heterorotula*, the morph "rotules with margins only crenulated" seems to be shared by only two species, *H. capewelli* and *H. lucasi* sp. nov. However, also the margins of the rotules of the Neotropical *Ephydatia facunda* (Pinheiro et al. 2004) resemble those of the new species but diverges from it for the shaft morphology, whereas the Middle Eocene fossil *E.* cf. *facunda* from Canada totally diverges from *H. lucasi* sp. nov. in the outline of the birotules (Pisera et al. 2016).

In contrast, it is noteworthy that the southeastern Nearctic harbors *Ephydatia millsi* (Potts, 1887), which is exclusively endemic to the type locality at Sherwood Lake near DeLand, Florida (Potts 1887; Penney and Racek 1968; Manconi and Pronzato 2016). This aberrant species, which does not match the diagnosis of *Ephydatia*, was considered "eventually to be an ecomorph of one of the more widely distributed *Ephydatia* species" (Penney and Racek 1968: 96) because it displays the morph "spiny, flat rotules with lacinulate margins" (Penney and Racek 1968; Reiswig et al. 2010). *Ephydatia millsii* must still be considered as rather insufficiently known.

The gemmuloscleres of *H. capewelli* are birotules with a slender, smooth shaft bearing sometimes few spines. Rotules with unequal diameter are flat with small spines and margins irregularly crenulated with rare teeth. In comparison the birotules of *H. lucasi* sp. nov. are smaller and with margins of rotules irregularly crenulate/incised and evidently spiny, with also a stouter shaft bearing abundant rosetta-shaped microspines and rare long spines. Diameter of rotules is unequal.

Summarizing, the new species is characterized by slimmer megascleres that are predominantly and abundantly microspiny to nearly spineless; smaller gemmuloscleres that are abundantly microspiny on rotules and the stout shaft (the latter with also spines in rosettas), and a very distant disjunct geographical range.

This biogeographic enclave in Tennessee contributes to the understanding of the origin of Nearctic inland water biodiversity, biogeographical patterns, and morphological adaptive traits in Nearctic sponges. The present record matches the recent discovery in Tennessee of *Cherokeesia armata* Copeland, Pronzato & Manconi, 2015, the first living Nearctic Potamolepidae (Copeland et al. 2015). Data confirm that the Appalachian region (Ordovician–Permian origin) of Tennessee and, in general, of southeastern North America have high levels of diversity and endemicity (Curtin et al. 2002; Hodkinson 2010; Erlandson et al. 2021).

With the present record of a new species in Tennessee, the biogeographic pattern of the genus *Heterorotula* matches the Australasian–Pacific Islands–Neotropical regions in the southern hemisphere (Australia n = 5 species; New Caledonia n = 2; New Zealand n = 1; Brazil n = 1 species), with one spot in a Nearctic enclave (n = 1 species, *H. lucasi* sp. nov.) and an alien species in the insular easternmost Palaearctic (Japan n = 1) (Fig. 9) (Manconi and Pronzato 2008; Manconi et al. 2016; Pronzato and Manconi 2019; de Voogd et al. 2022).

Not considering the Japanese record of an alien species, the scenario that arose with the unexpected discovery of a *Heterorotula* species in the southwestern Laurasian Nearctic (North America), and the subsequent tripartite geographic range, is anomalous but not unique to and shows a similitude with, for example, marsupials of which the majority of species is strictly Australasian but having some key species recorded from the Neotropical and Nearctic regions (e.g., Opossum, *Didelphis virginiana* (Voss 2022)).

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The complete mitogenome of the potentially invasive flatworm *Australopacifica atrata* (Platyhelminthes, Geoplanidae) displays unusual features common to other Rhynchodeminae

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Abstract

We sequenced the complete mitochondrial genome of the flatworm *Australopacifica atrata*. The species, originally described from New South Wales, Australia, has been found in various locations in the British Isles, New Zealand and in the United States of America; it is thus potentially invasive. The genome is 16513 bp long, encodes for 12 protein coding genes, two ribosomal RNA genes and 20 tRNA genes, and is completely colinear with the other two available Rhynchodeminae. In addition, it shares with them some unusual characters discriminating them from members of the other subfamilies of Geoplanidae, the most noticeable being the extra length of its *cox2* gene. The data allow a reliable multigene phylogeny to be derived, and also provide a means of accurate biomonitoring of possible invasiveness by *A. atrata*.

Keywords

Biomonitoring, Continenticola, *cox2* gene, multigene phylogeny, next generation sequencing, *Parakontikia ventrolineata*, Tricladida

Introduction

In the recent years, an increasing number of reports have emerged from Europe and abroad concerning invasive terrestrial flatworms of the family Geoplanidae Stimpson, 1857 (Winsor et al. 2004; Sluys 2016). The most recent classification of the Geoplanidae (Almeida et al. 2021) lists five subfamilies within the Geoplanidae, among which three might be of particular concern in the context of biological invasions. The most infamous species in these subfamilies are probably the 'hammerhead flatworm' *Bipalium kewense* Moseley, 1878 (Bipaliinae Von Graff, 1896) from South East Asia (Winsor 1983; Justine et al. 2018), followed by *Obama nungara* Carbayo, Álvarez-Presas, Jones & Riutort, 2016 (Geoplaninae Stimpson, 1857) from South America (Carbayo et al. 2016; Lago-Barcia et al. 2019; Justine et al. 2020b; Fourcade 2021) and *Platydemus manokwari* de Beauchamp, 1963 (Rhynchodeminae Von Graff, 1896) from Papua New Guinea (Justine et al. 2014; Justine et al. 2015; Gastineau et al. 2020; Justine et al. 2021). With their large size and their feeding habits, these predators of soil invertebrates have attracted most attention.

Two species, smaller than the aforementioned large ones and with a mostly scavenging behaviour, are also potentially invasive (Winsor et al. 2004). The first is *Parakontikia ventrolineata* (Dendy, 1892) Winsor 1991; the species is about 30 mm in length with a dark grey-black body, with longitudinal black stripes on the dorsum, and with the eponymous paired greyish stripes on its ventral surface. Originally from Queensland, Australia, it has been found elsewhere in Australia, and has also been reported in France (Justine et al. 2014; Gastineau et al. 2020), Spain (Álvarez-Presas et al. 2014) and possibly in South Africa (Jones et al. 1998). Also New Zealand, Hawaii, United Kingdom, Madeira, USA, and Mexico (records summarized in Winsor et al. 2004), Costa-Rica, Colombia, Ecuador, Chile, Argentina, and confirmed for South Africa (iNaturalist records, https://www.inaturalist.org/). The second species is *Australopacifica atrata* (Steel, 1897). Originally described as *Geoplana atrata* from New South Wales, Australia, it was transferred to *Parakontikia* on the basis of its external morphology and internal anatomy (Winsor 1991), and subsequently to *Australopacifica* (Jones, 2019). The formal definition of *Australopacifica* is "Geoplanidae, but not classifiable into the present taxonomic genera because of insufficient morphological information; geographical distribution largely in Australasia and Indo Pacific islands. A collective group to temporarily assign *species inquirendae* and *nomina dubia*" (Ogren and Kawakatsu 1991). It is also found in other Australian states and territories: Victoria (Winsor 1973), Queensland (Winsor 1997), the Australian Capital Territory, and Tasmania (Atlas of Living Australia records, https://www.ala.org.au/). The species has been recorded in New Zealand (Winsor et al. 2004), in Wales and South England (Jones 2019), and in California, USA (iNaturalist records). The species superficially resembles *Pa. ventrolineata*, except that it has a uniformly black dorsum, with a characteristic median dark stripe on its ventral surface (Figs 1, 2). Both species are gregarious, collectively predating upon slugs and snails and other soil species such as isopods (Smith 1979; Barker 1989; Winsor et al. 2004), and are also necrophagous scavengers (Smith 1979; Jones 2019). They have been recorded in France and the UK on various fallen fruit lying on the soil, and in cavities in growing strawberries; whether the

Figure 1. Living flatworm *Australopacifica atrata* (Steel, 1897), showing underside of mid body.

planarians are simply attracted to rotting fruit or actually feeding on the fruit remains has yet to be adequately explained (Jones 2019; Justine et al. 2020b).

In recent years, several complete mitochondrial genomes from different species of terrestrial invasive flatworms have been sequenced (Solà et al. 2015; Gastineau et al. 2019; Gastineau et al. 2020; Gastineau and Justine, 2020; Justine et al. 2020a; Justine et al. 2022). Full mitogenomes provide a complete sequence for the widely used molecular barcode COI or *cox1* (the gene of the cytochrome c oxidase subunit 1) and allows us to perform robust multigene phylogenies. Full mitogenomes may provide additional information such as those related to the gene order or the presence of pseudogenes resulting from duplications. It also prevents amplification of nuclear copies of mitochondrial pseudogenes by PCR, a problem that sometimes occurs in molecular barcoding (Song et al. 2008). In the case of terrestrial flatworms, earlier reports have noted that there are mitogenomic features common to several species of the same subfamily that are not present in other subfamilies (Solà et al. 2015; Gastineau et al. 2020; Gastineau and Justine 2020; Justine et al. 2020a). However, the number of available mitogenomes remains still too low to allow for more general conclusions. Prior to this study, GenBank did not contain any sequence corresponding to the genus *Australopacifica*.

Here we report the complete mitochondrial genome of a morphologically identified specimen of *A. atrata* and compare it with other species. We provide further evidence concerning the specific features of the mitogenomes of Rhynchodeminae

Figure 2. Living flatworm *Australopacifica atrata* (Steel, 1897), dorsal view.

compared to land flatworms in other subfamilies. We present a multigene phylogeny demonstrating its proximity with *Pa. ventrolineata*. Finally, we discuss the next steps that should take place in the investigation of invasive terrestrial flatworms by means of next generation sequencing.

Material and methods

Biological material

All specimens were collected from a native plant nursery in Maffra, Victoria, Australia (37°57'S, 146°59'E), from November 2019 to February 2020 and identified on the basis of external morphology and colour pattern (Steel 1897; Winsor 1973). They were killed in boiling water and preserved in 95% ethanol. Specimens were registered in the collection of the Muséum National d'Histoire Naturelle (MNHN; Paris, France) and in the collection of one of us (LW field number, as MNHN JL368 (2 specimens LW1874), JL372 (3 specimens LW1880) and JL374 (3 specimens LW1883). One specimen MNHN JL374 was destroyed for the molecular analysis.

Sequencing, assembly and annotation

A tissue sample in 95% ethanol was sent to the Beijing Genomics Institute (BGI) in Shenzhen, China, where DNA extraction and sequencing took place. A total of ca 40 million of 150 base pair clean paired-end reads was obtained on a DNBseq platform.

The reads were assembled using SPAdes 3.14.0 (Bankevich et al. 2012), with a k-mer of 125. The annotation was made with the help of MITOS (Bernt et al. 2013) and manually curated, using Sequin 15.50 and the genetic code 9. In particular, the ribosomal genes needed to be located by alignments with reference sequences of *O. nungara* (KP208777). In such cases, alignments were performed with MEGAX (Kumar et al. 2018). The tRNA were also verified with Arwen v1.2 command line, using the -gcflatworm option (Laslett and Canbäck 2008). The map of the mitogenome was generated using OGDRAW (Lohse et al. 2013).

Multigene phylogenies

A phylogeny was inferred with the amino-acid sequences of the conserved mitochondrial proteins, following a protocol adapted from Justine et al. (2021). Sequences were concatenated by alphabetic order, aligned using MAFFT 7 (Katoh and Standley 2013) using the -auto option and the resulting alignments was trimmed by trimAl (Capella-Gutiérrez et al. 2009), using the -automated1 option. Phylogenies were conducted using RaxML 8.0 (Stamatakis 2014), with the best tree out of 100 computed for 1000 bootstrap replicates and using the MtArt evolution model (Abascal et al. 2007) with the GAMMA model of rate heterogeneity and an estimate of proportion of invariable sites (PROTGAMMAIMTART option). *Prosthiostomum siphunculus* Delle Chiaje (KT363736) (Polycladida) was used as an outgroup.

Results

Mitogenome

The mitogenome was retrieved among the other contigs from the assembly file, in the form of a contig containing all conserved coding parts, with redundant extremities which allowed us to circularize it. The genome has been deposited on GenBank with accession number OM456243.

The mitogenome is 16513 bp long. Its composition is 4810 A, 1248 C, 2461 G and 7994 T. It encodes for 12 protein coding genes, 2 ribosomal RNA genes and 20 tRNA genes (Fig. 3). It is strictly colinear with the mitogenomes of the other Rhynchodeminae, *Pl. manokwari* and *Pa. ventrolineata*, including the tRNA. Most of the protein-coding genes start with an ATG, except *ND4*, which starts with a GTG overlapping *ND4L*, and *ND2*, for which no canonical start codon could be found. The *ND5* gene has a premature stop due to the presence of a *tRNA-Ser*. No *tRNA-Thr* could be detected. Similar to *Pl. manokwari* and *Pa. ventrolineata*, the *cox2* gene was found to be unusually long, with an extension fragment located in the central part of the open reading frame. Table 1 lists the size of the *cox2* genes for the available mitogenomes of Continenticola (Tricladida).

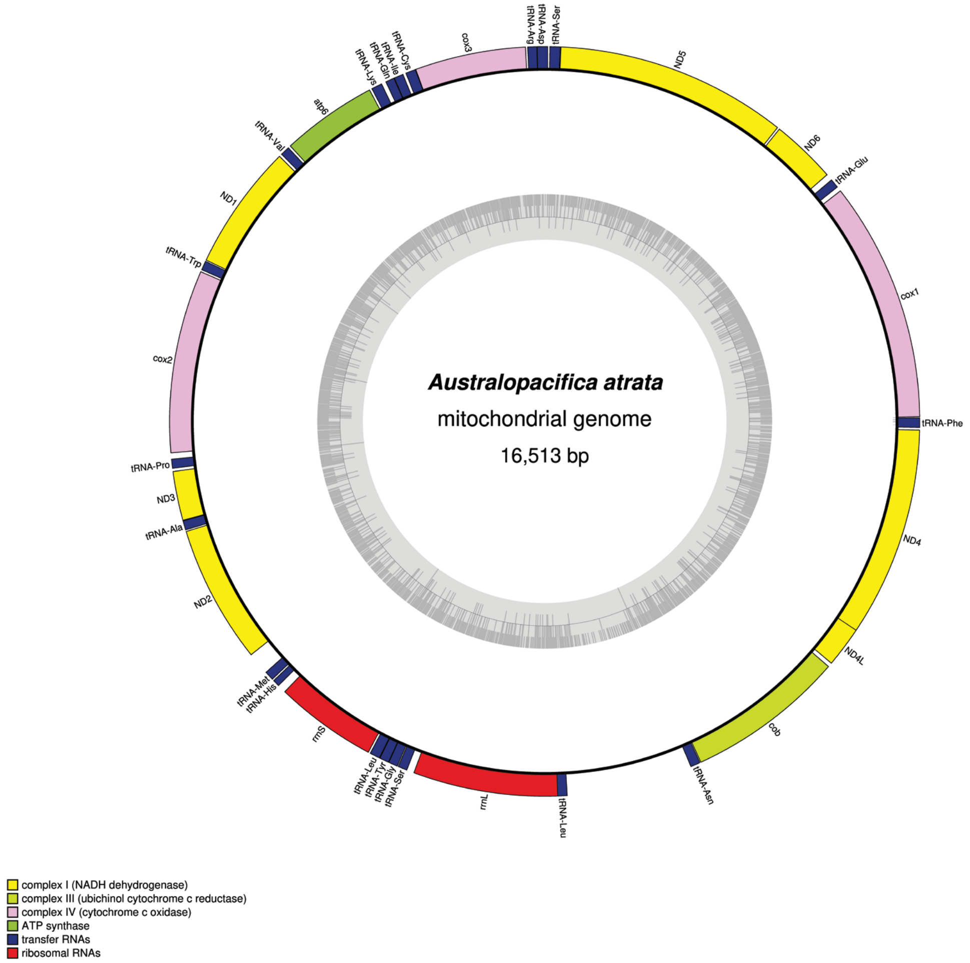

Figure 3. Map of the mitochondrial genome of *Australopacifica atrata* (Steel, 1897).

Phylogeny

The maximum likelihood phylogenetic tree (Fig. 4) easily distinguishes the three families in the Continenticola for which mitogenomes are available, namely the Planariidae, Dugesiidae and Geoplanidae. Within the cluster of Geoplanidae, two major groups emerge: the Geoplaninae, and a cluster containing the Bipaliinae and Rhynchodeminae. The tree also retrieved *A. atrata* as the sister species of *Pa. ventrolineata* with support of 100%.

Discussion

The mitogenome of *A. atrata* is shorter than those of the two other Rhynchodeminae *Pl. manokwari* (19959 bp, MT081580) and *Pa. ventrolineata* (17210 bp, MT081960),

Table 1. Sizes in amino acids (AA) of the *cox2* proteins encoded by the available mitogenomes of Continenticola (Tricladida).

Name	Family	GenBank accession number	Size of the putative *cox2* protein (in AA)	Specific features
Schmidtea mediterranea	Dugesiidae	JX398125	292	Start codon not determined
Girardia sp.	Dugesiidae	KP090061	389	Start codon not determined
Girardia tigrina	Dugesiidae	MW972220	389	Start codon not determined
Dugesia japonica	Dugesiidae	AB618487	227	NA
Dugesia ryukyuensis	Dugesiidae	AB618488	230	TAA stop codon completed by the addition of 3' A residues to the mRNA
Crenobia alpina	Planariidae	KP208776	239	NA
Phagocata gracilis	Planariidae	KP090060	297	NA
Obama nungara	Geoplanidae	KP208777	259	Start codon not determined
Amaga expatria	Geoplanidae	MT527191	260	Start codon not determined
Bipalium kewense	Geoplanidae	MK455837	225	NA
Bipalium vagum	Geoplanidae	MZ561468	229	NA
Bipalium adventitium	Geoplanidae	MZ561467	227	NA
Diversibipalium multilineatum	Geoplanidae	MZ561469	228	NA
Diversibipalium mayottensis	Geoplanidae	MZ561470	246	NA
Humbertium covidum	Geoplanidae	MZ561471, MZ561472	248	NA
Platydemus manokwari	Geoplanidae	MT081580	452	NA
Parakontikia ventrolineata	Geoplanidae	MT081960	433	NA
Australopacifica atrata	Geoplanidae	OM456243	434	NA

but larger than the largest recorded for a Bipaliinae, *Bipalium vagum* Jones & Sterrer, 2005 (17149 bp, MZ561468) or the Geoplaninae, *O. nungara* (14909 bp, KP208777) and *Amaga expatria* (14962 bp, MT527191). However, the presence of repeated sequences in the mitogenome of Geoplanidae has been strongly suggested (Solà et al. 2015). These repeated portions cannot be fully resolved by short reads sequencing, so the criteria of the length of the mitogenome should be taken with care.

So far, the Rhynchodeminae can be differentiated from Bipaliinae and Geoplaninae by the position of the *tRNA-Cys*. For *A. atrata*, *Pl. manokwari* and *Pa. ventrolineata*, it is located between the protein coding genes *cox3* and *atp6*, clustering with *tRNA-Ile*, *tRNA-Gln* and *tRNA-Lys*, as the first tRNA of this cluster. For *B. kewense* as well as *O. nungara* and *A. expatria*, it is located between the protein coding gene *ND2* and the 12S rRNA gene, clustering with *tRNA-Met* and *tRNA-His*, located after these two tRNA. Another difference regarding tRNA is the apparent lack of a *tRNA-Thr* among all Rhynchodeminae. Among the other species, *tRNA-Thr* has been found between the 16S rRNA gene and the protein-coding *cob* gene, clustering with *tRNA-Leu* and *tRNA-Asn*. It is worth emphasising here that there is a difference between the Geoplaninae, in which the order of this cluster is 16S, *tRNA-Thr*, *tRNA-Leu*, *tRNA-Asn*, *cob* and *B. kewense*, in which *tRNA-Leu* and *tRNA-Thr* exchange their positions. However, it might be noted that for the recently described species of Bipaliinae, *Humbertium covidum* Justine, Gastineau, Gros, Gey, Ruzzier, Charles & Winsor, 2022 (Justine et al. 2022), it was also impossible to find a *tRNA-Thr*. Therefore, it would be better to take a conservative view of this feature, and not to assign it a too high value for classification as a molecular synapomorphy.

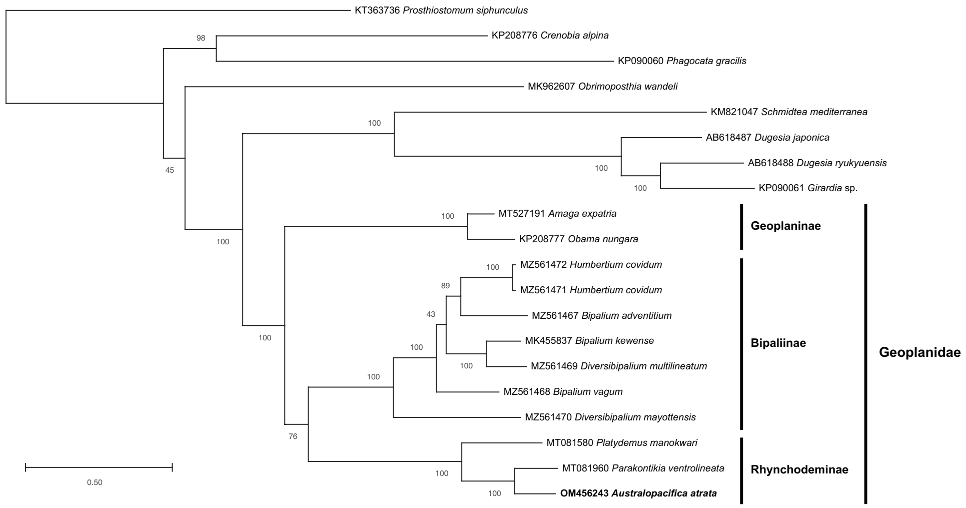

Figure 4. Maximum Likelihood phylogenetic tree obtained from concatenated amino-acid sequences of the mitochondrial proteins of *Australopacifica atrata* (Steel, 1897) and other flatworms. The tree with the best likelihood is shown (-75607.404300). Subfamilies of Geoplanidae are indicated on the right.

Several studies failed to find a start codon for various mitochondrial genes of terrestrial flatworms such as *O. nungara*, *A. expatria*, *Diversibipalium multilineatum* Makino & Shirasawa, 1983 and *B. vagum* (Solà et al. 2015; Justine et al. 2020a, 2022). This also seems to be the case for other members of the Continenticola, including Planariidae and Dugesiidae such as *Crenobia alpina* Dana (KP208776) (Solà et al. 2015), *Schmidtea mediterranea* Benazzi, Baguñà, Ballester, Puccinelli & Del Papa (JX398125) (Ross et al. 2016), *Dugesia ryukyuensis* Kawakatsu (AB618488), and *Dugesia japonica* Ichikawa & Kawakatsu (AB618487) (Sakai and Sakaizumi 2012).

For all three Rhynchodeminae, the *ND5* gene has an early termination because of the presence of a *tRNA-Ser* immediately following the last T residue of the gene, for which we suspect that the functional TAA stop codon is obtained by being completed by the addition of 3' A residues to the mRNA, while a canonical stop codon was found for all other species. Also, it is interesting to note that there is an overlap between the *ND4L* and the *ND4* genes, and that the size of this overlap is always 32 bp. This character, which is common to *Pl. manokwari* and *Pa. ventrolineata*, has been also reported in *O. nungara* and *C. alpina*. A rapid investigation of the mitogenomes of all available Bipaliinae shows that this overlap is totally lacking. Trying to simulate this overlap by extending the open reading frame at its N terminal ending leads to a fictious polypeptide with no initial methionine but that may comprise several stop codons in its early part.

As was observed in both the other Rhynchodeminae, the *cox2* gene has an important extra length. This extra length does not result from a missing stop codon, as it is located in the middle of the gene, and not on the 3' extremity. The size of the *cox2* putative protein of *A. atrata* is nearly identical to those of *Pa. ventrolineata* (434 and 433 amino acids respectively) (Table 1). The size is comparable to *Pl. manokwari* (452 amino acids), and far bigger than those observed among Geoplaninae and Bipaliinae, where this size ranges from 225 to 260 amino acids. It is noteworthy that *Girardia* spp. also display substantially larger *cox2* genes (KP090061, MW972220). However, an alignment performed with all the amino-acid sequences showed that this extra length is not located in the middle of the ORF, as for Rhynchodeminae, but at the C terminal ending. As verified on CDD/SPARCLE (Marchler-Bauer et al. 2017; accessed on 08/24/2021), the last conserved domain of the putative protein of *Girardia* spp. seems to be approximately at the amino-acid 227, for a total length of the predicted protein of 389 amino acids. CDD/SPARCLE positions this same conserved domain at the amino-acid 348 in *A. atrata*, for a total length of the predicted protein of 434 amino acids.

Following this work, we see three major paths of investigation. The first one will continue to focus on Rhynchodeminae. It will be interesting to compare all the characters considered here (colinearity, composition in tRNA, type of termination for the *ND5* gene, overlap between *ND4L* and *ND4* and of course the extra length of the *cox2* gene) with more species. Of the five tribes of Rhynchodeminae, we have now sequenced representatives of two: the Rhynchodemini (i.e. *Pl. manokwari*) and the Caenoplanini (*Pa. ventrolineata* and *A. atrata*). However, living examples of many of the other species assigned to the remaining tribes will be difficult to find considering their origin and repartition, but at least one of them, Anzoplanini *Marionfyfea adventor* Jones & Sluys, is present in Europe (Jones and Sluys 2016). Also, *Anzoplana trilineata* Winsor, can be found in south-eastern Australia (Winsor, 2006). Thus, we hope to obtain specimens of both species in the near future. The second would be to extend the search of shared mitogenomic characters among the subfamilies which have not been investigated until now, such as Timyminae and Microplaninae. The third and last path deals with the relationships between the genera *Parakontikia* and *Australopacifica*. Our results support the earlier provisional classification of *A. atrata* within the genus *Parakontikia* (Winsor 1991). We provide here the first sequence for a species presently assigned to the genus *Australopacifica*, which contributes towards the resolution of systematic relationships of species currently included in this heterogeneous collective genus.

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More discussion of minimalist species descriptions and clarifying some misconceptions contained in Meier et al. 2021

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Abstract

This is a response to a preprint version of "A re-analysis of the data in Sharkey et al.'s (2021) minimalist revision reveals that BINs do not deserve names, but BOLD Systems needs a stronger commitment to open science", https://www.biorxiv.org/content/10.1101/2021.04.28.441626v2. Meier et al. strongly criticized Sharkey et al.'s publication in which 403 new species were deliberately minimally described, based primarily on COI barcode sequence data. Here we respond to these criticisms. The following points are made: 1) Sharkey et al. did not equate BINs with species, as demonstrated in several examples in which multiple species were found to be in single BINs. 2) We reiterate that BINs were used as a preliminary sorting tool, just as preliminary morphological identification commonly sorts specimens based on color and size into unit trays; despite BINs and species concepts matching well over 90% of species, this matching does not equate to equality. 3) Consensus barcodes were used only to provide a diagnosis to conform to the rules of the International Code of Zoological Nomenclature just as consensus morphological diagnoses are. The barcode of a holotype is definitive and simply part of its cellular morphology. 4) Minimalist revisions will facilitate and accelerate future taxonomic research, not hinder it. 5) We refute the claim that the BOLD sequences of *Plesiocoelus vanachterbergi* are pseudogenes and demonstrate that they simply represent a frameshift mutation. 6) We reassert our observation that morphological evidence alone is insufficient to recognize species within species-rich higher taxa and that its usefulness lies in character states that are congruent with molecular data. 7) We show that in the cases in which COI barcodes code for the same amino acids in different putative species, data from morphology, host specificity, and other ecological traits reaffirm their utility as indicators of genetically distinct lineages.

Keywords
Barcode Index Numbers (BINs), biodiversity, Braconidae, consensus barcodes, DNA barcodes, Ichneumonoidea, taxonomic impediment

Introduction

The world is in crisis. Not only are we facing massive global species extinctions, primarily of species yet to be discovered, but every year we lose more and more of the taxonomic expertise needed to describe and record these species before they are gone. While neither the ongoing extreme loss of biodiversity (Cardinale et al. 2012; IPBES 2019) nor the loss of taxonomic expertise is questionable (Wheeler 2014), a satisfactory and viable solution has yet to be found.

To help address the taxonomic impediment and to address some of the many issues taxonomists struggle with in their efforts to describe hyper-diverse fauna before species are lost, Meierotto et al. (2019) proposed a new method to describe species more quickly, to give them handles with which all of humanity can diagnose and treat them. This method primarily uses COI barcodes as indicators of species, largely leaving time-consuming, and at times more ambiguous, morphological description up to future work if desired or needed. This protocol was later named the "Minimalist Technique" by Sharkey et al. (2021a) who employed it to describe 403 new species of Costa Rican braconids. All studies using the Minimalist Technique thus far have focused on taxa within the hyper-diverse hymenopteran superfamily Ichneumonoidea, a taxon in which COI barcoding has already been established as useful and often essential in delimiting species boundaries. As one may imagine, this primarily molecular-based approach has inspired intense debate within the taxonomic community, a community that traditionally regards morphological descriptive work as essential for species description. Not only has the value of the Minimalist Technique been debated, but the appropriateness of its use has been argued, despite having originally been proposed to try to address the taxonomic and biodiversity crises.

Meierotto et al.'s (2019) paper was quickly followed by criticisms from Zamani et al. (2020). This was then followed by the Sharkey et al. (2021b) reply addressing and refuting the points brought up by Zamani et al. (2020), and then by Sharkey et al.'s (2021a) paper publishing 403 new species using the Minimalist Technique. Sharkey et al.'s (2021a) paper was followed by a detailed response from Meier et al. (2021) strongly criticizing not just the method used to evaluate the molecular data used in both Sharkey et al. (2021a) and Meierotto et al. (2019), but in using BINs (Barcode Index Numbers) and Barcode of Life Data System (BOLD) services in general. Subsequent discussion recently published by Zamani et al. (2022) offers support of Meier et al.'s criticisms, yet neither paper offers a realistic alternative solution to the problems faced by the taxonomic community and its relation to the greater global populations on which it depends.

Here, we attempt to constructively continue the ongoing discussion. Despite the harsh criticism and lack of alternative solutions, we appreciate the considerable amount of time Meier et al. (2021) put into their critique and will address some of the points they brought up. We respond here to a preprint version of Meier et al. (2021). There are several other preprint versions available, and some of these can be found at https://doi.org/10.1101/2021.04.28.441626. The article was recently published in Cladistics (Meier et al. 2022) but our manuscript was too far along to incorporate the edits contained there. We also reiterate the value the Minimalist Technique has for many taxonomists, particularly those working in Ichneumonoidea and other diverse taxa; of course, like any other tool, it may not work for every taxonomist in every situation.

Discussion

Revision methods

Towards the end of their paper, Meier et al. (2021) stated, "Overall, we must conclude that the methods used in the revision are too poorly described to fully understand how the authors gathered and treated evidence." Yet, in their own paper, Meier et al. stated that Sharkey et al. (2021a) equated species with BINs. As the methods described in Sharkey et al. (2021a) clearly explained, the use of BINs in the Minimalist Technique is the first step in grouping together what are likely to be conspecific specimens. This is analogous to when physical specimens are sorted to morphospecies into unit trays in museum drawers, based on color, morphology, and size, before further inspection, when they may or may not be found to be conspecifics. The BIN is effectively a DNA-based unit tray. Subsequent to BIN sorting, morphology, NJ tree topology, sequence length, and often host and other ecological data, were considered to produce each species boundary in Sharkey et al. (2021a).

Consensus barcodes

Meier et al. (2021) spent a considerable amount of effort criticizing the consensus barcode approach to diagnosing species. It should be noted that the consensus barcode was not mentioned in the description of the Minimalist Technique (Meierotto et al. 2019). It was included in later papers (Sharkey et al. 2021a, 2021c) only to satisfy the rules of the International Code of Zoological Nomenclature (ICZN), just as consensus morphological character states are used for the same purpose. Consensus barcodes are simply an average of all the barcodes that belong to a species, just as "foretibia 0.02–

0.04 mm" is part of a morphological diagnostic. It is exactly analogous to species-level descriptions/diagnoses of colors patterns, which are famous for slight, and difficult to decipher variation. For example, if a species is polymorphic at a particular site, an International Union of Pure and Applied Chemistry (IUPAC) ambiguity code is used to indicate the polymorphism. The program employed to generate consensus barcodes can be found at https://www.phorid.net/DNAbarcode, and information on how it functions to produce a consensus barcode can be found here https://www.phorid.net/DNAbarcode/about_conSeq.php. The Sharkey et al. (2021a) revision was meant to describe species that were reared, and/or Malaise-trapped, in Costa Rica as part of Dan Janzen and Winnie Hallwachs's massive caterpillar and parasitoid rearing program. The barcode clusters generated, closely matching BINs, often contained specimens already on BOLD that the authors did not examine. This is the same as in the cases of many morphologically described species encountered in this or that museum, that the authors of the species never see. As mentioned, the consensus barcode is there only to satisfy the code, but it does represent the species as much as any consensus morphological trait, and the barcode of the holotype certainly falls within the BIN that contributes the consensus barcode. Much more collecting, barcoding, and examining of specimens from Costa Rica and additional locations will achieve more robust concepts, but no more so than for any classical morphological approach.

Rather than using consensus barcodes as the tool to identify a species, Sharkey et al. (2021a) and Meierotto et al. (2019) presented an example of how species identifications could proceed using COI barcode data. One of the primary aims for introducing the Minimalist Technique was to create the ability for anyone to quickly identify a specimen on BOLD after acquiring a COI barcode from the specimen (if a similar sequence exists in BOLD already). Whenever a sequence is submitted to BOLD the user is given an identification for that sequence. The level of the identification can vary depending on the length of the sequence submitted, but at a minimum it usually allows the user some degree of confidence that they sequenced the correct specimen and did not amplify the barcode of a contaminant. When something as precise as a species-level identification is returned, best practice is, of course, for users to double-check the quality of the determination. Users can do this by going through many of the same steps that Sharkey et al. (2021a) and Meierotto et al. (2019) went through to name species, e.g., checking nearest neighbors, images, and locality data. Perhaps the most effective and efficient method is to generate a simple neighbor-joining (NJ) tree on BOLD that includes the submitted sequence; this is an option given on BOLD. The user can then see how distant the submitted sequence is from a reliably identified specimen, preferably the holotype if it has been sequenced. The distance from one specimen's sequence to another, with a check of any similar pre-existing specimen image, will determine the degree of confidence the user may have.

The BIN code, in and of itself, is only meant as a communication and organization handle. This is not only because BINs do not equal species, but also because in rare cases the codes for BINs may change over time. One BIN could split into several BINs or be subsumed into another BIN and completely disappear. This happens, though rarely, when two narrowly separated BINs converge due to increased specimen sampling. One of the two BINs takes precedence, and the other is lost. This is especially true for under-sampled taxa, and that is the case for almost all species of hyper-diverse taxa such as most tropical Ichneumonoidea. Neither the BIN code nor the consensus sequence has any relationship with the new species described by Sharkey et al. (2021a) other than to give the specimens a preliminary sort and to satisfy the ICZN rules. While it would have been ideal to have better explained the use of BINs and consensus barcodes in the methods section of the original paper, the assumption during preparation was that it would be self-evident. As that was clearly a poor assumption herein we strive to correct our error.

Research depth

Meier et al. (2021) criticized the Minimalist Technique and Sharkey et al. (2021a) for leaving the heavy taxonomic work for future revisors and suggested that the authors should have done more in-depth research themselves. Unfortunately, Meier et al. (2021 took part of the paper out of context and thus misunderstood what was written. Meier et al. wrote, "The authors [referring to Sharkey et al. 2021a] stated: "… we view barcode-based descriptions as a first pass in an iterative approach to solve the taxonomic impediment of megadiverse and under-taxonomically resourced groups that standard technical and biopolitical approaches have not been able to tackle. This means that Sharkey et al. delegate the critical 'iterative' work to future generations of taxonomists. They will have to start revisions by first revisiting the species descriptions, types, and specimens of Sharkey et al.'s species to resolve species boundaries based on data that should have been collected and analyzed at the time of description."

To provide proper context, the following is the entirety of what was written in Sharkey et al. (2021a) that was unnecessarily criticized, "As we have made clear in the Meierotto et al. (2019) paper and here, we are not proposing that comprehensive revisions that include keys and morphological diagnoses be abandoned. Rather, we view barcode-based descriptions as a first pass in an iterative approach to solve the taxonomic impediment of megadiverse and under-taxonomically resourced groups that standard technical and biopolitical approaches have not been able to tackle. For example, if a taxonomist wishes to integrate these elements [morphological keys etc.] to *Zelomorpha* or any of the taxa that we review below they will have a great starting point (and resources). When a large number of specimens, from a wide geographic range, are barcoded, effective morphological keys may be written, and old museum specimens will regain their value to go along with their barcodes."

The Minimalist Technique first-pass approach does not preclude the addition of morphological data. If a future researcher wishes, for example, to write a key to the species of *Zelomorpha* of Costa Rica, they have a head start, with the species well-defined by sequences, images, and often host data. The entire reason this approach is termed "a first pass" is to suggest that it will not be perfect and that more collecting, and more barcodes will refine the species limits. Meier et al.'s suggestion that we should have collected and analyzed more data at the time of description seems naïve. The entire point of the approach used by Sharkey et al. (2021a) is to speed up the description process. If more data means more specimen collection, more sequence generation, and the addition of more morphological data, then the purpose is defeated, and in the case of hyper-diverse taxa there is no practical end point. It should be noted, however, for those unfamiliar with taxonomic processes, that anyone working on a deep revision of a taxonomic group, particularly those that include keys or new descriptions, should always revisit previous species descriptions, types, and other information previously documented. Therefore, taxonomists needing to work with species as described by Sharkey et al. (2021a) are starting at a huge advantage with the availability of sequences, images, often host information, and known type specimen locations.

Despite the clear explanation given in Sharkey et al. (2021a) on how beneficial the Minimalist Technique can be for producing more descriptive future works, we do not believe generating these products now to be a good use of resources. It is doubtful that writing keys and extensive morphological descriptions for many hyper-diverse groups is of value until there is a taxon-specific need. This is due to the fact that the keys to most species-rich taxa do not usually work, and likely never will. Likewise, the morphological descriptions are often ineffective in confirming species identity. They are rarely, if ever, used, despite the extensive amount of time required to produce them. The pointlessness of such keys is described in detail by Sharkey et al. (2021c). The high species-richness of these enormous genera make it unreasonable to imagine that at this point in our sampling all of the species and their potential discriminatory traits are adequately sampled and understood.

BIN stability

Meier et al. (2021) made the statement that BINs are unstable. This is not news to us and is why we do not simply name BINs. To demonstrate instability, Meier et al. compared the BINs supported by the BOLD algorithm with three other methods to group species. They then showed that as the size of the data sets increased, fewer and fewer BINs agreed with the other three methods of species delimitation. This is not at all surprising and is actually the expected result of the exercise. If more than three other methods were used to delimit species, the overlap would decrease even more. That is the nature of being different. We reiterate here that BINs were used as a preliminary sorting tool, just as a preliminary morphological approach might sort specimens by color and size. Interestingly, Meier and others investigated the species of Swedish Phoridae (Diptera) and concluded that the BIN algorithm was more efficient than the other three methods in delimiting species boundaries and that it matched 85% of their species concepts that were the result of morphological and COI-based data (Hartop et al. 2021). We also used morphology, but our concordance with the BIN sorts was well over 90%. This is not an unusually high number for the Braconidae. There are examples of conventional braconid revisions in which BIN concordance with species concepts is 100%, e.g., *Glyptapanteles* (Arias-Penna et al. 2019) with 136 species, and

Prasmodon (Fernandez-Triana et al. 2014) with 16 species. Both of these publications include morphological keys and descriptions. BINs vary in their effectiveness from taxon to taxon, and for a very few groups of organisms, BINs may not work at all. Author DHJ, who has spent thousands of hours reading multi-thousand-member NJ trees pre- and post-BIN application, has found that BINs are vastly more expeditious than any other kind of specimen sorting for later taxonomic study. For example, try to imagine the difficulty of adding 30 new species morphologically into an ichneumonid pool of 30,000 specimens representing at least 2,000 species, and their intraspecific variation, without using BINs.

In their figure 3 Meier et al. showed that 15 specimens that are currently in BOLD:ABY5286 (*Chelonus scottmilleri*) bounced around in six other BINs before settling in the current BIN in July 2013. Although it is appropriate to analyze BINs in the manner used by Meier et al., their utilization of BIN historical assignments from 2009 to 2013 is not. Assignment data is available in the BOLD audit trail from 2009, however it is only relevant beyond July 2013, when Plos One accepted the BIN publication (Ratnasingham and Hebert 2013). Before this publication, the system was in a state of flux. For example, assignments prior to 2011 were based on Single Linkage Clustering alone, and the RESL algorithm was deployed in 2012. Parameter and algorithmic optimization took place throughout this period. The BIN system was developed directly on the BOLD platform for only one reason: to gain feedback from users with taxonomic expertise. During this time, there was no expectation of BIN persistence, and the BIN database was reset multiple times. This methodological oversite in Meier et al.'s analysis results in the inflation of BIN splits, merges, mixtures, and deletions, all of which make little sense when reviewing actual sequence divergences.

As a final note on this topic, we point out that, like BINs, species names are also unstable, with concepts often changing substantially over time and names subsumed by synonymy and changed with homonymy. Meier (2016) himself demonstrated this using the example of *Drosophila melanogaster*.

BOLD Systems data quality

Meier et al. (2021 made several criticisms on the quality of the data on BOLD. The first of these concerns a set of barcodes in one of Sharkey et al.'s (2021a) species. Meier et al. stated, "BOLD:ABX6701 was described by Sharkey et al. (2021a) as *Plesiocoelus vanachterbergi*. The consensus barcode includes two single indels and was presumably obtained from the seven "barcode-compliant" sequences in BOLD Systems (as of April 18, 2021). Only one of the seven barcodes is translatable to amino acids with the remaining six having deletions. Sequences showing these attributes are often derived from pseudogenes, and it is conceivable that *P. vanachterbergi* was described based on paralogs. Note that this is likely due to a lapse in quality control because BOLD is supposed to check for shifts in reading frames (Ratnasingham and Hebert 2013)."

Contrary to the claim that the BOLD sequences of *P. vanachterbergi* are pseudogenes, they simply represent a frameshift mutation. Frameshift mutations in mitochondrial DNA have been uncovered in multiple taxa since the late 1990s (e.g., the ostrich: Harlid et al. 1997; Mindell et al. 1998). Single base-pair deletions are known characteristics for members of the braconid subfamily Agathidinae (e.g., Hrcek et al. 2011; Quicke et al. 2012; Hansson et al. 2015). While frameshifts (+1 or −1) are rare, they appear to be phylogenetically widespread as known examples include diatoms (Oudot-Le Secq and Green 2011), mollusks (Milbury and Gaffney 2005; Capt et al. 2020, ants (Beckenbach et al. 2005), turtles (Russell and Beckenbach 2008), birds (Harlid et al. 1997; Mindell et al. 1998), and parasitic wasps (as addressed in this paper). In the case of Agathidinae, we have consistently recovered the −1 frameshift in taxa from widely divergent localities, from species characterized by host-specific ecologies, and by using multiple primers in different combinations. For mitochondrial DNA sequences characterized by deletions, character removal from the frame can be a sign of pseudogene amplification; however, there are aspects of sequence composition that make this unlikely. The nucleotide composition in sequences with the 1 bp deletions remains highly AT biased, and those substitutions which do appear between species remain biased towards 3rd positions. In addition, the interspecific clusters formed by Agathidinae mtDNA align closely with their host-species biology and identity. Each of these three observations suggests that the sequences are functional rather than pseudogenes. If we were to conclude that the Agathidinae COI sequences on BOLD were pseudogenes, they would be both old (as they are widely geographically and taxonomically dispersed) and young (as they are host-specific), and odd in that they have retained the compositional bias (AT) patterns of substitution that characterize functional gene fragments. Thus, we have worked for over a decade under the more parsimonious hypothesis that the Agathidinae COI sequences are functional and characterized by a novel −1 frameshift mutation that may be cleaved in an unknown bit of RNA editing and that are a characteristic of this subfamily. It appears that Meier et al. (2021) downloaded and aligned COI sequences from BOLD for the BIN BOLD:ABX6701 without consideration for the frameshifts which characterize the subfamily.

Meier et al. (2021) did reveal a clear error “the consensus barcode for *Pseudorhysipolis mailyngonzalezae* contains 104 indels, which may be related to the fact that the BOLD fasta download for this BIN consists of data for multiple genes (COI, 28S, 16S, Ef1a). The correct consensus barcode for this species is TGTTTTGTATTTTATTTTTGGTATATGAGCTGGAATAGTTGGTTTATCTATAAGATTAATTATTCGATTAGAATTAGGGGTATCTGGAAGATTATTAGGGAATGATCAAATTTATAATACTATTGTTACATCTCATGCTTTTGTAATAATTTTTTTTATAGTTATACCTATTATATTAGGAGGATTTGGTAATTGATTAATTCCTTTAATATTAGGGGCTCCTGATATAGCATTTCCTCGAATAAATAATATAAGATTTTGATTATTGATTCCATCATTAATTTTATTATTTTTAAGTAGATCAATAAATTTAGGAGCTGGAACGGGGTGAACTATATATCCTCCTTTATCTTCAAGAATTGGTCATAGAGGAATATCTGTTGATTTAACAATTTTTTTCTTTACATTTAGCTGGTTGTTCTTCTATTATAGGATCAATTAATTTTATTTGTACAATTTTTAATATAAAAATTAATTTTTTAAAAATAGAACAATTAAGTTTATTTGTTTGGTCAGTTTTAATTACAACAATTTTAT-

TATTATTATCTTTACCAGTTTTAGCTGGTGCTATTACTATATTATTAACAGATCGTAATTTAAATACATCTTTTTTTGATTTTTCAGGTGGTGGTGATCCAATTTTATTTCAACATTTATTT.

Though as discussed, its value is limited, and the hybrid consensus sequence is also diagnostic for the species.

Taxon identification

Meier et al. (2021) wrote, "Depending on the time of BIN description, two of Sharkey et al.'s (2021a) wasp taxa would have been described as a fly or stonefly species, respectively" The explanation for this criticism is obviously contamination (Pentinsaari et al. 2020). Author MJS has morphologically sorted through thousands of Hymenoptera records on BOLD to identify entries to the lowest possible level and has previously flagged contaminants. Many others, including staff at BOLD have done the same. Nonetheless, a few contaminants are always possible and, just as using GenBank or in any other taxonomic endeavor, the revisor must check data quality. We note that Meier et al. (2021) did not find any flies and stoneflies in Sharkey et al.'s (2021a) treatment.

Morphology

Meier et al. (2021) confused comments made concerning the use of morphology by Sharkey et al. (2021a). Meier et al. wrote, "On the one hand, Sharkey et al. (2021a) argue that morphological evidence is not suitable for braconid taxonomy. On the other hand, they point out that subtle morphological differences often agree with barcode clusters, which would imply that the morphological evidence was misinterpreted." Our point was that morphological evidence alone is not sufficient to resolve species limits in many taxa. This is clearly the case in the morphological treatments of species of *Alabagrus* by Leathers and Sharkey (2003), as illuminated by Sharkey et al. (2021c). The problem is not that morphological evidence is non-existent, but rather that it is cryptic. There are multitudes of morphological characters, each of which may indicate different groupings of specimens, with no consensus. When COI barcode data are included, there are often one or more morphological characters that are congruent with them. It is more parsimonious to conclude that the morphological characters and COI sequences are congruent due to shared ancestry rather than random chance.

BINs vs species

Meier et al. (2021) demonstrated that in 16 publications employing barcodes as part of their evidence for determining species limits the species concepts and BINs did not always match, and wrote "We find that, for example, the barcodes for the 10 species in *Astraptes* belong to 5 BINs (Hebert et al. 2004)". This mismatch is simply because, as in the Sharkey et al. (2021a) publication, authors of these papers did not rely solely on BINs but rather they used larval morphology, microgeographic ecology, and food plants by authors DHJ and WH to determine species based on barcode clusters years before BINs were even a concept; in retrospect they were found to be perfect containers (6 species in one BIN, BOLD:ACE8393 with six obvious shallow-separated clusters on the NJ tree). That BINs do not equal species has been evident to everyone using COI barcodes and was even presented as a characteristic of the BIN system at its inception (Ratnasingham and Hebert 2013). MJS, DHJ and WH routinely sort by BINs, then inspect NJ trees with1–2 thousand specimens, watching for small clusters within BINs that match with other ecological traits. In this fashion they encounter many cryptic species within "well-known" species names (e.g., Hebert et al. 2004).

Amino acids

Meier et al. (2021) went to great lengths to show that there are 11 cases in which different species named by Sharkey et al. (2021a) had COI sequences that coded for the same amino acids. Meier et al. stated, "Given that most biologists associate speciation with the origin of biologically meaningful differences, describing such BINs as species rests on the hope that the correlation between time of divergence and the origin of new species is strong enough that biologically meaningful differences will later be found." It is true that Sharkey et al. (2021a) did not check if COI sequences coded for identical amino acids across species, but they did check morphology and host data, and an inspection of these 11 cases shows that the species in each group are distinct morphologically and in all but one case they have non-overlapping host ranges. Contrary to Meier et al., "biologically meaningful differences" are included in the Sharkey et al. (2021a) paper in the form of host data, and images that clearly show morphological differences between species with identical amino acids. One such example are the two species, *Triraphis camilocamargoi* (BIN BOLD:AAJ3968) and *T. martindohrni* (BIN BOLD:ABZ7672) (Fig. 1). The barcodes of these two species code for the same amino acids, yet, as the images in figure 1 demonstrate they are radically different morphologically, and they have quite different barcodes, which are the code combinations of relevance for all of this discussion, rather than amino acids. What is "biologically meaningful" is a quite different question from taxonomy. An enormous number of traits used in morphological-based taxonomy are of unknown "biological meaningfulness" and barcodes were deliberately chosen for their seeming freedom from natural selection for "use". Authors DHJ and WH became adherents of the use of barcodes and then BINs, as taxonomic tools in 2004 when it became obvious that for their inventory of very large numbers of species of undescribed tropical Lepidoptera, Hymenoptera, and Diptera, they were a major solution to the taxonomic impediment simply because they matched long established tropical named species. Barcodes and BINs also opened their eyes to other species hiding among the morphologically described species (e.g., Smith et al. 2006, 2007, 2008; Burns et al. 2008; Janzen et al. 2009, 2017; Janzen and Hallwachs 2016). This has continued until today, and the barcode libraries themselves will be major tools for everyone, in any society, rather than just where they currently reside.

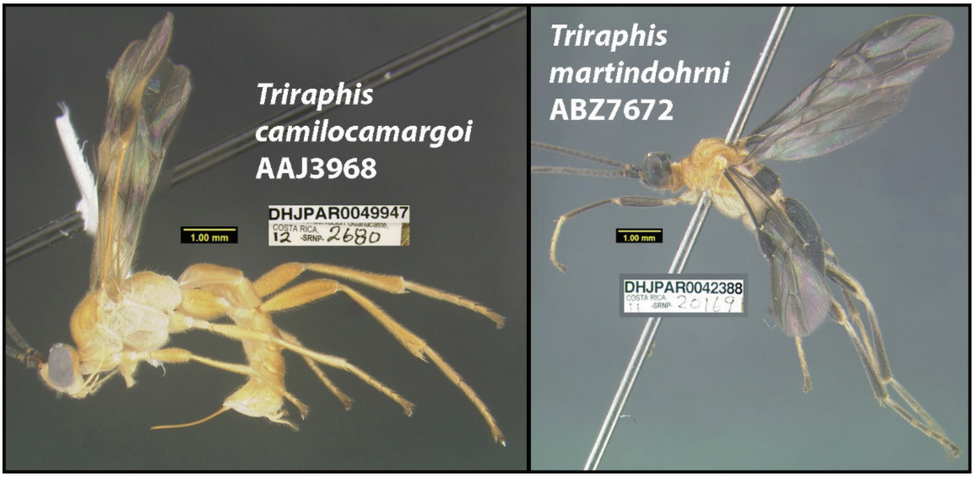

Figure 1. Two species with COI barcodes coding for identical amino acids. Specimen on the left is *Triraphis camilocamargoi* (BIN BOLD:AAJ3968); specimen on the right is *T. martindohrni* (BIN BOLD:ABZ7672).

Conclusions

We hope to have demonstrated that Sharkey et al. (2021a) did not equate BINs with species. This is exemplified by several cases in which multiple species were uncovered in single BINs. We reiterate that BINs were used as a preliminary sorting tool, just as a morphological approach might preliminarily sort specimens by color, size, or other traits. The fact that the match between BINs and species concepts was well over 90% does not equate to equality. We explain that consensus barcodes were used only to provide a diagnosis that conformed to the rules of the International Code of Zoological Nomenclature and agree with Meier et al. (2021) that they do not provide a functional diagnosis other than what is similarly provided by consensus morphology. We emphasize that minimalist revisions will facilitate future taxonomic research, not hinder it. We refute the claim that the BOLD sequences of *P. vanachterbergi* are pseudogenes and demonstrate that they simply represent a frameshift mutation. We reassert our claim that morphological evidence alone is insufficient to delineate many species, but its usefulness lies in traits that are congruent with molecular data. Finally, we show that in the cases in which COI barcodes code for the same amino acids, morphological and host use data reaffirm their utility as do the barcodes themselves in their various combinations of nucleotides.

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RESEARCH ARTICLE

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Three new genera and one new species of leaf insect from *Melanesia* (Phasmatodea, Phylliidae)

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Abstract

With the first large-scale Phylliidae molecular phylogeny recently published adding a great deal of clarity to phylliid diversity, several of the rarer species which could not be included were methodically and morphologically reviewed. This review resulted in identification of numerous substantial morphological features that suggest there are Melanesian clades that create polyphyletic groups within the phylliids which should instead be taxonomically recognized as unique. These rarer *Melanesia* species have historically been considered to be southern representatives of the *Pulchriphyllium* Griffini, 1898 sensu lato. However, there are notable morphological differences between the *Pulchriphyllium* sensu stricto and the "*schultzei*" group. Therefore, two new genera are erected, *Vaabonbonphyllium* **gen. nov.** from the Solomon Islands and Papua New Guinea and *Rakaphyllium* **gen. nov.** from New Guinea and the Aru Islands. Erection of these two new genera warrants the following new combinations: *Rakaphyllium schultzei* (Giglio-Tos, 1912), **comb. nov.**, *Rakaphyllium exsectum* (Zompro, 2001b), **comb. nov.**, and *Vaabonbonphyllium groesseri* (Zompro, 1998), **comb. nov.** Additionally, while reviewing material an undescribed *Vaabonbonphyllium* gen. nov. specimen was located and is herein described as *Vaabonbonphyllium rafidahae* **gen. et sp. nov.** from Mt. Hagen, Papua New Guinea. Additionally, a morphologically unique clade of several species recovered as sister to the *Nanophyllium* sensu stricto was recognized and their numerous unique morphological features and monophyly leads the authors to erect the new genus *Acentetaphyllium* **gen. nov.** which warrants the following new combinations: *Acentetaphyllium brevipenne* (Größer, 1992), **comb. nov.**, *Acentetaphyllium larssoni* (Cumming, 2017), **comb. nov.**, *Acentetaphyllium miyashitai* (Cumming et al. 2020), **comb. nov.**, and *Acentetaphyllium stellae* (Cumming, 2016), **comb. nov.** With the addition of several new genera, a key to phylliid genera is included for adult males and females.

Keywords
Aru Islands, camouflage, Indonesia, mimicry, new combination, Papua New Guinea, Phasmida, walking leaf

Introduction

The leaf insects (aka walking leaves) are masters of leaf masquerade, as both sexes have abdomen that are broad and thin with variable coloration to match their arboreal habitats (Fig. 1). Leaf insects are strongly dimorphic with males that are smaller and can fly (Fig. 1A) and females that are largely stationary, spending their lives in the canopy (Fig. 1B; Wedmann et al. 2007; Boisseau et al. 2021). This strong sexual dimorphism, coupled with their impressive camouflage means that leaf insects are unfortunately rather rare in collections despite having a significant range throughout most of Southeast Asia, and unless confirmed through molecular analyses or from captive rearing, pairing up the sexes is not always possible and can lead to significant identifications errors (Cumming et al. 2020a; Brock et al. 202).

Due to their notable rarity within collections, some clades are poorly known (composed only of singular holotype specimens or few representatives within a couple museums around the world) and have therefore been somewhat neglected/lumped in with other, better-known clades based upon limited morphological features. Recently the first phylliid-wide molecular based phylogeny was completed which added a great deal of clarity to the generic relationships by digging beyond limited, few feature morphological evidence (Bank et al. 2021). Interestingly, a relationship which was uncovered within Bank et al. (2021) was that when observing a singular morphological feature between genera, certain relationships could be recovered that immediately became unsupported when a different singular morphological feature was reviewed. Additionally, in many cases these single morphological feature-based relationships did not agree with well-supported molecular based phylogenies (for example, in females the ventral color of the coxae can either be the same color as the surrounding tissue or a vibrant different variety of colors (like is seen in *Chitoniscus* Stål, 1875 and *Phyllium* Illiger 1798); however, these clades have not been recovered as sister to each other (Bank et al. 2021)). Instead, it has become apparent that phylliid relationships must be examined from multiple lines of evidence whenever possible (such as the erection of the *Trolicaphyllium* Cumming et al. 2021a from the *Chitoniscus* Stål, 1875 which was supported by numerous molecular based studies and 23 morphological characters; see Cumming et al. 2021a and citations therein).

Unfortunately, certain phylliid clades are so rare that they have yet to be sequenced to be included within molecular phylogenies leaving only morphology to reveal higher level relationships at this time. One area which appears to be highly diverse and significant for phylliid evolution is *Melanesia* (an area roughly encompassing New Guinea, Solomon Islands, Fiji, New Caledonia, and other nearby islands) as several genera have been described from this area in recent years and *Melanesia* was recovered as the likely

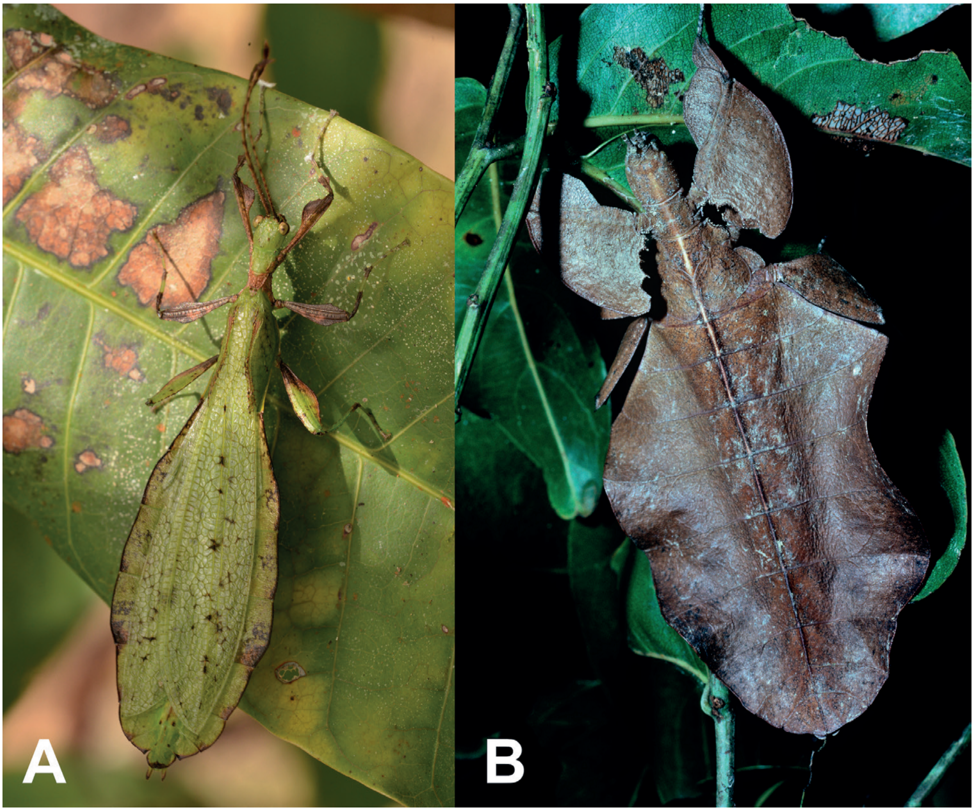


Figure 1. Live representatives from two of the three herein described genera **A** *Rakaphyllium schultzei* comb. nov. male photographed by Loïc Degen (Switzerland) on Wokam Island, Aru Islands, Indonesia **B** *Acentetaphyllium brevipenne* comb. nov. brown form female, photograph purchased from Alamy stock photo website, noted as simply from the "rainforest of New Guinea", no additional observational data could be traced.

ancestral range for all extant phylliids (Bank et al. 2021). With that in mind, several of the rarer and poorly known clades of *Melanesia* were systematically reviewed to correct their taxonomy to better reflect their recovered phylogenetic history.

All three of the herein described genera have previously been recognized (fully or partially) at one time or another as distinct to some degree. Within the substantial phylliid revision by Hennemann et al. (2009) their intrageneric systematizations recognized several Melanesian clades, namely the *schultzei* species group, *frondosum* species group, and the *brevipenne* species group (all of which at the time were nested within the *Pulchriphyllium* Griffini, 1898 sensu lato which was thought to be a subgenus of *Phyllium* Illiger, 1798). Most of the *frondosum* species group has since been found to be the previously unrecognized females to the *Nanophyllium* (suspected by Brock and Hasenpusch (2003); confirmed by Cumming et al. (2020a)) but the other clades have not yet been extensively reviewed and are therefore the focus of this work.

One group which is rare in collections is the *schultzei* group of *Pulchriphyllium* sensu lato, which were extensively reviewed because of their disjunct geographic distribution from the known *Pulchriphyllium* sensu lato range and the unique morphology of having a two lobed exterior protibia. Unfortunately, this clade has not been sequenced for molecular analyses yet, so only morphological review was possible. Interestingly numerous morphological features of this clade differentiate the *schultzei* group species from the *Pulchriphyllium* sensu stricto (such as the genitalia in females and alae wing venation in males). Additionally, within the species of the *schultzei* group, it was found that these same features which are disjunct from the *Pulchriphyllium* sensu stricto also separate the *schultzei* group into two morphologically distinct clades.

An additional species which has been included within molecular analyses was *Nanophyllium brevipenne* (Größer, 1992), which was recovered as sister to all other sampled *Nanophyllium* Redtenbacher, 1906 species. Upon further review numerous morphological features were found that allow simple morphological distinction from the *Nanophyllium* and agree with the recovery of *brevipenne* and several related species within their own clade, sister to the *Nanophyllium*. This led the authors to review the genus concept with the phylliids to determine (based upon how other genera are classified within the phylliids) just how unique must a clade be to differentiate it taxonomically from another.

Materials and methods

The following collection acronyms are used:

BPBM	Bishop Museum, Honolulu, Hawaii, USA;
Coll DG	Private collection of Detlef Größer, Berlin, Germany;
Coll RC	Private collection of Royce T. Cumming, California, USA;
Coll SLT	Private collection of Stéphane Le Tirant, Québec, Canada;
Coll TM	Private collection of Tetsuo Miyashita, Japan;
ANIC (CSIRO)	Australian National Insect Collection, Canberra, Australia;
IMQC	Insectarium de Montréal, Montréal, Québec, Canada;
MAMU	Macleay Collections, Sydney University Museums, Sydney, Australia;
MNHN	Muséum National d'Histoire Naturelle, Paris, France;
MNHU	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany;
NHMUK	Natural History Museum United Kingdom, London, United Kingdom;
RBINS	Royal Belgian Institute of Natural Sciences, Brussels, Belgium;
SDEI	Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany;
SDNHM	San Diego Natural History Museum, California, USA;
SMTD	Staatliches Museum für Tierkunde, Dresden, Germany;
ZFMK	Zoological Research Museum Alexander Koenig, Bonn, Germany.

Specimens

Specimens reviewed within this study are from various institutional and private collections and when specimens could not be reviewed in person, high-resolution images from The World of Stick Insects website (Phasmatodea.com) and the Phasmida Species Files Online (Brock et al. 2021) were reviewed. Specimen data and depositions are presented in Suppl. material 1 which was utilized to generate the distribution map (Fig. 5) and the discussion sections of each species herein reviewed.

Phylogenetic analyses

As several important taxa that are the focus of this work are poorly known and presently lack molecular data for phylogenetic analyses, combined molecular and morphological matrices were generated and analyzed to provide as much support as possible but also include these rare taxa. Additionally, because several of the species of significance for this work are poorly known, the opposite sex associations are only based upon morphological similarities (but not yet confirmed through rearing (as was done in Cumming et al. (2020a) for *Nanophyllium asekiense* (Größer, 2002) or through molecular analyses like in Bank et al. (2021) for *Pseudomicrophyllium pusillulum* (Rehn and Rehn, 1934)). Therefore, phylogenetic analyses were carried out on separate male and female morphological matrices to take into consideration the possibility of erroneously associating the opposite sexes of different species which in these strongly sexually dimorphic insects is a possibility.

Where positively associated opposite sexes have been confirmed from past studies, molecular data from a single specimen (and therefore a single sex) was used for both male and female molecular datasets. For example, as *Pulchriphyllium pulchrifolium* (Audinet-Serville, 1838) is well-known and the opposite sex has been confirmed through captive rearing, the molecular data from only one specimen (in the case of this study, female specimen ID Coll RC 16-030) was utilized for both the male and female datasets molecular portion. All molecular data utilized for this study is that which was utilized within Bank et al. (2021) with GenBank accession numbers and specimen data outlined in Suppl. material 2. The molecular data was organized into 12 subsets based on three ribosomal genes (16 S, 18 S, 28 S) and three protein-coding genes (COI, COII, H3) resulting in a supermatrix of 4694 bp (see Suppl. material 3).

Morphological features with their codable states and the resulting morphological matrices are separated by sex and are outlined in Suppl. materials 4 and 5, respectively. Wherever possible the specimen which was originally sequenced within Bank et al. (2021) was reviewed for the morphological dataset, but when not possible/applicable specimens from the authors collections, museums, and online images were utilized.

As Phylliidae has repeatedly been recovered as monophyletic (Buckley et al. 2009; Robertson et al. 2018; Bank et al. 2021; Bank and Bradler 2022) and the focus of this work was on little known clades represented almost exclusively by morphological data, only one outgroup taxon was included to root our Phylliidae focused tree (see Suppl. material 2 for taxa details). As the focus of this work was on the arbitrary rank of genus, the focus of the morphological matrices was features which are commonly associated with generic differentiation and have been the focus of past revisionary works/keys to phylliid genera. The list of morphological features utilized is limited as the focus was not on the species level for morphological differentiation (which would require a great deal more fine detail characters) and only features considered significant for higher taxonomic level differentiation were included. In total, for both male and female analyses, there were 27 phylliid taxa for females and 28 males were included representing all currently known and herein described genera.

Phylogenetic trees (Fig. 2) were inferred via Bayesian inference (BI) performed using MrBayes v. 3.2.6 × 64 (Ronquist et al. 2012) implemented in the online CIPRES Science Gateway v. 3.3 (https://www.phylo.org, Miller et al. 2010). Two runs of four chains each were sampled for 30 million generations with samples taken every 5,000 generations with the first 25% of the samples discarded as burn-in. For additional parameters and summary statistics see Suppl. material 6. Resulting tree files were visualized and edited using Figtree v. 1.4.4 (https://github.com/rambaut/figtree/) and Adobe Illustrator v. 16.0.0 (Adobe Inc., San Jose, USA). Parsimony analyses were also conducted using TNT (Tree Analysis Using New Technology) v. 1.5 (Goloboff and Catalano 2016) made available by the Willi Hennig Society (Goloboff et al. 2008) downloaded for use on Windows. The search strategy to find the shortest trees used a combination of sectorial searches, ratchet (with the perturbation phase set to eight up-weighting probability and four down-weighting probability), drift, and tree fusing.

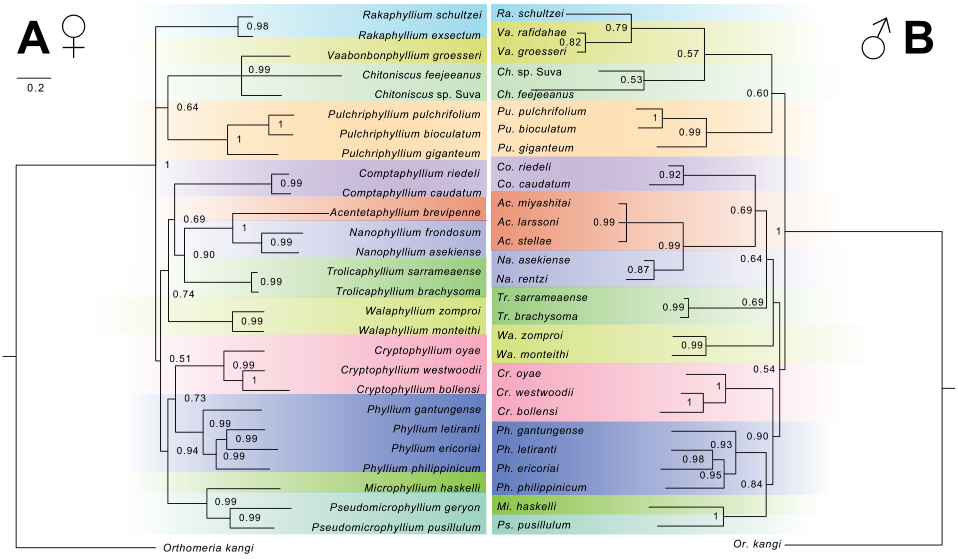


Figure 2. Phylogenetic reconstruction utilizing Bayesian Inference (BI) of representative taxa from all Phylliidae genera (morphology + DNA). Values presented are the posterior probability. Reconstruction based on a supermatrix of 4694 bp of molecular data and morphological characters (33 characters for females **A** and 25 characters for males **B** Branch length is proportional to relative divergence with the scale bar in the upper left indicating 0.2 units of divergence. Genera are color-coded to match between the female and male trees.

The minimum length was set to be found 15 times and Tree Bisection and Reconnection (TBR) method of branch swapping was enacted to search the available tree space. Support values were estimated using 1,000 replicates for both the standard bootstrap and jackknife and these parsimony results are available in Suppl. material 7.

Photography

Photographs of specimens deposited within the IMQC, Coll SLT, and Coll TM were taken by René Limoges (IMQC) using a Nikon D850 DSLR camera (Nikon Corporation, Tokyo, Japan) with Nikon Micro-Nikkor 200 mm f/4 lens on Manfrotto 454 micrometric positioning sliding plate (Manfrotto, Casolla, Italy). Lighting was provided by two Nikon SB-25 flash units with a Cameron Digital diffusion photo box (Henry's, Vancouver, Canada). Photographs of specimens within the first authors collection (Coll RC) were taken by the first author using a Canon 5D Mark II and a MP-E 65 mm macro lens and stacked using Zerene Stacker (Zerene Systems LLC, Richland, USA). Photographs not taken by René Limoges (IMQC) are accompanied by citation to their photographers and were taken using a variety of equipment. All images were edited using Adobe Photoshop Elements 13 (Adobe Inc., San Jose, USA) to remove their backgrounds and correct brightness/contrast. For wing illustrations (Figs 3, 4) specimens were chosen with the tegmina and alae already pinned flat, and the wings were photographed and simply cropped in the photos from the bodies of the specimens, not dissected from the specimens. Wing venation terminology follows Burt (1932) and Ragge (1955).

Morphological abbreviations (listed morphologically anterior to posterior)

C	costa
Sc	subcosta
R	radius
R1	radius 1
Rs	radial sector
R–M	radius to media crossvein
M	media
MA	media anterior
MP	media posterior
MA+MP	fused media anterior and media posterior
Rs+MA	fused radial sector and media anterior
Cu	cubitus
CuA	cubitus anterior
CuP	cubitus posterior
Cu+1AA	cubitus and first anterior anal
1A	first anal
1AA–7AA	first–seventh anterior anal
1PA–5PA	first–fifth posterior anal

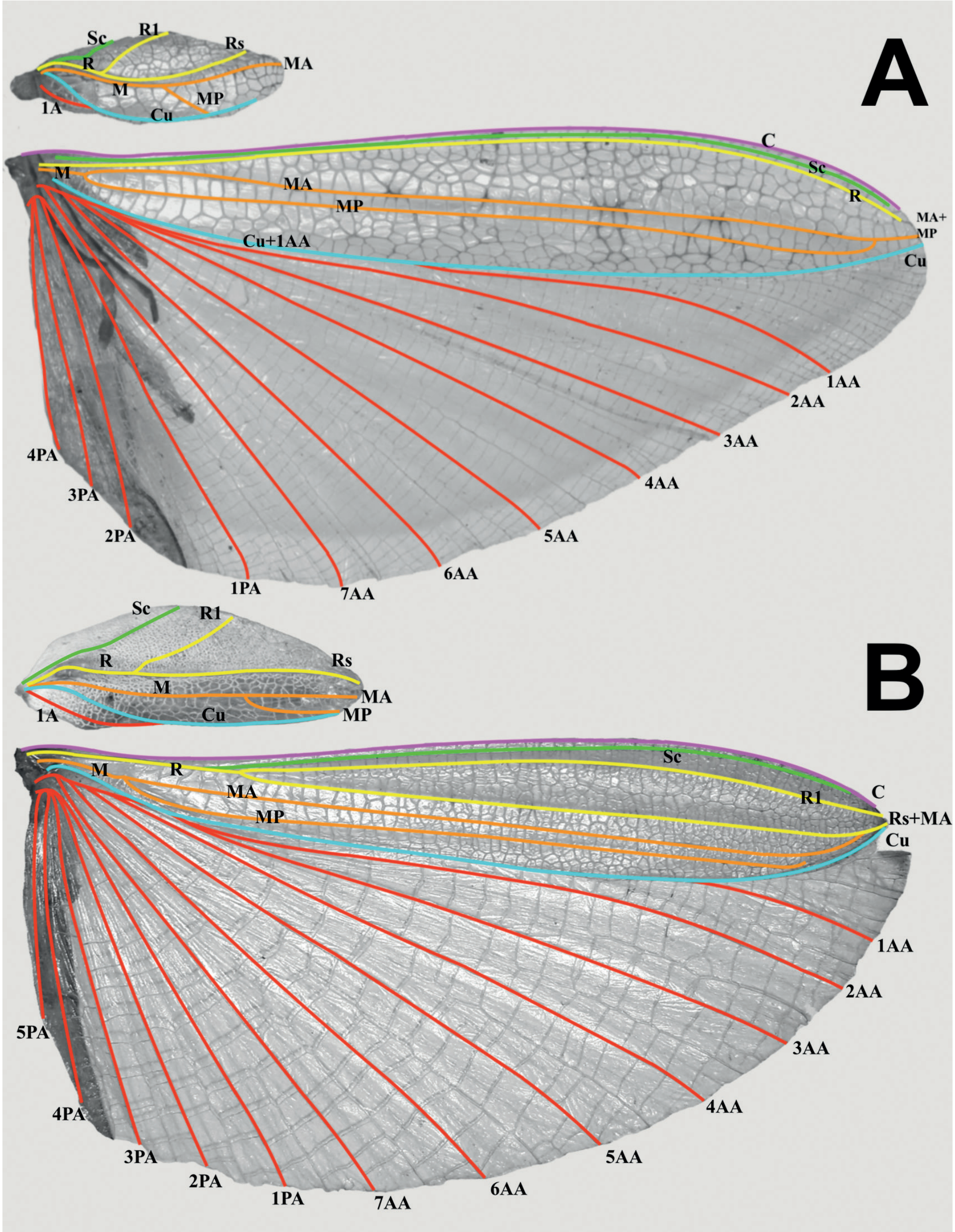


Figure 3. Male tegmina venation for two of the herein described genera **A** *Rakaphyllium schultzei* comb. nov. (MNHU) **B** *Vaabonbonphyllium rafidahae* (IMQC) (Coll RC 19-106). Abbreviations: Sc (subcosta); R (radius); R1 (radius 1); Rs (radial sector); Rs+MA (fused radial sector and media anterior); M (media); MA (media anterior); MP (media posterior); Cu+1AA (fused cubitus and first anterior anal); 1AA–7AA (first through seventh anterior anal); 1PA–5PA (first through fifth posterior anal); 1A (first anal).

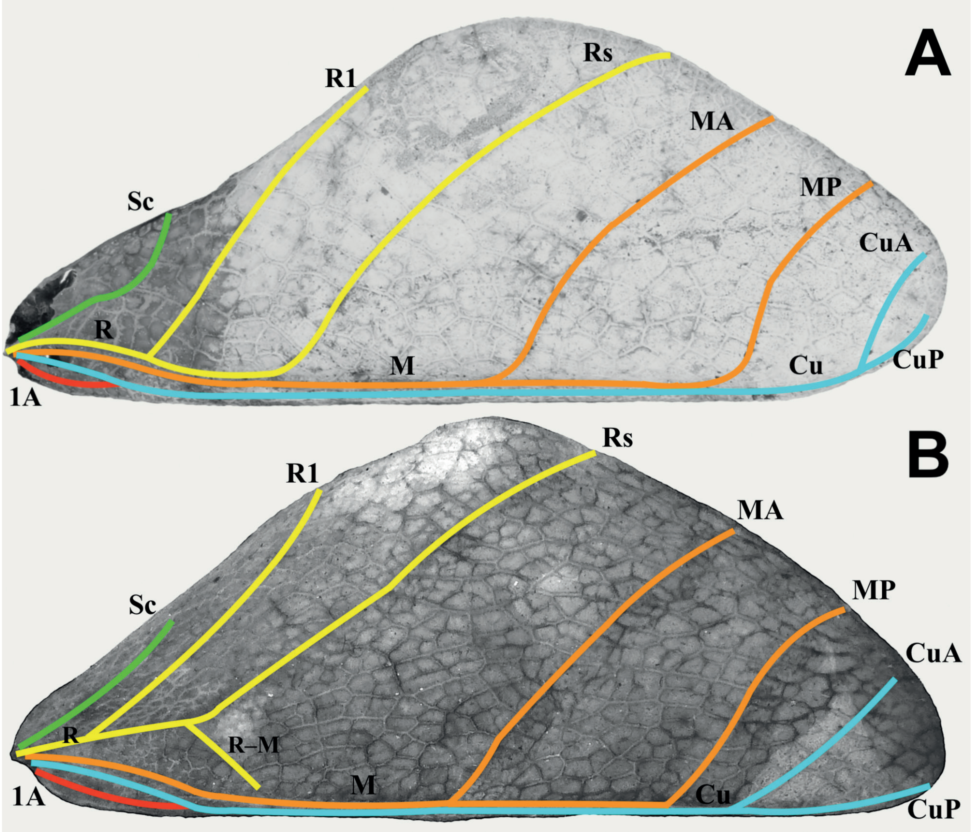


Figure 4. Female tegmina venation for two of the herein described genera **A** *Rakaphyllium schultzei* comb. nov. (SDEI) **B** *Vaabonbonphyllium groesseri* comb. nov. (SMTD). Abbreviations: Sc (subcosta); R (radius); R1 (radius 1); Rs (radial sector); R–M (radius to media crossvein); M (media); MA (media anterior); MP (media posterior); Cu (cubitus); CuA (cubitus anterior); CuP (cubitus posterior); 1A (first anal).

Results

Both the parsimony analyses based solely upon morphology data (Suppl. material 7) and the Bayesian analyses based upon the combined molecular and morphological datasets (Fig. 2) gave similar relationships regarding the taxa of focus. However, when reviewing these results, it should be remembered that two of the clades of focus are only represented by morphological data (therefore in the combined molecular and morphology datasets a great deal of information is lacking). Despite this, the within clade relationships were almost always strongly supported and only the deeper recovered relationships were less reliable (Fig. 2).

Most of the species of focus for this study were only represented by morphological data which presents a challenge. This is because most morphological features when viewed singularly appear to unite a subset of clades, but as soon as a different feature is reviewed a different subset of clades appear more closely linked (see the supplementary discussion in Bank et al. 2021 for further discussion on this point). This creates clades which, when lacking molecular support, have notably low support values. Interestingly though even within the analyses of Bank et al. (2021) which only included species which had molecular data, some clades still showed somewhat low support values.

Within the male-based analyses of this study, *Pulchriphyllium schultzei* Giglio-Tos, 1912 was recovered as sister to a clade formed by *Pulchriphyllium groesseri* (Zompro, 1998) and an undescribed species with moderately high support (Fig. 2). Interestingly, within the female-based analyses *Pulchriphyllium groesseri* was instead recovered as sister to the *Chitoniscus* Stål, 1875 while *Pulchriphyllium schultzei* and *Pulchriphyllium exsectum* (Zompro, 2001b) were recovered as external to this clade and instead were part of a polytomy deeper within the recovered phylogeny (Fig. 2). The important takeaway from these analyses is that historically the species *Pulchriphyllium groesseri*, *Pulchriphyllium schultzei*, and *Pulchriphyllium exsectum* are considered to belong within the *Pulchriphyllium* sensu lato simply due to the presence of exterior tibial lobes (regardless of the fact that *Pulchriphyllium* sensu stricto has a single large lobe and these other species have two smaller lobes; Fig. 7C). The results of these phylogenetic analyses instead place these “two-lobed” species as distinctly outside of the reviewed *Pulchriphyllium* sensu stricto species. Therefore, as the taxonomy currently stands, *Pulchriphyllium* sensu lato is polyphyletic when the morphologically unique species *Pulchriphyllium groesseri*, *Pulchriphyllium schultzei*, and *Pulchriphyllium exsectum* are included.

Additionally, in reviewing the *Nanophyllium* sensu lato, this distinct clade was recovered as bifurcate with significant divergence distance between two internal sister clades. These sister clades correspond to two morphologically distinct groups which have been recognized by past authors as significant (Hennemann et al. 2009; Cumming et al. 2020a). Essentially taxonomic ranks above the species level are arbitrarily assigned in order to facilitate communication about natural groups, therefore these two morphologically distinct sister clades based upon the current treatment of genera within the phylliids clearly align themselves with the genus concept well. A designation which can facilitate future species differentiation within either of these clades.

In summary, the phylogenetic analyses recovered two distinct clades which are currently taxonomically treated as *Pulchriphyllium* sensu lato members and one morphologically unique clade which is sister to the *Nanophyllium* sensu stricto. All three of these clades are morphologically unique to an extensive degree (with such significant differences that within the phylliids such levels of morphological uniqueness are treated by taxonomists as genera) and therefore in order to recognize these three clades with autapomorphic morphological features as unique and to correct the current polyphyly, the following three genera are erected below.

Taxonomy

Phylliinae Brunner von Wattenwyl, 1893
Phylliini Brunner von Wattenwyl, 1893

Rakaphyllium gen. nov.

https://zoobank.org/2C2C6B6B-83F4-4341-954C-351E92119BEA

Type species. *Pulchriphyllium schultzei* Giglio-Tos, 1912: 56, herein designated.

Taxonomic hierarchy. This genus has a combination of features which link it to several genera, thus making a higher-level taxonomic placement difficult and requiring molecular confirmation in the future. The thorax and tegmina venation in the females are reminiscent of some *Pulchriphyllium* sensu stricto species and the profemoral and protibial lobes are reminiscent of some *Phyllium* Illiger, 1798 and *Comptaphyllium* Cumming et al., 2019 species, whereas the male thorax is similar to *Trolicaphyllium* Cumming et al., 2021. At present within Phylliidae there are two recognized tribes, the Nanophylliini Zompro and Größer 2003 (which contains only the *Nanophyllium* Redtenbacher, 1906) and the Phylliini Brunner von Wattenwyl, 1893 (which contains all other genera). Therefore, at this time this genus is placed within the tribe Phylliini as notable features such as a two lobed posteromedial tubercle and a prescutum which is wider than long (features which help to define the *Nanophyllium*) are absent, suggesting a closer relationship to other genera instead.

Discussion. The selected type species for this new genus is *Pulchriphyllium schultzei* Giglio-Tos, 1912 (= *Rakaphyllium schultzei* (Giglio-Tos, 1912), comb. nov.) as this was the first species described within this new genus, the holotype is from an exact collection locality, and this species appears to be the more commonly encountered of the two species within this new genus.

This new genus has been recognized as unique in the past, as this clade was designated as the *schultzei* species group within Hennemann et al. (2009) based upon the shorter tegmina and the two lobes on the exterior protibiae. While these features are helpful to differentiate this genus from others, the below noted autapomorphic features allow differentiation from all phylliid genera.

Autapomorphic features. Within each sex there is an easily observed morphological feature which supports their monophyly and readily separates them from other phylliid genera. For females, the gonapophyses VIII are exceptionally long, with approximately half of their length projecting from under the terminal abdominal segment (Figs 7G, 9D), a feature not seen in any other phylliid as typically gonapophyses VIII only reach to the apex of the terminal abdominal segment or at most only exceed the tip slightly (Fig. 10E). For males the alae venation is unique as the radius vein is simple (Fig. 3A), not bifurcate as is seen in all other phylliid genera (Fig. 3B). These autapomorphic features help to define the new genus *Rakaphyllium* gen. nov. within the phylliids as well as differentiate them from the *Pulchriphyllium* sensu stricto.

Generic characteristics. The *Rakaphyllium* gen. nov. are average sized phylliids, with females ranging from ca. 80 mm to 90 mm long and males ca. 60 mm long. Typical coloration appears to be green, but with so few specimens known and color variation a common occurrence in phylliids it is possible that this genus may also exhibit color forms (such as possibly *Rakaphyllium exsectum* comb. nov. but this brown coloration may simply be due to the age and preservation technique (Fig. 9)).

Antennae. Females have antennae with nine segments with the terminal antennomere not notably long (only as long as the previous one to two segments combined) and segments IV through VIII all of a similar length (Figs 6D, 7F, 9E). Males have antennae which range from 20 to 23 segments with most segments covered densely in setae, and overall antennomere shape somewhat flattened.

Head capsule. Males have well-developed ocelli (Fig. 8B), females do not have ocelli (Figs 6D, 7H). Males have compound eyes which are strongly protruding and occupy ca. 2/5 of the head capsule lateral margins (Fig. 8B) versus females which have compound eyes which are notably smaller, only occupying less than 1/3 of the head capsule lateral margins and which do not strongly protrude from the capsule (Fig. 7H). Both sexes have head capsules which are marked by irregularly sized and space granulation.

Thorax. The thorax is similar in both sexes with mesopleurae that are narrowly diverging from the anterior to the posterior (evenly so in females, almost parallel in males for the anterior half and then more prominently on the posterior half) are marked on the anterior half with three to four small tubercles with granulation interspersed with the posterior half relatively smooth or with only minimal granulation (Figs 6D, 7H). In both sexes the prescutum is about the same length as the width of the anterior margin, with a posterior margin that is slightly narrower giving the prescutum a slight isosceles trapezoid appearance. The margins of the prescutum are marked with granulation and the prescutum surface is covered with granulation with those along the sagittal plane slightly larger and in males a weak sagittal crest is present (Figs 6D, 8B, 9C). When viewed laterally, both sexes have a weakly formed prescutum anterior rim with a granular surface (Figs 7B, 8B).

Legs. Both sexes have interior protibial lobes which do not span the full length of the shaft, instead they are only situated on the proximal half (Figs 6B, 7C) and exterior protibiae which are marked by two lobes, one on the proximal and one on the distal end (Figs 6B, 7C). The exterior meso- and metatibiae are notably reduced, but if lobes are present its just as small spurs (sometimes just a distal spur or sometimes one on each end of the shaft) never as prominent lobes. Profemoral exterior lobes are rather variable as within *Rakaphyllium schultzei* comb. nov. females they are simply arcing smoothly from end to end without a strong angle (Fig. 6B), but in *Rakaphyllium exsectum* comb. nov. females the exterior lobe is distinctly boxy with a right angle (Fig. 9B). Males are only known for *Rakaphyllium schultzei* comb. nov. in which the femoral morphology is similar to the female, simply arcing tightly along the profemoral shaft (Fig. 8B).

Wings. Female tegmina are average in length, only reaching onto abdominal segments V or VI and male tegmina are moderate in length, only reaching onto abdominal segment II or III. Females have rudimentary alae. Male alae are fully developed in an oval-fan configuration and reach onto abdominal segments VIII to X. Female tegmina have a subcoastal vein; radial vein which runs parallel with the media and splits into the first radial about halfway through its length and terminates in a radial sector which bends distinctly away from the media and arcs to the wing margin; a bifurcate medial vein; a bifurcate cubitus vein; and a first anal vein which fuses with the cubitus early on (Fig. 4A). Male tegmina (Fig. 3A) have a simple subcoastal vein; radial vein which runs parallel/subparallel with the media almost throughout the full length of the wing and branches into the first radial about one third of the way through the wing length and terminates as the radial sector; the media runs parallel/subparallel with the radius and has the media posterior split near the middle of the wing and terminates as the media anterior; the cubitus is unbranched; and there is a first anal which fuses with the cubitus early on. Male alae (Fig. 3A) have a costal vein running along the anterior margin; a subcostal vein which runs parallel with the costal vein for the full length; the radial vein is the most unique feature of the alae as it is simple, running parallel to the subcostal vein; the media splits early into the media anterior and posterior which run parallel until the media posterior fuses with the media anterior near the wing margin and they run fused to the apex of the wing; the cubitus is fused with the first anterior anal for ca. half of the length until the first anterior anal splits and runs to the wing margin; the anal veins are split into two groups, the anterior anals and the posterior anals (with seven anterior anals and four or five posterior anals).

Abdomen. Both sexes have variable abdominal shapes, but all forms are broad in the middle with the widest segments V or VI; in both sexes the anterior halves are uniformly broadening to the middle segments, and then the posterior half of the abdomen is variable either with smooth margins giving the abdomen an ovoid appearance, or with the posterior segments gently or strongly undulating giving the abdomen a lobed appearance. Female subgenital plate is short and relatively narrow with the apex slightly reaching onto the anterior margin of the terminal abdominal segment and ending in a fine point; gonapophyses VIII are exceptionally long (with ca. half of their length projecting out from underneath the abdomen) with a uniform width through most of their length; the cerci are relatively flat, marked sparsely with a granular surface, and end in blunt points (Figs 7G, 9D). Males have a long, relatively narrow, triangular vomer which is singularly pronged, hooking up into the paraproct (Fig. 8C).

Egg. Egg morphology is not yet known for this rare genus, but with such unusually long gonapophyses to hold the eggs before they are flung away, the eggs must have a unique shape to require such ungainly gonapophyses.

Etymology. *Rakaphyllium* meaning "walking leaf". This generic epithet is a compound of the Latinized name *Phyllium* the type genus for the family (from Greek φυλλον, -ου (*phyllon*, *-oy*) + -um; Poitout 2007), coupled with the prefix *raka* from the Hiri Motu language of New Guinea which means "to walk" (Chatterton 1975; Dutton and Voorhoeve 1975). We wish to honor the original inhabitants of this area by using the traditional language of Hiri Motu which is one of the official languages of Papua New Guinea and as Papua New Guinea pushed towards sovereignty in 1975, Hiri Motu was seen as a unifying force which was instrumental in the awakening of national pride (Dutton and Voorhoeve 1975). We chose this name because of the amazing camouflage these insects possess, allowing them to appear miraculously as a leaf that simply stands up and walks away when disturbed. This new genus is neuter in gender, following *Phyllium*.

Distribution. At present our knowledge of the *Rakaphyllium* gen. nov. is rather limited due to its rarity, however, interestingly despite it being rarely collected, this genus appears to be somewhat widespread, with records from throughout New Guinea, and even a record from the Aru Islands (Fig. 1A) off the western coast of Papua Province, Indonesia (Fig. 5).

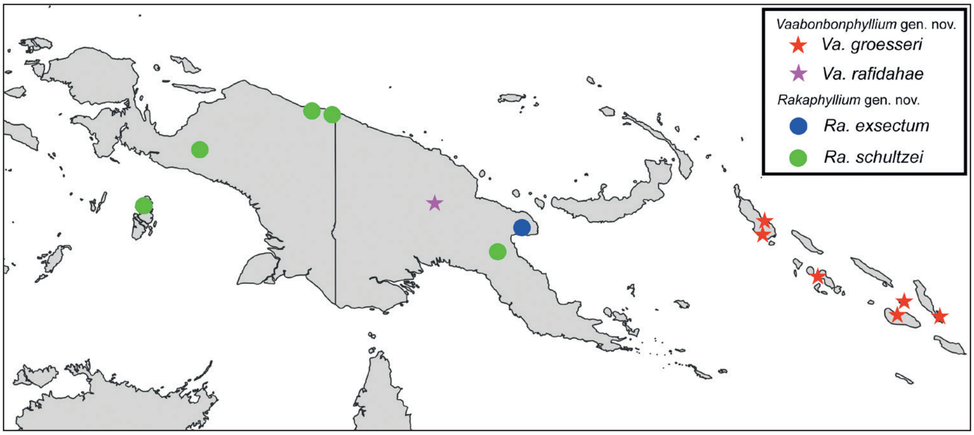


Figure 5. Distribution map noting all presently known records of *Vaabonbonphyllium* gen. nov. (denoted as star symbols) and *Rakaphyllium* gen. nov. (denoted as circle symbols) which were located and had data that could accurately be mapped. See Supplementary File 1 for full details for all records presented.

New combinations

Rakaphyllium schultzei **(Giglio-Tos, 1912), comb. nov.**
Rakaphyllium exsectum **(Zompro, 2001b), comb. nov.**

Rakaphyllium schultzei (Giglio-Tos, 1912), comb. nov.

Figs 1A, 3A, 4A, 6, 7, 8

Material examined. (3 ♀♀, 3 ♂♂, 3 ♂♂ nymphs, 2 ♀♀ nymphs): ***Holotype*** (♀): "New Guinea (No 131) VIII. 1910 L. Schultze L.J.; *Pulchriphyllium schultzei* Giglio-Tos, E. Giglio-Tos, det.; Mossu südl von Germainhuk; Holotypus; DEI Hemimetabola #100122" (SDEI; Fig. 6). See Suppl. material 1 for additional specimens reviewed, their collection data, and depositories.

Remarks. This rarely encountered species was described from a female from northern New Guinea (Fig. 6), and since its description over a century ago few additional specimens have been collected. At present due to the rarity of material and lack of fresh material for molecular comparison, the male specimens associated with

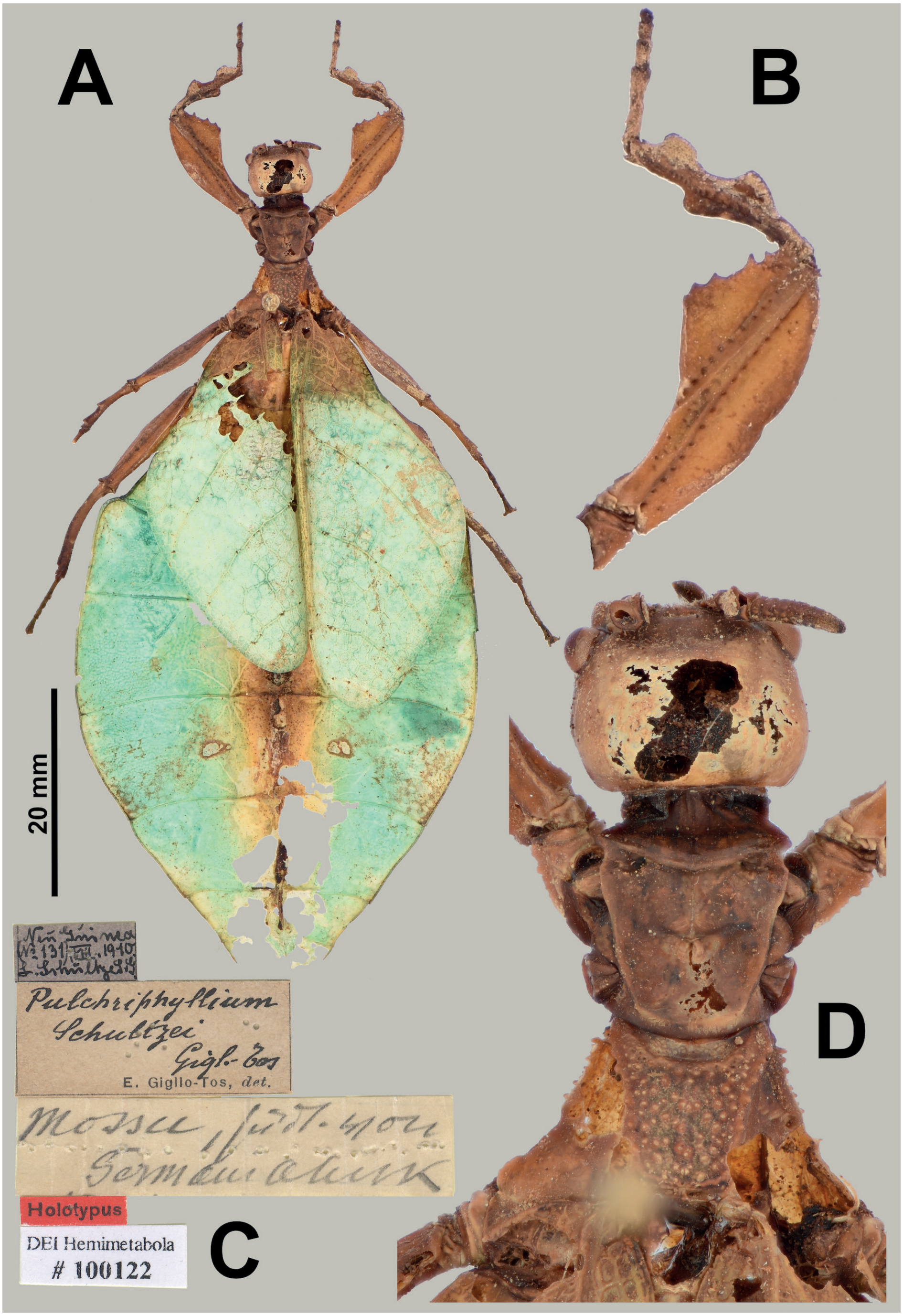


Figure 6. *Rakaphyllium schultzei* comb. nov. holotype female. Note that the holotype female is heavily damaged in several places, especially the abdomen which has numerous holes through which the background can be seen, this is not an artifact of image postprocessing **A** habitus, dorsal **B** right front leg, dorsal **C** associated data labels **D** details of head through thorax, dorsal. Scale bar only associated with **A**. Photographs by Arne Köhler (SDEI) and Mandy Schröter (SDEI).

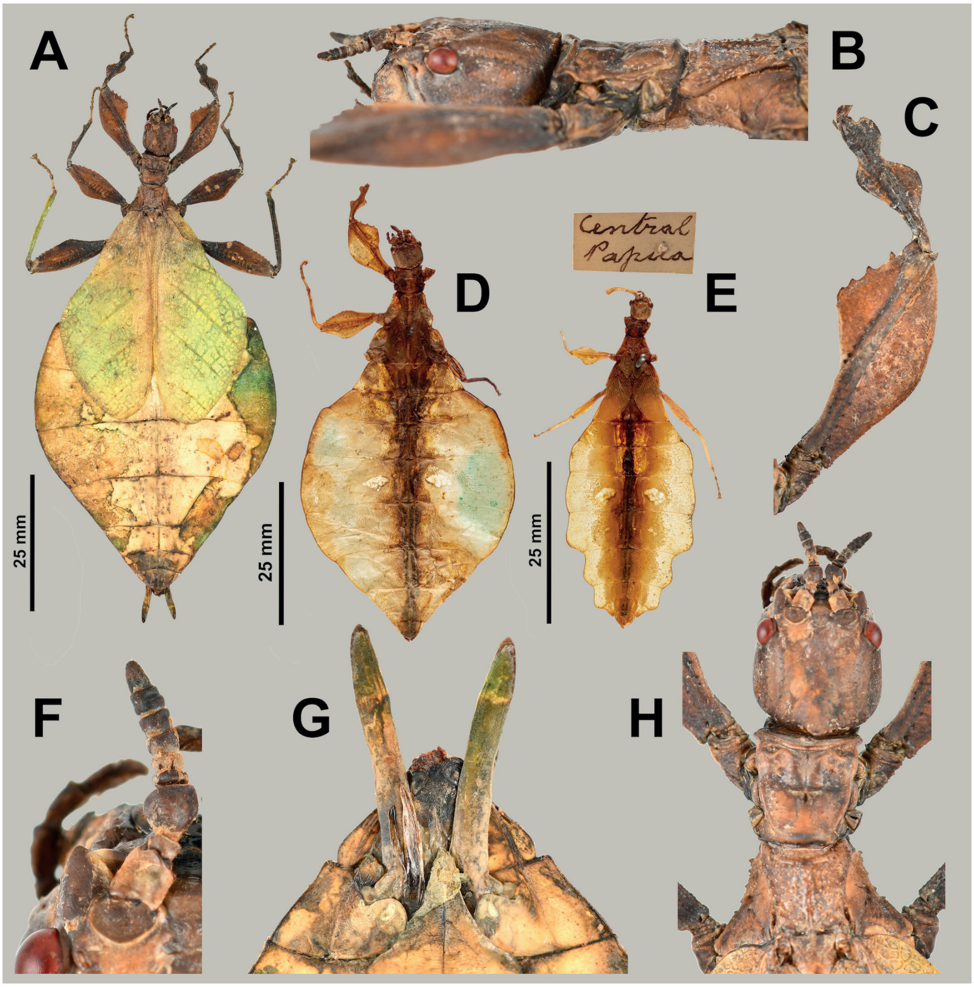


Figure 7. *Rakaphyllium schultzei* comb. nov. non-type material specimens **A–C, F–H** adult female from Coll SLT, Indonesia: Jayapura, Klaisu **D, E** nymphs from MAMU **A** habitus, dorsal **B** details of head through thorax, lateral **C** profemora, dorsal **D** female nymph without collection data (MAMU) **E** male nymph with slightly undulating abdomen with associated data label above (MAMU) **F** details of antenna, dorsal **G** genitalia, ventral **H** details of head through thorax, dorsal. Scale bars only associated with images **A, D, E**. Photographs **A–C, F–H** taken by Rene Limoges (IMQC) **D, E** courtesy of Chau Chak Wing Museum, the University of Sydney, and photographs taken by David James.

this species are only assumed based on shared morphology (Fig. 8) but have not been confirmed yet.

Differentiation. For female *Rakaphyllium schultzei* comb. nov., the abdominal shape and profemoral lobes easily differentiate it from *Rakaphyllium exsectum* comb. nov. Within *Rakaphyllium schultzei* comb. nov. the profemoral exterior lobe arcs smoothly from end to end without a strong angle (Fig. 6B), but in *Rakaphyllium exsectum* comb. nov. the exterior lobe is distinctly boxy with a right angle (Fig. 9B). Additionally,

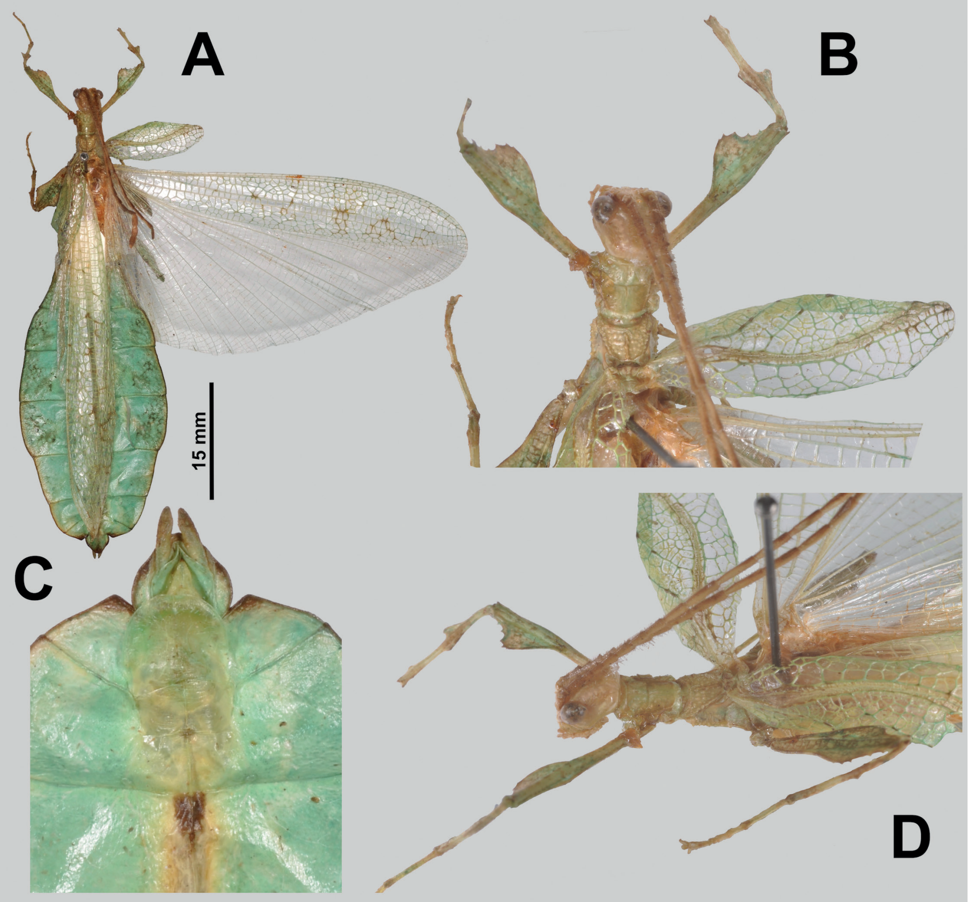


Figure 8. *Rakaphyllium schultzei* comb. nov. male from the MNHU collection **A** habitus, dorsal **B** details of front legs, base of antennae, head, thorax, and tegmina, dorsal **C** genitalia, ventral **D** details of front legs, base of antennae, head, thorax, and tegmina, dorsolateral. Scale bar only associated with **A**. Photographs by Frank Hennemann (Germany).

abdominal shape appears to be reliable for differentiation, but some abdominal variation has been observed in a few *Rakaphyllium schultzei* comb. nov. females to include perfectly smooth margins and some with slight undulations on the terminal abdominal segments (although none have been observed to be as extreme as in *Rakaphyllium exsectum* comb. nov.) therefore abdominal shape may also be useful for differentiation.

Distribution. At present we are aware of records from several locations throughout New Guinea (both on the Indonesian and Papuan sides) and records from the Aru Islands which visually appear to be this species despite the geographic disconnect from mainland New Guinea (see Fig. 5 and Suppl. material 1 for details of these records). The distribution of this species is vague as a thorough knowledge of morphological variation (due to limited material) and molecular analyses are lacking at present, and therefore our identification assumptions are only based upon general morphology. Unfortunately, without positive confirmation of the males we assume are this species, this distribution may be over expansive (possibly representing several species instead of one), and especially for the observational record from the Aru Islands (Fig. 1A), the specimen could not be examined and although the habitus appears to match the morphology of the mainland New Guinea male the fine details may suggest otherwise if a specimen could be examined.

Rakaphyllium exsectum (Zompro, 2001b), comb. nov.

Fig. 9

Material examined. ***Holotype*** (♀): "HOLO-TYPE; Rawlinson Mts. inland Huon Gulf. (Keysser).; Rothschild Bequest B. M. 1939-I.; Rawlinson Mt; *Phyllium exsectum* ♀ Zompro n. sp. HOLOTYPUS det. O. Zompro X.2000; BMNH(E) #845235" (NHMUK; Fig. 9).

Remarks. This remarkable looking species is unfortunately only known from the singular holotype female from the little accessed Rawlinson Mountains of the Huon Peninsula (Fig. 5). Even in other phylliid species with prominent lobes on the abdomen, they pale in comparison to the unique habitus of this species, which likely evolved to mimic some strongly lobed host plant. Unfortunately, as this is the only specimen known and it lacks ecological data, nothing is known about a possible plant that the unique shape of this species may have evolved to resemble through advergent evolution.

Differentiation. At present only the female is known for this species, no tentative males have been found which could represent the opposite sex. This species can be differentiated from its single other congenic by the elaborate lobed abdomen (although some *schultzei*-like females have been observed with slight lobes suggesting morphological plasticity within that species) or more reliably by the profemoral exterior lobe, which in *Rakaphyllium exsectum* comb. nov. is right angled (Fig. 9B), versus *Rakaphyllium schultzei* which always has the profemoral exterior lobe roundly arcing from end to end without a distinct angle present (Fig. 6B).

Distribution. The type locality of Rawlinson Mts. on the Huon Gulf, Morobe Province, Papua New Guinea is the only record known at present (Fig. 5).

Phylliinae Brunner von Wattenwyl, 1893

Phylliini Brunner von Wattenwyl, 1893

Vaabonbonphyllium gen. nov.

https://zoobank.org/0D8A7944-2461-4FFF-A041-A3A90E696398

Type species. *Phyllium groesseri* Zompro, 1998: 159, herein designated.

Taxonomic hierarchy. Several features link this genus to the Phylliini genera and not the Nanophylliini, such as males with the alae radius split near the wing base (not splitting on the distal half of the wing as in *Nanophyllium* and *Acentetaphyllium* gen. nov.), females with tegmina venation similar to *Chitoniscus* due to the diverging

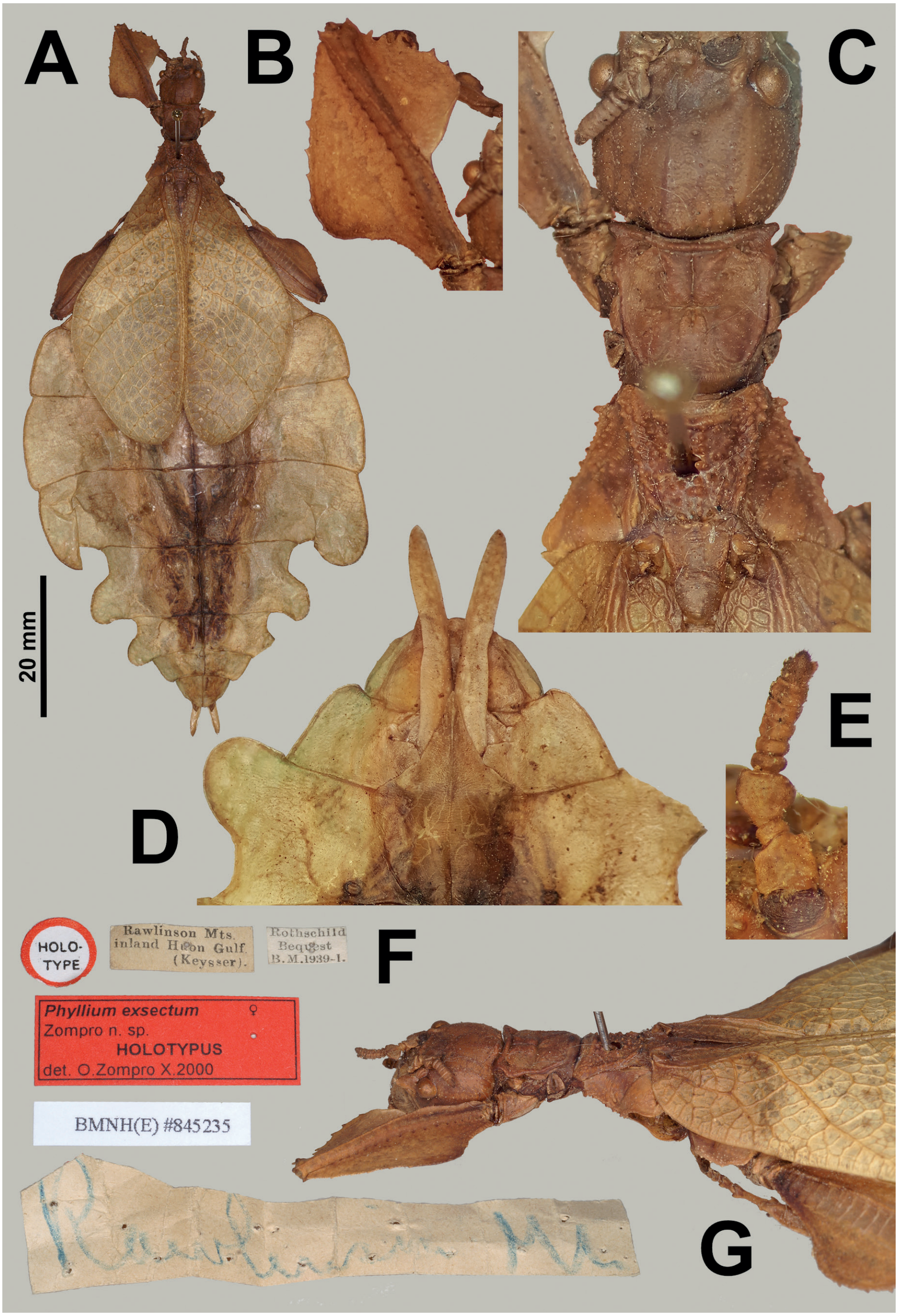


Figure 9. *Rakaphyllium exsectum* comb. nov. holotype female (NHMUK) **A** habitus, dorsal **B** profemora, dorsal **C** details of head through thorax, dorsal **D** genitalia, ventral **E** details of antenna, dorsal **F** associated data labels **G** details of head through thorax, dorsolateral. Scale bar only associated with **A**. Photographs **A, F, G** taken by Paul Brock (United Kingdom), other photographs taken by first author.

radius and media veins and distinct cubitus anterior and cubitus posterior (cubitus only weakly bifurcate or not bifurcate in *Nanophyllium*), and both sexes have a singular posteromedial head tubercle (not bilobed as in *Nanophyllium* or *Acentetaphyllium* gen. nov.). Due to these notable morphological features, we place this herein described genus within the tribe Phylliini Brunner von Wattenwyl, 1893.

Discussion. The selected type species for this new genus is *Phyllium groesseri* Zompro, 1998 (= *Vaabonbonphyllium groesseri* (Zompro, 1998), comb. nov.) as this was the first species described from within this new genus and this species is well represented by multiple specimens from the geographically isolated Solomon Islands (Fig. 4). Unfortunately, fresh specimens of either species within this genus have not yet been sequenced so hopefully this species can be collected one day and sequenced to add clarity to the higher-level taxonomy.

Autapomorphic features. Within females, the first radial vein (R1) of the tegmina splits from the radius early on, ca. one third of the way between the wing base and the radius to media crossvein (R–M)/the bend in the radial sector (Fig. 4B) versus all other phylliids which have the first radius splitting from the radius midway or more than midway from the wing base to the radius to media cross vein (R–M)/the bend in the radial sector or even after this point.

For males, the media (M) vein of the tegmina runs parallel with the radial sector (Rs) several vein widths away and the media posterior (MP) splits on the distal half of the tegmina, bends immediately and runs parallel with the media anterior (MA) to the wing apex (Fig. 3B) versus in other genera typically the media and radial veins run side by side touching or nearly so, and the posterior branch(es) of the media split on the proximal half or near the middle of the length and run diverging (not parallel) from the media posterior and run to the wing margin, not the apex (Fig. 3A).

Generic characteristics. The *Vaabonbonphyllium* gen. nov. are small to medium, with females ca. 70.0 mm in length (in *Vaabonbonphyllium groesseri* comb. nov.) and with males from ca. 43.0 mm (*Vaabonbonphyllium groesseri* comb. nov.) to ca. 53.0 mm (in *Vaabonbonphyllium rafidahae* gen. et sp. nov.).

Antennae. Females have antennae with nine segments with segments I, III, and IV notably broader than the other segments and the terminal antennae segment is as long as the preceding three and a half segments (Fig. 10D). Males have antennae with around 22 segments (in *Vaabonbonphyllium groesseri* comb. nov. both known male specimens have broken antennae that are missing segments, therefore they only have 19 or 20 segments, but in *Vaabonbonphyllium rafidahae* gen. et sp. nov. there are 22 segments that are not broken; Fig. 11F) and most segments covered in setae which are longer than the segment is wide.

Head capsule. Males have well-developed ocelli (Figs 11C, 12G) and female lack ocelli (Fig. 10C). Males have compound eyes which are strongly protruding and occupy ca. 2/5 of the head capsule lateral margins (Fig. 11C) versus females which have compound eyes which are notably smaller, only occupying ca. ¼ of the head capsule lateral margins and which to not strongly protrude from the capsule (Fig. 10C). In both sexes the head capsules are marked throughout by weak (Fig. 10C) to distinct granulation (Fig. 12G) which is relatively evenly spaced. The posteromedial head tubercle is singular and distinctly raised from the head capsule (Figs 11E, 12H).

Thorax. The thorax is similar in both sexes with the pronotum notably longer than wide (Figs 10C, 11B, 12G). The mesopleurae are gradually diverging from the anterior to the posterior and are marked with four to six tubercles and some minor granulation throughout (Figs 10C, 11B, 12G). In both sexes the prescutum is ca. 2X wider on the anterior than long with lateral margins marked by three to five nodes/tubercles, and a prescutum surface which can be relatively smooth or slightly granular. When viewed laterally, both sexes have the prescutum anterior rim marked with a raised sagittal spine (Fig. 12H).

Legs. Both sexes have interior tibial lobes on the protibiae which do not span the full length of the shaft (only occupying the proximal ½ to 4/5, but not fully reaching), have two lobes on the protibial exterior (one on the distal end which is smaller and one near the middle which is larger; Figs 10F, 11B, 12C), and the meso-, metatibiae are mostly bare except for the exterior distal ends which can be variable, by either being slightly raised or with small to medium sized lobes present (Fig. 12D, 12E). In both sexes the profemoral interior lobe is broader than the exterior lobe and marked with three or four broad teeth which can be more sharply pointed (Fig. 11B) or dulled (Fig. 12C). Both sexes have the interior mesofemoral lobes slightly broader or about even in width to the exterior lobes, and both lobes can have serration on the distal ½ to 1/3 of the lobe, but the interior lobe serration is more prominent than the exterior lobe. Both sexes have the interior metafemoral lobes several times broader than the exterior lobes and the interior lobes are prominently marked by serration.

Wings. Female tegmina are always long, reaching onto abdominal segments VIII or IX and male tegmina are moderate in length, reaching onto abdominal segment III. Female alae are highly reduced to no more than just a nub and male alae are fully developed in an oval-fan configuration reaching slightly past the apex of the terminal abdominal segment. Female tegmina have a subcostal (Sc) vein which is simple and runs parallel with the wing margin for ca. ⅕ of the wing length before fading; radial vein which diverges steadily away from the media and splits into the first radial vein (R1) which diverges from the radius (R) early on, ca. ⅓ of the way between the wing base and the radius to media crossvein (R–M)/the bend in the radial sector, the primary vein terminates as the radial sector (Rs) on the wing margin just past the middle of the wing length, and as a small radial to medial crossvein (R–M) which does fully connect; the media vein (M) runs side by side the cubitus vein (Cu) and splits into the media anterior (MA) ca. halfway through the wing length and the media posterior (MP) diverges from the cubitus near the distal third of the wing length; the cubitus vein runs along the wing margin until it splits into the cubitus anterior (CuA) and cubitus posterior (CuP) near the distal quarter of the wing; and a first anal vein fuses with the cubitus early on (Fig. 4B). Male tegmina have a simple subcoastal vein; a radial vein (R) which runs parallel with the media (M) throughout the full length of the wing and branches into the first radial (R1) ca. 2/5 of the way through the wing length and the primary vein terminates as the radial sector (Rs) at the wing apex; the media runs parallel with the radius and splits into the media posterior (MP) near the distal 2/5 of the wing, bends immediately and runs parallel with the media anterior (MA) until reaching the wing margin; the cubitus is unbranched and runs along the wing margin; and there is a first anal which fuses with the cubitus ca. ⅓ of the way through the wing length (Fig. 3B). Male alae (Fig. 3B) have a costal vein (C) running along the anterior margin and a subcostal vein (Sc) which runs for nearly the same length parallel with the costal vein; the radial vein (R) is bifurcate when it splits slightly less than 1/3 of the way through the wing length where they gradually diverge, run parallel/subparallel, then converge gradually to the apex where they terminating very near each other but not touching; the media (M) is also bifurcate, splitting about 1/8 of the way through the alae length and these veins run parallel for most of their length until the media posterior (MP) fades near the wing apex and the media anterior (MA) fuses with the radial sector near the wing apex; the cubitus (Cu) is fused with the first anterior anal (1AA) for ca. ¾ of the length until the first anterior anal splits and runs to the wing margin and the cubitus runs singularly to the wing apex; the anal veins are split into two groups, seven anterior anals (1AA–7AA) and five posterior anals (1PA–5PA).

Abdomen. Abdominal shapes are variable within this genus; females are only known for *Vaabonbonphyllium groesseri* (Zompro, 1998), comb. nov. and have a boxy abdomen (with segments IV through VI parallel sided) and segment VII strongly lobed and tapering strongly to a narrower segment VIII which is weakly lobed (Fig. 10B). For the males, *Vaabonbonphyllium groesseri* comb. nov. have segment II converging to the narrowest point of the abdomen, III diverging (giving the abdomen a pinched waist appearance), IV weakly projecting and converging, V diverging, VI strongly diverging and converging giving it a notable lobe, VII appears variable as one male known has this segment converging (Fig. 11A) and the other male has a notable lobe on this segment as well (Fig. 11D), the remainder of the segments converge to the apex. In *Vaabonbonphyllium rafidahae* gen. et sp. nov. the abdominal shape is simple with all segments similar in width and margins all nearly parallel sided giving it a narrow, blade-like appearance (Fig. 12B).

Female subgenital plate is moderate in length and width, projecting slightly less than halfway under the terminal abdominal segment; gonapophyses VIII are long, broad, and parallel sided for most of their length, with an apex which projects from under the terminal abdominal segment about as much as the cerci do; the cerci are flat and marked sparsely with a granular surface (Fig. 10E). Males have an approximately equilateral triangular vomer which is singularly pronged, hooking up into the paraproct (Figs 11G, 12A).

Egg. Egg morphology is not yet known for this rare genus.

Etymology. *Vaabonbonphyllium* meaning “leaf that waits for the night to come”. This generic epithet is a compound of the Latinized name *Phyllium* the type genus for the family (from Greek φυλλον, -ου (*phyllon*, *-oy*) + -um; Poitout 2007), coupled with the prefix *vaabonbon* from the Teop (Tiop) language phrase which means “wait for the night to come” (Mosel 2019). We wished to honor the original inhabitants of this area by using a traditional language from Bougainville Island, the type locality of the genus.

The Teop language is little known and considered a threatened language with as few as 6,000 speakers left in the autonomous region of Bougainville Island (Mosel 2014). We chose this name as these insects are exceptionally camouflaged and nocturnal, holding still during the day, thus hiding from view and only venturing out at night, making them exceptionally rare in collections and little known. This new genus is neuter in gender, following *Phyllium*.

Distribution. At present *Vaabonbonphyllium* gen. nov. is primarily known from the Solomon Islands (*Vaabonbonphyllium groesseri* comb. nov.) and a single record from Western Highlands Province, Papua New Guinea (*Vaabonbonphyllium rafidahae* gen. et sp. nov.; Fig. 5).

New combination and new species

***Vaabonbonphyllium groesseri* (Zompro, 1998), comb. nov.**
***Vaabonbonphyllium rafidahae* gen. et sp. nov.**

Vaabonbonphyllium groesseri (Zompro, 1998), comb. nov.

Figs 4B, 10, 11

Material examined. (15 ♀♀, 2 ♂♂, 3 ♀♀ nymph, 2 ♂♂ nymph, 2 unsexed nymph): ***Holotype*** (♀): “Buin I. Bougainville Arch. Salomons; 1941 4; *Chitoniscus lobiventris* Blanch. K. Gunther det.; *lobiventris* Blanch.; *Chitoniscus* Stal; *Phyllium groesseri* Zompro 1997 HOLOTYPUS det. O. Zompro X. 1996; HOLOTYPUS; *Phyllium groesseri* ♀ Zompro n. sp. HOLOTYPUS det. O.Zompro V.1998” (SMTD; Fig. 10A). See Suppl. material 1 for additional specimens reviewed, their collection data, and depositories.

Remarks. At present this is the only known phylliid species from the Solomon Islands, although the phylliid knowledge of these islands is rather limited as even this species is rarely collected so if additional species were present but not yet formally described it would not be surprising.

Only two males are known to us at the present (from within the NHMUK and CSIRO collections) both of which are notably damaged (Fig. 11). These males have not yet been positively confirmed as the male *Vaabonbonphyllium groesseri* comb. nov. via molecular comparison or from captive breeding but based upon shared morphology between the sexes and the current knowledge that only one species of phylliid is present on the Solomon Islands it is safe to assume these are the male for this species.

This species was originally described simply as a *Phyllium* species (not placed within a subgenus) and was placed within the *Pulchriphyllium* subgenus by Größer (2001) due to the presence of exterior lobes of the tibiae. During the intrageneric revision of Hennemann et al. (2009) this species was placed within the *frondosum* species group to which this species has a strong general resemblance (when reviewing the size, abdominal shape, and female wing venation). In 2020 the *frondosum* species group was found to represent the unknown female *Nanophyllium*; however, this species was not

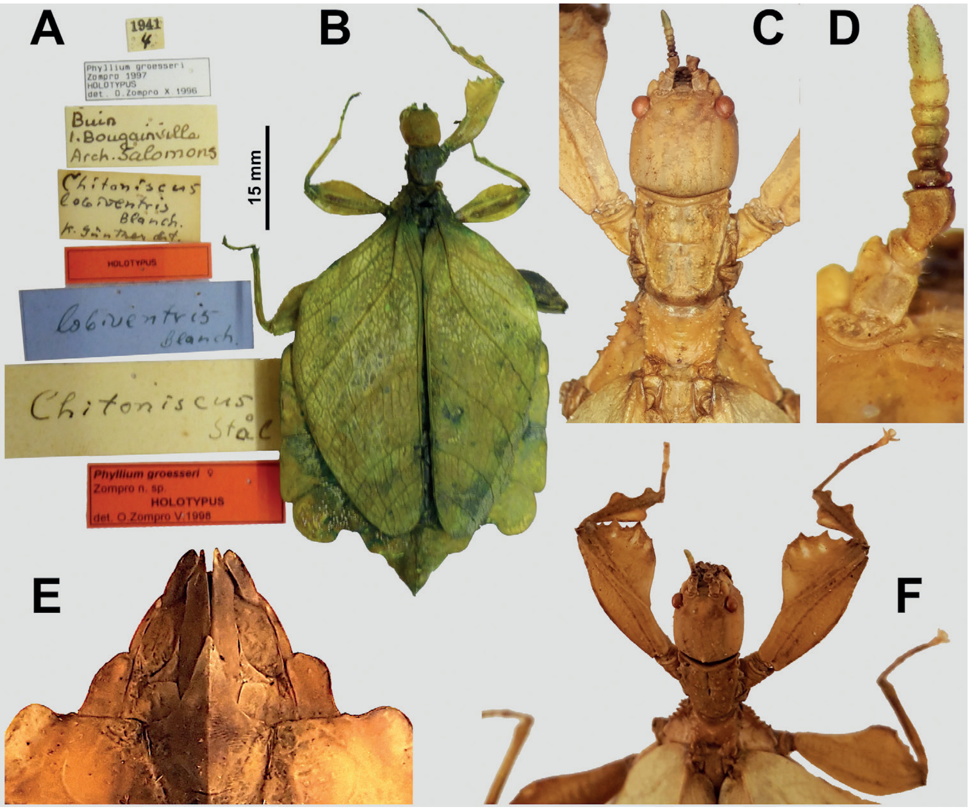
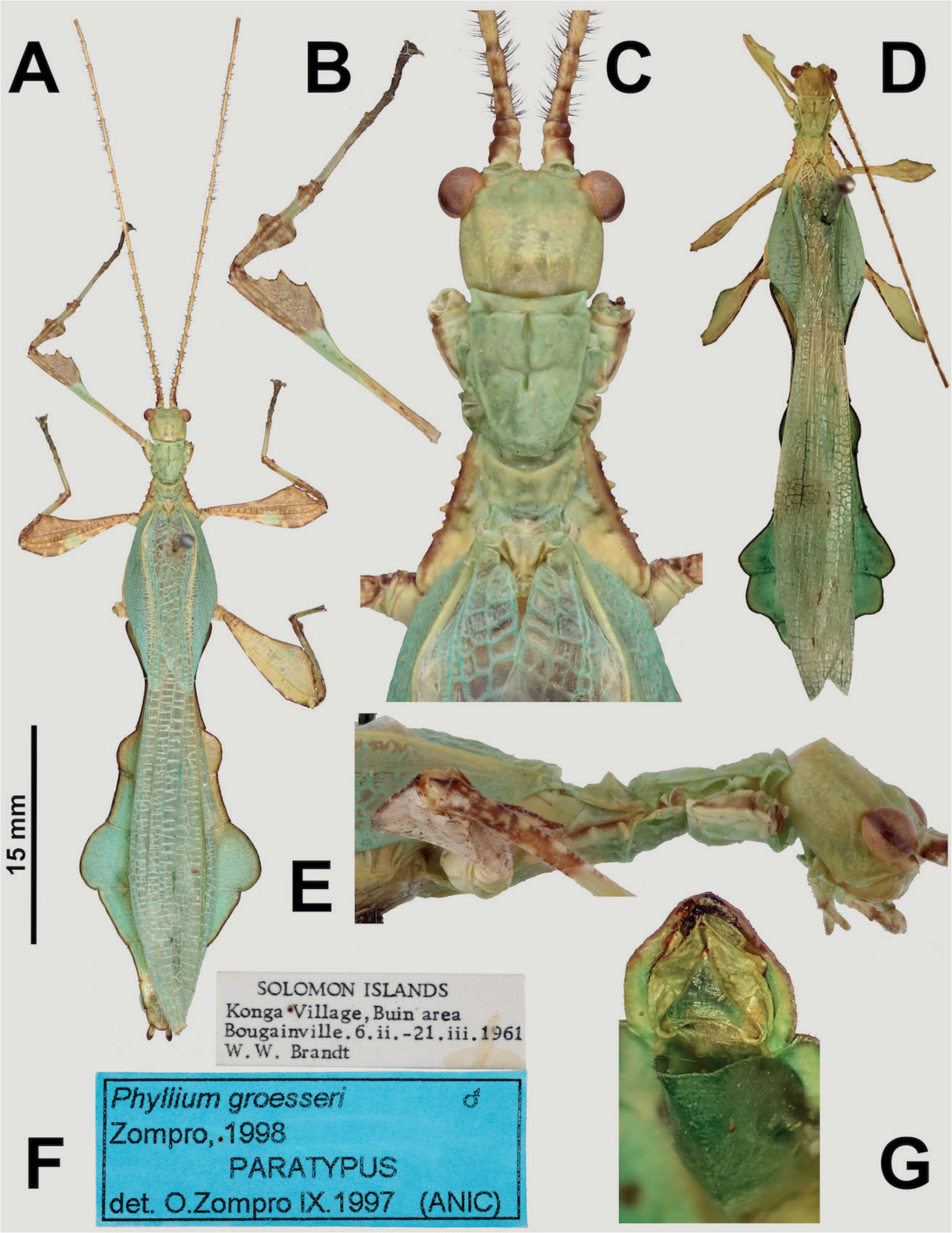


Figure 10. *Vaabonbonphyllium groesseri* comb. nov. females **A** data labels associated with the holotype **B** holotype, habitus, dorsal **C** details of head through thorax, dorsal **D** details of antennae, dorsal **E** details of genitalia, ventral **F** front legs, head, and thorax, anterodorsal. Scale bar associated with **A** and **B**. **A** and **B** photographs by Christian Schmidt (SMTD), other images taken by the first author of specimens from the NHMUK.

transferred to the *Nanophyllium* but was instead moved to the *Pulchriphyllium* subgenus *schultzei* species group based upon the bilobed protibiae (Cumming et al. 2020a). Now thanks to a full review of morphology and review of multiple specimens, the fine details which differentiate this clade from others allows its formal recognition as a unique genus.

Differentiation. Female *Vaabonbonphyllium groesseri* comb. nov. are the only females known for this genus, so differentiation from congenerics is not possible until the unknown female *Vaabonbonphyllium rafidahae* gen. et sp. nov. is located and described. Male *Vaabonbonphyllium groesseri* comb. nov. can be differentiated from *Vaabonbonphyllium rafidahae* gen. et sp. nov. based upon the abdominal shape as *Vaabonbonphyllium groesseri* comb. nov. has notably differing abdominal segment widths, with the middle segments many times wider than the anterior and distal



SOLOMON ISLANDS
Konga Village, Buin area
Bougainville. 6. ii.-21. iii. 1961
W. W. Brandt

Phyllium groesseri ♂
Zompro, 1998
PARATYPUS
det. O.Zompro IX.1997 (ANIC)

Figure 11. *Vaabonbonphyllium groesseri* comb. nov. males **A–C, E, F** paratype male, (ANIC (CSIRO)), photographs by Bonnie Koopmans (CSIRO) **D, G** non-type male (NHMUK), photographs by first author **A** habitus, dorsal (left front leg is physically detached, photoshopped onto the specimen for this figure) **B** left front leg, dorsal **C** details of head through thorax, dorsal **D** habitus, dorsal **E** details of head through thorax, lateral **F** associated data labels **G** genitalia, ventral. Scale bar only associated with **A**.

segments (Fig. 11A, D) versus *Vaabonbonphyllium rafidahae* gen. et sp. nov. which has an abdomen with all segments relatively even in width giving it a smooth margined appearance (Fig. 12B). Also, the lobes of the pro- and mesofemora allow differentiation as *Vaabonbonphyllium groesseri* comb. nov. has the profemoral exterior lobe highly reduced (Fig. 11B) and the interior mesofemoral lobe is notably broader than the mesofemoral exterior lobe versus *Vaabonbonphyllium rafidahae* gen. et sp. nov. which has a profemoral exterior lobe which is slightly wider than the profemoral shaft width (Fig. 12C) and the mesofemoral interior and exterior lobes which are approximately the same width (Fig. 12E). Additionally, the prosternum allows for differentiation as in *Vaabonbonphyllium groesseri* comb. nov. the prosternum is relatively smooth, lacking a distinct protrusion (Fig. 11E) versus *Vaabonbonphyllium rafidahae* gen. et sp. nov. which has a distinct protrusion with a granular surface (Fig. 12H).

Distribution. This species was originally only recorded from Bougainville Island, but since then additional records have been recovered from numerous collections representing previously unknown localities (see Suppl. material 1 for a full list of known specimens and their data). Additional islands recorded are (north to south): Kolombangara, Nggela, Guadalcanal, and Small Malaita (Fig. 5). With how rarely this species is collected, and from the wide range that it can be found from throughout the Solomon Islands, it is likely that this species can be found on several of the other larger islands as well, but just has not been formally recorded yet.

Vaabonbonphyllium rafidahae **gen. et sp. nov.**

https://zoobank.org/D8265C9B-18D2-4C41-9697-829CCD2A0655
Figs 3B, 12

Material examined. ***Holotype*** ♂: "Papua New Guinea: Western Highlands Province (Highlands Region). Mt. Hagen Dist. (New Guinea Island) Mt. Hagen, along Highlands Hwy. (5 51'46.20"S, 144 13'30.75"E) Elev. 1730 m 23-VI-1989 Coll. L D. Munsey. (Coll RC 19-106)". Deposited in the Montreal Insectarium (IMQC).

Remarks. This mainland New Guinea species is presently only known from the singular holotype male (Fig. 12). This species is tentatively placed within *Vaabonbonphyllium* gen. nov. due to the protibial exterior being bilobed (a feature only known from *Vaabonbonphyllium* gen. nov. and *Rakaphyllium* gen. nov.) but the wing venation of this male differs notably from *Rakaphyllium* gen. nov. and therefore, this species appears to be related to *Vaabonbonphyllium groesseri* comb. nov. based upon our poor knowledge of male morphology within that species. Unfortunately, for the *Vaabonbonphyllium* gen. nov. only three males are known (two *Vaabonbonphyllium groesseri* comb. nov. and this herein described species) so our knowledge on the intrageneric variability is severely limited and until specimens of both species can be sequenced a confident placement cannot be made beyond this initial morphology-based review.

Differentiation. Females unknown. Male *Vaabonbonphyllium rafidahae* gen. et sp. nov. can be differentiated from *Vaabonbonphyllium groesseri* comb. nov. based upon

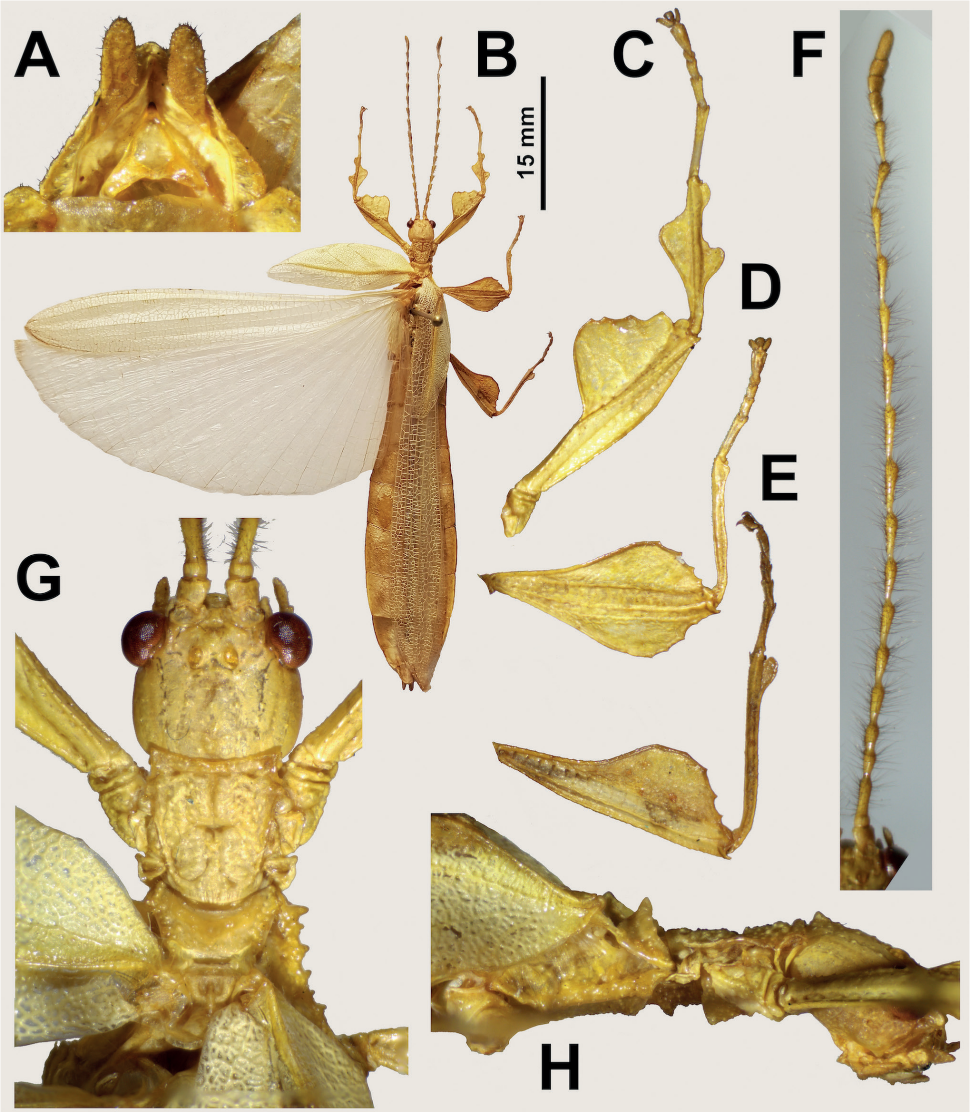


Figure 12. Holotype male *Vaabonbonphyllium rafidahae* gen. et sp. nov., (IMQC) (Coll RC 19-106) photographs by the first author **A** genitalia, ventral **B** habitus, dorsal **C** details of the protibial and profemoral lobes **D** details of the mesotibial and mesofemoral lobes **E** details of the metatibial and metafemoral lobes **F** antenna, dorsal **G** details of the head and thorax, dorsal **H** head–thorax, lateral. Scale bar only associated with **B**.

the abdominal shape as *Vaabonbonphyllium rafidahae* gen. et sp. nov. has an abdomen with all segments relatively even in width giving it a smooth margined appearance (Fig. 12B), versus *Vaabonbonphyllium groesseri* comb. nov. which have notably differing abdominal segment widths, with the middle segments many times wider than the anterior and distal segments (Fig. 11A, D). The lobes of the femora allow differentiation as *Vaabonbonphyllium rafidahae* gen. et sp. nov. has a profemoral exterior lobe which is slightly wider than the profemoral shaft width (Fig. 12C) and mesofemoral interior and exterior lobes which are approximately the same width (Fig. 12D) versus *Vaabonbonphyllium groesseri* comb. nov. which has the profemoral exterior lobe highly reduced (Fig. 11B) and the interior mesofemoral lobe notably broader than the mesofemoral exterior lobe. Additionally, the prosternum allows for differentiation as in *Vaabonbonphyllium rafidahae* gen. et sp. nov. there is a distinct protrusion (Fig. 12H), and in *Vaabonbonphyllium groesseri* comb. nov. the prosternum is relatively smooth, lacking a distinct protrusion (Fig. 11E).

Distribution. Currently only known from the type locality of Mt. Hagen, Western Highlands Province, Papua New Guinea (Fig. 5).

Male. *Coloration.* The coloration description is based on the single dried holotype specimen which appears to have been collected in alcohol which turns phylliids yellow (Fig. 12). In life the specimen likely was green, but color variation is known within phylliids so a definitive coloration description cannot be given at this time for possible living coloration. The overall coloration of the holotype specimen is straw yellow throughout with the only feature which is distinctly different in color being the compound eyes which are a dark red (Fig. 12G). Abdominal segment V has a set of eye spots which are ovular and slightly darker in color than the remainder of the abdomen.

Morphology. Head. Head capsule approximately as long as wide, with a vertex that is marked throughout by nodes which are relatively evenly spaced and of uniform size (Fig. 12G). The posteromedial tubercle is singularly pointed, large, and notably raised above the head capsule, many times larger than any of the capsule granules (Fig. 12H). Frontal convexity stout and bluntly pointed with several short setae on the apex. Compound eyes large and bulbous, occupying ca. ⅖ of the head capsule lateral margins (Fig. 12G). Between and slightly posterior to the compound eyes are three ocelli that are well-developed (Fig. 12G). Antennal fields are slightly wider than and approximately as long as the scapus.

Antennae. Antennae (including the scapus and pedicellus) consist of 22 segments. The scapus and pedicellus are bare, segments III through XIX have dark setae which are mostly around two times longer than the segments are wide and are not densely packed, the terminal three segments are covered by fine, densely packed, transparent setae (Fig. 12F).

Thorax. Pronotum is slightly longer than the greatest width, with anterior margin concave and lateral margins that are straight and converge to a gently convex posterior margin that is ca. ⅗ the width of the anterior rim (Fig. 12G). Anterior and lateral margins of the pronotum have distinct rims and the posterior margin lacks a rim (Fig. 12G). Face of the pronotum is notably lumpy, marked with a distinct sagittal furrow on the anterior half, and a distinct pit in the center (Fig. 12G). Prosternum has a notably granulose surface with a distinctly projecting area in the center and an additional smaller projection near the posterior margin, which is smaller than the central projection, both projections are marked with granulation (Fig. 12H). Mesosternum has a notably granulose surface similarly marked as the prosternum with uniform spacing between granules which are all nearly even in size. Metasternum not as heavily marked with granulation, but instead has slightly wrinkled anterior areas and a nearly smooth central and posterior area. Prescutum on the anterior is wider than long, with lateral margins converging uniformly to the posterior which is ca. ⅗ as wide as the anterior rim width (Fig. 12G). Lateral rims with a lumpy texture due to irregularly sized tubercles/nodes (ca. nine distinct but some weakly formed) with the largest near the anterior margin (Fig. 12G). The surface of the prescutum is marked with a few nodes but it is mostly smooth as there is no distinct sagittal crest, instead the anterior margin is the only distinct feature as the rim is prominently formed with a granular surface and raised into a distinct sagittal point (Fig. 12G, H). Mesopleura narrow, only slightly diverging from the anterior to the posterior giving the mesosternum a rectangular appearance when viewed ventrally. Lateral margin with one notably larger tubercle on the anterior margin with three setae protruding from it followed by four or five moderately sized tubercles which in some cases have a seta or two protruding from them, with these tubercles evenly spaced throughout the length (Fig. 12G). Face of the mesopleura with a weakly lumpy surface (most areas are relatively smooth) and marked with two weakly formed pits near the middle of the length (Fig. 12G, H).

Wings. Tegmina moderate length, extending ca. ⅓ of the way onto abdominal segment III (Fig. 3B). Tegmina wing venation: the subcosta (Sc) is the first vein, it runs relatively straight and terminates slightly less than ½ of the way through the overall tegmina length. The radius (R) spans the entire length of the tegmina with the first radius (R1) branching slightly greater than ⅓ of the way through the wing length and running to the wing margin where it terminates ca. ⅗ of the way through the wing length, and then the radial sector (Rs) runs straight from the branching point to the wing apex. The media (M) spans the entire length of the tegmina, terminating at the wing apex as the media anterior (MA). The media posterior (MP) branches ca. ⅔ of the way through the wing length, immediately bends and runs parallel with the media anterior until it terminates near the wing apex. The cubitus (Cu) runs through the wing surface angled towards the margin which it meets ca. ⅓ of the way through the tegmina length at the same location where the first anal (1A) vein fuses with it and the cubitus runs along the wing margin until it fades near the point where the media posterior terminates near the wing margin. Alae well-developed in an oval fan configuration, reaching beyond the apex of the abdominal segments, instead reaching to the apex of the cerci. Alae wing venation (Fig. 3B): the costa (C) is present along the entire forewing margin. The subcosta (Sc) runs along the costa throughout almost its entire length. The radius (R) branches ca. ¼ of the way through the wing length into the first radius (R1) and radial sector (Rs) which run for half of their length slightly diverging, then parallel/subparallel until the wing apex where thy converge and terminate very near each other at the wing apex. The media (M) branches early, ca. ⅛ of the way through the wing length into the media anterior (MA) and the media posterior (MP) which run parallel with each other until the media anterior bends towards the radial sector and fuses with it near the wing apex, and the media posterior bends towards the media anterior but fades before fusing with it. The cubitus (Cu) runs unbranched and terminates at the wing apex. Of the anterior anal veins, the first anterior anal (1AA) fuses with the cubitus near the point where the media branches into the media anterior and media posterior and then the first anterior anal branches from the cubitus ca. ¼ of the way through the wing length where it uniformly diverges from the cubitus until it terminates at the wing margin. The anterior anal veins two–seven (2AA–7AA) have a common origin and run unbranched in a folding fan pattern of relatively uniform spacing to the wing margin. The posterior anal veins (1PA–5PA) share a common origin separate from the anterior anal veins and run unbranched to the wing margin.

Abdomen. The general shape of the abdomen is long and slender with margins that are relatively parallel and segments which are nearly even in width, varying little from one to the next (Fig. 12B). Abdominal segments II through IV diverge only slightly, segments V and VI are parallel sided, and VII through the apex converge to the apex.

Genitalia. Poculum roundly rectangular in shape with anterior and lateral margins relatively straight and the posterior margin only slightly passing onto the terminal abdominal segment as it gently arcs towards the abdomen apex. Cerci long and slender (approximately ⅖ as wide as long), with slightly more than ½ of their length extending out from under the anal abdominal segment. The cerci are relatively flat and covered in a granulose surface with numerous short setae evenly spaced throughout (Fig. 12A). The vomer general shape is an equilateral triangle with straight sides evenly converging to the apex, which is armed with a singular upward turning hook that is broad and notably darker than the rest of the vomer (Fig. 12A).

Legs. Profemoral exterior lobe arcing gently with a width only slightly greater than the greatest width of the profemoral shaft with the margin marked slightly with granulation (Fig. 12D). Profemoral interior lobe roundly right angled with a greatest width ca. three times that of the exterior lobe and the distal margin is marked with three or four stout and blunted teeth with varying spacing between them (Fig. 12D). Mesofemoral exterior lobe arcs unevenly end to end with the widest point on the distal ⅓, and the greatest width slightly wider than the mesofemoral shaft or mesofemoral exterior lobe widths (Fig. 12D). The mesofemoral interior lobe is marked by four dulled teeth on the distal ⅓ of the lobe (Fig. 12D). The mesofemoral exterior lobe is very similar to the interior lobe in terms of shape, size, and dentition, with the only notable difference being that at its greatest width it is slightly thinner than the interior lobe (Fig. 12D). Metafemoral exterior lobe has a slightly granular margin but lacks dentition, and the margin is straight running along the metafemoral shaft (Fig. 12E). Metafemoral interior lobe is unevenly weighted on the shaft with the majority occupying the distal ⅔ of the length with the distal ⅓ marked with three or four teeth, with the proximal most rather dulled and the distal most teeth more finely pointed (Fig. 12E). Protibiae with two exterior lobes, one small lobe on the distal end and a larger lobe (ca. three times as large as the small distal lobe) slightly proximal to halfway through the length (Fig. 12D). Protibiae with a single interior lobe which is the shape of a rounded scalene triangle which occupies the proximal ¾ of the protibial length and at its maximum width is ca. two and a half times the width of the protibial shaft width (Fig. 12D). Mesotibiae and metatibiae lack interior lobes but both have singular small exterior lobes situated on the distal end, with the mesotibial exterior lobe only weakly formed (not as wide as the shaft width: Fig. 12D) and the metatibial exterior lobe more distinctly formed (ca. as wide as the metatibial shaft width: Fig. 12E).

Measurements of holotype male [mm]. Length of body (including cerci and head, excluding antennae) 52.8, length/width of head 3.1/3.0, antennae 21.5, pronotum 2.5, mesonotum 2.3, length of tegmina 17.2, length of alae 45.6, greatest width of abdomen 10.5, profemora 7.7, mesofemora 7.4, metafemora 7.4, protibiae 4.8, mesotibiae 4.8, metatibiae 6.8.

Etymology. The specific epithet of the new species is selected to honor the wife of Larry D. Munsey, the individual who collected the specimen upon which the new species description is based. Rafidah, a native of West Malaysia, and Larry, a native Californian, live in Borneo where they jointly have been studying the megadiverse cerambycid fauna of the island for the past 17 years.

Phylliinae Brunner von Wattenwyl, 1893
Nanophylliini Zompro & Größer, 2003

Acentetaphyllium gen. nov.

https://zoobank.org/A5CCCEC7-1112-4961-A9EA-503B393B8CFB

Type species. *Phyllium brevipennis* Größer, 1992: 164, herein designated.

Taxonomic hierarchy. This clade, based upon molecular analyses, was recovered as sister to the *Nanophyllium* a placement which is supported by the linked morphological features of the wider than long prescutum, two lobed posteromedial tubercle, and similar antennae morphology (Bank et al. 2021). Therefore, this new genus is included within the tribe Nanophylliini Zompro & Größer, 2003 along with its sister genus *Nanophyllium*.

Discussion. The selected type species for this new genus is *Phyllium brevipennis* Größer, 1992 (= *Acentetaphyllium brevipenne* (Größer, 1992), comb. nov.) as this was the first species described from this group and is represented by a good condition holotype female with detailed collection data so little confusion surrounds the type species. Additionally, this species has been sequenced and included in molecular phylogenies (Bank et al. 2021) while the other species are still lacking.

Again, the uniqueness of this clade was recognized by Hennemann et al. (2009), with their designation of the *brevipenne* species group, and they even recognized this clade as closely related to the *Nanophyllium* (which as the time were still considered the *Phyllium* (*Pulchriphyllium*) *frondosum* species group, not the opposite sex of the *Nanophyllium* yet which would not be until a decade later in Cumming et al. 2020a).

Within the phylliid-wide phylogeny of Bank et al. (2021) *Acentetaphyllium brevipenne* comb. nov. was recovered as sister to all sampled *Nanophyllium* species with high support. Despite the drastic morphological differences between this new genus (Fig. 13A) and the *Nanophyllium* sensu stricto (Fig. 13B), there are still several morphological features which help to link these two genera. Most notably the two lobed

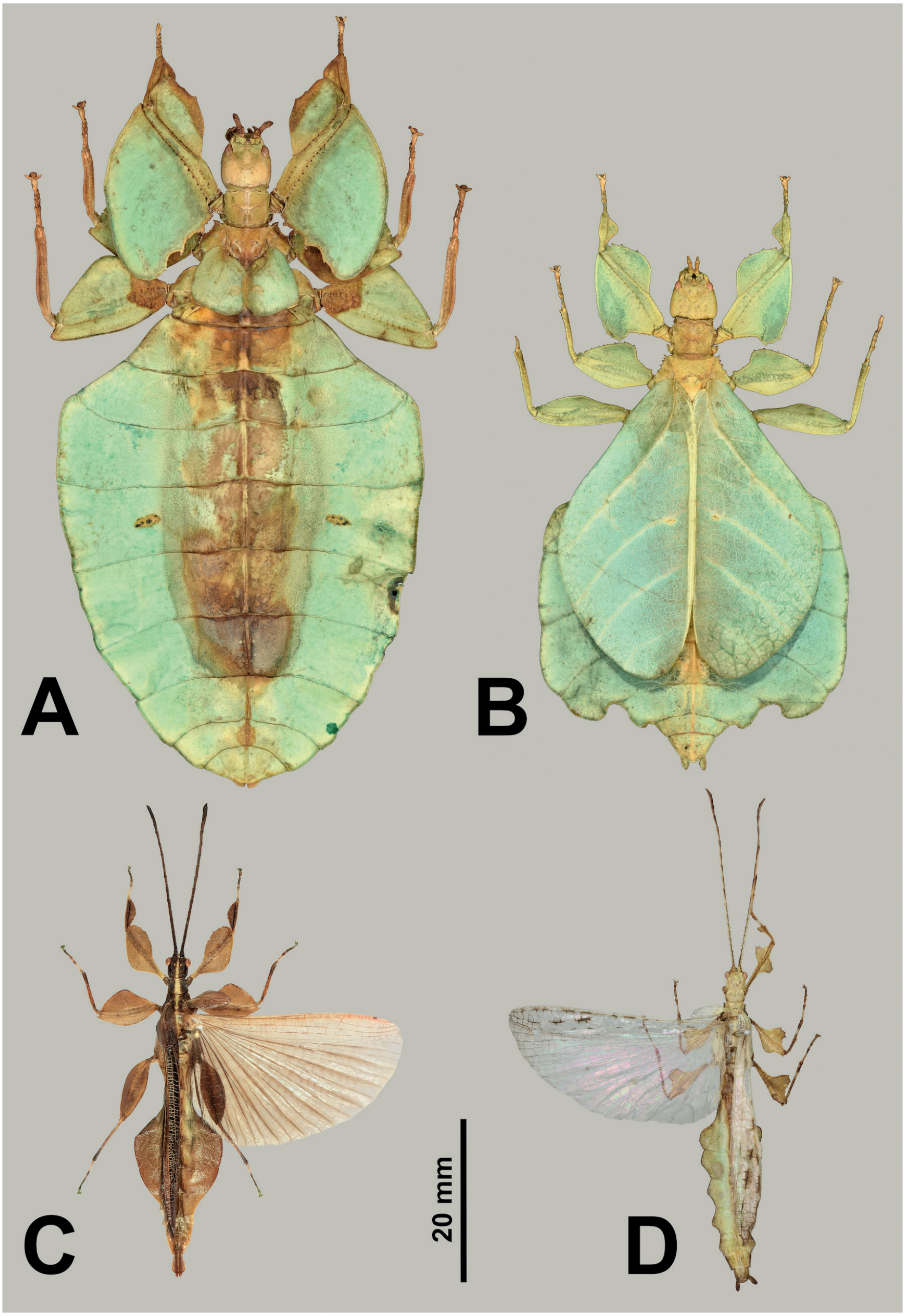


Figure 13. Dorsal habitus of adult *Acentetaphyllium* gen. nov. and *Nanophyllium* to help illustrate the morphological differences/similarities between these two genera. Images scaled to relative size; scale bar associated with all images **A** *Acentetaphyllium brevipenne* female (Coll TM) **B** *Nanophyllium chitoniscoides* (Größer, 1992) female (Coll TM) **C** *Acentetaphyllium stellae* comb. nov. holotype male (SDNHM) **D** *Nanophyllium rentzi* holotype male (NHMUK) **A, B** photographs by Rene Limoges (IMQC) **C** photograph by first author **D** photograph by Paul Brock (NHMUK).

posteromedial tubercle of the head capsule and the similar mesothorax morphology, which is notably wider than long, immediately link these two genera.

Little is known about the *Acentetaphyllium* gen. nov. ecology except for labels included with two females from within the BPBM collection noting their host plant as "Eucalyptus" (Fig. 14). Several other genera/species are known to accept *Eucalyptus* spp. as a host within captivity (pers. comm. Tomas Stijnts (Belgium; Flanders)). Additionally, these BPBM specimens also note an elevation of collection at 750 meters, whereas a specimen in the Coll SLT from Wau, Morobe Province was collected at 1,200 meters, suggesting this species is not restricted to a limited elevation range.

Within the *Nanophyllium* the association of opposite sexes has always been problematic due to limited material and notable age of many specimens not allowing molecular comparison. Only recently was the confusion surrounding the "missing" *Nanophyllium* females for the most part been resolved (Cumming et al. 2020a). However, even within that work it was recognized that there were two distinct clades known for the males (noted within Cumming et al. 2020a as the *pygmaeum* species

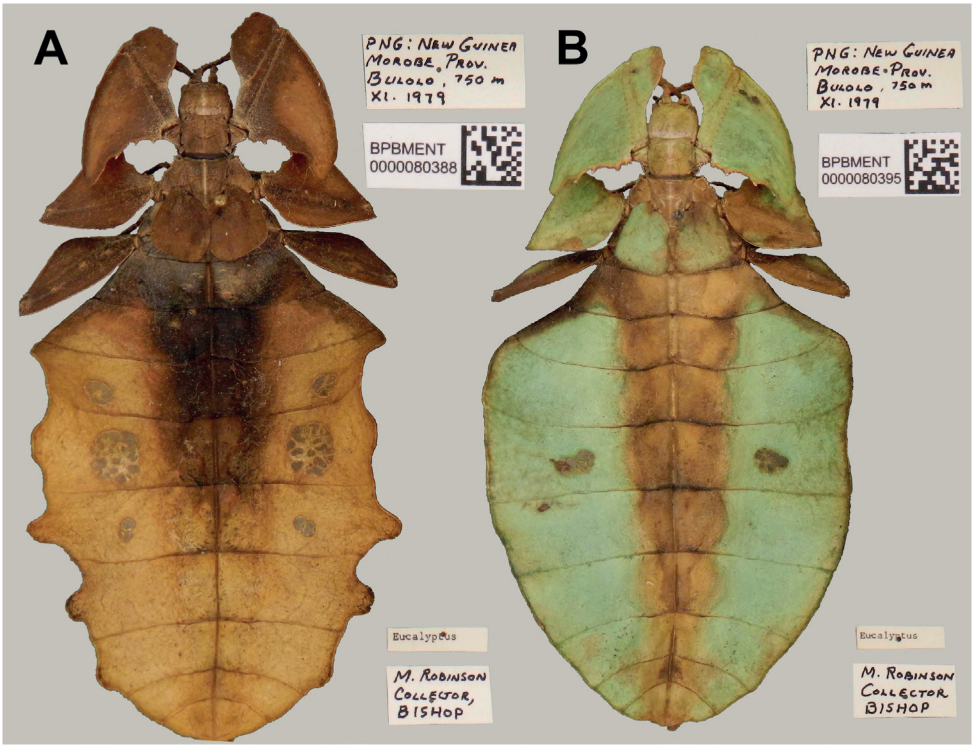


Figure 14. Examples of abdominal variation observed within *Acentetaphyllium brevipenne* comb. nov. adult females from within the BPBM collection, dorsal habitus, with their associated data labels to the right of each specimen. Photographs by Miho Maeda, Jerilynn Chun, and James Boone, 2020 (BPBM) **A** brown form with distinct eye spots on three of the abdominal segments, and a notably lobed abdomen **B** green form female with eye spots on a single abdominal segment and an abdominal shape which has smoother margins.

group and the *stellae* species group, see therein figures and side-by-side morphological comparisons) but females were only confirmed for the *pygmaeum* species group. The *stellae* species group was recognized as having numerous morphological similarities and differences to the *pygmaeum* species group but no tentative female had been linked with clarity. Now with the molecular results of Bank et al. (2021) recovering *brevipenne* as sister to the *Nanophyllium*, the males of the *stellae* species group can with higher confidence be associated with these morphologically unique females (see Fig. 13 for a side by side of these two clades). Although not yet confirmed by breeding or from molecular comparison, the numerous shared morphological features of the males of the *stellae* species group and *brevipenne* strongly suggest these are opposite sexes of the same clade (Fig. 13A, C).

For detailed discussion and figures of the non "*brevipenne*" *Acentetaphyllium* gen. nov. species which were originally placed within *Nanophyllium*, see the recent work of Cumming et al. (2020a) as these species (*Acentetaphyllium larssoni* (Cumming, 2017), comb. nov.; *Acentetaphyllium miyashitai* (Cumming et al., 2020), comb. nov.; and *Acentetaphyllium stellae* (Cumming, 2016), comb. nov.) are not discussed below as no additional information can be added at this time to supplement the recent publication and their original descriptions.

Morphological differentiation from *Nanophyllium sensu stricto*. Features which link these two genera are their antennae morphology (in females), thorax shape and spination (both sexes), and genitalia (both sexes). There are several notable differences between the genera, for female *Acentetaphyllium* gen. nov. the tegmina are highly sclerotized and rudimentary (Fig. 15D), at most reaching onto the anterior of abdominal segment II leaving the dorsal of the abdomen fully exposed versus tegmina which are fully developed and covering most of the abdomen in *Nanophyllium*. For male *Acentetaphyllium* gen. nov. the shape of the femoral lobes differentiates them as the profemoral interior lobes are rounded without a sharp angle and the mesofemoral interior lobes have a large rounded triangular lobe, reaching from end to end and lacking prominent spination (versus *Nanophyllium* which have profemoral interior lobes which are angular and mesofemoral interior lobes which are heavily weighted towards the distal end and are marked by distinct serrate teeth on the distal half). Also, the alae of the male *Acentetaphyllium* gen. nov. allow differentiation as the media anterior and the media posterior do not fuse, instead they both run to the wing margin, and the cubitus after splitting from the first anterior anal fuses with the media posterior near the wing margin and they run fused to the wing apex (versus *Nanophyllium* which have the media posterior fusing to the media anterior and then they run fused to the wing margin without fusing with other veins (see Cumming et al. 2020a: figs. 10–13 for side by side comparisons)).

Autapomorphic features. For females the tegmina are highly sclerotized and rudimentary, no longer than the anterior of abdominal segment II (Fig. 14), whereas all other phylliids have well-developed tegmina which at least reach halfway onto the abdomen and in most cases cover most of the abdomen. For males the alae venation is autapomorphic as the media anterior and the media posterior do not fuse, instead the media anterior runs alone to the wing margin and the cubitus (after splitting from the

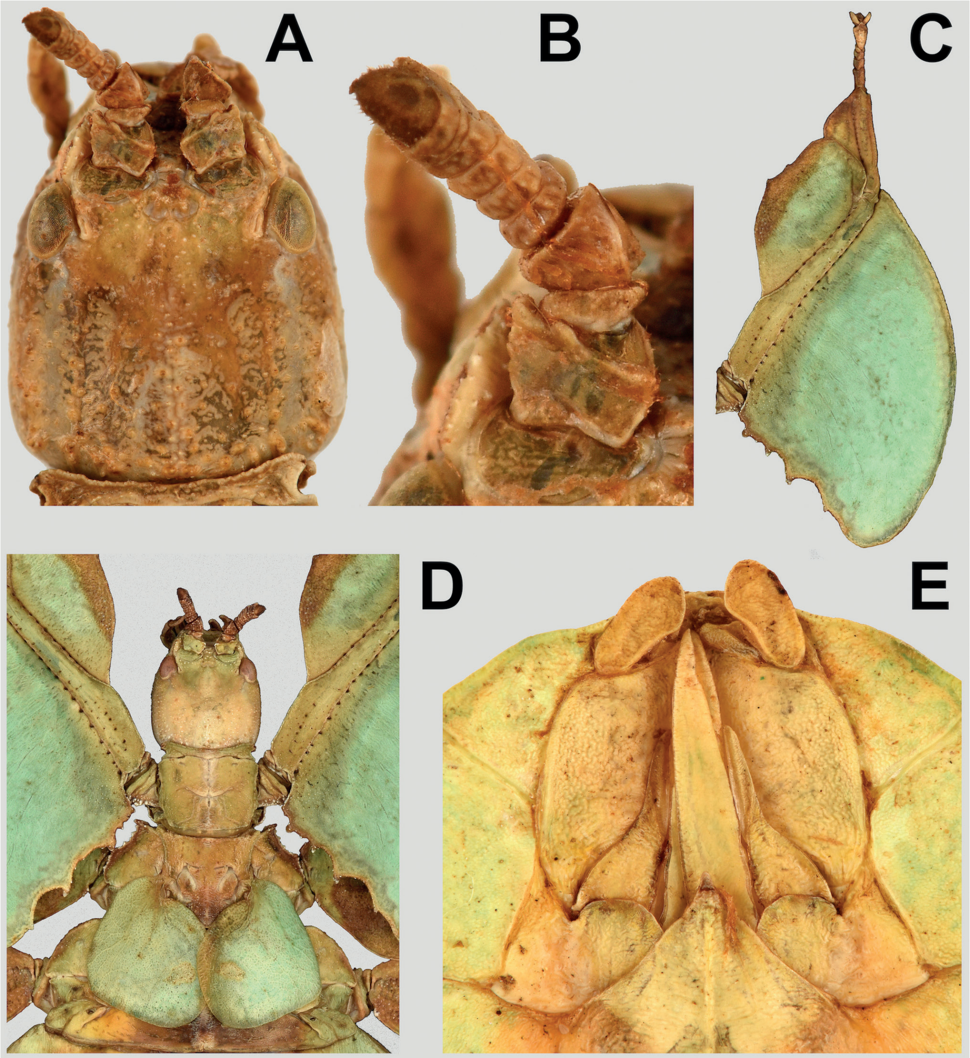


Figure 15. *Acentetaphyllium brevipenne* comb. nov. adult female details **A, B, E** from Coll SLT **C, D** from Coll TM Photographs by Rene Limoges (IMQC) **A** details of head, dorsal **B** details of antennae, dorsal **C** details of protibiae and profemora, dorsal **D** details of head through thorax, dorsal **E** details of genitalia, ventral.

first anterior anal) fuses with the media posterior near the wing margin and then they run fused to the wing apex as one (see Cumming et al. 2020a: fig. 13B for an illustration of this venation pattern).

Generic characteristics. The *Acentetaphyllium* gen. nov. have females that for phylliids are average length (ca. 80 mm) and males which are on the smaller end (ca. 40 mm), which gives a male to female length ratio of ca. 1.0:2.0 between the sexes for this genus which is the most extreme known in the family (Fig. 13A, C) as typically a male to female ratio of 1.0:1.2 to 1.0:1.6 is common. Interestingly for

Acentetaphyllium gen. nov. coloration, males are only known to have brown forms but within females brown, green, and yellow are all known to exist. Hopefully as more males are located it will become apparent if males are exclusively brown or also come in different color forms.

Antennae. Females have antennae with nine segments with segments IV through VIII which are uniform in length, disk-like, marked with granulation, and the terminal segment is stout and densely covered in setae (Fig. 15B). Males have antennae with 21 to 22 segments which are relatively flat, and most segments are covered by setae which are longer than the segment is wide.

Head capsule. Males have well-developed ocelli and females lack ocelli. Males have compound eyes which are strongly protruding and occupy ca. 2/5 of the head capsule lateral margins versus females which have compound eyes which are notably smaller, only occupying ca. ¼ of the head capsule lateral margins and which to not strongly protrude from the capsule (Fig. 15A). Both sexes have head capsules which are marked throughout by distinct but somewhat irregularly sized granulation which is relatively evenly spaced (Fig. 15A).

Thorax. In both sexes the prescutum can be two to three times wider on the anterior than long with lateral margins that are granular, and a prescutum surface which is only slightly granular, lacking a prominent anterior rim or sagittal crest (Fig. 15D). In both sexes the mesopleurae begin notably wider than the prescutum anterior rim and weakly diverge gradually and can be marked with a single anterior tubercle or by as many as nine warty tubercles (Fig. 15D).

Legs. Both sexes have interior protibial lobes which span the full length in a broadly rounded triangle, lack lobes on the protibial exterior, and the meso-, metatibiae are simple, lacking both interior and exterior lobes. In both sexes the profemoral interior lobe is notably smaller than the exterior lobe and is rounded without a strong angle and marked by two to four dulled weakly formed teeth (Fig. 15C). The profemoral exterior lobe is somewhat variable in males (being roundly obtuse or with a slight recurve giving the lobe a boxier appearance) but in females it appears to be more stable as it is notably recurving posteriorly and marked with a strongly serrate posterior margin and a smoothly arcing anterior margin (Fig. 15C). Both sexes have the interior meso-, and metafemoral lobes ca. 1.5 to 2.0 times as broad as the exterior lobes, with both arcing smoothly, giving the meso-, and metafemora smooth, pointed leaflet-like appearances which tuck in nicely to the anterior of the abdomen helping to give the habitus a full ovoid appearance (Fig. 14).

Wings. In both sexes the tegmina are highly reduced and heavily sclerotized, with a maximum length of reaching the anterior margin of abdominal segment II, but mostly shorter, only reaching the metanotum (Fig. 15D). Females lack alae but in males the alae are fully developed, reaching onto abdominal segment IX or X. Male alae have a costal vein running along the anterior margin; a subcostal vein which runs alongside the costal vein for most of its length until it fuses with the costal vein near the wing margin; the radial vein is bifurcate, splitting into the first radial and radial sector near the distal third of the alae and these run to the wing margin without fusing to other veins or each other; the media splits early on into the media anterior and media posterior which run parallel to the wing apex, with the media anterior arriving alone, but the media posterior has the cubitus fuse to it near the distal 1/8 of the wing apex; the cubitus is fused with the first anterior anal for ca. half of the wing length until the first anterior anal splits and runs to the wing margin and the cubitus fuses with the media posterior; the anal veins are split into two groups, the anterior anals and the posterior anals (with seven anterior anals and six posterior anals).

Abdomen. Abdominal shapes are somewhat variable; females are often perfectly spade-shaped (Fig. 14B) but in some forms the abdominal segments can have slightly undulating margins, giving them a wavy appearance (Fig. 14A); males have similar appearances as they can be smoothly spade-shaped (Fig. 14C) or can have abdomen which are strongly undulating (see *Acentetaphyllium larssoni* comb. nov., for an example of a strongly undulating abdomen in males). Female subgenital plate is short and stout with the apex only reaching halfway onto abdominal segment IX and ending in a fine point; gonapophyses VIII are long and slender, but not quite reaching to the apex of the terminal abdominal segment; gonapophyses IX are located to either side of the gonapophyses VIII and have broad bases for ca. ½ their length which taper quickly to slender points; the cerci are relatively flat and rounded, with a slightly wrinkled surface (Fig. 15E). Males have a narrow, triangular vomer which is ca. two times longer than the greatest width and terminates in a single hook. Male cerci are notably marked with granulation and distinct setae along the margins.

Egg. Egg morphology is not yet known for this rare genus.

Etymology. *Acentetaphyllium* meaning "flawless leaf". This generic epithet is a compound of the Latinized name *Phyllium* the type genus for the family (from Greek φυλλον, -ου (*phyllon*, *-oy*) + -um; Poitout 2007), coupled with the Latin prefix *acenteta* meaning "flawless". One of the most remarkable features of this genus is how the abdomen and legs are shaped so that when the legs are at rest the habitus is a perfect oval, giving this genus a flawless leaf-like shape (Fig. 14B). This new genus is neuter in gender, following *Phyllium*.

Distribution. At present only known from a handful of records as this appears to be a rarely collected genus. However, it appears as though this genus is found throughout the island of New Guinea as an undescribed *Acentetaphyllium* gen. nov. is known to us from Fak Fak, Indonesia, there are multiple records from northern New Guinea from the Cyclops Mountains (Indonesia side) and Bewani Mountains (Papua New Guinea side), and multiple records from Morobe Province, Papua New Guinea. See Suppl. material 1 for details on known records.

New combination

***Acentetaphyllium brevipenne* (Größer, 1992), comb. nov.,**
***Acentetaphyllium larssoni* (Cumming, 2017), comb. nov.,**
***Acentetaphyllium miyashitai* (Cumming et al., 2020), comb. nov.,**
***Acentetaphyllium stellae* (Cumming, 2016), comb. nov.**

Discussion

The genus concept within Phylliidae

Due to a lack of scientific objectivity for determination of taxonomic ranks, taxonomic levels are inherently arbitrary, opening up a great deal of personal preference by the participating taxonomists (de Queiroz and Gauthier 1992). In many cases it is likely the historical precedent set forth by past taxonomists which have persisted through the ages that have defined the various taxonomic rankings that are arbitrarily yet habitually followed today across the plethora of organisms and their respective scholars. Therefore, the results of taxonomic rank determinations vary with author preference and often across organismal groups.

To be explicit with our taxonomic intentions, the goal of the current authors is for the phylliid taxonomy to: 1) reflect our understanding of their recovered phylogenetics, 2) that the arbitrary taxonomic ranks be assigned to monophyletic clades, and 3) that these ranks carry with them meaningful differentiation points for communications sake. This of course results in some taxonomic ranks having different degrees of differentiation (such as inferred clade age or degree of species diversity within a clade). Therefore, sometimes different clades assigned to the same taxonomic rank may not be perfectly comparable in a phylogenetic sense. Even though taxonomic ranks are arbitrary, our goal with adjusting the supraspecific phylliid taxonomy is to reflect natural groupings which carry with them human interpretable (and therefore useful) meaning.

For example, if someone were to discuss a male *Acentetaphyllium* one would immediately know they were referencing a male that morphologically the alae have the media anterior which runs alone to the alae apex, that the media posterior fuses with the cubitus and these fused veins run to the apex as MP+Cu, and phylogenetically that this specimen falls within a clade that is molecularly sister to the *Nanophyllium* yet morphologically distinct (as *Nanophyllium* males have alae where the media posterior fuses with the media anterior and this runs to the wing apex as MA+MP). Despite the arbitrary nature of the genus rank in this case, a great deal of pertinent information can easily be gleaned.

Alternatively, as taxonomic ranks are arbitrary, one could just as easily suggest making the *Nanophyllium* and *Acentetaphyllium* “subgenera”. This conundrum was explored within the supplementary materials of Bank et al. (2021) for how “genera” versus “subgenera” might be treated in the revision of paraphyletic clades. This issue was discussed because historically *Phyllium* Illiger, 1798 contained two subgenera which were just as morphologically and phylogenetically unique as other historic genera described around the same time. In light of the phylogenetic results recovering sister clades which were not nested within each other but instead were rather well-defined (and because the taxonomic rank of “subgenus” implies a certain degree of nestedness) instead it was decided that these easily morphologically differentiated groups be given the arbitrary rank of genus. As the phylliids currently stand (based upon precedent within the group set forth by past author preferences) significant wing venation differences coupled with notable phylogenetic distances often correspond to the artificial rank of genus.

So, for simplicity's sake, due to the significant wing venation differences and notable molecular distance between various clades such as the herein discussed *Nanophyllium* and *Acentetaphyllium* gen. nov. we are treating them as genera. Even though clades such as *Microphyllium*, *Pseudomicrophyllium*, *Nanophyllium*, and *Acentetaphyllium* gen. nov. are not evolutionarily as old as the *Chitoniscus* or *Walaphyllium*, all of these clades are monophyletic and carry with them associated morphological features which are easily observed. Thus, these taxonomic units carry with them certain diagnostic and useful information with them despite their arbitrary nature. To summarize the current treatment of genera within the Phylliidae, a summary table is presented to highlight key morphological features which allow differentiation of all phylliid genera (Table 1).

Table 1. Summary of key morphological features for differentiating phylliid genera and well as the clade geographic distribution.

Clade / Author	Geographic distribution	Males	Females
Nanophyllium Redtenbacher, 1906	Southern Indonesia to New Guinea and Northeastern Australia.	Alae, the media posterior fuses to the media anterior and then they run fused to the wing margin without fusing with other veins and bilobed posteriormedial tubercle of the head capsule.	Posteriormedial tubercle of the head capsule split into two lobes and tegmina well-developed, reaching onto abdominal segment VII or VIII.
Acentetaphyllium gen. nov.	New Guinea.	Bilobed posteriormedial tubercle of the head capsule and in the alae, the media anterior and the media posterior do not fuse, instead they both run to the wing margin, and the cubitus after splitting from the first anterior anal fuses with the media posterior near the wing margin and they run fused to the wing apex.	Bilobed posteriormedial tubercle of the head capsule and tegmina rudimentary, at most only reaching onto the anterior of abdominal segment II.
Walaphyllium Cumming et al., 2020	New Guinea and Northeastern Australia.	Abdominal shape rectangular, with segments V and VI with fully parallel-sided margins (segments IV and VII with only half parallel-sided and the remainder curved) and alae radial sector, media anterior, and media posterior not fusing with the cubitus; metafemora exterior simple, lacking a lobe.	Tegmina venation with the posterior cubitus split into an anterior cubitus (CuA), first posterior cubitus (CuP1), and second posterior cubitus (CuP2).
Vaabonbonphyllium gen. nov.	New Guinea and Solomon Islands.	Profemora exterior with two lobes, one near the middle of the length and one on the distal end and in the alae the radius is bifurcate.	Protibiae exterior with two lobes (one on the distal end and one closer to the proximal end) and gonapophyses VIII average in length, with a majority of their length under the terminal abdominal segment, only the tips project from the apex.
Rakaphyllium gen. nov.	New Guinea and Aru Islands.	Protibiae exterior with two lobes (one on the distal end and one closer to the proximal end) and alae, radius vein is simple, does not split.	Protibiae exterior with two lobes (one on the distal end and one closer to the proximal end) and gonapophyses VIII long, with ca. half of their length projecting from under the terminal abdominal segment.
Pulchriphyllium Griffini, 1898	Seychelles, India to mainland Asia, to western Indonesia islands.	Alae radial sector, media anterior, and media posterior veins fusing to the cubitus at different locations along the vein and running together to the wing margin and metafemora exterior with a prominent lobe spanning the full length of the shaft.	Pro-, meso-, and metatibiae exterior with lobes; and tegmina with media and cubitus veins running side by side, touching throughout most of their lengths.
Pseudomicrophyllium Cumming, 2017	Philippines (Luzon).	Antennae notably longer than the outstretched front legs, with antennomeres 4–5× longer than wide and profemoral interior lobes reduced to only a single anterior spine.	Posterior most spine of the prescutum is the most prominent, gonapophyses VIII which are long, reaching to the apex of the anal abdominal segment and third antennomere with stridulatory file.
Microphyllium Zompro, 2001	Philippines (Luzon).	Antennae short (only ca. the length of the outstretched front legs), with bead-like antennomeres that are no more than 2× longer than they are wide and profemoral interior lobes which are narrow and marked with three distinct teeth.	Middle most spine along the prescutum is the largest spine and the posterior most spine is highly reduced and third antennomere lacking stridulatory file.
Comptaphyllium Cumming et al., 2019	Southern Indonesia to New Guinea.	Tegmina media vein splits into the anterior media vein (MA) and posterior media vein (MP) very early on, immediately or at most 1/4 of the way through the wing length and they run unbranched and subparallel through the wing length; protibial interior lobe not reaching from end to end of the shaft, only restricted to the proximal ½ to ⅔ but never more; a head capsule with clearly defined nodes arranged in evenly spaced patterns.	Terminal antennomere as long as the preceding three to five segments combined; pprescutum sagittal plane either with: the spine on the anterior rim most prominent.

Clade / Author	Geographic distribution	Males	Females
Chitoniscus Stål, 1875	Fiji.	Ocelli absent, alae media anterior (MA) runs unfused to the wing margin and media posterior (MP) fades without fusing or reaching the wing margin, and prescutum stout, ca. 2× wider than long.	Coxae ventrally are sky blue in color and prescutum anterior rim strongly protruding and distinctly angled posteriorly.
Trolicaphyllium Cumming et al. 2021	New Caledonia.	Ocelli well-developed and in the alae, media anterior (MA) and media posterior (MP) veins fuse with the cubitus (Cu) at different locations along the cubitus and run fused to the wing margin.	Coxae ventral color is the same color as the legs and thorax, prescutum anterior rim and spine not strongly protruding posteriorly, only slightly raised and vertical, and tegmina radial vein (R) runs parallel with media (M) until the split of the radial sector (Rs), at which point the radial sector (Rs) bends away distinctly.
Cryptophyllium Cumming et al., 2021	Mainland Asia to north/central Indonesia, Southern Philippines, and Micronesia.	Vomer with two apical hooks.	Third antennomere with the proximal end broad and often slightly recurved, making the segment a similar width throughout, or broader on the proximal end; fourth antennomere short and disk-like at least 3× wider than long and notably shorter than any of the following three segments, or rarely a similar length to the following segment, but still at least 2× as wide as long.
Phyllium Illiger, 1798	Malaysia, throughout Indonesia and Philippines, to New Guinea and offshore islands.	Tegmina media vein running unbranched for the first 1⁄3 to 2⁄5 of the wing length, and then branching with either a single short media posterior running to the wing margin or two short media posteriors branching from the notably longer media anterior and running to the margin; protibial interior lobe variable, either fully spanning the full length or only 1⁄2 of the length; head capsule at most with random granulation but frequently bare; Abdominal shape variable, either spade-shaped (with the margins of V parallel or strongly converging and segment VI strongly converging), ovular (with margins expanding and then contracting, no segments parallel-sided), thin and slender with converging margins, bell-shaped (with margins expanding until segment VI then strongly converging) or boxy with only segment V parallel-sided (segments IV and VI only partially parallel-sided, the remainder rounded	Third antennomere narrowest on the proximal end, broadening to the distal end; fourth antennomere typically as long as wide and of a similar length to each of the following three segments length, not notably shorter; tibiae lacking exterior lobes; many other features rather variable

Keys to Phylliidae genera

These keys are adapted from Cumming et al. (2021b) and the supplementary materials of Bank et al. (2021). Unfortunately, the egg and freshly hatched nymph morphology is unknown for the three herein described genera, therefore only keys to adult females and males are revised.

Key to females

1 Posteriormedial tubercle of the head capsule split into two lobes................**2**
– Posteriormedial tubercle of the head capsule singular, not split into two lobes **3**
2 Tegmina well-developed, reaching onto abdominal segment VII or VIII....... ....***Nanophyllium* Redtenbacher, 1906**
– Tegmina rudimentary, at most only reaching onto the anterior of abdominal segment II..***Acentetaphyllium* gen. nov.**
3 Tegmina venation with the posterior cubitus split into an anterior cubitus (CuA), first posterior cubitus (CuP1), and second posterior cubitus (CuP2). .... ***Walaphyllium* Cumming et al., 2020b**
– Tegmina cubitus venation simple (unsplit) or bifurcate (into an anterior cubitus (CuA) and posterior cubitus (CuP1) only) **4**

4 Protibiae exterior with two lobes (one on the distal end and one closer to the proximal end) **5**

– Protibiae exterior simple (lacking lobes) or with a singular lobe that can be present partially on the distal portion only or fully spanning the shaft **6**

5 Gonapophyses VIII average in length, with a majority of their length under the terminal abdominal segment, only the tips project from the apex; tegmina venation has the radius and the media diverging immediately, not running parallel for any portion of their lengths; tegmina venation has a distinct radius to media crossvein .... ***Vaabonbonphyllium*** **gen. nov.**

– Gonapophyses VIII long, with ca. half of their length projecting from under the terminal abdominal segment; tegmina venation has the radius running alongside the media until the radial sector arcs away; tegmina venation, no radius to media crossvein present .... ***Rakaphyllium*** **gen. nov.**

6 Pro-, meso-, and metatibiae exterior with lobes; and tegmina with media and cubitus veins running side by side, touching throughout most of their lengths .... ***Pulchriphyllium*** **Griffini, 1898**

– Pro-, meso-, and meatibiae simple, lacking exterior lobes (but if exterior lobes are present they are usually only partially present only on the distal portion or very rarely fully spanning the shaft (in which case the tegmina media and cubitus veins are distinctly separated with several vein width distances between them throughout the length, not touching)) **7**

7 Prescutum spination along the sagittal plane with the middle or posterior spine the most prominent (therefore not equal in size the other spines) **8**

– Prescutum sagittal plane either with: the spine on the anterior rim most prominent, an anterior rim followed by granulation, or with spination of equal size from the anterior to the posterior (no single spine notably more prominent than the others) **9**

8 Posterior most spine of the prescutum is the most prominent; gonapophyses VIII which are long, reaching to the apex of the anal abdominal segment; third antennomere with stridulatory file..........
.... ***Pseudomicrophyllium*** **Cumming, 2017**

– Middle most spine along the prescutum is the largest spine and the posterior most spine is highly reduced; gonapophyses VIII are notably short, only slightly protruding from under the subgenital plate; third antennomere lacking stridulatory file.......... ***Microphyllium*** **Zompro, 2001a**

9 Terminal antennomere as long as the preceding three to five segments combined .... ***Comptaphyllium*** **Cumming et al., 2019**

– Terminal antennomere as long as the preceding one or two segments combined **10**

10 Tegmina with media and cubitus veins running side by side, touching throughout the majority of their lengths or fused throughout most of their length **11**

– Tegmina with media and cubitus veins with significant spacing between them (several vein widths away) not touching **12**

11 Coxae ventrally are sky blue in color; prescutum anterior rim strongly protruding and distinctly angled posteriorly; tegmina radial vein (R) diverges steadily from media (M) for the full length, therefore the split of the radial sector (Rs) is not a significant bend.......................... ***Chitoniscus*** **Stål, 1875**

– Coxae ventral color is the same color as the legs and thorax; prescutum anterior rim and spine not strongly protruding posteriorly, only slightly raised and vertical; tegmina radial vein (R) runs parallel with media (M) until the split of the radial sector (Rs), at which point the radial sector (Rs) bends away distinctly....................................... ***Trolicaphyllium*** **Cumming et al. 2021**

12 Third antennomere with the proximal end broad and often slightly recurved, making the segment a similar width throughout, or broader on the proximal end; fourth antennomere short and disk-like at least 3× wider than long and notably shorter than any of the following three segments, or rarely a similar length to the following segment, but still at least 2× as wide as long............. .... ***Cryptophyllium*** **Cumming et al., 2021**

– Third antennomere narrowest on the proximal end, broadening to the distal end; fourth antennomere typically as long as wide and of a similar length to each of the following three segments length, not notably shorter .... ***Phyllium*** **Illiger, 1798**

Key to males

1 Posteriormedial tubercle of the head capsule split into two lobes................ **2**

– Posteriormedial tubercle of the head capsule singular, not split into two lobes **3**

2 The profemoral interior lobes are angular; mesofemoral interior lobes are heavily weighted towards the distal end and are marked by distinct serrate teeth on the distal half; alae, the media posterior fuses to the media anterior and then they run fused to the wing margin without fusing with other veins .... ***Nanophyllium*** **Redtenbacher, 1906**

– The profemoral interior lobes are rounded without a sharp angle; mesofemoral interior lobes have a large rounded triangular lobe, reaching from end to end and lacking prominent spination; alae, the media anterior and the media posterior do not fuse, instead they both run to the wing margin, and the cubitus after splitting from the first anterior anal fuses with the media posterior near the wing margin and they run fused to the wing apex ....***Acentetaphyllium*** **gen. nov.**

3 Alae, radius vein is simple, does not split .... ***Rakaphyllium*** **gen. nov.**

– Alae, radius vein is bifurcate, creating the radial sector (Rs) and first radius (R1) **4**

4 Small (< 30.0 mm in length); protibiae lacking an interior lobe; restricted to the Philippines **5**

– Medium to large (35.0 mm to > 80.0 mm); protibiae almost always with a half to fully developed interior lobe, or rarely highly reduced to a sliver on the proximal half only. **6**

5 Antennae short (only ca. the length of the outstretched front legs), with bead-like antennomeres that are no more than 2× longer than they are wide; profemoral interior lobes which are narrow and marked with three distinct teeth .... ***Microphyllium*** **Zompro, 2001a**

– Antennae notably longer than the outstretched front legs, with antennomeres 4–5× longer than wide; profemoral interior lobes reduced to only a single anterior spine................................ ***Pseudomicrophyllium*** **Cumming, 2017**

6 Prescutum stout, ca. 2× wider than long **7**

– Prescutum as long as wide or notably longer than wide.............................. **8**

7 Ocelli absent; alae, media anterior (MA) runs unfused to the wing margin and media posterior (MP) fades without fusing or reaching the wing margin .... ***Chitoniscus*** **Stål, 1875**

– Ocelli well-developed; alae, media anterior (MA) and media posterior (MP) veins fuse with the cubitus (Cu) at different locations along the cubitus and run fused to the wing margin.......... ***Trolicaphyllium*** **Cumming et al. 2021**

8 Vomer with two apical hooks.......... ***Cryptophyllium*** **Cumming et al., 2021**

– Vomer with a single apical hook... **9**

9 Profemora exterior with two lobes, one near the middle of the length and one on the distal end .... ***Vaabonbonphyllium*** **gen. nov.**

– Profemora exterior either with one lobe or simple, lacking lobes............... **10**

10 Alae radial sector, media anterior, and media posterior veins fusing to the cubitus at different locations along the vein and running together to the wing margin; metafemora exterior with a prominent lobe spanning the full length of the shaft.. ***Pulchriphyllium*** **Griffini, 1898**

– Alae radial sector, media anterior, and media posterior not fusing with the cubitus; metafemora exterior simple, lacking a lobe **11**

11 Tegmina media vein splits into the anterior media vein (MA) and posterior media vein (MP) very early on, immediately or at most 1/4 of the way through the wing length and they run unbranched and subparallel through the wing length; protibial interior lobe not reaching from end to end of the shaft, only restricted to the proximal 1/2 to 2/3 but never more; a head capsule with clearly defined nodes arranged in evenly spaced patterns...................... .... ***Comptaphyllium*** **Cumming et al., 2019**

– Tegmina media vein running unbranched for the first 1/3 to 2/5 of the wing length, and then branching with either a single short media posterior running to the wing margin or two short media posteriors branching from the notably longer media anterior and running to the margin; protibial interior lobe variable, either fully spanning the full length or only 1/2 of the length; head capsule at most with random granulation but frequently bare................... **12**

12 Abdominal shape rectangular, with segments V and VI with fully parallel-sided margins (segments IV and VII with only half parallel-sided and the remainder curved).......................... ***Walaphyllium*** **Cumming et al., 2020b**

– Abdominal shape variable, either spade-shaped (with the margins of V parallel or strongly converging and segment VI strongly converging), ovular (with margins expanding and then contracting, no segments parallel-sided), thin and slender with converging margins, bell-shaped (with margins expanding until segment VI then strongly converging) or boxy with only segment V parallel-sided (segments IV and VI only partially parallel-sided, the remainder rounded)..***Phyllium* Illiger, 1798**

Conclusions

Melanesia has been recovered as the likely origin of all extant phylliids with a possible origin around ~ 50 million years ago (MYA) during the Eocene (Bank et al. 2021). Given this recovered history it logically follows that as this region is home to older lineages which have had significantly more time for morphological diversification, this region has more distinct phylliid clades (which based upon their prominent morphological distinctiveness are treated by taxonomists as genera). In comparison, the regions which were more recently colonized (~ 20 to 25 MYA) such as mainland Asia, Sundaland, and the Philippines have a high diversity of younger lineages and not as many lineages treated as genera due to their more recent diversification and significant morphological similarities (Bank et al. 2021). As the phylliids of *Melanesia* have been reviewed in the past few years, through detailed morphological examination of eggs, freshly hatched nymphs, and fine details of adults, coupled with molecular analyses, the significant morphological diversity of this region is now being described with more clarity.

This region is, however, severely under-collected as many of the species of *Melanesia* are only known from type specimens or only a few representatives of each species. Likely the species diversity of *Melanesia* is also notably diverse, but due to this limited sampling species ranges and diversity are only beginning to be unraveled. Hopefully future research efforts in *Melanesia* will recover representatives of these herein described genera which have yet to be sequenced to include them in molecular phylogenies to clarify the higher-level taxonomic classifications of the leaf insects.

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Supplementary material 1

Material examined

Authors: Royce Cumming, Stéphane Le Tirant

Data type: specimen data

Explanation note: Material examined (specimen data and deposition/ observational records) for localities used within the distribution map (Fig. 5) and to accompany the discussion of each species.

Link: https://doi.org/10.3897/zookeys.1110.80808.suppl1

Supplementary material 2

List of species/specimens included within the phylogenetic analyses of this study (molecular data portion)

Authors: Royce Cumming, Stéphane Le Tirant

Data type: molecular sequence data accession numbers

Explanation note: List of species/specimens included within the phylogenetic analyses of this study (molecular data portion). Taxonomic information, specimen collection locality, sample ID, and collection deposition are provided. GenBank accession numbers are listed for each sequence used.

Link: https://doi.org/10.3897/zookeys.1110.80808.suppl2

Supplementary material 3

Nexus file containing the molecular sequences and morphological data

Authors: Royce Cumming, Stéphane Le Tirant

Data type: molecular and morphological sequence data

Explanation note: Nexus file containing the molecular sequences and morphological data utilized within the phylogenetic analyses.

Link: https://doi.org/10.3897/zookeys.1110.80808.suppl3

Supplementary material 4

List of adult morphological characters and their states

Authors: Royce Cumming, Stéphane Le Tirant

Data type: morphological features and their available states

Explanation note: List of adult morphological characters and their states used to code the morphological matrices.

Link: https://doi.org/10.3897/zookeys.1110.80808.suppl4

Supplementary material 5

List of species and matrix of morphological characters for adults of outgroup phasmid and representatives from all Phylliidae genera for phylogenetic analyses

Authors: Royce Cumming, Stéphane Le Tirant

Data type: morphological matrix data

Explanation note: List of species and matrix of morphological characters for adults of outgroup phasmid and representatives from all Phylliidae genera for phylogenetic analyses.

Link: https://doi.org/10.3897/zookeys.1110.80808.suppl5

Supplementary material 6

Mr. Bayes run logs for the male and female matrices

Authors: Royce Cumming, Stéphane Le Tirant

Data type: phylogenetic analyses run logs with settings

Explanation note: Mr. Bayes run logs for the male and female matrices.

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Supplementary material 7

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Description of *Alvania wangi* Xu, Qi & Kong, sp. nov. (Mollusca, Gastropoda, Littorinimorpha, Rissoidae) from the East China Sea

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Abstract

Alvania wangi Xu, Qi & Kong, **sp. nov.** (Mollusca, Gastropoda, Littorinimorpha, Rissoidae) was discovered within the intertidal zone in the Nanji Islands and Zhoushan Islands, Zhejiang Province, China. It has a radula characteristic of *Alvania* Risso, 1826, a protoconch sculptured with micro pits and lamellae between spiral lirae, and a teleoconch with growth lines and subobsolete cords. Specimens were examined using an integrative taxonomic approach incorporating morphological observations and phylogenetic analyses of concatenated mitochondrial 16S rRNA and nuclear 28S rRNA gene sequences. The findings suggest that the new species is sister to *Alvania circinata* A. Adams, 1861 and is probably endemic to the shallow waters of the East China Sea.

Keywords

Micromolluscs, morphology, new species, phylogenetics, systematics

Introduction

Rissoidae Gray, 1847 is a family of highly diversified and widespread microgastropods (Ponder 1985; Hasegawa 2014; Criscione et al. 2017). Comprising hundreds of species, *Alvania* is one of the most diverse genera within Rissoidae. It is found worldwide, except in the Antarctic and sub-Antarctic regions (Ponder 1985), and in shallow to bathyal waters (e.g., Ávila et al. 2012; Hasegawa 2014; Hoffman and Freiwald 2021). *Alvania* is especially abundant in the Mediterranean Sea (Ávila et al. 2012; Criscione et al. 2017) and the North Atlantic (e.g., Warén 1973, 1974), and many new species from these regions have been described in the last five years (Amati and Chiarelli 2017; Bitlis and Öztürk 2017; Villari 2017; Amati et al. 2018, 2019, 2020a, b; Hoffman and Freiwald 2021). The Indo-West Pacific region (Ekman 1934, 1935, 1953) has the most diverse marine molluscan fauna (Lozouet and Plaziat 2008). However, only a few species of *Alvania* have been described or recorded from the Western Indian Ocean (e.g., Bozzetti 2017; Perugia 2021), Australasia (e.g., Laseron 1956; Ponder 1967), the Philippines (e.g., Poppe et al. 2018), Thailand (Bu-on and Dumgrongrojwattana 2019), China (e.g., Sowerby 1894; Zhang et al. 2016), and Japan (Adams 1861; Higo et al. 1999; Hasegawa 2014; Okutani 2017). Studies on *Alvania* from the East China Sea have been rarely reported. Only *Alvania circinata* A. Adams, 1861 was recorded from Kyushu (Okutani 2017); however, its type locality is Sado Island in the Sea of Japan (Adams 1861).

Classification of *Alvania* species based on their external features is rendered difficult by considerable convergence in shell characteristics and variations in the degree of development of the upper oviduct gland in females and the number of seminal receptacles in males (Johansson 1955; Fretter and Patil 1961; Golikov and Starobogatov 1975; Ponder 1985). Criscione et al. (2017) conducted the most comprehensive molecular phylogenetic study of *Alvania* to date, and provided a useful phylogenetic approach to better identify these rissoids. During two field studies conducted by the Laboratory of Shellfish Genetics and Breeding (hereafter LSGB) in 2020–2021, microgastropod samples were collected from the Nanji Islands and Zhoushan Islands, China. By integrating morphological observations and phylogenetic analyses based on the mitochondrial 16S rRNA gene fragment (hereafter 16S) and partial nuclear 28S rRNA gene (hereafter 28S), several rissoiform gastropods with peculiar shell characteristics were identified as belonging to a new species, described herein as *Alvania wangi* Xu, Qi & Kong, sp. nov.

Methods

Algae were scraped from intertidal rocks at Dalei Island and Miaozihu Island (Table 1; Figs 1, 2) in the East China Sea. The collected algae were washed manually and vigorously with seawater to obtain specimens of micromolluscs. The specimens were then subjected to the boiling method (Fukuda et al. 2007), fixed in 95% ethanol, and stored at –30 °C. Specimens mixed with debris were segregated, observed under a stereomicroscope (Nikon SMZ 800N; Nikon, Tokyo, Japan), and picked out using fine-tip forceps. Well-preserved specimens were then placed into 1.5 mL cryogenic vials containing 95% ethanol. The specimens were sonicated at a frequency of 40kHz for 2 minutes and selected as the type material on which further analysis and the new species description were based.

Table 1. Sampling information.

Location	Locality name	Collection date	Collector	Geo-coordinates
1	Dalei Island, Nanji Islands National Nature Reserve, Wenzhou, China	23 Jul. 2020	Biyang Xu, Lu Qi	27°29.82'N, 121°06.17'E
2	Miaozihu Island, Zhongjieshan Islands Special Marine Reserve, Zhoushan, China	09 Apr. 2021	Biyang Xu, Lu Qi	30°11.77'N, 122°41.41'E

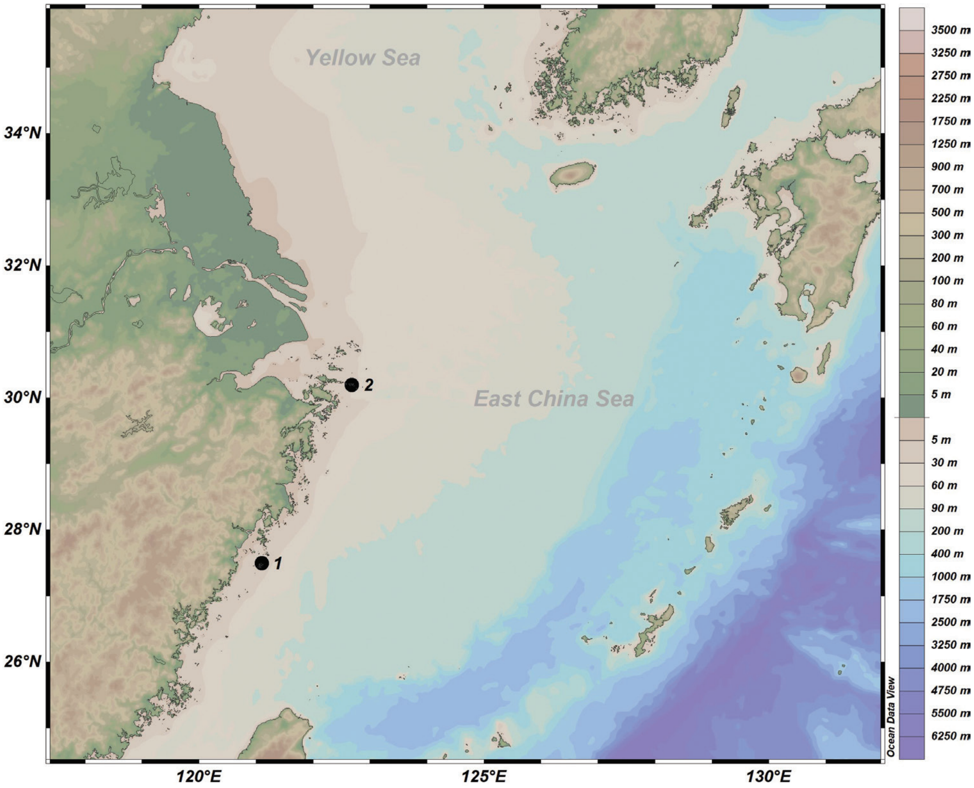

Figure 1. Map of sampling sites.

Shells were photographed with a DS-Fi2 digital camera (Nikon, Tokyo, Japan) mounted on a stereomicroscope. The image stacks of standard views of the shells (Callomon 2019) were produced and combined using Helicon Focus 8.0.2. For scanning electron microscopy (SEM) studies, the radula was collected during DNA extraction following the method described by Qi et al. (2020), cleaned using 10% NaOH for 0.5 h, and rinsed in double-distilled water (ddH$_2$O). The shells, opercula, and radulae were then gilded and examined using a Tescan Vega3 scanning electron microscope (Tescan, Brno, Czech Republic). The sampling locations were mapped using Ocean Data View 5.5.2 (Schlitzer 2021).

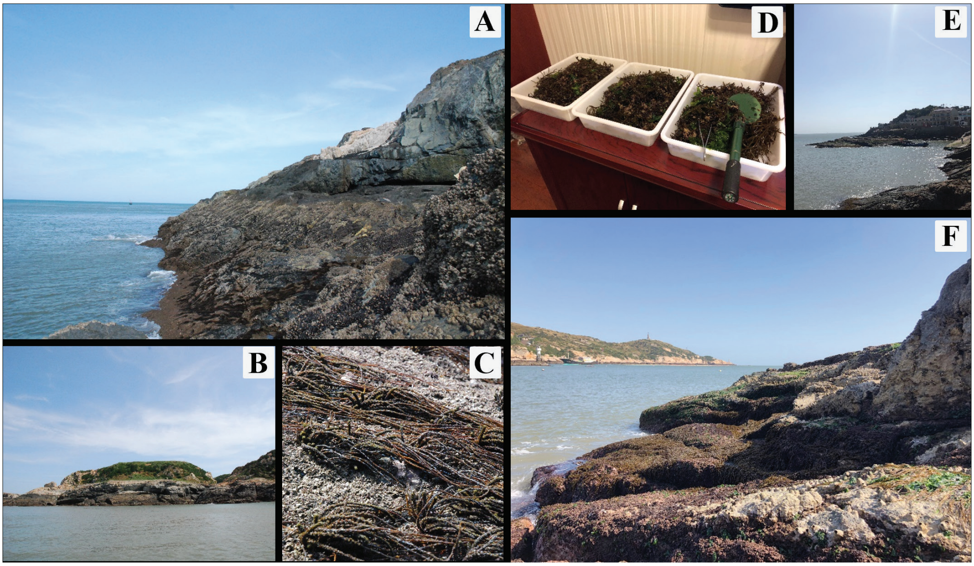

Figure 2. Pictures of the two sampling sites **A** rocky intertidal zonation on the coast of Dalei Island (location 1) **B** distant view of part of Dalei Island **C** algae growing on the lower intertidal zone of Dalei Island **D** algae collected from Miaozihu Island **E** distant view of part of Miaozihu Island **F** rocky intertidal zonation on the coast of Miaozihu Island (location 2) (photographs by Biyang Xu).

Genomic DNA was extracted from the specimens using the TIANamp Marine Animals DNA Kit (Tiangen, Beijing, China), following the manufacturer's protocol. DNA was extracted in elution buffer (25 μL) and stored at 4 °C for short-term use. The 28S and 16S sequences were then amplified (Table 2). These gene markers have been widely and effectively used for phylogenetic studies of the family Rissoidae (Hausdorf et al. 2003; Criscione and Ponder 2013; Takano and Kano 2014; Baptista 2017; Criscione et al. 2017; Baptista et al. 2019). The target gene sequences were amplified using PCR; each sample (10 μL) contained 4 μL of DNA extract and 6 μL of PCR mix (0.2 μL ddH$_2$O, 5 μL of 2× Taq Plus Master Mix II (Dye Plus; Vazyme, Nanjing, China), 0.4 μL of 10 μM forward primer, and 0.4 μL of 10 μM reverse primer). The amplified products were verified using 2% (w/v) agarose gel electrophoresis. After running the gel at 115V for 0.5 h, the products were stained with ethidium bromide, visualized using a UV transilluminator (Peiqing, Shanghai, China), and, finally, Sanger sequenced using the PCR primer pairs (Table 2). The sequences obtained were manually corrected for misreads, and the forward and reverse strands were both primer-trimmed and merged into contigs using SeqMan v.6 (DNASTAR, Madison, WI, USA). The assembled 28S and 16S sequences were BLAST searched to check for contamination and then deposited in GenBank (for accession numbers, see Suppl. material 1). The 28S and 16S sequences of related Rissoidae species were retrieved

Table 2. Target gene, primer details and PCR conditions (temperature, time, and number of cycles) applied in the present study.

Gene	Primer	Sequence 5’-3’	Reference	PCR conditions
28S	28SDKF LSU 1600R	F: GATCGGACGAGATTACCCGCTGAA R: AGCGCCATCCATTTTCAGG	Strong et al. 2011 Williams et al. 2003	94 °C (7’), 58 °C (1’), 72 °C (2’) [x1]; 94 °C (1’), 52 °C (1’), 72 °C (2’) [x35]; 94 °C (1’), 52 °C (1’), 72 °C (7’) [x1]
16S	16SARis 16SBRis	F: TGCCTGTTTAGCAAAAACAT R: CCGGTCTGAACTCAGATCATGT	Criscione and Ponder 2013	94 °C (5’), 52 °C (30”), 72 °C (1’) [x1]; 94 °C (30”), 52 °C (30”), 72 °C (1’) [x40]; 94 °C (30”), 52 °C (30”), 72 °C (7’) [x1]

from GenBank (Suppl. material 1). *Amphithalamus fulcira* (Laseron, 1956) (family Anabathridae) was selected as the outgroup.

The 16S and 28S sequences were aligned independently using MUSCLE v3.8.31 (Edgar 2004). Areas of uncertain alignment were removed using Gblocks 0.91b (Castresana 2000) with the parameters -t=d -b1=36 -b2=45 -b3=3 -b4=5 -b5=a and -t=d -b1=25 -b2=41 -b3=8 -b4=10 -b5=a for 16S and 28S sequences, respectively. Substitution saturation of 16S and 28S was tested using DAMBE 7 (Xia 2018). The aligned 16S and 28S sequences from the same species were concatenated into one sequence with PhyloSuite 1.2.2 (Zhang et al. 2020) for two-gene analysis. Phylogenetic analyses were conducted for concatenated sequences using maximum likelihood (ML) and Bayesian inference (BI) methods. Prior to the phylogenetic analyses, the Bayesian information criterion (Schwarz 1978) was calculated using jModelTest 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012) and GTR + G + I was identified as the best-fit model of nucleotide substitution. ML analyses were performed using RAxML v8.2.12 (Stamatakis 2014) and node support was assessed using 1000 ML bootstrap replicates. BI analyses were conducted using MrBayes v3.2.3 (Ronquist et al. 2012). Bayesian posterior probabilities were estimated by running 10,000,000 generations of four Markov chain Monte Carlo chains, including one cold chain and three heated chains, in two parallel runs. Trees were sampled every 1000 generations. The initial 25% of the trees was discarded as burn-in, and the remaining trees were summarized as 50% majority-rule trees. Stationarity was reached when the average standard deviation of split frequencies (Ronquist et al. 2012) was less than 0.01 and the potential scale reduction factor (Gelman and Rubin 1992) approached 1.0. Trees were graphed using FigTree v1.4.4 (Rambaut 2018). Pairwise distances of 16S (Table 3) and 28S (Table 4) within the *Alvania* clade were computed with MEGA X (Kumar et al. 2018), using the substitution model K2P.

All the materials analyzed in this study are deposited in the Laboratory of Shellfish Genetics and Breeding, Fisheries College, Ocean University of China, Qingdao, China.

Table 3. K2P pairwise sequence distances (in percentage) between the analyzed specimens based on 16S rRNA.

Species	1	2	3	4	5	6	7	8	9	10	11	12
Alvania wangi sp. nov.	–											
Alvania aeoliae	12.93	–										
Alvania circinata	8.69	13.37	–									
Alvania discors	13.19	7.16	13.40	–								
Alvania lanciae	13.46	6.00	14.20	8.36	–							
Alvania lineata	12.93	0.00	13.37	7.16	6.00	–						
Alvania novarensis	6.78	11.83	8.64	12.59	12.84	11.83	–					
Alvania ogasawarana	7.74	12.63	9.11	12.86	13.66	12.63	5.33	–				
Alvania scabra	14.83	8.21	15.27	9.39	8.92	8.21	13.40	14.47	–			
Alvania tenera	15.58	14.26	18.33	14.13	12.31	14.26	14.20	15.02	13.97	–		
Crisilla galvagni	13.78	12.19	15.24	13.73	11.96	12.19	13.66	12.89	12.75	14.55	–	
Cingula trifasciata	13.90	10.77	14.14	12.26	11.25	10.77	12.29	13.65	12.32	12.35	12.60	–

Table 4. K2P pairwise sequence distances (in percentage) between the analyzed specimens based on 28S rRNA.

Species	1	2	3	4	5	6	7	8	9	10	11	12
Alvania wangi sp. nov.	–											
Alvania aeoliae	4.51	–										
Alvania circinata	1.14	5.25	–									
Alvania discors	5.89	2.69	6.06	–								
Alvania lanciae	4.67	1.29	5.32	2.30	–							
Alvania lineata	4.51	0.00	5.25	2.69	1.29	–						
Alvania novarensis	3.22	5.56	3.38	6.29	5.47	5.56	–					
Alvania ogasawarana	3.15	5.39	3.15	6.13	5.23	5.39	1.44	–				
Alvania scabra	5.00	1.29	5.73	3.56	1.75	1.29	5.96	5.80	–			
Alvania tenera	4.43	2.45	5.08	3.55	2.53	2.45	5.38	5.14	3.15	–		
Crisilla galvagni	4.75	2.37	5.24	3.79	2.53	2.37	5.71	5.38	3.00	2.60	–	
Cingula trifasciata	4.27	3.63	4.67	4.43	3.23	3.63	5.30	5.06	3.87	2.69	2.85	–

Results

The 16S (489 bp) and 28S (1414 bp) regions of *Alvania wangi* Xu, Qi & Kong, sp. nov. were successfully amplified and sequenced. No cross-contamination or substitution saturation of 16S or 28S was detected. The K2P distances between *Alvania wangi* Xu, Qi & Kong, sp. nov. and the analyzed species ranged from 6.78% to 15.58% and 1.14% to 5.89% for 16S and 28S, respectively (Tables 3, 4). *Alvania wangi* Xu, Qi & Kong, sp. nov. is closely related to other *Alvania* species. BI (Fig. 5) and ML (Fig. 6) phylogenetic trees indicated that it is placed in the *Alvania* clade and sister to *Alvania circinata* A. Adams, 1861, with 100% nodal support from both BI and ML methods.

Systematics

Family Rissoidae Gray, 1847
Genus *Alvania* Risso, 1826

Alvania wangi Xu, Qi & Kong, sp. nov.

https://zoobank.org/680409A3-C0A8-4571-98E5-3B28D80A7C86
Figs 3, 4

Type locality. China, Zhejiang: Pingyang County, the Nanji Islands National Nature Reserve, Dalei Island, 27°29.82'N, 121°06.17'E.

***Holotype*:** Alcohol-fixed, photographed by SEM; original label: "CN, ZJ, Pingyang, Dalei, 27°29.82'N, 121°06.17'E, 23 Jul. 2020, B.Y. Xu & L. Qi" "LSGB mg325408 0601".

***Paratypes*:** Alcohol-fixed, five specimens, original label: "CN, ZJ, Pingyang, Dalei, 27°29.82'N, 121°06.17'E, 23 Jul. 2020, B.Y. Xu & L. Qi" "LSGB mg325408 0602 to 0606"; alcohol-fixed, ten specimens, original label: "CN, ZJ, Zhoushan, Miaozihu, 30°11.77'N, 122°41.41'E, 09 Apr. 2021, B.Y. Xu & L. Qi" "LSGB mg316141 0601 to 0610".

Diagnosis. Shell minute, ovate-conical, thin, with weakly convex whorls, non-umbilicate. Protoconch paucispiral, sculptured with micro pits and lamellae between spiral lirae. Teleoconch with subobsolete cords and growth lines. Umbilicus chink very narrow and slit-like. Aperture oval, broadly rounded anteriorly, slightly angled posteriorly; peristome simple; outer lip orthocline, without varix. Periostracum thin.

Description. ***Shell*:** (Figs 3A–D, 4A–D) minute, ovate-conical, semitransparent, thin but not fragile. Protoconch (Figs 3E, F, 4E, F) dome-shaped, with 1.5 whorls, height ~190 µm, diameter 353 µm, translucent; nucleus apparently smooth, followed by half whorl ornamented with dense and irregular micro pits (Fig. 3F) which subsequently fuse into micro-lamellae between 9–10 spiral lirae (Fig. 3E, F), roughly three times wider than interspaces. Protoconch-teleoconch border simple, marked by a shallow depression of the spire (Fig. 4F). Teleoconch with 2 whorls, slightly convex, with 3–4 subobsolete spiral cords on penultimate whorl, 9–10 on body whorl, 3–4 relatively distinct spiral cords on base, roughly equidistant and broader than interspaces, crossing with exceedingly fine growth lines, and few weak spiral furrows in the periumbilical area. Periphery of body whorl broadly rounded. Suture impressed, simple. Aperture oval, with simple peristome, angled posteriorly, rounded anteriorly; inner lip narrow, anteriorly slightly separated from lower base, posteriorly attached to base; outer lip orthocline, without external varix and internal lirae. Umbilical chink represented by a narrow and short groove. Periostracum very thin, barely visible. Background color yellowish and brownish, forming interlaced band.

***Operculum*:** (Fig. 3G, H) subovate, horny, simple, thin, smooth, posteriorly broadly angled, anteriorly rounded; nucleus eccentric; last whorl long and large, yellowish, and translucent.

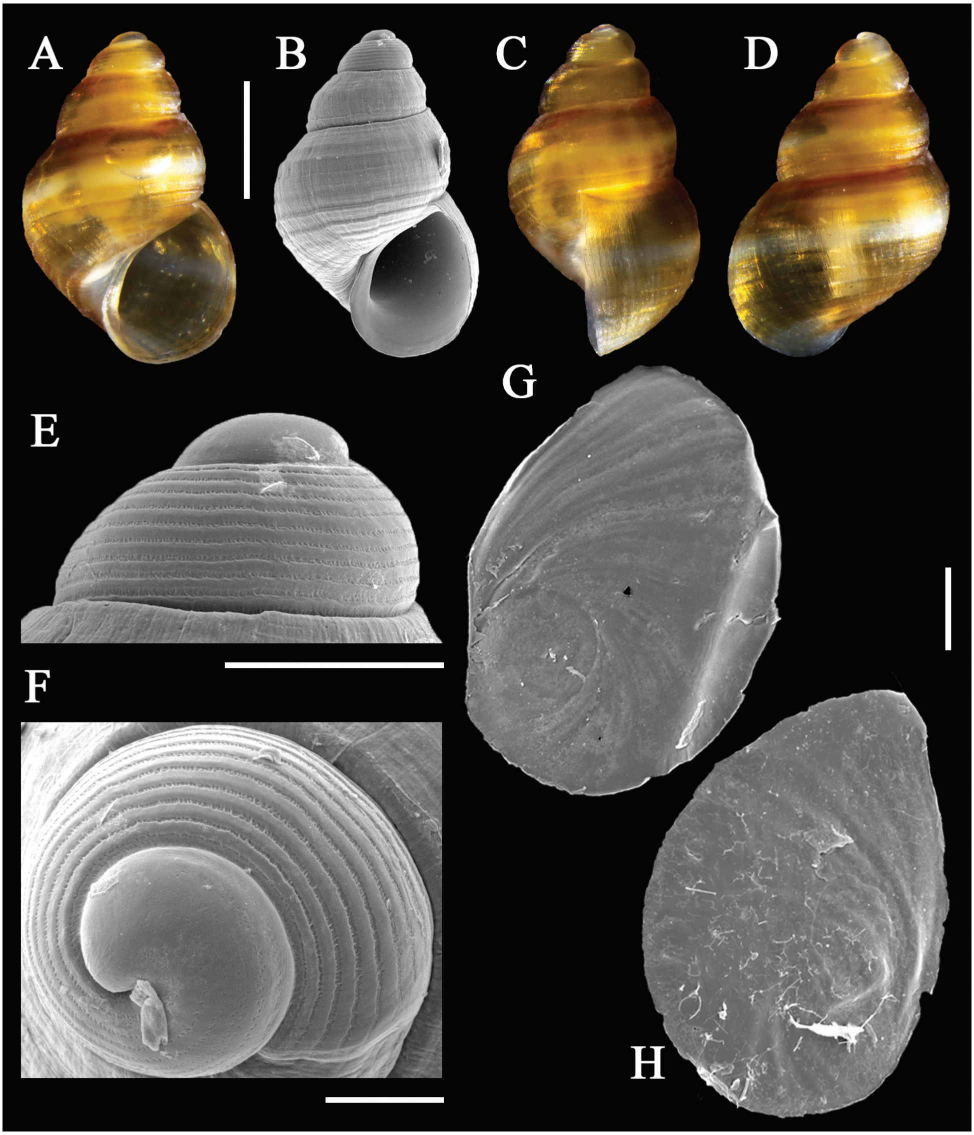

Figure 3. Holotype of *Alvania wangi* Xu, Qi & Kong, sp. nov. (**A–D**) shell **A** apertural view of shell **B** scanning electron micrographs of apertural view of shell **C** lateral view of shell **D** dorsal view of shell **E** protoconch **F** apical view of protoconch (**G, H**) operculum **G** outer face of operculum **H** inner face of operculum. Scale bars: 500 µm (**A–D**); 200 µm (**E**); 100 µm (**F–H**).

***Radula*:** (Fig. 4G–J) typical of *Alvania*. Central teeth $\frac{2-3+1+2-3}{1+11+1}$, with long triangular cutting edge, small cusps, a single pair of basal denticles and a pair of smaller denticles produced from the thickened lateral margins; U-shaped ventral extension poorly to not developed. Lateral teeth 3 + 1 + 6–7, elongate, each with triangular and asymmetric cusps: larger primary cusp long and wide; 3 inner and 6–7 outer, smaller

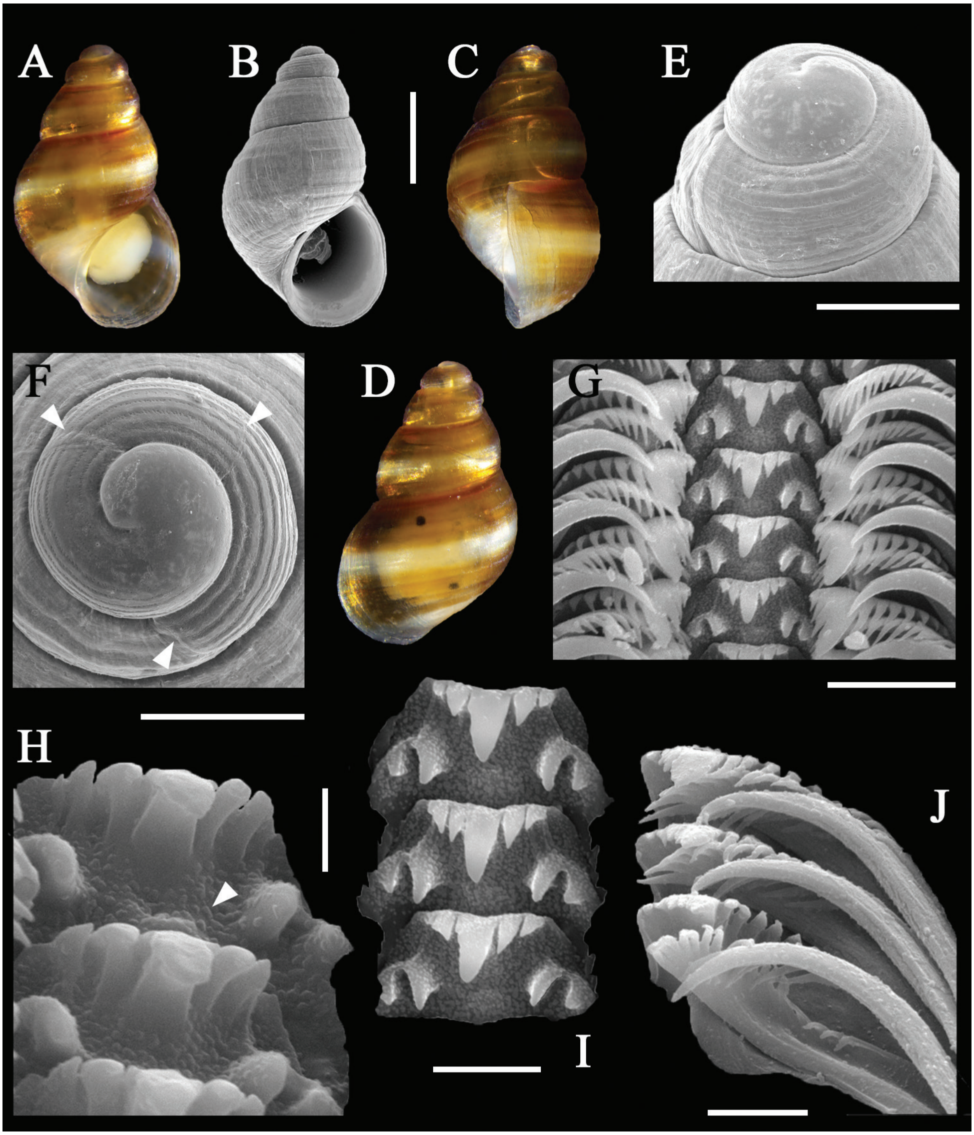

Figure 4. Paratype of *Alvania wangi* Xu, Qi & Kong, sp. nov. (**A–D**) shell **A** apertural view of shell **B** scanning electron micrographs of apertural view of shell **C** lateral view of shell **D** dorsal view of shell **E** protoconch **F** apical view of protoconch (top two arrowheads show the two growth lines of the protoconch; bottom arrowhead indicates demarcation between protoconch and teleoconch) **G** radula **H** oblique view of central teeth (arrowhead indicates pustules on base of central teeth) **I** central teeth **J** lateral and marginal teeth. Scale bars: 500 µm (**A–D**); 200 µm (**E, F**); 10 µm (**G**); 2 µm (**H**); 5 µm (**I, J**).

pointed denticles at the sides. Marginal teeth elongated, cusps subequal; inner marginal teeth with ~17 cusps on outer 1/3 of teeth, outer marginal teeth with ~6–7 cusps on inner distal 1/3.

***Soft parts*:** Yellowish head and foot. A pair of black-pigmented eyes (Fig. 4D) can be seen through the translucent shell. Cephalic tentacles yellow, behind the eyes.

Etymology. The species is named after Prof. Rucai Wang, who established LSGB and was one of the founders of shellfish culture in China.

Known distribution. In addition to the type locality, this species can also be found in the middle intertidal zone of Miaozihu Island, the northeastern part of Zhoushan City, China, 30°11.77'N, 122°41.41'E.

Remarks. The characteristics of *Alvania wangi* Xu, Qi & Kong, sp. nov. are consistent with those of *Alvania* described by Risso (1826) and Ponder (1967, 1985). It also possesses some unique features, such as the subobsolete spiral cords and the protoconch sculptured with pits, lirae, and micro-lamellae. The new species can be clearly distinguished from other *Alvania* species in adjacent waters (Suppl. material 2). Additionally, it resembles *A. carinata* (da Costa, 1778), *A. cimex* (Linnaeus, 1758), *A. lineata* Risso, 1826, *A. punctura* (Montagu, 1803), and *A. scabra* (Philippi, 1844) in radula morphology, but differs both in clathrate sculpture and protoconch features. Among all species of Rissoidae, the new species may be most closely related to *Crisilla simulans* (Locard, 1886) and *C. perminima* (Manzoni, 1868), which are found independently in the Mediterranean Sea (Morena and Luigi 2005) and northwestern Africa (Oliver et al. 2019). It shares similar color patterns, sizes, the general outline of the shell, and the lack of conspicuous axial sculpture with the two *Crisilla* species. However, it differs from these species in the presence of 9–10 relatively clear spiral cords on the body whorl and in the spiral microsculptures of the protoconch. Furthermore, the new species has a similar protoconch sculpture to those of *Cingula aequa* (E. A. Smith, 1890) and *C. farquhari* (E. A. Smith, 1910) in terms of the number and sculpture of spiral lirae with axial micro-lamellae. However, the two *Cingula* species lack the rows of pits and smooth zones found in the protoconch of *Alvania wangi* Xu, Qi & Kong, sp. nov. (Fig. 3E, F). Moreover, the central tooth of the new species (Fig. 4G–I) is similar to that of *Cingula trifasciata* (J. Adams, 1800), the type species of *Cingula* Fleming, 1818, which, however, has a moderately developed "U-shaped" ventral extension (Ponder 1985) and lacks pustules (Fig. 4H) on the base. *Alvania wangi* Xu, Qi & Kong, sp. nov. can be distinguished from the above-mentioned species based on its peculiar characteristics and is therefore regarded as a distinct species of *Alvania*.

Discussion

The new species described in the present study differs from the previously reported *Alvania* species with respect to the sculptures on its shells. Molecular evidence supports the morphological identification. Moreover, phylogenetic reconstruction revealed consistent topologies of *Alvania* in both BI (Fig. 5) and ML (Fig. 6) analyses, and confirmed that *Alvania wangi* Xu, Qi & Kong, sp. nov. is a valid species within the *Alvania* lineage.

Notably, the addition of *Alvania wangi* Xu, Qi & Kong, sp. nov. changes the topology of some subclades within the *Alvania* clade (Criscione et al. 2017).

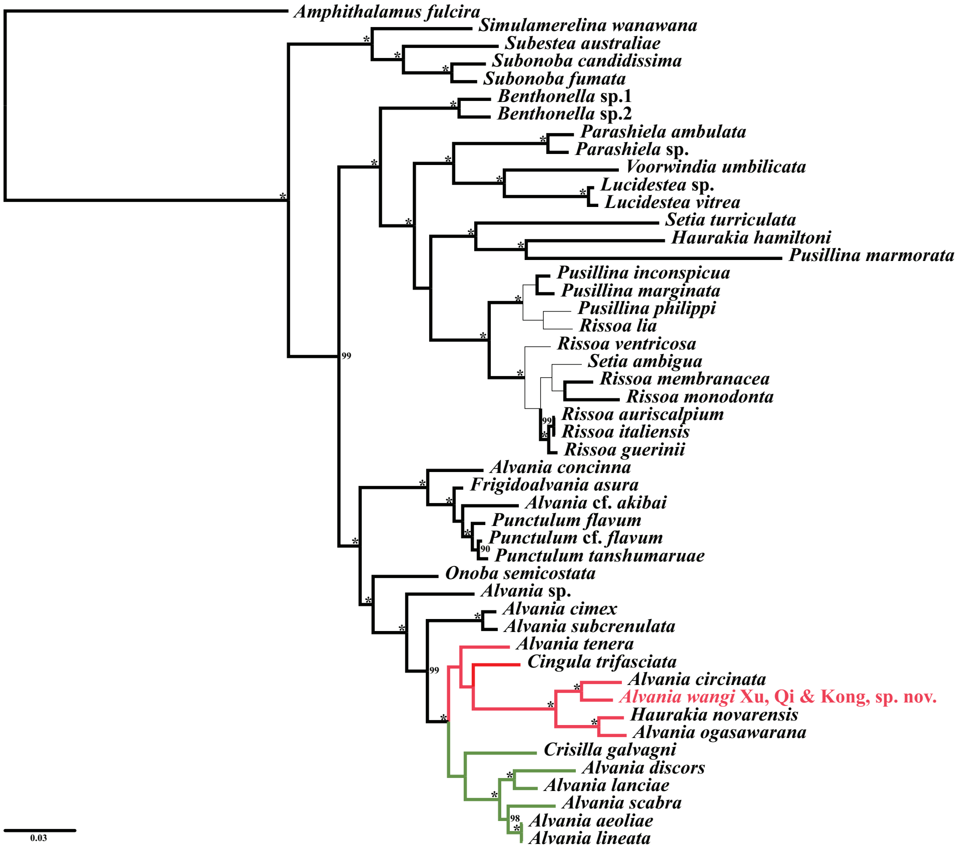

Figure 5. Bayesian consensus phylogram based on analysis of the concatenated 16S and 28S sequences. Numbers on branches indicate nodal support (in percentage) by Bayesian posterior probabilities (BPP; only values ≥ 90% are shown; values of 100% are represented by asterisks). Thick lines mark branches that are consistent with the topology of the ML tree.

Alvania tenera (Philippi, 1844) is sister to the clade that includes *Cingula trifasciata* (J. Adams, 1800), *A. circinata* A. Adams, 1861, *Alvania wangi* Xu, Qi & Kong, sp. nov., *A. ogasawarana* (Pilsbry, 1904), and *Haurakia novarensis* (Frauenfeld, 1867). The clade marked in green (Figs 5, 6) includes *Crisilla galvagni* (Aradas & Maggiore, 1844), *A. discors* (T. Brown, 1818), *A. lanciae* (Calcara, 1845), *A. scabra* (Philippi, 1844), *A. aeoliae* Palazzi, 1988, and *A. lineata* Risso, 1826. *Crisilla galvagni* did not cluster with *A. tenera* in the subclade marked in red (Figs 5, 6), although both species have an ovate-conical shell sculptured with spiral cords and weaker growth lines. Instead, *C. galvagni* is sister to the five *Alvania* species mentioned above, which have conical shells with strong axial ribs but weaker spirals. This indicates that *C. galvagni* is closely related to *Alvania* (Ponder 1985). However, the *Alvania* clade remains polyphyletic (Criscione et al. 2017), which is particularly reflected in the subclade marked in red (Figs 5, 6).

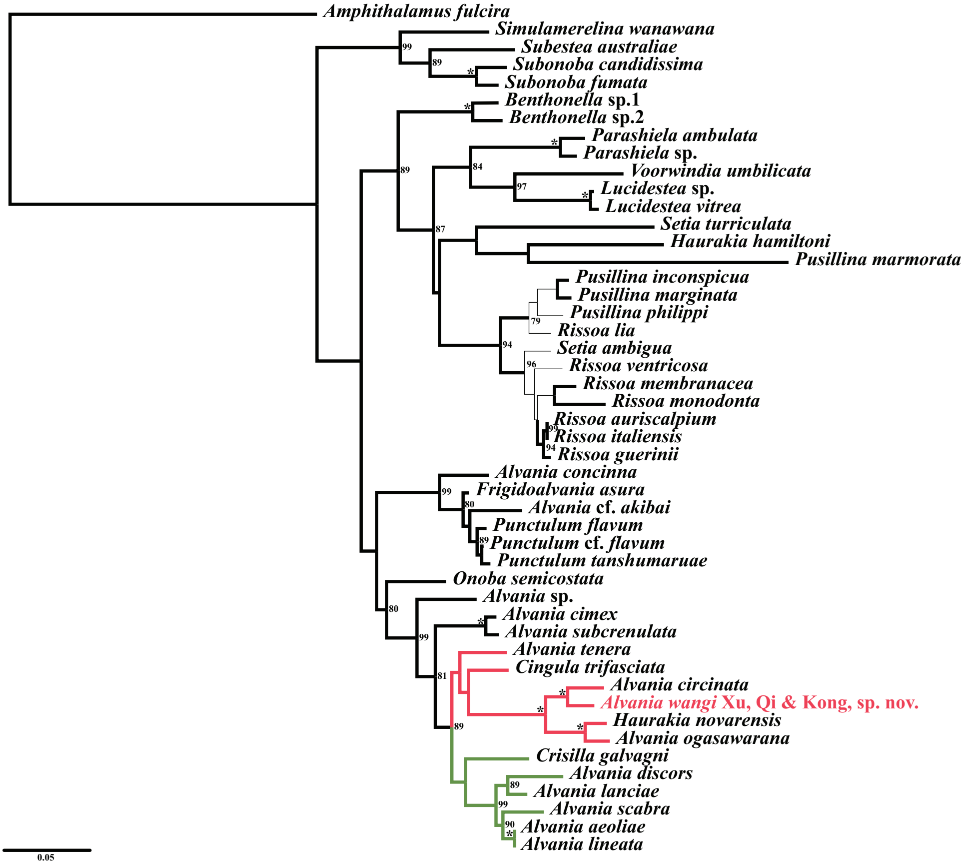

Figure 6. Maximum-likelihood phylogram based on analysis of the concatenated 16S and 28S sequences. Numbers on branches indicate nodal support (in percentage) by ML bootstrap (BTSP; only values ≥ 70% are shown; values of 100% are represented by asterisks). Thick lines mark branches that are consistent with the topology of the BI tree.

In the subclade marked in red (Figs 5, 6), *Alvania wangi* Xu, Qi & Kong, sp. nov. shows a close relationship with *A. circinata* A. Adams, 1861, with 100% nodal support (Figs 5, 6). *Alvania circinata* was originally described from Sado Island (Adams 1861) in the Sea of Japan, and was later found in the intertidal zone of the Boso Peninsula, Oga Peninsula, and Kyushu (Okutani 2017). *Alvania circinata* is currently regarded as taxon inquirendum (Criscione et al. 2017) owing to inadequate descriptions, undesignated types, and scattered or even lost potential syntypes (Ponder and De Keyzer 1992; Hasegawa 2014). Thus, the new species described in the present study provides genetic support for reassigning *A. circinata* to *Alvania* and further clues for solving the taxonomic uncertainty surrounding this species.

The protoconch of *Alvania wangi* Xu, Qi & Kong, sp. nov. is not sculptured with granules between a few spiral lirae like that of most *Alvania* species (Ponder 1985). It has rows of shallow pits and a smooth area in protoconch I and lamellae between the lirae in protoconch II (Figs 3E, F, 4E, F). This structure might be a remnant of the early stages of the evolution of *Alvania* (Ponder 1985). *Alvania wangi* Xu, Qi & Kong, sp. nov. shows modifications of the general pattern of the protoconch and is probably a direct developer (Ponder 1985) with limited dispersal ability to achieve an extensive geographical distribution (Thorson 1950; Shuto 1974; Hansen 1980). Notably, it does not cluster with bathyal rissoid gastropods (Hasegawa 2014; Criscione et al. 2017) and possesses distinct black eyes, which are indicative of its shallow water origin (Hasegawa 2014). Moreover, this species has not been recorded in Japan (Takenori Sasaki, personal communication), and other species of *Alvania* in Japan are currently known to inhabit only the bathyal depths of the Sea of Japan and adjacent waters (Hasegawa 2014). Considering that this deep basin is a barrier for the dispersal of shallow water lecithotrophic species (Shuto 1974), *Alvania wangi* Xu, Qi & Kong, sp. nov. may be endemic to the East China Sea.

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Supplementary material 1

GenBank accession numbers for species included in the molecular analyses

Authors: Biyang Xu, Lu Qi, Lingfeng Kong, Qi Li

Data type: docx file

Explanation note: GenBank accession numbers for species included in the molecular analyses.

Link: https://doi.org/10.3897/zookeys.1110.82173.suppl1

Supplementary material 2

A comparison among *Alvania* species found in the East China sea and adjacent waters

Authors: Biyang Xu, Lu Qi, Lingfeng Kong, Qi Li

Data type: docx file

Explanation note: A comparison among *Alvania* species found in the East China sea and adjacent waters.

Link: https://doi.org/10.3897/zookeys.1110.82173.suppl2