Tropical Natural History, Supplement 7 (2023): 242–250
Date of publication: 6 May 2023

Discovery of a New Endangered Freshwater Mussel Species in the Genus *Chamberlainia* Simpson, 1900 (Bivalvia: Unionidae) from Mekong Basin

BANGON KONGIM[1], CHIRASAK SUTCHARIT[2] AND EKGACHAI JERATTHITIKUL[3]*

[1]Biodiversity and Traditional Knowledge Research Unit, Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham, 44150, THAILAND
[2]Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, THAILAND
[3]Animal Systematics and Molecular Ecology Laboratory, Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, THAILAND
*Corresponding author. Ekgachai Jeratthitikul (ekgachai.jer@mahidol.ac.th)
Received: 20 February 2023; Accepted: 27 April 2023
http://zoobank.org/urn:lsid:zoobank.org:pub:5F155F6F-F0D6-4ED9-9C79-0A17EE6D36AB

ABSTRACT.– *Chamberlainia somsakpanhai* **sp. nov.**, the largest freshwater bivalve (Unionidae) in the Mekong Basin, is described using morphological characters and molecular analysis. The new species differs from its congeners by having an ovate shell outline and obtuse and distinct sub-biangulate posterior margin. A multi-locus phylogenetic tree (mitochondrial COI and 16S, and nuclear 28S genes) recognized the new species along with other two members of the genus, namely *C. hainesiana* and *C. duclerci*. Average genetic divergence between the new species and its congeners is 13.8–14.1% by an uncorrected COI p-distance.

KEYWORDS: conservation, endanger, pearl mussel, endemic, Thailand, Indochina

INTRODUCTION

The freshwater mussel family Unionidae in Thailand has a long history of exploration, i.e., by the pioneer missionaries J. B. Pallegoix (1805–1862) and S. R. House (1817–1899), and the naturalists H. Mouhot (1826–1861) and A. Pavie (1847–1925). The mussels they collected were brought back, and several well-known zoologists studied these specimens and described more than 20 nominal species in the early- to mid-19th century, e.g., Lea (1845, 1852, 1856, 1863), Martens (1860), and Morlet (1889), for instance. One remarkable species among these earliest described mussels is *Unio hainesianus* Lea, 1856, which is distinct by its large and thick shell, and is probably the largest unionid species in Indochina (Brandt, 1974). In Thailand, it is reportedly used as a protein source by local peoples and well known for its production of good quality freshwater pearls (Brandt, 1974; Nagajinda et al., 2005; Kovidtvadhi and Kovidtvadhi, 2014; Goncalves et al., 2022). Almost fifty years after Lea's (1856) description of *U. hainesianus*, it was transferred to the genus *Chamberlainia* Simpson, 1900, a genus endemic to the Indochina Peninsula. Later, this genus was a subject to multiple systematic revisions with the number of species included to it varying from one to seven (Rochebrune, 1904; Simpson, 1914; Haas, 1924; Brandt, 1974; Graf and Cummings, 2007; Goncalves et al., 2022). For Thailand's freshwater mussel fauna, the most comprehensive publication on the unionid classification and identification is the classical work by Brandt (1974), which synonymized all the previously known *Chamberlainia* species from Indochina drainages with *C. hainesiana*. However, all of these early classifications were based solely on morphological evidences.

In the past two decades, over-harvesting, water pollution, and habitat loss are major causes for the decline of unionid populations at the global scale, and some populations have become locally extinct (Lydeard et al., 2004; IUCN, 2015; Zieritz et al., 2018). Meanwhile, molecular phylogeny has developed rapidly and is now a popular tool to elucidate the systematics and evolutionary relationships of various organisms. The unionids have been studied in various aspects, and their systematics has been subjected to revision with continued effort to clarify relationships among taxa using new data and techniques (i.e., Graf and Cummings, 2007, 2021; Pfeiffer et al., 2018, 2019, 2021; Jeratthitikul et al., 2021a, b, 2022; Konopleva et al., 2023). However, *C. hainesiana* has not received much attention in terms of its molecular systematics (Goncalves et al., 2022). It has appeared only in a more general phylogenetic study at the family level (Lopes-Lima et al., 2017; Pfeiffer et al 2019). With the development of robust molecular phylogeny, the tribe Chamberlainiini has been proposed to include three genera: *Chamberlainia*; *Caudiculatus* Simpson, 1900; and *Sinohyriopsis* Starobogatov, 1970 (Lopes-Lima et al., 2017; Graf and Cummings, 2021; Goncalves et al., 2022). A recent species-level revision of *Chamberlainia* proposed that this genus comprises of three distinct species-level clades (Goncalves et al., 2022). By this scheme, the first two sister clades are *C.*

hainesiana s. str., which distributed in the Chao Phraya Basin, and *C. duclerci* (Rochebrune, 1882), which is restricted to the Mae Klong Basin. The other, more distant clade is an unnamed lineage, and was known only from DNA sequences which were impossible to trace back to the shell specimen that the tissue was taken from (Goncalves et al., 2022). Furthermore, the authors also indicated that there is another unnamed species possibly distributed in the Mekong Basin (Goncalves et al., 2022). This Mekong population was previously recognized under the name *C. hainesiana* and its karyotype has been published; however, no associated molecular data exist (Kongim et al., 2015).

Fortunately, a freshwater unionid monitoring survey in Northeast Thailand recently yielded an additional live specimen of *Chamberlainia* from the Chi River. We have re-analyzed the specimen previously used in karyotype study (Kongim et al., 2015) together with this newly obtained specimen by means of molecular examination, and found that these are identical to the unnamed lineage previously recognized as an undescribed species in Goncalves et al. (2022); therefore, we formally describe them herein.

MATERIALS AND METHODS

Specimen collecting

Two living specimens were collected from natural habitat along the bottom of the Chi River at Maha Sarakham Province, Thailand (16.195644, 103.224933) during the periods of exceptional drought. One of the specimens had previously been used for the chromosome preparation (Kongim et al., 2015). Specimens were subjected to euthanization by a two-step method (AVMA, 2020). The mussels were laid in a container and anaesthetized by the gradual addition of 95% (v/v) ethanol to the container from 5% (v/v) concentration until the adductor muscles and foot fully relaxed. Adductor muscle tissues were cut and preserved in 95% (v/v) ethanol at -20 °C until DNA extraction. The remaining soft tissues were separated from the shells and preserved in 70% (v/v) ethanol for fixation and storage. Voucher specimens, including soft parts and shells, are deposited in the Zoology Museum of Mahasarakham University, Department of Biology, Faculty of Science, Mahasarakham University, Thailand (ZMMSU).

Molecular analysis

Total genomic DNA was isolated using NucleoSpinR Tissue kit (MACHEREY-NAGEL, Germany) from alcohol-preserved adductor muscle tissue in the newly collected specimen, and from dry adductor muscle tissue in the specimen from Kongim et al. (2015). Two mitochondrial genes, the protein-coding cytochrome c oxidase subunit I (COI) and the large ribosomal subunit rRNA gene (16S rRNA), and the 28S large ribosomal subunit rDNA nuclear gene (28S rRNA) were amplified using polymerase chain reaction (PCR) following our previous protocol (Jeratthitikul et al., 2021a, 2021b). 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 and D2 (Jovelin and Justine, 2001). We suc-cessfully amplified only the16s gene in the specimen from Kongim et al. (2015), while the newly collected specimen yielded all three gene fragments. The PCR products were purified by the PEG precipitation method and sequenced on the ABI 3730XL DNA Analyzer (BIONEER, Republic of Korea). Obtained sequences were deposited in the NCBI's GenBank database under accession numbers as shown in Table1.

Sequences were edited and aligned using the ClustalW option in MEGA v7.0.26 (Kumar et al. 2016). In total, sequence data from 20 unionids were used in the phylogenetic tree reconstruction. Eight *Chamberlainia* specimens were used as ingroups, and the remainder were unionids from other genera of the tribe Chamberlainiini, Lamprotulini, Gonideini, and Pseudodontini, serving as outgroups (Table 1). The final concatenated alignment contained 1,920 bp (660 bp of COI, 494 bp of 16S, and 766 bp of 28S).

Two phylogenetic methods were used to infer the relationship among *Chamberlainia*. Firstly, maximum likelihood (ML) method was performed using RAxML v.8.2.10 (Stamatakis, 2014), with GTRGAMMA as the nucleotide substitution model for all gene partitions. One thousand ML bootstrap replicates were performed to assess the topology support. Secondly, the Bayesian inference (BI) method was carried out using MrBayes v3.2.6 (Ronquist et al., 2012) under the best-fit model of nucleotide substitution as suggested by PartitionFinder2 v.2.3.4 (Lanfear et al., 2016). The best-fit models for each partition were HKY+I for the first codon position of COI; GTR+G for the second codon position of COI and 16S; and GTR+I+G for the third codon position of COI and 28S. The BI trees were estimated by running a 10 million generation Metropolis-coupled Markov chain of Monte Carlo (MC-MCMC). Each MCMC consisted of two runs with four chains, one of which was heated. Tree samples were selected 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. Both ML and BI were run through the on-line CIPRES Science Gateway (Miller et al., 2010).

TABLE 1. List of taxa used in phylogenetic analysis with details of specimens and GenBank accession numbers.

Taxa/ Voucher	Locality	COI	16S	28S	Reference
Tribe Chamberlainiini Bogan et al., 2017					
Chamberlainia somsakpanhai sp. nov.					
BIV46	Thailand	NC_044110	NC_044110	KX822592	Froufe et al., 2019, Lopes-Lima et al., 2017
ZMMSU 00491 Holotype	Thailand: Chi River, Maha Sarakham	n/a	OQ455721	n/a	This study
ZMMSU 00490 Paratype	Thailand: Chi River, Maha Sarakham	OQ455718	OQ455722	OQ455724	This study
Chamberlainia hainesiana (Lea, 1856)					
MUMNH-UNI0944	Thailand: Yom River, Phrae	OQ455719	OQ4557223	OQ455725	This study
UF 507722 (ICH-00675)	Thailand: Yom River, Sukhothai	MZ820771	n/a	n/a	Goncalves et al., 2022
UF 507722 (ICH-00676)	Thailand: Yom River, Sukhothai	MZ820772	n/a	n/a	Goncalves et al., 2022
UF 507722 (ICH-00677	Thailand: Yom River, Sukhothai	MZ820773	n/a	n/a	Goncalves et al., 2022
Chamberlainia duclerci (Rochebrune, 1882)					
UF 507872 (ICH-02092)	Thailand: Khwae Noi River, Kanchanaburi	MZ820770	n/a	n/a	Goncalves et al., 2022
Outgroups					
Sinohyriopsis cumingii (Lea, 1852)					
16_NCU_XPWU_SU175	China: Jiangxi, Poyang Lake	MG463085	n/a	MG595612	Huang at al., 2019
Sinohyriopsis goliath (Rolle, 1904)					
CIIMAR BIV3632	Viet Nam	MT020704	n/a	n/a	Genbank
Sinohyriopsis schlegelii (Martens, 1861)					
Hs21-05f	Lake Anenuma, Aomori, Japan	LC519007	LC224006	LC519071	Sano et al., 2017, 2020
Tribe Lamprotulini Modell, 1942					
Lamprotula leaii (Gray in Griffith & Pidgeon, 1833)					
RMBH biv0200_1	Vietnam	MN402616	MN396727	MN396723	Bolotov et al., 2020
Potomida littoralis (Cuvier, 1798)					
RMBH biv0177_10	Turkey: Karasu River	MN402617	MN396728	MN396724	Bolotov et al., 2020
Pronodularia japanensis (Lea, 1859)					
	Japan	KX822659	AB055625	KX822615	Lopes-Lima et al., 2017, Genbank
Tribe Gonideini Ortmann, 1916					
Sinosolenaia carinata (Heude, 1877)					
n/a	China	KX822669	NC_023250	KX822626	Huang et al., 2013, Lopes-Lima et al., 2017
Gonidea angulata (Lea, 1838)					
RMBH biv0294_1	USA: Okanagan Lake	MN402615	MN396726	MN396722	Bolotov et al., 2020
Leguminaia wheatleyi (Lea, 1862)					
RMBH biv0177_7	Turkey: Karasu River	MN402614	MN396725	MN396721	Bolotov et al., 2020
Tribe Pseudodontini					
Namkongnaia inkhavilayi Jeratthitikul et al., 2021					
MUMNH-UNI2704	Laos: Local market near Xe Bangfai River	MZ822395	MZ822895	MZ822917	Jeratthitikul et al., 2021
Pseudodon cf. *inoscularis* (Gould, 1844)					
RMBH biv0110_5	Myanmar: Tributary of Lake Indawgyi	KX865858	KX865629	KX865730	Bolotov et al., 2017
Pilsbryoconcha exilis (Lea, 1838)					
MUMNH-UNI2481	Indonesia: Bogor Botanical Gardens, West Java	MZ822408	MZ822908	MZ822930	Jeratthitikul et al., 2021

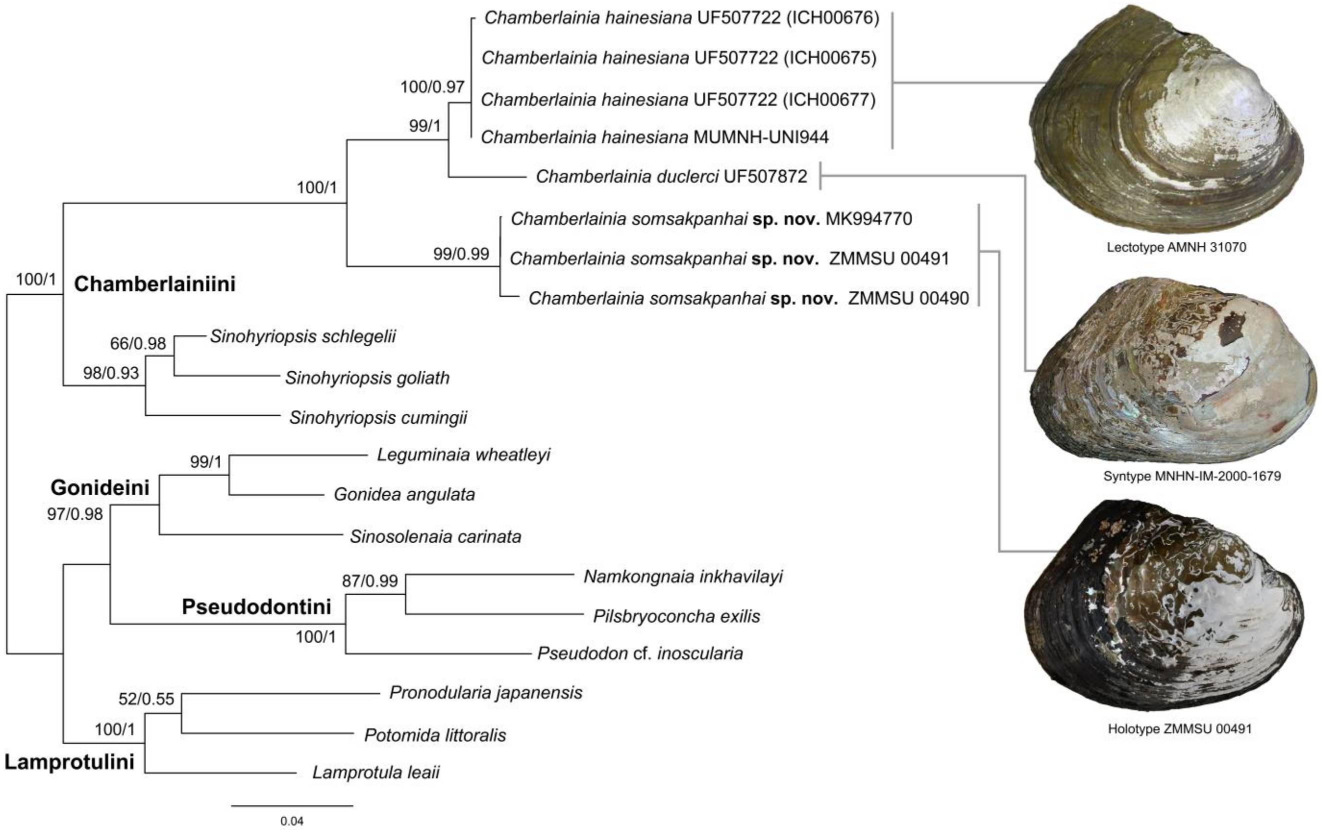

FIGURE 1. Maximum likelihood tree (ML) of *Chamberlainia* and other related freshwater mussel tribes based on 1,920 bp concatenated dataset of COI + 16S + 28S genes. Numbers on nodes are bootstrap support values (BS) from ML analysis and Bayesian posterior probabilities (BPP) from Bayesian inference analysis (shown as BS/BPP). Shells are not to scale. Photo credit: D. Graf and K. Cummings; *C. duclerci*, https://mussel-project.uwsp.edu/fmuotwaolcb/specimen_12306.html; and *C. hainesiana*, https://mussel-project.uwsp.edu/fmuotwaolcb/specimen_7596.html.

In addition, uncorrected pairwise genetic distances (p-distance) were calculated in MEGA v7.0.26 (Kumar et al., 2016) to estimate genetic distance.

RESULTS

Molecular analysis

Both phylogenetic analyses gave similar topology and congruently retrieved *Chamberlainia* as a monophyletic clade regarding to the outgroups (Fig. 1). Three specimens of the new species were recovered as a monophyletic group and placed basally to the clade comprising of *C. hainesiana* and *C. duclerci*. The average uncorrected COI p-distance between the new species and *C. hainesiana* was 13.82±1.32% (mean ± standard error), while the distance between the new species and *C. duclerci* was 14.08±1.34%. In contrast, the divergence between *C. hainesiana* and *C. duclerci* was 4.02±0.74%.

Systematics

Family Unionidae Rafinesque, 1820
Subfamily Gonideinae Ortmann, 1916

Tribe Chamberlainiini Bogan, Froufe & Lopes-Lima in Lopes-Lima et al., 2017

Genus *Chamberlainia* Simpson, 1900

Type species.– *Unio hainesianus* Lea, 1856 (by original designation).

Remarks.– The genus currently contains three valid species, including a new species, described herein. They are ranged in the Mae Klong Basin (*C. duclerci*), the Chao Phraya Basin (*C. hainesiana*) and the Mekong Basin (the new species) of Thailand. The nominal taxon *Dysnomia pavonina* Rochebrune, 1882 (= junior synonym of *C. hainesiana*) was described from 'Battambang' (the type locality) based on M. Bocourt's collection record. Later, Rochebrune (1904: 464) made a re-description and stated 'Battambang, de la province du Salabury (Siam)' as the collection locality. However, Goncalves et al. (2022) examined the syntype and found that the locality as stated in the original label could be interpreted more precisely as 'Saraburi Province in Chao Phraya River Basin'. In addition, the shell morphology of the syntypes aligns well with the typical *C. hainesiana* (see Goncalves et al., 2022), and we agree with this interpretation.

Chamberlainia hainesiana (Lea, 1856)

Unio hainesianus Lea, 1856: 92. Type locality: Siam. Lea, 1857: 289, pl. 21, fig. 1.
Unio imperialis Morelet, 1862: 480. Type locality: in paludibus prope fluvium Meïnam.
Dysnomia pavonina Rochebrune, 1882: 43. Type locality: Battambang.
Unio paviei Morlet, 1891: 238, 241, pl. 7, fig. 3. Type locality: Rivière de Ménam-Pinh.
Chamberlainia hainesiana—Brandt, 1974: 276–278, pl. 23, fig. 45 (in part). Goncalves et al., 2022: 4–5, figs 1–4.

Remarks.– For a complete history of the name usage and synonymies, see Haas (1969), Brandt (1974), and Goncalves et al. (2022).

Chamberlainia duclerci (Rochebrune, 1882)

Unio duclerci Rochebrune, 1882: 27, pl. 1. Type locality: Mekkong.
Chamberlainia duclerci—Goncalves et al., 2022: 5, 6.

Remarks.– For the taxonomic status and synonym usage of this species, see Goncalves et al. (2022).

Chamberlainia somsakpanhai sp. nov.

http://zoobank.org/urn:lsid:zoobank.org:act:CCFD3D19-5E51-4AC1-8E11-FEF5674E2211

(Figs 1–3)

Chamberlainia hainesiana—Kongim et al., 2015: fig. 2a (non Lea, 1856).
Chamberlainia sp. Goncalves et al., 2022: 6, 7.

Type material.– Holotype ZMMSU 00491 (shell length 140 mm, height 110 mm, width 27.5 mm; Fig. 2) from the Chi River, Ban Thasongkorn, Muang, Maha Sarakham, Thailand (16.195644, 103.224933); paratypes ZMMSU 00490 (length 211 mm, height 146 mm, width 37.5 mm) same collection data as for holotype.

Other material examined.– ZMMSU 00650 (2 incomplete shells; length 183 and 190 mm, height 136 and 138 mm, width 30.5 and 31.5 mm, respectively) from the Chi River, Ban Tha Khonyang, Kantharawichai, Maha Sarakham, Thailand (16.232374, 103.268253).

Etymology.– The specific name refers to Professor Dr. Somsak Panha, the research pioneer of invertebrate systematics in Thailand, especially for land and freshwater molluscs, and in recognition of his 65th birthday.

Diagnosis.– Shell greenish-brown, very large and very thick, ovate, posterior end obtuse and sub-biangulate. This new species differs from *C. hainesiana* s. str. in having a more elongate, straighter ventral margin, and obtuse posterior margin with biangulate posterior end. In contrast, *C. hainesiana* has suborbicular shell, distinct circular posterior and curved ventral margins. It also differs from *C. duclerci* in having a more ovate shell, more curved ventral margin, and obtuse posterior margin. In turn, *C. duclerci* has an elongated shell, almost straight ventral margin, and rounder posterior margin.

Description.– Shell very thick, very large, length 140–211 mm, height 110–146 mm, width 27.5–37.5 mm, ovate in outline, inequilateral, inflated. Dorsal margin slightly curved; anterior end low, elevated to posterior end, forming posterior wing; posterior wing high. Umbonal area elevated, eroded, and sloping upwards posteriorly, posterior ridges obtuse. Anterior margin rounded; posterior margin obtuse and sub-biangulate. Ventral margin curved. Periostracum think, greenish-brown with dark brown on posterior margin, distinctly eroded on anterior part and decreasingly and irregularly eroded towards posterior portion, umbo and anterior part with prismatic layer.

Inner side of the shell mostly bluish-white, with iridescence on posterior outer pallial line, ligament and shell margin black, ventral part very thick especially between pallial line and ventral margin (thickness 22 mm). Hinge plate well developed, pseudocardinal teeth forming a much thickened plate; one large triangulate pseudocardinal tooth on the right valve, with a small plate that is curved and sharp; two large triangulate and rough pseudocardinal teeth on the left valve. Lateral teeth well-developed, long and raised lamelliform; one on the right valve, two on the left valve, the outer one on the left valve more swollen than the inner one. Umbonal cavities deep; with tiny muscle scars in the cavity. Anterior adductor muscle scar deeply impressed, medusa like, filled with a cluster of tiny scars, distinct stacked nacreous layers on ventral; anterior pedal retractor muscle scar smaller and connected to anterior adductor muscle scar; pedal protractor scar small, horseshoe like, separate from anterior adductor muscle scar; posterior adductor muscle scar shallow, droplet-shaped, 23 mm in diameter, filled with iridescent nacreous layers.

Excurrent and incurrent siphons creamy white inside, black or dark brown on edges. Excurrent aperture smooth, shorter than incurrent. Incurrent with

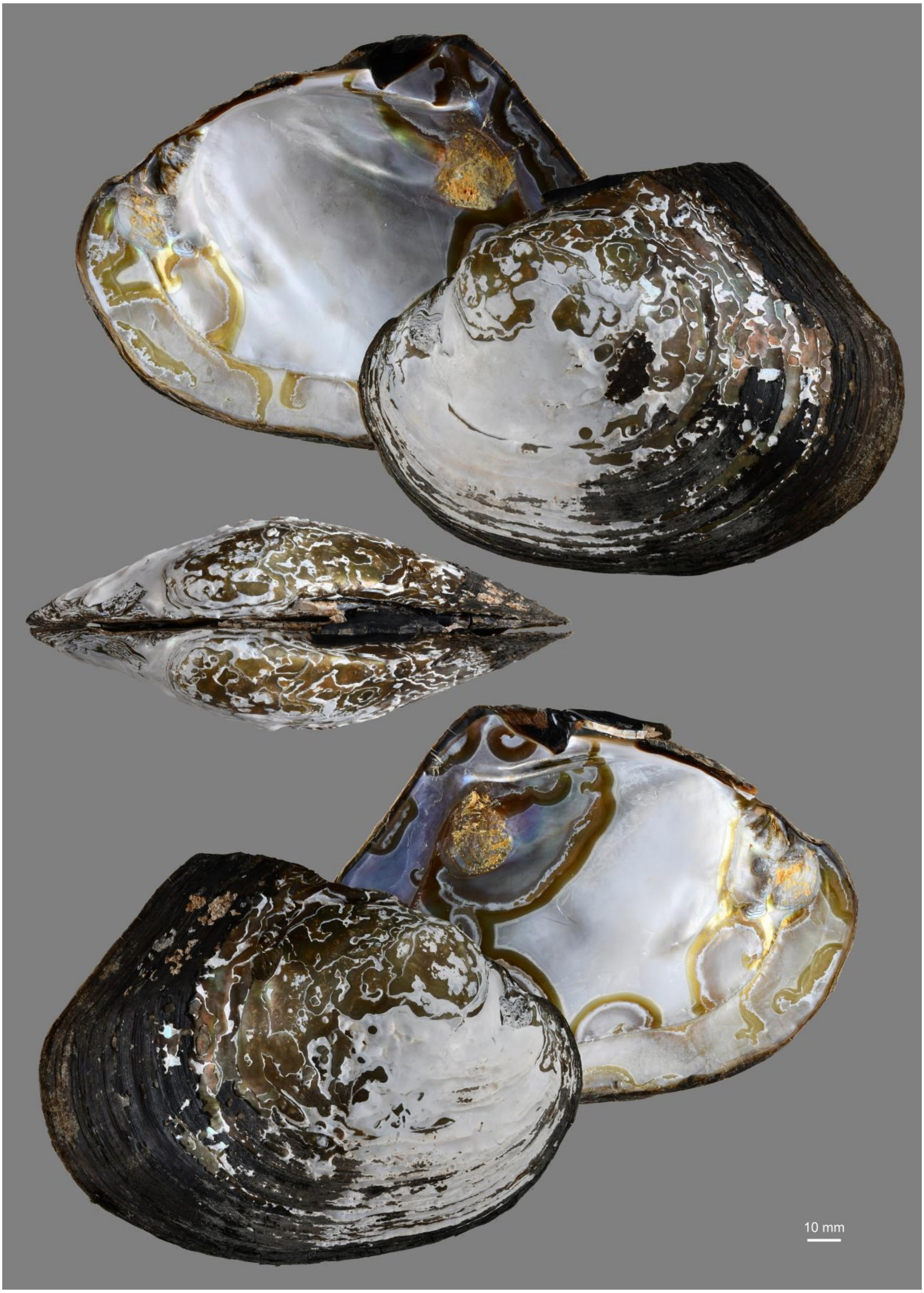

FIGURE 2. Holotype of *Chamberlainia somsakpanhai* sp. nov.

1–2 rows of conical papillae; inner papillae white, cone-shaped, thickened at base; outer papillae shorter, more numerous. Gills distinctly brown, elongated, anterior part broader than posterior, inner gills longer and wider than outer gills.

Distribution.– The new species is known to occur in the Chi River, Northeastern Thailand (Fig. 3) and the Mekong mainstream (based on museum records, see Goncalves et al., 2022). It is also known from the Mae Klong drainage based on the DNA sequences (KX 822635 and MK994770; Lopes-Lima et al. 2017; Froufe et al. 2020), but that population is hypothesized to be introduced (see discussion below).

Habitat.– The new species was collected from the bottom of the Chi River (2–3 m depth in the dry season). The mussel was found in the upper layer of substrate, which was hard clay mixed with mud.

Remarks.– Before the revision by Goncalves et al. (2022), the identification of *Chamberliania* species in Thailand heavily relied on Brandt (1974), who recognized only a single species from this genus, *C. hainesiana*. Therefore, nearly all the previous works related to glochidiosis (Panha, 1993), mantle transplantation (Panha and Kosavititkul, 1997), growth and survival (Kovitvadhi and Kovitvadhi, 2013, 2014; Kovitvadhi et al., 2017) are attributed to *C. hainesiana* s. lato. The sole exception is the karyotype analysis (Kongim et al., 2015), which is attributed to the new species.

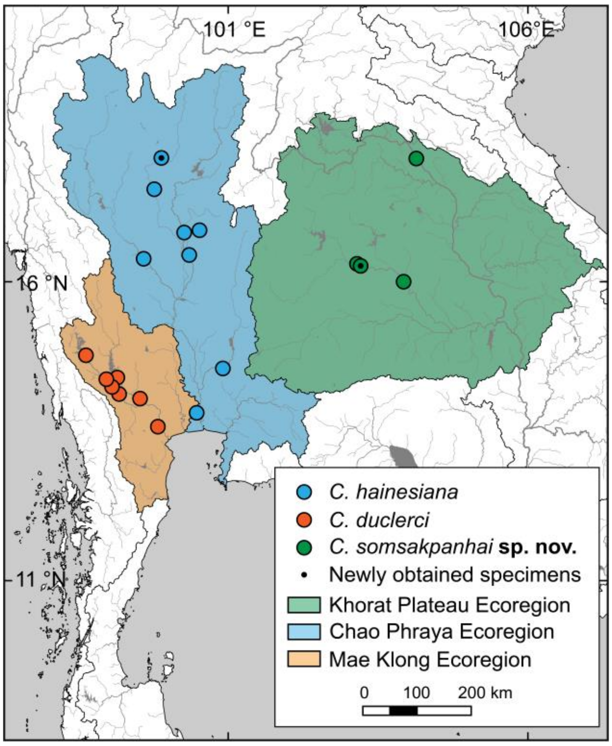

FIGURE 3. Sampling localities and distribution map of *Chamberlainia*. Map is updated from Goncalves et al. (2022) by adding occurrences newly obtained in this study. Map was generated using QGIS v3.24.3 by compiling freshwater river ecoregions map from the Freshwater Ecoregions of the World (Abell et al., 2008), and river and lake topology from the HydroSHEDS database (https://www.hydrosheds.org).

Two GenBank sequences (KX822635 and MK 994770) used in our analysis were said to be from ‘Thailand’ (e.g. Lopes-Lima et al. 2017; Froufe et al. 2020), without precise locality or drainage, or even voucher specimen deposition. Goncalves et al. (2022) concluded that these two sequences represented a divergent lineage of ‘*Chamberlainia*’. Our phylogenetic results cluster these sequences with those of our new species. Thus, these sequences should be recognized as *C. somsakpanhai* sp. nov.

DISCUSSION

Goncalves et al. (2022) recognized four possible lineages in the *Chamberlainia*: (1) the more circular shell outline from the Chao Phraya drainage is recognized as *C. hainesiana*, (2) the elongated shell outline from the Mae Klong drainage is recognized as *C. duclerci*. (3) the historical records from the Mekong drainage in Thailand are recognized as ‘*Chamberlainia* sp.’, and (4) the two sequences without voucher specimens are recognized as a ‘divergent *Chamberlainia*’. The latter lineage was labeled as from the Mae Klong River, Thailand. Interestingly, the two specimens of the new species examined here clustered with the ‘divergent *Chamberlainia*’ clade without genetic structure (Fig. 1). This disjunct distribution appears to be the result of a deliberate non-native introduction associated with pearl culture.

In fact, these two DNA sequences (KX822635 and MK994770; Lopes-Lima et al., 2017; Frouse et al., 2020) have arisen from an unregistered voucher specimen. Lopes-Lima et al. (2017) roughly state only ‘Thailand’ as the source of the sequencing specimen, and state that the tissue sample was provided by U. Kovitvadhi. We consulted with U. Kovitvadhi about the origin of that sequenced specimen, which she stated was from the captive breeding stock from Kanchanaburi Inland Fisheries Research and Development Centre (=RFA-Kanchanaburi). Around 1987, a number of *C. hainesiana* (believed to be the sole species) were gathered from the Chao Phraya and Mekong watersheds and sent to RFA-Kanchanaburi, where they were raised and bred with the purpose of enhancing the quality and production of pearl culture (W. Kitimasak and U. Kovitvadhi, per. comm. May 2022). Therefore, the tissue sample examined by Lopes-Lima et al. (2017) possibly did not originate from the Mae Klong River, but rather from somewhere else, and was gathered during the breeding program. Our phylogenetic results suggest a high possibility that the specimen came from the Mekong watershed population.

Currently, all three *Chamberlainia* species occur only in Thailand, with highly fragmented populations and very low densities (Fig. 3). Over-harvesting for the pearl production process is one of the leading causes of the declining populations (see Goncalves et al., 2022 and reference therein). As is the case for other freshwater mussels, anthropogenic activities, especially water pollution and habitat loss, seem to be the primary factors putting *Chamberlainia* at risk of extinction. Regarding *C. somsakpanhai* sp. nov., which is known only from the type locality and two historical specimen records, this species should be considered Endangered, as Gonclaves et al. (2022) has suggested. In addition, like many other freshwater mussel species, unseasonal fluctuations in the water level of the Mekong River due to dams can negatively affect mussels' life history and lead to population decrease.

ACKNOWLEDGMENTS

We thank U. Kovitvadhi and W. Kitimasak for their deep insight into the history of pearl culture at the Kanchanaburi Inland Fisheries Research and Development Centre. We also thank D. J. Anderson for grammar checking and comments. This research was financially supported by Mahasarakham University, Thailand, and the Center of Excellence on Biodiversity (BDC-PG4-163009). Animal uses in this study have been approved by the Faculty of Science, Mahidol University Animal Care and Use Committee (SCMU-ACUC) under approval number MUSC65-013-606.

LITERATURE CITED

Abell, R., Thieme, M.L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B., Mandrak, N., Balderas, S.C., Bussing, W., Stiassny, M.L.J., Skelton, P., Allen, G.R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T.J., Wikramanayake, E., Olson, D., López, H.L., Reis, R.E., Lundberg, J.G., Sabaj Pérez, M.H. and Petry, P. 2008. Freshwater ecoregions of the World: A new map of biogeographic units for freshwater biodiversity conservation. BioScience, 58(5): 403–414.

AVMA. 2020. AVMA guidelines for the euthanasia of animals. American Veterinary Medical Association. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf (Accessed 13 January 2023).

Bolotov, I.N., Kondakov, A.V., Vikhrev, I.V., Aksenova, O.V., Bespalaya, Y.V., Gofarov, M.Y., Kolosova, Y.S., Konopleva, E.S., Spitsyn, V.M., Tanmuangpak, K. and Tumpeesuwan, S. 2017. Ancient river inference explains exceptional Oriental freshwater mussel radiations. Scientific Reports, 7: 2135.

Bolotov, I.N., Konopleva, E.S., Vikhrev, I.V., Gofarov, M.Y., Lopes-Lima, M., Bogan, A.E., Lunn, Z., Chan, N., Win, T., Aksenova, O.V., Tomilova, A.A., Tanmuangpak, K., Tumpeesuwan, S. and Kondakov, A.V. 2020. New freshwater mussel taxa discoveries clarify biogeographic division of Southeast Asia. Scientific Reports, 10: 6616.

Brandt, R.A.M. 1974. The non-marine aquatic Mollusca of Thailand. Archiv für Molluskenkunde, 105: 1–423.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294–299.

Froufe, E., Bolotov, I., Aldridge, D.C., Bogan, A.E., Breton, S., Gan, H.M., Kovitvadhi, U., Kovitvadhi, S., Riccardi, N., Secci-Petretto, G., Sousa, R., Teixeira, A., Varandas, S., Zanatta, D., Zieritz, A., Fonseca, M.M. and Lopes-Lima, M. 2020. Mesozoic mitogenome rearrangements and freshwater mussel (Bivalvia: Unionoidea) macroevolution. Heredity, 124: 182–196.

Goncalves, A., Zieritz, A., Lopes-Lima, M., Deein G. and Pfeiffer, J. 2022.Taxonomic revision and conservation assessment of the Southeast Asian freshwater mussel genus *Chamberlainia* Simpson, 1900. Journal of Molluscan Studies, 88: eyac008.

Graf, D.L. and Cummings, K.S. 2021. A 'big data' approach to global freshwater mussel diversity (Bivalvia: Unionoida), with an updated checklist of genera and species. Journal of Molluscan Studies, 87: eyaa034.

Graf, D.L. and Cummings, K.S. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). Journal of Molluscan Studies, 73: 291–314.

Haas, F. 1924. Beiträge zu einer Monographie der asiatischen Unioniden. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft, 38: 129–203.

Haas, F. 1969. Superfamilia Unionacea. Das Tierreich. 88: 1–663.

Huang, X.C., Rong, J., Liu, Y., Zhang, M.H., Wan, Y., Ouyang, S., Zhou, C.-H. and Wu, X.-P. 2013. The complete maternally and paternally inherited mitochondrial genomes of the endangered freshwater mussel *Solenaia carinatus* (Bivalvia: Unionidae) and implications for Unionidae taxonomy. PLoS ONE, 8(12): e84352.

Huang, X.C., Su, J.H., Ouyang, J.X., Ouyang, S., Zhou, C.H. and Wu, X.P. 2019. Towards a global phylogeny of freshwater mussels (Bivalvia: Unionida): Species delimitation of Chinese taxa, mitochondrial phylogenomics, and diversification patterns. Molecular Phylogenetics and Evolution, 130: 45–59.

IUCN. 2015. The IUCN Red List of Threatened Species. Version 2015-4. (http://www.iucnredlist.org).

Jeratthitikul, E., Paphatmethin, S., Sutcharit, C., Ngor, P.B., Inkhavilay, K. and Prasankok, P. 2022. Phylogeny and biogeography of Indochinese freshwater mussels in the genus *Pilsbryoconcha* Simpson, 1900 (Bivalvia: Unionidae) with descriptions of four new species. Scientific Reports, 12: 20458.

Jeratthitikul, E., Paphatmethin, S., Zieritz, A., Lopes-Lima, M. and Ngor, P.B. 2021a. *Hyriopsis panhai*, a new species of freshwater mussel from Thailand (Bivalvia: Unionidae). Raffles Bulletin of Zoology, 69: 124–136.

Jeratthitikul, E., Sutcharit, C., Ngor, P.B. and Prasankok, P. 2021b. Molecular phylogeny reveals a new genus of freshwater mussels from the Mekong River Basin (Bivalvia: Unionidae). European Journal of Taxonomy, 775: 119–142.

Jovelin, R. and Justine, J.-L. 2001. Phylogenetic relationships within the polyopisthocotylean monogeneans (Platyhelminthes) inferred from partial 28S rDNA sequences. International Journal for Parasitology, 31(4): 393–401.

Kongim, B., Sutcharit, C. and Panha, S. 2015. Cytotaxonomy of unionid freshwater mussels (Unionoida, Unionidae) from northeastern Thailand with description of a new species. ZooKeys, 514: 93–110.

Konopleva, E.S., Lheknim, V., Sriwoon, R., Kondakov, A.V., Tomilova, A.A., Gofarov, M.Y., Vikhrev, I.V. and Bolotov, I.N. 2023. Diversity and phylogenetics of freshwater mussels (Unionidae) from Southern Thailand with the description of one new genus and five new species-group taxa. Diversity, 15: 10.

Kovitvadhi, S. and Kovitvadhi, U. 2013. Effects of rearing density and sub-sand filters on growth performance of juvenile freshwater mussels (*Chamberlainia hainesiana*) reared under recirculating system conditions. Science Asia, 39: 139–149.

Kovitvadhi, S. and Kovitvadhi, U. 2014. The biology and in vitro culturing of the freshwater pearl mussel glochidia to adulthood in Thailand. In: Medical and applied malacology crossing boundaries: integrative approaches to malacology (A.R. de Toledo Piza, L. de Freitas Tallarico & G.O. Introíni, eds), pp. 165–197. Cambridge Scholars Publishing, Newcastle upon Tyne, UK.

Kovitvadhi, U., Kovitvadhi, S. and Meejui, O. 2017. Growth and survival of juvenile freshwater mussel, *Chamberlainia hainesiana* (Lea, 1856), at three densities in the natural reservoir and river environments, Thailand. Journal of the World Aquaculture Society, 48: 623–633.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870–1874.

Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T. and Calcott, B. 2016. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution, 34(3): 772–773.

Lea, I. 1845. Description of new fresh water and land shells. Transactions of the American Philosophical Society, 9: 275–282.

Lea, I. 1852. Descriptions of new species of the family Unionidæ. Transactions of the American Philosophical Society, 10: 253–294.

Lea, I. 1856. Description of twenty-five new species of exotic uniones. Proceedings of the Academy of Natural Sciences of Philadelphia, 8: 92–95.

Lea, I. 1863. Description of a new species of *Unio* and a *Monocondylœa*. Proceedings of the Academy of Natural Sciences of Philadelphia, 15: 190.

Lopes-Lima, M., Froufe, E., Do, V.T., Ghamizi, M., Mock, K.E., Kebapçi, Ü., Klishko, O., Kovitvadhi, S., Kovit- Vadhi, U., Paulo, O.S., Pfeiffer, J.M., Raley, M., Riccardi, N., ¸S Erefli ¸S An, H., Sousa, R., Teixeira, A., Varandas, S., Wu, X., Zanatta, D.T., Zieritz, A. and Bogan, A.E. 2017. Phylogeny of the most species-rich freshwater bivalve family (Bivalvia: Unionida: Unionidae): defining modern subfamilies and tribes. Molecular Phylogenetics and Evolution, 106: 174–191.

Lydeard, C., Cowie, R.H., Ponder, W.F., Bogan, A.E., Bouchet, P., Clark, S.A., Cummings, K.S., Frest, T.J., Gargominy, O., Herbert, D.G., Hershler, R., Perez, K.E., Roth, B., Seddon, M., Strong, E.E. and Thompson, F.G. 2004. The global decline of nonmarine mollusks. Bioscience, 54: 321–330.

Lydeard, C., Mulvey, M. and Davis, G.M., 1996. Molecular systematics and evolution of reproductive traits of North American freshwater unionacean mussels (Mollusca: Bivalvia) as inferred from 16S rRNA gene sequences. Philosophical Transaction of the Royal Society of London. Series B, Biological Science, 351: 1593–1603.

Martens, E. von. 1860. On the Mollusca of Siam. Proceedings of the Zoological Society of London, 28: 6–18.

Miller, M.A., Pfeiffer, W. and Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop (GCE): 1–8.

Morelet, A. 1862. Diagnoses testarum Indo-Sinarum. — Series prima. Revue et Magasin de Zoologie, 14: 477–481.

Morlet, L. 1889. Catalogue des coquilles recueillies, par M. Pavie dans le Cambode et le Royaume de Siam, et description d’espèces nouvelles (1). Journal de Conchyliologie, 37: 121–199.

Morlet, L. 1891. Contributions à la Faune malacologique de l’Indo-Chine. Journal de Conchyliologie, 39: 230–1254.

Nagajinda, A., Meejui, O., Kovitvadhi, S., Kovitvadhi, U. and Chatchavalvanich, K. 2005. Freshwater pearl culture from Thai amblemid mussel. In: Report on development of freshwater pearl culture in Thailand, pp. 108–186. National Research Council of Thailand, Bangkok, Thailand.

Ortmann, A.E., 1916. The anatomical structure of *Gonidea angulata* (Lea). Nautilus, 30: 50–53.

Panha, S. 1993. Glochidiosis and juvenile production in a freshwater pearl mussel, *Chamberlainia hainesiana*. Invertebrate Reproduction & Development, 24: 157–160.

Panha, S. and Kosavititkul, P. 1997. Mantle transplantations in freshwater pearl mussel in Thailand. Aquaculture International, 5: 267–276.

Pfeiffer, J.M., Breinholt, J.W. and Page, L.M. 2019. Unioverse: A phylogenomic resource for reconstructing the evolution of freshwater mussels (Bivalvia, Unionoida). Molecular Phylogenetics and Evolution, 137: 114–126.

Pfeiffer, J.M., Graf, D.L., Cummings, K.S. and Page, L.M. 2018. Molecular phylogeny and taxonomic revision of two enigmatic freshwater mussel genera (Bivalvia: Unionidae incertae sedis: *Harmandia* and *Unionetta*) reveals a diverse clade of Southeast Asian Parreysiinae. Journal of Molluscan Studies, 84: 404–416.

Pfeiffer, J.M., Graf, D.L., Cummings, K.S. and Page, L.M. 2021. Taxonomic revision of a radiation of Southeast Asian freshwater mussels (Unionidae: Gonideinae: Contradentini+Rectidentini). Invertebrate Systematics, 35: 394–470.

Rafinesque, C.S. 1820. Monographie des coquilles bivalves fluviatiles de la Riviere Ohio, contenant douze genres et soixante-huit especes. Annales Générales des Sciences Physiques, 5(13): 287–322.

Rochebrune, A.-T. de. 1882. Documents sur la faune malacologique de la Cochinchine et du Cambodge. Bulletin de la Société Philomathique de Paris, 7(1): 35–74.

Rochebrune, A.-T. de. 1904. Essai monographique sur le genre *Chamberlainia* Simpson. Bulletin du Muséum National d’Histoire Naturelle, 10: 463–466.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology, 61(3): 539–542.

Sano, I., Saito, T., Miyazaki, J.-I, Shirai, A., Uechi, T., Kondo, T. and Chiba, S. 2020. Evolutionary history and diversity of unionoid mussels (Mollusca: Bivalvia) in the Japanese Archipelago. Plankton and Benthos Research, 15(2): 97–111.

Sano, I., Shirai, A., Kondo, T. and Miyazaki, J.-I. 2017. Phylogenetic relationships of Japanese unionoida (Mollusca: Bivalvia) based on mitochondrial 16S rDNA sequences. Journal of Water Resource and Protection, 9(5): 493–509.

Simpson, C.T. 1900. Synopsis of the naiades, or pearly fresh-water mussels. Proceedings of the United States National Museum, 22: 501–1044.

Simpson, C.T. 1914. A descriptive catalogue of the naiades, or pearly fresh-water mussels. Parts I–III. 1540 pp.

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9): 1312–1313.

Zieritz, A., Bogan, A.E., Froufe, E., Klishko, O., Kondo, T., Kovitvadhi, U., Kovitvadhi, S., Lee, J.H., Lopes-Lima, M., Pfeiffer, J.M., Sousa, R., Do, V.T., Vikhrev, I. and Zanatta, D.T. 2018. Diversity, biogeography and conservation of freshwater mussels (Bivalvia: Unionida) in East and Southeast Asia. Hydrobiologia, 810: 29–44.