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Molecular Phylogeny Reveals a New Snorkel Snail, *Rhiostoma panhai* sp. nov., from Thailand (Gastropoda: Caenogastropoda: Cyclophoridae)

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ABSTRACT.– The present study used two mitochondrial gene fragments (COI and 16S rRNA) and morphology to reveal a new species of *Rhiostoma* in Thailand: *Rhiostoma panhai* **sp. nov.** Genetic divergence between the new species and other *Rhiostoma* species was high, ranged from 14 to 19% *p*-distance. Phylogenetic analyses confirmed the novelty of this new species. The new species differs from other *Rhiostoma* species by having a detached whorl shorter than the aperture width (mean detached-whorl length/aperture width: 0.89±1.40), a small, curved, and slender tubular breathing device, and dark brown with paler zigzag streaks periostracum.

KEYWORDS: DNA barcoding, mitochondrial DNA, operculate land snail, taxonomy

INTRODUCTION

Snorkel snails are ground dwellers that belong to Cyclophoridae, a family of operculate terrestrial snails. The genus *Rhiostoma* Benson, 1860 is characterized either by a peculiar-shaped shell in which the last whorl is free due to weaker physical contact with the penultimate whorl and a circular aperture (Chen et al., 2022), or the detached whorl is absent and the shell has a unique cup-shaped operculum. The last whorl has a breathing device in the form of an incision or tube, allowing gas exchange (Pall-Gergely et al., 2016). The snails are widely distributed in Indo-China and the Malay Peninsula (Benson, 1860; Gude, 1921). The classification of *Rhiostoma* was traditionally based on morphology (Benson, 1860; Fischer, 1891; Kobelt, 1902; Gude, 1921; Habe, 1965; Fischer, 1973). However, a high degree of intraspecific variation within the genus confounds their taxonomy. Therefore, the classification of these snails has recently been based on a combination of morphological and molecular phylogeny data, enabling the discovery of a rapidly growing number of species (Tongkerd et al., 2023; Prasankok et al., 2023). Currently, around 20 species of *Rhiostoma* are known from Thailand, out of these, more than 30 species are in Indochina and the Malay Peninsula.

The present study describes a new species of *Rhiostoma* based on morphology and genetic divergence from congeners based on two mitochondrial (mtDNA) gene fragments (COI and 16S rRNA).

MATERIALS AND METHODS

Sample collection

Specimens were collected from Chao Pho Pratu Pha Shrine, Lampang Province, northern Thailand (Fig. 1). Voucher specimens were deposited in the Museum of Zoology, Chulalongkorn University (CUMZ, Table 1). Suranaree University of Technology Animal Care and Use Committee approved the animal protocols used in this study.

DNA extraction and sequencing

Four specimens from Chao Pho Pratu Pha Shrine were used for sequencing. Genomic DNA from foot tissues was extracted using the NucleoSpin Tissue kit (MACHEREY-NAGEL Inc.). Gene fragments were amplified using the primer pairs of LCO1490 and HCO2198 (Folmer et al., 1994) for COI and 16Sar and 16Sbr for 16S rRNA (Kessing et al., 1989). PCR conditions followed those reported by Prasankok et al. (2023). PCR products were sent for sequencing at Macrogen, Inc. Sequences were aligned in MUSCLE as implemented in MEGA X. COI sequences were translated against amino acid to check for stop codons. For 16S, gaps were excluded by complete deletion. Sequences were deposited in GenBank with accession number OQ918562–OQ918565 for COI and OQ919148–OQ919151 for 16S rRNA (Table 1).

Phylogenetic analysis

Phylogenetic trees based on concatenated COI and 16S rRNA data were constructed using Bayesian inference (BI) and maximum likelihood (ML). Additional sequences of other *Rhiostoma* and *Pterocyclos* species were obtained from GenBank and included as outgroups (Tongkerd et al., 2023; Prasankok et al., 2023) for phylogenetic analyses, as presented in Table 1. The BI tree was built in MrBayes 3.2 (Ronquist et al., 2012) and run for 10,000,000 generations, sampling

TABLE 1. List of samples of *Rhiostoma panhai* sp. nov. and related species and *Pterocyclos* species used in this study, with CUMZ voucher number and their GenBank accession number of COI and 16S rRNA.

Species /locality	CUMZ no.	Specimen code	GenBank Accession No. COI	GenBank Accession No. 16S rRNA
R. panhai sp. nov.				
Chao Pho Pratu Pha Shrine, Lampang, Thailand (18°31'0.01"N, 99°49'28.75"E)	14386	MRU35-1	OQ918562	OQ919148
	14387	MRU35-2	OQ918563	OQ919149
	14387	MRU35-3	OQ918564	OQ919150
	14387	MRU35-4	OQ918565	OQ919151
R. phunangense				
Doi Phunang, Phayao, Thailand (18°51'22.6"N, 100°10'54.9"E)	3977	PN1	OP680760*	OP681317*
		PN3	OP680762*	OP681319*
R. housei				
Tham Sriwilai, Saraburi, Thailand (14°42'43.9"N, 100°51'58.6"E)	3982	SL1	OP491242**	OP681326*
Khao Lom Muak, Prachuap Khiri Khan, Thailand (11°47'4"N, 99°48'53.9"E)	3987	LM2	OP491240**	OP681328*
R. khoratense				
Khao Lukchang, Nakhon Ratchasima, Thailand (14°31'33.3"N, 101°21'36.2"E)	3981	LC1	OP680766*	OP681329*
Khao Wong, Nakhon Ratchasima, Thailand (14°34'20"N, 101°20'58"E)	10215	R2-4	OP680771*	OP681334*
R. nakwangense				
Tham Nakwang, Phetchaburi, Thailand (12°51'25.9"N, 99°56'28.8"E)	3988	NW2	OP680774*	OP681337*
		NW29	OP680776*	OP681339*
R. abletti				
Hot Springs, Meuang Hiam, Houaphanh, Laos (20°5'43.2"N, 103°22'19.6"E)	10206	MRU22	OP491196**	OP681341*
R. asiphon				
Koh Wua Talab, Koh Samui, Surat Thani, Thailand (9°38'6"N, 99°40'11.8"E)	4767	MRU16-1	OP491198**	OP681342*
Koh Sam Sao, Koh Samui, Surat Thani, Thailand (9°39'32"N, 99°41'1"E)	4756	SS2	OP491200**	OP681344*
R. breviocollar				
Wat Khao Smokon, Ban Mi District, Lopburi, Thailand (14°54'25.9"N, 100°30'21.9"E)	3975	KB10	OP491201**	OP681345*
		KB11	OP491202**	OP681346*
R. cambodjense				
Tham Khao Chakan, Khao Chakan, Sa Kaeo, Thailand (13°39'37.8"N, 102°5'6.7"E)	4714	MRU11-1	OP491203**	OP681347*
		MRU11-2	OP491204**	OP681348*
R. cheliopegma				
Khao Cha-Ang, Bo Thong, Chonburi, Thailand (13°12'29.8"N, 101°39'6.5"E)	4886	CA1	OP491208**	OP681352*
Tham Takein, Khao Chamao, Rayong, Thailand (12°56'49.27"N, 101°42'34.01"E)	3985/2	TT4	OP491217**	OP681361*
R. dalyi				
Hill Near Tham Air Thammachart, Long, Phrae, Thailand (18°17'16.6"N, 100°0'43.8"E)	3979	AT1	OP491219**	OP681363*
		AT9	OP491223**	OP681367*
R. furfurosum				
Noen Maprang, Phitsanulok, Thailand (16°41'37.9"N, 100°40'44.9"E)	3901	NP3	OP491227**	OP681371*
		NP18	OP491230**	OP681374*
R. hainesi				
Makok Waterfall, Khlung, Chanthaburi, Thailand (12°35'12"N, 102°15'21"E)	4814	MRU8-2	OP491234**	OP681378*
Khao Soi Dao Breeding Centre, Pong Nam Ron, Chanthaburi, Thailand (12°55'19.8"N, 102°14'39.7"E)	4457	SD	OP491235**	OP681379*
R. haughtoni				
Dhammatat Cave, Mawlamyine, Mon, Myanmar (16°30′4.9"N, 97°49′16.6"E)	10048	MRU4	OP491236**	OP681380*
R. jalorensis				
Tham Sra Yoon Thong, Ao Luek, Krabi, Thailand (8°23'36.6"N, 98°46'24.8"E)	3819	MRU7	OP491244**	OP681381*
Tharn Bok Khorani, Ao Luek, Krabi, Thailand (8°23'18"N, 98°44'4"E)	10146	MRU13-2	OP491245**	OP681382*
R. lannaense				
Ban Ping Klong (village), Chiangdao, Chiang Mai, Thailand (19°30'48.6"N, 99°3'21.1"E)	4701	MRU1	OP491251**	OP681387*
		MRU2	OP491252**	OP681388*
R. morleti				
Ban Na Wid, Vieng Xai, Houaphanh, Laos (20°26'59.5"N, 104°10'51.2"E)	1004/2	UHL1	OP491258**	OP681393*
R. platymorpha				
Tham Muang On, Mae On, Chiang Mai, Thailand (18°47'10.6"N, 99°14'17.1"E)	4763	MO47	OP491262**	OP681396*
		MO48	OP491263**	OP681397*
R. rhothonotaphrosa				
Tham Sri Thong, Klong Hat, Sa Kaeo, Thailand (13°28'43.6"N, 102°16'53.8"E)	3858	RST1	OP491264**	OP681398*
Tham Phet Pho Thong, Klong Hat, Sa Kaeo, Thailand (13°24'49"N, 102°19'31"E)	10172	MRU17-2	OP491265**	OP681399*
R. samuiense				
Khao Huay Hang, Huay Yod, Trang, Thailand (7°47'38.6"N, 99°38'38.5"E)	4774	MRU6-1	OP491268**	OP681402*
Tham Wang Thong, Kanom, Nakhon Sri Thamarat, Thailand (9°12'16"N, 99°46'26.6"E)	4708	WT	OP491273**	OP681405*
R. simplicilabre				
Sapan Waterfall, Bo Kluea, Nan, Thailand (19°11'25"N, 101°11'55"E)	4868	MRU18-1	OP491274**	OP681406*
		MRU18-2	OP491275**	OP681407*
R. tigrina				
Tham Saohin Prayanak, Mae Sai, Chiang Rai, Thailand (20°19'24.6"N, 99°51'51.5"E)	10193	MRU31-2	OP491278**	OP681410*
Wat Tham Pum Tham Pla, Mae Sai, Chiang Rai, Thailand (20°20'53.2"N, 99°51'29.3"E)	3909	MRU34	OP491279**	OP681411*
R. ebenozostera				
Wat Tham Pha Pu, Loei, Thailand (17°34'43.2"N, 101°42'41.2"E)	14388	MRU5-1	-	OQ919152
	14388	MRU32-1	-	OQ919153
	14388	MRU36-1	-	OQ919154
Pterocyclos blandi				
Teluic Ewa, Langkawi, Kedah, Malaysia (6°25'2"N, 99°45'44"E)	4582	TU	OP491280**	OP681412*
P. diluvium				
Tam Puttha Kodome, Srinagarindra, Patthalung, Thailand (7°33'36.5"N, 99°53'10.5"E)	3812	PK2	OP491281**	OP681413*
Tham Sumano, Srinagarindra, Patthalung, Thailand (7°35'12"N, 99°52'4"E)	4588	TS	OP491282**	OP681414*
Gua Cenderawasih, Kangar, Perlis, Malaysia (6°24'45.8"N, 100°11'33.7"E)	4592	GU	OP491283**	OP681415*

* sequences from Prasankok et al. (2023) and ** sequences from Tongkerd et al. (2023)

TABLE 2. Uncorrected *p*-genetic distance of *Rhiostoma panhai* sp. nov. and related species based on COI gene.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. *R. panhai* sp. nov.	-																					
2. *R. dalyi*	0.154	-																				
3. *R. phunangense*	0.144	0.149	-																			
4. *R. platymorpha*	0.142	0.151	0.145	-																		
5. *R. trigrina*	0.147	0.165	0.149	0.062	-																	
6. *R. haughtoni*	0.161	0.156	0.155	0.145	0.145	-																
7. *R. lannaense*	0.152	0.150	0.139	0.166	0.165	0.141	-															
8. *R. abletti*	0.190	0.167	0.182	0.179	0.187	0.190	0.184	-														
9. *R. simplicilabre*	0.165	0.149	0.162	0.165	0.172	0.164	0.166	0.082	-													
10. *R. cambodjense*	0.148	0.143	0.135	0.153	0.162	0.152	0.159	0.179	0.167	-												
11. *R. furfurosum*	0.174	0.159	0.170	0.161	0.169	0.178	0.177	0.184	0.173	0.153	-											
12. *R. khoratense*	0.172	0.168	0.165	0.171	0.177	0.166	0.162	0.180	0.168	0.141	0.144	-										
13. *R. housei*	0.167	0.156	0.165	0.184	0.181	0.165	0.170	0.188	0.169	0.153	0.135	0.103	-									
14. *R. breviocollar*	0.186	0.159	0.165	0.164	0.174	0.168	0.161	0.195	0.162	0.147	0.142	0.119	0.088	-								
15. *R. cheliopegma*	0.177	0.145	0.174	0.175	0.174	0.163	0.165	0.168	0.146	0.136	0.138	0.115	0.116	0.126	-							
16. *R. jalorensis*	0.162	0.156	0.152	0.146	0.163	0.174	0.164	0.178	0.160	0.143	0.149	0.144	0.149	0.151	0.148	-						
17. *R. nakwangense*	0.154	0.151	0.151	0.148	0.165	0.160	0.161	0.185	0.170	0.135	0.142	0.147	0.156	0.169	0.151	0.119	-					
18. *R. hainesi*	0.178	0.179	0.175	0.171	0.174	0.184	0.166	0.180	0.159	0.158	0.167	0.153	0.179	0.171	0.160	0.133	0.129	-				
19. *R. rhothonotaphrosa*	0.179	0.183	0.177	0.174	0.176	0.195	0.169	0.195	0.171	0.161	0.176	0.164	0.180	0.179	0.170	0.139	0.133	0.036	-			
20. *R. asiphon*	0.186	0.187	0.171	0.176	0.179	0.192	0.170	0.198	0.175	0.160	0.172	0.159	0.182	0.175	0.174	0.136	0.129	0.038	0.038	-		
21. *R. samuiense*	0.179	0.178	0.170	0.171	0.175	0.194	0.170	0.190	0.167	0.159	0.169	0.159	0.179	0.184	0.161	0.134	0.129	0.038	0.031	0.037	-	
22. *R. morleti*	0.167	0.137	0.159	0.172	0.175	0.173	0.155	0.097	0.073	0.157	0.172	0.176	0.167	0.165	0.149	0.170	0.173	0.160	0.168	0.174	0.159	-

FIGURE 1. Locality of *Rhiostoma panhai* sp. nov. Chao Pho Pratu Pha Shrine, Ban Dong subdistrict, Mae Moa district, Lampang Province, Thailand.

every 1,000 generations. The first 1,000 trees were discarded as burn-in. The ML tree was constructed in RAxML v.7.6.3 (Stamatakis, 2006) with 1,000 replicates. The phylogenetic trees were built on the CIPRES Science Gateway (Miller et al., 2015).

Uncorrected pairwise *p*-distances were calculated among *Rhiostoma* species based on COI. Ninety-five sequences of 21 *Rhiostoma* species were included from the study of Prasankok et al. (2023).

Morphological study

Shell and operculum were photographed with a digital camera. Shell size of Chao Pho Pratu Pha Shrine was compared with closely related species *R. dalyi* and *R. phunangense*. Four shell measurement (Fig. 3) including shell height (SH), shell width (SW), detached whorl length (DWL), and aperture width (AW) were measured with a Vernier caliper. Boxplots based on SH, SW, DWL, AW and DWL/AW were assessed the difference among those three species. Analysis of variance (ANOVA) was performed to assess the significant differences between species; when the test proved significant, a pairwise comparison between pairs of species was performed using Tukey's honestly significant difference (HSD) test.

Shell shape was analyzed based on morphometric geometric by compared with closely related species *R. phunangense* and *R. dalyi*. A total of 60 shells were included in the analysis, containing 25 shells from Chao Pho Pratu Pha Shrine, Lampang Province, Thailand, and 35 shells of closely related species, *R. dalyi* and *R. phunangense*. Each shell was photographed from an aperture view with a tripod-mounted Nikon D7200 digital camera. Ten artificial outlines based on 2-dimensional Cartesian coordinates were generated by MakeFan8. The specimen photographs were randomly ordered in tpsUtil v.1.61 (Rohlf, 2015). The images of the specimens were digitized into ten landmarks and four semilandmarks (Fig. 4) by the program tpsDig2 v.2.26 (Rohlf, 2005). Geometric morphometric analyses based on Procrustes methods (Dryden and Mardia, 2016) to estimate shell shape variation were performed in MORPHOJ v1.06d (Klingenberg, 2011). Canonical variate analysis (CVA) was analyzed to explore the differences of shell shapes among Chao Pho Pratu Pha Shrine, *R. phunangense* and *R. dalyi* specimens. The pairwise differences in shell shape based on Mahalanobis and Procrustes distances were calculated to validate the difference among species. The significant differences among pairwise comparisons were tested using permutation (10,000 iterations).

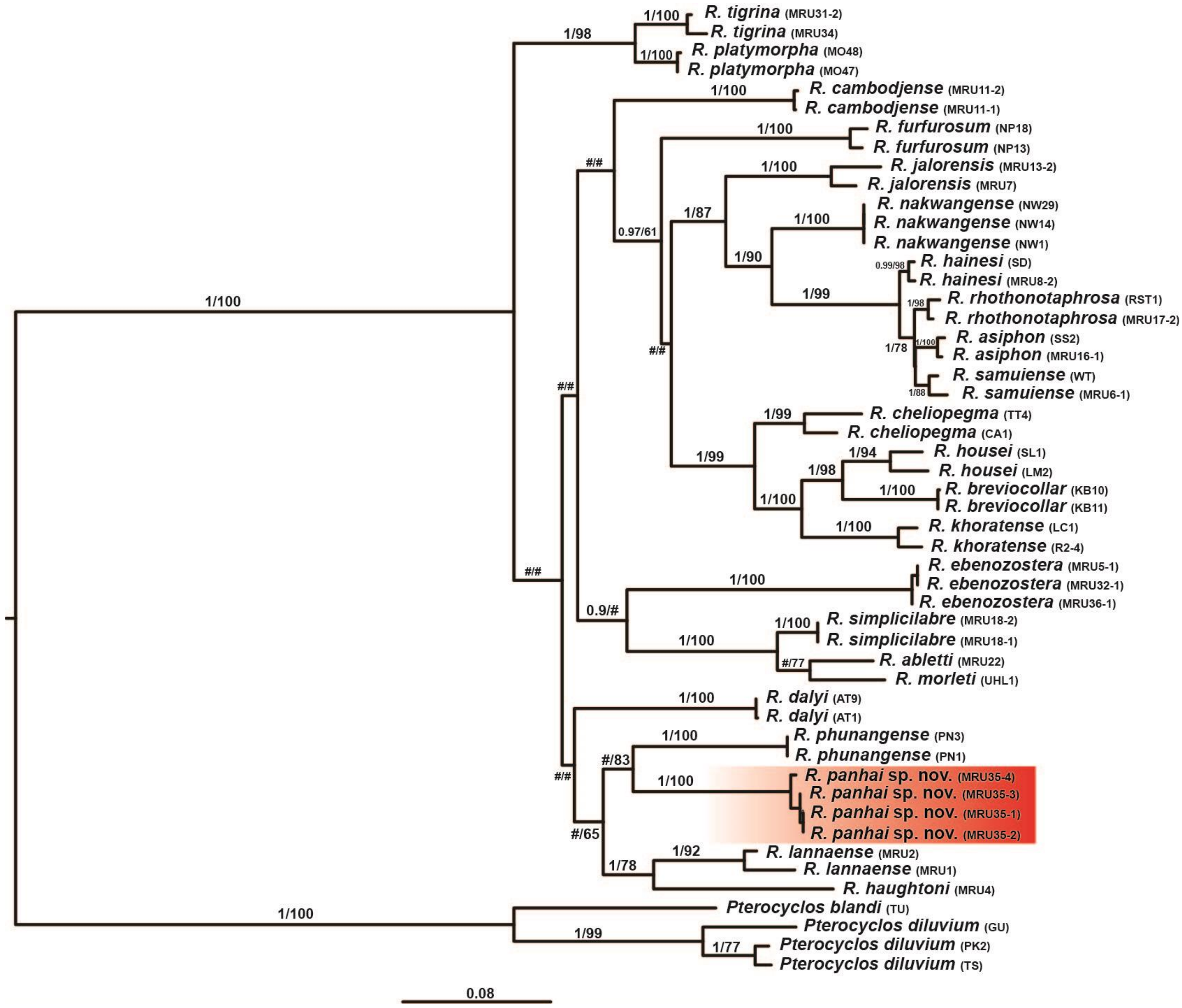

FIGURE 2. Maximum likelihood (ML) phylogenetic tree of *Rhiostoma panhai* sp. nov., and related species derived from a concatenated COI and 16S rRNA data. Node bars represent posterior probability/ML bootstrap support. # indicates branch with <50% ML bootstrap support and < 0.90 posterior probability. Red color represents *Rhiostoma panhai* sp. nov. clade.

RESULTS

Molecular study

The aligned sequences consisted of 660 bp for COI and 495 bp for 16S rRNA. The COI gene sequence contained 217 (32.9%) variable sites, whereas the 16S rRNA contained 249 (50.0%) variable sites. The concatenated data of COI and 16S rRNA consisted of 1,155 bp, of which 553 (48.1%) were variable sites.

The phylogenetic analysis (Fig. 2) showed that specimens from Chao Pho Pratu Pha Shrine were strongly supported by both the bootstrap value (100) and posterior probability (1). This clade was described here in as *Rhiostoma panhai* sp. nov. The *p*-distance based on COI also indicated high divergence between this new species and other *Rhiostoma* species, ranging from 14 to 19%. Overall, the *p*-distance among *Rhiostoma* species ranged from 3 to 19% (Table 2).

Morphological study

All five boxplots differed across all shell size measurements among *R. panhai* sp. nov., *R. dalyi*, and *R. phunangense* (Fig. 3). Tukey's honestly significant difference test exhibited significant differences among three pairwise of *R. panhai* sp. nov., *R. dalyi*, and *R. phunangense* across all five measurements (Table 3). The discriminant characters of *R. panhai* sp. nov. were SW, SH, AW, and DWL. Moreover, *R. panhai* sp. nov. shell also appeared smaller than *R. dalyi* and *R. phunangense* shell for SW, SH, and AW (Table 4).

Canonical variate analysis (Fig. 4) of *R. panhai* sp. nov., *R. phunamgense* and *R. dalyi* provided a graphic display of the shape differences by the relation between

TABLE 3. Tukey's honestly significant differences (HSD) test for five shell measurements (in bold Tukey's pos-hoc $p \leq 0.01$).

Pairs	SW	SH	AW	DWL	DWL/ AW
R. panhai sp. nov. vs *R. phunangense*	**0.000**	**0.000**	**0.000**	**0.002**	0.478
R. panhai sp. nov. vs *R. dalyi*	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
R. phunangense vs *R. dalyi*	0.072	0.355	0.782	**0.000**	**0.000**

TABLE 4. Summary of the shell measurements for *Rhiostoma panhai* sp. nov. and closely related species.

	R. panhai **sp. nov. Mean ± S.D. (range)**	***R. dalyi*** **Mean ± S.D. (range)**	***R. phunangense*** **Mean ± S.D. (range)**
No.	25	10	25
SW	21.28 ± 1.02 (19.20–22.90)	26.82 ± 1.49 (23.90–29.00)	25.54 ± 1.89 (22.30–28.80)
SH	12.68 ± 0.99 (11.00–14.60)	15.84 ± 1.80 (11.80–17.60)	16.55 ± 1.49 (13.30–19.20)
AW	7.90 ± 0.38 (7.20–8.60)	9.83 ± 0.38 (8.90–10.20)	9.94 ± 0.51 (9.00–10.90)
DWL	7.10 ± 1.22 (4.60–11.00)	12.49 ± 1.09 (10.80–13.80)	8.50 ± 1.53 (6.00–12.40)
DWL/AW	0.89 ± 1.40 (0.64–1.38)	1.28 ± 0.11 (1.10–1.42)	0.85 ± 0.13 (0.63–1.20)

TABLE 5. Mahalanobis distances (below diagonal) and Procrustes distances (above diagonal) of *Rhiostoma panhai* sp. nov. and closely related species with *p*-values (shown in parentheses).

	R. panhai **sp. nov**	***R. dalyi***	***R. phunangense***
R. panhai sp. nov	–	0.0584 (<0.0001)	0.0412 (0.0001)
R. dalyi	5.5563 (<0.0001)	–	0.0609 (<0.0001)
R. phunangense	3.9695 (<0.0001)	6.3023 (<0.0001)	–

the first two CV variables. The first canonical axis (CV1) illustrated 61.0% (Eigenvalues = 4.75226697) of the shape variability. The second canonical axis (CV2) accounted for 38.9% (Eigenvalues = 3.03555128). Overall, individuals from *R. panhai* sp. nov., *R. dalyi*, and *R. phunangense* showed distinct shell shape separation (Fig. 4). The CV1 showed differences in the coiled whorl by a shift of landmarks 7 and 8 and a different aperture shape principally represented by landmarks 9 and 12. The CV2 exhibited the differences in the aperture by changing landmarks 11. Significant differences also existed among the shell shapes for both Mahalanobis and Procrustes distances ($p<0.0001$; to 0.0001; Table 5).

Taxonomy

Family Cyclophoridae Gray, 1847
Subfamily Cyclophorinae Gray, 1847
Tribe Pterocyclini Kobelt and Möllendorff, 1897

Genus *Rhiostoma* Benson, 1860

For a list of the synonyms and usage of the name, see Tongkerd et al. (2023).

Type species.– *Rhiostoma haughtoni* Benson, 1860 by original designation in Benson (1860: 96).

Remarks.– *Rhiostoma* has been diagnosed as small to large, and with heliciform to depressed shell. Detached whorl absent or with short to long detached whorl, curved and descending. Breathing device prominently present with various types. Peristome double; lip thickened and expanded. Shell color varying from uniform color to zigzag pattern. Operculum calcareous, cup-shaped and anticlockwise multispiral with elevated lamella.

Rhiostoma housei group

Species with long detached whorl and tubular-shaped breathing device

Rhiostoma panhai sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:BD26F768-FDA5-4A8D-A089-43F87FA82703
(Figs 1–5)

Rhiostoma dalyi—Tongkerd et al., 2023: 50 [*partim*].

Type locality.– Chao Pho Pratu Pha Shrine, Ban Dong Subdistrict, Mae Moa District, Lampang Province, Thailand (18°31'0.01"N, 99°49'28.75"E; Fig. 1).

Holotype.– CUMZ 14386 (female, height 13.6 mm, width 21.0 mm, detached-whorl length 6.9 mm; Fig. 5A).

Paratypes.– From the type locality CUMZ 14387 (3 females and 4 unknown gender shells). Measurement of 7 shells: height 12.4–13.8 (std±0.51 mm), width 21.1–23.6 (std±0.89 mm) and detached-whorl length 6.6–8.3 (std±0.48 mm).

Etymology.– The species is named after and dedicated to Dr. Somsak Panha, who has greatly contributed to the taxonomy of terrestrial snails in Thailand and Indochina.

Other material examined.– Chao Pho Pratu Pha Shrine, Ban Dong Subdistrict, Mae Moa District, Lampang Province, Thailand, CUMZ 14387 (18 shells) (18°31'0.01"N, 99°49'28.75"E).

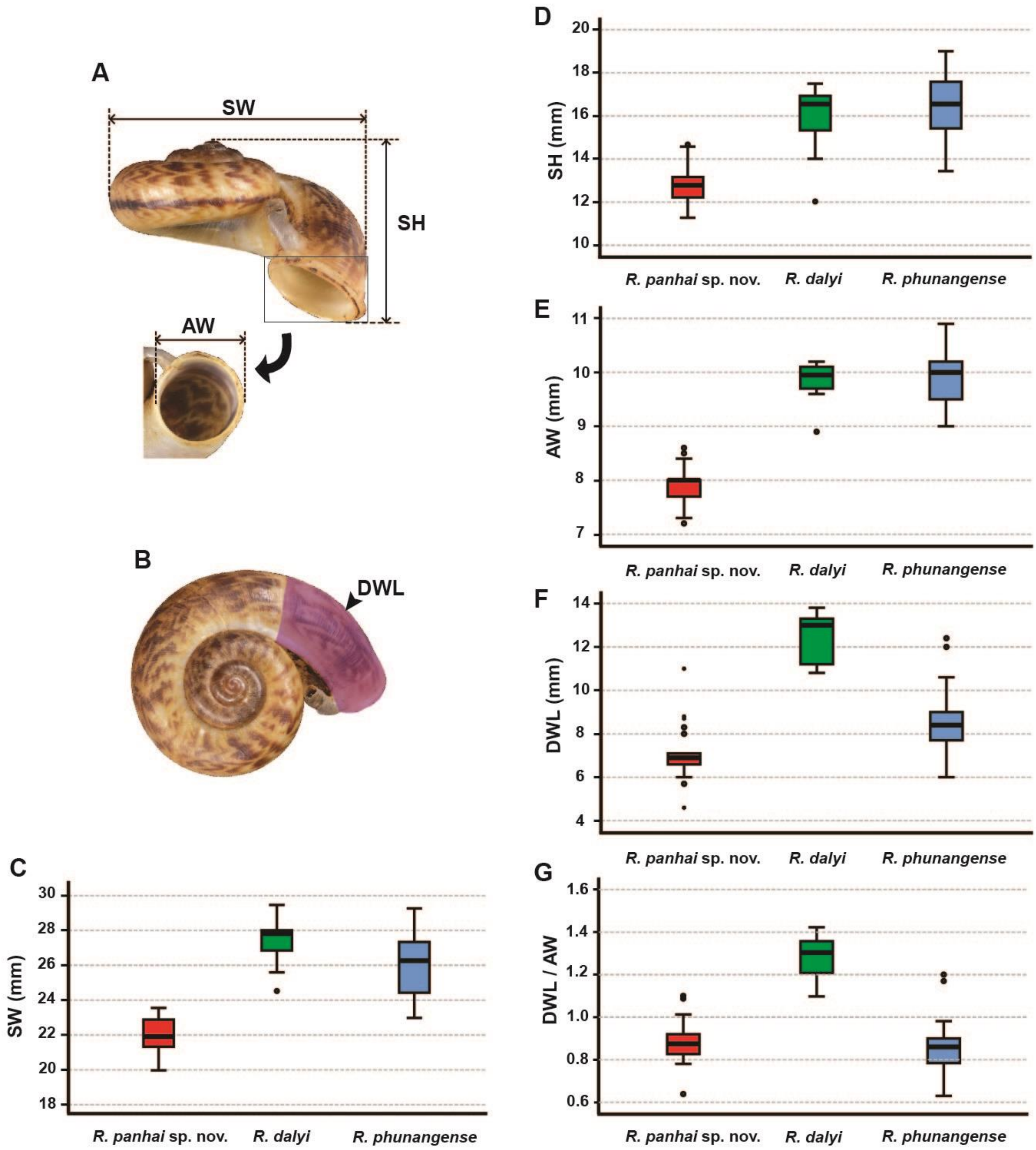

FIGURE 3. Shell size measurements and boxplots showing the difference of five shell measurements for *Rhiostoma panhai* sp. nov. (n=25) and closely related species: *R. dalyi* (n=10) and *R. phunangense* (n=25). **A.** Shell size measurements: SW, shell width; SH, shell height and AW, aperture width. **B.** DWL, detached whorl length. **C–G.** Boxplots of five shell measurements.

Diagnosis.– Shell medium, detached whorl medium length; detached whorl shorter than the aperture width (mean detached-whorl length/aperture width: 0.89), breathing device small, curved, slender and tubular. Periostracum dark brown with paler zigzag streaks.

Differential diagnosis.– *Rhiostoma panhai* sp. nov. is closely related to *R. phunangense*. *Rhiostoma panhai* sp. nov. differs from *R. phunangense* by having a smaller shell, paler apex, shorter detached whorl, slender tubular breathing device, and tall cup-shape operculum, whereas *R. phunangense* has a bigger shell, longer detached whorl, thick tubular breathing device, and low cup-shape operculum. This new species is also supported by the phylogenetic tree (Fig. 2).

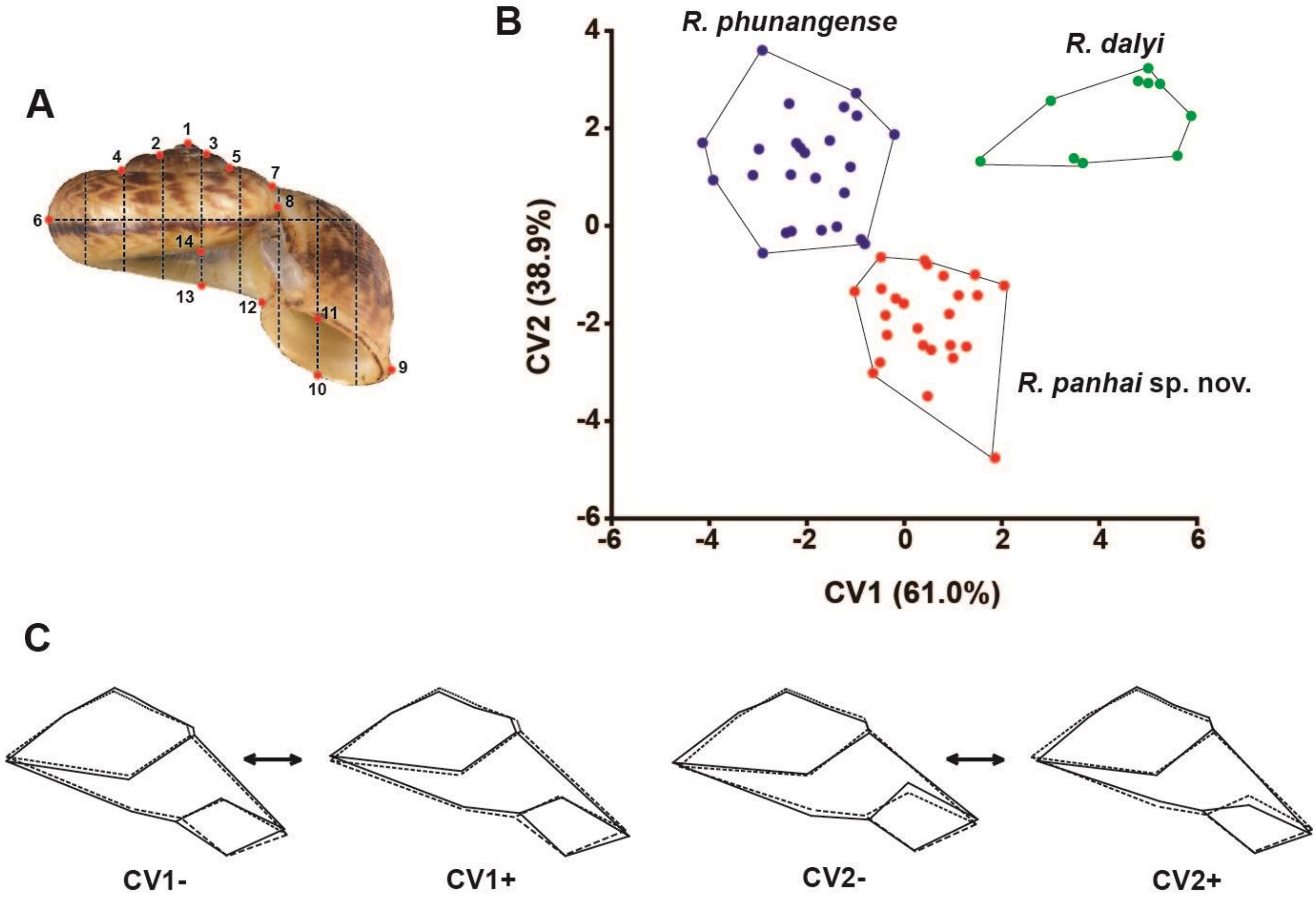

FIGURE 4. Geometric morphometric study of shell shape variation in *Rhiostoma panhai* sp. nov. and closely related species *R. dalyi* and *R. phunangense*. **A.** Shell outlines and landmarks used in this study. **B.** Plots of individual scores for the two canonical variates (CVs) derived from canonical variate analysis (CVA). Colors represent clades: red = *R. panhai* sp. nov.; green = *R. dalyi*; blue = *R. phunangense*. **C.** Wireframes showing the shape deformations (dotted line) from the consensus configuration (solid line) to each extreme negative and positive CVs. Shape changes along CV1 are shown on the left and CV2 on the right.

Description.– Shell medium-sized (width: 19.2–22.9 mm; height: 11.0–14.6 mm; detached-whorl length 4.6–11.0 (std±1.22 mm); n=25, thick, discoidal, widely umbilicate. Apex acute; spire little elevated to nearly flat. Whorls 4 to 5 convex, increasing regularly; suture wide and depressed; last whorl rounded; detached whorl medium and almost same length as apertural width, curved and descending. Shell surface with thin growth lines. Shell color dark brown with paler zigzag streaks; thin dark brown spiral band on periphery. Periostracum thick corneous, brownish. Aperture rounded; lip thickened and expanded, multilayered. Breathing device small, slender, tubular, medium length, curved; wide hole. Operculum calcareous, cup-shaped, and anticlockwise multispiral.

DNA barcode.– The GenBank accession number of the COI barcode of the holotype is OQ918562.

Distribution and habitats.– This new species is known only from the type locality. Most occur under leaf litter in limestone areas.

Remark.– Specimens from Chao Pho Pratu Pha Shrine identified as '*Rhiostoma dalyi* Blanford, 1902' in Tongkerd et al. (2023) due to its resemblance to *R. dalyi*, but are phylogenetically placed in this new species. The new species differs from *R. dalyi* by a smaller shell (Fig. 3), a detached whorl shorter than the aperture width, and a dark brown periostracum with zigzag streak. In contrast, *R. dalyi* has a larger shell, a detached whorl longer than the aperture width, and a thinner periostracum with a light brown zigzag streak. Moreover, the characters of *R. panhai* sp. nov. are distinguished in shell shape by morphometric geometric analysis (Fig. 4).

DISCUSSION

The phylogenetic tree and genetic distance analyses strongly suggest *Rhiostoma panhai* sp. nov. as a distinct species. The mean interspecific divergence based on COI between *Rhiostoma panhai* sp. nov. and the other *Rhiostoma* species was deep (14 to 19%)

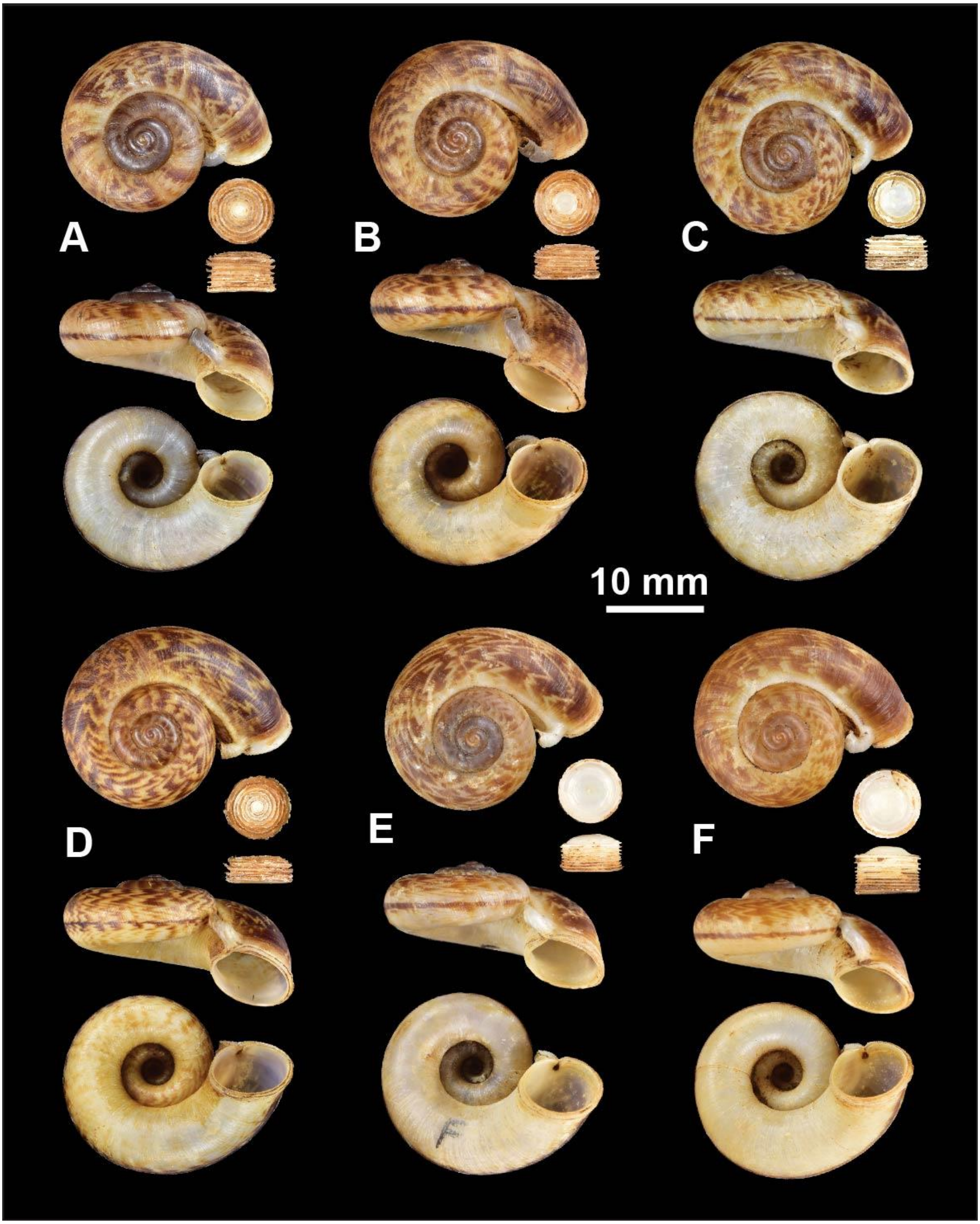

FIGURE 5. Shell of *Rhiostoma panhai* sp. nov. **A.** Holotype CUMZ 14386 from Chao Pho Pratu Pha Shrine, Lampang Province and **B–F.** paratypes CUMZ14387/1–5 from the type locality.

when compared to divergence among other *Rhiostoma* species (3.3 to 19.5%, mean: 9.6%, Tongkerd et al., 2023), and two other cyclophorid genera, namely *Cyclophorus* (mean: 10.9%, Nantarat et al., 2014; 5.8–17.3%, Hirano et al., 2022) and *Japonia* (mean: 11.4%, Lee et al., 2008b). Divergence scores for the new species and its congeners are more similar to those of *Chondrocyclus* (range: 10.3–20.8%, Cole et al., 2019), *Cyathopoma* (15.1%, Lee et al., 2008a), and *Pilosphaera* (mean: 16.0%, Lee et al., 2008b).

Rhiostoma panhai sp. nov. can be classified within the *Rhiostoma housei* group (Tongkerd et al., 2023) by having a long detached whorl and tubular-shaped breathing device. The characters supporting this new species are a detached whorl shorter than the aperture width (mean detached-whorl length/aperture width: 0.89), and a small, curved, and slender tubular breathing device, and dark brown with paler zigzag streaks Periostracum.

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