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Multi-locus Phylogeny Reveals a New Freshwater Mussel in the Genus *Bineurus* Simpson, 1900 (Unionidae: Pseudodontini) from Thailand

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ABSTRACT.– A new freshwater mussel species is described here as *Bineurus panhai* **sp. nov.** Morphological investigation and multi-locus phylogeny based on concatenated data of COI, 16S, and 28S gene sequences reveal that the new species is similar to the *Bineurus exilis* (Morelet, 1866), but it can be distinguished from the latter taxon by having a curved dorsal margin and a lower posterior end (vs. straight dorsal margin and higher posterior end). They also differ from each other by 2.32% of uncorrected COI p-distance. *Bineurus panhai* **sp. nov.** is distributed in the Bang Pakong Basin of eastern Thailand and in headwater tributaries of the Tonle Sap Basin in Thailand and Cambodia. Because of its restricted range, this new species may represent a rare endemic lineage and, hence, is a valuable target for conservation.

KEYWORDS: freshwater mussels, Indochina, multi-locus phylogeny, new species, Unionida

INTRODUCTION

In recent years, freshwater mussels (Bivalvia: Unionida) have been the subject of taxonomic investigation and reclassification. Traditional classification based solely on shell morphology has been challenged by modern systematic approaches, and as a result, nearly one thousand nominal species are now recognized worldwide (Graf and Cummings, 2021). Mainland Southeast Asia, also known as Indochina, is one of the most important biodiversity hotspots for freshwater mussels, both in terms of its high species richness and endemism (Graf and Cummings, 2021), and has been described as one of the world's most exceptional locations for the origin of freshwater mussels (Bolotov et al., 2017a). The diversity of freshwater mussels in Indochinese river systems currently comprises 131 species from 38 genera that are recorded in the global database (i.e., MUSSELp; Graf and Cummings, 2022). These include several new taxa that have been discovered recently (Kongim et al., 2015; Bolotov et al., 2017b, 2018, 2019, 2020, 2022; Jeratthitikul et al., 2019a, 2021a, b, 2022; Konopleva et al., 2019a, b, 2020, 2021, 2023; Pfeiffer et al., 2021). Furthermore, 80% of the recognized species are considered as endemic to the region (Graf and Cummings, 2022).

Pseudodontini is the largest tribe in terms of species diversity among the Indochinese freshwater mussels, consisting of 36 extant species (Graf and Cummings, 2022). The genus *Bineurus* Simpson, 1900 is one of nine pseudodontinid genera which were recently resurrected (Bolotov et al., 2017b). *Bineurus* can be distinguished from other genera by having elongated rhomboid or kidney-shaped, inequilateral and compressed shell, and a small tubercle-like pseudocardinal tooth (Konopleva et al., 2021). Historically, *Bineurus* was first described as a section (= subgenus) of the *Pseudodon* Gould, 1844, with *Monocondyloea mouhotii* Lea, 1863 as the type species, along with three other species (Simpson, 1900, 1914). Later, Haas (1920) revised the subgenus and recognized seven nominal species within it. Subsequently, *Bineurus* was subjected to taxonomic revision again by Haas (1969), who synonymized several nominal species and recognized only *P. mouhotii* and *P. hageni* (Strubell, 1897) as valid. However, during study of *Pseudodon* in the Mekong River drainage, Brandt (1974) did not recognize any previous subordinate classification, and considered only *P. mouhotii* as a valid species among the members previously recognized by Haas (1920, 1969). This classification by Brandt (1974) had been accepted for a half century, until Bolotov et al. (2017b) resurrected *Bineurus* as a valid genus on the basis of their integrative taxonomic approach combining morphology and molecular phylogenetic analyses. At present, *Bineurus* comprises four valid species (Bolotov et al., 2017b; Konopleva et al., 2021), and their distribution range is limited to tributaries in the Middle Mekong Basin in Laos and Thailand, and the Lower Mekong Basin in Cambodia and southern Vietnam (Konopleva et al., 2021).

In this paper, we provide description of another new species of *Bineurus* from Indochina. The new species is supported by its conchological distinction and molecular phylogenetic analysis. This finding expands the known range of the genus beyond the Mekong

TABLE 1. Locality and GenBank accession numbers for specimens used in phylogenetic analysis. Sequences marked with '*' were newly obtained in this study.

Taxa / Voucher ID	Localities	Genbank accession			References
		COI	16S	28S	
Bineurus panhai sp. nov.					
MUMNH-UNI2840	Thailand: Bang Pakong Basin, Phra Sathueng Stream, Wang Thong, Wang Sombun, Sa Kaeo	OQ108585*	OQ110575*	OQ110565*	This study
MUMNH-UNI2841		OQ108586*	OQ110576*	OQ110566*	This study
MUMNH-UNI2096		OQ108587*	OQ110577*	OQ110567*	This study
MUMNH-UNI2097		OQ108588*	OQ110578*	OQ110568*	This study
MUMNH-UNI2660	Cambodia: Tole Sap Basin, Sangker River, Traeng, Rotanak Mondol, Battambang	MZ822405	MZ822905	MZ822927	Jeratthitikul et al., 2021b
MUMNH-UNI2728		MZ822406	MZ822906	MZ822928	Jeratthitikul et al., 2021b
MUMNH-UNI2730		MZ822407	MZ822907	MZ822929	Jeratthitikul et al., 2021b
Bineurus mouhotii (Lea, 1863)					
biv182_2	Laos: Mekong Basin, Nam Long River	KX865876	KX865647	KX865747	Bolotov et al., 2017a
biv182_19		KX865878	KX865649	KX865749	Bolotov et al., 2017a
biv183_4	Laos: Mekong Basin, Nam Pe River	KX865877	KX865648	KX865748	Bolotov et al., 2017a
biv201_2	Laos: Mekong Basin, a tributary of Nam Fa River near Vieng Phou Kha	KY561623	KY561641	KY561655	Bolotov et al., 2017a
biv201_3		KY561624	KY561642	KY561656	Bolotov et al., 2017a
Bineurus exilis (Morelet, 1866)					
UF 507440 (ICH-00609)	Cambodia: Mekong Basin, Tributary of Tonle Sap River	MW603663	n/a	n/a	Konopleva et al., 2021
UF 507440 (ICH-00610)		MW603664	n/a	n/a	Konopleva et al., 2021
biv474_1	Thailand: Mekong Basin, Mun River, upstream of upper reservoir	MN275052	MN307243	MN307184	Bolotov et al., 2020
UMMZ:304649	Laos: Mekong Basin	KP795026	KP795051	KP795009	Pfeiffer and Graf, 2015
UF 567737 (ICH-00468)	Cambodia: Mekong Basin, Tonle Kong River	MW603648	n/a	MW647151	Konopleva et al., 2021
Bineurus anodontinum (Rochebrune, 1882)					
UF 507391 (ICH-00445)	Cambodia: Mekong Basin, Tonle Sekong River	MW603639	n/a	n/a	Konopleva et al., 2021
UF 507391 (ICH-00447)		MW603641	n/a	n/a	Konopleva et al., 2021
UF 507419 (ICH-00553)	Cambodia: Mekong Basin, upstream from Sambour	MW603660	n/a	n/a	Konopleva et al., 2021
UF 507424 (ICH-00564)	Cambodia: Mekong Basin, downstream of Sandan	MW603661	n/a	n/a	Konopleva et al., 2021
UF 507408 (ICH-00525)	Cambodia: Mekong Basin, tributary just north of Phumi Prêk Preah on AH11	MW603651	n/a	n/a	Konopleva et al., 2021
Bineurus loeiensis Konopleva et al., 2021					
biv119_1	Thailand: Mekong Basin, Loei River	KX865879	KX865650	KX865750	Konopleva et al., 2021
biv119_2		KX865880	KX865651	KX865751	Konopleva et al., 2021
biv119_3		KX865881	KX865652	KX865752	Konopleva et al., 2021
biv119_4		KX865882	KX865653	KX865753	Konopleva et al., 2021
Outgroups					
Monodontina vondembuschiana (Lea, 1840)					
BIV1822	Malaysia	MK994774	MK994775	n/a	Froufe et al., 2020
Pilsbryoconcha exilis (Lea, 1838)					
MUMNH-UNI2481	Indonesia: Java Basin, Bogor Botanical Gardens	MZ822408	MZ822908	MZ822930	Jeratthitikul et al., 2021b
Thaiconcha callifera (Martens, 1860)					
RMBH biv0120_3	Thailand: Mekong Basin, Phong River	KX865865	KX865636	KX865737	Konopleva et al., 2021
Sundadontina cumingii (Lea, 1850)					
X115	Malaysia	KX051295	n/a	n/a	Zieritz et al., 2016
Nyeinchanconcha nyeinchani Bolotov et al., 2020					
UMMZ 304648	Laos: Mekong Basin, Nam Phiat River	KP795025	KP795050	KP795008	Pfeiffer and Graf, 2015
Namkongnaia inkhavilayi Jeratthitikul et al., 2021					
MUMNH-UNI2704	Laos: Mekong Basin, Local market near Xe Bangfai River	MZ822395	MZ822895	MZ822917	Jeratthitikul et al., 2021b
Pseudodon cf. *inoscularis* (Gould, 1844)					
RMBH biv0110_5	Myanmar: Ayeyarwady Basin, a tributary of Lake Indawgyi	KX865858	KX865629	KX865730	Bolotov et al., 2017a
Pseudodon kayinensis Bolotov et al., 2020					
RMBH biv0618_3	Myanmar: Ataran Basin, Winyaw River	MN275045	MN307240	MN307181	Bolotov et al., 2020

Basin, and sheds light on the biogeographical history of freshwater mussels in Indochinese aquatic systems.

MATERIALS AND METHODS

Animal use protocol was approved by the Faculty of Science, Mahidol University Animal Care and Use Committee, SCMU-ACUC (MUSC65-013-606). Newly obtained specimens of *Bineurus* were collected by hand. They were subjected to euthanization by the two-step method (AVMA, 2020). Specimens were placed in a container filled with fresh water, then 95% (v/v) ethanol was gradually added to the container, starting from approximately 5% (v/v) concentration until the mussels were fully anesthetized. The anesthetized specimens were then moved to 95% (v/v) ethanol for tissue fixation. Small pieces from the foot tissues were cut and used for DNA extraction. The remaining soft parts and their dry shells were kept together and deposited as vouchers into Mahidol University Museum of Natural History, Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand (MUMNH).

TABLE 2. Average uncorrected p-distance of the 660-bp COI gene fragment sequences (%±S.E.) among *Bineurus* species (below diagonal) and within each *Bineurus* species (in bold).

Taxa	*B. panhai* sp. nov.	*B. anodontinum*	*B. exilis*	*B. loeiensis*	*B. mouhotii*
B. panhai sp. nov.	**0.56 ± 0.19**				
B. anodontinum	2.62 ± 0.56	**0.79 ± 0.25**			
B. exilis	2.32 ± 0.52	2.79 ± 0.60	**0.21 ± 0.12**		
B. loeiensis	5.03 ± 0.79	5.86 ± 0.88	5.62 ± 0.85	**0.57 ± 0.19**	
B. mouhotii	4.05 ± 0.67	4.37 ± 0.68	3.91 ± 0.67	6.11 ± 0.88	**0.10 ± 0.09**

Shell morphology of newly obtained specimens was compared with the type series of other *Bineurus* species, photographs from museum collections available from the online MUSSELp database (Graf and Cummings, 2022), and with the original description from literature (Lea, 1863; Morelet, 1875; Rochebrune, 1882; Konopleva et al., 2021). The comparative analysis was based on the shell shape, shell size, umbo position, teeth, and the morphology of adductor muscle scars. Shell dimensions were measured for the shell length, shell width, and shell height using a digital Vernier caliper (±0.01 mm).

Partial sequences of the mitochondrial protein-coding cytochrome c oxidase subunit I gene (COI), the mitochondrial large ribosomal subunit rRNA gene (16S rRNA), and the nuclear 28S large ribosomal subunit rDNA gene (28S rRNA) were amplified and used for phylogenetic reconstruction. The DNA extraction, PCR amplification, sequencing, sequence editing, and sequence alignment were performed using protocols described in our previous works (Jeratthitikul et al., 2021a; 2021b). The newly obtained nucleotide sequences in this study were deposited in the GenBank database under accession numbers OQ108585 – OQ108588 for COI, OQ110575 – OQ110-578 for 16S, and OQ110565 – OQ110568 for 28S.

The final concatenated alignment contained 1,938 bp (660 bp of COI, 488 bp of 16S r RNA, and 790 bp of 28S r RNA) from all valid *Bineurus* species and eight representative taxa from all genera of the tribe Pseudodontini as outgroups (Table 1). Phylogenetic trees were constructed using the maximum likelihood (ML) and Bayesian inference (BI) methods at the San Diego Supercomputer Center through the online CIPRES Science Gateway (Miller et al., 2010). The ML analysis was run in RAxML v8.2.10 (Stamatakis, 2014) with 1,000 ML bootstrap replicates and the GTRGAMMA model for all gene partitions. The BI tree was performed in MrBayes v3.2.6 (Ronquist et al., 2012) with the best-fit model of nucleotide substitution as suggested by PartitionFinder2 v.2.3.4 (Lanfear et al., 2016). The models were selected as follows: GTR+I+G for the first and the third codon positions of COI, and 16S rRNA; F81+I for the second codon position of COI; and HKY+I for 28S rRNA. Two runs of three heated and one cold Markov chains were conducted for 10 million generations. Trees were sampled every 1,000 generations. The first 25% of obtained trees were discarded as burn-in. The remaining trees were used to estimate the consensus tree topology, bipartition posterior probability (BPP), and branch lengths. The effective sample size (ESS) values from the MCMC analysis were greater than 100 for all parameters. In addition, uncorrected pairwise genetic distances (p-distance) of the COI dataset were calculated in MEGA v7.0.26 (Kumar et al., 2016) to unveil the genetic distance among *Bineurus* species.

RESULTS

Phylogenetic position and genetic divergence

Both ML and BI analyses gave identical tree topology and retrieved *Bineurus* as a strongly supported monophyletic clade when compared with other genera in the Pseudodontini (100% bootstrap support for ML, and 1 BPP for BI; Fig. 1). All four valid *Bineurus* species and one undescribed lineage, which is described below as a new species, were recovered as monophyletic and with high support values (99 to 100% bootstrap support for ML, and 0.94 to 1 BPP for BI). However, phylogenetic relationships among *Bineurus* species were not clearly resolved. *Bineurus mouhotii* was placed at the basal position, followed by *B. loeiensis*, and an unsupported clade of *B. exilis*, *B. anodontinum*, and the new species.

The level of genetic divergence as revealed by the uncorrected COI p-distance among *Bineurus* species varied from 2.32 to 6.11% (Table 2). The nearest neighbor to the new species was *B. exilis*. They were genetically separated by 2.32%. The distance between two populations of the new species (Phra Sathueng Stream in Thailand and Sangker River in Cambodia) was 0.89%.

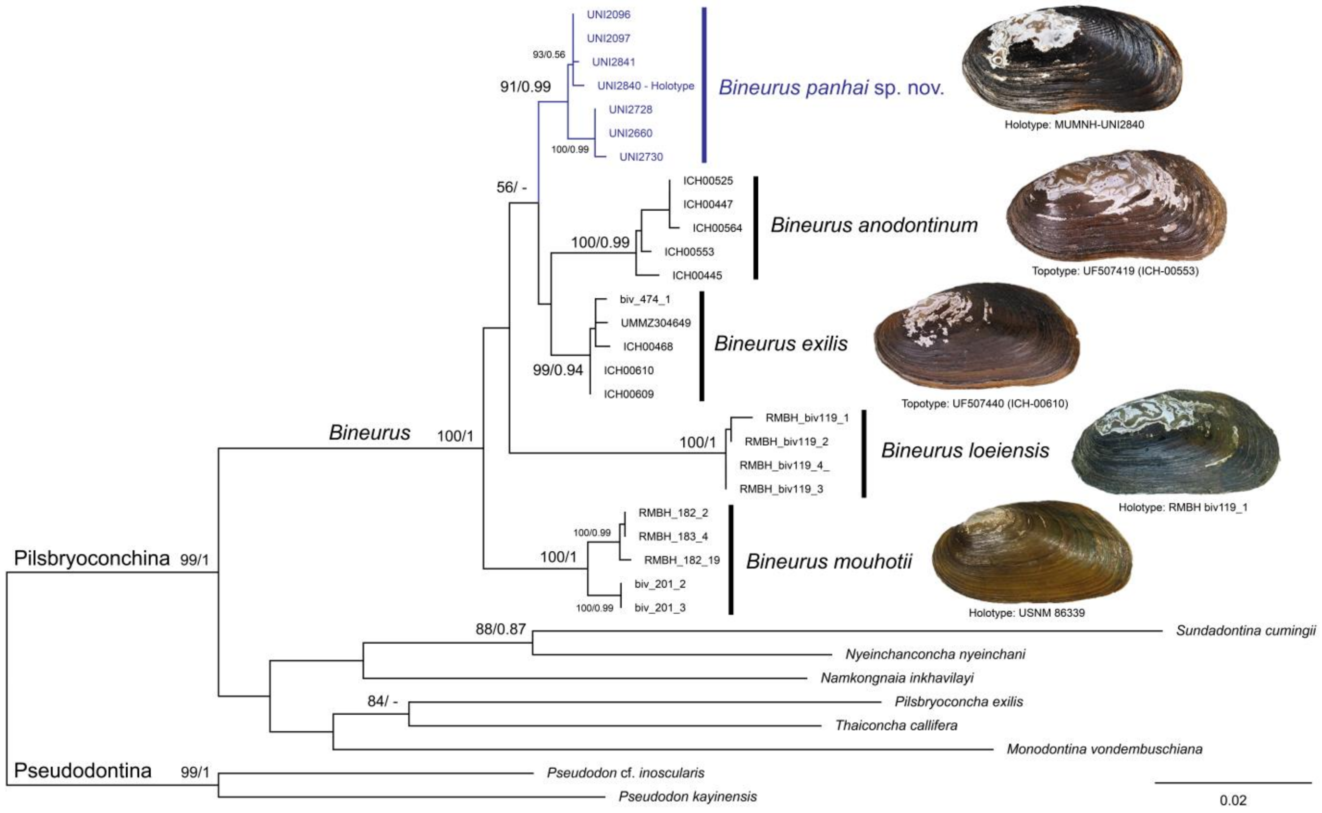

FIGURE 1. Phylogenetic tree of *Bineurus* and other members from the tribe Pseudodontini recovered from the maximum likelihood analysis (ML) of the 1,938-bp concatenated alignment dataset of three loci (COI and 16S mitochondrial genes, and 28S nuclear gene). Numbers on nodes are bootstrap values from ML analysis and Bayesian posterior probabilities from Bayesian inference analysis (BI), and are shown as ML/BI. Scale bar indicates the branch length. The clade of *B. panhai* sp. nov. is blue. Shell images, except for this new species, are reproduced from Konopleva et al. (2021). Shells are not to scale.

Systematics

Family Unionidae Rafinesque, 1820
Subfamily Unioninae Rafinesque, 1820
Tribe Pseudodontini Frierson, 1927
Subtribe Pilsbryoconchina Bolotov et al., 2017

Genus *Bineurus* Simpson, 1900

Type species.– *Monocondyloea mouhotii* Lea, 1863 (by original designation)

Remarks.– The genus consists of five species, namely *B. mouhotii* (Lea, 1863), *B. exilis* (Morelet, 1866), *B. anodontinum* (Rochebrune, 1882), *B. loeiensis* Konopleva et al., 2021, and a new species described below. Their distribution range covers the Middle and Lower Mekong basins, with some populations extending to the Bang Pakong Basin in eastern Thailand (Fig. 2).

Bineurus panhai sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act: F8252E69-CFD0-4BB0-9C61-8CABC27D5445

(Figs 1–3)

Bineurus mouhotii—Ng et al., 2020: 116, 117, fig. 3b.
Bineurus exilis—Jeratthitikul et al., 2021b: 123, fig. 1, 2.

Type materials.– THAILAND • Holotype: MUMNH-UNI2840 (Fig. 3A, length 90.62 mm, height 43.54 mm, width 24.50 mm); Phra Sathueng Stream, Bang Pakong Basin, Wang Thong Subdistrict, Wang Sombun District, Sa Kaeo Province; 13°20'04.7"N, 102°07' 58.4"E; E. Jeratthitikul, C. Sutcharit, and K. Macharoenboon leg. Paratypes: 4 shells, MUMNH-UNI2841, UNI2096 (Fig. 3B), UNI2097, and UNI2936 (empty shell); same collection data as for the holotype.

Other material examined.– THAILAND • 10 shells, MUMNH-UNI2937–46 (empty shells); same collection data as for the holotype. • 2 shells and 1 valve, INHS 25290; tributary of Khlong Phra Phong, 20 km E Klong Hat Subdistrict, Sa Kaeo Province; 13°26' 19.5"N, 102°04'49.8"E; M. H. Sabaj and M. Hardman leg. • 1 shell, UF 507530; Yang River at Rt 3004 in Na Di District, Prachin Buri Province; 14°08'11.4"N, 101°51'27.0"E; J. Pfeiffer and L. Page leg. • 1 shell, UF 507544; Sai Khao River at Rt. 3139 in Sai Khao Subdistrict, Soi Dao District, Chanthaburi Province;

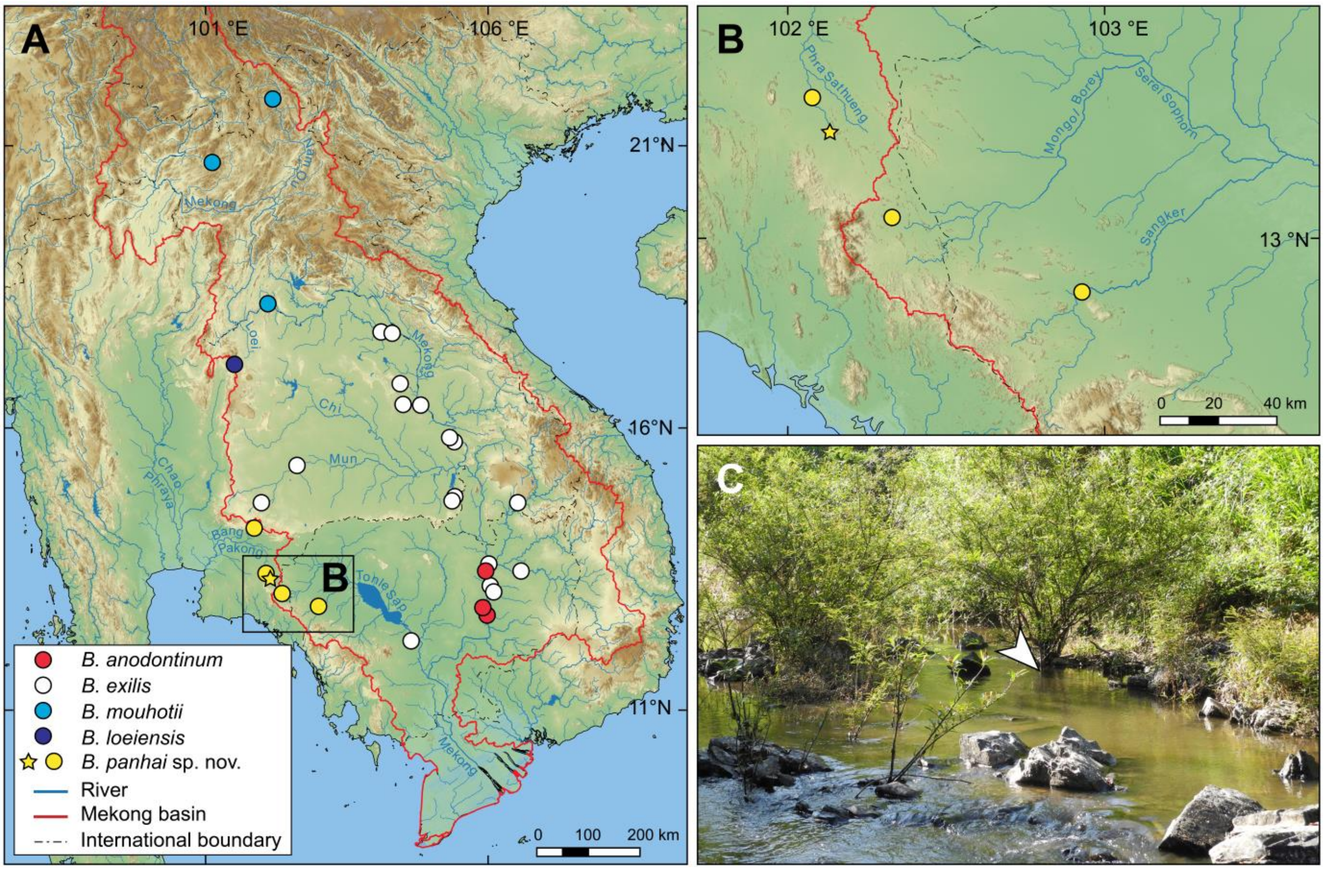

FIGURE 2. A. Map showing the distribution of all *Bineurus* species. Distribution data, except for this new species, are from Konopleva et al. (2021). **B.** Type locality (yellow star) and distribution of *Bineurus panhai* sp. nov. **C.** Habitat at the type locality of *Bineurus panhai* sp. nov., where mussels were collected from soft substrate near tree roots, and between cobble-sized rocks (white arrow head). Maps were generated using QGIS v3.24.3 by compiling topographic base map of freshwater river basins from the Freshwater Ecoregions of the World (Abell et al., 2008), river and lake topology from the HydroSHEDS database (https://www.hydrosheds.org), and map raster data from the NASA EARTHDATA (https://www.earthdata.nasa.gov/).

13°03'43.0"N, 102°19'37.0"E; J. Pfeiffer and L. Page leg. CAMBODIA • 3 shells, MUMNH-UNI2660, UNI2728, and UNI2730; Sangker River, Tonle Sap Basin, Traeng Commune, Rotanak Mondol District, Battambang Province; 12°49'51.4"N, 102°55'44.3"E; sold by local people.

Etymology.– The specific name ‘*panhai*’ is to honor Professor Dr. Somsak Panha, a famous Thai zoologist from the Center of Excellence on Biodiversity, Faculty of Science, Chulalongkorn University, Thailand, who has made outstanding contributions to the systematic study of the invertebrate fauna of Thailand.

Diagnosis.– Shell elongate, anterior end of dorsal margin at the same level as posterior end, and ventral margin concave in middle. *Bineurus panhai* sp. nov. is the most elongate species among its congeners. The new species is similar to *B. exilis*, but it can be distinguished from the latter taxon by having a curved dorsal margin and a lower posterior end (vs. straight dorsal margin and higher posterior end). It can also be distinguished from its congeners by fixed nucleotide substitutions of base C on the 243rd and 558th positions in the COI gene fragment.

Description.– Shell medium-sized, rather thin, elongate (H/L ratio = 0.46–0.51), very inequilateral, kidney-like shell outlines, and rather compressed. Dorsal margin slightly curved, anterior end at the same level as posterior end. Umbonal area strongly eroded, not elevated. Anterior margin round; posterior margin round or slightly truncated. Ventral margin concave in middle. Posterior ridges low, wide and obtuse, not prominent. Periostracum dark brown to black, eroded part white and coppery-brown. Shell surface with fine to rough growth lines, rougher near shell margin; posterior slope with or without parallel diagonal ridges; if present, parallel diagonal ridges running from posterior ridges to dorsal margin. Ligament long and narrow; posterior end of hinge structure with V-shaped fossette. Pseudocardinal teeth small, tubercle-like, one on each valve. Anterior adductor muscle scar deep, droplet-shape, fused with pedal retractor muscle scars;

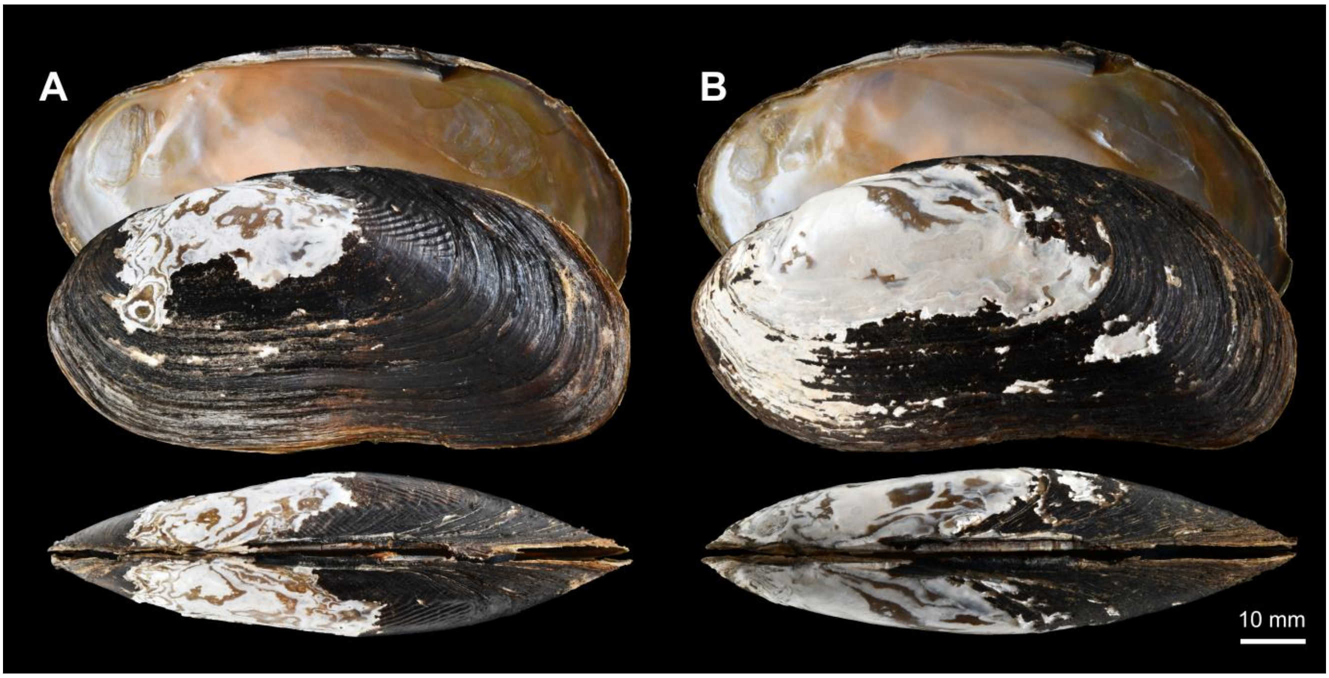

FIGURE 3. Shells of *Bineurus panhai* sp. nov., showing the inner side of the right valve, the outer side of the left valve, and the dorsal view of both valves. **A.** Holotype MUMNH-UNI2840 from Phra Sathueng Stream, Sa Kaeo, Thailand. **B.** Paratype MUMNH-UNI2096 from the type locality.

posterior adductor muscle scars rounded, rather shallow. Pallial line very faint. Nacre iridescent, yellowish-white, salmon towards umbo.

Variation.– Specimens from Sanker River in Cambodia are much smaller, about half the shell length of the holotype (see Ng et al., 2020: fig. 3b for comparison).

Distribution.– Bang Pakong Basin, Eastern Thailand and headwater tributaries of Tonle Sap Basin in Thailand and Cambodia (Fig. 2A, B).

Habitat.– The new species was collected from soft substrate near tree roots, and between cobble-sized rocks (Fig. 2C) in slow-flowing sections of a small stream.

Remarks.– Specimens from the Tonle Sap Basin identified as '*Bineurus mouhotii*' in Ng et al. (2020) and '*Bineurus exilis*' in Jeratthitikul et al. (2021b) are phylogenetically placed in this new species.

DISCUSSION

The new species unambiguously belongs to the genus *Bineurus*, as it has the following generic diagnostics: the rhomboidal or kidney-like shell outlines; very inequilateral, rather thin and compressed shells; and each valve with a tubercle-like pseudocardinal tooth (Konopleva et al., 2021). Species in the genus *Bineurus* exhibit highly similar shell morphology and are difficult to distinguish based on conchological traits alone (Brandt, 1974; Konopleva et al., 2021). Nevertheless, the new species is unique among its congeners by having the most elongated shell (Fig. 1). In addition, the posterior end of the new species is more rounded, and relatively low and at the same level as the anterior end. In contrast, other members of the genus have a more truncated and higher posterior end of the shell, especially *B. exilis*, for which the posterior end is much higher than the anterior end (Fig. 1).

Phylogenetic analyses recovered all *Bineurus* members as separate well-supported clades, suggesting that these species are essentially reproductively isolated and also justifying their recognition as biological species. However, the genetic distances among them were moderate, 2.32 to 6.11% (4.27% average), when compared to other Indochinese unionids, e.g., 6.5–12.3% in *Ensidens* (Muanta et al., 2019), 6.2–9.9% in *Hyriopsis* (Jeratthitikul et al., 2021a), 4.7–11.5% in *Lens* (Jeratthitikul et al., 2019a; Konopleva et al., 2019a), 5.1% in *Namkongnaia* (Jeratthitikul et al., 2021b), 3.6–10.0% in *Pilsbryoconcha* (Jeratthitikul et al., 2022), and 2.0–10.9% in *Scabies* (Jeratthitikul et al., 2019b). The minimum level of genetic divergence was 2.32%, between the new species and *B. exilis*. This level of divergence is still sufficient for species delimitation in unionid mussels (Prié and Puillandre, 2014; Smith et al., 2019).

Besides shell morphology and molecular diagnostic characters, the allopatric distribution of *Bineurus* species also could be useful in their identification. Members of this genus are mainly distributed in the Middle and Lower Mekong basins (Konopleva et al., 2021), and the ranges of most species do not seem to overlap (Fig. 2A). Apart from *B. exilis*, which has a wide distribution range in the Middle Mekong Region and does co-occur with its congeners, *B. anodontinum* is found in the Lower Mekong upstream of Sambour in Cambodia, *B. mouhotii* has its geographic distribution further upstream in the Mekong River in Thailand and Laos, *B. loeiensis* is restricted to the Loei River in Northeast Thailand, and *B. panhai* sp. nov. is distributed in the Bang Pakong Basin of eastern Thailand and in headwater tributaries of the Tonle Sap Basin in Thailand and Cambodia. The allopatric distribution among congeners of unionid mussels can also be seen in other Indochinese unionids, which reflects the complex history of hydrographical change of river systems in the region (Jeratthitikul et al., 2022; Pfeiffer et al., 2021).

Interestingly, the new species is distributed across two separate river basins, the Bang Pakong and Tonle Sap basins. This disjunct distribution may be explained by the stream capture phenomenon, in which the eastern part of the Bang Pakong Basin captured some portions of the Tonle Sap drainage. The molecular divergence between these two populations is relatively shallow (0.89% of the uncorrected COI p-distance), suggesting that this stream capture event occurred relatively recently. Although the timing of this event is still not known, there is evidence of connection between these two basins in the past (Rainboth et al., 2012). Similar disjunct distribution patterns in the same area were also discovered in other unionid species including *Ensidens telus* (see Pfeiffer et al., 2021), and *Pilsbryoconcha linguaeformis* and *P. carinifera* (see Jeratthitikul et al., 2022), suggesting that these taxa may have undergone the same geomorphological event. In addition, it is interesting that whether *Bineurus* is distributed in the Chao Phraya Basin, since several unionid taxa that inhabit the Bang Pakong Basin also extend their range to the Chao Phraya Basin (Jeratthitikul et al., 2019a; Pfeiffer et al., 2021). In fact, these two basins were connected during the exposure of the Sunda continental shelf through the Paleo-Siam River system when the sea levels were low (Voris, 2000).

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