Integrative systematics reveals the new land-snail genus *Taphrenalla* (Eupulmonata: Ariophantidae) with a description of nine new species from Thailand

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Abstract

The endemic terrestrial Crown Snail from Southern Thailand formerly in *Macrochlamys* Gray, 1847 is now described as *Taphrenalla* Pholyotha & Panha gen. nov., based on comparative morphology and molecular data. Overall, eleven *Taphrenalla* species are now recognized, including two nominal species: *T. asamurai* and *T. diadema*. A total of nine new species are proposed: *T. alba* sp. nov., *T. conformis* sp. nov., *T. corona* sp. nov., *T. dalli* sp. nov., *T. incilis* sp. nov., *T. macrosulcata* sp. nov., *T. parversa* sp. nov., *T. pygmaea* sp. nov. and *T. zemia* sp. nov. The molecular phylogeny constructed from the mitochondrial cytochrome oxidase c subunit I (COI) and 16S rRNA gene fragments plus the nuclear 28S rDNA gene fragments revealed that *Taphrenalla*

gen. nov. is monophyletic with a well-supported clade. The diagnostic characters of *Taphrenalla* gen. nov. are the shell sculpture with several radial grooves, body with well-developed colourful stripes running from the head to tail, and genitalia similar to *Macrochamys* but with an un-coiling epiphallic caecum. The spermatophore has one or two spines near the sperm sac and a spineless tail.

Keywords

Southeast Asia – limestone – endemic species – Limacoidei – Helicarionoidea – morphology – anatomy – phylogeny

Introduction

Thailand, especially the southern part, is rich in biodiversity of malacofauna and is recognized as a biodiversity hotspot (Myers et al., 2000). The topography of Southern Thailand is a peninsula between the Gulf of Thailand and the Andaman Sea. The long ridge of two mountain ranges (Phuket Range and Nakhon Si Thammarat Range) separated by low-lying areas of less than 200 m above mean sea level (amsl) extend to this part. The Phuket Range runs parallel to the west coast of upper Southern Thailand which extends northwards to the Tenasserim Range, whereas the Nakhon Si Thammarat Ranges runs from Surat Thai southwards to the Malaysian border (Gupta, 2005; Ridd et al., 2011; Gardner et al., 2015; Department of Mineral Resources, 2018).

The diversity of land snails in Southern Thailand is due to several interrelated factors. There is a high relative humidity, a short dry season (2–4 months) and a great amount of rainfall (Thai Meteorological Department, 2015). According to the key zoogeographical position of Thailand, the snail fauna has dispersed from Indo-Himalayan, Indo-Chinese, and Malaysian regions (Naggs et al., 2006). In addition, there are many extensive ranges of limestone karsts and outcrops scattered along this area. Limestones in Southern Thailand are formed from the Permian Ratburi Limestone and, to a lesser extent, the Ordovician-Devonian Satun

Group limestone (Gupta, 2005; Ridd et al., 2011; Gardner et al., 2015; Department of Mineral Resources, 2018). Many documents show that land snail diversity is associated with limestone karsts and outcrops (Clements et al., 2006; Naggs et al., 2006). The larger karsts probably contain a higher number and diversity of microhabitats that are conducive for land snail speciation and endemism (Clements et al., 2008; Foon et al., 2017).

The land snail family Ariophantidae Godwin-Austen, 1888 is recognized as one of the most diverse families and contains a remarkably high number of species with various shell characteristics from snails to semi-slugs (Blanford & Godwin-Austen, 1908; Schileyko, 2002, 2003). Ariophantid taxa are only characterized by the absence of the penial nerve fibres running through the cerebral ganglion (Hausdorf, 1998). Later, the definitions of Ariophantidae are given by Schileyko (2002), who made extensive changes to the classification of Limacoidea s.l. The characters of Ariophantidae are shell helicoid to plate-like, animal has no frontal organ, caudal foss and caudal horn well-developed, genitalia with various length flagellum, a straight, spirally coiled or missing epiphallic caecum attached by penial retractor, penis with or without verge, dart apparatus absent or present (when present, it is vaginal, with apical retractor). Ariophantidae is comprised of three subfamilies (Bouchet et al., 2017).

Among these, the Macrochlamydinae is widely distributed throughout Asia, especially South and Southeast Asia (Blanford & Godwin-Austen, 1908). Due to the similarity in their shell morphology (glossy, thin, and semi-transparent), early malacologists attributed most Asian species to *Macrochlamys* (Godwin-Austen, 1883, 1907; Blanford & Godwin-Austen, 1908). The type genus of this subfamily is *Macrochlamys* Gray, 1847, which is recognized as the most species-rich genus in Ariophantidae (Blanford & Godwin-Austen, 1908; Pholyotha et al., 2018, 2020; Sajan et al., 2019). Subsequently, many genera, such as *Taphrospira* Blanford, 1905, *Sarika* Godwin-Austen, 1907, *Sakiella* Godwin-Austen, 1908, *Syama* Godwin-Austen, 1908, and *Rasama* Laidlaw, 1932, have been described as different genera on the basis of their reproductive anatomy or some unique characters of their shells (Blanford, 1905; Blanford & Godwin-Austen, 1908; Laidlaw, 1932).

Taxonomic studies of the two Crown Snails, *Nanina* (*Macrochlamys*) *diadema* Dall, 1897 and *Macrochlamys* *asamurai* (Panha, 1997), are still problematic in their generic placement because of the striking appearance of many radial grooves on their shell sculpture, leading to their name of “Crown Snails”, and their remarkable body colouration. Early authors placed them in *Macrochlamys* (e.g., Dall, 1897; Panha, 1996, 1997; Maneevong, 2000; Hemmen & Hemmen, 2001), while they were transferred to *Sarika* by later authors (e.g., Maassen, 2001; Sutcharit & Panha, 2008; Pholyotha et al., 2018, 2020). Although Maassen (2001) suggested that a new genus should be established for these two species, Sutcharit & Panha (2008) still included them in *Sarika*. During a re-examination of all specimens having remarkable radial grooves in our collection, many peculiar specimens were found that were distinct in their shell sculpture, animal colouration, and genital anatomy.

Moreover, molecular analysis suggested that they formed a monophyletic group of a different lineage to *Sarika*. Therefore, Maassen's suggestion was agreed upon.

Consequently, several studies have documented that an integrative taxonomic approach, including molecular, morphological, and anatomical data, and including information of the reproductive biology should be used when proposing species entities (Huang et al., 2019; Jeratthitikul et al., 2019; Jirapatrasilp et al., 2019). For inferring molecular phylogenies, the mitochondrial cytochrome *c* oxidase subunit I (COI) and the large subunit ribosomal (r)RNA gene (16S) have been widely and successfully used to study putatively closely related land snails (Hyman et al., 2007, 2017; Wu et al., 2008; Liew et al., 2009; Köhler & Criscione, 2015; Hyman & Köhler, 2018). The partial 28S nuclear rDNA (28S) sequences are more conserved and become useful to resolve deep relationships within the family (Wade et al., 2001, 2006; Páll-Gergely et al., 2017; Nantararat et al., 2019).

In this present study, several newly collected specimens of crown snails from Southern Thailand were examined. The new genus is proposed based on the distinct morphological characters and molecular data. The descriptions of nine new species are included herein and the status of two previously described species is updated. Additionally, phylogenetic trees constructed from concatenated sequences of three gene fragments (COI, 16S, and 28S) were used to infer the evolutionary relationship and distribution patterns among members of this genus in Southern Thailand.

Materials and methods

Sampling and morphological studies

This study is based on voucher specimens deposited in Chulalongkorn University

FIGURE 1 Map showing the sampling sites of the 11 *Taphrenalla* species along Southern Thailand. Each colour of the distribution range follows the phylogenetic tree of all *Taphrenalla* species in fig. 3.

Museum of Zoology (CUMZ) and new samples collected throughout Southern Thailand from 2015 to 2019 (fig. 1). The living snails were photographed and euthanised (American Veterinary Medical Association, 2013). Then, specimens from each locality were fixed and preserved in 70% (v/v) ethanol for anatomical studies and duplicate specimens and/or tissues were preserved in 95% (v/v) ethanol for molecular analysis. The genitalia of 3–10 specimens of each species were dissected and examined under an Olympus SZX2-TR30 stereoscopic light microscope. Shells and genitalia were imaged using a digital camera (DSLR D850 - Nikon) and a stereo microscope with Cell'D Imaging Software. Radulae were

imaged by scanning electron microscopy (SEM; JEOL, JSM-6610 LV).

Species identification followed Dall (1897), Blanford & Godwin-Austen (1908), Panha (1997), and Sutcharit & Panha (2008), and was subsequently compared to the relevant type specimens. In the examined material sections, shells refer to empty shells, while specimens refer to specimens preserved in ethanol. Descriptions of a new genus and all new species herein are attributed to the first and fifth authors, Pholyotha & Panha, respectively. The general vouchers and type specimens were deposited in the CUMZ and the Natural History Museum, London, United Kingdom (NHM).

Molecular studies

DNA extraction, amplification, and sequencing

We extracted DNA from a total of 41 individuals of 11 *Taphrenalla* species. Our outgroup sampling included a total of 21 specimens representing six genera, including five ariophantid genera: *Macrochlamys*, *Sarika*, *Hemiplecta* Albers, 1850, *Cryptozona* Mörch, 1872 and

Megaustenia Cockerell, 1912, and one helicariionid genus *Durgella* Blanford, 1863 (table 1). We choose these genera because their distributions are in Thailand and some members of the genera *Macrochlamys* and *Sarika* had been hypothesized previously to be closely related genera (Blanford & Godwin-Austen, 1908; Solem, 1966; Sutcharit & Panha, 2008) as well as to the new genus.

TABLE 1 Samples used in the molecular analyses with GenBank accession numbers

Species	Specimen code	Voucher code	Locality name	GenBank accession number		
				COI	16S	28S
Family Ariophantidae						
Genus <i>Taphrenalla</i> gen. nov						
<i>T. alba</i> sp. nov.	S6	CUMZ7223	Limestone outcrops near Than Bok Khorani, Ao Luek, Krabi, Thailand	MT364971	MT365754	MT365698
	S45	CUMZ7219	Wat Tham Seu, Mueang, Krabi, Thailand	MT364972	MT365755	MT365699
	S45-2	CUMZ7219	Wat Tham Seu, Mueang, Krabi, Thailand	MT364973	MT365756	MT365700
	S127	CUMZ7221	Limestone outcrops at Ao Luek Tai, Ao Luek, Krabi, Thailand	MT364974	MT365757	MT365701
<i>T. asamurai</i>	S18	CUMZ7153	Wat Tham Wararam, Phanom, Surat Thani, Thailand	MT364934	MT365723	MT365667
	S22	CUMZ7159	Limestone outcrops at Khlong Sok, Phanom, Surat Thani, Thailand	MT364935	MT365724	MT365668
	S34	CUMZ7153	Wat Tham Wararam, Phanom, Surat Thani, Thailand	MT364936	MT365725	MT365669
<i>T. conformis</i> sp. nov.	S21	CUMZ7203	Tham Phung Chang, Mueang, Phang-nga, Thailand	MT364959	MT365742	MT365686
	S120	CUMZ7205	Tham Phung Chang, Mueang, Phang-nga, Thailand	MT364960	MT365743	MT365687

TABLE 1 Samples used in the molecular analyses with GenBank accession numbers (*cont.*)

Species	Specimen code	Voucher code	Locality name	GenBank accession number		
				COI	16S	28S
	S126	CUMZ7206	Tham Phung Chang, Mueang, Phang-nga, Thailand	MT364961	MT365744	MT365688
<i>T. corona</i> sp. nov.	S5	CUMZ7192	Limestone outcrops near Than Bok Khorani, Ao Luek, Krabi, Thailand	MT364953	MT365736	MT365680
	S5-2	CUMZ7192	Limestone outcrops near Than Bok Khorani, Ao Luek, Krabi, Thailand	MT364954	MT365737	MT365681
	S47	CUMZ7195	Limestone outcrops at Ao Luek Tai, Ao Luek, Krabi, Thailand	MT364955	MT365738	MT365682
<i>T. dalli</i> sp. nov.	S52-2	CUMZ7184	Tham Lord, Nopphitam, Nakhon Si Thammarat, Thailand	MT364948	MT365733	MT365677
	S61	CUMZ7185	Wat Khiri Rat Phatthana, Wiang Sa, Surat Thani, Thailand	MT364949	-	-
	S65	CUMZ7187	Wat Na San, Ban Na San, Surat Thani, Thailand	MT364950	-	-
	S71	CUMZ7189	Wat Tham Kanlayanamit, Tham Phannara, Nakhon Si Thammarat, Thailand	MT364951	MT365734	MT365678
	S73	CUMZ7190	Wat Tham Thong Panara, Tham Phannara, Nakhon Si Thammarat, Thailand	MT364952	MT365735	MT365679
<i>T. diadema</i>	S46	CUMZ7175	Wat Tham Sumano, Srinagarindra, Phatthalung, Thailand	MT364940	MT365729	MT365673
	S62	CUMZ7181	Limestone outcrops at Khlong Chaloem, Kong Ra, Phatthalung, Thailand	MT364941	MT365730	MT365674
	S63	CUMZ7180	Tham Phaya Hong, Kong Ra, Phatthalung, Thailand	MT364942	MT365731	MT365675
	S70	CUMZ7167	Tham Rad, Thung Song, Nakhon Si Thammarat, Thailand	MT364943	-	-

TABLE 1 Samples used in the molecular analyses with GenBank accession numbers (*cont.*)

Species	Specimen code	Voucher code	Locality name	GenBank accession number		
				COI	16S	28S
	S72	CUMZ7177	Khao Ok Talu Khuha Sawan, Mueang, Phatthalung, Thailand	MT364944	MT365732	MT365676
	S75	CUMZ7164	Wat Khuha Santayaram, Ron Phibun, Nakhon Si Thammarat	MT364945	-	-
	S76	CUMZ7168	Wat Tham Phraphut, Ratsada, Trang, Thailand	MT364946	-	-
	S77	CUMZ7165	Tham Talot, Thung Song, Nakhon Si Thammarat, Thailand	MT364947	-	-
<i>T. incilis</i> sp. nov.	S2	CUMZ7208	Tham Khao Ting, Palian, Trang, Thailand	MT364962	MT365745	MT365689
	S69	CUMZ7209	Tham Khao Ting, Palian, Trang, Thailand	MT364963	MT365746	MT365690
	S69-2	CUMZ7209	Tham Khao Ting, Palian, Trang, Thailand	MT364964	MT365747	MT365691
<i>T. macrosulcata</i> sp. nov.	S38	CUMZ7162	Limestone outcrops at Khlong Sok, Phanom, Surat Thani, Thailand	MT364937	MT365726	MT365670
	S38-2	CUMZ7162	Limestone outcrops at Khlong Sok, Phanom, Surat Thani, Thailand	MT364938	MT365727	MT365671
	S38-3	CUMZ7162	Limestone outcrops at Khlong Sok, Phanom, Surat Thani, Thailand	MT364939	MT365728	MT365672
<i>T. parversa</i> sp. nov.	S40	CUMZ7200	Tham Suwan Khuha, Takua Thung, Phang-nga, Thailand	MT364956	MT365739	MT365683
	S40-2	CUMZ7200	Tham Suwan Khuha, Takua Thung, Phang-nga, Thailand	MT364957	MT365740	MT365684
	S40-3	CUMZ7200	Tham Suwan Khuha, Takua Thung, Phang-nga, Thailand	MT364958	MT365741	MT365685
<i>T. pygmaea</i> sp. nov.	S67	CUMZ7212	Wat Sathit Khirirom, Khiri Rat Nikhom, Surat Thani, Thailand	MT364965	MT365748	MT365692

TABLE 1 Samples used in the molecular analyses with GenBank accession numbers (*cont.*)

Species	Specimen code	Voucher code	Locality name	GenBank accession number		
				COI	16S	28S
	S119	CUMZ7211	Tham Wang Badan Priest's Camp Site, Khiri Rat Nikhom, Surat Thani, Thailand	MT364966	MT365749	MT365693
	S119-2	CUMZ7211	Tham Wang Badan Priest's Camp Site, Khiri Rat Nikhom, Surat Thani, Thailand	MT364967	MT365750	MT365694
<i>T. zemia</i> sp. nov.	S64	CUMZ7214	Wat Khiriwong, Thap Put, Phang-nga, Thailand	MT364968	MT365751	MT365695
	S66	CUMZ7216	Wat Tham Bang Toei, Mueang, Phang-nga, Thailand	MT364969	MT365752	MT365696
	S68	CUMZ7215	Tham Pha Sua Bureau of Monks, Mueang, Phang-nga, Thailand	MT364970	MT365753	MT365697
Genus <i>Macrochlamys</i>						
<i>M. aspides</i>	MY8	CUMZ7135	Lun-Nya Mountain, Hpa-an, Kayin, Myanmar	MT364986	MT365761	MT365705
<i>M. caverna</i>	C1	CUMZ7109	Wat Khao Samo Khon, Tha Wung, Lopburi, Thailand	MT364989	MT365758	MT365702
	C15	CUMZ7113	Khao Mon Ing Dharma Practice Place, Ban Mi, Lopburi, Thailand	MT364988	MT365759	MT365703
	C16	CUMZ7111	Wat Tham Chang Pueak, Tha Wung, Lopburi, Thailand	MT364987	MT365760	MT365704
<i>M. tanymentula</i>	W16	CUMZ7119	Limestone outcrops at Khao Lok, Thong Pha Phum, Kanchanaburi, Thailand	MT364985	MT365762	MT365706
Genus <i>Sarika</i>						
<i>S. heptagyra</i>	W19	CUMZ7231	Tham Khao Noi Bureau of Monks, Thong Pha Phum, Kanchanaburi, Thailand	MT364980	MT365765	MT365709

TABLE 1 Samples used in the molecular analyses with GenBank accession numbers (*cont.*)

Species	Specimen code	Voucher code	Locality name	GenBank accession number		
				COI	16S	28S
	W25	CUMZ7232	Tham Dao Wadung, Sai Yok, Kanchanaburi, Thailand	MT364981	MT365766	MT365710
<i>S. obesior</i>	MY2	CUMZ7141	Forest on Kala Island, Myeik, Tanintharyi, Myanmar	MT364979	MT365769	MT365713
	MY2-2	CUMZ7141	Forest on Kala Island, Myeik, Tanintharyi, Myanmar	MT364978	MT365770	MT365714
	W65	CUMZ7233	Wat Nong Phlap, Hua Hin, Prachuap Khiri Khan, Thailand	MT364977	MT365768	MT365712
<i>S. resplendens</i>	W4	CUMZ7234	Khao Cha Ngum, Photharam, Ratchaburi, Thailand	MT364982	MT365763	MT365707
	W23	CUMZ7235	Limestone outcrops near Song Karia, Sangkhla Buri, Kanchanaburi, Thailand	MT364983	MT365764	MT365708
<i>Sarika</i> sp. 1	S116	CUMZ7236	Wat Sathit Khirirom, Khiri Rat Nikhom, Surat Thani, Thailand	MT364975	MT365771	MT365715
<i>Sarika</i> sp. 2	E2-2	CUMZ7237	Pang Sida Waterfall, Watthana Nakhon, Sa Kaeo, Thailand	MT364984	MT365772	MT365716
<i>Sarika</i> sp. 3	W58	CUMZ7238	Tham Khao Ma-Rong, Bang Saphan, Prachuap Khiri Khan, Thailand	MT364976	MT365767	MT365711
Genus <i>Cryptozona</i>						
<i>C. siamensis</i>	NE7	CUMZ7239	Garden in Khon Kaen University, Mueang, Khon Kaen, Thailand	MT364993	MT365773	MT365717
	S129	CUMZ7240	Wat Tha Mai Lai, Mueang, Chumphon, Thailand	MT364992	MT365774	MT365718

TABLE 1 Samples used in the molecular analyses with GenBank accession numbers (cont.)

Species	Specimen code	Voucher code	Locality name	GenBank accession number		
				COI	16S	28S
Genus <i>Hemiplecta</i>						
<i>H. humphreysiana</i>	H7	CUMZ	Singapore	MT364994	MT365775	MT365719
<i>H. pluto</i>	H63	CUMZ	Laos	MT364995	MT365776	MT365720
Genus <i>Megaustenia</i>						
<i>Megaustenia</i> sp.	C19	CUMZ7241	Pa Ma-muang Bureau of Monks, Noen Maprang, Phitsanulok, Thailand	MT364990	MT365778	MT365722
Family Helicarionidae						
Genus <i>Durgella</i>						
<i>Durgella</i> sp.	N15	CUMZ7242	Tham Lom Tham Wang, Si Samrong, Sukhothai, Thailand	MT364991	MT365777	MT365721

Whole genomic DNA was extracted from a small piece of the foot muscle tissue using the NucleoSpin® Tissue Kit (Macherey-Nagel, Germany), following the manufacturer's standard instructions. The two partial mitochondrial genes (COI and 16S rRNA) were amplified using the LCO1491 and HCO2198 universal primers for COI (Folmer et al., 1994) and the 16Sar and 16Sbr universal primers for 16S (Palumbi et al., 1991), while the 28S rDNA was amplified using the LSU2 and LSU4 primers (Wade & Mordan, 2000). The PCR thermal cycling was performed under the following conditions: (i) COI: initial 94°C for 2 min followed by 36 cycles of 94°C for 30 s, 50 or 52°C for 120 s, and 72°C for 120 s, and then followed by a final 72°C for 5 min; (ii) 16S rRNA: 94°C for 2 min, followed by 40 cycles of 94°C for 30 s, 50°C for 60 s, and 72°C for 120 s, and then followed by a final 72 °C for 5 min; (iii) 28S rDNA: 94°C for 1 min, followed by 36 cycles of 94°C for 30 s, 62°C for 90 s, and 72°C

for 120 s, and then followed by a final 72°C for 5 min. The size of the PCR products was checked under UV transillumination after gel electrophoresis. The PCR products were sent for commercial sequencing at Bioneer Co., Korea.

Sequence alignment and phylogenetic analyses

Chromatograms were manually corrected for misreads using MEGA v. 7.0 (Kumar et al., 2016). To achieve a single consensus sequence, individual forward and reverse sequence traces were aligned and edited using MUSCLE, as implemented in MEGA v. 7.0. The newly generated sequences have been deposited in the GenBank databases with accession numbers: MT364934–MT364995 and MT365667–MT365778 (table 1).

The sequence alignments of the COI, 16S rRNA, and 28S rDNA gene fragments were performed separately using MAFFT v. 7 in

the MAFFT online service (<https://mafft.cbrc.jp/alignment/server/index.html>) with the default options (Kato et al., 2017). The regions of ambiguous alignment were checked and cleaned with Gblocks v. 0.91b using relaxed parameters and allowing all gap positions (Castresana, 2000; Talavera & Castresana, 2007). To check for substitution saturation, we performed the test implemented in DAMBE (Xia, 2013). The phylogenetic trees were then constructed using maximum likelihood (ML) and Bayesian inference (BI). Prior to the ML and BI analyses, the best-fit models of nucleotide substitution were identified for each gene separately with five partitions (16S rRNA, 28S rDNA and the three codon positions of COI) using Kakusan4 (Tanabe, 2007, 2011). The ML phylogenetic analysis was performed by applying the GTRGAMMA model to all three genes at the default settings of RAxML-HPC2 on XSEDE v. 8.2.10 (Stamatakis, 2014) in the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010). Nodal support values of ML were assessed with 1000 bootstrap (BS) replicates.

The BI analysis was performed using MrBayes on XSEDE v. 3.2.6 (Ronquist et al., 2012) in the CIPRES Science Gateway. The analysis was performed using two simultaneous runs consisted of running four simultaneous chains for 10 million generations. Every 500 generations were sampled and the first 50% of sampled trees were discarded as burn-in. The other settings of the analysis followed the developer's recommendation (Ronquist et al., 2012). For ML, BS support values of $\geq 70\%$ were considered highly supported and BI posterior probabilities (PP) of ≥ 0.95 were considered statistically significant (Hillis & Bull, 1993; Felsenstein, 2004; Huelsenbeck & Rannala, 2004; Mauro & Agorreta, 2010; Hirano et al., 2018). Pairwise genetic distances based on the COI and 16S sequences were calculated using Kimura's two-parameter model (K2P) in MEGA v. 7.

Molecular species delimitation

We conducted species delimitation analyses using two methods: (i) the Automatic Barcode Gap Discovery (ABGD) method (Puillandre et al., 2012) as a distance-based method and (ii) the Poisson Tree Processes (PTP) method (Zhang et al., 2013; Kapli et al., 2017) as a phylogeny-based method. We applied two approaches only to the COI dataset because COI-based DNA barcoding can delimit diverse animal species (Hebert et al., 2003). To apply ABGD, the sequence alignment of the COI gene was uploaded at <https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html> (last modification date: 7 December 2018) and then the K2P model was chosen as the distance type with the following settings: $P_{min} = 0.001$, $P_{max} = 0.1$, Steps = 40, X (relative gap width) = 1, and Nb bins (for distance distribution) = 20. The analysis with Jukes-Cantor distance (JC69) was employed with the same settings, except for the relative gap width ($X = 0.8$). The PTP species delimitation analyses using the single-rate PTP (sPTP) were processed thorough the online PTP web server (<http://mptp.h-its.org>). A phylogenetic input tree was obtained from the ML analysis, which was conducted based on an alignment of the 41 COI sequences from all *Taphrenalla* species with an additional two sequences of *Sarika resplendens* as the out-group using RAxML-HPC2 on XSEDE v. 8.2.10. The molecular operational taxonomic units (MOTUs) delimited by the two species delimitation analyses were regarded as preliminary hypothesis, then the distinction based on morphological and anatomical characters were observed to treat them as either distinct species or conspecific lineages.

Abbreviations

In the descriptions of the reproductive organs, 'proximal' refers to the region closest to the genital pore and 'distal' refers to the region furthest away. The genital terminology and

abbreviations follow Blanford & Godwin-Austen (1908) and Pholyotha et al. (2018, 2020): ant-ldl, anterior left dorsal lobe; an, anus; at, atrium; da, dart apparatus; ch, caudal horn; e, epiphallus; ec, epiphallic caecum; fl, flagellum; fo, free oviduct; gd, gametolytic duct; gs, gametolytic sac; h, heart; hf, head filament; hg, hindgut; kd, kidney; lsl, left shell lobe; mc, mantle collar; ov, oviduct; p, penis; pc, penial caecum; pg, prostate gland; post-ldl, posterior left dorsal lobe; pp, penial pilaster; ppv, principal pulmonary vein; prm, penial retractor muscle; pv, penial verge; rdl, right dorsal lobe; rsl, right shell lobe; ss, sperm sac; tf, tail filament; ur, ureter; v, vagina, and vd, vas deferens.

Collections

CUMZ, Chulalongkorn University Museum of Zoology, Bangkok; NHM, The Natural History Museum, London; SMF, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main; USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC.

Results

Molecular phylogeny

From a total of 56 individuals used (including outgroups), we obtained COI, 16S, and 28S sequences for 35 specimens from all *Taphrenalla* species. The final concatenated dataset of the aligned sequences of the three gene fragments had a total length of 1672 bp (COI: 655 bp, 16S: 478 bp, and 28S: 539 bp), after 41 bp were excluded as ambiguously aligned by Gblocks (COI: 1 bp, 16S: 40 bp). Using DAMBE, no substitution saturation was detected in all datasets (Iss < Iss.c with $p < 0.01$). The best-fit models of evolution for each gene fragment (1st COI; 2nd COI; 3rd COI; 16S; 28S) based on the Bayesian Information

Criterion (BIC) test were the Kimura 1980 model with gamma distribution (K80+G) for the 1st COI codon, the Felsenstein (1981) model with gamma distribution (F81+G) for the 2nd COI codon, and the general time reversible model with time-reversible gamma distribution (GTR+G) for the 3rd COI codon, the 16S gene, and the 28S gene.

Phylogenetic relationships

The phylogenetic trees (fig. 2) based on the concatenated datasets using ML and BI methods recovered 11 species of *Taphrenalla* forming a well-supported clade (BS = 89%, PP = 1). Focusing on the sister-group relationships among genera, *M. tanymentula* Pholyotha & Panha, 2018 was retrieved as the closest sister group to *Taphrenalla*, but with poor support (BS = 67%, PP = 0.88). The two other *Macrochlamys* species in this analysis, which are endemic in Thailand and Myanmar, formed a sister clade to the *Taphrenalla* + *M. tanymentula* clade with strong support (BS = 96%, PP = 0.99). The genera *Sarika* and the *Cryptozona* were retrieved as monophyletic with very strong support (BS = 100%, PP = 1). The *Cryptozona* + *Sarika* clade was also a sister group to the *Taphrenalla* + *Macrochlamys* clade with high support (BS = 97%, PP = 1). In addition, compared with the other outgroups, the large land snail genus *Hemiplecta* and the semi-slug genus *Megaustenia* positioned as basal clades with strong support (BS = 94%, PP = 1).

With respect to the monophyly of all *Taphrenalla* species, the phylogenetic trees (figs 2, 3) were clearly divided into two well-separated clades (clade A and B). Clade A contained *T. diadema* and eight new species with good support (BS = 85%, PP = 0.99), while clade B contained *T. macrosulcata* and *T. asamurai* with very strong support (BS = 100%, PP = 1). Although the species relationships among clade A were not well resolved,

FIGURE 2 Representative ML tree of the *Taphrenalla* gen. nov., *Macrochlamys*, *Sarika*, *Cryptozona*, *Hemiplecta*, *Megaustenia*, and *Durgella* based on the combined partial sequences of the COI, 16S, and 28S genes. The code in the parenthesis next to the species name refers to voucher numbers of the analysed samples. Numbers on the branches indicate the ML BS and BI PP values.

FIGURE 3 A representative ML tree showing only the *Taphrenalla* clade. The synopsis of the radial groove on shell sculpture, genitalia showing location of dart apparatus, and live specimens of *Taphrenalla* spp. Results of species delimitation analyses based on the COI gene using ABGD and PTP. Black circles represent nodes with both ML (when $\geq 70\%$) and BI (when ≥ 0.95) support. White circles represent nodes with ML BS support but no BI PP support (when < 0.95). Each clade colour refers to the distribution range in fig. 1.

this clade was further divided into five subclades (A1 to A5). Four new species (*T. parversa*, *T. corona*, *T. zemia*, and *T. conformis*) formed clade A1 with good support (BS = 78%, PP = 1). Moreover, within clade A1, *T. parversa* was very closely related to *T. corona* with high support (clade A1/1; BS = 94%, PP = 0.99). Interestingly, clade A1 showed various degrees of the radial grooves on shell sculpture, ranging from distinct to absent. The strongly supported clade A2 (BS = 100%, PP = 1) consisted of one species, *T. dalli*. Within clade A3, the type species *T. diadema* was the sister lineage of *T. incilis* with moderate support (BS = 79%, PP = 0.93). Clades A4 and A5 consisted of *T. pygmaea* and *T. alba*, respectively, both of which were highly supported (BS = 100%, PP = 1). Taxa with a similar shell morphology were not always retrieved as monophyletic, e.g. *T. dalli* (clade A2) and *T. diadema* (clade A3).

Pairwise genetic distances

The aligned COI sequence data matrix of 62 specimens, including 41 specimens of *Taphrenalla* species, and the 16S data matrix of 56 specimens, including 35 specimens of *Taphrenalla* species, were used to calculate the mean sequence divergences. The mean genetic divergence observed among the genera of the family Ariophantidae ranged between 10.0–16.7% in COI and 12.2–19.5% in 16S (table 2),

while the interspecific divergences among the 11 species of *Taphrenalla* ranged from 3.4–7.7% for COI and from 1.0–9.2% for 16S (table 3). The *Taphrenalla* intraspecific divergences ranged from 0–2.4% for COI and from 0–1.5% for 16S (table 4). The highest intraspecific mean sequence divergence of COI and 16S within *Taphrenalla* was observed in *T. alba*. No variation in the COI was observed in *T. parversa*, and no variation in the 16S was observed in *T. parversa*, *T. incilis*, and *T. conformis*.

Species delimitation

A total of 41 COI sequences, representing all *Taphrenalla* species were assembled for the molecular species delimitation to identify species boundaries. The results of the ABGD species delimitation approach using the JC69 model and the K2P model recovered 11 and 12 MOTUs, respectively (fig. 3). The other method, the single-rate PTP analysis recovered 13 MOTUs (fig. 3). Therefore, the two species delimitation analyses (ABGD and PTP) suggested there were between 11 and 13 MOTUs in *Taphrenalla* gen. nov. By comparing them with morphology-based delimitation, we concluded that there were 11 confirmed candidate species being treated as distinct species, and the two remaining MOTUs were treated as the conspecific lineages of *T. asamurai* and *T. alba* sp. nov., respectively.

TABLE 2 Mean genetic divergences between *Taphrenalla* gen. nov. and other genera estimated by the K2P model from the mitochondrial COI (lower-left) and 16S (upper-right) gene sequences

Genera	1	2	3	4	5	6	7
1 <i>Taphrenalla</i> gen. nov.		0.123	0.142	0.136	0.131	0.195	0.214
2 <i>Macrochlamys</i>	0.100		0.136	0.139	0.134	0.173	0.222
3 <i>Sarika</i>	0.122	0.116		0.122	0.150	0.158	0.190
4 <i>Cryptozona</i>	0.134	0.127	0.114		0.155	0.147	0.205
5 <i>Hemiplecta</i>	0.120	0.123	0.136	0.150		0.149	0.172
6 <i>Megaustenia</i>	0.141	0.134	0.144	0.167	0.144		0.196
7 <i>Durgella</i>	0.148	0.145	0.144	0.145	0.145	0.173	

TABLE 3 Mean intra- and inter-specific genetic divergences among species within *Taphrenalla* gen. nov. from the mitochondrial COI (lower-left) and 16S (upper-right) gene sequences estimated by the K2P model. Taxa in **bold** are the new species described herein

<i>Taphrenalla</i> spp.	1	2	3	4	5	6	7	8	9	10	11
1 <i>asamurai</i>		0.026	0.086	0.087	0.078	0.080	0.077	0.088	0.083	0.079	0.089
2 <i>macrosulcata</i>	0.045		0.085	0.079	0.077	0.079	0.082	0.092	0.081	0.078	0.085
3 <i>diadema</i>	0.075	0.066		0.050	0.035	0.036	0.033	0.028	0.055	0.038	0.068
4 <i>dalli</i>	0.061	0.063	0.051		0.044	0.051	0.047	0.042	0.068	0.047	0.083
5 <i>corona</i>	0.068	0.063	0.052	0.045		0.010	0.014	0.025	0.055	0.014	0.063
6 <i>parversa</i>	0.076	0.073	0.057	0.046	0.035		0.017	0.030	0.062	0.013	0.070
7 <i>conformis</i>	0.060	0.070	0.056	0.045	0.043	0.053		0.030	0.060	0.019	0.061
8 <i>incilis</i>	0.055	0.064	0.051	0.052	0.046	0.055	0.046		0.052	0.031	0.059
9 <i>pygmaea</i>	0.071	0.067	0.072	0.059	0.062	0.070	0.069	0.073		0.056	0.073
10 <i>zemia</i>	0.062	0.053	0.044	0.034	0.034	0.038	0.037	0.047	0.057		0.065
11 <i>alba</i>	0.077	0.077	0.068	0.058	0.057	0.067	0.058	0.068	0.075	0.054	

TABLE 4 Mean intra- and inter-specific genetic divergences among species within *Taphrenalla* gen. nov. from the mitochondrial COI (lower-left) and 16S (upper-right) gene sequences estimated by the K2P model. Taxa in **bold** are the new species described herein

	<i>asamurai</i>	<i>macro-</i>	<i>diadema</i>	<i>dalli</i>	<i>corona</i>	<i>parversa</i>	<i>conformis</i>	<i>incilis</i>	<i>pygmaea</i>	<i>zemia</i>	<i>alba</i>
		<i>sulcata</i>									
COI	0.016	0.001	0.018	0.017	0.009	0	0.001	0.004	0.015	0.011	0.024
16S	0.005	0.002	0.005	0.011	0.003	0	0	0	0.011	0.008	0.015

In general, the results from the ABGD and PTP approaches recognized higher number of species than morphological distinctiveness (Jirapatrasilp et al., 2019; Nantarat et al., 2019). The ABGD analysis using the K2P model also yielded higher numbers of MOTUs than the JC69 model (Jirapatrasilp et al., 2019), which is similar to this study. Therefore, the K2P model, which is popular for COI barcoding may yield biased results (Collins et al., 2012; Srivathsan & Meier, 2012; Jirapatrasilp et al., 2019). Also similar to our study, in the recent analysis of the operculated land snails, Nantarat et al. (2019) reported that the PTP species delimitation revealed additional MOTUs relative to the ABGD method.

Discussion

Phylogeny and taxonomic implications

Based on two mitochondrial and one nuclear gene fragments, the phylogenetic analyses supported that both *Taphrenalla* gen. nov. and all the recognized species were monophyletic (fig. 2). Previously, the two species of the Crown Snail (*T. asamurai* and *T. diadema*) were placed in the genus *Macrochlamys* s.l. because of their resemblance in having a depressed and thin shell with a shiny shell surface (Dall, 1897; Panha, 1996). Generally, taxonomic confusion in ariophantid snails has arisen when principally relying on shell morphology, since this is highly similar between

TABLE 5 Comparison of the shell sculpture and genitalia of *Taphrenalla* gen. nov. and the related genera. Data are taken from the original descriptions and additional references: Blanford & Godwin-Austen (1908) and Schileyko (2002, 2003)

Genera	Type species	Shell		Genitalia		
		Shell width (mm)	Sculpture	Penial verge	Epiphallic caecum	Dart apparatus
<i>Taphrenalla</i> gen. nov.	<i>Nanina diadema</i> Dall, 1897	9.6 – 21.3	Radial grooves / rarely smooth	Present	Straight or bent	Present
<i>Macrochlamys</i> Gray, 1847	<i>Helix vitrinoides</i> Deshayes, 1831	7.0 – 27.0	Generally smooth	Present	Spirally coiled	Present
<i>Sarika</i> Godwin-Austen, 1907	<i>Helix resplendens</i> Philippi, 1846	14.0 – 25.5	Generally smooth	Absent / rarely present	Straight	Present
<i>Syama</i> Godwin-Austen, 1908	<i>Macrochlamys prona</i> Nevill, 1878	10.0 – 23.0	Generally smooth	Present	Spirally coiled	Absent
<i>Taphrospira</i> Blanford, 1905	<i>Macrochlamys convallata</i> Benson, 1856	10.0 – 15.0	Generally smooth	Present	Straight	Absent
<i>Rasama</i> Laidlaw, 1932	<i>Macrochlamys kala</i> Godwin-Austen, 1883	7.0 – 8.5	Generally smooth	Present	Absent	Absent
<i>Cryptozona</i> Mörch, 1872	<i>Helix ligulata</i> Ferussac, 1819	20.0 – 40.0	Spiral and radial striae	Absent	Straight	Present
<i>Hemiplecta</i> Albers, 1850	<i>Helix humphreysiana</i> Lea, 1841	37.0 – 56.0	Smooth / spiral wrinkles / spiral striae	Present / absent	Straight	Present

species. The snail's anatomy is now required in later taxonomic works, especially the reproductive organs that show a significant difference among genera and species (Blanford & Godwin-Austen, 1908; Schileyko, 2002, 2003). For example, *Macrochlamys* has a spirally coiled epiphallic caecum, *Sarika* has a straight epiphallic caecum, *Taphrenalla* gen. nov. has a straight or slightly bent epiphallic caecum and *Rasama* has no epiphallic caecum (Blanford & Godwin-Austen, 1908). Thus, *T. asamurai* and *T. diadema* were then transferred to *Sarika* based on their straight epiphallic caecum (Sutcharit & Panha, 2008).

However, *Taphrenalla* gen. nov. and *Sarika* did not appear to be closely related in this study (fig. 2). Two anatomical features supported that this new genus is closer to the Indochinese *Macrochlamys* even though they have a straight epiphallic caecum. The shared characters between them are a penial verge and penial caecum in the male genitalia. Interestingly, *T. corona* sp. nov. and *T. parversa* sp. nov. do not develop the penial caecum as well as *M. tanymentula* (Pholyotha et al., 2018). On the other hand, *Cryptozona siamensis* is closer to *Sarika* in the molecular phylogeny.

The genitalia without penial verge and penial caecum of *C. siamensis* showed a strong similarity with *Sarika*, even though the shell with a reticulated sculpture was an obvious difference (Godwin-Austen, 1907; Pholyotha et al., 2020). Several reports (e.g., Hausdorf, 1998; Hirano et al., 2014; Hyman et al., 2017) have suggested that genital traits are more likely to reflect the actual phylogenetic relationships than morphological appearances. On one hand, phenotypic plasticity in snail shells is a well-documented phenomenon and is probably a response to environmental variation (Emberton, 1991, 1995; Gustafson et al., 2014; Proćków et al., 2017). The other is convergence and parallelism in the character states of the shell morphology and other anatomical structures that are generally known in land snails (Hirano et al., 2014, 2015; Köhler & Criscione, 2015; Hyman et al., 2017). On the other hand, if they are sister relationships based on molecular data, the result of the similarity in shell morphology in the close species groups is best explained by retention of the ancestral characters (Köhler & Criscione, 2015).

In addition, our results suggested that the shell features with radial grooves on the shell sculpture of *Taphrenalla* gen. nov., appeared to be suitable for differentiating them from ariophantid genera. Moreover, the anatomical characters of (i) having a penial verge and straight to bent epiphallic caecum, (ii) one or two spines on tail filament of the spermatophore, and (iii) well-developed colourful lateral stripes are the important diagnostic characters found in this new genus (table 6). Even though *T. zemia* sp. nov. and *T. alba* sp. nov. have no radial grooves, they have the other unique features of this new genus. The comparative information of genitalia of these genera is mentioned in the remarks of the genus.

The spermatophore morphology is unique in this new genus. Variation can only be

found in the tail filament close to the sperm sac bearing one or two spines, whereas the other parts and head filament are spineless. In contrast, the spermatophore of the *Sarika*, *Macrochlamys*, and *Cryptozona* species have a series of complex spine arrangements on the tail filament (Blanford & Godwin-Austen, 1908; Maneevong, 2000; Pholyotha et al., 2018; Pholyotha, unpubl. data).

In the case of the soft-body colouration, most of the ariophantid snails generally have a black or dark grey to milky colour even though some species have a bright (attractive) colour, such as red, orange, or yellow (Blanford & Godwin-Austen, 1908; Pholyotha et al., 2018; Inkhavilay et al., 2019; Sajan et al., 2019). For example, the Indochina *M. aurantia* Pholyotha & Panha, 2018 and *Hemiplecta pluto* (Pfeiffer, 1863) have a bright reddish-orange soft-body colour (Pholyotha et al., 2018; Inkhavilay et al., 2019). Remarkably, colourful stripes on the body only occurs in *Taphrenalla* species.

Endemism and biogeographical patterns

Interestingly, *Taphrenalla* nov. gen. is one of the high endemism genera and is found in limestone areas along the two main mountain ranges of Southern Thailand. Our results also found that (i) the species distributed near the Phuket and Nakhon Si Thammarat mountain ranges are likely to present strong radial grooves (*T. asamurai*, *T. diadema*, *T. macrosulcata* sp. nov., and *T. dalli* sp. nov.), while (ii) the species distributed further away or near the coastal areas may be prone to independent reduction (*T. parversa* sp. nov., *T. conformis* sp. nov., *T. incilis* sp. nov., and *T. pygmaea* sp. nov.) or loss (*T. zemia* sp. nov. and *T. alba* sp. nov.) of the radial grooves on the shell sculpture. However, all *Taphrenalla* species are conspicuous in this hypothesis presented here except for *T. corona* sp. nov. This new species has strong radial grooves but can be found on

TABLE 6 Comparison of the shell and genitalia morphology of *Taphrenalla* species. Taxa in bold are the new species described herein; n.a. means data not available

Species	Shell width (mm), range, means $\pm$ SD	Shell height (mm), range, means $\pm$ SD	Radial grooves	Suture	Epiphallic caecum	Penial caecum	Penial sculpture ratio (smooth / wrinkled)	Location of dart apparatus	Spine on spermatophore
<i>T. asamurai</i>	16.0 – 21.1, 18.5 $\pm$ 1.24, n = 32	8.3 – 11.7, 10.0 $\pm$ 0.83, n = 32	37 – 64 grooves on last whorl	Deep impressed	Bent	Present	1:1	Opposite of penis	One
<i>T. macrosulcata</i>	14.7 – 18.7, 16.4 $\pm$ 1.05, n = 24	8.2 – 9.8, 8.8 $\pm$ 0.45, n = 24	42 – 58 grooves on last whorl	Deep impressed	Straight	Present	1:1	On vagina	One
<i>T. diadema</i>	13.4 – 17.8, 15.7 $\pm$ 1.33, n = 39	7.8 – 10.6, 9.2 $\pm$ 0.74, n = 39	24 – 43 grooves on last whorl	Wide channel shape	Straight	Present	1:1	On vagina	Two
<i>T. dalli</i>	13.4 – 18.3, 15.9 $\pm$ 1.33, n = 33	7.3 – 11.3, 9.4 $\pm$ 1.01, n = 33	23 – 37 grooves on last whorl	Very wide channel shape	Straight	Present	1:2	On vagina	Two
<i>T. corona</i>	10.8 – 16.0, 13.7 $\pm$ 1.53, n = 26	5.7 – 9.3, 7.8 $\pm$ 1.01, n = 26	26 – 47 grooves on last whorl	Wide channel shape	Straight	Absent	1:2	On vagina	Two
<i>T. parversa</i>	11.2 – 14.4, 12.7 $\pm$ 1.09, n = 7	6.5 – 8.1, 7.2 $\pm$ 0.55, n = 7	Present / uncountable	Shallow impressed	Straight	Absent	1:1	On vagina	Two
<i>T. conformis</i>	14.2 – 17.8, 16.5 $\pm$ 0.92, n = 19	8.2 – 10.5, 9.2 $\pm$ 0.61, n = 19	Present / uncountable	Shallow impressed	Bent	Present	1:3	On vagina	Two
<i>T. incilis</i>	14.2 – 16.1, 15.3 $\pm$ 0.74, n = 6	6.9 – 8.8, 7.9 $\pm$ 0.62, n = 6	Present / uncountable	Narrow channel shape	Bent	Present	2:1	Opposite of penis	Two

TABLE 6 Comparison of the shell and genitalia morphology of *Taphrenalla* species. Taxa in bold are the new species described herein; n.a. means data not available (cont.)

Species	Shell width (mm), range, means ± SD	Shell height (mm), range, means ± SD	Radial grooves	Suture	Epiphallallic caecum	Penial caecum	Penial sculpture ratio (smooth / wrinkled)	Location of dart apparatus	Spine on spermatophore
<i>T. pygmaea</i>	9.6 – 12.3, 10.9 ± 0.74, n = 22	5.5 – 6.8, 6.2 ± 0.35, n = 22	Present / uncountable	Narrow channel shape	Straight	Present	2:1	Opposite of penis	n.a.
<i>T. zemia</i>	16.4 – 21.3, 18.3 ± 1.20, n = 12	9.1 – 11.9, 10.0 ± 0.71, n = 12	Absent	Shallow impressed	Straight	n.a.	n.a.	On vagina	n.a.
<i>T. alba</i>	15.0 – 18.5, 16.0 ± 0.91, n = 17	8.6 – 10.6, 9.2 ± 0.55, n = 17	Absent	Shallow impressed	Bent	Present	2:1	Opposite of penis	Two

limestone outcrops near the Andaman sea northwards to the southern part of the Phuket Range.

In support, firstly the populations of *T. diadema*, found on limestone outcrops in Phatthalung close to the Gulf of Thailand, have shallower radial grooves on the shell sculpture and are smaller than the other populations distributed along the Nakhon Si Thammarat Range. Secondly, *T. incilis* sp. nov. is distributed near the southern part of Nakhon Si Thammarat Range but its localities are close (within 7 km) to coastal areas. Thirdly, the isolated limestone outcrops of the southern part of the Phuket Range, not far from the Andaman sea, contain three allopatric species with a different form of radial groove; two species with a weak radial groove (*T. parversa* sp. nov. and *T. conformis* sp. nov.) and one species without a radial groove (*T. zemia* sp. nov.). Fourthly, the smooth-shelled species *T. alba* sp. nov. was found in the limestone hills along the shore of the Andaman sea at Krabi.

In general, the influence of convergent shifts in the morphology of terrestrial gastropod shells is possibly caused by several factors, such as habitat, isolation, surrounding soil type, natural predators, moisture levels, and geological age (Voris, 2000; Nekola, 2003; Chiba, 2004; Clements et al., 2008; Woodruff, 2010; Stankowski, 2011; Köhler & Criscione, 2015; Nantararat et al., 2019). Furthermore, several studies have also documented that occupying similar ecological niches with similar selective forces can cause species in the same area to undergo convergent evolution (Alizon et al., 2008; Clements et al., 2008; Wu et al., 2008; Hirano et al., 2014; Nantararat et al., 2019). The mechanism of diversification may be related to adaptation to new environmental conditions (Wellborn & Langerhans, 2015; Cameron, 2016), or may simply be the result of habitat fragmentation and isolation (Delicado et al., 2013).

In tropical landmasses, limestone forests are considered de facto habitat islands because of their isolation from one another by habitats without limestone substrata, and this spatial configuration limits gene flow and induces high levels of species endemism (Clements et al., 2008; Foon et al., 2017). However, it is not clear whether the high level of endemic species or the degree of radial grooves on the sculpture of *Taphrenalla* shells are also caused by adaptation to these factors because insufficient environmental data was obtained in the present work. To fill this gap, further analyses incorporating the habitat condition of these *Taphrenalla* species are needed.

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APPENDIX

Systematics

Family: **Ariophantidae** Godwin-Austen, 1888

Subfamily: **Macrochlamydinae** Godwin-Austen, 1888

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

Type species. Nanina (Macrochlamys) diadema Dall, 1897

Etymology. *Taphrenalla* is derived from 'Taphr' (the Greek word 'Taphros') meaning 'trench or ditch' and 'enalla' (the Greek word 'enallax') meaning crosswise. Therefore, the generic name means the snail with transverse ditches or radial grooves on the shell sculpture. The gender of the new generic name is feminine.

Description

Shell. Shell dextral, depressed to globosely depressed, thin, translucent, corneous to dark brown colour. Shell surface shiny, smooth (fig. 4A), with very fine growth lines or sculptured, very weak (fig. 4B) to strong (figs 4C, D) radial grooves starting from suture. Suture shallow impressed (figs 4A, B), deep impressed (fig. 4C), or wide and deep channel-shaped (fig. 4D). Whorls 5–6, increasing regularly. Last whorl rounded to slightly shouldered. Aperture crescentic with simple lip. Columellar margin simple and little expanded near umbilicus. Umbilicus perforate, narrowly opened.

Genital organs. Genitalia with short to long penis; thick or thin penial sheath. Epiphallic caecum usually straight, rarely a little bent at tip (not spirally coiled); penial retractor muscle attached to head of epiphallic caecum (rarely attached near the head). Flagellum relatively short and enlarged at base. Penial verge well-developed and small to large; penial caecum usually present and short

or rarely absent. Female genital organ well-developed; dart apparatus located on vagina or at vagina and penis junction. Spermatophore long, needle-shaped and translucent. Head filament smooth, shorter than sperm sac and gradually thinning to terminal point. Sperm sac enlarged, capsule-shaped and contained whitish sperm mass. Tail filament very long and thin tube; region close to sperm sac bearing one or two spines.

Radula. Teeth arranged in U-shape with very wide angle. Central tooth symmetrical tricuspid; mesocone lanceolated-shaped; ectocones short and located at middle of tooth height. Lateral teeth asymmetrical tricuspid; mesocone triangular shaped; endocone smaller than ectocone; ectocone located below endocone. Marginal teeth with obliquely bicuspid, elongate and narrower teeth, and outermost teeth shorter and smaller than inner teeth (fig. 4H).

External features. Living snails usually covered with reticulated skin; three colourful stripes running from head to tail (one at middle of body and two on each side of body near foot sole). Eye stalks and head usually with dark colour; tail region usually with lighter colour than head region. Pallial cavity and some internal organs visible through translucent shell (figs 4F, G).

Mantle collar large, well developed and composed of two shell lobes and three dorsal lobes (figs 4E–G); shell lobes usually slender and longer than dorsal lobes. Right shell lobe placed on upper shell surface and longer than left shell lobe placed below periphery. Right dorsal lobe larger than left dorsal lobe; left dorsal lobe divided into anterior left dorsal lobe and posterior left dorsal lobe. Foot sole tripartite; caudal foss long and narrow; caudal horn raised and large. Colouration of soft body usually variable from reddish, orange, yellow, milky-white to greyish, and colour disappeared in preserved specimens.

FIGURE 4 Schematic of shell sculpture, mantle lobes, body terminology and radular of *Taphrenalla* gen. nov. A–D. radial grooves and suture types on upper shell surface: A. no radial grooves with shallow impressed suture. B. weak to nearly absent radial grooves with impressed suture. C. deep radial grooves with deep impressed suture. D. deep radial grooves with wide and deep channel shaped suture. E. dorsal view of mantle collar. F. right-hand side of snail shows mantle lobes and colour stripes on body. G. left-hand side of snail shows mantle lobes. H. representative SEM images of radular.

Distribution range. Endemic to Malay Peninsula: Southern Thailand (fig. 1) and probably in Peninsular Malaysia.

Remarks. *Taphrenalla diadema* is the type species of the new genus because this species somewhat represents the morphology in general among congeneric species and is also the oldest valid species. Although the absence of radial grooves is evolved in *T. zemia* sp. nov. and *T. alba* sp. nov., they present the other characteristics of yellowish stripes on the body and a simple spermatophore. The spermatophore of *T. alba* sp. nov. has two spines near the sperm sac but is spineless in other regions. In addition, the molecular analysis grouped them within the *Taphrenalla* clade with strong support.

For comparison with the other closely related ariophantid genera, *Taphrenalla* gen. nov. can be distinguished from *Sarika*, *Macrochlamys*, *Taphrospira*, *Syama*, and *Rasama* by the shell sculpture having radial grooves, while the others have a smooth shell without radial grooves (table 5). The shell morphology of *Hemiplecta* and *Cryptozona* are clearly different from the new genus in being much larger, with a smooth shell surface and without a radial groove. Regarding the genitalia anatomy, *Taphrenalla* gen. nov. have a penial verge, dart apparatus, and straight or slightly bent epiphallic caecum. Compared to the other genera, *Macrochlamys* and *Syama* both have a spirally coiled epiphallic caecum, but only *Syama* has no dart apparatus. The genus *Taphrospira* also has a straight epiphallic caecum and is without a dart apparatus, while the genus *Rasama* has neither an epiphallic caecum nor a dart apparatus (Blanford & Godwin-Austen, 1908; Schileyko, 2002, 2003). Thus, the genitalia of the genus *Sarika* is similar to *Taphrenalla* gen. nov. However, all species of the genus *Sarika*, except for *S. consepta* (Benson, 1860), have no penial verge (Pholyotha et al., 2020), while this character is present in all *Taphrenalla* species. The taxonomic status of *S. consepta* is needed to

be investigated in the future using a molecular approach.

In addition, most *Taphrenalla* species have bright body colouration patterns of the left, middle, and right stripes, either restricted to the head section or running from head to tail, while all *Sarika* species have a monochrome greyish to blackish body colour (Pholyotha et al., 2020).

Taphrenalla asamurai (Panha, 1997)

(figs 1, 3, 5, 6A, 7A–C, table 6)

Macrochlamys asamurai Panha, 1997: 101–105, figs 2a, 2b1, 3–4. Type locality: Takun Village, Suratthani, Thailand.

Macrochlamys asamurai: Maneevong, 2000: 26–27, fig. 4-1.

Sarika asamurai: Sutcharit & Panha, 2008: 95–100, figs 2c, d, 3f–i, 4d–f.

Material examined. Type material: not examined. Non-type material: Wat Tham Wararam, Phanom District, Surat Thani (98°53'07.3"N 98°40'01.0"E): CUMZ 7153 (fig. 6A; 26 shells and 15 specimens in ethanol), CUMZ 7154 (six specimens in ethanol), CUMZ 7155 (14 shells and eight specimens in ethanol), and CUMZ 7156 (four shells). Limestone outcrops at Khlong Sok, Phanom District, Surat Thani (8°53'39.3"N 98°33'10.7"E): CUMZ 7157 (three shells) and CUMZ 7158 (one shell). Limestone outcrops at Khlong Sok, Phanom District, Surat Thani (8°50'51.2"N 98°44'18.0"E): CUMZ 7159 (31 shells and 12 specimens in ethanol). Wat Tham Phanthurat, Phanom District, Surat Thani (8°54'01.3"N 98°31'30.2"E): CUMZ 7160 (three specimens in ethanol).

Remarks. *Taphrenalla asamurai* had been re-described and illustrated by Sutcharit & Panha (2008). The unique characters are a depressed conic shell of medium size (width up to 21.1 mm; height up to 11.7 mm), deep and narrow impressed suture, and long radial grooves that appeared on

FIGURE 5 Frequency of radial grooves numbers on the last whorl of *Taphrenalla asamurai*, *T. macrosulcata* sp. nov., *T. diadema*, *T. dalli* sp. nov., and *T. corona* sp. nov.

FIGURE 6 Shells. A. *Taphrenalla asamurai* specimen CUMZ 7153. B. *T. macrosulcata* sp. nov. holotype CUMZ 7161. Inset SEM image showing shell sculpture of each species.

the last 37–64 grooves ($n = 32$; figs 5, 6A). The genitalia are bent at the epiphallal caecum, and the proximal part of dart apparatus is with a thick and swollen sac-like structure (figs 7A, 7B). Spermatophore has one spine on the tail filament close to the sperm sac (fig. 7C). Living snails have monochrome red to dark red on tail (fig. 3).

Taphrenalla macrosulcata Pholyotha & Panha, sp. nov.

(figs 1, 3, 5, 6B, 7D–F, table 6)

Type material examined. Holotype: CUMZ 7161 (fig. 6B). Paratypes. Same data as holotype: CUMZ 7162 (15 shells and five specimens in ethanol), CUMZ 7163 (three shells and nine specimens in ethanol), NHMUK (two shells) and SMF (two shells).

Type locality. Limestone outcrops at Khlong Sok, Phanom District, Surat Thani, Thailand ($8^{\circ}53'20.5''N$ $98^{\circ}40'27.7''E$).

Etymology. The specific epithet is derived from ‘macro’ (the Greek word ‘makros’) meaning ‘long’ and ‘sulcata’ (the Latin word ‘sulcus’) meaning ‘furrow, groove’ with reference to the characteristic of having numerous long radial grooves on shell sculpture.

Diagnosis. Shell with a narrow and deep impressed suture and deep and long radial grooves. Epiphallal caecum straight and short. Penial caecum short. Inner sculpture of proximal penis with fine wrinkled longitudinal folds; distally with oblique longitudinal folds and transverse-folded surrounding penial verge. Dart apparatus located on vagina.

FIGURE 7 Genitalia and spermatophore. A–C. *Taphrenalla asamurai* specimen CUMZ 7153. A. whole reproductive system. B. internal wall of penis with penial verge. C. spermatophore; inset showing one spine located close to the sperm sac. D–F. *T. macrosulcata* sp. nov. paratype CUMZ 7162. D. whole reproductive system. E. internal wall of penis with penial verge. F. spermatophore; inset showing one spine located close to the sperm sac.

Spermatophore with one spine on tail filament close to sperm sac.

Description

Shell (fig. 6B). Shell comparatively depressed conic, medium-sized (width up to 18.7 mm; height up to 9.8 mm) and pale brown. Whorls 5–6; suture deep and narrow impressed. Upper shell surface with strong and long radial grooves continuing from suture, gradually inconspicuous and then disappear below periphery. Radial grooves starting between whorl 3 to 4 and with 42–58 grooves at last whorl ($n = 24$; fig. 5). Spire depressed conic; apex raised with a spire angle of about $143\text{--}164^\circ$. Last whorl shouldered and well-rounded at periphery. Aperture lip simple; umbilicus narrowly opened.

Genital organs (figs 7D–F). Atrium short. Penis elongate cylindrical and same length as total vagina length; penial caecum present. Inner sculpture of proximal penial wall has very fine wrinkled, longitudinal penial pilasters, with thick and oblique longitudinal folds in middle, and then transformed to a thin, transverse-folded, surrounded penial verge. Epiphallus slender tube and about two times total penis length. Epiphallic caecum very short; thick penial retractor muscle attached at tip. Flagellum large and irregular slender tube. Vas deferens thin tube. Vagina long and cylindrical tube. Dart apparatus located on vagina away from penis joining to atrium. Spermatophore long and needle-shaped. Tail filament very long tube; region close to sperm sac bearing a very small spine.

External features (fig. 3). Animal dark gray mixed with pale yellow on head and body and monochrome bright yellowish on tail.

Remarks. *Taphrenalla macrosulcata* sp. nov. is similar to *T. asamurai* in shell morphology but can be differentiated by a paler brown shell and a pale yellowish soft body. Whereas, *T. asamurai* has a dark brown shell and reddish to dark red soft body.

Regarding the genital anatomy, this new species has a short flagellum, straight epiphallic caecum, and dart apparatus connecting to the vagina away from the penis junction and without thick connective tissue surrounding the proximal end. For comparison, *T. asamurai* has a long flagellum, bent epiphallic caecum, and dart apparatus located opposite the penis junction and with thick connective tissue at the proximal end.

The presence of one spine on the tail filament of the spermatophore is the remarkable character of *T. macrosulcata* sp. nov. and *T. asamurai*, while other *Taphrenalla* species have two spines.

Taphrenalla diadema (Dall, 1897)

(figs 1, 3, 5, 8A–C, 9A–C, table 6)

Nanina (*Macrochlamys*) *diadema* Dall, 1897: 37–38. Type locality: Prang, Malay Peninsula; Dall, 1902: 499, pl. 27 figs 1–3; Boss, Rosewater, & Ruhoff, 1968: 104.

Macrochlamys diadema: Gude, 1903: 50.

Syama diadema: Abbott, 1989: 23, 133 (text figure); Panha, 1996: 102–104, fig. 2b2 (Holotype, USNM 150277); Maneevong, 2000: 28–29, fig. 4-2.

Sarika diadema: Sutcharit & Panha, 2008: 95–100, figs 2a, b, 3a–e, 4a–c.

Material examined. Type material: holotype USNM 150277. Non-type material: Wat Khuha Santayaram (Wat Tham Khao Daeng), Ron Phibun District, Nakhon Si Thammarat ($8^\circ14'39.5''\text{N}$ $99^\circ52'00.1''\text{E}$): CUMZ 7164 (one specimen in ethanol). Tham Talot, Thung Song District, Nakhon Si Thammarat ($8^\circ09'32.3''\text{N}$ $99^\circ40'41.6''\text{E}$): CUMZ 7165 (16 shells and three specimens in ethanol). Tham Rad, Thung Song District, Nakhon Si Thammarat ($8^\circ02'47.2''\text{N}$ $99^\circ43'45.0''\text{E}$): CUMZ 7166 (10 shells) and CUMZ 7167 (11 specimens in ethanol). Wat Tham Phraphut, Ratsada District, Trang ($7^\circ57'57.3''\text{N}$ $99^\circ44'41.3''\text{E}$): CUMZ 7168 (four shells and two specimens in ethanol). Tham Chang Hai, Mueang District, Trang ($7^\circ35'26.6''\text{N}$ $99^\circ40'05.3''\text{E}$): CUMZ 7169 (nine shells) and CUMZ

FIGURE 8 Shells. A–C. *Taphrenalla diadema*. A. specimen CUMZ 7175. B. specimen CUMZ 7177. C. specimen CUMZ 7171 showing channel-shaped suture with wide bottom. D, E. *T. dalli* sp. nov. D. holotype CUMZ 7183. E. specimen CUMZ 7189 showing channel-shaped suture with very wide bottom. Inset SEM image showing shell sculpture of each species.

FIGURE 9 Genitalia and spermatophore. A–C. *Taphrenalla diadema* specimen CUMZ 7180. A. whole reproductive system, B. internal wall of penis with penial verge. C. spermatophore; inset showing two spines located close to sperm sac. D–F. *T. dalli* sp. nov. paratype CUMZ 7184. D. whole reproductive system; inset showing a small penial caecum. E. internal wall of penis with penial verge. F. spermatophore of *T. dalli* sp. nov. specimen CUMZ 7186; inset showing two spines located close to the sperm sac.

7170 (two shells). Khao Pu Chao Bureau of Monks, Na Yong District, Trang ($7^{\circ}33'53.0''\text{N}$ $99^{\circ}46'36.2''\text{E}$): CUMZ 7171 (fig. 8C; 18 shells) and CUMZ 7172 (four shells). Wat Khao Huai Haeng, Huai Yot District, Trang ($7^{\circ}47'36.5''\text{N}$ $99^{\circ}38'37.0''\text{E}$): CUMZ 7173 (16 specimens in ethanol). Wat Tham Sumano, Srinagarindra District, Phatthalung ($7^{\circ}35'14.0''\text{N}$ $99^{\circ}52'06.8''\text{E}$): CUMZ 7174 (10 specimens in ethanol) and CUMZ 7175 (fig. 8A; four shells and 33 specimens in ethanol). Khao Ok Talu Khuha Sawan, Mueang District, Phatthalung ($7^{\circ}37'33.7''\text{N}$ $100^{\circ}05'23.7''\text{E}$): CUMZ 7176 (three shells) and CUMZ 7177 (fig. 8B; 55 shells and 18 specimens in ethanol). Wat Khuha Sawan, Mueang District, Phatthalung ($7^{\circ}37'15.0''\text{N}$ $100^{\circ}04'48.7''\text{E}$): CUMZ 7178 (one shell). Tham Phaya Hong, Kong Ra District, Phatthalung ($7^{\circ}27'48.4''\text{N}$ $99^{\circ}57'53.3''\text{E}$): CUMZ 7179 (36 shells) and CUMZ 7180 (22 shells and 137 specimens in ethanol). Limestone outcrops at Khlong Chaloem, Kong Ra District, Phatthalung ($7^{\circ}23'50.6''\text{N}$ $99^{\circ}58'45.2''\text{E}$): CUMZ 7181 (five shells and 31 specimens in ethanol). Tham Phra Non, Mueang District, Phatthalung ($7^{\circ}40'52.1''\text{N}$ $100^{\circ}03'42.4''\text{E}$): CUMZ 7182 (10 shells).

Remarks. *Taphrenalla diadema* had been recently re-described and illustrated by Sutcharit & Panha (2008). The distinguishing characters are a depressed conic shell, medium-sized (width up to 17.8 mm; height up to 10.6 mm), a wide and deep channel-shaped suture, and short radial grooves appeared on last about 24–43 grooves ($n = 39$; figs 5, 8A–C). The genitalia have a short and straight epiphallic caecum, and dart apparatus located on vagina away from vagina and penis junction (figs 9A, B). Spermatophore has one short spine on tail filament close to sperm sac (fig. 9C). Living snail has yellowish stripes on head and tail (fig. 3).

The specimens from Khao Ok Talu, Phatthalung Province (fig. 8B) tended to have a smaller diameter and shallower radial grooves than the other populations (figs 8A, C). However, all the genital characters and spermatophores from all localities

appear to be identical. In addition, the molecular data also confirmed that the shell variations were all grouped within the *T. diadema* clade.

***Taphrenalla dalli* Pholyotha & Panha, sp. nov.**

(figs 1, 3, 5, 8D, E, 9D–F, table 6)

Type material examined. Holotype: CUMZ 7183 (fig. 8D). Paratypes. Same data as holotype: CUMZ 7184 (31 shells and nine specimens in ethanol), CUMZ 7188 (one shell), NHMUK (two shells) and SMF (two shells).

Other material examined. Wat Khiri Rat Phatthana, Wiang Sa District, Surat Thani ($8^{\circ}31'36.5''\text{N}$ $99^{\circ}23'00.2''\text{E}$): CUMZ 7185 (63 shells and 88 specimens in ethanol). Wat Na San, Ban Na San District, Surat Thani, Thailand ($8^{\circ}48'30.7''\text{N}$ $99^{\circ}22'16.1''\text{E}$): CUMZ 7186 (four shells and 11 specimens in ethanol) and CUMZ 7187 (11 shells and 24 specimens in ethanol). Wat Tham Kanlayanamit, Tham Phannara District, Nakhon Si Thammarat ($8^{\circ}30'49.3''\text{N}$ $99^{\circ}22'53.9''\text{E}$): CUMZ 7189 (fig. 8E; 39 shells and 27 specimens in ethanol). Wat Tham Thong Panara, Tham Phannara District, Nakhon Si Thammarat ($8^{\circ}25'19.1''\text{N}$ $99^{\circ}22'48.4''\text{E}$): CUMZ 7190 (20 shells and one specimen in ethanol).

Type locality. Limestone outcrop at Tham Lord, Nopphitam District, Nakhon Si Thammarat, Thailand ($8^{\circ}47'16.7''\text{N}$ $99^{\circ}38'29.9''\text{E}$).

Etymology. This species is named after William Healey Dall, an American malacologist, who discovered the first species of Crown Snail, *T. diadema*.

Diagnosis. Shell with a very wide channel-shaped suture and with deep and short radial grooves. Epiphallic caecum straight. Penial caecum very short. Inner sculpture of proximal penis with very small, irregular and longitudinal folds, and then with obliquely and wrinkled to very fine pilasters distally. Dart apparatus connected on vagina.

Spermatophore with two spines on tail filament close to sperm sac.

Description

Shell (figs 8D, E). Shell depressed conic to globosely depressed, medium-sized (width up to 18.3 mm; height up to 11.3 mm) and pale to dark brown. Whorls 5–6; suture very wide, deep and channeled-shape. Upper shell surface with strong and short radial grooves continuing from suture to inconspicuous at periphery and then disappear below periphery. Radial grooves beginning between whorl 3 to 4 and having 23–37 grooves at last whorl ($n = 33$; fig. 5). Spire depressed conic; apex raised with a spire angle of about 135° – 155° . Last whorl shouldered and well-rounded at periphery. Aperture lip simple; umbilicus narrowly opened.

Genital organs (figs 9D–F). Atrium slightly short. Penis elongate cylindrical and nearly two times that of total vagina length; penial caecum present. Inner sculpture of proximal penial wall supported by penial pilasters starting proximally with very small irregular longitudinal folds, then gradually, obliquely, wrinkled pilasters in middle, to very fine pilasters surrounding penial verge. Epiphallus slender tube, approximately one and a half times total penis length. Epiphallic caecum slightly prolonged, and attached with thick penial retractor muscle (prm). Flagellum enlarged at base and tapering with irregular shape to tip. Vas deferens slender tube. Vagina long and cylindrical tube. Dart apparatus located on proximal vagina away from penis joining to atrium. Spermatophore long and needle-shaped. Tail filament very long tube; region close to sperm sac bearing two spines; spine close to sperm sac shorter than the other.

External features (fig. 3). Animal dark gray in body colour with three pale orange stripes running from head to caudal horn. One stripe at middle of the body and others two stripes on side of body close to foot sole; stripes on tail wider than anterior.

Remarks. Shell morphology of this new species looks similar to *T. diadema*, but can be distinguished by having a wider base of the suture than in *T. diadema*. In comparison, *T. dalli* sp. nov. shows a small penial caecum, shorter flagellum, and large and coarse penial sculpture pilaster at the middle of penis. Whereas, *T. diadema* has a large penial caecum, longer flagellum, and inner wall of penis is sculptured with very fine wrinkled pilasters. In addition, the penial sculpture at the middle of the penis of *T. dalli* sp. nov. has larger, more obliquely wrinkled pilasters than *T. diadema*.

This new species shows variation in size and shell sculpture, the like of which also appears in *T. diadema*. However, the shell suture and genitalia of all variants are identical, and are also clustered together in the *T. dalli* clade in the molecular analysis.

Taphrenalla corona Pholyotha & Panha, sp. nov.
(figs 1, 3, 5, 10A, 11A–C, table 6)

Type material examined. Holotype: CUMZ 7191 (fig. 10A). Paratypes. Same data as holotype: CUMZ 7192 (two shells and nine specimens in ethanol), CUMZ 7193 (21 shells), CUMZ 7194 (one specimen in ethanol), NHMUK (two shells), and SMF (two shells).

Other material examined. Limestone outcrops at Ao Luek Tai, Ao Luek District, Krabi ($8^{\circ}24'09.9''\text{N}$, $98^{\circ}44'16.3''\text{E}$): CUMZ 7195 (five specimens in ethanol). Tham Sa Yuan Thong, Ao Luek District, Krabi ($8^{\circ}21'44.6''\text{N}$, $98^{\circ}44'45.9''\text{E}$): CUMZ 7196 (one shell). Tham Phet, Ao Luek District, Krabi ($8^{\circ}23'33.9''\text{N}$, $98^{\circ}46'24.0''\text{E}$): CUMZ 7197 (one shell). Wat Khao Hua Sing, Plai Phraya District, Krabi ($8^{\circ}30'47.7''\text{N}$, $98^{\circ}45'30.1''\text{E}$): CUMZ 7198 (29 shells). Tham Nalaking Bureau of Monks, Plai Phraya District, Krabi ($8^{\circ}33'35.3''\text{N}$, $98^{\circ}51'37.5''\text{E}$): CUMZ 7225 (eight shells and three specimens in ethanol). Wat Khao Phra, Phrasaeng District, Surat Thani ($8^{\circ}37'33.0''\text{N}$, $98^{\circ}56'53.5''\text{E}$): CUMZ

FIGURE 10 Shells. A. *Taphrenalla corona* sp. nov. holotype CUMZ 7191. B. *T. parversa* sp. nov. holotype CUMZ 7199. C. *T. conformis* sp. nov. holotype CUMZ 7202. D. *Taphrenalla incilis* sp. nov. holotype CUMZ 7207. Inset SEM image showing shell sculpture of each species.

FIGURE 11 Genitalia and spermatophore. A–C. *Taphrenalla corona* sp. nov. paratype CUMZ 7192. A. whole reproductive system. B. internal wall of penis with penial verge. C. spermatophore; inset showing two spines located close to the sperm sac. D–F. *T. parversa* sp. nov. paratype CUMZ 7200. D. whole reproductive system. E. internal wall of penis with penial verge. F. spermatophore; inset showing two spines located close to the sperm sac. White arrowheads indicate the position of penial verge.

7226 (15 shells). Tham Namlod Bureau of Monks, Phrasaeng District, Surat Thani ($8^{\circ}40'40.2''\text{N}$ $98^{\circ}56'41.9''\text{E}$): CUMZ 7227 (six shells).

Type locality. Limestone outcrops near Than Bok Khorani, Ao Luek District, Krabi, Thailand ($8^{\circ}22'28.7''\text{N}$ $98^{\circ}44'07.9''\text{E}$).

Etymology. The specific epithet “*corona*” is from the Latin word “*corona*” meaning “crown” and is in reference to the characteristic radial grooves on the shell sculpture of this genus.

Diagnosis. Shell with wide channel-shaped suture, and slightly deep and short radial grooves. Penis with thick penial sheath. Penial caecum absent. Epiphallic caecum straight. Inner sculpture of proximal penis with fine irregular longitudinal folds and then with obliquely longitudinal folds distally. Dart apparatus connected on vagina. Spermatophore with two spines on tail filament close to sperm sac.

Description

Shell (fig. 10A). Shell depressed conic, medium-sized (width up to 16.0 mm; height up to 9.3 mm) and slightly milky to brownish. Whorl 5–6; suture wide and deep groove, channel-shaped. Upper shell surface with rather strong and short radial grooves continuing from suture to inconspicuous at periphery and disappears below periphery. Radial grooves starting between whorl 3 to 4 and having 26–47 grooves at last whorl ($n = 26$; fig. 5). Spire slightly depressed conic; apex raised with a spire angle of about 140° – 155° . Last whorl barely shouldered and well-rounded at periphery. Aperture lip simple; umbilicus narrowly opened.

Genital organs (figs 11A–C). Atrium short. Penis enlarge cylindrical and same length as vagina; penial caecum absent. Inner sculpture of proximal penis with fine irregularly longitudinal penial

pilasters, gradually transforming to obliquely longitudinal folds surrounding penial verge. Epiphallus slender tube, about two and a half times total penis length and smaller diameter than penis. Epiphallic caecum short, and attached with thick penial retractor muscle. Flagellum prolonged with irregular cylindrical tip. Vas deferens slim tube. Vagina prolonged, cylindrical-shaped and distal part larger diameter than proximal part. Dart apparatus located on proximal vagina away from penis joining to atrium. Spermatophore long and needle-shaped. Tail filament very long tube; region near sperm sac bearing two spines, spine close to sperm sac very small and shorter than the other.

External features (fig. 3). Animal very dark gray in body colour with one creamy to pale orange stripe in middle of body running from head to caudal horn; stripe on tail wider than on body to head.

Remarks. Although the shell morphology of this new species is close to those of *T. diadema* and *T. dalli* sp. nov., its genitalia is unique. The absence of a penial caecum and presence of a thick penial sheath covering a proximal portion of penis distinguished *T. corona* sp. nov. from the other *Taphrenalla* species.

Taphrenalla parversa Pholyotha & Panha, sp. nov. (figs 1, 3, 10B, 11D–F, table 6)

Type material examined. Holotype: CUMZ 7199 (fig. 10B). Paratypes. Same data as holotype: CUMZ 7200 (one shell and five specimens in ethanol), CUMZ 7201 (five shells), NHMUK (two shells), and SMF (two shells).

Type locality. Tham Suwan Khuha, Takua Thung District, Phang-nga, Thailand ($8^{\circ}25'45.0''\text{N}$ $98^{\circ}28'19.6''\text{E}$).

Etymology. This species is derived from ‘*parv*’ (the Latin word ‘*parvus*’) meaning ‘little’ and ‘*versa*’

(the Latin word ‘*versus*’) meaning ‘line, furrow’ with reference to the characteristic numerous little radial grooves on the shell sculpture.

Diagnosis. Shell with shallowly impressed suture and very weakly radial grooves. Penis with thick penial sheath. Penial caecum absent. Epiphallallic caecum straight. Inner sculpture of proximal penis with fine, irregular and longitudinal folds, and then transition to irregular and zig-zag, longitudinal folds distally. Dart apparatus connected on vagina. Spermatophore with two spines on tail filament close to sperm sac.

Description

Shell (fig. 10B). Shell comparatively depressed conic, medium-sized (width up to 14.4 mm; height up to 8.1 mm) and pale brown. Whorls 5–6; suture shallowly impressed. Upper shell surface with numerous weak radial grooves continuing from suture and disappear before periphery. Spire depressed conic; apex raised with a spire angle of about 142–164°. Last whorl barely shouldered and rounded periphery. Aperture lip simple; umbilicus narrowly opened.

Genital organs (figs 11D–F). Atrium very short. Penis stout cylindrical and about same length as vagina; penial caecum absent. Inner sculpture of proximal penial wall with fine irregular longitudinal penial pilasters and then gradually irregular, zig-zag, longitudinal folds in middle and surrounded penial verge. Epiphallus slender tube, around two times total penis length, and smaller diameter than penis; proximal epiphallus larger than distal portion. Epiphallallic caecum short, same diameter as proximal epiphallus, and attached with thick penial retractor muscle. Flagellum short with irregular shape. Vas deferens small tube. Vagina very enlarged, cylindrical-shaped, and distal part enlarged. Dart apparatus located on proximal vagina away from penis joining to atrium. Spermatophore elongate and needle-shaped. Tail filament very long tube;

region close to sperm sac bearing two spines; spine located close to sperm sac smaller and shorter than the other.

External features (fig. 3). Animal dark gray colour on head to body with three bright orange stripes running from head to body (two stripes near foot sole and one at middle of body), and then gradually only bright orange stripes on body to tail.

Remarks. *Taphrenalla parversa* sp. nov. is similar to *T. corona* sp. nov. because of the absence of the penial caecum and a thick penial sheath. However, they can be discriminated by their flagellum, epiphallus, vagina, and shell morphology. *Taphrenalla parversa* sp. nov. has a shorter flagellum, longer epiphallus, and larger vagina than *T. corona* sp. nov. In shell comparison, *T. parversa* sp. nov. has a weakly impressed suture and very weakly radial grooves on the shell sculpture, whereas *T. corona* sp. nov. exhibits a wide and deep groove at suture and deep radial grooves on the shell sculpture.

Taphrenalla conformis Pholyotha & Panha, sp. nov. (figs 1, 3, 10C, 12A–C, table 6)

Type material examined. Holotype: CUMZ 7202 (fig. 10C). Paratypes. Same data as holotype: CUMZ 7203 (15 shells and 52 specimens in ethanol), CUMZ 7230 (three specimens in ethanol), CUMZ 7204 (eight specimens in ethanol), CUMZ 7205 (11 shells and six specimens in ethanol), CUMZ 7206 (24 specimens in ethanol), NHMUK (two shells), and SMF (two shells).

Type locality. Tham Phung Chang, Mueang District, Phang-nga, Thailand (8°26'33.0"N, 98°30'55.4"E).

Etymology. The specific name is from the Latin ‘*conformis*’ meaning “like” or “similar” and name refers to the new species possessing a superficially similar shell to *T. parversa* sp. nov.

FIGURE 12 Genitalia and spermatophore. A–C. *Taphrenalla conformis* sp. nov. paratype CUMZ 7230. A. whole reproductive system; inset showing a small penial caecum. B. internal wall of penis with penial verge. C. spermatophore; inset showing two spines located close to thesperm sac. D–F. *T. incilis* sp. nov. paratype CUMZ 7208. D. whole reproductive system. E. internal wall of penis with penial verge. F. spermatophore; inset showing two spines located close to the sperm sac.

Diagnosis. Shell having shallowly impressed suture, weakly radial grooves. Penis covered with thick penial sheath. Epiphallic caecum short and bent at tip. Penial caecum short. Inner sculpture of proximal penis with very small longitudinal folds; distally narrow and obliquely cuboidal folds. Dart apparatus connected on vagina. Spermatophore with two spines on tail filament close to sperm sac.

Description

Shell (fig. 10C). Shell comparatively depressed conic, medium-sized (width up to 17.7 mm; height up to 10.3 mm) and pale brown. Whorls 5–6; suture shallowly impressed. Upper shell surface with numerous weak radial grooves continuing from suture to gradually disappear before periphery. Spire depressed conic; apex raised with a spire angle of about 138–148°. Last whorl well-rounded periphery. Aperture lip simple; umbilicus narrowly opened.

Genital organs (figs 12A–C). Atrium slightly prolonged. Penis cylindrical and nearly three times total vagina length; penial caecum present. Inner sculpture of proximal penis with small longitudinal folds; distal portion with narrow obliquely cuboidal pilasters, then reduced in size and surrounding penial verge. Epiphallus slender tube, around two times total penis length and smaller diameter than penis. Epiphallic caecum short and bent at the tip; thick penial retractor muscle connecting near the tip. Flagellum short, tapering and irregular slender tube. Vas deferens thin tube. Vagina long and cylindrical-shaped. Dart apparatus located near proximal vagina away from penis join to atrium. Spermatophore long and needle-shaped. Tail filament very long tube; region close to sperm sac bearing two spines; spine close to sperm sac small and shorter than the other.

External features (fig. 3). Animal dark gray in body colour with three pale milky yellow stripes running from head to caudal horn. One stripe at middle of the body and other two stripes above foot sole.

Remarks. Shell characters in *T. conformis* sp. nov. and *T. parversa* sp. nov. are very similar. They have a weakly impressed suture and weakly radial grooves on shell sculpture. However, the body colouration and reproductive organ can be used to separate them. In *T. conformis* sp. nov., the body has three pale milky-yellow bands running from the head to caudal horn, and the genitalia have a penial caecum. Whereas, the body colouration of *T. parversa* sp. nov. contains three bright orange stripes running from head to body, and only bright orange stripes on the body to tail. The genitalia of *T. parversa* sp. nov. do not have a penial caecum.

Taphrenalla incilis Pholyotha & Panha, sp. nov.
(figs 1, 3, 10D, 12D–F, table 6)

Type material examined. Holotype: CUMZ 7207 (fig. 10D). Paratypes. Same data as holotype: CUMZ 7208 (four shells and five specimens in ethanol), CUMZ 7209 (six shells and six specimens in ethanol), NHMUK (two shells), and SMF (two shells).

Type locality. Tham Khao Ting, Palian District, Trang, Thailand (7°09'30.0"N, 99°48'06.0"E).

Etymology. The specific epithet "*incilis*" is from the Latin word "*incile*" meaning "ditch, trench", which refers to the characteristic small radial grooves in the shell sculpture.

Diagnosis. Shell with narrow and deep channel-shaped suture, and weak and short radial grooves. Epiphallic caecum short and bent at tip. Penial caecum short. Inner sculpture of proximal penis with very small longitudinal folds; distally narrow and obliquely cuboidal folds. Dart apparatus located at vagina and penis junction. Spermatophore with two spines on tail filament close to sperm sac.

Description

Shell (fig. 10D). Shell comparatively depressed conic, moderate-sized (width up to 16.1 mm; height

up to 8.8 mm) and pale to dark brown. Whorls 5–6; suture narrow, deep grooved, and channel-shaped. Upper shell surface with numerous, very short and weak radial grooves near suture and then suddenly disappear. Spire depressed conic; apex raised with a spire angle of about $134\text{--}151^\circ$. Last whorl barely shouldered, and periphery almost rounded. Aperture lip simple; umbilicus narrowly opened.

Genital organs (figs 12D–F). Atrium short. Penis cylindrical and around three times total vagina length; penial caecum present. Inner sculpture of proximal and middle part of penis with fine longitudinal folds; distal portion with narrow obliquely cuboidal penial pilasters surrounding penial verge. Epiphallus slender tube and slightly longer than penis. Epiphallic caecum short and bent at tip; thick penial retractor muscle connecting near the tip. Flagellum short, irregular curved and with obtuse tip. Vas deferens thin tube. Vagina prolonged, cylindrical-shaped. Dart apparatus located at junction of vagina and penis. Spermatophore long and needle-shaped. Tail filament very long tube; region close to sperm sac bearing two spines; spine near sperm sac very small and shorter than the other.

External features (fig. 3). Animal dark gray in body colour with a pale milky yellow stripe at middle of the body running from head to caudal horn. Posterior part (body to tail) with pale milky yellow colour and wider stripe than anterior part (head to body).

Remarks. The weak radial grooves on the upper shell surface of *T. incilis* sp. nov. is similar to *T. parversa* sp. nov., *T. conformis* sp. nov., and *T. pygmaea* sp. nov. However, together with the suture shape and genitalia, *T. incilis* sp. nov. have a deeper and wider suture than *T. parversa* sp. nov. and *T. conformis* sp. nov. The dart apparatus of *T. parversa* sp. nov. and *T. conformis* sp. nov. is located on the proximal vagina away from the penis junction,

while *T. incilis* sp. nov. has the dart apparatus joining at the junction of vagina and penis.

While the suture shape and position of dart apparatus of *T. incilis* sp. nov. is similar to *T. pygmaea* sp. nov., *T. incilis* sp. nov. have a bent epiphallic caecum, and short flagellum and vagina, whereas *T. pygmaea* sp. nov. have a straight epiphallic caecum, and long flagellum and vagina.

Taphrenalla pygmaea Pholyotha & Panha, sp. nov. (figs 1, 3, 13A, 14A, B, table 6)

Type material examined. Holotype: CUMZ 7210 (fig. 13A). Paratypes. Same data as holotype: CUMZ 7211 (19 shells and 12 specimens in ethanol), NHMUK (two shells), and SMF (two shells).

Other material examined. Wat Sathit Khirirom, Khiri Rat Nikhom District, Surat Thani ($9^\circ 01' 48.2''\text{N}$ $98^\circ 59' 12.5''\text{E}$): CUMZ 7212 (75 shells and nine specimens in ethanol).

Type locality. Tham Wang Badan Priest's Camp Site, Khiri Rat Nikhom District, Surat Thani, Thailand ($8^\circ 54' 21.9''\text{N}$ $98^\circ 56' 53.9''\text{E}$).

Etymology. The specific epithet '*pygmaea*' is from the Latin word '*pygmaeus*' meaning 'dwarf', which refers to it being the smallest sized snail in this genus.

Diagnosis. Shell small to medium-sized, narrow and deep channel-shaped suture, and weak, diminutive radial grooves. Epiphallic caecum straight. Penial caecum short. Inner sculpture of proximal penis with very fine longitudinal folds and then transition to oblique and transverse folds distally. Dart apparatus located at junction of vagina and penis.

Description

Shell (fig. 13A). Shell moderately depressed conic, small to nearly medium-sized (width up to 12.3

FIGURE 13 Shells. A. *Taphrenalla pygmaea* sp. nov. holotype CUMZ 7210. B. *T. zemia* sp. nov. holotype CUMZ 7213. C. *T. alba* sp. nov. holotype CUMZ 7217. Inset SEM image showing shell sculpture of each species.

mm; height up to 6.8 mm) and pale brown. Whorls 5–6; suture narrow, deep, and channel-shaped. Upper shell surface with weak and diminutive radial grooves that appear only near suture. Spire deressed conic; apex raised with a spire angle of about 131–151°. Last whorl barely shouldered and

periphery almost rounded. Aperture lip simple; umbilicus narrowly opened.

Genital organs (figs 14A, B). Atrium short. Penis funnel-shaped and slightly longer than vagina; penial caecum present. Inner sculpture of proximal penis

FIGURE 14 Genitalia. A, B. *Taphrenalla pygmaea* sp. nov. paratype CUMZ 7211. A. whole reproductive system. B. internal wall of penis with penial verge. C. *T. zemia* sp. nov. paratype CUMZ 7214, whole reproductive system from juvenile specimen.

with very fine longitudinal penial pilasters, gradually transforming to oblique and transverse folds surrounding penial verge. Epiphallus cylindrical-shaped, about one and half times total penis length. Epiphallic caecum short, and attached with thin penial retractor muscle. Flagellum prolonged and cylindrical with blunt tip. Vas deferens small tube. Vagina long and cylindrical-shaped. Dart apparatus located at junction of proximal vagina and proximal penis.

External features (fig. 3). Animal very dark gray in body colour with indistinct pale milky stripe at middle of the body running from head to caudal horn.

Remarks. *Taphrenalla pygmaea* sp. nov. is distinctive in being the smallest of the known *Taphrenalla* species. Compared to *T. incilis* sp. nov., this new species is distinguished by a small shell with narrow body whorl. Moreover, the internal distal

penial surface of *T. pygmaea* sp. nov. has oblique and transverse folds whereas the distal portion of *T. incilis* sp. nov. has narrow obliquely cuboidal pilasters.

Taphrenalla zemia Pholyotha & Panha, sp. nov.
(figs 1, 3, 13B, 14C, table 6)

Type material examined. Holotype: CUMZ 7213 (fig. 13B). Paratypes. Same data as holotype: CUMZ 7214 (25 shells and 30 specimens in ethanol), NHMUK (two shells), and SMF (two shells).

Other material examined. Tham Pha Sua Bureau of Monks, Mueang District, Phang-nga (8°28'30.9"N 98°32'20.8"E): CUMZ 7215 (five specimens in ethanol). Wat Tham Bang Toei, Mueang District, Phang-nga (8°27'47.9"N 98°34'11.6"E): CUMZ 7216 (five specimens in ethanol).

Type locality. Wat Khiriwong, Thap Put District, Phang-nga, Thailand (8°31'53.2"N 98°34'41.4"E).

Etymology. The specific epithet is from the Greek word 'zemia' meaning 'loss', which refers to the shell being without radial grooves on the upper surface.

Diagnosis. Shell moderate to large-sized, shallowly impressed suture, and without radial groove. Epiphallallic caecum straight. Dart apparatus located on vagina.

Description

Shell (fig. 13B). Shell globosely depressed, moderate to nearly large (width up to 21.3 mm; height up to 11.9 mm) and yellowish-brown. Whorls 5–6; suture shallowly impressed. Upper shell surface smooth without radial grooves. Spire moderately elevated; apex raised with a spire angle of about 137–151°. Last whorl slightly shouldered and almost rounded periphery. Aperture lip simple; umbilicus narrowly opened.

Genital organs (fig. 14C). Atrium long. Penis slender tube and slightly longer than vagina. Epiphallus cylindrical tube, nearly one and a half times total penis length. Epiphallallic caecum short, and attached with penial retractor muscle. Flagellum large and slender tube. Vas deferens thin tube connected between distal end of epiphallus and free oviduct. Vagina long and cylindrical-shaped. Dart apparatus located near proximal vagina away from penis joining to atrium.

External features (fig. 3). Animal dark gray in body colour with three pale milky yellow stripes running from head to caudal horn. One stripe at middle of the body and two stripes close to foot sole.

Remarks. *Taphrenalla zemia* sp. nov. is distinguished from all other *Taphrenalla* species by the shell having a shallowly impressed suture without a subsutural band and no radial grooves on shell sculpture. Although we surveyed three localities during the wet season, only juvenile specimens were found for the examination and illustrated herein.

Taphrenalla alba Pholyotha & Panha, sp. nov.
(figs 1, 3, 13C, 15, table 6)

Type material examined. Holotype: CUMZ 7217 (fig. 13C). Paratypes. Same data as holotype: CUMZ 7218 (12 shells and 22 specimens in ethanol), CUMZ 7219 (13 specimens in ethanol), NHMUK (two shells), and SMF (two shells).

Other material examined. Limestone outcrops at Khlong Thom Nuea, Khlong Thom District, Krabi (7°55'47.9"N 99°16'23.3"E): CUMZ 7220 (six shells). Limestone outcrops at Ao Luek Tai, Ao Luek District, Krabi (8°24'09.9"N, 98°44'16.3"E): CUMZ 7221 (four specimens in ethanol). Limestone outcrops near Than Bok Khorani, Ao Luek District, Krabi (8°22'28.7"N 98°44'07.9"E): CUMZ 7222 (one shell), CUMZ 7223 (10 shells and four specimens in ethanol), and CUMZ 7224 (five specimens in ethanol). Tham

FIGURE 15 Genitalia and spermatophore of *Taphrenalla alba* sp. nov. specimen CUMZ 7223. A. whole reproductive system. B. internal wall of penis with penial verge. C. spermatophore; inset showing two spines located close to the sperm sac.

Nalakiring Bureau of Monks, Plai Phraya District, Krabi (8°33'35.3"N 98°51'37.5"E): CUMZ 7228 (two specimens in ethanol). Wat Khao Phra, Phrasaeng District, Surat Thani (8°37'33.0"N 98°56'53.5"E): CUMZ 7229 (one specimen in ethanol).

Type locality. Wat Tham Seu (Tiger Cave Temple), Mueang District, Krabi, Thailand (8°07'32.9"N, 98°55'27.1"E).

Etymology. The specific epithet '*alba*' is from the Greek word '*albus*' meaning 'white, whitish', and

refers to the characteristics of the shell having a white line or white subsutural band below the suture.

Diagnosis. Suture shallowly impressed and with white subsutural band. Epiphallic caecum short and bent at tip. Penial caecum short. Inner sculpture of proximal penis with very soft longitudinal folds then transition to small cuboidal pilasters distally. Dart apparatus located at vagina and penis junction. Spermatophore with two spines on tail filament close to sperm.

Description

Shell (fig. 13C). Shell globosely depressed, medium-sized (width up to 18.5 mm; height up to 10.6 mm) and reddish-brown. Whorls 5–6; suture shallowly impressed and with narrow whitish subsutural band. Upper shell surface smooth and without radial grooves. Spire conic; apex raised with a spire angle of about 126–144°. Last whorl well-rounded at periphery. Aperture lip simple; umbilicus narrowly opened.

Genital organs (fig. 15). Atrium short. Penis cylindrical and nearly one and a-half times total vagina length; penial caecum present. Internal sculpture of proximal penis with thin longitudinal folds then transformed to small cuboidal penial pilasters in middle and surrounding penial verge in distal penis. Epiphallus slender tube, slightly longer than total penis length. Epiphallic caecum short and bent at the tip, and penial retractor muscle connected near tip. Flagellum slightly long (about half-length of epiphallus) with blunt tip. Vas deferens small and tube-shaped. Vagina prolonged

and cylindrical-shaped. Dart apparatus located at junction between vagina and penis. Gametolytic sac elongate bulbous; gametolytic duct long and tube-shaped. Spermatophore elongate and needle-shaped. Tail filament very long tube; region close to sperm sac bearing two spines; furthest spine from sperm sac large and longer than the other.

External features (fig. 3). Animal very dark gray in body colour with three milky yellow stripes running from head to caudal horn. One stripe at middle of the body and the other two above foot sole.

Remarks. *Taphrenalla alba* sp. nov. is similar to *T. zemia* sp. nov., but can be distinguished by having a reddish-brown shell with narrow whitish subsutural band, whereas *T. zemia* sp. nov. has a yellowish-brown shell and without subsutural band. Furthermore, the dart apparatus of *T. alba* sp. nov. is located at the vagina and penis junction, whereas in *T. zemia* sp. nov. it is located on vagina away from the vagina and penis junction.