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 Aenigmatoconcha 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.

Pholyotha A., Sutcharit C., Tongkerd P & Panha S. 2021. Systematic revision of the limestone karst-restricted land snail genus *Aenigmatoconcha* (Eupulmonata: Helicarionidae), with description of a new species. *European Journal of Taxonomy* 767: 55-82. https://doi.org/10.5852/ejt.2021.767.1487

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 & Tannuangpak 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 karts, 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 karts 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 congeneres 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 *Aenigmatoconcha* 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 *Aenigmatoconcha* 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**  |           |                               |                |
| **Aenigmatoconcha**    |           |                               |                |
| Tumpeesuwan & Tumpeesuwan, 2017 | | | |
| *A. clivicola*         |           |                               |                |
| 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       |
| *A. mitis* (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      |
| *A. sumonthai*         |           |                               |                |
| 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       |
| *A. eunetis* (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**  |           |                               |                |
| *Sophina schistostelis schistostelis* (Benson, 1859) | | | MN897023 |
| *Sophina conjungens*   | Stoliczka, 1871 | | | MN897033 |
| *Sophina bensoni*      | Blanford & Godwin-Austen, 1908 | | | MN897051 |
| *Sophina discoidalis*  | Stoliczka, 1871 | | | MN897041 |
| *Chalepotaxis infantilis* (Gredler, 1881) | | | KX027275 |
| **Ariophantidae**      | Godwin-Austen, 1883 | | | MT364982 |
| *Sarika resplendens*   | (Philippi, 1846) | | | MT364982 |
| *Sarika resplendens*   | (Philippi, 1846) | | | MT364982 |
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’-TAAACTTCTAGGGTGACCAAAAAATCA-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 ≥ 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 *Aenigmatoconcha* together with other helicarionoids 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 *Aenigmaconcha* 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 |
|-----------------------|------------------|---------------------------|-------------------------------------------------|-----------|----------------|-------------------|-----------|------------------------|
| *A. clivicola*²       | 5                | Strongly depressed to depressed | V-shaped rows / monocuspid with spatulate shape | Absent    | Long           | Present           | Oblique   | trapezoid              |
| *A. mitis*³           | 5                | Depressed to globously depressed | V-shaped rows / monocuspid with spatulate shape | Absent    | Short          | Present           | Small     | oblique wrinkled       |
| *A. sumonthai*³       | 5                | Strongly depressed to depressed | V-shaped rows / monocuspid with spatulate shape | Absent    | Short          | Present           | Small     | conical                |
| *A. eunetis sp.* nov.³ | 5              | Depressed to globously depressed | Wide-angle U-shape rows / monocuspid with oblong shape | Absent    | Short          | Present           | Small     | conical                |
| *Chalepotaxis*²       | –                | Depressed conical          | V-shaped rows / monocuspid with spatulate shape | Absent    | Short          | Absent            | –         | –                      |
| *Sophina*³            | 4                | Depressed to globously depressed | V-shaped rows / monocuspid with spatulate shape | Present   | Short          | Absent            | –         | –                      |

**Taxonomy**

Phylum Mollusca Linnaeus, 1758  
Class Gastropoda Cuvier, 1795  
Superfamily Helicarionioidea 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 globously 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.
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.

| Genera          | Aenigmatoconcha | Chalepotaxis | Sophina | Sarika |
|-----------------|-----------------|--------------|---------|--------|
| Aenigmatoconcha | 0.087           | --           | --      | --     |
| Chalepotaxis    | 0.130           | --           | --      | --     |
| Sophina         | 0.125           | 0.141        | 0.095   | --     |
| Sarika          | 0.119           | 0.140        | 0.123   | 0.008  |

**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.

**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).
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 *Aenigmatococona* 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 toptype 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* Stanisic, 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 (Tompa 1984; Baur 2010). However, during this study no spermatophores were observed in *A. clivicola*.

*Aenigmatoconcha 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* – Inkhavilay *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 Faet; 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 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 Phuang 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.
Description

Shell (Fig. 4C–F). Shell depressed to globose 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 *Aenigmatococoncha 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 *Aenigmatococoncha 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.

*Aenigmatoconcha 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).

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

*Aenigmatoconcha 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°01′58.5″ 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 *Aenigmatoconcha* 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).

*Euneta* *eunetis* Pholyotha & Panha sp. nov.

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, monocuspoid.

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 globose 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 *Aenigmaconcha 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 papillate. 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 *Aenigmaconcha 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 epiphallic 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.](image)

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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