

Description of a new freshwater mussel species of *Pletholophus*, Simpson, 1900 (Bivalvia, Unionidae) from Guangdong, China

Yu-Ting Dai^{1*}, Zhong-Guang Chen^{1*}, Cheng-Lin Hu¹, Shan Ouyang¹, Xiao-Chen Huang¹, Xiao-Ping Wu¹

¹ School of Life Sciences, Nanchang University, Nanchang 330031, China

<https://zoobank.org/C9F1030C-0564-4960-9378-38D9848ACE05>

Corresponding authors: Xiao-Chen Huang (xiaochenhuang@hotmail.com), xchuang@ncu.edu.cn); Xiao-Ping Wu (xpwu@ncu.edu.cn)

Academic editor: Matthias Glaubrecht ♦ Received 2 July 2024 ♦ Accepted 5 August 2024 ♦ Published 28 August 2024

Abstract

The Pearl River Basin, China's second-largest freshwater basin, hosts a significant diversity of species and a highly endemic freshwater mussel fauna. In this study, a new species from the Liuxi River in Guangzhou, Guangdong, China, *Pletholophus guangzhouensis* **sp. nov.**, is described based on morphological diagnostic features and molecular phylogenetics. The glochidia shells of the new species are subtriangular, medium-sized, and have a styliform hook on the ventral angle of each valve. Phylogenetic analyses based on the *COI* and 28S rRNA gene fragments indicated that *Pletholophus guangzhouensis* **sp. nov.** is the sister to *Pletholophus tenuis* + *Pletholophus reinianus*. The pairwise uncorrected *COI* *p*-distance analysis demonstrated genetic distances ranging from 5.27% (between *P. guangzhouensis* **sp. nov.** and *P. tenuis*) to 11.06% (between *P. guangzhouensis* **sp. nov.** and *P. honglinhensis*). Our findings suggest a significant underestimation of the diversity of freshwater mussel species in Guangdong. Further field collections and systematic studies are necessary to fully explore the biodiversity of this region. Furthermore, integrative classification methods and genetic research are essential for informing the development of effective conservation strategies.

Key Words

conservation, glochidia, molecular systematics, morphological characters, taxonomy

Introduction

Freshwater bivalves (Bivalvia, Unionidae) are well-known for providing important ecosystem functions and services, including nutrient cycling, habitat structure, substrate and food web modification, and serving as environmental monitors (Vaughn 2018). Furthermore, their stable biogeography, characterized by low dispersal and restriction to freshwater habitats, makes them invaluable for elucidating past geological and hydrological events (Zieritz et al. 2021). The life cycle of Unionidae is unique, involving parasitic larvae (glochidia) that must attach to vertebrate hosts, primarily freshwater fish, before becoming sessile

adults. This distinctive life cycle has likely contributed significantly to the rapid diversification of this group (Barnhart et al. 2008). However, freshwater mussels represent one of the most threatened faunal groups on a global scale (Böhm et al. 2021), as they are highly impacted by human activities, climate change, and water loss (Aldridge et al. 2022). In recent decades, freshwater mussels have experienced a significant decline, with both species loss and reductions in abundance (Karatayev et al. 2012; Vaughn 2018). This highlights the importance of further research into their diversity, distribution, and evolution.

China exhibits both high species diversity and a highly endemic mussel fauna (Zieritz et al. 2018; Liu et al.

* These authors contributed equally to this paper.

2022). Nevertheless, field investigations and studies of freshwater bivalves in China exhibit a geographic bias, with the majority of research concentrated in the middle and lower reaches of the Yangtze River (Liu et al. 2022; Wu et al. 2022). In recent years, an expanding body of research has revealed that the Pearl River Basin, China's second-largest freshwater basin, hosts numerous previously unidentified and distinct species (Dai et al. 2023). For example, several new species have recently been discovered in the Guangxi Zhuang Autonomous Region, situated along the Pearl River Basin, including *Postolata guangxiensis* Dai, Huang, Guo & Wu, 2023; *Pseudocuneopsis yangshuoensis* Wu & Liu, 2023; *P. wuana* Liu & Wu, 2023; and *P. longjiangensis* Liu & Wu, 2024 (Dai et al. 2023; Dai et al. 2024; Liu et al. 2024). This observation prompted the hypothesis that Guangdong Province, another significant region through which the Pearl River Basin flows, may also be rich in unique species. However, there is a paucity of mussel diversity surveys and studies in Guangdong, particularly over the past decade (Liu and Duan 1991; Hu 2005; He and Zhuang 2013; Zhang et al. 2013; Dong et al. 2017).

Pletholophus Simpson, 1900, belongs to the Unioninae Rafinesque, 1820, in the family Unionidae Rafinesque, 1820. This genus was established by Simpson (1900) as a subgenus of *Cristaria* Schumacher, 1817, with *Cristaria (Pletholophus) discoidea* (Lea, 1834) (by original designation) designated as the type species. Đặng et al. (1980) elevated *Pletholophus* to the generic level and included three species: *Pletholophus swinhoei* (Adams, 1866), *Pletholophus inangulatus* (Haas, 1910a), and *Pletholophus discoideus* (Lea, 1834). All of these are considered synonyms of *Cristaria tenuis* (He & Zhuang, 2013). Based on the *COI* + *28S* rRNA phylogenies, the species *Cristaria tenuis* (Griffith & Pidgeon, 1833) has been recently reassigned to *Pletholophus* and separated from *Cristaria* (Lopes-Lima et al. 2017). Lopes-Lima et al. (2020) considered *Pletholophus reinianus* (Martens, 1875) to be a valid species based on the analysis of *COI* and *28S* rRNA gene fragments. Recently, Bogan et al. (2023) summarized the taxonomy and diversity of Anodontini in Vietnam, identifying a new species, *Pletholophus honglinhensis* Bogan, Do, Froufe & Lopes-Lima, 2023, based on molecular and morphological evidence. Currently, the genus is recognized to comprise three valid species: *Pletholophus tenuis* (Griffith & Pidgeon, 1833), *Pletholophus reinianus* (Martens, 1875), and *Pletholophus honglinhensis* Bogan, Do, Froufe & Lopes-Lima, 2023. *Pletholophus tenuis* has been recorded as widespread in southeastern Asia, ranging from the Yangtze River south to Cambodia (Bogan et al. 2023). In contrast, the distribution of *P. reinianus* and *P. honglinhensis* is more restricted, with *P. reinianus* endemic to southern Japan and *P. honglinhensis* found exclusively in a coastal basin south of Hanoi, Vietnam (Lopes-Lima et al. 2020; Bogan et al. 2023).

In this study, we discovered a distinct species of freshwater mussel in the Liuxi River, Guangzhou, China. After

examining the shell morphology of this unique species, as well as referring to the literature (e.g., Heude 1875, 1877a, 1877b, 1878, 1879, 1880a, 1880b, 1881, 1883, 1885; Simpson 1900, 1914; Haas 1969; Brandt 1974; Liu et al. 1979; He and Zhuang 2013) and MUSSELp online database (see <http://mussel-project.uwsp.edu>), we were unable to match it to any of the recorded species. Subsequently, a new species of *Pletholophus* was described based on a combination of morphological characters and the *COI* + *28S* rRNA gene phylogenies. We provide morphological descriptions, glochidia descriptions, localities, and photographs for this new species.

Materials and methods

Specimen sampling, identification, and deposition

In January 2021, a total of 10 specimens were collected from the Liuxi River (23°32'02"N, 113°35'03"E) in Guangzhou City, Guangdong, China (Figs 1, 2). A digital vernier caliper with an accuracy of ± 0.01 mm was used to measure the length, height, and width of the type series of the new taxa. Live specimens were euthanized with 100% ethanol and then separated into soft tissue and shells. The adductor muscle was used for subsequent DNA extraction. The remaining soft parts were preserved at -80 °C. All voucher specimens were deposited in the Museum of Biology, Nanchang University (NCUMB), China.

Scanning electron microscopy of glochidia

The glochidial mass was stored in 96% ethanol and subsequently washed with deionized water. It was then transferred into a 5% NaOH solution and allowed to rest for approximately two hours to remove any residual tissue. Following the deionized water wash, the glochidia were observed under an optical microscope to ascertain their cleanliness and the integrity of their shells. The sample preparation process was completed using anhydrous ethanol for storage purposes. Prior to scanning electron microscopy, the samples were dried in a clean environment for a minimum of eight hours, after which their surfaces were sprayed with gold. Subsequently, the samples were subjected to examination via scanning electron microscopy (SEM) (Quanta 200FEG03040702, USA) (Shu and Wu 2005b; Sayenko et al. 2023).

Molecular phylogenetic analyses

The Qiagen Genomic DNA kit (Qiagen, Hilden, Germany) was employed to extract total genomic DNA from the excised tissue following the instructions provided by the manufacturer. The quality and concentration of the DNA



Figure 1. Distribution map of *Pletholophus guangzhouensis* sp. nov.

were checked using 1% agarose gel electrophoresis and NanoDrop 2000 (Thermo Scientific, USA). We amplified and sequenced fragments from the mitochondrial cytochrome *c* oxidase subunit-I gene (*COI*) (LCO22me2 + HCO700dy2) (Walker et al. 2007) and the nuclear 28S ribosomal RNA gene (28S) (D23F + D4RB) (Park and Foighil 2000). The polymerase chain reaction (PCR) was conducted using a 25 μ L mixture of 2 \times Taq Plus Master MixII (Vazyme, China) (12.5 μ L), ddH₂O (9.5 μ L), 10 μ M primers (1 μ L each), and genomic DNA (1 μ L, about 100 ng/ μ L). Thermal cycling was started at 98 $^{\circ}$ C for 10 s, followed by 35 cycles of 94 $^{\circ}$ C for 1 min, annealing at 50 $^{\circ}$ C for 1 min, extension at 72 $^{\circ}$ C for 1 min, and then a final extension at 72 $^{\circ}$ C for 7 min. The PCR products were sequenced commercially by Sangon Biotech (Shanghai, China). The newly obtained sequences have been deposited in GenBank, and their accession numbers are provided in Table 1.

Two datasets were constructed in this study: (i) the *COI* dataset (11 sequences; 600 bp); and (ii) the *COI* + 28S rRNA dataset (67 sequences; 1,009 bp) (Table 1).

All PCGs were codon-aligned by MUSCLE ver. 3.6 (<https://www.drive5.com/muscle/>; Edgar 2004) implemented in MEGA ver. 10.1.6 (<http://www.megasoftware.net>; Kumar et al. 2018), whereas 28S rRNA were aligned in MAFFT ver. 7 (<https://mafft.cbrc.jp/alignment/software/>; Katoh et al. 2019) using the Q-INS-i algorithm. We used Gblocks ver. 0.91b (<http://gensoft.pasteur.fr/docs/gblocks/0.91b/>; Castresana 2000) to ex-

clude ambiguous areas of the alignment for each gene. DnaSP ver. 6 (<http://www.ub.edu/dnasp/>; Rozas et al. 2017) was used to calculate the number of haplotypes. The best-fit model for each gene and gene partition was calculated by PartitionFinder2 ver. 2.3.4 (Lanfear et al. 2017), based on the corrected Akaike Information Criterion (AICc) and using a heuristic search algorithm. The program proposed the division of the concatenated dataset into three partitions, comprising partitions for the 28S gene and each of the three codon positions of the *COI* gene. The best-fit model was determined to be GTR + I + G for the first and third codon positions of *COI*, GTR for the second position of *COI*, and GTR + G for 28S.

Maximum-likelihood (ML) analyses were performed in IQ-TREE (Nguyen et al. 2015) with the ML + rapid bootstrapping method and 10,000 replicates. Bayesian inference (BI) analyses were conducted in MrBayes (Ronquist et al. 2012). Four simultaneous runs with four independent Markov Chain Monte Carlo (MCMC) algorithms were implemented for 10 million generations, and trees were sampled every 1000 generations with a burn-in of 25%. The convergence was checked with the average standard deviation of split frequencies < 0.01 and the potential scale reduction factor (PSRF) \sim 1.

Inter- and intra-specific distances based on the *COI* dataset were calculated in MEGA X using the uncorrected *p*-distance. Standard error estimates were obtained by 1000 bootstrapping replicates.

Table 1. List of sequences used in phylogenetic analyses. (*) Sequenced from this study.

Taxon	COI	28S rRNA	Country
UNIONIDAE Rafinesque, 1820			
Unioninae Rafinesque, 1820			
Cristariini Lopes-Lima, Bogan, & Froufe, 2017			
<i>Amuranodonta kijaensis</i> Moskvicheva, 1973	MK574204	MK574473	Russia
<i>Anemina arcaeformis</i> (Heude, 1877)	MG462936	MG595463	China
<i>Beringiana beringiana</i> (Middendorff, 1851)	MT020557	MT020799	Japan
<i>Beringiana japonica</i> (Clessin, 1874)	MT020576	MT020803	Japan
<i>Beringiana fukuharai</i> San, Hattori & Kondo, 2020	MT020567	MT020801	Japan
<i>Beringiana gosannensis</i> San, Hattori & Kondo, 2020	MT020584	MT020802	Japan
<i>Buldowskia flavotincta</i> (Martens, 1905)	MT020537	MT020804	South Korea
<i>Buldowskia suifunica</i> (Lindholm, 1925)	MK574190	MK574460	Russia
<i>Buldowskia iwakawai</i> (Suzuki, 1939)	MT020523	MT020806	Japan
<i>Buldowskia kamiyai</i> San, Hattori & Kondo, 2020	MT020525	MT020808	Japan
<i>Buldowskia shadini</i> (Moskvicheva, 1973)	MK574197	MK574467	Russia
<i>Cristaria bellua</i> (Morelet, 1866)	ON704642	ON695893	Laos
<i>Cristaria clessini</i> (Kobelt, 1879)	MT020592	MT020810	Japan
<i>Cristaria plicata</i> (Leach, 1814)	MG462956	MG595484	China
<i>Cristaria truncata</i> Dang, Thai & Pham, 1980	OP491287	OP499826	Vietnam
<i>Pletholophus honglinhensis</i> Bogan, Do, Froufe & Lopes-Lima, 2023	OR912962	OR913009	Vietnam
<i>Pletholophus reinianus</i> (Martens, 1875)	MT020603	n/a	Japan
<i>Pletholophus tenuis</i> (Griffith & Pidgeon, 1833)	KX822658	KX822614	Vietnam
<i>Pletholophus tenuis</i> (Griffith & Pidgeon, 1833)	MT020599	LC519084	Japan
<i>Pletholophus tenuis</i> (Griffith & Pidgeon, 1833)	MT020600	LC519085	Japan
<i>Pletholophus tenuis</i> (Griffith & Pidgeon, 1833)	MT020601	KX822614	Japan
<i>Pletholophus tenuis</i> (Griffith & Pidgeon, 1833)	MT020602	KX822614	Japan
<i>Pletholophus guangzhouensis</i> Dai, Chen, Huang & Wu, sp. nov.*	PP945818	PP956591	China
<i>Pletholophus guangzhouensis</i> Dai, Chen, Huang & Wu, sp. nov.*	PP945819	PP956591	China
<i>Pletholophus guangzhouensis</i> Dai, Chen, Huang & Wu, sp. nov.*	PP945820	PP956591	China
<i>Pletholophus guangzhouensis</i> Dai, Chen, Huang & Wu, sp. nov.*	PP945821	PP956591	China
<i>Sinanodonta angula</i> (Tchang, Li & Liu, 1965)	MG463053	MG595580	China
<i>Sinanodonta calipygos</i> (Kobelt, 1879)	MT020623	MT020833	Japan
<i>Sinanodonta lauta</i> (Martens, 1877)	MT020616	MT020834	Japan
<i>Sinanodonta lucida</i> (Heude, 1877)	MG463066	MG595589	China
<i>Sinanodonta schrenkii</i> (Lea, 1870)	MT020618	MT020837	South Korea
<i>Sinanodonta tumens</i> (Haas, 1910)	MT020622	MT020838	Japan
<i>Sinanodonta pacifica</i> (Heude, 1878)	MG463052	MG595599	China
<i>Sinanodonta woodiana</i> (Lea, 1834)	MG463080	MG595608	China
Parreysiinae Henderson, 1935			
<i>Scabies crispata</i> (Gould, 1843)	MG288632	MG552824	Thailand
<i>Trapezidens exolescens</i> (Gould, 1843)	KX230532	KX230559	Thailand
MARGARITIFERIDAE Henderson, 1929			
<i>Gibbosula laosensis</i> (Lea, 1863)	JX497731	KT343741	Laos
<i>Margaritifera margaritifera</i> (Linnaeus, 1758)	KX550089	KX550093	Russia

Results

Molecular analyses

Four *COI* haplotypes and one *28S* haplotype were identified in the 10 sequenced specimens from Guangzhou, Guangdong. The *COI* dataset had an aligned length of 600 characters, with 95 variable sites and 42 parsimony informative sites. The *COI* + *28S* dataset, which had undergone trimming and concatenation, consisted of 1,009 characters, comprising 600 bp of *COI* and 409 bp of *28S*. There were 383 variable sites and 307 parsimony informative sites.

The ML and BI trees based on the *COI* + *28S* dataset exhibited largely congruent topologies, except for two nodes containing polytomies in the BI tree (Fig. 3). In both trees, *Pletholophus* Simpson, 1900, occupied a

distinct position in the subfamily Unioninae and was the sister group with *Sinanodonta* + *Beringiana* (BS/BPP = 98/1.0) (Fig. 3). Within *Pletholophus*, specimens from Guangzhou, Guangdong, represent a distinct taxon and were recovered as sisters to *Pletholophus tenuis* + *Pletholophus reinianus*, with high nodal support (BS/BPP = 98/1). The pairwise uncorrected *COI* *p*-distance analysis demonstrated genetic distances ranging from 5.27% (between this species and *P. tenuis*) to 11.06% (between this species and *P. honglinhensis*) (Table 2). This species shared a closer relationship with *P. tenuis*. It occupies a unique phylogenetic position and displays distinctive morphological characteristics (Fig. 3; Table 3), which are described herein as *Pletholophus guangzhouensis* sp. nov. Moreover, our results resolved the phylogenetic relationship within *Pletholophus* as (*P. honglinhensis* + (*P. guangzhouensis* sp. nov. + (*P. tenuis* + *P. reinianus*))).

Table 2. Average intraspecific (bold) and interspecific uncorrected *p*-distance (% ± S.E.) for *COI* sequences of species in *Pletholophus* Simpson, 1900.

Taxa	1	2	3	4
1. <i>P. guangzhouensis</i> sp. nov.	0.42 ± 0.18			
2. <i>P. tenuis</i>	5.27 ± 0.89	0.67 ± 0.20		
3. <i>P. reinianus</i>	5.30 ± 0.93	5.68 ± 0.93	n/c	
4. <i>P. honglinhensis</i>	11.06 ± 1.32	10.75 ± 1.25	11.52 ± 1.32	n/c

Table 3. Analyzed conchological characters of *Pletholophus* species. Characteristic descriptions of *P. tenuis*, *P. reinianus*, and *P. honglinhensis* are referenced from published works (Simpson 1900; Haas 1969; He and Zhuang 2013; Bogan et al. 2023) and the MUSSELp online database.

Conchological features	<i>P. guangzhouensis</i>	<i>P. honglinhensis</i>	<i>P. reinianus</i>	<i>P. tenuis</i>
Shell shape	Oval	Slightly rectangular to elongate oval	Elliptical or slightly rhomboid	Evenly elliptical
Shell thickness	Thin	Thin	Rather thin	Thin but strong
Shell color	Greenish-yellow in young individuals, darkish-brown in old individuals	Brown to black	Greenish or brownish	Yellowish-green
Umbo	1/4 of shell length, compressed, as high as hinge line	1/3 of shell length, inflated, not elevated above the dorsal margin	1/3 of shell length, compressed, as high as hinge line	1/3 of shell length, compressed, as high as hinge line
Umbo cavity	Rather shallow, open	Shallow, open	Rather shallow, open	Shallow, open
Posterior ridge	Developed	Prominent but not sharp	Developed	Almost wanting
Surface sculpture	Fine and dense growth lines; two faint ridge on the posterior dorsal; a few elegant, feebly rays	Growth lines	Three faint darker ridges; on the posterior slope with a few slight plications; finer growth lines	Feebly rayed throughout; finer growth lines
Pseudocardinal teeth	Reduced to mere raised threads	One long, thin lamellar tooth	Linear pseudocardinal incach valve	Wanting or reduced to mere raised threads
Lateral teeth	One tooth on both valves, long and narrow	Right valve with a long, narrow lateral tooth; left valve with a straight and well developed tooth	Anterior tooth well developed, posterior tooth reduced	One tooth, high and triangular
Nacre colour	Bluish-white, iridescent	White, becoming bluish-iridescent toward the posterior margin	One tooth on both valves, slender Bluish-white	One tooth on both valves scarcely developed Bluish-white, iridescent behind

Taxonomy

Family Unionidae Rafinesque, 1820

Subfamily Unioninae Rafinesque, 1820

Tribe Cristariini Lopes-Lima, Bogan & Froufe, 2017

Genus *Pletholophus* Simpson, 1900

Type species. *Pletholophus tenuis* (Griffith & Pidgeon, 1833)

***Pletholophus guangzhouensis* Dai, Chen, Huang & Wu, sp. nov.**

<https://zoobank.org/E435D35E-CE14-4F27-A726-6346AA0ECF3A>

Fig. 2

Material examined. *Holotype* CHINA • ♀; Guangdong, Guangzhou City, Conghua District, Liuxi River; 23°32'02"N, 113°35'03"E; 9 January 2021; leg. local people; ex. Y. T. Dai & L. Guo; 24_NCU_XPWU_PGU01.

Paratypes CHINA • 9 shells; same collection data as for the holotype; specimen vouchers are shown in Table 4.

Diagnosis. Periostracum greenish-yellow in young individuals, darkish-brown in old individuals; with fine and dense growth lines and two faint ridges on the posterior dorsal; periostracum often painted with a few elegant, feebly rays. Hinge undeveloped. Beak cavities shallow, open. In both valves, only one pseudocardinal and lateral tooth. Pseudocardinal teeth reduced to mere raised threads, lateral teeth long and narrow. Nacre bluish-white, iridescent. Glochidia hooked, subtriangular in shape, medium size, shell length less than shell height. The surface of glochidia have deep and dense small holes.

Shell description. Shell medium-sized, not inflated, thin but strong. Length 34.47–51.19 mm, width 6.55–16.21 mm, height 22.86–33.76 mm (Table 4). Shell ovoid, anterior rounded, short, posterior long and wide, slightly obtuse angle, posterior ridge developed. Umbo not prominent, compressed, as high as dorsal margin, located at 1/4 of the dorsal margin, and often eroded. Dorsal margin straight, rear end curved down-wards,

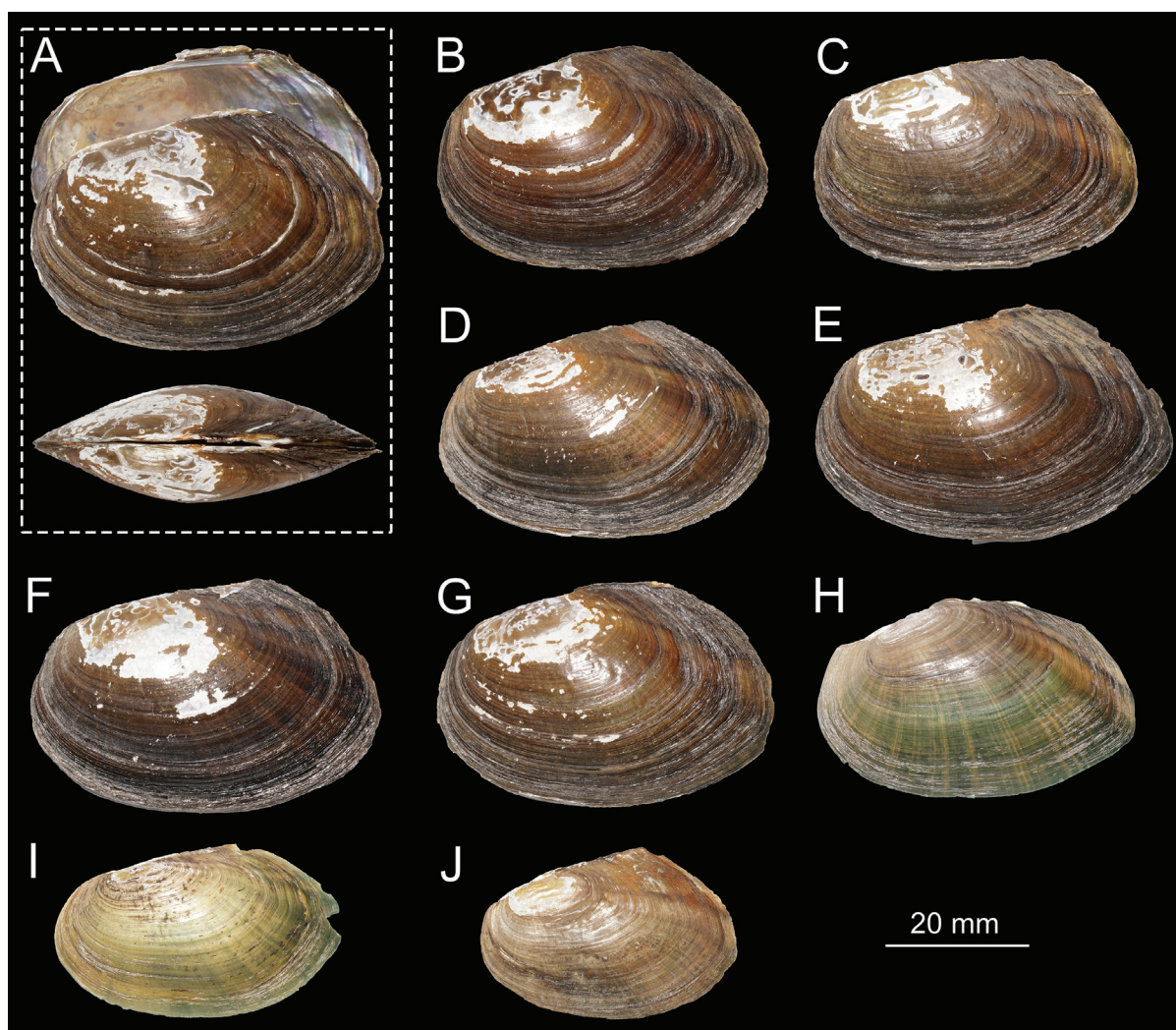


Figure 2. The shell morphology of *Pletholophus guangzhouensis* sp. nov. **A.** holotype; **B–J.** paratypes.

with a low wing behind; ventral margin weakly curved. Periostracum greenish-yellow in young individuals, darkish-brown in old individuals; with fine and dense growth lines and two faint ridges on the posterior dorsal; periostracum often painted with a few elegant, feebly rays. Lines arranged in irregular concentric circles. Hinge undeveloped. Beak cavities shallow, open. In both valves, only one pseudocardinal and lateral tooth. Pseudocardinal teeth reduced to mere raised threads, and lateral teeth long and narrow. Mantle attachment scars on the edge of shells obvious. Both anterior adductor muscle scars and posterior adductor muscle scars shallow, irregularly crescent-shaped. Nacre bluish-white, iridescent.

Glochidia morphology description. Glochidial shells typically anodontin hooked shells and subtriangular in shape, with the ventral angle slightly protruding dorsally. Medium size, length 0.226 ± 0.003 mm, height 0.247 ± 0.015 , shell length less than shell height. The ventral angle of each glochidia valve with an anchor-shaped styli-form hook. The hook covered by lanceolate macrospines arranged in 2–3 diagonal rows near the ventral terminus and reduced to a single row distally. Microspines and micropoints cover the entire ventral terminus and less than

one-third of the hook lateral lobes. The fossae on the shell surface deep and dense, with distinct small holes.

Etymology. The name of this species is derived from Guangzhou City, in which its type locality is located. For the common name of *Pletholophus guangzhouensis*, we recommend “Guangzhou micro tooth mussel” (English) and “Guang Zhou Wei Chi Bang” (广州微齿蚌) (Chinese).

Table 4. Shell measurements of *Pletholophus guangzhouensis* sp. nov. Measurements are in millimeters (mm).

Status of specimen	Specimen voucher	Shell length	Shell width	Shell height
Holotype	24_NCU_XPWU_PGU01	50.86	15.51	26.72
Paratype	24_NCU_XPWU_PGU02	50.56	15.42	33.76
Paratype	24_NCU_XPWU_PGU03	45.71	11.96	29.87
Paratype	24_NCU_XPWU_PGU04	49.32	15.41	33.11
Paratype	24_NCU_XPWU_PGU05	48.85	15.70	32.54
Paratype	24_NCU_XPWU_PGU06	51.19	16.21	33.22
Paratype	24_NCU_XPWU_PGU07	48.95	15.19	32.38
Paratype	24_NCU_XPWU_PGU08	47.46	14.98	31.02
Paratype	24_NCU_XPWU_PGU09	40.87	11.58	23.98
Paratype	24_NCU_XPWU_PGU10	34.47	6.55	22.86

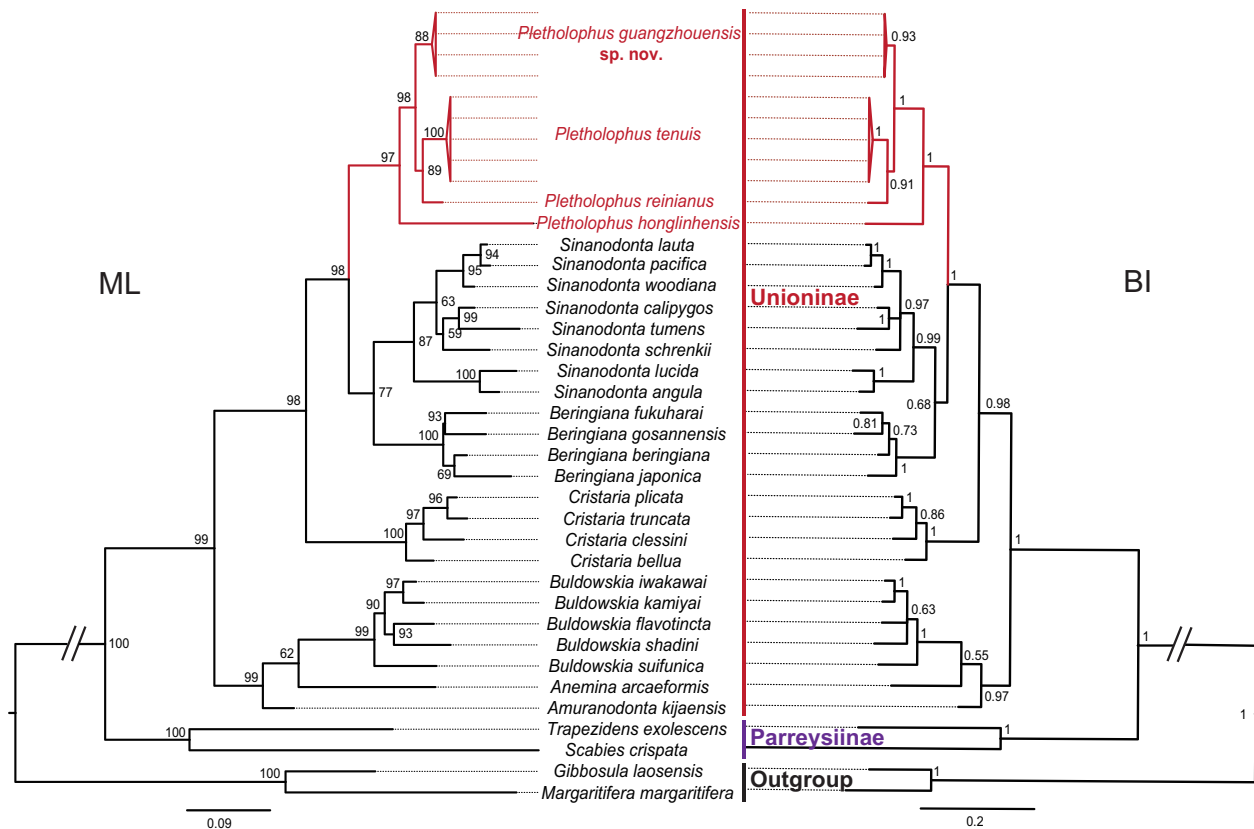


Figure 3. Maximum likelihood (ML) and Bayesian inference (BI) trees of Unionidae based on the *COI + 28S* dataset. *Gibbosula laosensis* and *Margaritifera margaritifera* from the family Margaritiferidae were used as outgroups. Support values above the branches are the posterior probability and bootstrap support, respectively.

Distribution. The species is endemic to the Liuxi River, located in Conghua District, Guangzhou City, Guangdong Province.

Discussion

Our morphological and molecular analyses provide compelling evidence that the freshwater mussels from Guangzhou, Guangdong, represent a new species of *Pletholophus* within the tribe Cristariini of the subfamily Unioninae. Species belonging to the Cristariini exhibit high levels of cryptic diversity, rendering it challenging to distinguish them based solely on morphological characteristics (He and Zhuang 2013; Lopes-Lima et al. 2020; Bogan et al. 2023). Our study has once again highlighted the importance of utilizing an integrative approach in generic classification. In our phylogenetic trees, *Pletholophus guangzhou* sp. nov. formed a well-supported clade in *Pletholophus* and has large genetic distances from its congeneric species, supporting it as a distinct species (uncorrected *COI* *p*-distance = 5.27% ~ 11.06%; Table 2). The phylogenetic relationships of genera in the Cristariini align with previous studies in most topologies (Lopes-Lima et al. 2020; Bogan et al. 2023). Our *COI + 28S* phylogenies showed the position of *Buldowskia*, *Anemina*, and *Amuranodonta* at the base of Cristariini (Fig. 3).

Nevertheless, previous studies have inferred from the *COI + 28S* dataset that *Cristaria* was placed at the base of the clade in Cristariini (Lopes-Lima et al. 2020; Bogan et al. 2023). The incongruencies between topologies are likely due to incomplete lineage sorting, insufficient taxon sampling, and varying rates of genome evolution and mutation (Perkins et al. 2017). To resolve the intergeneric relationships within this tribe, it is recommended that more comprehensive taxon sampling and an increased number of informative loci be utilized.

The morphologic analysis is in alignment with the molecular data. *Pletholophus* is distinguished from other genera in Cristariini by its slender pseudocardinal teeth. For example, *Sinanodonta* lacks any evidence of hinge teeth, while *Cristaria* typically possesses only well-developed lateral teeth (Simpson 1914; Bogan et al. 2023). The new species, *Pletholophus guangzhou* sp. nov., can be distinguished from its congeneric species by its oval shell shape, weakly curved ventral margin, faint rays, and two faint ridges on the posterior dorsal (Fig. 2; Table 3). *Pletholophus tenuis* is taller and has a more rounded ventral margin compared to other species within *Pletholophus*. In contrast, *P. honglinhensis* possesses a more elongated shell. Therefore, *P. tenuis* and *P. honglinhensis* can be readily distinguished from their congeneric species based on shell morphology. *Pletholophus guangzhou* sp. nov. is morphologically similar to *P. reinianus* but can be distinguished

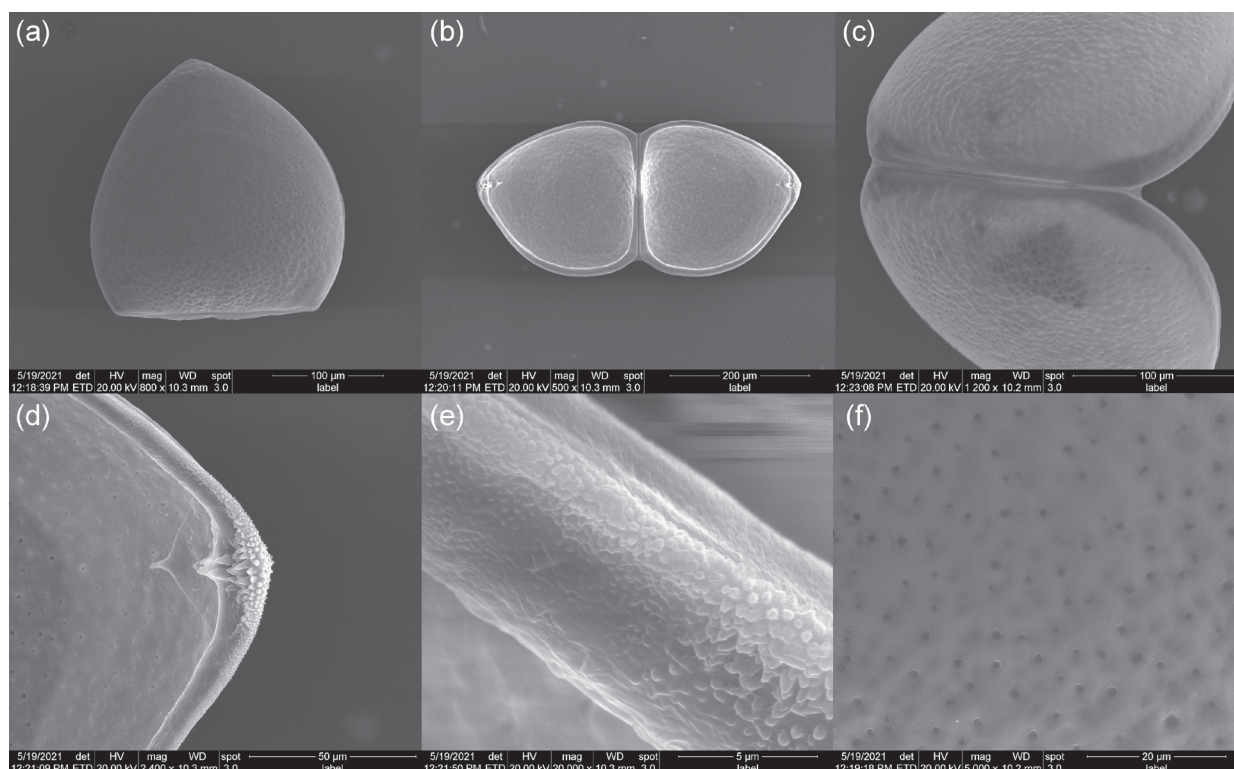


Figure 4. SEM microphotographs of *Pletholophus guangzhouensis* sp. nov. glochidia. **A.** Closed valves of glochidia; **B.** Open valves of glochidia; **C.** Hinge of glochidia; **D.** Hook of glochidia; **E.** Microspines on the ventral margin of glochidia; **F.** Pores on exterior glochidial valve surfaces.

by its more developed pseudocardinal teeth and the presence of two faint ridges (versus reduced pseudocardinal teeth and three faint darker ridges in *P. reinianus*).

In this study, we provide morphological descriptions of the glochidia of *Pletholophus guangzhou* sp. nov., which have proven useful for interpreting the phylogenetic relationships among freshwater mussels (Hoggarth 2000; Sayenko 2006; Sayenko et al. 2020). The glochidia shells of *P. guangzhou* are subtriangular, medium-sized, and have a styliform hook on the ventral angle of each valve (Fig. 4). These characteristics are consistent with those observed in the majority of species within the subfamily Unioninae (Wu et al. 1999a, 1999b; Ćmiel et al. 2021; Sayenko et al. 2023). The majority of Margaritiferidae species, as well as the Ambleminae and Gonideinae within the Unionidae, lack hooks (Shu and Wu 2005a; Xu et al. 2013; Wu et al. 2018; Vikhrev et al. 2019; Ćmiel et al. 2021). Furthermore, the glochidia of Margaritiferidae are notably small and semicircular, as observed in *Margaritifera dahurica* (Ćmiel et al. 2021) and *Gibbosula rochechouartii* (unpublished data from our laboratory). The size of glochidia can aid in taxonomic classification (Ćmiel et al. 2021), while their shape (including aspects such as symmetry and vertical/horizontal elongation) provides valuable taxonomic characteristics that can be utilized in the reconstruction of paleoenvironments (Pfeiffer and Graf 2015; Chernyshev et al. 2020). Given the plasticity of freshwater mussel shells, it is increasingly necessary to incorporate glochidia morphology and anatomical characters into mussel taxonomic studies.

In light of the ongoing global biodiversity loss, the assessment and monitoring of species, along with the detection of new species, are of paramount significance (Dai et al. 2024). The discovery of the new freshwater mussel taxon serves to confirm the high diversity and endemic nature of the mussel fauna in Guangdong. Nevertheless, the high levels of urbanization in the area may result in significant habitat loss for the mussels, thereby threatening their survival. Integrative classification methods and genetic research will inform the development of effective conservation strategies, enabling management based on a more accurate understanding of the unique evolutionary relationships of imperiled freshwater organisms.

Acknowledgments

We are grateful to Prof. Matthias Glaubrecht as well as two reviewers, Dr. Arthur Bogan and Dr. Ivan N. Bolotov, for their helpful comments. This study was supported by the National Natural Science Foundation of China (No. 32100354 and No. 31772412) and the Jiangxi Provincial Natural Science Foundation (No. 20232BAB205067).

References

- Đặng N, Thái T, Phạm V (1980) Identification of freshwater invertebrates of North Vietnam. Hanoi. Sciences and Technology Publishing Co, Vietnam.

- Aldridge DC, Ollard IS, Bernalaya YV, Bolotov IN, Douda K, Geist J, Haag WR, Klunzinger MW, Lopes-Lima M, Mlambo MC, Riccardi N, Sousa R, Strayer DL, Torres SH, Vaughn CC, Zajac T, Zieritz A (2022) Freshwater mussel conservation: A global horizon scan of emerging threats and opportunities. *Global Change Biology* 29(3): 575–589. <https://doi.org/10.1111/gcb.16510>
- Barnhart MC, Haag WR, Roston WN (2008) Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society* 27(2): 370–394. <https://doi.org/10.1899/07-093.1>
- Bogan AE, Do VT, Froufe E, Lopes-Lima M (2023) The Anodontini of Vietnam (Mollusca: Bivalvia: Unionidae: Unioninae) with the description of a new species. *Diversity* 15(6): 710. <https://doi.org/10.3390/d15060710>
- Böhm M, Dewhurst-Richman NI, Seddon M, Ledger SE, Albrecht C, Allen D, Bogan AE, Cordeiro J, Cummings KS, Cuttelod A (2021) The conservation status of the world's freshwater molluscs. *Hydrobiologia* 848(12): 3231–3254. <https://doi.org/10.1007/s10750-020-04385-w>
- Brandt RAM (1974) The non-marine aquatic Mollusca of Thailand. *Archiv für Molluskenkunde* 105: 1–423.
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular biology and evolution* 17(4): 540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- Chernyshev AV, Sayenko EM, Bogatov VV (2020) Superspecific Taxonomy of the Far Eastern Unionids (Bivalvia, Unionidae): Review and Analysis. *Biology Bulletin* 47(3): 267–275. <https://doi.org/10.1134/S1062359020010045>
- Ćmiel AM, Dolega J, Aldridge DC, Lipinska A, Tang F, Zajac K, Lopes-Lima M, Zajac T (2021) The size and shape of parasitic larvae of naiads (Unionidae) are not dependent on female size. *Scientific Reports* 11(1): 23755. <https://doi.org/10.1038/s41598-021-03143-9>
- Dai YT, Huang XC, Wu CHZ, Chen ZG, Guo L, Shu FY, Ouyang S, Wu XP (2023) Multilocus and mitogenomic phylogenetic analyses reveal a new genus and species of freshwater mussel (Bivalvia: Unionidae) from Guangxi, China. *Invertebrate Systematics* 37(2): 152–166. <https://doi.org/10.1071/IS22048>
- Dai YT, Chen ZG, Peng KJ, Ouyang S, Huang XC, Wu XP (2024) Revisiting the genus *Pseudocuneopsis* (Bivalvia, Unionidae): Morphology, mitochondrial phylogenomics, and the description of a new species. *Zoologica Scripta* 53 (3): 323–337. <https://doi.org/10.1111/zsc.12647>
- Dong XX, Xing YM, Shu FY (2017) A Preliminary Study on Species and Distributions of Freshwater Mollusca in Zhongshan City, South China. *Journal of Qufu Normal University* 43(1): 76–80.
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Haas F (1969) Superfamilia Unionacea. Walter de Gruyter, Germany.
- He J, Zhuang Z (2013) The Freshwater Bivalves of China. ConchBooks, Germany.
- Heude PM (1875) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Premier Fascicule. Librairie F. Savy, France. <https://doi.org/10.5962/bhl.title.14450>
- Heude PM (1877a) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Deuxième Fascicule. Librairie F. Savy, France.
- Heude PM (1877b) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Troisième Fascicule. Librairie F. Savy, France.
- Heude PM (1878) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Quatrième Fascicule. Librairie F. Savy, France.
- Heude PM (1879) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Cinquième Fascicule. Librairie F. Savy, France.
- Heude PM (1880a) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Sixième Fascicule. Librairie F. Savy, France.
- Heude PM (1880b) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Dixième Fascicule. Librairie F. Savy, France.
- Heude PM (1881) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Septième Fascicule. Librairie F. Savy, France.
- Heude PM (1883) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Huitième Fascicule. Librairie F. Savy, France.
- Heude PM (1885) Conchyliologie fluviatile de la province de Nanking et de la Chine centrale. Neuvième Fascicule. Librairie F. Savy, France.
- Hoggarth M (2000) Descriptions of some of the glochidia of the Unionidae (Mollusca: Bivalvia). *Malacologia* 41(1): 1–118.
- Hu ZQ (2005) Geographical Distribution of Endemic Species of Chinese Freshwater Bivalves. *Chinese Journal of Zoology* 40: 80–83.
- Karatayev AY, Miller TD, Burlakova LE (2012) Long-term changes in unionid assemblages in the Rio Grande, one of the World's top 10 rivers at risk. *Aquatic Conservation: Marine and Freshwater Ecosystems* 22(2): 206–219. <https://doi.org/10.1002/aqc.2226>
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20(4): 1160–1166. <https://doi.org/10.1093/bib/bbx108>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution* 34(3): 772–773. <https://doi.org/10.1093/molbev/msw260>
- Liu YY, Duan YH (1991) Two new species of freshwater mussel of the genus *Scabies* from China. *Acta Zootaxonomica Sinica* 16(3): 278–281.
- Liu YY, Zhang WZ, Wang YX, Wang EY (1979) Economic fauna of China: Freshwater mollusc. Science Press, China.
- Liu XJ, Liu YY, Wu RW, Zanatta DT, Lopes-Lima M, Goncalves DV, Bogan AE, Ouyang S, Wu XP (2022) Systematics, distribution, biology, and conservation of freshwater mussels (Bivalvia: Unionida) in China. *Aquatic Conservation: Marine and Freshwater Ecosystems* 32(5): 859–895. <https://doi.org/10.1002/aqc.3799>
- Liu L, Zhang L, Hou K, Ning L, Wu R (2024) Addition to the known diversity of Chinese freshwater mussels: integrative description of a new species of *Postolata* Dai et al., 2023 (Bivalvia, Unionidae, Gonideinae). *Zoosystematics and Evolution* 100(3): 769–778. <https://doi.org/10.3897/zse.100.126069>

- Lopes-Lima M, Froufe E, Do VT, Ghamizi M, Mock KE, Kebapçı Ü, Klishko O, Kovitvadhi S, Kovitvadhi U, Paulo OS, Pfeiffer JM III, Raley M, Riccardi N, Şerefişan H, Sousa R, Teixeira A, Varandas S, Wu X, Zanatta DT, Zieritz A, Bogan AE (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. <https://doi.org/10.1016/j.ympev.2016.08.021>
- Lopes-Lima M, Hattori A, Kondo T, Hee Lee J, Ki Kim S, Shirai A, Hayashi H, Usui T, Sakuma K, Toriya T, Sunamura Y, Ishikawa H, Hoshino N, Kusano Y, Kumaki H, Utsugi Y, Yabe S, Yoshinari Y, Hiruma H, Tanaka A, Sao K, Ueda T, Sano I, Miyazaki JI, Gonçalves DV, Klishko OK, Konopleva ES, Vikhrev IV, Kondakov AV, Yu Gofarov M, Bolotov IN, Sayenko EM, Soroka M, Zieritz A, Bogan AE, Froufe E (2020) Freshwater mussels (Bivalvia: Unionidae) from the rising sun (Far East Asia): phylogeny, systematics, and distribution. *Molecular Phylogenetics and Evolution* 146: 106755. <https://doi.org/10.1016/j.ympev.2020.106755>
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–74. <https://doi.org/10.1093/molbev/msu300>
- Park JK, Foighil DÓ (2000) Sphaeriid and Corbiculid Clams Represent Separate Heterodont Bivalve Radiations into Freshwater Environments. *Molecular Phylogenetics and Evolution* 14(1): 75–88. <https://doi.org/10.1006/mpev.1999.0691>
- Perkins MA, Johnson NA, Gangloff MM (2017) Molecular systematics of the critically-endangered North American spinymussels (Unionidae: Elliptio and Pleurobema) and description of *Parvaspina* gen. nov. *Conservation Genetics* 18(4): 745–757. <https://doi.org/10.1007/s10592-017-0924-z>
- Pfeiffer JMI, Graf DL (2015) Evolution of bilaterally asymmetrical larvae in freshwater mussels (Bivalvia: Unionida: Unionidae). *Zoological Journal of the Linnean Society* 175(2): 307–318. <https://doi.org/10.1111/zoj.12282>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Molecular Biology and Evolution* 34(12): 3299–3302. <https://doi.org/10.1093/molbev/msx248>
- Sayenko E (2006) Morphology of glochidia (Bivalvia: Unionidae: anodontinae, pseudanodontinae) of Russia. *Dal'nauka. Vladivostok, Primorye Territory, Russia*.
- Sayenko EM, Soroka M, Akiyama YB, Uechi T, Ito K, Kondo M (2020) Taxonomic status of genera *Nodularia*, *Middendorffinaia* and *Inversiumio* (Bivalvia: Unionidae) from South-East Asia: morphometric, genetic and GenBank data. *Systematics and Biodiversity* 19(1): 54–73. <https://doi.org/10.1080/14772000.2020.1844817>
- Sayenko EM, Nikischenko VE, Dyachuk VA (2023) Morphological description of the glochidia of *Buldowskia suifunica* (Bivalvia: Unionidae): External and internal structures and organ systems. *Scientific Reports* 13(1): 19777. <https://doi.org/10.1038/s41598-023-46894-3>
- Shu FY, Wu XP (2005a) A comparative observation on glochidial morphology of two unionid species (unionidae). *Shandong Science* 18(1): 14–15.
- Shu FY, Wu XP (2005b) Observation on Shell Surface of Glochidia of Two Freshwater Mussel Species (Unionidae) by SEM. *Life Science Research* 9(1): 73–76.
- Simpson CT (1900) Synopsis of the Naiades: or pearly fresh-water mussels. US Government Printing Office, USA. <https://doi.org/10.5962/bhl.title.935>
- Simpson CT (1914) Descriptive Catalogue Of The Naiades, Or Pearly Freshwater Mussels. Bryant Walker, USA. <https://doi.org/10.5962/bhl.title.10910>
- Vaughn CC (2018) Ecosystem services provided by freshwater mussels. *Hydrobiologia* 810(1): 15–27. <https://doi.org/10.1007/s10750-017-3139-x>
- Vikhrev IV, Makhrov AA, Artamonova VS, Ermolenko AV, Gofarov MY, Kabakov MB, Kondakov AV, Chukhchin DG, Lyubas AA, Bolotov IN (2019) Fish hosts, glochidia features and life cycle of the endemic freshwater pearl mussel *Margaritifera dahurica* from the Amur Basin. *Scientific Reports* 9(1): 8300. <https://doi.org/10.1038/s41598-019-44752-9>
- Walker JM, Bogan AE, Bonfiglio EA, Campbell DC, Christian AD, Currole JP, Harris JL, Wojtecki RJ, Hoeh WR (2007) Primers for amplifying the hypervariable, male-transmitted COII-COI junction region in ambelmine freshwater mussels (Bivalvia: Unionoidea: Ambelminae). *Molecular Ecology Notes* 7(3): 489–491. <https://doi.org/10.1111/j.1471-8286.2006.01630.x>
- Wu XP, Liang Y, Wang H (1999a) A comparative study on glochidial morphology of Unionidae (Bivalvia)-I. *Unio douglasiae*, *Cuneopsis pisciulus*, *Acuticosta chinensis* and *Acuticosta ovata*. *Acta Hydrobiologica Sinica* 23: 145–149. <https://doi.org/10.3724/issn1000-3207-1999-2-141-1>
- Wu XP, Liang Y, Wang H, Ouyang S (1999b) Morphological characters of glochidia of Unionidae and their taxonomic significance. *Acta Hydrobiologica Sinica* 23: 139–147. <https://doi.org/10.3724/issn1000-3207-1999-2-141-1>
- Wu RW, Chen TH, Zanatta DT, Ouyang S, Wu XP (2018) Reproductive traits of nine freshwater mussel species (Mollusca: Unionidae) from Poyang Lake, China. *Journal of Molluscan Studies* 84(3): 324–332. <https://doi.org/10.1093/mollus/eyy016>
- Wu XP, Dai YT, Yin N, Shu FY, Chen ZG, Guo L, Zhou CH, Ouyang S, Huang XC (2022) Mitogenomic phylogeny resolves *Cuneopsis* (Bivalvia: Unionidae) as polyphyletic: The description of two new genera and a new species. *Zoologica Scripta* 51(2): 173–184. <https://doi.org/10.1111/zsc.12527>
- Xu L, Wu XP, Ling C, Ouyang S, Xie GL (2013) Reproductive traits and glochidium Morphology of *Lamprotula leai* (Gray). *Journal of Nanchang University* 37(3): 263–265.
- Zhang MH, Xie GL, Xu L, Liu YY, Wu XP (2013) New Records of a Genus and a Species of Unionidae from China (*Pilsbryconcha compressa* von Martens, 1860). *Life Science Research* 17(6): 499–501.
- Zieritz A, Bogan AE, Froufe E, Klishko O, Kondo T, Kovitvadhi U, Kovitvadhi S, Lee JH, Lopes-Lima M, Pfeiffer JM, Sousa R, Van Do T, Vikhrev I, Zanatta DT (2018) Diversity, biogeography and conservation of freshwater mussels (Bivalvia: Unionida) in East and Southeast Asia. *Hydrobiologia* 810: 29–44. <https://doi.org/10.1007/s10750-017-3104-8>
- Zieritz A, Froufe E, Bolotov I, Gonçalves DV, Aldridge DC, Bogan AE, Gan HM, Gomes-Dos-Santos A, Sousa R, Teixeira A (2021) Mitogenomic phylogeny and fossil-calibrated mutation rates for all F- and M-type mtDNA genes of the largest freshwater mussel family, the Unionidae (Bivalvia). *Zoological Journal of the Linnean Society* 193(3): 1088–1107. <https://doi.org/10.1093/zoolin/zlaa153>