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Hyriopsis panhai, a new species of freshwater mussel from Thailand (Bivalvia: Unionidae)

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Abstract. A freshwater mussel in the genus *Hyriopsis* Conrad, 1853 is described from the Chao Phraya Basin in central Thailand as *Hyriopsis panhai*, new species. This new species was once included in *Hyriopsis bialata* Simpson, 1900, but morphological investigations and multi-locus phylogeny based on concatenated data of COI, 16S, and 28S genes retrieved it as a distinct species, being separated from *H. bialata* 8.30% by uncorrected COI p-distance. The new species is conchologically similar to its sister *H. bialata* sensu stricto, but can be distinguished by being more laterally compressed in anterior portion; a more elongated and rounded posterior margin; and anterior adductor muscle scars that are distinctly separated from pedal retractor muscle scars.

Key words. Chao Phraya Basin, freshwater mussels, multi-locus phylogeny, taxonomy

INTRODUCTION

Freshwater mussels in the family Unionidae are exceptionally diverse in the Indotropical region, accounting for approximately 30% of all described species worldwide (Lopes-Lima et al., 2018; Zieritz et al., 2018a). Recently, a number of systematics studies have dramatically increased the body of knowledge in taxonomy and systematics of freshwater mussels in Southeast Asia (Bolotov et al., 2017a, b, 2018, 2019, 2020; Konopleva et al., 2017, 2019a, b; Pfeiffer et al., 2018, 2021; Jeratthitikul et al., 2019a, b; Muanta et al., 2019). However, the taxonomic revision of all extant taxa in the region is still far from being completed (Zieritz et al., 2018a). This is particularly true for taxa from the Chao Phraya and Mekong basins, the two main drainage systems in Indochina, where only a few studies are available (Pfeiffer et al., 2018, 2021; Jeratthitikul et al., 2019a, b; Muanta et al., 2019; Bolotov et al., 2020).

Hyriopsis Conrad, 1853 is one of the economically important freshwater bivalves in Southeast Asia, exhibiting high potential for pearl production and providing a protein source for animals and humans (Chatchavalvanich et al., 2010; Rath, 2011). These freshwater pearl mussels are characterised by their lanciform or elongate shells with a distinctive, large posterior dorsal wing and a smaller anterior dorsal wing. The hinge of both valves contains lamelliform lateral teeth, one in the right and two in the left. Pseudocardinal teeth are either incised or crenulate. The muscle scars are anteriorly deep but posteriorly shallow (Brandt, 1974).

Nine valid species of *Hyriopsis* are currently recognised, which are mainly distributed in the drainages of Indochina, with some populations and species being present in Sundaland (Pfeiffer et al., 2021). Two species are known from the Chao Phraya Basin in Thailand, namely *Hyriopsis desowitzi* Brandt, 1974, and *Hyriopsis myersiana* (Lea, 1856) (Brandt, 1974; Pfeiffer et al., 2021). *Hyriopsis bialata* Simpson, 1900 is recorded from Peninsular Malaysia, Singapore, Tonle Sap Lake in Cambodia, and the lower Mekong River Basin in Vietnam (Zieritz et al., 2016; Zieritz & Lopes-Lima, 2018; Ng et al., 2020; Pfeiffer et al., 2021). *Hyriopsis delaportei* (Crosse & Fischer, 1876) is abundant in the Tonle Sap Lake in Cambodia (Brandt, 1974; Ng et al., 2020) and the Mekong Delta in Vietnam (Bogan & Do, 2014; Do et al., 2018). Recently, Pfeiffer et al. (2021) revised the systematics of the genus *Hyriopsis* based on museum specimens, phylogenomic analysis, and COI sequence data, and described four additional new taxa from the Mekong River Basin, i.e., *Hyriopsis kratiensis* Pfeiffer, Graf, Cummings & Page, 2021, *Hyriopsis khoratensis* Pfeiffer, Graf, Cummings & Page, 2021, *Hyriopsis phuphaniensis* Pfeiffer, Graf, Cummings & Page, 2021, and *Hyriopsis sakhonensis* Pfeiffer, Graf, Cummings & Page, 2021. Finally, *Hyriopsis velthuizeni*

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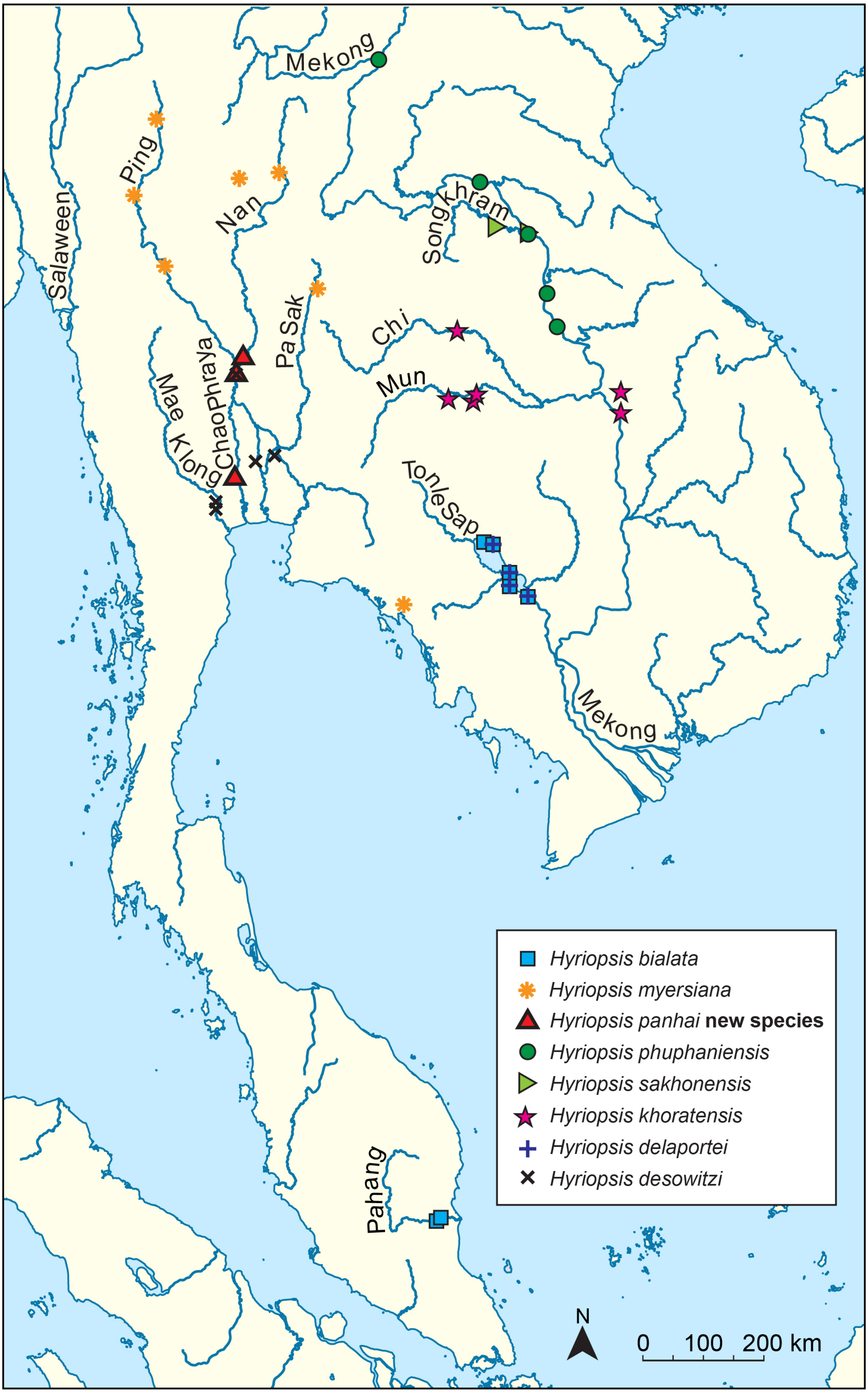

Fig. 1. Map of mainland Southeast Asia showing the main river systems and sampling sites.

(Schepman, 1896) from Western Borneo and Sumatra (Schepman, 1896; Zieritz et al., 2020) is known only from a few shell specimens, with its molecular entity having never been examined (Pfeiffer et al., 2021).

There is another *Hyriopsis* population from the Chao Phraya Basin which has been recognised as *H. bialata* (Brandt, 1974). However, it has been retrieved as a separated lineage in several molecular phylogenetic reconstructions (Zieritz et al., 2016, 2020; Bolotov et al., 2020; Pfeiffer et al., 2021), and should be recognised as a distinct species. In the present study, we used an integrative approach based on analysis of shell morphology and multi-locus molecular phylogeny to confirm the identity of this lineage and describe it as a new species.

MATERIAL AND METHODS

Specimens sampling. Specimens were obtained from natural habitat in mainland Southeast Asia (Fig. 1; Table 1). We successfully obtained fresh materials for all the valid species of *Hyriopsis,* except the rare *H. velthuizeni* from Sundaland and the recently described *H. kratiensis* from Sambour, Cambodia. All specimens were sampled by hand, cleaned with tap water, and subjected to euthanisation by a two-step method (AVMA, 2020). Specimens were placed in a container filled with fresh water and anaesthetised by the gradual addition of 95% (v/v) ethanol to the container, starting from approximately 5% (v/v) concentration, until the foot and adductor muscles fully relaxed. Specimens were then moved to 70% (v/v) ethanol for tissue fixation, and subsequently separated into soft bodies and shells. Foot and mantle tissues were cut and preserved in 95% (v/v) ethanol at –20°C until DNA extraction. Remaining soft parts were preserved in 70% (v/v) ethanol and kept together with their shells as vouchers. Voucher specimens, including type series, are deposited in Mahidol University Museum of Natural History, Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand (MUMNH) and Museum of Zoology, Universiti Malaya, Kuala Lumpur, Malaysia (MZUM). Animal use protocol was approved by the Faculty of Science, Mahidol University Animal Care and Use Committee, SCMU-ACUC (MUSC62-009-473).

DNA extraction, amplification, and sequencing. Total genomic DNA was extracted from a part of the foot or mantle using NucleoSpin® Tissue kit (MACHEREY-NAGEL, Germany). The quality and quantity of DNA was checked via agarose gel electrophoresis. Two mitochondrial genes, the protein-coding cytochrome c oxidase subunit I (COI) and the 16S large ribosomal subunit rRNA gene (16S), and the 28S large ribosomal subunit rDNA nuclear gene (28S) were used as molecular markers to reconstruct the phylogeny. Gene fragments were amplified using polymerase chain reaction (PCR). Each PCR was prepared for 30 µl as a final volume containing deionised water, 100 µM of forward and reverse primers, 15 µl of EmeraldAmp GT PCR Master Mix (Takara Bio Inc., Japan), and at least 10 ng of template DNA. Primers used for the COI gene were LCO1490 and HCO2198 (Folmer et al., 1994); 16S gene were 16sar-L-myt and 16Sbr-H-myt (Lydeard et al., 1996); and 28S gene were C1 & D2 (Jovelin & Justine, 2001). The PCR reaction was accomplished at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 42–52°C (depending on samples and gene) for 60 s, extension at 72°C for 90 s, then followed by a final 72°C for 5 min. The PCR products were electrophoresed in 1.0% agarose gel containing the nucleic acid staining RedSafe™ (iNtRon Biotechnology) in 0.5× Tris-borate-EDTA (TBE) buffer, and were subsequently purified by the PEG precipitation method. The purified products were sent for sequencing with the same primers used for amplification on the ABI 3730XL DNA Analyzer (BIONEER, Republic of Korea). Nucleotide sequences obtained in this study were deposited in the GenBank database, under GenBank Acession numbers as shown in Table 1.

Phylogenetic analyses. Sequences were edited and aligned using the ClustalW option in the program MEGA v7.0.26 (Kumar et al., 2016). The final concatenated alignment used in the phylogenetic tree construction contained 1,967 bp: 660 bp of COI (199 informative sites), 501 bp of 16S (123 informative sites), and 806 bp of 28S (41 informative sites). These sequences came from 43 *Hyriopsis* specimens, along with specimens from other genera of the tribe Rectidentini (*Rectidens* Simpson, 1900 and *Ensidens* Frierson, 1911). Members of the tribe Contradentini were used as outgroup. Details of taxon sampling used in phylogenetic analysis are shown in Table 1. The best-fit model of nucleotide substitution and the best partitioning scheme were identified by the program PartitionFinder2 v.2.3.4 (Lanfear et al., 2016), by using a heuristic search algorithm under the corrected Akaike Information Criterion (AICc).

The partitioned concatenated dataset was then used to infer the phylogenetic relationship by using the Bayesian inference (BI) and maximum likelihood (ML) methods via the on-line CIPRES Science Gateway (Miller et al., 2010). The ML analysis was performed using RAxML v.8.2.10 (Stamatakis, 2014). The GTR+G was set as the model for all gene partitions. One thousand ML bootstrap replicates were performed to assess topology support. The BI trees were estimated by running a 10 million generation Metropolis-coupled Markov chain of Monte Carlo (MC-MCMC) as implemented in MrBayes 3.2.6 (Ronquist et al., 2012). Each MCMC consisted of two runs with four chains, three heated chains and one cold chain. A data partition was applied that allowed parameters to be estimated separately for each partition. Sampling rate of the trees was every 1,000 generations. Stationarity was considered to have been reached when the average standard deviation of split frequencies shown in MrBayes was less than 0.01 and the log likelihood of sampled trees reached a stationary distribution. The first 25% of the obtained trees were discarded as burn-in. The remaining trees were used to estimate the consensus tree topology, posterior probability (pp), and branch lengths. Posterior probabilities of BI ≥ 0.95 were considered as statistically significant and ML bootstrap support values ≥ 70 were considered as highly supported (Huelsenbeck & Hillis, 1993; Larget & Simon, 1999); support values below

Table 1. Locality information, including geographic coordinates, Voucher ID, and GenBank accession numbers for specimens used in phylogenetic analysis.

Taxa	Voucher ID	Localities	Geographic coordinates	GenBank acession		
				COI	16S	28S
UNIONIDAE Rafinesque, 1820						
GONIDEINAE Ortmann, 1916						
RECTIDENTINI Modell, 1942						
Ingroups						
Hyriopsis bialata Simpson, 1900	MZUM(BIV)0024 (=BIV1774)	Pahang River, Kampung Pelak, Pahang, Malaysia	3°29′09.6″N 103°05′16.8″E	KX051274	MT993644	MT993697
Hyriopsis bialata Simpson, 1900	NCSM_113709 (=BIV1775)	Pahang River, Kampung Pelak, Pahang, Malaysia	3°29′09.6″N 103°04′26.4″E	KX051273	MT993645	MN902295
Hyriopsis bialata Simpson, 1900	MUMNH:UNI2622	Kampong Chhnok Tru, Chhnok Tru, Kampong Chhnang, Cambodia	12°30′36.5″N 104°27′18.2″E	MT993500	MT993646	MT993698
Hyriopsis bialata Simpson, 1900	MUMNH:UNI2677	Tonle Sap Lake Kampong Thom, Cambodia	12°42′42.5″N 104°11′58.2″E	MT993501	MT993647	MT993699
Hyriopsis bialata Simpson, 1900	MUMNH:UNI2680	Tonle Sap Lake in Krakor, Pursat, Cambodia	12°38′20.2″N 104°12′12.1″E	MT993502	MT993648	MT993700
Hyriopsis bialata Simpson, 1900	MUMNH:UNI2650	Tonle Sap Lake in Chong Khneas, Siem Reap, Cambodia	13°14′03.1″N 103°49′45.3″E	MT993503	MT993649	MT993701
Hyriopsis bialata Simpson, 1900	MUMNH:UNI2696	Tonle Sap Lake in Kampong Phluk, Prasat Bakong, Siem Reap, Cambodia	13°11′16.2″N 103°57′25.4″E	MT993504	MT993650	MT993702
Hyriopsis panhai, **new species**	MUMNH:UNI0201	Bang Ta Khian, Song Phi Nong, Suphan Buri, Thailand	14°10′49.5″N 100°06′07.2″E	MT993505	MT993651	MT993703
Hyriopsis panhai, **new species**	MUMNH:UNI1210	Bueng Boraphet, Mueang, Nakhon Sawan, Thailand	15°42′17.8″N 100°09′50.6″E	MT993506	MT993652	MT993704
Hyriopsis panhai, **new species**	MUMNH:UNI2494	Huai San River in Bang Khian, Chum Saeng, Nakhon Sawan, Thailand	15°53′47.3″N 100°13′09.2″E	MT993507	MT993653	MT993705
Hyriopsis panhai, **new species**	MUMNH:UNI2495	Huai San Stream in Bang Khian, Chum Saeng, Nakhon Sawan, Thailand	15°53′47.3″N 100°13′09.2″E	MT993508	MT993654	MT993706
Hyriopsis phuphaniensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI0906	Songkharm River at Chai Buri, Tha Uthen, Nakhon Phanom, Thailand	17°39′05.5″N 104°27′45.1″E	MT993509	MT993655	MT993707
Hyriopsis phuphaniensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1698	Bang I River at confluence with Mekong River, Tan, Mukdahan, Thailand	16°20′44.5″N 104°53′25.1″E	MT993510	MT993656	MT993708

Taxa	Voucher ID	Localities	Geographic coordinates	GenBank acession		
				COI	16S	28S
Hyriopsis phuphaniensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1818	Mekong River at Nam Kam, That Phanom, Nakhon Phanom, Thailand	16°48′41.5″N 104°44′07.3″E	MT993511	MT993657	MT993709
Hyriopsis phuphaniensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI2082	Nam Ou River at Hatgna, Luang Prabang, Laos	20°05′11.3″N 102°15′51.0″E	MT993512	MT993658	MT993710
Hyriopsis phuphaniensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI2155	Mekong River at Wat Tha Pho, Mueang, Bueng Kan, Thailand	18°20′19.8″N 103°43′23.7″E	MT993513	MT993659	MT993711
Hyriopsis sakhonensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI0903	Songkharm River at Chai Buri, Tha Uthen, Nakhon Phanom, Thailand	17°39′05.5″N 104°27′45.1″E	MT993514	MT993660	MT993712
Hyriopsis sakhonensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI0908	Songkharm River at Chai Buri, Tha Uthen, Nakhon Phanom, Thailand	17°39′05.5″N 104°27′45.1″E	MT993515	MT993661	MT993713
Hyriopsis sakhonensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI2204	Songkharm River at Tha Kon, Akat Amnuai, Sakon Nakhon, Thailand	17°46′43.2″N 103°57′10.2″E	MT993516	MT993662	MT993714
Hyriopsis khoratensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI0646	Chi River at Lat Phatthana, Mueang, Maha Sarakham, Thailand	16°13′58.8″N 103°25′50.2″E	MT993517	MT993663	MT993715
Hyriopsis khoratensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1180	Mun River at Krabueang, Chumphon Buri, Surin, Thailand	15°18′55.1″N 103°17′12.2″E	MT993518	MT993664	MT993716
Hyriopsis khoratensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1188	Mun River at Ban Krasang, Tha Tum, Surin, Thailand	15°18′51.9″N 103°37′52.0″E	MT993519	MT993665	MT993717
Hyriopsis khoratensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1192	Mun River at Ban Tha Tum, Tha Tum, Surin, Thailand	15°19′28.7″N 103°40′28.2″E	MT993520	MT993666	MT993718
Hyriopsis khoratensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1213	Xe Don River at Ban Photok, Pakse, Laos	15°07′54.0″N 105°48′37.2″E	MT993521	MT993667	MT993719
Hyriopsis khoratensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1294	Mun River at Ban Tha Tum, Tha Tum, Surin, Thailand	15°19′28.7″N 103°40′28.2″E	MT993522	MT993668	MT993720
Hyriopsis khoratensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI1882	Xe Don River at Ban Nong Du, Champasak, Laos	15°21′37.7″N 105°49′52.9″E	MT993523	MT993669	MT993721
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI0049	Yom River at Huai Mai, Song, Pharae, Thailand	18°25′39.0″N 100°10′20.4″E	MT993524	MT993670	MT993722
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI0122	Nan River at Ban San, Wiang Sa, Nan, Thailand	18°31′54.6″N 100°46′03.6″E	MT993525	MT993671	MT993723
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI0165	Pa Sak River at Tha I-bun, Lom Sak, Phetchabun, Thailand	16°52′41.5″N 101°19′45.5″E	MT993526	MT993672	MT993724

Taxa	Voucher ID	Localities	Geographic coordinates	GenBank acession		
				COI	16S	28S
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI0307	Ping River at Ban Tan, Hot, Chiang Mai, Thailand	18°10′41.5″N 98°37′50.8″E	MT993527	MT993673	MT993725
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI0309	Wang River at Wang Man, Sam Ngao, Tak, Thailand	17°12′15.4″N 99°05′54.0″E	MT993528	MT993674	MT993726
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI0336	Ping River, Inthakhin, Mae Taeng, Chiang Mai, Thailand	19°15′37.7″N 98°58′17.1″E	MT993529	MT993675	MT993727
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI0815	Huai Raeng River at Huai Raeng, Mueang, Trat, Thailand	12°22′34.3″N 102°34′55.2″E	MT993530	MT993676	MT993728
Hyriopsis myersiana (Lea, 1856)	MUMNH:UNI2029	Ping River at Kosamphi, Kosamphi Nakhon, Kamphaeng Phet, Thailand	16°41′02.8″N 99°16′28.7″E	MT993531	MT993677	MT993729
Hyriopsis desowitzi (Brandt, 1974)	MUMNH:UNI0200	Chao Phra Ya River at Phongpheng, Pa Mok, Ang Thong, Thailand	14°26′38.0″N 100°27′29.7″E	MT993532	MT993678	MT993730
Hyriopsis desowitzi (Brandt, 1974)	MUMNH:UNI0227	Man-made cannel at Cham Pa, Tha Ruea, Ayutthaya, Thailand	14°31′48.8″N 100°44′17.1″E	MT993533	MT993679	MT993731
Hyriopsis desowitzi (Brandt, 1974)	MUMNH:UNI0295	Mae Klong River at Boek Phrai, Ban Pong, Ratchaburi, Thailand	13°50′34.8″N 99°51′40.7″E	MT993534	MT993680	MT993732
Hyriopsis desowitzi (Brandt, 1974)	MUMNH:UNI0304	Mae Klong River at Khlong Takhot, Photharam, Ratchaburi, Thailand	13°43′14.9″N 99°50′50.4″E	MT993535	MT993681	MT993373
Hyriopsis desowitzi (Brandt, 1974)	MUMNH:UNI1209	Bueng Boraphet, Mueang, Nakhon Sawan, Thailand	15°42′17.8″N 100°09′50.6″E	MT993536	MT993682	MT993734
Hyriopsis delaportei (Crosse & Fischer, 1876)	MUMNH:UNI2682	Kampong Chhnok Tru, Chhnok Tru, Kampong Chhnang, Cambodia	12°30′36.5″N 104°27′18.2″E	MT993537	MT993683	MT993735
Hyriopsis delaportei (Crosse & Fischer, 1876)	MUMNH:UNI2628	Tonle Sap Lake Kampong Thom, Cambodia	12°42′42.5″N 104°11′58.2″E	MT993538	MT993684	MT993736
Hyriopsis delaportei (Crosse & Fischer, 1876)	MUMNH:UNI2631	Tonle Sap Lake in Krakor, Pursat, Cambodia	12°38′20.2″N 104°12′12.1″E	MT993539	MT993685	MT993737
Hyriopsis delaportei (Crosse & Fischer, 1876)	MUMNH:UNI2653	Tonle Sap Lake in Kampong Phluk, Prasat Bakong, Siem Reap, Cambodia	13°11′16.2″N 103°57′25.4″E	MT993540	MT993686	MT993738
Outgroups						
Rectidens sumatrensis (Dunker, 1852)	n/a	Perak river, Perak, Malaysia	n/a	KX822664	n/a	KX822620
Rectidens sumatrensis (Dunker, 1852)	RMBH biv0211_1	Perak river, Perak, Malaysia	n/a	MF352288	n/a	MF352405
Ensidens ingallsianus (Lea, 1852)	UMMZ 304651	Mekong River basin, Cambodia		KP795033	KP795053	KP795015

Taxa	Voucher ID	Localities	Geographic coordinates	GenBank acession		
				COI	16S	28S
Ensidens ingallsianus (Lea, 1852)	MUMNH:UNI0074	Song River, Ban Klang, Song, Phrae, Thailand	18°27′47.2″N 100°11′01.8″E	MT993541	MT993687	MT993739
Ensidens ingallsianus (Lea, 1852)	NCSM 84889	Mekong River basin, Laos		KX822641	n/a	KX822598
Ensidens jaculus (Rochebrune, 1882)	MUMNH:UNI0259	Kam River at Na Khu, Na Kae, Nakhon Phanom, Thailand	16°57′29.2″N 104°30′16.3″E	MT993542	MT993688	MT993740
CONTRADENTINI Modell, 1942						
Physunio superbus (Lea, 1843)	MUMNH:UNI0199	Chao Phraya River at Krok Phra, Nakhon Sawan, Thailand	15°33′04.5″N 100°05′52.9″E	MG582020	MT993689	MT993741
Physunio modelli Brandt, 1974	MUMNH:UNI0557	Songkhram River at Ban Ya, Nong Han, Udon Thani, Thailand	17°23′44.5″N 103°18′01.7″E	MT993543	MT993690	MT993742
Physunio pellucidus Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI0258	Kam River at Na Khu, Na Kae, Nakhon Phanom, Thailand	16°57′29.2″N 104°30′16.3″E	MT993544	MT993691	MT993743
Trapezoideus foliaceus (Gould, 1843)	MUMNH:UNI0057	Yom River at Huai Mai, Song, Pharae, Thailand	18°25′39.0″N 100°10′20.4″E	MT993545	MT993692	MT993744
Trapezoideus foliaceus (Gould, 1843)	UF507879 (=ICH-02104)	Mae Klong River basin, Pachee River, Thailand	13°55′05.3″N 99°22′56.2″E	MH345984	MH346024	MH346004
Yaukthwa nesemanni (Konopleva et al., 2017)	RMBH biv0144_14	Sittaung River basin, Thauk Ye Kupt River, Myanmar	n/a	KX865906	KX865663	KX865777
Yaukthwa panhai Konopleva et al., 2017	RMBH biv0138_4	Sittaung River basin, Kyan Hone River, Myanmar	n/a	KX865909	KX865666	KX865780
Lens contradens (Lea, 1838)	MUMNH:UNI0197	Tak Daet River at Sakae Krang, Mueang, Uthai Thani, Thailand	15°25′43.6″N 100°03′10.9″E	MG581991	MT993693	MT993745
Lens maenamensis Pfeiffer, Graf, Cummings & Page, 2020	MUMNH:UNI0253	Songkhram River at Thung Fon, Udon Thani, Thailand	17°27′06.5″N 103°16′50.4″E	MG582007	MT993694	MT993746
Lens rolfbrandti (Jeratthitikul & Panha, 2019)	MUMNH:UNI0213	Huai Luang River at Don Kloi, Phibun Rak, Udon Thani, Thailand	17°28′34.9″N 102°58′31.5″E	MG582016	MT993695	MT993747
Lens novoselovi (Konopleva et al., 2019)	RMBH biv0203_4	A tributary of Nam Fa River near Vieng Phou Kha, Laos	20°40′55.2″N 101°04′45.8″E	KY561632	KY561647	KY561664

MZUM: Museum of Zoology, Universiti Malaya, MUMNH: Mahidol University Museum of Natural History, NCSM: North Carolina Museum of Natural Sciences, RMBH: Russian Museum of Biodiversity Hotspots, UF: Florida Museum of Natural History, UMMZ: University of Michigan Museum of Zoology.

this significance level were not considered significant. In addition, uncorrected pairwise genetic distances were also calculated in MEGA v7.0.26 (Kumar et al., 2016) to unveil the genetic distance among taxa and clades.

TAXONOMY AND SYSTEMATICS

Family Unionidae Rafinesque, 1820

Subfamily Gonideinae Ortmann, 1916

Tribe Rectidentini Modell, 1942

Hyriopsis Conrad, 1853

Hyriopsis panhai, new species

(Figs. 1, 2C, D, 3C, D, 4)

Hyriopsis (*Hyriopsis*) *bialatus* – Brandt, 1974: 272, 273, pl. 21, fig. 36. (in part).

Hyriopsis undescribed Pfeiffer et. al., 2021: 450, 457, 458, fig. 35.

Type material. Holotype (length 104.83 mm, height without wing 38.58 mm, width 20.73 mm) (MUMNH:UNI0201) Bang Ta Khian, Song Phi Nong, Suphan Buri, Thailand, 14°10′49.5″N 100°06′07.2″E; Paratypes 3 shells (length 85.63–121.99 mm, height without wing 33.18–46.37 mm, width 17.09–26.30 mm): 1 shell (MUMNH:UNI1210) Bueng Boraphet, Mueang, Nakhon Sawan, Thailand, 15°42′17.8″N 100°09′50.6″E. 2 shells (MUMNH:UNI2494, 2495) Huai San River in Bang Khian, Chum Saeng, Nakhon Sawan, Thailand (15°53′47.3″N 100°13′09.2″E).

Diagnosis. Shell winged, narrow, elongate and tapering towards the posterior end. Posterior ridges high and obtuse. Ventral margin almost straight. This new species differs from *H. bialata* in having a more elongated and rounded posterior margin (vs. obtused and somewhat biangulated posterior end), and a distinct separation of anterior adductor muscle scar from pedal retractor muscle scar (vs. a connection between anterior adductor muscle and pedal retractor muscle scars).

Description. Shell moderately thick, winged, compressed and elongate lanciform. Posterior wing prominent and triangular; anterior wing small and pointed. Dorsal margin somewhat curved; umbonal area low. Posterior ridges high and obtuse, running from umbo to posterior end, and with two secondary faint ridges dorsally. Anterior margin round and curved towards anterior wing; posterior margin elongated and rounded. Ventral margin almost straight and slightly curved upward posteriorly. Periostracum greenish to brownish or blackish. Shell surface with fine growth lines. Ligament strong and covered by wing. Pseudocardinal teeth crenulated or vertically striated; one in left valve, two in right; outer tooth in right valve subobsolete. Lateral teeth long and straight or slightly curved; two in left valve, one in right. Anterior adductor muscle scar round, deep and distinctly separated from pedal retractor muscle scar. Posterior adductor muscle scars very shallow. Excurrent aperture smooth, shorter than incurrent. Incurrent with 1–2 rows of short, conically shaped papillae. Small epithelial fold divides excurrent and incurrent aperture. Gills elongated and slightly ribbed. Anterior margin of inner gills slightly longer and wider than that of inner gills. Ectobranchous brooding. Glochidia unknown.

Distribution. This new species is restricted to the Chao Phraya Basin in central Thailand. Its distribution probably extends to the northern part of the basin in Pitsanulok Province based on the record by Brandt (1974).

Habitat. Specimens were collected from muddy and/or sandy substrates. They were found in sympatry with *H. desowitzi*, *Ensidens ingallsianus* (Lea, 1852), *Pilsbryoconcha exilis* (Lea, 1838), *Lens contradens* (Lea, 1838), and *Corbicula* spp.

Etymology. This species is dedicated to Prof. Dr. Somsak Panha, a famous Thai zoologist, who has made great contributions to the systematics of invertebrate taxa in Thailand.

Remarks. *Hyriopsis bialata* recorded by Brandt (1974) as collected from central Thailand and the figured specimen (pl. 21, fig. 36) should be recognised as this new species.

Molecular phylogenies and genetic divergence. The programme PartitionFinder2 suggested dividing the dataset into five partitions, consisting of partitions for 16S and 28S genes, and for each of the three codon positions of the COI gene. The best-fit models for each partition were GTR+I+G for the first codon position of COI, 16S, and 28S; GTR+I for the second codon position of COI; and GTR+G for the third codon position of COI. Multi-locus phylogenies as constructed by Bayesian inference (BI) and maximum likelihood (ML) gave similar topology and congruently recovered *Hyriopsis* as paraphyletic, consisting of two well-supported clades (77% and 100% bootstrap support for ML, and 0.98 and 1 pp for BI; Fig. 4). The first clade contained the majority of *Hyriopsis* members, consisting of *H. bialata* (the type species of the genus), *H. myersiana*, *H. phuphaniensis*, *H. sakhonensis*, *H. khoratensis*, and the new species *H. panhai*. All of these taxa were each recovered as highly supported monophyletic clades (99–100% bootstrap support for ML and 1 pp for BI) and separated from each other by high mean uncorrected COI p-distances (6.15–9.92%; Table 2). However, the phylogenetic relationships among them were unclear. Only a sister relationship between *H. bialata* and *H. panhai* was highly supported (81% bootstrap support for ML and 0.95 pp for BI), while three taxa from Mekong River Basin, *H. phuphaniensis*, *H. sakhonensis*, and *H. khoratensis* (Fig. 2), were nested together with non-significant support (50% bootstrap support for ML and 0.91 pp for BI). The genetic divergence between *H. bialata* and *H. panhai* was 8.30% uncorrected COI p-distance. Another clade of *Hyriopsis* consisted of two species, *H. desowitzi* and *H. delaportei.* These two species are genetically different by 3.21% uncorrected COI p-distance. The paraphyly of *Hyriopsis* is separated by the Sundaland genus *Rectidens,* but was only moderately supported by ML analysis (71–77% bootstrap support for ML).

Table 2. Average interspecific genetic divergence (uncorrected p-distance: % ± S.E.) matrix for the 660 bp COI gene fragment sequences between species in the tribe Rectidentini (below diagonal). Average intraspecific distances within each taxon are shown in bold.

Taxa	1	2	3	4	5	6	7	8	9	10	11
1. *Hyriopsis bialata*	**0.57 ± 0.21**										
2. *Hyriopsis panhai*, **new species**	8.30 ± 1.10	**0.08 ± 0.08**									
3. *Hyriopsis myersiana*	8.39 ± 1.05	8.54 ± 1.07	**1.46 ± 0.30**								
4. *Hyriopsis khoratensis*	7.43 ± 1.00	7.24 ± 1.01	8.84 ± 1.07	**0.65 ± 0.19**							
5. *Hyriopsis phuphaniensis*	6.93 ± 0.96	8.31 ± 1.08	8.26 ± 1.08	6.15 ± 0.87	**0.54 ± 0.19**						
6. *Hyriopsis sakhonensis*	8.12 ± 1.05	9.52 ± 1.19	9.92 ± 1.14	7.85 ± 1.02	8.09 ± 1.06	**0.34 ± 0.19**					
7. *Rectidens sumatrensis*	12.87 ± 1.32	11.43 ± 1.33	13.09 ± 1.32	11.19 ± 1.26	11.78 ± 1.28	12.87 ± 1.31	**0.00 ± 0.00**				
8. *Hyriopsis desowitzi*	10.32 ± 1.20	9.69 ± 1.23	10.59 ± 1.25	9.55 ± 1.17	9.76 ± 1.16	11.01 ± 1.22	11.12 ± 1.29	**0.25 ± 0.14**			
9. *Hyriopsis delaportei*	11.05 ± 1.26	9.63 ± 1.22	11.35 ± 1.30	10.50 ± 1.22	10.50 ± 1.27	11.34 ± 1.26	11.93 ± 1.32	3.21 ± 0.66	**0.10 ± 0.10**		
10. *Ensidens ingallsianus*	10.66 ± 1.13	10.159 ± 1.14	11.66 ± 1.19	10.145 ± 1.14	9.94 ± 1.09	11.71 ± 1.22	12.90 ± 1.32	10.886 ± 1.13	11.715 ± 1.23	**4.69 ± 0.60**	
11. *Ensidens jaculus*	11.53 ± 1.21	10.93 ± 1.22	12.37 ± 1.23	11.99 ± 1.23	12.28 ± 1.30	13.11 ± 1.39	12.73 ± 1.33	12.83 ± 1.38	13.36 ± 1.39	9.21 ± 0.10	**N/A**

DISCUSSION

The present new species, *H. panhai*, is morphologically similar to its sister *H. bialata* (Fig. 2), but can be distinguished by being more laterally compressed in the anterior portion, and a more elongated and rounded posterior margin (vs. obtuse and somewhat biangulated posterior end in *H. bialata*). The new species also possesses deep anterior adductor muscle scars that are distinctly separated from pedal retractor muscle scars, which is in contrast to *H. bialata*, where anterior adductor muscle and pedal retractor muscle scars are fused (Fig. 3).

Hyriopsis desowitzi can be found in the same habitat as *H. panhai*. However, it can be distinguished from the new species by having a much higher, somewhat triangular, and more inflated shell (Fig. 4). Another congener that occurs in the same river basin as *H. panhai* is *H. myersiana*, which can be distinguished by its larger, laterally inflated shell, elliptical shape with rounder ventral margin and broader posterior ridges (Fig. 4). Moreover, distribution of *H. myersiana* seems to be limited to the upper reaches of the Chao Phraya, while *H. panhai* is restricted to the most downstream reaches of this river basin in central Thailand (Fig. 1).

In this study, *Hyriopsis* was not recovered as monophyletic, but grouped into two clades separated by the genus *Rectidens* (Fig. 4). These two clades are conchologically distinguishable. The clade of *H. desowitzi* and *H. delaportei* share the synapomorphic characteristic of having much higher, somewhat triangular and inflated shells, and curved lateral teeth. The other clade has elongated lanciform and compressed shells, and somewhat straight lateral teeth. Our tree topology is in accordance with previous studies (Bolotov et al., 2020; Zieritz et al., 2020). However, a recent phylogenomic analysis revealed a monophyletic *Hyriopsis* and placed *H. delaportei* at the basal position in relation to other *Hyriopsis* species (Pfeiffer et al., 2021).

Interestingly, *Hyriopsis* lineages from the Tonle Sap Lake in the Lower Mekong River Basin are genetically more similar to those of the Chao Phraya Basin than those of the Middle Mekong, i.e., *H. bialata* vs. *H. panhai*, and *H. desowitzi* vs. *H. delaportei*. Faunal similarities between these two basins are documented in other unionid genera, for example, *Ensidens* and *Lens* Simpson, 1900 (Muanta et al., 2019; Pfeiffer et al., 2021), as well as other freshwater taxa (de Bruyn & Mather, 2007; de Bruyn et al., 2013). This pattern may have been caused by extensive lowland connections between the Chao Phraya and Lower Mekong basins through the Paleo-Siam River system when sea levels were low (Attwood & Johnston, 2001; Adamson et al., 2012), and subsequent isolation when sea level was high. This sea-level fluctuation in Indochina has been occuring since the Early Miocene (Hall, 2013).

Unionid mussels are among the most threatened freshwater molluscs, exhibiting a global trend of steep decline in both diversity and abundance (Bogan, 2008, 2015; Lopes-Lima et al., 2018). Comparing recent (last five years) field survey

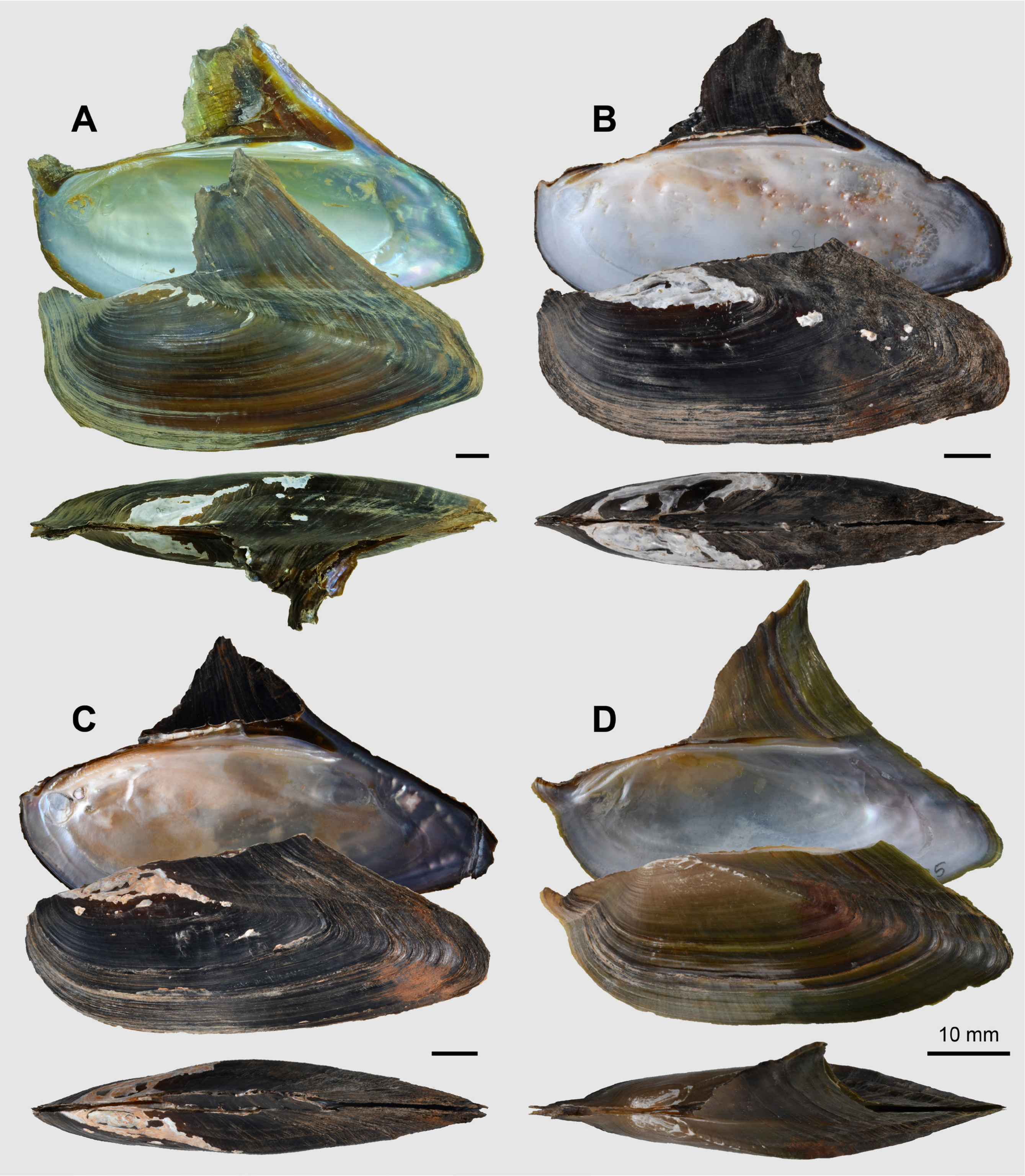

Fig. 2. Shells of *Hyriopsis* species, showing internal view of the right valve, external view of the left valve and dorsal view of both valves. **A**, *H. bialata*, specimen MZUM(BIV)T0001-x230 from Pahang, Malaysia; **B**, *H. bialata*, specimen MUMNH-UNI2622 from Tonle Sap, Cambodia; **C**, *H. panhai*, new species, holotype, MUMNH-UNI0201 from Suphan Buri, Thailand; **D**, *H. panhai*, new species, paratype, MUMNH-UNI2495 from Nakhon Sawan, Thailand. Scale bar = 10 mm.

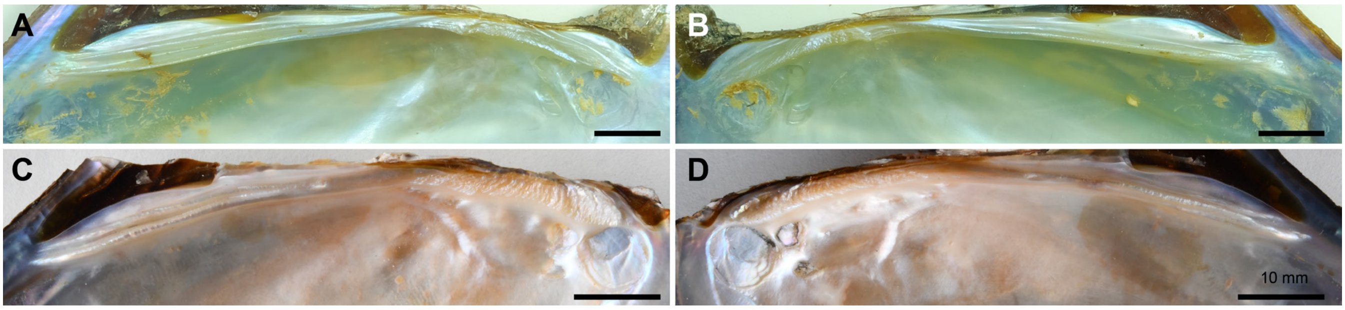

Fig. 3. Morphology of hinge teeth and muscle scars on the left (**A** and **C**) and right (**B** and **D**) valves of *Hyriopsis* species. **A**, **B**, *H. bialata*, specimen MZUM(BIV)T0001-x230 from Pahang, Malaysia. **C**, **D**, *H. panhai*, new species, holotype, MUMNH-UNI0201 from Suphan Buri, Thailand. Scale bar = 10 mm.

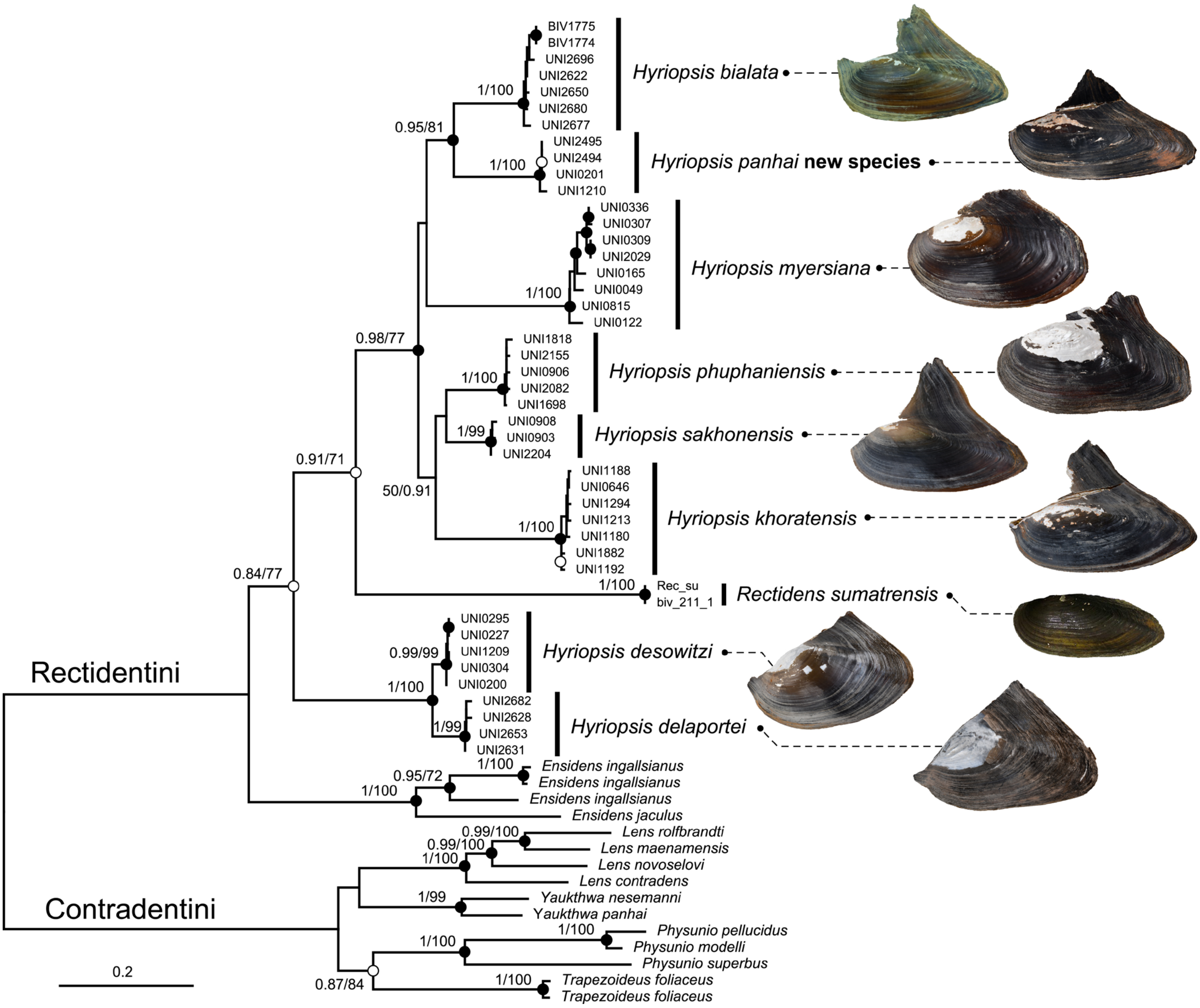

Fig. 4. Bayesian inference tree based on 1,967 bp concatenated alignment dataset of COI + 16S + 28S genes. Numbers on nodes indicate bootstrap values from maximum likelihood (ML) and bipartition posterior probabilities from Bayesian inference analysis (BI), and are shown as BI/ML. Black circles on nodes indicate high support by BI (≥ 0.95) and ML (≥ 70); white circles indicate high support by BI. Shells are not to scale.

records by our team to historical data (Brandt, 1974; Pfeiffer et al., 2021) indicates that the distribution range of *H. panhai*, new species, has decreased and is now restricted to a few locations in the lower Chao Phraya Basin (Fig. 1). Sadly, these remaining habitats of *H. panhai* are currently suffering from anthropogenic activities (i.e., water pollution, habitat destruction and modifications; Singkran et al., 2018) that have caused severe reductions in freshwater bivalve populations worldwide (Haag & Williams, 2014; Bogan, 2015; Lopes-Lima et al., 2018). Moreover, due to its highly localised distribution, *H. panhai* will be particularly susceptible to further environmental changes (Bogan, 2015; Zieritz et al., 2016, 2018b). Therefore, this newly discovered species may be under severe threat, and comprehensive field surveys, including a long-term population monitoring program, will be needed to evaluate and monitor its distribution and conservation status.

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