

Research article

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Systematic revision of the limestone karst-restricted land snail genus *Aenigματοconcha* (Eupulmonata: Helicarionidae), with description of a new species

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Abstract. Thai limestone karsts are known to contain a rich biodiversity of animals, especially terrestrial snails, but still require further intensive exploration to evaluate their biodiversity. To date, only a few studies on the limestone karst-inhabiting land snail genera have been published. The present work focuses on the species diversity and phylogenetic relationships of the limestone karst-restricted land snail genus *Aenigματοconcha* from Thailand, based on comparative morphology and molecular evidence. The results yielded three known species (*A. clivicola* Tumpeesuwan & Tumpeesuwan, 2017, *A. sumonthai* Tumpeesuwan & Tumpeesuwan, 2018, and *A. mitis* (Pfeiffer, 1863) comb. nov.), plus a new species (*A. eunetis* Pholyotha & Panha sp. nov.). The phylogenetic analyses of partial fragments of the mitochondrial cytochrome oxidase c subunit I (COI) gene confirmed the monophyly of all recognized species and congruence with the traditional morphology-based species designations. Average uncorrected p-distances of COI sequences between species were 9.7–12.0% and within species were 0.2–4.2%. This study also provides the re-description of penial sculpture, penial sheath, flagellum, penial caecum, and mantle lobe morphology that were neglected from the type species description. The present discovery of a new species increases the known diversity of Thai land snails and will support the conservation planning to protect karst biodiversity.

Keywords. Endemic, Indochina, limestones, COI gene, DNA barcoding.

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Introduction

Situated in the Indo-Burmese biodiversity hotspot region, Thailand is one of the most bio-diverse countries in mainland Southeast Asia (Myers *et al.* 2000). The country boasts many unique limestone formations (Naggs *et al.* 2006; Ridd *et al.* 2011; Latinne *et al.* 2013). Thus, Thailand houses many endemic cave-dwelling animals, such as various groups of vertebrates (e.g., Latinne *et al.* 2013; Pauwels *et al.* 2014; Sumontha *et al.* 2017) and invertebrates (e.g., Sedgwick & Schwendinger 1990; Srisonchai *et al.* 2018; Likhitrakarn *et al.* 2020), especially several families of land snails (e.g., Tongkerd *et al.* 2004; Siriboon *et al.* 2014; Dumrongrojwattana & Tanmuangpak 2020; Pholyotha *et al.* 2020b, 2021; Sutcharit *et al.* 2020b). Although the recent studies of karst-associated land snail species in Southeast Asia have flourished, the number of new species discovered has increased every year, supporting that the Thai fauna on limestone karsts, especially land snails, still remains mostly unexplored.

Native to Thailand, the helicarionid snail genus *Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017 occurs in limestone karst areas only. The specific characteristics of this genus include a medium-sized and umbilicated shell, radula with spatulate-shaped teeth, and genitalia without a dart apparatus (Tumpeesuwan & Tumpeesuwan 2017, 2018). Until now, two species have been included in this genus (*A. clivicola* Tumpeesuwan & Tumpeesuwan, 2017 from Northeast Thailand, and *A. sumonthai* Tumpeesuwan & Tumpeesuwan, 2018 from Southern Thailand). Although *Aenigmatoconcha* was recently described including its genital and radula features, several other diagnostic characters have never been mentioned for either species. These key characters are important for identification at both the specific and generic levels. Thus, the genitalia and mantle lobe morphology were re-examined in this study. Moreover, as the type localities of the two known *Aenigmatoconcha* species are distantly separated by approximately 900 km, it is expected that *Aenigmatoconcha* species may also occur in limestone karsts along the Tenasserim Range from North to South Thailand (Naggs *et al.* 2006; Ridd *et al.* 2011; Latinne *et al.* 2013; Gardner *et al.* 2015).

During a recent intensive field survey throughout Thailand, many helicarionid specimens have been classified as members of *Aenigmatoconcha* on the basis of their shell morphology and genital anatomy. Some populations shared similarities in shell morphology, but the genitalia tended to differ from congeners and were not identical to any currently described species. Generally, helicarionoid snails tend to have a diverse shell form but conserved reproductive organs and mantle lobes. These conserved characters appear to be systematically informative following integrative taxonomic approaches, such as in the Southeast Asian ariophantids (Pholyotha *et al.* 2020b, 2021; Sutcharit *et al.* 2020b) and helicarionids (Páll-Gergely *et al.* 2016; Sutcharit *et al.* 2020a), and in the Australian helicarionids (Hyman & Ponder 2010; Hyman & Köhler 2018, 2019).

In recent years, the mitochondrial cytochrome c oxidase subunit I (COI) gene has been widely used as a standard barcoding marker to delineate species, for systematic revision, and to investigate phylogenetic relationships in various groups of land snails (i.e., Liew *et al.* 2009; Hyman & Ponder 2010; Köhler & Criscione 2015; Hyman & Köhler 2018, 2019; Zhang *et al.* 2020; Siriboon *et al.* 2020). This study integrates morphological and COI gene data to clarify the species boundaries within the genus *Aenigmatoconcha*. The aims of this research are to 1) clarify the species boundaries and relationships within the genus, 2) revise the genitalia and mantle lobe morphology of *A. clivicola* and *A. sumonthai*, 3) re-describe the

long-overlooked species, *A. mitis* (Pfeiffer, 1863), and 4) describe a new species based on morphological (living snails, shells, genitalia, and radula) information and COI gene sequence analyses.

Material and methods

Specimen sampling and morphological studies

Several limestone and non-limestone areas in Thailand were surveyed. All *Aenigματοconcha* specimens were found on limestone areas only (Fig. 1) and were hand-collected. Living snails were euthanized by a two-step method following the AVMA Guidelines for the Euthanasia of Animals (American Veterinary Medical Association 2020) and then fixed in 95% (v/v) ethanol for morphological and DNA studies.

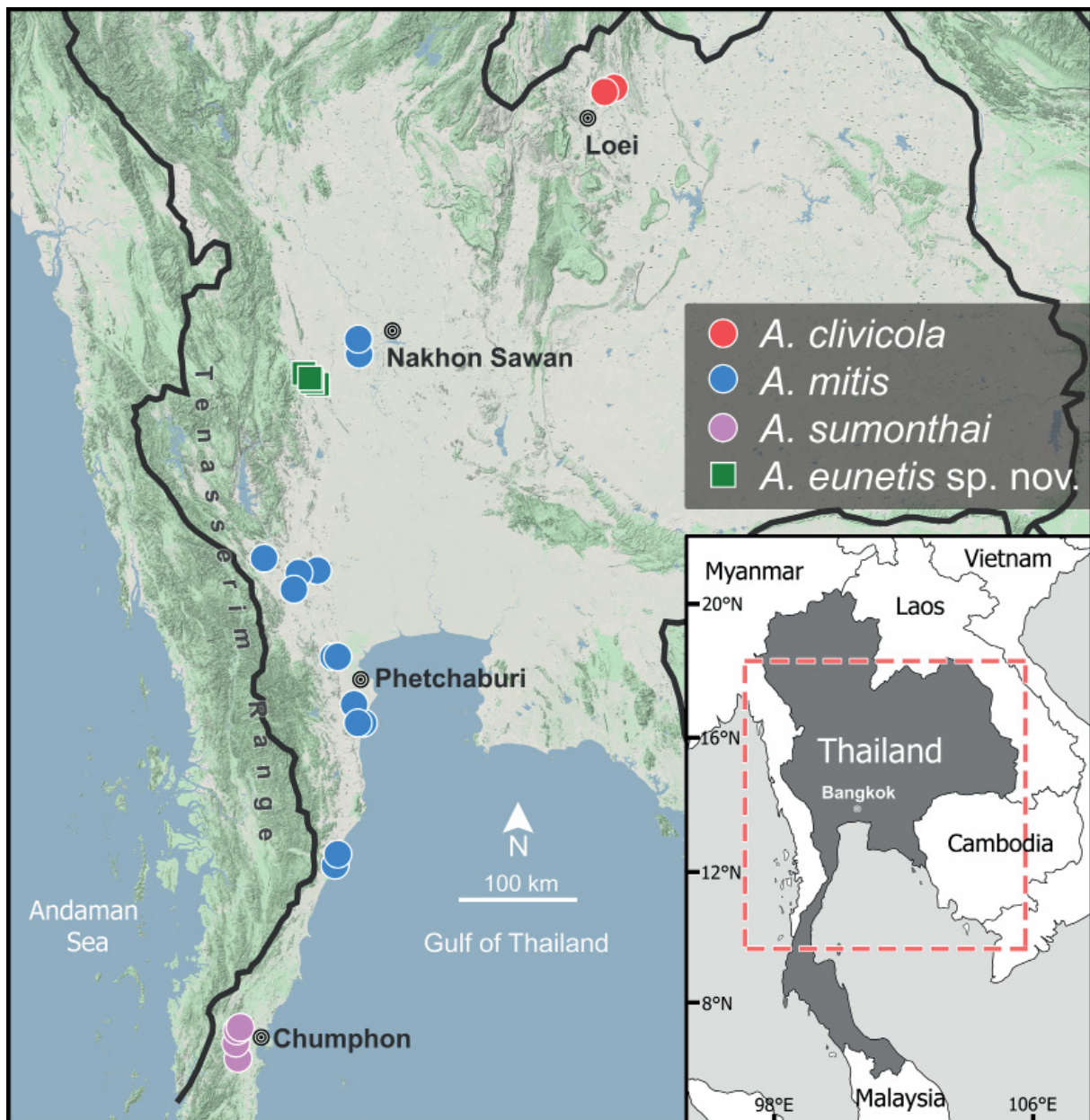


Fig. 1. Geographic distribution of four species of *Aenigματοconcha* Tumpeesuwan & Tumpeesuwan, 2017 based on specimens examined herein.

Table 1. Information of all specimens used in the molecular phylogenetic study.

Species/ specimen code	CUMZ code	Locality	GenBank Number
Helicarionidae Bourguignat, 1877			
<i>Aenigmatoconcha</i> Tumpeesuwan & Tumpeesuwan, 2017			
<i>A. clivicola</i> Tumpeesuwan & Tumpeesuwan, 2017			
NE12	7928	Loei Province, Thailand	MN897082
NE68-1	7929.1	Loei Province, Thailand	MW703614
NE68-2	7929.2	Loei Province, Thailand	MW703615
NE68-3	7929.3	Loei Province, Thailand	MW703616
<i>A. mitis</i> (Pfeiffer, 1863)			
C25	7885	Nakhon Sawan Province, Thailand	MW703617
C26	7849	Nakhon Sawan Province, Thailand	MW703618
W6	7920	Phetchaburi Province, Thailand	MW703619
W8	7914	Kanchanaburi Province, Thailand	MW703620
W47	7921.1	Phetchaburi Province, Thailand	MW703621
W50	7921.2	Phetchaburi Province, Thailand	MW703622
W56	7916	Prachuap Khiri Khan Province, Thailand	MW703623
W57	7915	Prachuap Khiri Khan Province, Thailand	MW703624
W88	7917	Phetchaburi Province, Thailand	MW703625
W89	7918	Phetchaburi Province, Thailand	MW703626
<i>A. sumonthai</i> Tumpeesuwan & Tumpeesuwan, 2018			
S79	7926	Chumphon Province, Thailand	MW703627
S80	7922	Chumphon Province, Thailand	MN897083
S109	7925	Chumphon Province, Thailand	MW703628
S162-2	7927.2	Chumphon Province, Thailand	MW703629
<i>A. eunetis</i> Pholyotha & Panha sp. nov.			
C8	7935	Uthai Thani Province, Thailand	MW703630
C10-1	7933.1	Uthai Thani Province, Thailand	MW703631
C10-3	7933.3	Uthai Thani Province, Thailand	MW703632
C24	7936	Uthai Thani Province, Thailand	MW703633
Other genera			
Helicarionidae Bourguignat, 1877			
<i>Sophina schistostelis schistostelis</i> (Benson, 1859)			MN897023
<i>Sophina conjungens</i> Stoliczka, 1871			MN897033
<i>Sophina bensoni</i> Blanford & Godwin-Austen, 1908			MN897051
<i>Sophina discoidalis</i> Stoliczka, 1871			MN897041
<i>Chalepotaxis infantilis</i> (Gredler, 1881)			KX027275
Ariophantidae Godwin-Austen, 1883			
<i>Sarika resplendens</i> (Philippi, 1846)			MT364982
<i>Sarika resplendens</i> (Philippi, 1846)			MT364983

Identification of species followed the original descriptions (Pfeiffer 1863; Tumpeesuwan & Tumpeesuwan 2017, 2018) and were then compared to the relevant type specimens. For the descriptive work, living snails, shells, and whole genitalia were imaged using a Nikon camera (DSLR D850) with a Nikon 105 Macro lens (AF-S VR Micro-Nikkor 105mm f/2.8G IF-ED). The number of shell whorls was counted and shells were measured using a Vernier calliper. Three to 20 specimens of each species were examined under an Olympus SZX2-TR30 stereoscopic light microscope. The inner sculpture of genitalia was imaged by a stereo microscope with the Cell'D Imaging Software. Radulae were extracted, soaked in 10% (w/v) sodium hydroxide, cleaned with distilled water, and imaged by scanning electron microscopy (SEM; JEOL, JSM-6610 LV).

Molecular studies

Genomic DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the foot tissue using a NucleoSpin Tissue kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. For sequencing the partial COI gene fragment, the universal primer pair LCO1491 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994) were used for PCR amplification. PCR cycling was performed as 94°C for 1 min, followed by 40 cycles of 98°C for 10 s, 51°C for 30 s, and 72°C for 90 s, and then followed by a final 72°C for 5 min. The PCR products were commercially sequenced by Bioneer Co., Korea. To achieve a single consensus sequence, individual forward and reverse sequence traces were aligned and edited using ClustalW, as implemented in the MEGA7 software (Kumar *et al.* 2016). A total of 20 new COI gene sequences were subsequently uploaded and stored in GenBank under accession numbers: MW703614–MW703633. Information of all samples included in this analysis is given in Table 1.

COI analyses

The 20 new COI gene sequences from this study and nine sequences from GenBank were aligned using ClustalW, as implemented in the MEGA7 software (Kumar *et al.* 2016). The partitioning and substitution model choice for the COI alignment were done in the program Kakusan4 (Tanabe 2011), using a heuristic search algorithm and under the Akaike Information Criterion (AIC). The Kakusan4 program suggested dividing the dataset into three partitions (the three codon positions of COI) and gave the best-fit models for each partition as the general time reversible model with gamma distribution for the first and the second COI codon positions, and the HKY model with gamma distribution for the third COI codon position.

Maximum likelihood (ML) and Bayesian inference (BI) were used to estimate the phylogenetic relationships and were performed online through the Cyber Infrastructure for Phylogenetic Research (CIPRES) Science Gateway (Miller *et al.* 2010). The RAXML-HPC2 program on XSEDE v. 8.2.12 software (Stamatakis 2014) was used to carry out the ML analysis, with 1000 bootstrap replicates, by applying the GTRCAT model. The BI analysis was performed in MrBayes on XSEDE v. 3.2.7a (Ronquist *et al.* 2012) with two independent Markov chain Monte Carlo (MCMC) simulations. The analysis ran the MCMC simulation for 10 million generations (default heating parameters) with sampling every 1000 generations. All parameters from both runs were checked for convergence and stationarity by visualizing the plot of generation vs the log-probability and checking the values of the estimated sample size (ESS) were more than 200 using Tracer v.1.6 (Rambaut *et al.* 2014). The BI trees generated during the first 25% of the generations were discarded as burn-in. Nodes having ML bootstrap support values (BS) of $\geq 70\%$ and BI posterior probabilities (PP) of ≥ 0.95 were considered to be significantly supported (Hillis & Bull 1993; Felsenstein 2004; Huelsenbeck & Rannala 2004; Mauro & Agorreta 2010; Hirano *et al.* 2018). Genetic sequence divergences in the COI gene among and within the species of *Aenigmatonconcha* together with other helicariionoids were calculated using uncorrected *p*-distances as implemented in the MEGA7 software (Kumar *et al.* 2016).

Abbreviations

In the descriptions, the terminology and abbreviations used here follow those of Tumpeesuwan & Tumpeesuwan (2017), Pholyotha *et al.* (2018), and Sutcharit *et al.* (2020a).

ant-ldl	=	anterior left dorsal lobe
at	=	atrium
e1	=	portion of epiphallus nearer to penis
e2	=	portion of epiphallus nearer to retractor muscle
ec	=	epiphallic caecum
fl	=	flagellum
fo	=	free oviduct
gd	=	gametolytic duct
gs	=	gametolytic sac
lsl	=	left shell lobe
p	=	penis
pc	=	penial caecum
post-ldl	=	posterior left dorsal lobe
pp	=	penial pilaster
prm	=	penial retractor muscle
ps	=	penial sheath
rdl	=	right dorsal lobe
rsl	=	right shell lobe
sh	=	dead shells
sp	=	specimens (preserved in alcohol)
v	=	vagina
vd	=	vas deferens

Institutional abbreviations

CUMZ	=	Chulalongkorn University, Museum of Zoology, Bangkok, Thailand
NHM	=	The Natural History Museum, London, United Kingdom (NHMUK—when citing specimen lots deposited in the NHM)

Results

COI phylogeny

The final sequence dataset contained sequences of 22 *Aenigmatoconcha* specimens together with five helicarionid specimens (one sequence of *Chalepotaxis* Ancey, 1887 and four sequences of *Sophina* Benson, 1859) included as related taxa based on a molecular phylogeny of some Asian Helicarionidae (Sutcharit *et al.* 2020a). In addition, two specimens of *Sarika* Godwin-Austen, 1907 from the family Ariophantidae were used to root the trees. The final COI alignment had a total length of 655 aligned nucleotides, containing 209 variable sites and 177 parsimony informative sites. The COI dataset did not resolve the phylogenetic relationships among *Aenigmatoconcha*, *Chalepotaxis*, and *Sophina* (Fig. 2), yet it did retrieve each of the four *Aenigmatoconcha* species as well-supported clades (Fig. 2). The relationships between the four species, however, remain unclear.

The mean uncorrected p-distance of the COI gene among *Aenigmatoconcha*, *Chalepotaxis*, and *Sophina* ranged from 12.5% to 14.1% (Table 3). Among *Aenigmatoconcha* lineages, the average uncorrected p-distance of the COI gene ranged from 9.7% to 12.0% (Table 4). The intraspecific genetic distances within each lineage ranged from 0.2% to 4.2% (Table 4).

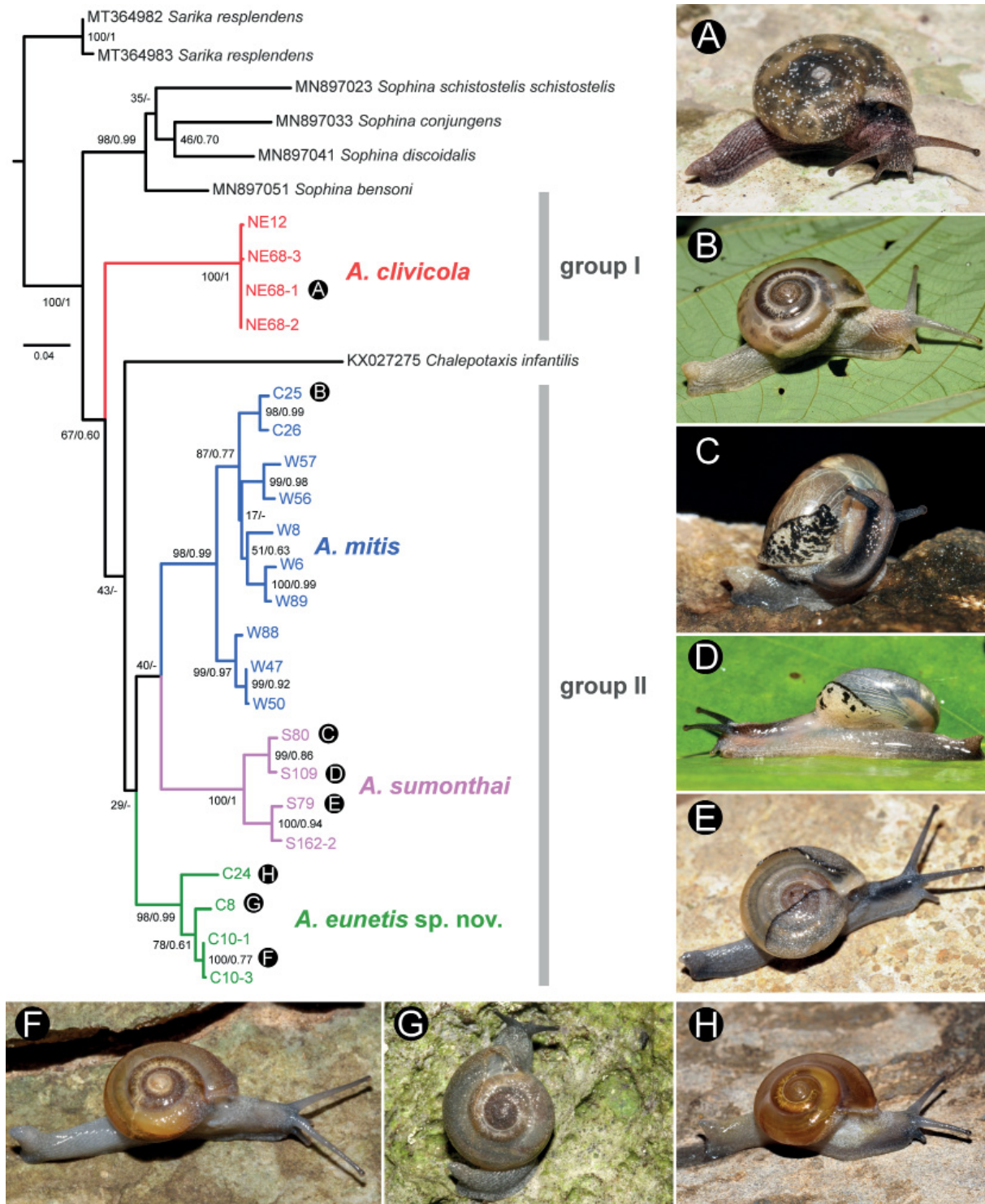


Fig. 2. Maximum likelihood tree of *Aenigματοconcha* Tumpeesuwan & Tumpeesuwan, 2017 based on the COI gene dataset. Numbers on nodes indicate the ML bootstrap values (left) and Bayesian posterior probabilities (right) values. Pictures of living snails are not to scale.

Table 2. Comparison of the morpho-anatomical characteristics of *Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017: species, *Chalepotaxis* Ancey, 1887 and *Sophina* Benson, 1859. Superscript numbers indicate the source reference: ¹ this study, ² Páll-Gergely *et al.* (2016) and ³ Sutcharit *et al.* (2020a).

Taxa	No. mantle lobes	Shell shape	Radular teeth	Genitalia			
				Dart apparatus	Epiphallic caecum	Flagellum	Inner penial sculpture
<i>A. clivicola</i> ¹	5	Strongly depressed to depressed	V-shaped rows / monocuspid with spatulate shape	Absent	Long	Present	Oblique trapezoid
<i>A. mitis</i> ¹	5	Depressed to globosely depressed	V-shaped rows / monocuspid with spatulate shape	Absent	Short	Present	Small oblique wrinkled
<i>A. sumonthai</i> ¹	5	Strongly depressed to depressed	V-shaped rows / monocuspid with spatulate shape	Absent	Short	Present	Small conical
<i>A. eunetis</i> sp. nov. ¹	5	Depressed to globosely depressed	Wide-angle U-shape rows / monocuspid with oblong shape	Absent	Short	Present	Small conical
<i>Chalepotaxis</i> ²	–	Depressed conical	V-shaped rows / monocuspid with spatulate shape	Absent	Short	Absent	–
<i>Sophina</i> ³	4	Depressed to globosely depressed	V-shaped rows / monocuspid with spatulate shape	Present	Short	Absent	–

Taxonomy

Phylum Mollusca Linnaeus, 1758
 Class Gastropoda Cuvier, 1795
 Superfamily Helicarionoidea Bourguignat, 1877
 Family Helicarionidae Bourguignat, 1877
 Subfamily Durgellinae Godwin-Austen, 1888

Genus *Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017

Aenigmatoconcha Tumpeesuwan & Tumpeesuwan, 2017: 182–184.

Aenigmatoconcha – Tumpeesuwan & Tumpeesuwan 2018: 171.

Type species

Aenigmatoconcha clivicola Tumpeesuwan & Tumpeesuwan, 2017, by original designation.

Description

Shell dextral with 5–5½ convex whorls, strongly depressed to globosely depressed, medium-sized, thin to slightly solid, translucent, and pale milky to whitish-horny in colour. Shell surface smooth, glossy, and varix usually present. Body whorl well-rounded to slightly-shouldered. Suture shallow. Aperture slightly to very crescentic in shape with simple lip. Umbilicus open and deep.

Table 3. Average genetic divergences among *Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017, *Sophina* Benson, 1859, *Chalepotaxis* Ancey, 1887, and *Sarika* Godwin-Austen, 1907 (Ariophantidae Godwin-Austen, 1883) and among species within each of these genera from the mitochondrial COI gene fragment sequences estimated by uncorrected *p*-distances.

Genera	<i>Aenigmatoconcha</i>	<i>Chalepotaxis</i>	<i>Sophina</i>	<i>Sarika</i>
<i>Aenigmatoconcha</i>	0.087	–	–	–
<i>Chalepotaxis</i>	0.130	–	–	–
<i>Sophina</i>	0.125	0.141	0.095	–
<i>Sarika</i>	0.119	0.140	0.123	0.008

Animal with reticulated skin and whitish, yellowish, pale fleshy grey to dark brown body with tiny whitish dots irregularly scattered over entire body. Mantle lobes well-developed (two shell lobes and three dorsal lobes; see Figs 3, 5A). Shell lobes can cover most, if not all of the shell and are retracted when disturbed. Left and right shell lobes very thin, translucent, ovate to triangular in shape; right shell lobe (rsl) smaller than left shell lobe (lsl). Dorsal lobes enlarged, crescent-shaped, covering body, and smaller than shell lobes. Anterior left dorsal lobe (ant-ldl) and posterior left dorsal lobe (post-ldl) smaller than right dorsal lobe (rdl). Sole tripartite, lateral foot margin, caudal fossa, and caudal horn present.

Genitalia with moderately long to very long penis, thick penial sheath, short to long epiphallus, small flagellum, and short vagina. Gametolytic organ with short gametolytic duct and bulbous gametolytic sac. Oviduct with large lobules; prostate gland running alongside oviduct.

Radular teeth arranged in anteriorly V-shaped or wide-angle U-shaped rows; central tooth symmetrical monocuspid and spatulate or oblong in shape; lateral and marginal teeth undifferentiated, asymmetrical monocuspid and spatulate or oblong in shape, and outermost teeth gradually reduced in size.

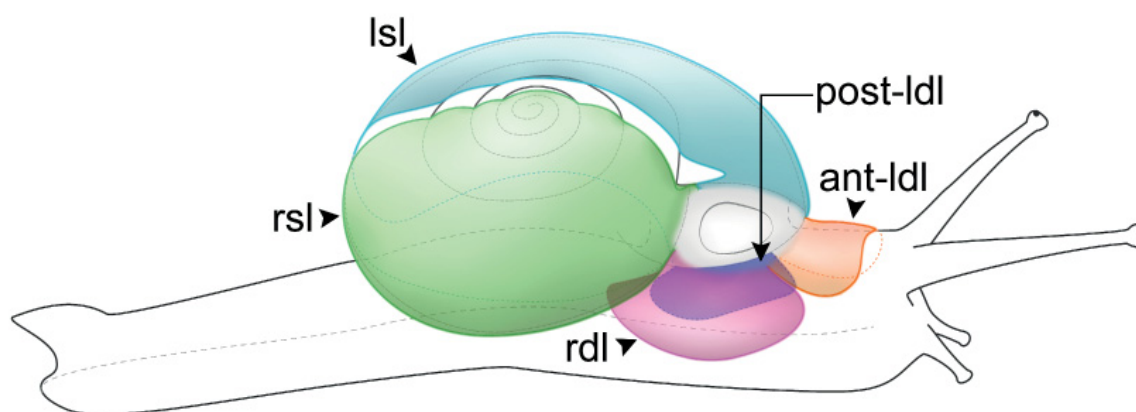


Fig. 3. Schematic drawing of living species of *Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017, emphasizing the relative size, position and arrangement of mantle lobes (two shell lobes and three dorsal lobes).

Table 4. Average inter- and intra-specific *p*-distances of the mitochondrial COI gene fragment sequences on species of *Aenigmatoconcha* Tumpeesuwan & Tumpeesuwan, 2017.

<i>Aenigmatoconcha</i> spp.	<i>A. clivicola</i>	<i>A. mitis</i>	<i>A. sumonthai</i>	<i>A. eunetis</i> sp. nov.
<i>A. clivicola</i>	0.002	–	–	–
<i>A. mitis</i>	0.112	0.042	–	–
<i>A. sumonthai</i>	0.120	0.102	0.034	–
<i>A. eunetis</i> sp. nov.	0.106	0.099	0.097	0.027

Distribution

Species of *Aenigmatoconcha* exhibit allopatric distributions and are restricted to limestone karsts in Thailand (Fig. 1).

Aenigmatoconcha clivicola Tumpeesuwan & Tumpeesuwan, 2017
Figs 1, 2A, 4A–B, 5, 10A

Aenigmatoconcha clivicola Tumpeesuwan & Tumpeesuwan, 2017: 184–187, figs 2–5. Type locality: Phu Pha Lom Limestone Hill in Mueang District, Loei Province, northeastern Thailand.

Aenigmatoconcha clivicola – Tumpeesuwan & Tumpeesuwan 2018: 171.

Material examined

THAILAND • 39 sh, 22 sp; Loei Province, Mueang District, Phu Pha Lom Limestone Hill; 17°33'16.6" N, 101°52'05.4" E; CUMZ 7928 • 6 sh, 12 sp; same collection data as for preceding; CUMZ 7929 • 48 sh; Loei Province, Na Duang District, Limestone outcrops at Wat Tham Pha Ya; 17°34'40.1" N, 101°53'35.1" E; CUMZ 7930.

Description

SHELL (Fig. 4A–B). Shell strongly depressed to depressed, medium-sized (shell width 17.2–20.1 mm, shell height 8.6–10.0 mm), rather thin to slightly solid and translucent, milky to pale whitish-horny colour, well-rounded body whorl, little elevated spire, impressed suture, obvious varix, and open umbilicus.

EXTERNAL FEATURES (Figs 2A, 5A). Animal with five well-developed mantle lobes. Left and right shell lobes thin, pale yellowish to fleshy-grey colour and spread with small whitish dots. Three dorsal lobes crescent-shaped and smaller than shell lobes.

GENITALIA (Fig. 5B–D). Atrium (at) enlarged and very short. Penis (p) rather long cylindrical, penial sheath (ps) very thick and covering entire penis, and penial caecum (pc) rather small protruding. Inner wall of penis covered with trapezoid-shaped penial pilasters (pp) arranged in oblique rows. Epiphallus (e1 + e2) as long as penis: e1 long and slender, and e2 bulbous and about half e1 length. Inner sculpture of e1 with very small and thin longitudinal folds to nearly smooth surface. Inner sculpture of e2 trapezoid to conical pilasters. Epiphallic caecum (ec) long with thick penial retractor muscle (prm) attached at tip. Flagellum (fl) small and short.

RADULA (Fig. 10A). Teeth arranged in anteriorly V-shaped rows with half row consisting of about 70–76 teeth at middle plate. Central, lateral, and marginal teeth monocuspid and spatulate-shaped with curved cusp.

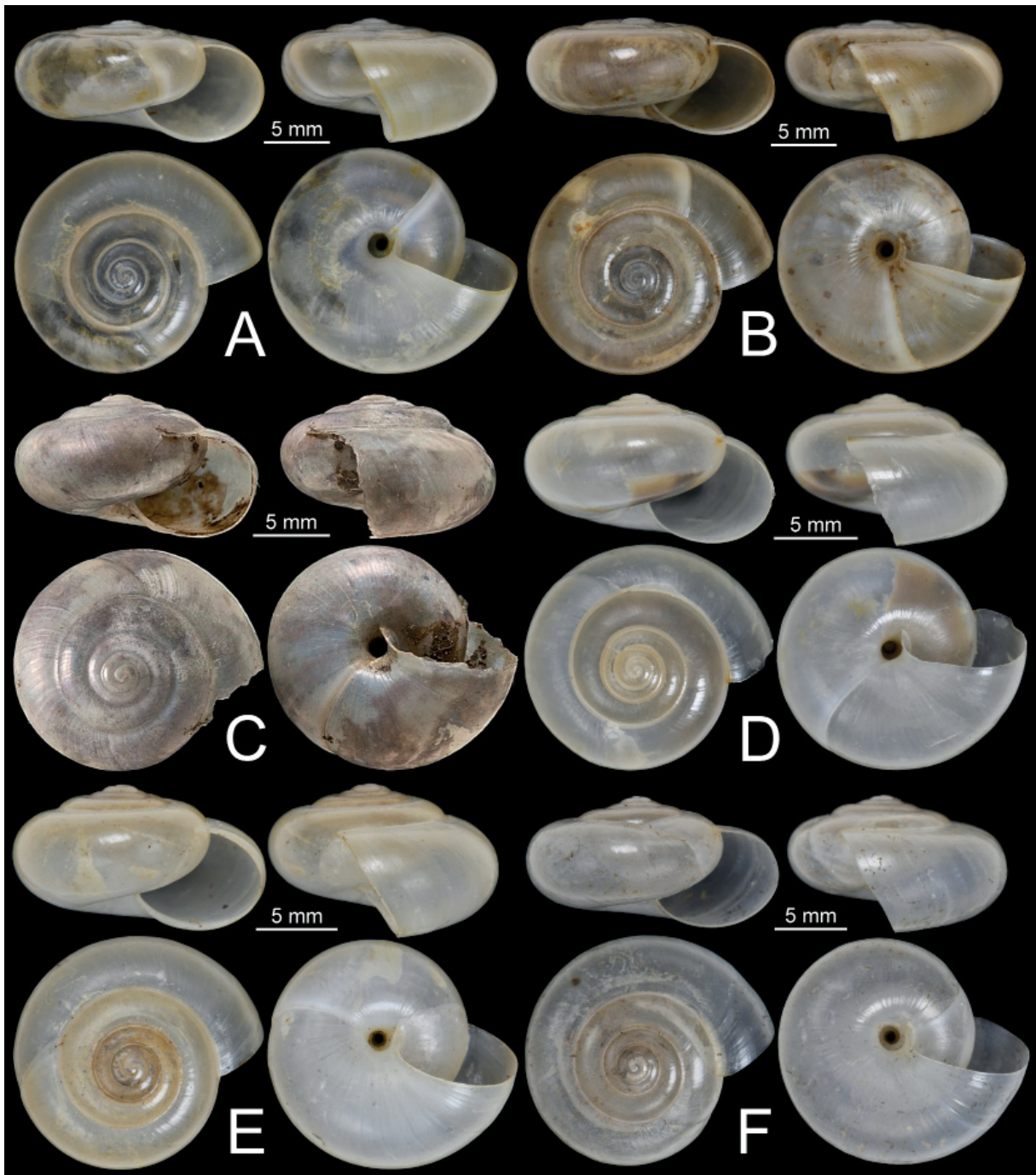


Fig. 4. Shells of *Aenigmatoconcha* spp. **A–B.** *A. clivicola* Tumpeesuwan & Tumpeesuwan, 2017, specimen CUMZ 7928 from the type locality (A) and specimen CUMZ 7930 from Loei Province (B). **C–F.** *A. mitis* (Pfeiffer, 1863) comb. nov., syntypes NHMUK ex. Cuming collection (C), specimen CUMZ 7921 from Phetchaburi Province (D), specimen CUMZ 7687 from Kanchanaburi Province (E), and specimen CUMZ 7885 from Nakhon Sawan Province (F).

Distribution

This species is currently known only from 2 localities: the type locality (Phu Pha Lom) and limestone hills in Loei Province (Fig. 1).

Remarks

The genitalia were originally described but without examination of the internal sculpture of penis. In this study, we examined 20 adult topotypic specimens to provide descriptions of the penial sheath, penial caecum, and flagellum that were not included in the original description. The penial sheath is very large and covers the entire penis (Fig. 5B). The short penial caecum, an extension of the penis, is located near the penis and epiphallus junction (Fig. 5B). This character is visible when penial sheath is removed. Its internal sculpture is rather smooth, unlike the penial sculpture, with its trapezoid-shaped pilasters (Fig. 5C). The Australian helicarionids, *Nitor whitneyae* Stanisc, 2010 has a penial caecum while other *Nitor* taxa do not have a penial caecum (Hyman & Köhler 2018). In Southeast Asian ariophantids, the presence or absence of a penial caecum is a discriminating character among species in genera such as *Macrochlamys* Gray, 1847 and *Taphrenalla* Pholyotha & Panha, 2020, and is supported by molecular studies (Pholyotha *et al.* 2018, 2021). The flagellum of *A. clivicola* is an extension of the epiphallus and is located near the insertion point of the vas deferens. It is somewhat small and short, and bound to the vas deferens by thin connective tissue. This feature is important for spermatophore formation before copulation (Tompia 1984; Baur 2010). However, during this study no spermatophores were observed in *A. clivicola*.

Aenigματοconcha mitis (Pfeiffer, 1863) comb. nov.

Figs 1, 2B, 4C–F, 6, 10B

Helix mitis Pfeiffer, 1863[1862]: 268. Type locality: Lao Mountains, Camboja [Lao Mountains, Cambodia].

Helix mitis – Pfeiffer 1868: 141. — Tryon 1886: 171.

Ariophanta (Kaliella) mitis – Fischer 1891: 21.

Hyalinia mitis – Fischer & Dautzenberg 1904: 396.

Macrochlamys (?) *mitis* – Inkavilay *et al.* 2019: 78, 79, fig. 37b.

Material examined

Syntypes

CAMBODIA • 2 sh; “Lao Mountains, Camboja” [Lao Mountains, Cambodia]; NHMUK ex. Cuming collection.

Other material

THAILAND • 37 sh, 16 sp; Nakhon Sawan Province, Mueang District, Limestone outcrops at Wat Tham Bo Ya; 15°43'47.3" N, 99°56'44.7" E; CUMZ 7708 • 30 sh, 15 sp; same collection data as for preceding; CUMZ 7885 • 8 sh, 21 sp; Nakhon Sawan Province, Krok Phra District, Limestone outcrops at Wat Khao Tham Phra; 15°33'30.2" N, 99°57'28.1" E; CUMZ 7849 • 3 sp; Kanchanaburi Province, Sai Yok District, Limestone outcrops at Wat Thep Thepa Satthatham; 14°03'56.6" N, 99°11'45.7" E; CUMZ 7913 • 21 sh, 24 sp; Kanchanaburi Province, Mueang District, Limestone outcrops at Wat Tham Charoentham; 13°55'59.1" N, 99°27'59.9" E; CUMZ 7687 • 3 sp; Kanchanaburi Province, Tha Muang District, Limestone outcrops at Wat Tham Faet; 13°57'52.5" N, 99°34'56.1" E; CUMZ 7914 • 30 sh, 1 sp; Kanchanaburi Province, Dan Makham Tia District, Limestone outcrops at Wat Tham Khao Cha Ang; 13°48'08.4" N, 99°26'33.2" E; CUMZ 7247 • 4 sh, 3 sp; Phetchaburi Province, Khao Yoi District, Limestone outcrops at Wat Khiri Wong; 13°20'03.2" N, 99°45'19.0" E; CUMZ 7917 • 1 sh, 15 sp; Phetchaburi Province, Khao Yoi District, Limestone outcrops at Wat Puang Malai; 13°18'46.0" N,

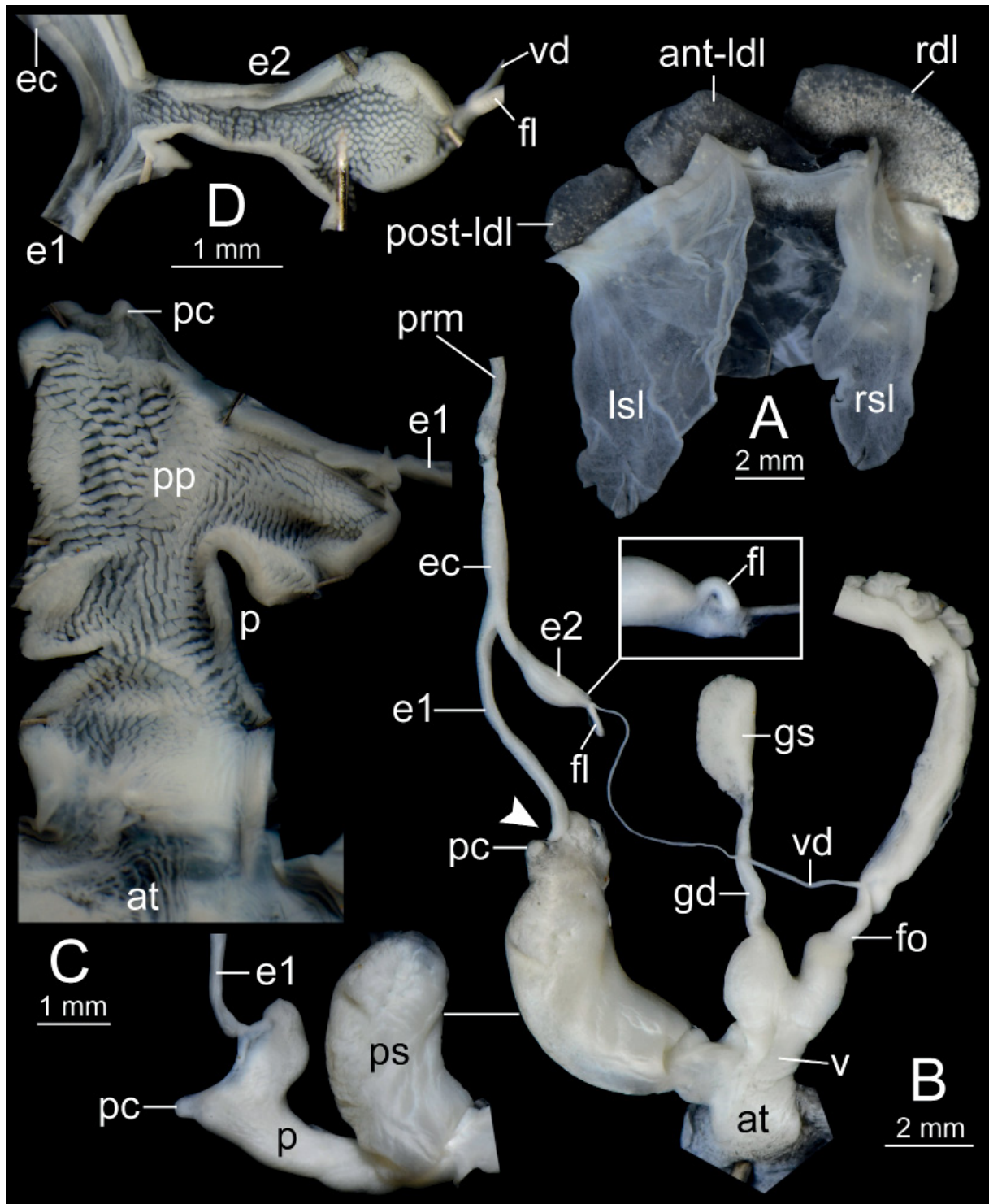


Fig. 5. Genital system and mantle lobes of *Aenigmatoconcha clivicola* Tumpeesuwan & Tumpeesuwan, 2017, specimen CUMZ 7929 from the type locality. **A.** Mantle lobes. **B.** General view of genitalia; inset showing flagellum encircled with a loose tissue before clearing it out. **C.** Internal structure of penis. **D.** Internal structure of epiphallus and epiphallic caecum. White arrow indicates the junction between penis and epiphallus.

99°47'02.2" E; CUMZ 7921 • 9 sh; same collection data as for preceding; CUMZ 7938 • 5 sh, 14 sp; Phetchaburi Province, Tha Yang District, Limestone outcrops at Wat Khao Krachiu; 12°57'41.3" N, 99°54'49.3" E; CUMZ 7918 • 3 sh, 16 sp; Phetchaburi Province, Cha-am District, Limestone outcrops at Khao Nang Panthurat; 12°50'20.5" N, 99°57'11.6" E; CUMZ 7920 • 2 sh, 2 sp; Phetchaburi Province, Cha-am District, Limestone outcrops at Tham Chaeng Bureau of Monks; 12°49'44.3" N, 99°56'27.1" E; CUMZ 7919 • 4 sh, 7 sp; Prachuap Khiri Khan Province, Mueang District, Limestone outcrops at Khao Lom Muak; 11°47'03.0" N, 99°48'56.3" E; CUMZ 7915 • 3 sh, 8 sp; Prachuap Khiri Khan Province, Mueang District, Limestone outcrops at Khao Ta Mong Lai; 11°50'07.3" N, 99°49'50.1" E; CUMZ 7916.

Description

SHELL (Fig. 4C–F). Shell depressed to globosely depressed, medium-sized (width 12.3–18.1 mm, height 6.9–9.9 mm), rather thin, and translucent. Shell surface smooth and glossy. Shell colour whitish to very pale horny-white. Whorls: 5–5½, regularly increasing in size; varix present; suture rather wide and shallow. Spire rather elevated. Last whorl broad and well-rounded. Aperture obliquely oval-lunate in shape; peristome simple. Columellar margin simple, slightly expanded near umbilicus. Umbilicus open and deep.

EXTERNAL FEATURES (Fig. 2B). Living snails with reticulated skin and pale yellowish to dark grey body. Five well-developed mantle lobes; left and right shell lobes thin, pale yellowish, spread with small whitish dots, and left shell lobe larger than right shell lobe. Three dorsal lobes broad and crescent-shaped; right dorsal lobe larger than anterior and posterior left dorsal lobes. Caudal fossa present; caudal horn raised, rather large, and whitish to pale fleshy-grey in colour.

GENITALIA (Fig. 6). Atrium (at) enlarged and very short. Penis (p) long, cylindrical, and with slightly thick penial sheath (ps) encircling about half of penis length. Inner sculpture of penis with very small and oblique wrinkled penial pilasters (pp), and one large longitudinal fold running the length of the entire penis chamber. Epiphallus (e1 + e2) approximately as long as penis: e1 slender and narrower than penis, and e2 shorter and bulbous shape. Inner sculpture of e1 with very small thin longitudinal folds to nearly smooth surface with one thickened longitudinal fold, and inner sculpture of e2 with large papillae arranged in oblique rows. Epiphallic caecum (ec) very short; penial retractor muscle (prm) thin and attached at tip. Flagellum (fl) small and rather short. Vas deferens (vd) very long and thin. Vagina (v) very short and enlarged. Gametolytic duct (gd) long, slender, and enlarged near vagina; gametolytic sac (gs) very large and oblong shape. Free oviduct (fo) cylindrical, long, and encircled with thick tissue near vagina.

RADULA (Fig. 10B). Teeth arranged in anteriorly V-shaped rows with half row consisting of about 76–79 teeth at middle plate. Central teeth symmetrical monocuspid, and spatulate-shaped with curved cusp. Lateral and marginal teeth undifferentiated, asymmetrical monocuspid, spatulate-shaped with curved cusp, and outermost teeth gradually reduced in size.

Distribution

The distribution of *Aenigmatoconcha mitis* is wider than all other recognised species. This species can be found in limestone areas ranging from central (Nakhon Sawan Province) to southern (Prachuap Khiri Khan Province) Thailand (Fig. 1).

Remarks

This species was originally described by L. Pfeiffer (1863) based on specimens in the collection of H. Cuming obtained from Henry Mouhot. The collection locality was brief: “Lao Mountains, Camboja”. However, Mouhot’s recorded localities were generally imprecise and referred to a wide geographical area, for example “Siam”, “Lao Mountains, Camboja” and “Camboja”. This has made it difficult to

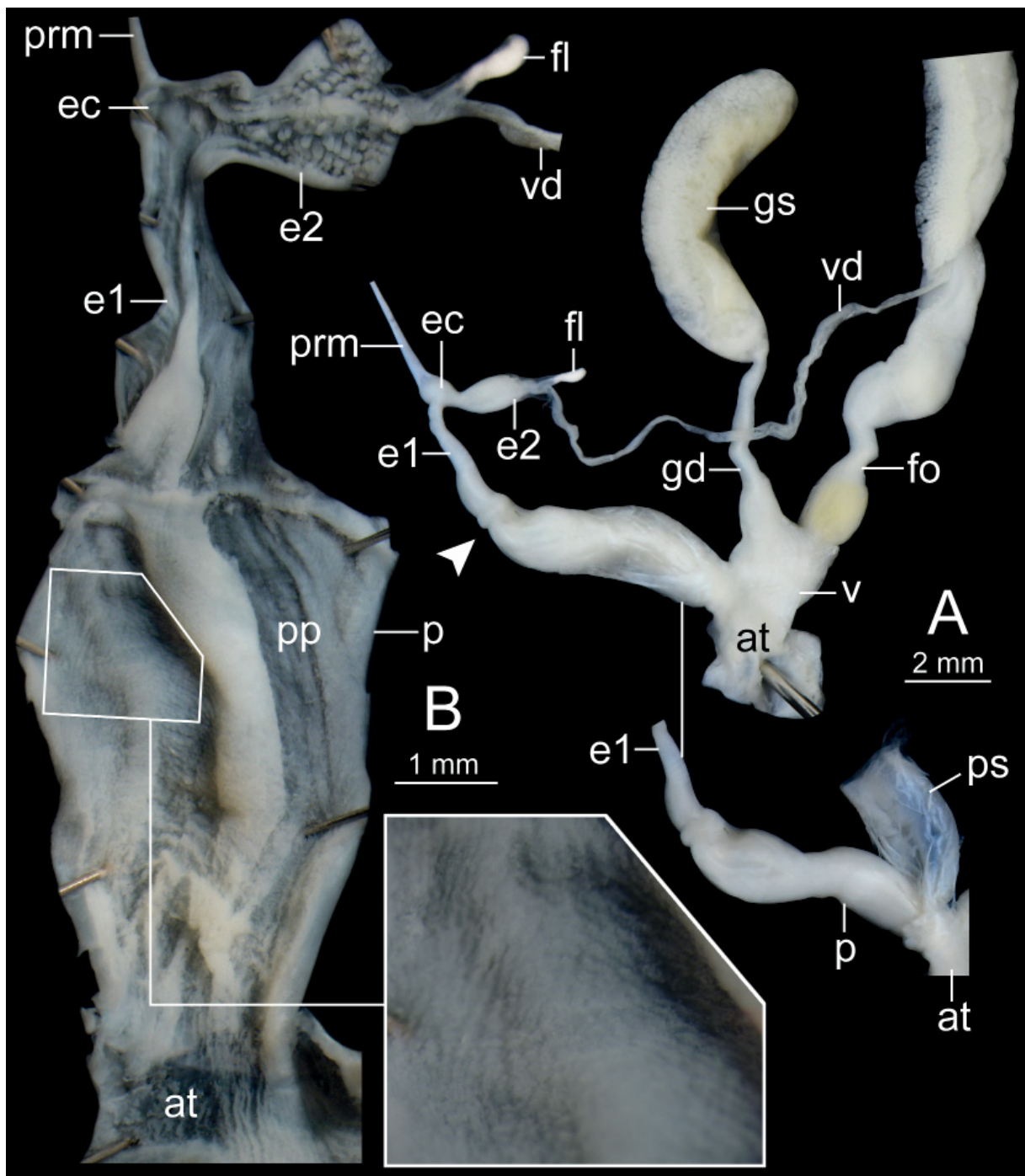


Fig. 6. Genital system of *Aenigματοconcha mitis* (Pfeiffer, 1863) comb. nov., specimen CUMZ 7687 from Kanchanaburi Province. **A.** General view of genitalia. **B.** Internal structure of penis, epiphallus, and epiphallic caecum; inset showing penial sculpture. White arrow indicates the junction between the penis and epiphallus.

infer more precise type localities of several land snail species described from Mouhot's specimens. No additional specimen records or literature references are available for this species until now. The most recent works on land snails from Laos and southern Cambodia confirmed the existence of *A. mitis* (Inkhavilay *et al.* 2019; Sutcharit *et al.* 2020c). Based on the recorded itinerary, H. Mouhot had travelled to “Pechaburi” [Petchaburi Province] in 1861, and clearly stated that he had visited caves and several hills during his four-month stay (Mouhot 1864: 57; Ashburton 1864: map). We have surveyed several limestone hills in western and peninsular Thailand and encountered numbers of empty shells and living specimens that well-matched with the type specimens of “*Helix mitis* Pfeiffer, 1863” (Fig. 4C). Therefore, peninsular Thailand (Petchaburi Province; Fig. 1) might be the area where H. Mouhot collected this species.

Aenigματοconcha mitis exhibits a rather wide range of shell shape variation from depressed (Fig. 4F) to somewhat globose (Fig. 4C, D). However, the genitalia of these shell morphs are identical and the COI phylogeny also supports that all shell morphs are grouped together within the *A. mitis* clade (Fig. 2).

Aenigματοconcha sumonthai Tumpeesuwan & Tumpeesuwan, 2018
Figs 1, 2C–E, 7A–D, 8, 10C

Aenigματοconcha sumonthai Tumpeesuwan & Tumpeesuwan, 2018: 171–173, figs 2–6. Type locality: Tham Chang Phueak limestone range, Mueang District, Chumphon Province, southern Thailand.

Material examined

THAILAND • 45 sh, 20 sp; Chumphon Province, Mueang District, Limestone outcrops at Tham Chang Phueak Bureau of Monks; 10°26'50.0" N, 99°02'07.1" E; CUMZ 7922 • 40 sh; same collection data as for preceding; CUMZ 7923 • 18 sh, 10 sp; same collection data as for preceding; CUMZ 7937 • 45 sh, 6 sp; Chumphon Province, Mueang District, Limestone outcrops at Wat Tham Sanook; 10°28'51.3" N, 99°04'28.3" E; CUMZ 7924 • 10 sh, 7 sp; same collection data as for preceding; CUMZ 7925 • 22 sh, 11 sp; Chumphon Province, Sawi District, Limestone outcrops at Tham Nam Lod Thepnimit Bureau of Monks; 10°22'39.5" N, 99°00'39.5" E; CUMZ 7927 • 4 sh, 8 sp; Chumphon Province, Sawi District, Limestone outcrops at Wat Nam Cha; 10°17'57.0" N, 99°01'58.5" E; CUMZ 7926.

Description

SHELL (Fig. 7A–B). Shell strongly depressed to depressed, medium-sized (width 14.4–16.6 mm, height 7.0–8.2 mm), thin, translucent, whitish colour, well-rounded to slightly shouldered body whorl, elevated spire, impressed suture, obvious varix, and open umbilicus.

EXTERNAL FEATURES (Fig. 2C–E). Animal with five well-developed mantle lobes. Left and right shell lobes pale yellowish to fleshy-grey colour, usually with black margin, and with or without small to large black spots or blotches. Three dorsal lobes crescent-shaped and smaller than the other two shell lobes. Black stripes behind long tentacles.

GENITALIA (Fig. 8). Atrium (at) enlarged and very short. Penis (p) long, cylindrical with thick penial sheath (ps) extending to half of penis length. Inner sculpture of penis with small conical penial pilasters (pp). Epiphallus (e1 + e2) as long as penis: e1 slender, and e2 bulbous. Inner sculpture of e1 with small thin longitudinal folds, while e2 with small papillae arranged in oblique rows. Epiphallic caecum (ec) short with thin penial retractor muscle (prm) attached at tip. Flagellum (fl) small and short.

RADULA (Fig. 10C). Teeth arranged in anteriorly V-shaped rows with half row consisting of about 63–65 teeth at the middle plate. All teeth monocuspid and spatulate-shaped with curved cusp.

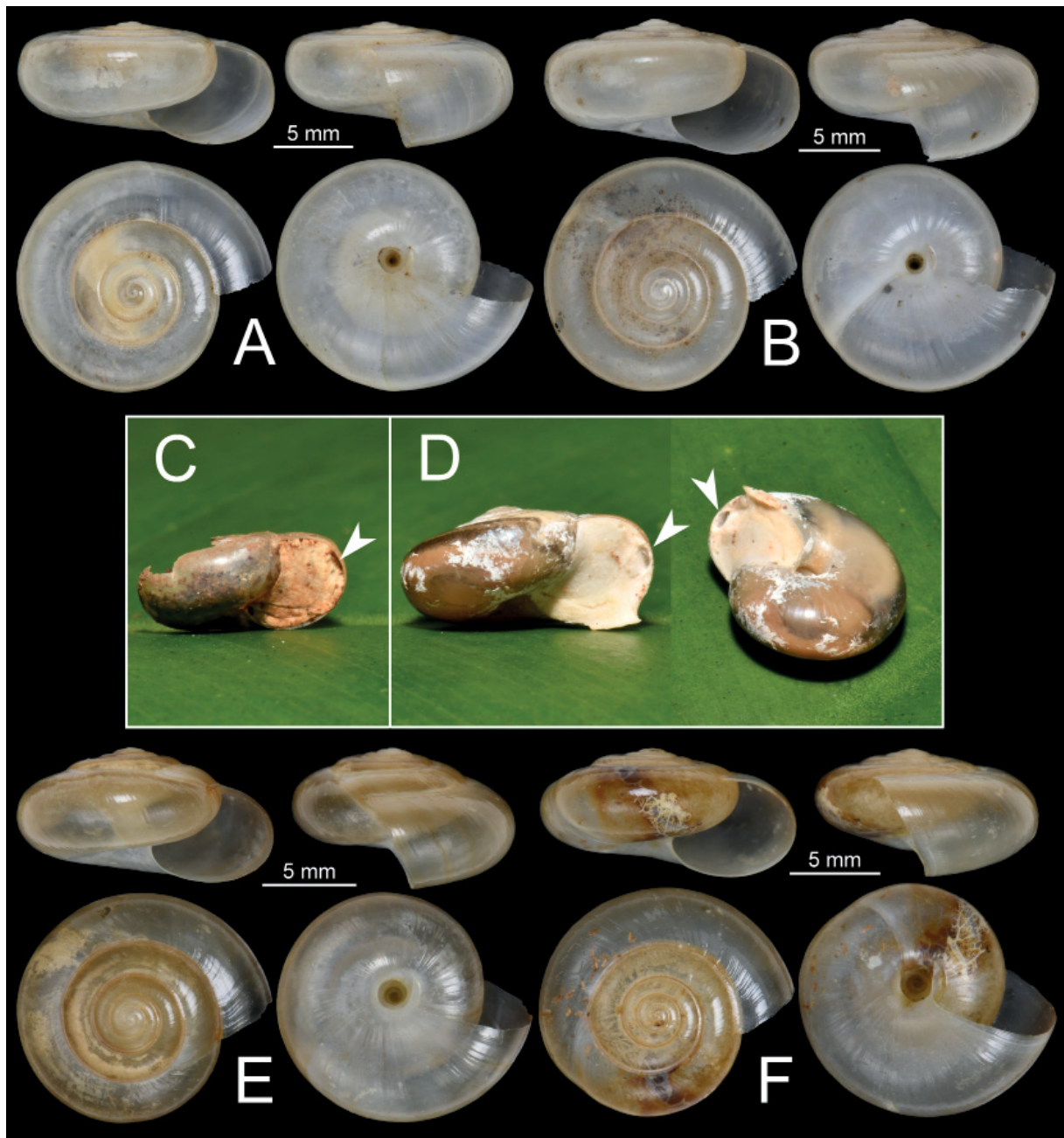


Fig. 7. Shells of *Aenigματοconcha* spp. **A–D.** *A. sumonthai* Tumpeesuwan & Tumpeesuwan, 2018. **A–B.** Specimen CUMZ 7922 from the type locality. **C–D.** Specimen CUMZ 7937 from the type locality showing the calcareous epiphragm with a small perforation (white arrows). Only shell (C) and living (D) specimens in both lateral and ventral views. **E–F.** *A. eunetis* Pholyotha & Panha sp. nov. **E.** Holotype CUMZ 7931. **F.** Paratype CUMZ 7933 from Uthai Thani Province.

Distribution

Aenigmatoconcha sumonthai has a narrow distribution, with populations living on a few limestone hills in Chumphon Province (Fig. 1). We extended our survey, especially among limestone sites about 200 km southwards down to southern peninsular Thailand, but we could not find this species elsewhere.

Remarks

The lack of a penial sheath and flagellum in the male reproductive organs of *A. sumonthai* was originally reported to be similar to *A. clivicola* (Tumpeesuwan & Tumpeesuwan, 2018: 174, fig. 6). In this study, based on topotypic specimens, however, *A. sumonthai* was found to have a large and thickened penial sheath and small flagellum encircled with loose tissue (Fig. 8).

Aenigmatoconcha sumonthai shows variation in the black blotches on both shell lobes ranging from absent (Fig. 2E) to the lobes almost entirely covered (Fig. 2C). The DNA sequence analysis suggested that these variations formed a clade of *A. sumonthai* (Fig. 2). In addition, this species develops a calcareous epiphragm with a small perforation to limit body-water evaporation but allowing respiratory gas exchange during dormancy (Fig. 7C, D).

Aenigmatoconcha eunetis Pholyotha & Panha sp. nov.

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Figs 1, 2F–H, 7E–F, 9, 10D

Diagnosis

Shell medium-sized and pale yellowish white. Aperture ovate-lunate in shape and vertically open. Genitalia with very long and slender penis with many tiny conical penial pilasters inside. Radular teeth arranged in wide-angle U-shaped row, teeth with oblong shape, monocuspid.

Etymology

The specific name ‘*eunetis*’ is from the Greek word meaning ‘spouses’, honouring the authors of genus *Aenigmatoconcha*, who are married.

Material examined

Holotype

THAILAND • 1 sh (width 13.9 mm, height 7.4 mm); Uthai Thani Province, Lan Sak District, Limestone outcrops at Tham Namthip Bureau of Monks; 15°26′00.3″ N, 99°35′18.7″ E; CUMZ 7931.

Paratypes

THAILAND • 1 sh, 7 sp; same collection data as for holotype; CUMZ 7933 • 12 sp; same collection data as for holotype; CUMZ 7932 • 2 sh; same collection data as for holotype; NHMUK.

Other material

THAILAND • 1 sp; Uthai Thani Province, Lan Sak District, Limestone outcrops at Hup Pa Tat; 15°22′36.5″ N, 99°37′49.5″ E; CUMZ 7934 • 6 sp; same collection data as for preceding; CUMZ 7935 • 3 sh, 27 sp; Uthai Thani Province, Nong Chang District, Limestone outcrops at Wat Khao Bang Kraek; 15°18′07.9″ N, 99°41′04.5″ E; CUMZ 7936.

Description

SHELL (Fig. 7E–F). Depressed to globosely depressed, medium-sized (width 12.6–14.2 mm, height 6.1–7.1 mm), thin and translucent. Shell surface smooth, and polished, and pale yellowish white. Whorls: 5–5½, regularly increasing in size, separated by shallow suture. Spire rather elevated; varix usually

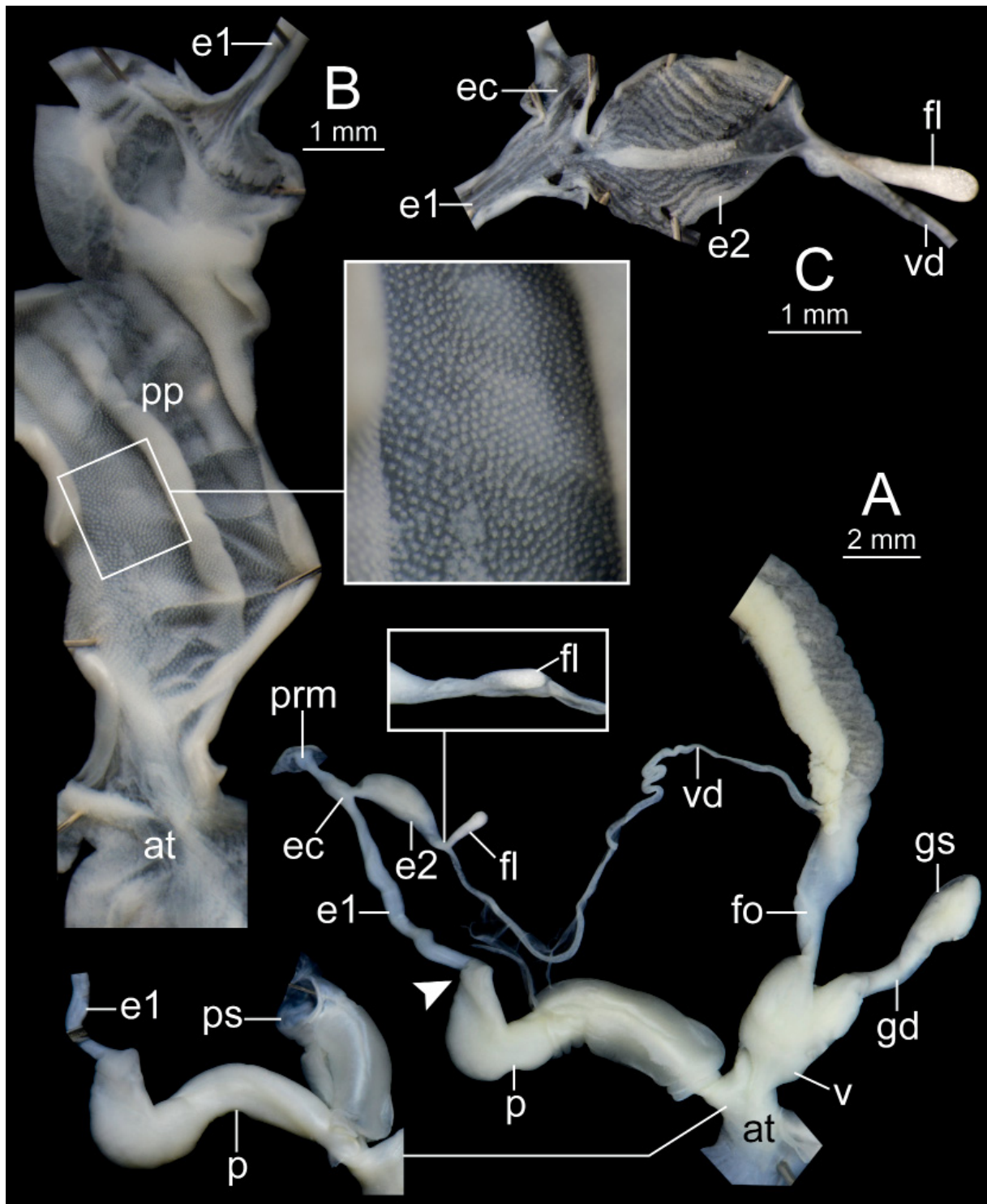


Fig. 8. Genital system of *Aenigmatoconcha sumonthai* Tumpeesuwan & Tumpeesuwan, 2018, specimen CUMZ 7922 from the type locality. **A.** General view of genitalia; inset showing flagellum encircled with a loose tissue before clearing it out. **B.** Internal structure of penis; inset showing penial sculpture. **C.** Internal structure of epiphallus and epiphallic caecum. White arrow indicates the junction between the penis and epiphallus.

present; last whorl well-rounded. Aperture very obliquely oval-lunate in shape; peristome simple. Columellar margin simple and slightly expanded near umbilicus. Umbilicus open and deep.

EXTERNAL FEATURES (Fig. 2F–H). Living snails with reticulated skin and pale yellowish to slightly dark grey body. Five well-developed mantle lobes: left and right shell lobes translucent, same colour as body, covered by tiny whitish dots, right shell lobe smaller than left shell lobe. Dorsal lobes broad and crescent-shaped: right dorsal lobe larger than both anterior and posterior left dorsal lobes. Caudal fossa present; caudal horn raised and rather large, and same colour as body.

GENITALIA (Fig. 9). Atrium (at) enlarged and very short. Penis (p) very long and slender with rather thick penial sheath (ps) covering from atrium to almost middle of penis. Inner sculpture of penis with small conical penial pilasters (pp), and three prominent longitudinal folds along entire length of penis chamber. Epiphallus (e1 + e2) approximately half of penis length: e1 elongate and slender, and e2 very short and bulbous. Inner sculpture of e1 nearly smooth with small, thin, and longitudinal folds, and e2 densely papillate. Epiphallic caecum (ec) straight, short, and approximately as long as e2; penial retractor muscle (prm) thin and attached at tip. Flagellum (fl) small, rather short, and approximately as long as e2. Vas deferens (vd) very long, thin, and convoluted. Vagina (v) short, enlarged, thickened and cylindrical. Gametolytic sac (gs) short and bulbous; gametolytic duct (gd) long, enlarged near vagina then becoming smaller and very slender. Free oviduct (fo) long, cylindrical, and encircled with dense tissue near vagina.

RADULA (Fig. 10D). Teeth arranged in wide-angle U-shape with half row consisting of about 97–98 teeth at middle plate. Central tooth symmetrical monocuspid, elongated oblong-shaped with curved cusp, and slightly smaller than lateral tooth. Lateral and marginal teeth undifferentiated, asymmetrical monocuspid and elongated oblong-shaped with curved cusp; outermost teeth gradually becoming smaller.

Distribution

Aenigmatoconcha eunetis sp. nov. occurs in a few isolated limestone hills in Uthai Thani Province. This new species lives on limestone karsts, where snails tend to hide themselves in rock crevices and shelter during the daytime.

Remarks

Aenigmatoconcha eunetis sp. nov. clearly differs from all congeners in having 1) the longest penis, 2) penial internal sculpture consisting of longitudinal folds and small conical penial pilasters, and 3) inner sculpture of epiphallus (e2) densely papillae. For comparison, *A. clivicola* has oblique trapezoid-shaped penial pilasters, *A. mitis* has oblique wrinkled penial pilasters with a longitudinal fold, and *A. sumonthai* has very small conical penial pilasters without longitudinal fold. In addition, for the inner sculpture of epiphallus (e2), *A. clivicola* has irregularly oblique trapezoid and small conical papillae. *Aenigmatoconcha mitis* and *A. sumonthai* have loose papillae arranged in oblique rows, but the former has relatively fewer rows and papillae are significantly larger in size than the latter. Moreover, radular teeth of only *A. eunetis* sp. nov. are arranged in wide-angle U-shaped row and oblong-shaped teeth, while radular teeth of other *Aenigmatoconcha* species are arranged in V-shaped rows and spatulate in shape.

Discussion

Each of the four *Aenigmatoconcha* species forms a well-defined clade in the COI phylogeny. *Aenigmatoconcha* can be divided into two groups based on the genital characters (Figs 1–2; Table 2). Group I (Fig. 2) has a long epiphallic caecum and short penial caecum, and contains one species, *A. clivicola*, which is restricted to northeastern Thailand (Fig. 1). Group II (Fig. 2) has a short epiphallic caecum and no penial caecum; this group contains *A. mitis*, *A. sumonthai*, and *A. eunetis* sp. nov.

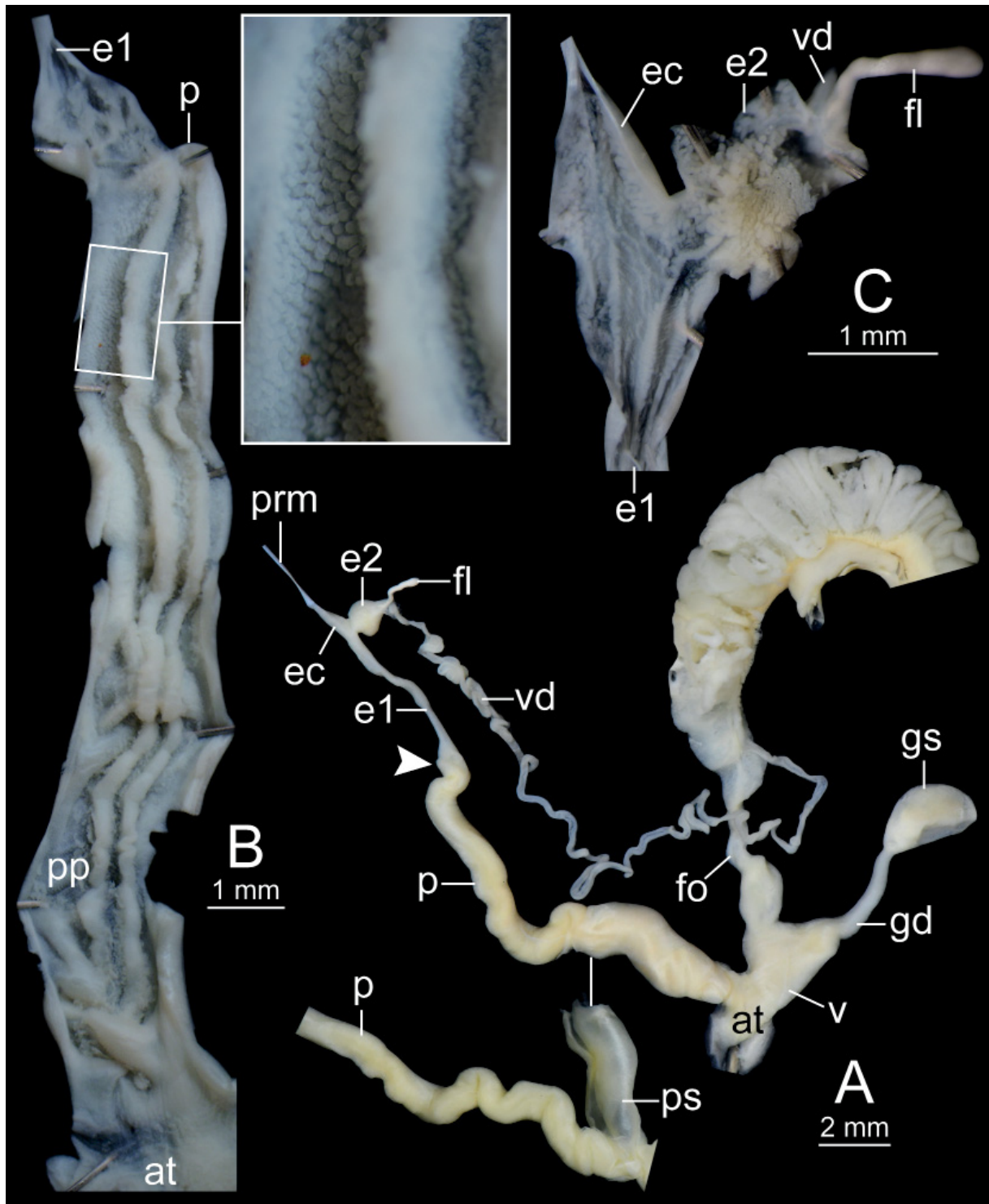


Fig. 9. Genital system of *Aenigmatoconcha eunetis* Pholyotha & Panha sp. nov., paratype CUMZ 7933 from Uthai Thani Province. **A.** General view of genitalia. **B.** Internal structure of penis; inset showing penial sculpture. **C.** Internal structure of epiphallus and epiphallal caecum. White arrow indicates the junction between the penis and epiphallus.

This group occurs from central to southern Thailand along the Tenasserim Range (Fig. 1). The COI tree showed very low nodal support for the relationships among the two *Aenigmatoconcha* groups and the other two helicarionid genera. Although the phylogenetic relationships among *Aenigmatoconcha*, *Sophina* and *Chalepotaxis* remain unresolved, the genital characters of both groups of *Aenigmatoconcha* were clearly distinct from *Sophina* and *Chalepotaxis*. In the phylogenetic tree of the 28S gene, moreover, these three genera were confirmed as different genera, but the systematic position of two *Aenigmatoconcha* species (*A. clivicola* and *A. sumonthai*) is unresolved (Sutcharit *et al.* 2020a). Further research should include on more genes and more taxa of the Southeast Asian helicarionoids to better understand the phylogenetic relationships and morphological evolution of these groups.

Aenigmatoconcha can be distinguished from almost all other Southeast Asian helicarionoid genera by their unique milky to pale whitish-horny and umbilicate shell. Other helicarionoids with a whitish shell include *Macrochlamys psyche* Vermeulen *et al.*, 2019, *Sarika lactoconcha* Pholyotha & Panha, 2020, and *Sarika consepta* (Benson, 1860). However, the relatively medium to large-sized shells (shell width larger than 15 mm), narrowly perforate umbilicus, genital structure, and radula with triangular teeth of these three species clearly differentiate them from all *Aenigmatoconcha* species (Vermeulen *et al.* 2019; Pholyotha *et al.* 2020a, 2020b). The greatest similarity in shell characters

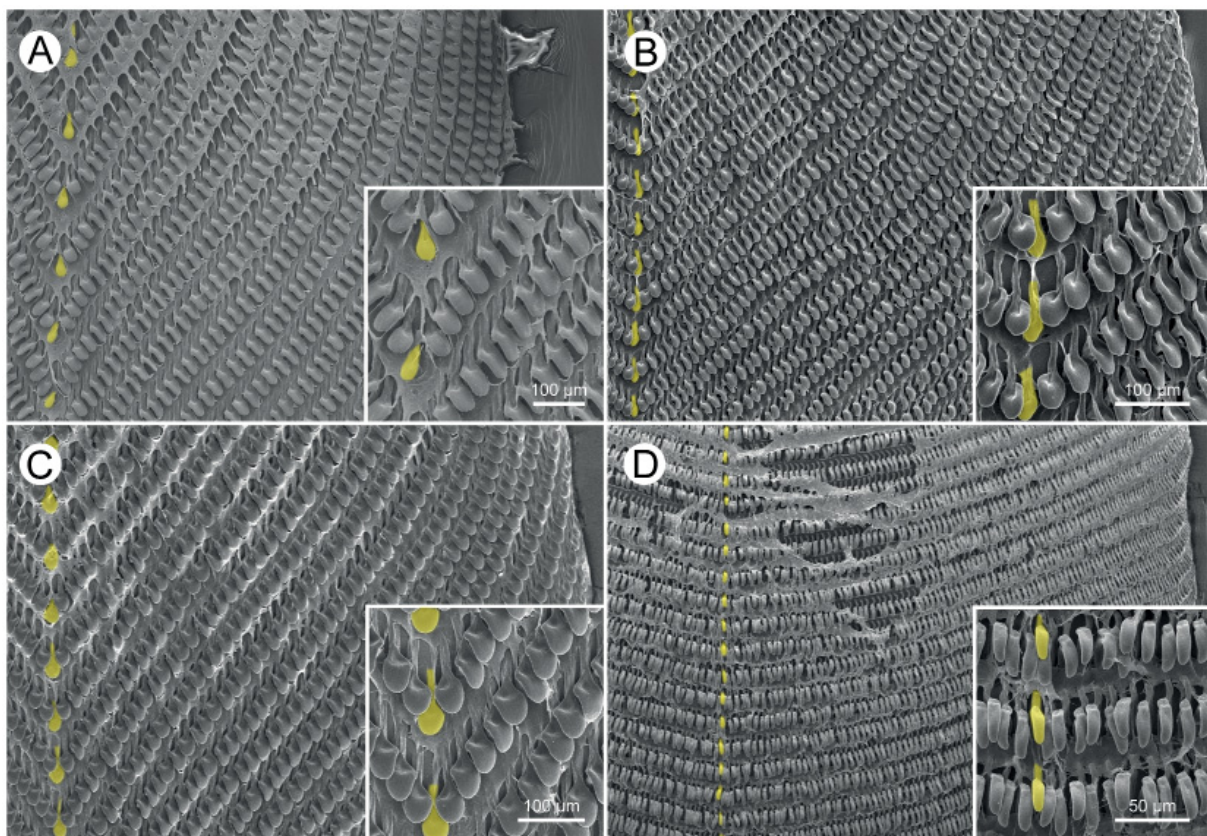


Fig. 10. Radula morphology of *Aenigmatoconcha* spp. **A.** *A. clivicola* Tumpeesuwan & Tumpeesuwan, 2017, specimen CUMZ 7929 from Loei Province. **B.** *A. mitis* (Pfeiffer, 1863) comb. nov., specimen CUMZ 7687 from Kanchanaburi Province. **C.** *A. sumonthai* Tumpeesuwan & Tumpeesuwan, 2018, specimen CUMZ 7922 from Chumphon Province. **D.** *A. eunetis* Pholyotha & Panha sp. nov., paratype CUMZ 7933 from Uthai Thani Province. Central tooth in yellow. Scale bars: A–C = 100 µm; D = 50 µm.

occurred in *Sophina* and *Chalepotaxis*, but their genitalia are obviously different. The distinctive characters between *Aenigmatoconcha* and *Chalepotaxis* have been reported (Tumpeesuwan & Tumpeesuwan 2017; Sutcharit *et al.* 2020a). However, Tumpeesuwan and Tumpeesuwan (2017) did not report the presence of a “penial sheath” and a “flagellum” in *A. clivicola* (the type species). In fact, they stated that the absence of a penial sheath in *Aenigmatoconcha* is a diagnostic difference between *Aenigmatoconcha* and *Chalepotaxis*. Yet, in the present work, the four *Aenigmatoconcha* species clearly exhibited a well-developed penial sheath and flagellum. Hence, the only difference between *Chalepotaxis* and *Aenigmatoconcha* is the presence of a flagellum in the latter (Páll-Gergely *et al.* 2016). In addition to the genitalia without a dart apparatus, the presence of well-developed anterior and posterior left dorsal lobes is a significant character of *Aenigmatoconcha*, while *Sophina* has an undivided left dorsal lobe and a dart apparatus (Blanford & Godwin-Austen 1908; Sutcharit *et al.* 2020a). However, information on the mantle lobe morphology of *Chalepotaxis* was not available for comparison.

Among Southeast Asian helicarionoid genera with uniform and spatulate-shaped radula, *Sophina* is the only genus that a dart apparatus present in genitalia, but a flagellum absent. While genitalia of *Aenigmatoconcha* lack dart apparatus, but contain the flagellum, and genitalia of *Chalepotaxis* lack both organs. Generally, some characters of genital anatomy, such as a penial caecum, penial verge, dart apparatus, or flagellum, have been hypothesized to evolve repeatedly during the evolution of land snails, and have been noticed in many groups of terrestrial pulmonate snails (Solem 1966; Hausdorf 1998; Hyman & Ponder 2010; Hirano *et al.* 2014; Köhler & Criscione 2015; Köhler *et al.* 2020; Sutcharit *et al.* 2020a). However, the classification of these three genera based on the presence or absence of the dart apparatus and flagellum is consistent with the molecular phylogeny (Sutcharit *et al.* 2020a).

From the East of Tenasserim Range to northeastern Thailand, three *Aenigmatoconcha* species occurred in a few limestone karsts, while only *A. mitis* occurs in many limestone karsts from central to western Thailand (Fig. 1). *Aenigmatoconcha clivicola* is confined to Loei Province, northeastern Thailand, while *A. eunetis* sp. nov. to Uthai Thani Province, central Thailand, and *A. sumonthai* to Chumphon Province, southern Thailand. The isolation explains the degree of endemism and the very high genetic divergence among sister lineages (9.7% to 12.0%) within *Aenigmatoconcha*. Regarding the West of Tenasserim Range, *Sophina* also reveals a high degree of endemism and localization with a pattern of one outcrop for one lineage in the Salween Basin, Southern Myanmar. These phenomena can be generally observed in karst-restricted animals because a very large number of ecological niches in karst ecosystems promote their evolutionary diversification and evolution of remarkably different lifestyles (Clements *et al.* 2006; Foon *et al.* 2017; Grismer *et al.* 2020; Sutcharit *et al.* 2020a). Therefore, our discovery enhances the understanding of karst biodiversity and supplements the information on terrestrial snails in Thailand available for efforts to establish well-planned and knowledge-based conservation procedures for Thai limestone protection in the future.

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References

- American Veterinary Medical Association. 2020. *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*. Available from <https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>. [accessed 12 Feb 2020]
- Ashburton J. 1864. Paper read at the Royal Geographical Society 10th March, 1862. In: Mouhot H. (ed.) *Travels in the Central Parts of Indo-China (Siam), Cambodia, and Laos, During the Years 1858, 1859, and 1860*: 296–300. Murray, London.
- Baur B. 2010. Stylommatophoran Gastropods. In: Leonard J.L. & Córdoba-Aguilar A. (eds) *The Evolution of Primary Sexual Characters in Animals*: 197–217. Oxford University Press, USA.
- Blanford W.T. & Godwin Austen H.H. 1908. Mollusca: Testacellidae and Zonitidae. In: Bingham C.T. (ed.) *The Fauna of British India including Ceylon and Burma*. Taylor and Francis, London.
- Clements R., Sodhi N.S., Schilthuizen M. & Ng P.K. 2006. Limestone karsts of Southeast Asia: Imperiled arks of biodiversity. *Bioscience* 56 (9): 733–742. <https://doi.org/fq9mjv>
- Dumrongrojwattana P. & Tanmuangpak K. 2020. The terrestrial microsnail genus *Aulacospira* Möllendorff, 1890 (Eupulmonata, Stylommatophora, Hypselostomatidae) in Thailand with key to Thai species. *ZooKeys* 980: 23–42. <https://doi.org/10.3897/zookeys.980.54100>
- Felsenstein J. 2004. *Inferring Phylogenies*. Massachusetts: Sinauer Associates, Sunderland.
- Fischer H. & Dautzenberg P. 1904. *Catalogue des Mollusques terrestres et fluviatiles de l'Indo-Chine orientale cités jusqu'à ce jour*. Mission Pavie, Études diverses 3: 390–450. <https://gallica.bnf.fr/ark:/12148/bpt6k8814596> [accessed 22 July 2021]
- Fischer P. 1891. *Catalogue et distribution géographique des Mollusques terrestres, fluviatiles & marins d'une partie de l'Indo-Chine (Siam, Laos, Cambodge, Cochinchine, Annam, Tonkin)*. Imprimerie Dejeussieu Père et Fils, Autun. <https://doi.org/10.5962/bhl.title.14809>
- Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Foon J.K., Clements G.R. & Liew T.-S. 2017. Diversity and biogeography of land snails (Mollusca, Gastropoda) in the limestone hills of Perak, Peninsular Malaysia. *ZooKeys* 682: 1–94. <https://doi.org/10.3897/zookeys.682.12999>
- Gardner S., Sidisunthorn P. & Chayamarit K. 2015. *Forest Trees of Southern Thailand Volume 1 (Acanthaceae to Escalloniaceae)*. Kobfai Publishing Project, Bangkok.
- Grismer L.L., Wood P.L., Le M.D., Quah E.S.H. & Grismer J.L. 2020. Evolution of habitat preference in 243 species of Bent-toed geckos (Genus *Cyrtodactylus* Gray, 1827) with a discussion of karst habitat conservation. *Ecology and Evolution* 10: 13717–13730. <https://doi.org/10.1002/ece3.6961>
- Hausdorf B. 1998. Phylogeny of the Limacoidea sensu lato (Gastropoda: Stylommatophora). *Journal of Molluscan Studies* 64: 35–66. <https://doi.org/10.1093/mollus/64.1.35>
- Hillis D.M. & Bull J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192. <https://doi.org/10.1093/sysbio/42.2.182>
- Hirano T., Kameda Y., Kimura K. & Chiba S. 2014. Substantial incongruence among the morphology, taxonomy, and molecular phylogeny of the land snails *Aegista*, *Landouria*, *Trishoplita*, and *Pseudobuliminus* (Pulmonata: Bradybaenidae) occurring in East Asia. *Molecular Phylogenetics and Evolution* 70: 171–181. <https://doi.org/10.1016/j.ympev.2013.09.020>

- Hirano T., Wada S., Mori H., Uchida S., Saito T. & Chiba S. 2018. Genetic and morphometric rediscovery of an extinct land snail on oceanic islands. *Journal of Molluscan Studies* 84: 148–156.
<https://doi.org/10.1093/mollus/eyy003>
- Huelsenbeck J.P. & Rannala B. 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. *Systematic Biology* 53: 904–913.
<https://doi.org/10.1080/10635150490522629>
- Hyman I.T. & Köhler F. 2018 Reconciling comparative anatomy and mitochondrial phylogenetics in revising species limits in the Australian semislug *Helicarion* Férussac, 1821 (Gastropoda: Stylommatophora). *Zoological Journal of the Linnean Society* 184: 933–968.
<https://doi.org/10.1093/zoolinnean/zly017>
- Hyman I.T. & Köhler F. 2019. Phylogeny and systematic revision of the helicarionid semislugs of eastern Queensland (Stylommatophora, Helicarionidae). *Contributions to Zoology* 88 (4): 351–451.
<https://doi.org/10.1163/18759866-20191416>
- Hyman I.T. & Ponder W.F. 2010. A morphological phylogenetic analysis and generic revision of Australian Helicarionidae (Gastropoda: Pulmonata: Stylommatophora), and an assessment of the relationships of the family. *Zootaxa* 2462: 1–148. <http://doi.org/10.11646/zootaxa.2462.1.1>
- Inkhavilay K., Sutcharit C., Bantaowong U., Chanabun R., Siriut W., Srisonchai R., Pholyotha A., Jirapatrasilp P. & Panha S. 2019. Annotated checklist of the terrestrial molluscs from Laos (Mollusca, Gastropoda). *ZooKeys* 834: 1–166. <http://doi.org/10.3897/zookeys.834.28800>
- Köhler F. & Criscione F. 2015. A molecular phylogeny of camaenid land snails from northwestern Australia unravels widespread homoplasy in morphological characters (Gastropoda, Helicoidea). *Molecular Phylogenetics and Evolution* 83: 44–55. <https://doi.org/10.1016/j.ympev.2014.11.009>
- Köhler F., Criscione F. & Shea M. 2020. A mitochondrial phylogeny uncovers taxonomic ambiguity and complex phylogeographic patterns in the eastern Australian land snail *Austrochloritis* (Stylommatophora, Camaenidae). *Journal of Zoological Systematics and Evolutionary Research* 58: 1005–1020.
<https://doi.org/10.1111/jzs.12366>
- Kumar S., Stecher G. & Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874.
<https://doi.org/10.1093/molbev/msw054>
- Latinne A., Waengsothorn S., Rojanadilok P., Eiamampai K., Sribuarod K. & Michaux J.R. 2013. Diversity and endemism of Murinae rodents in Thai limestone karsts. *Systematics and Biodiversity* 11: 323–344. <https://doi.org/10.1080/14772000.2013.818587>
- Liew T.S., Schilthuizen M. & Vermeulen J.J. 2009. Systematic revision of the genus *Everettia* Godwin-Austen, 1891 (Mollusca: Gastropoda: Dyakiidae) in Sabah, northern Borneo. *Zoological Journal of the Linnean Society* 157: 515–550. <https://doi.org/10.1111/j.1096-3642.2009.00526.x>
- Likhitrakarn N., Golovatch S.I., Jeratthitikul E., Srisonchai R., Sutcharit C. & Panha S. 2020. A remarkable new species of the millipede genus *Trachyjulus* Peters, 1864 (Diplopoda, Spirostreptida, Cambalopsidae) from Thailand, based both on morphological and molecular evidence. *ZooKeys* 925: 55–72. <https://doi.org/10.3897/zookeys.925.49953>
- Mauro D.S. & Agorreta A. 2010. Molecular systematics: a synthesis of the common methods and the state of knowledge. *Cellular & Molecular Biology Letters* 15: 311–341.
<https://doi.org/10.2478/s11658-010-0010-8>

- Miller M.A., Pfeiffer W. & Schwartz T. 2010. *Creating the CIPRES science gateway for inference of large phylogenetic trees*. Proceedings of the Gateway Computing Environments Workshop (GCE), IEEE, New Orleans, Louisiana. <https://doi.org/10.1109/GCE.2010.5676129>
- Mouhot H. 1864. *Travels in the Central Parts of Indo-China (Siam), Cambodia, and Laos, During the Years 1858, 1859, and 1860*. Murray, London.
- Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A.B. & Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. <https://doi.org/10.1038/35002501>
- Naggs F., Panha S. & Raheem D. 2006. Developing land snail expertise in South and Southeast Asia, a new Darwin Initiative Project. *The Natural History Journal of Chulalongkorn University* 6: 43–46.
- Páll-Gergely B., Fehér Z., Otani J. U. & Asami T. 2016. An integrative taxonomic approach to infer the systematic position of *Chalepotaxis* Ancey, 1887 (Gastropoda: Stylommatophora: Helicarionidae). *Molluscan Research* 37: 113–119. <https://doi.org/10.1080/13235818.2016.1234996>
- Pauwels O.S.G., Sumontha M., Panitvong N. & Varaguttanonda V. 2014. *Cyrtodactylus khelangensis*, a new cave-dwelling Bent-toed Gecko (Squamata: Gekkonidae) from Lampang Province, northern Thailand. *Zootaxa* 3755 (6): 584–594. <https://doi.org/10.11646/zootaxa.3755.6.5>
- Pfeiffer L. 1863. Descriptions of thirty-six new land shells from the collection of H. Cuming, Esq. *Proceedings of the Zoological Society of London* 30: 268–278. [Published in parts, dates follow Duncan (1937)].
- Pfeiffer L. 1868. *Monographia heliceorum viventium supplementum tertium: sistens descriptiones systematicas et criticas omnium hujus familiae generum et specierum hodie cognitarum, volume quintum*. F.A. Brockhaus, Lipsiae.
- Pholyotha A., Sutcharit C. & Panha S. 2018. The land snail genus *Macrochlamys* Gray, 1847 from Thailand, with descriptions of five new species (Pulmonata: Ariophantidae). *Raffles Bulletin of Zoology* 66: 763–781.
- Pholyotha A., Sutcharit C., Thach P., Chhuoy S., Ngor P.B. & Panha S. 2020a. Land snail genus *Sarika* Godwin-Austen, 1907 (Eupulmonata: Ariophantidae) from Cambodia, with description of three new species. *European Journal of Taxonomy* 674: 1–21. <https://doi.org/10.5852/ejt.2020.674>
- Pholyotha A., Sutcharit C., Tongkerd P. & Panha S. 2020b. Integrative taxonomic revision of the land snail genus *Sarika* Godwin-Austen, 1907 in Thailand, with descriptions of nine new species (Eupulmonata, Ariophantidae). *ZooKeys* 976: 1–100. <https://doi.org/10.3897/zookeys.976.53859>
- Pholyotha A., Sutcharit C., Tongkerd P., Jeratthitikul E. & Panha S. 2021. Integrative systematics reveals the new land-snail genus *Taphrenalla* (Eupulmonata: Ariophantidae) with a description of nine new species from Thailand. *Contributions to Zoology* 90(1): 21–69. <https://doi.org/10.1163/18759866-BJA10013>
- Rambaut A., Suchard M.A., Xie D. & Drummond A.J. 2014. Tracer v1.6. <http://tree.bio.ed.ac.uk/software/tracer/>
- Ridd M.F., Barber A.J. & Crow M.J. 2011. Introduction to the geology of Thailand. In: Ridd M.F., Barber A.J. & Crow M.J. (eds) *The Geology of Thailand*: 1–18. Geological Society, London.
- Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Sedgwick W.C. & Schwendinger P.J. 1990. On a new cave-dwelling *Liphistius* from Thailand (Araneae: Liphistiidae). *Bulletin of the British Arachnological Society* 8:109–112.

- Siriboon T., Sutcharit C., Naggs F., Rowson B. & Panha S. 2014. Revision of the carnivorous snail genus *Discartemon* Pfeiffer, 1856, with description of twelve new species (Pulmonata, Streptaxidae). *ZooKeys* 401: 45–107. <https://doi.org/10.3897/zookeys.401.7075>
- Siriboon T., Naggs F., Wade C.M., Jeratthitikul E., Tongkerd P., Jirapatrasilp P., Panha S. & Sutcharit C. 2020. Phylogenetic relationships of the carnivorous terrestrial snail family Streptaxidae (Stylommatophora: Achatinina) in Thailand and surrounding areas of Southeast Asia. *Systematics and Biodiversity* 18: 720–738. <https://doi.org/10.1080/14772000.2020.1783384>
- Solem A. 1966. Some non-marine mollusks from Thailand, with notes on classification of the Helicarionidae. *Spolia Zoologica Musei Hauniensis* 24: 1–110.
- Srisonchai R., Enghoff H., Likhitrakarn N. & Panha S. 2018. A revision of dragon millipedes I: genus *Desmoxytes* Chamberlin, 1923, with the description of eight new species (Diplopoda, Polydesmida, Paradoxosomatidae). *ZooKeys* 761: 1–177. <https://doi.org/10.3897/zookeys.761.24214>
- Stamatakis A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Sumontha M., Chomngam N., Phanamphon E., Pawangkhanant P., Viriyapanon C., Thanaprayotsak W. & Pauwels O.S.G. 2017. A new limestone-dwelling leaf-toed gecko (Gekkonidae: *Dixonius*) from Khao Sam Roi Yot massif, peninsular Thailand. *Zootaxa* 4247 (5): 556–568. <https://doi.org/10.11646/zootaxa.4247.5.2>
- Sutcharit C., Jeratthitikul E., Pholyotha A., Lin A. & Panha S. 2020a. Molecular phylogeny reveals high diversity and endemism in the limestone karst-restricted land snail genus *Sophina* Benson, 1859 from Myanmar (Eupulmonata: Helicarionidae), with description of four new species. *Journal of Zoological Systematics and Evolutionary Research* 58: 957–981. <https://doi.org/10.1111/jzs.12420>
- Sutcharit C., Jeratthitikul E., Tongkerd P. & Panha S. 2020b. Reassessment and systematic position of the sinistral snails of genus *Hemiplecta* from Thailand (Eupulmonata: Ariophantidae), with description of two new species. *Contributions to Zoology*: 1–33. <https://doi.org/10.1163/18759866-BJA10016>
- Sutcharit C., Thach P., Chhuoy S., Ngor P.B., Jeratthitikul E., Siriut W., Srisonchai R., Ng T.H., Pholyotha A., Jirapatrasilp P. & Panha S. 2020c. Annotated checklist of the land snail fauna from southern Cambodia (Mollusca, Gastropoda). *ZooKeys* 948: 1–46. <https://doi.org/10.3897/zookeys.948.51671>
- Tanabe A.S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources* 11: 914–921. <https://doi.org/10.1111/j.1755-0998.2011.03021.x>
- Tompa A.S. 1984. Land snails (Stylommatophora). In: Tompa A.S. & Verdonk N.H. (eds) *The Mollusca, Vol. 7: Reproduction*: 47–140. Academic Press, London.
- Tongkerd P., Lee T., Panha S., Burch J.B. & O’Foighil D. 2004. Molecular phylogeny of certain Thai gastrocoptine micro land snails (Stylommatophora: Pupillidae) inferred from mitochondrial and nuclear ribosomal DNA sequences. *Journal of Molluscan Studies* 70: 139–147. <https://doi.org/10.1093/mollus/70.2.139>
- Tryon Jr. G.W. 1886. *Manual of Conchology; Structural and Systematic. With illustrations of the species. Second Series: Pulmonata. Volume II. Zonitidae*. Academy of Natural Sciences, Philadelphia.
- Tumpeesuwan C. & Tumpeesuwan S. 2017. Discovery of an overlooked helicarionid land snail (Helicarionidae: Durgellinae) from northeastern Thailand, with description of a new genus and new species, and a note on radula morphology and genital system. *Raffles Bulletin of Zoology* 65: 174–181.

Tumpeesuwan C. & Tumpeesuwan S. 2018. *Aenigmatoconcha sumonthai*, a new helicarionid land snail from Chumphon Province, Southern Thailand (Helicarionidae: Durgellinae). *Raffles Bulletin of Zoology* 66: 170–176. <http://doi.org/10.5281/zenodo.4504598>

Vermeulen J.J., Luu H.T., Theary K. & Anker K. 2019. New species of land snails (Mollusca: Gastropoda: Caenogastropoda and Pulmonata) of the Mekong Delta Limestone Hills (Cambodia, Vietnam). *Folia Malacologica* 27: 7–41. <https://doi.org/10.12657/folmal.027.001>

Zhang L.-J., Zhu Y.-J. & Lyu Z.-T. 2020. A new sinistral species of the land-snail genus *Satsuma* (Pulmonata: Camaenidae) from China. *Molluscan Research* 40 (1): 93–100. <https://doi.org/10.1080/13235818.2019.1644721>

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