BMC Genomics

RESEARCH

Open Access

Comparative morphology and mitogenomics of freshwater mussels *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* (Bivalvia: Unionidae: Gonideinae)

Liping Zhang[1], Xiaoyan Liu[1], Xinyan Hu[2], Junli Jia[1], Xiongjun Liu[3] and Ruiwen Wu[1*]

Abstract

Background Amidst the escalating loss of global biodiversity, freshwater mussels (family Unionidae) have become one of the most imperiled animal groups. Acquiring more biological and phylogenetic information on understudied taxa constitutes a pivotal aspect of conservation biology. Consequently, a comprehensive examination was conducted on *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* from China encompassing morphology, anatomy, distribution, and molecular systematics to provide theoretical support for future species endangerment assessments and biodiversity conservation.

Results The shell characteristics of *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* were clearly distinct, and the soft-body morphology could also be easily distinguished from each other. The papillae of the incurrent aperture of *Sinosolenaia iridinea*, *Sinosolenaia recognita*, and *Sinosolenaia oleivora*, which were previously described as difficult, exhibited significant variations that could be utilized for species diagnosis. Furthermore, both incurrent and excurrent apertures of the *Sinosolenaia* species had small cysts on their dorsal surfaces which may be unique to this particular group. Comparative analysis of six mitochondrial genomes (*Parvasolenaia rivularis*, *Koreosolenaia sitgyensis*, *Sinosolenaia iridinea*, *Sinosolenaia recognita*, *Sinosolenaia carinata*, and *Sinosolenaia oleivora*) revealed a completely consistent gene arrangement pattern. Additionally, there was a high consistency in nucleotide base content and skewness, amino acid usage, and relative synonymous codon usage among the six complete mitochondrial genomes. Mitochondrial phylogenomics of these genomes with additional taxa within Gonideinae robustly supported the generic relationships as follows: (*Inversidens* + ((*Microcondylaea* + *Sinosolenaia*) + (*Parvasolenaia* + (*Koreosolenaia* + (*Ptychorhynchus* + (*Postolata* + *Cosmopseudodon*)))))).

Conclusions The present study provided significant data on the shell morphology and soft-body anatomy of *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia*, thereby clarifying the diagnostic characteristics for these challenging taxa. Additionally, we established a robust phylogenetic framework at both the generic and species levels based on mitochondrial genomics.

Keywords Shell morphology, Soft-body anatomy, Freshwater mussels, Unionidae, Gonideinae, Mitochondrial phylogenetics

*Correspondence:
Ruiwen Wu
wurw@sxnu.edu.cn
Full list of author information is available at the end of the article

Introduction

Freshwater mussels (order Unionida) are of practical and aesthetic interest due to their role as indicators of threatened inland waterways worldwide [1–4], as well as being ornamental, long-lived invertebrates with unique parasitic life cycles adapted for existence in flowing waters [5–8]. Unionidae is the most diverse family within Unionida, with more than 800 known species worldwide [8, 9]. The habitats of these organisms display a wide diversity, with some groups thriving in environments characterized by slow water flow, clear waters, and muddy substrates containing gravel particles, while others prefer habitats with fast currents, crystal-clear waters, and firm sedimentary substrate [10–13]. Adult unionids generally have limited mobility and mainly feed on plankton and other organic matter in the water [14]. Within the family Unionidae, there are groups that exhibit minimal movement throughout their lives by consistently embedding themselves in firm substrates, which is the case for *Solenaia* sensu lato [15–17]. This peculiar lifestyle makes this group more vulnerable to threats from habitat changes and climate extremes, such as drought [8, 18–20]. Accurate taxonomic and phylogenetic information can provide insights into species diversity levels and evolutionary history, which is of great significance for formulating strategies to conserve biodiversity [3, 21–23]. Over the past few years, molecular systematics, morphological anatomy, and reproductive biology have been studied for certain species in this group [17, 24, 25]; however, phylogenetic data is still lacking for other species, limiting our ability to understand the fundamental biology of these species that require conservation attention. Therefore, it is crucial to collect more biological information on understudied taxa to ensure that endangered biodiversity is not overlooked.

The genus *Solenaia sensu lato* was established by Conrad with the assignment of *Mycetopus emarginatus* Lea, 1860, as the type species [26]. Currently, the genus is recognized to include nine species (Table 1), which are endemic to East Asia and are distributed in China, India, Myanmar, and South Korea [27]. Initially, malacologists primarily focused on species descriptions and synonymy analyses based on shell morphological features [28–33]. However, the inherent variability of shell morphology has resulted in ongoing disputes regarding the species validity due to subjective interpretations by different scholars

Table 1 Classification history for *Solenaia sensu lato* species. Boldfaces indicate the taxa of this study

Simpson, 1900, 1914 [28, 29]	Haas, 1969 [30]	Graf & Cummings (2007) [32]	He & Zhuang, 2013 [33]	Huang et al. (2019) [35]	Lopes-Lima et al. (2020) [36]	Graf & Cummings (2021) [9]	Bolotov et al. (2021) [37]
Solenaia emarginatus (Lea, 1860) [26]	*Solenaia emarginata*	*Solenaia emarginata*				*Solenaia emarginata*	*Solenaia emarginata*
		Solenaia khwaenoiensis Panha & Deein, 2004				*Solenaia khwaenoiensis*	
Solenaia carinata (Heude, 1877) [45]	*Solenaia iridinea*		*Solenaia carinata*	*Solenaia carinata*	*Solenaia carinata*	*Solenaia carinata*	*Sinosolenaia carinata*
Solenaia iridinea (Heude, 1874) [46]		*Solenaia iridinea*	*Solenaia iridinea*			*Solenaia iridinea*	*Sinosolenaia iridinea*
Solenaia oleivora (Heude, 1877) [45] = *Micetopus recognitus* Heude, 1877				*Solenaia oleivora*	*Solenaia* cf. *oleivora*		*Sinosolenaia oleivora*
					Solenaia sp.		*Sinosolenaia recognita*
Solenaia rivularis (Heude, 1877) [45]			*Solenaia rivularis*	*Parvasolenaia rivularis*		*Parvasolenaia rivularis*	
Solenaia triangularis (Heude, 1885) [35]	*Solenaia triangularis*	*Solenaia triangularis*	*Solenaia triangularis*		*Parvasolenaia triangularis* 'China'	*Parvasolenaia triangularis*	
			Solenaia neotriangularis	*Parvasolenaia neotriangularis*		*Parvasolenaia neotriangularis*	
				Parvasolenaia triangularis 'South Korea'	*Koreosolenaia sitgyensis*		

when analyzing synonymy and proposing new species based on subtle differences in shell morphology [30, 32–34]. Starting from the 2020s, with increasing attention and the development of molecular techniques, significant advancements were achieved in the systematic investigation of this group [35–37]. Huang et al. [35] conducted a comprehensive phylogeny for Chinese freshwater mussels, and revealed that the genus *Solenaia* was actually polyphyletic. In light of shell morphology and phylogenetic position, a new genus named *Parvasolenaia* Huang & Wu, 2019 was established, with *Parvasolenaia rivularis* (Heude, 1877) designated as the type species. Additionally, *Parvasolenaia neotriangularis* (He & Zhuang, 2013) and *Parvasolenaia triangularis* (Heude, 1885) were reassigned to this newly proposed genus [35]. Subsequently, based on discernible disparities in shell morphology and geographical distribution patterns, Lopes-Lima et al. [36] proposed that *P. triangularis* found in China's Yangtze River Basin and South Korea should not be considered as the same species; instead, the population inhabiting South Korea represented an undescribed new species. Consequently, Lopes-Lima et al. [36] introduced a new genus and a new name for previously presumed *P. triangularis* in South Korea, i.e. *Koreosolenaia sitgyensis* Lee et al. 2020. The recent phylogenetic analyses revealed that both *Parvasolenaia* and *Koreosolenaia* belonged to the tribe Gonideini within the subfamily Gonideinae of the family Unionidae [36, 38–40]. However, with the addition of molecular data available for the type species *Solenaia emarginata*, molecular systematics revealed that this species was a member of the tribe Contradentini [34, 41], while the other *Solenaia* species, such as *Solenaia carinata* and *Solenaia oleivora*, belonged to the tribe Gonideini [35, 36, 42–44]. Therefore, based on the differences in tribe rank, Bolotov et al. [37] established a new genus called *Sinosolenaia* Bolotov et al. 2021 for the Chinese *Solenaia* lineage, with *Sinosolenaia recognita* (Heude, 1877) designated as the type species. Additionally, *Sinosolenaia carinata* (Heude, 1877), *Sinosolenaia iridinea* (Heude, 1874), and *Sinosolenaia oleivora* (Heude, 1877) were also assigned to this newly established genus [37]. Finally, by establishing multiple new genera (Table 1), the issue of polyphyly within *Solenaia sensu lato* has been successfully resolved.

However, the phylogenetic relationships of this group remain controversial. The phylogenetic tree (COI + 28S) constructed by Lopes-Lima et al. [36] revealed a sister relationship between *Koreosolenaia* and *Parvasolenaia*, which was consistent with the findings of Dai et al. [38] and Liu et al. [47], who utilized three genes (COI + 16S + 28S). However, Wu et al. [39, 40] established a phylogenetic relationship using five molecular markers (COI + ND1 + 16S + 18S + 28S), indicating that the two genera did not exhibit a sister relationship. These inconsistent phylogenetic relationships significantly impede our comprehension of the evolutionary interconnections among species. Furthermore, Bolotov et al. [37] reaffirmed the validity of *Sinosolenaia* species based on barcode data from Genbank; however, the interspecific phylogenetic relationships remain unresolved. Therefore, it is crucial to utilize substantial molecular data (e.g., mitochondrial genome) in order to clarify the systematic relationships at both the generic and species levels.

It is widely acknowledged that the morphological characteristics of mussels exhibit significant plasticity, leading to substantial disagreements regarding the classification of different taxa at various hierarchical levels and the validity of species [48–53]. *Sinosolenaia recognita*, *Sinosolenaia iridinea*, and *Sinosolenaia oleivora* were considered to be synonyms for a long time due to their similar shell morphology [15, 28–30, 33, 36]. Recently, Bolotov et al. [37] confirmed the validity of these three species based on COI barcode. However, more information about the three species, such as comparative conchology, anatomy and systematics, is still lacking.

Furthermore, there is a lack of precise species distribution information for these groups. The genera *Koreosolenaia*, *Sinosolenaia*, and *Parvasolenaia* exhibit distinct endemic patterns [35–37]. As far as is known, *Koreosolenaia* is endemic to Korea, while *Parvasolenaia* and *Sinosolenaia* are primarily distributed in China, specifically within the Yangtze River basin [27, 35–37]. Due to its vast geographical area, diverse water types, and complex hydrological environment, China serves as the primary hub for the distribution of freshwater mussels [8, 54]. This has resulted in a remarkable array of mussel species, including numerous endemic ones [38, 39, 55–57]. However, previous research on Chinese freshwater mussels predominantly focused on the Yangtze River basin, neglecting investigations into the diversity and distribution of these organisms across different regions of China [20, 35, 58]. This knowledge gap hampers our comprehensive understanding of precise species distribution patterns and paleobiogeography pertaining to specific endemic taxa.

Hence, this study conducted a comprehensive investigation on the species distribution of these groups in China, and achieved the following research objectives by integrating mitochondrial genomics, shell morphology, and soft-body anatomy: (1) to clarify the species distribution information of *Koreosolenaia*, *Sinosolenaia*, and *Parvasolenaia* in China; (2) to compare the morphology, anatomy, and mitogenomics distinguishing among genera and species; (3) to reconstruct the phylogenetic relationships based on mitochondrial genomics.

Materials and methods

Taxon sampling and morphological examination

In 2021–2024, specimens with tissues of *Sinosolenaia iridinea* [鸢尾华蛏蚌 (Chinese common name), Iris Solen Mussel (English common name)], *Sinosolenaia oleivora* [橄榄华蛏蚌 (Chinese common name), Olive Solen Mussel (English common name)], *Sinosolenaia recognita* [真华蛏蚌 (Chinese common name), Solen Mussel (English common name)], *Parvasolenaia rivularis* [河小蛏蚌 (Chinese common name), River Solen Mussel (English common name)], and *Koreosolenaia sitgyensis* [溪格韩蛏蚌 (Chinese common name), Sitgy Solen Mussel (English common name)] were collected from Liaoning, Henan, Hunan, Anhui, and Jiangxi provinces in China (Fig. 1; specific locations are shown in Supplementary Table S1). Only shells of *Sinosolenaia carinata* [龙骨华蛏蚌 (Chinese common name), Carinate Solen Mussel (English common name)] were collected from Gan River (Nanchang City) in Jiangxi Province. Species identification was based on the published literatures [28–30, 33, 45, 46, 59] and COI barcode data [36, 37]. Conchological and anatomical features were visually examined with the naked eye and under a stereoscopic microscope (SZX10, Olympus), including shell shape, surface sculpture, hinge structure, muscle attachment, and papillae in the incurrent and excurrent aperture. A Digital Vernier Calliper was used to measure shell length (L), shell width (W), and shell height (H1 and H2) with an accuracy of 0.01 mm (Fig. S1). Log-transformed variables were converted into three ratios: (H1-H2)/W, (H1-H2)/L, and W/L for the subsequent morphometric analyses. The definition of morphometric characteristics and soft-body anatomy was illustrated in Fig. S1. All voucher specimens were stored at the Museum of Zoology, Shanxi Normal University, Taiyuan City, China.

DNA extraction, PCR, sequencing, and mitogenome assembly

Doubly mitochondrial inheritance in freshwater mussels involves two types of mitochondrial DNA (mtDNA): maternal (F-type) and paternal (M-type) mtDNA [60–62]. The M-type is found predominantly in male gonads, while the F-type is more abundant in somatic tissues. Here, we selected only F-type mitochondrial genome by extracting DNA from foot tissue and conducted a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the target sequences. The total genomic DNA was extracted using the TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. DNA quality and concentration were assessed using 1% agarose gel electrophoresis and NanoDrop 2000 (Thermo Scientific). We performed amplification and sequencing of the COI gene using the previously known universal primer (LCO22me2 + HCO700dy2) [63]. The PCR was performed using a 25 μL mixture of 2 × Taq Plus Master Mix (Vazyme, China) (12.5 μL), ddH_2O (9.5 μL), 2 μM primers (1 μL each), and genomic DNA (1 μL, approximately 100 ng/μL). Thermal cycling started at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 50 °C for 30 s, extension at 72 °C for 1 min, and then a final extension step at 72 °C for 5 min. The purification and sequencing of the PCR products were outsourced to Sangon Biotech (Shanghai, China). The newly obtained sequences were uploaded to GenBank (Accession Numbers: *Koreosolenaia sitgyensis*: PQ062536—PQ062543; *Parvasolenaia rivularis*: PQ062544; *Sinosolenaia iridinea*: PQ062545—PQ062562; *Sinosolenaia oleivora*: PQ062563—PQ062567; *Sinosolenaia recognita*: PQ062568—PQ062572).

The high-quality genomic DNAs of four species (*Sinosolenaia iridinea*, *Sinosolenaia recognita*, *Parvasolenaia rivularis*, and *Koreosolenaia sitgyensis*) were sent to Novogene Co., Ltd. (China) for library construction and sequencing. The sequencing procedure was performed on an Illumina Novaseq 6000 platform following the manufacturer's instructions. The libraries had average insert sizes of approximately 300 bp and were sequenced as 150 bp paired-end. Each library generated approximately 5 Gb of raw data. Raw reads were filtered using fastp ver. 0.20.0 [64] by removing reads containing adaptor sequences and low-quality reads (i.e. reads containing unknown nucleotides 'N' higher than 10%, more than 50% bases with quality scores less than 5) to obtain clean reads. Clean reads were then de novo assembled using the CLC Genomic Workbench (https://digitalinsights.qiagen.com/). Contigs identified as mitogenome sequences were manually checked for overlap at the beginning and end, resulting in a circular mitogenome. Geneious [65] was used to examine the entire mitogenome and perform an analysis of nucleotide composition. The assembled mitogenome sequence was annotated using the MITOS WebServer (see http://mitos.bioinf.uni-leipzig.de/index.py) [66] with the invertebrate genetic code. Protein-coding genes (PCGs) were manually corrected using BLAST searches analysis (see http://blast.ncbi.nlm.nih.gov/). The positions and secondary structures of tRNAs were confirmed using ARWEN (see http://130.235.244.92/ARWEN/index.html) [67]. The mitogenome maps were generated using the online program Chloroplot (see https://irscope.shinyapps.io/Chloroplot/) [68]. The obtained mitogenome sequences have been submitted to GenBank under accession number (*Koreosolenaia sitgyensis*: OR933732; *Parvasolenaia rivularis*: OR933733;

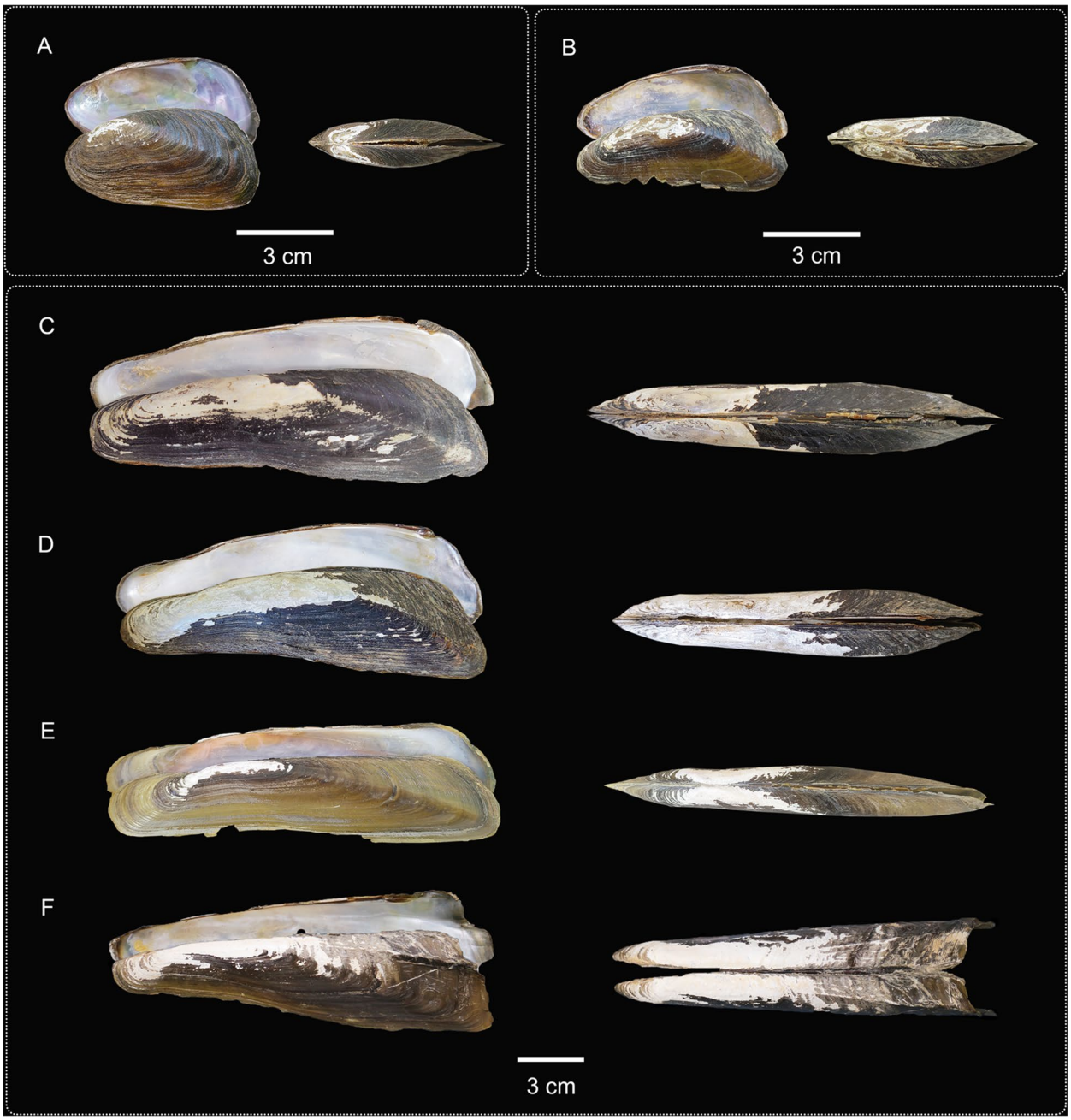

Fig. 1 Shells of *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* species. **A**: *Koreosolenaia sitgyensis*; **B**: *Parvasolenaia rivularis*; **C**: *Sinosolenaia iridinea*; **D**: *Sinosolenaia oleivora*; **E**: *Sinosolenaia recognita*; **F**: *Sinosolenaia carinata*. For each species, the left valve is located above with the shell surface facing upwards, and the right valve is located below with the nacre side facing upwards (left side); the shell umbo view is composed of both left and right valves (right side)

Sinosolenaia iridinea: OR933734; *Sinosolenaia recognita*: PQ063995).

Mitogenomic analysis

The mitochondrial genomes and annotation files of *Sinosolenaia oleivora* (KF296320) and *Sinosolenaia carinata* (NC_023250) were retrieved from GenBank for comparison with the mitochondrial genomes sequenced in this study. Sequence length, nucleotide composition, and AT content of the whole mitogenomes, PCGs, tRNAs, rRNAs and control regions (CRs) were calculated using the built-in EditSeq program in DNAstar (https://www.

dnastar.com/) [69]. Strand asymmetry was calculated using the following formulas: AT skew = (A − T)/(A + T) and GC skew = (G − C)/(G + C) [70]. The amino acid usage and relative synonymous codon usage (RSCU) values of PCGs were calculated using MEGA (ver. 7.0, see http://www.megasoftware.net) [71]. The potential secondary structure of the CR was predicted using the RNAfold web server with default parameters (see http://rna.tbi.univie.ac.at//cgibin/RNAWebSuite/RNAfold.cgi) [72]. Structural elements such as the stem-ring structure and tandem repeat sequences were determined based on visualization.

Phylogenetic analysis

Two datasets were constructed in this study: (i) COI dataset (Table S2); (ii) mitogenome dataset (containing 12 PCGs (*atp8* was excluded due to high sequence variation) and two rRNA genes; 28 taxa) (Table S3). The nucleotide sequence of all PCGs was aligned separately by the built-in MUSCLE algorithm [73] with default settings implemented in PhyloSuite (ver. 1.2.3, http://phylosuite.jushengwu.com/) [74]. The rRNAs were individually aligned by MAFFT (ver. 7.2, see https://mafft.cbrc.jp/alignment/server/) [75] with the L-INS-i algorithm. Ambiguous alignment areas were trimmed by Gblocks (ver. 0.91b, see http://molevol.cmima.csic.es/castresana/Gblocks.html) [76]. The parameter ribosomal gene block with a minimum length was set to 2 base pairs (bp), allowed gap position was selected with half; the minimum length of PCG block was set to 3 bp, allowed gap position was selected none. For the barcoding dataset, the COI sequence fragment was aligned and trimmed to a length of 567 base pairs (bp). The mitogenomic dataset was concatenated using PhyloSuite ver. 1.2.3, resulting in a total of 12,424 bp.

Based on the COI dataset, the neighbour-joining tree was constructed using the Kimura 2-parameter (K2P) model in MEGA (ver. 7.0, see http://www.megasoftware.net) with 1000 bootstrap replicates [71, 77].

Before constructing phylogenetic trees based on mitochondrial data, substitution saturation tests were performed using DAMBE [78, 79]. The results showed that ISS < ISS.c with significant difference ($p < 0.05$), indicating that the sequence substitutions were not saturated and could be used to construct trees. The mitogenomic dataset was analyzed with partition schemes based on genes and codons. The partition scheme and the best model for Bayesian inference (BI) and maximum likelihood (ML) were selected using PartitionFinder (ver. 2.1.1, see http://www.robertlanfear.com/partitionfinder/) [80], and ModelFinder (ver. 1.4.2, see http://www.iqtree.org/ModelFinder/) [81], respectively. The substitution models assigned to each partition by PartitionFinder and ModelFinder were listed in Table S4.

BI analyses were carried out in MrBayes (ver. 2.01, see http://nbisweden.github.io/MrBayes/) [82] with models generated in PartitionFinder. Four independent Markov Chain Monte Carlo (MCMC) chains were run simultaneously for ten million generations, and sampling was conducted every 1000 generations. When the average standard deviation of splitting frequency falls below 0.01, the process can be terminated. The total number of generations was 357,000, and the likelihood value converged after 28,000 generations. Therefore, the burn-in was set to 30 (> 28). ML analyses were implemented in the IQTREE web server (see http://iqtree.cibiv.univie.ac.at/) [83] based on models generated in ModelFinder, using 1000 ultrafast bootstraps [77]. The generated phylogenetic trees were viewed and edited using iTOL online software (http://itol.embl.de/itol.cgi) [84].

Morphometric analyses

A total of 46 specimens were used for shell morphometric analyses. The species' morphological measurements information was shown in Table S5. Principal component analysis (PCA) was conducted using an online data analysis and visualization platform (https://www.bioinformatics.com.cn) [85] to describe the morphological variation among species. Univariate analysis of variance (ANOVA) was performed in SPSS 24.0 [86] to examine differences among different groups. To further compare the specific differences between the groups, pairwise comparisons were conducted between PC1 and PC2 using the LSD (least significant difference) method [87], with statistical significance set at $p < 0.05$.

Results

Comparative morphological analysis

We retrieved all available COI sequences of *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* from Genbank and combined them with the sequences obtained from this study to compile a total of 152 sequences for the COI dataset. The six branches of the NJ tree, based on the COI dataset, provided good support for the validity of the currently recognized species: *Koreosolenaia sitgyensis*, *Parvasolenaia rivularis*, *Sinosolenaia iridinea*, *Sinosolenaia oleivora*, *Sinosolenaia recognita*, and *Sinosolenaia carinata* (Fig. 2).

We conducted a detailed morphological examination of all collected specimens (Fig. 1). The shells of *Koreosolenaia* and *Parvasolenaia* were subtriangular, while the shells of *Sinosolenaia* were narrow and elongated. Compared to *Parvasolenaia rivularis*, *Koreosolenaia sitgyensis* had a wider anterior margin and posterior margin. The shells of *Sinosolenaia* species resembled razor

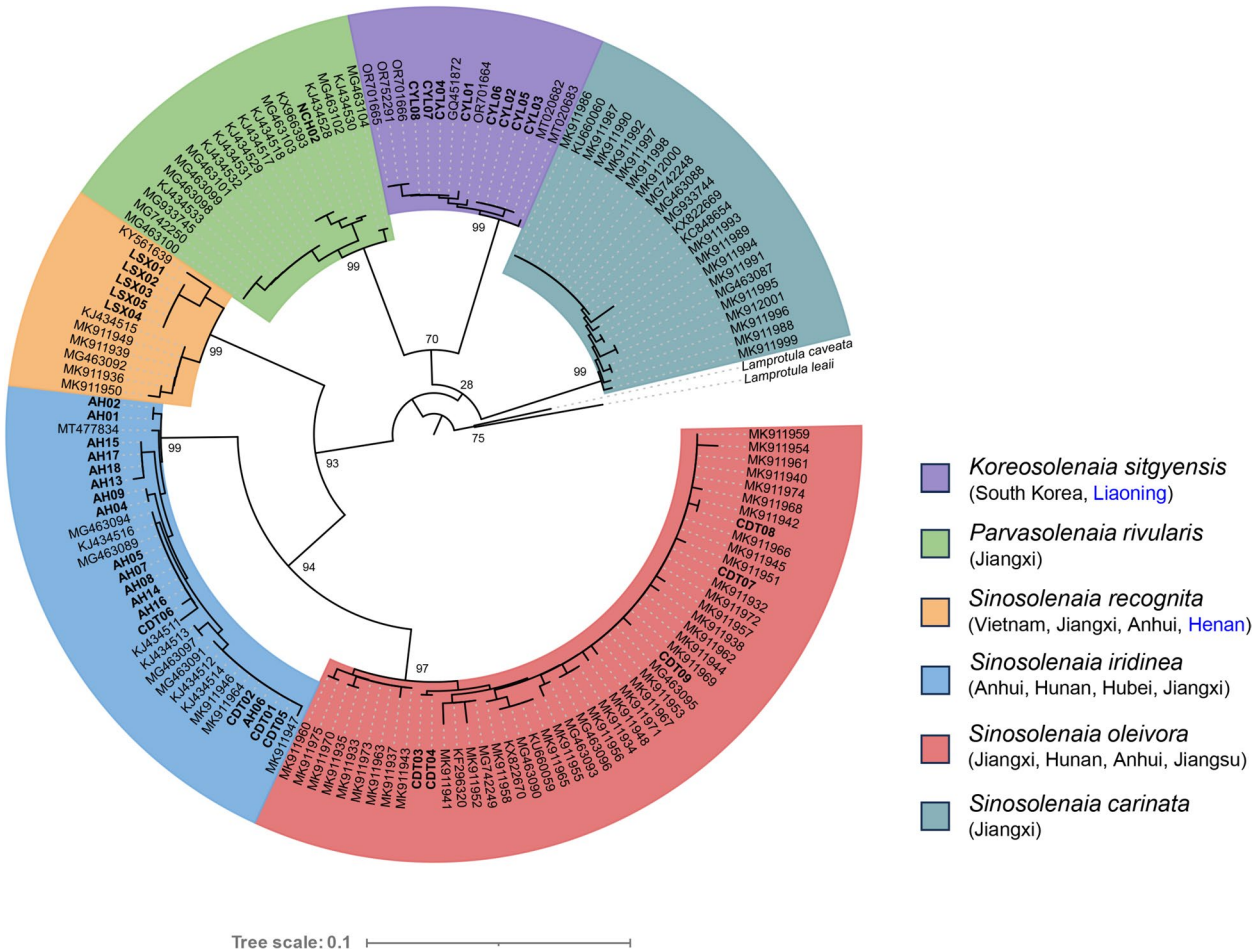

Fig. 2 Neighbour-joining tree inferred from 152 COI sequences of *Koreosolenaia, Parvasolenaia,* and *Sinosolenaia* species based on the Kimura 2-parameter model. Each branch is labelled with the GenBank accession numbers and specimen codes (bold type) from this study. Colored taxon blocks represent the different species in this study. The number on the branches represents the support values of the primary node. The distribution of species is shown in parentheses, with the new distribution found in this study highlighted in blue font

clams. *Sinosolenaia carinata* could be distinguished from other congeneric species (*Sinosolenaia oleivora*, *Sinosolenaia iridinea*, *Sinosolenaia recognita*) by a distinct keel-like protrusion on the dorsal ridge, which prevented shells from closing completely and resulted in an atractoid opening at the posterior edge. In contrast, in other congeneric species, the shells could be tightly closed. Shell characteristics, such as shape, sculpture, and size of *S. oleivora*, *S. iridinea*, and *S. recognita* were very similar, often making it difficult to distinguish them based on shell morphology. Although there were apparent variations in shell color (Fig. 1C, D, E), it should be noted that shell color is intricately linked to both age and habitat [33, 88], rendering it impractical to accurately identify these three species solely based on shell color.

The soft-body anatomical characteristics of *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* species were depicted in Fig. 3 and Fig. S2. In *Koreosolenaia sitgyensis*, the papillae of the incurrent aperture were short and arrange densely in a row, while the excurrent aperture was smooth and lacked papillae, but both apertures displayed evident pigmentation (Fig. 3A). In *Parvasolenaia rivularis*, the papillae of the incurrent aperture were short and conical, unevenly arranged in two rows with no papillae observed in the excurrent aperture, and both apertures exhibited shallow pigmentation (Fig. 3B).

Although the shell characteristics of *Sinosolenaia iridinea*, *Sinosolenaia oleivora*, and *Sinosolenaia recognita* exhibited remarkable similarities, distinct differences could be observed in the incurrent aperture of these three species. In *S. iridinea*, the papillae of the incurrent aperture were short and thick, arranged in two to three rows, and branched near the base (Fig. 3C1). In *S. oleivora*, the papillae were elongated and spindle-shaped, arranged in two to three rows with branching at the pointed end (Fig. 3D1). In *S. recognita*, the papillae were thick and

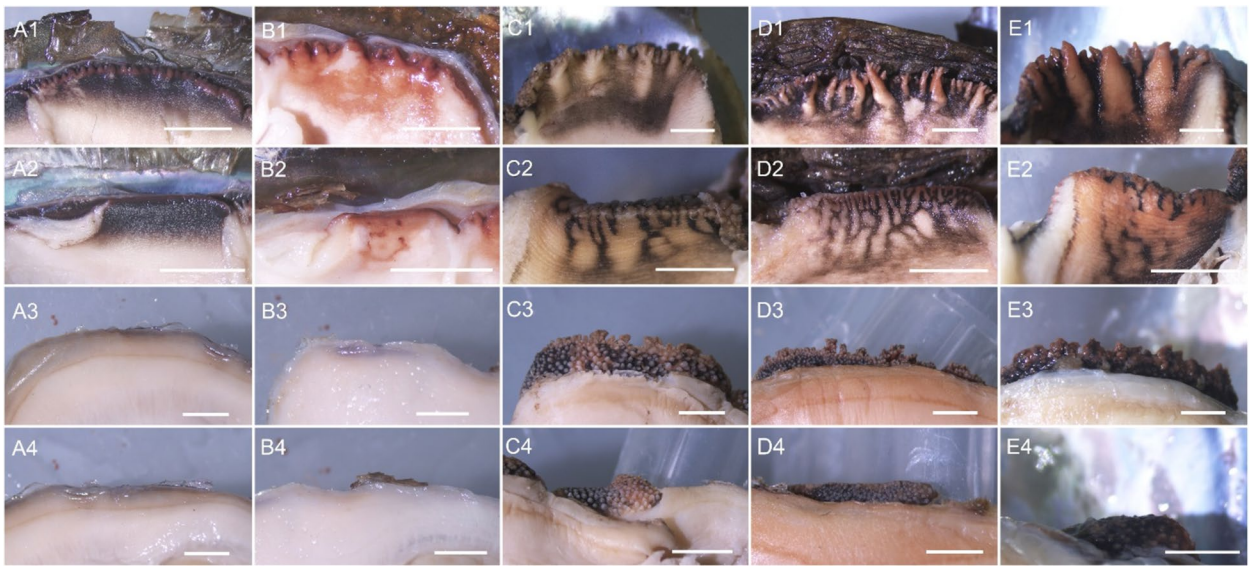

Fig. 3 Anatomical features of excurrent aperture and incurrent aperture. **A**: *Koreosolenaia sitgyensis*; **B**: *Parvasolenaia rivularis*; **C**: *Sinosolenaia iridinea*; **D**: *Sinosolenaia oleivora*; **E**: *Sinosolenaia recognita*; 1: incurrent aperture; 2: excurrent aperture; 3: the dorsal surface of incurrent aperture; 4: the dorsal surface of excurrent aperture. Scale = 3 mm

short, conical in shape, arranged in two rows without any branching (Fig. 3E1). These three species had no papillae in their excurrent aperture, but they had tightly packed small cysts along their margins (Fig. 3C2, D2, E2). It was worth noting that *Sinosolenaia* species possessed small cysts on the dorsal surface of both incurrent and excurrent apertures, which were closely connected to each other—a unique feature not observed in other genera (Fig. 3C3, C4, D3, D4, E3, E4).

We also observed the labial palps of the five species (*Koreosolenaia sitgyensis*, *Parvasolenaia rivularis*, *Sinosolenaia iridinea*, *Sinosolenaia oleivora*, and *Sinosolenaia recognita*), which had similar morphologies characterized by a nearly triangular shape (Fig. S2). The anal opening was located on the dorsal margin of the posterior adductor muscle and was connected to the apertures, displaying a type I morphology as described by Shu & Wu [89] (Fig. S2).

Morphometric analyses

The principal component analysis (PCA) yielded two distinct eigenvalues that accounted for over 99% of the total observed variation among individuals (Fig. 4). The first principal component (PC1) explained 67% of the overall variation, while the second principal component (PC2) accounted for 32.6% of the total variation. Although there was only one specimen of *Parvasolenaia rivularis*, the PCA map clustering showed that each species significantly formed non-overlapping groups ($p < 0.05$).

The PC1 axis revealed a significant difference among species ($p < 0.05$), except for the absence of a significant difference for *Sinosolenaia carinata* vs. *Sinosolenaia oleivora* and *Sinosolenaia carinata* vs. *Parvasolenaia rivularis* ($p > 0.05$). On the PC2 axis, *Koreosolenaia sitgyensis* could be well separated from all other species except *Parvasolenaia rivularis* ($p < 0.05$). Additionally, significant differences existed between some other species as well (Table S6).

Comparative mitogenome characteristics

Whole mitochondrial genome and protein-coding genes

We compared the F-type mitochondrial genomic features of *Koreosolenaia sitgyensis*, *Parvasolenaia rivularis*, *Sinosolenaia iridinea*, and *Sinosolenaia recognita* obtained in our study with the complete mitochondrial genomes of *Sinosolenaia oleivora* and *Sinosolenaia carinata* downloaded from GenBank. The six complete mitochondrial genomes ranged from 16,045 bp to 16,716 bp (Table 2), all of which contained the typical 37 genes (13 PCGs, 2 rRNAs, and 22 tRNAs) (Fig. 5A; Table S7). The A + T content of all six complete mitochondrial genomes was higher compared to the G + C content. Additionally, their mitochondrial nucleotide compositions exhibited a positive AT skew and a negative GC skew (Table 2). The gene arrangement in the mitochondrial genome sequences of the six species was identical (Fig. 5A). The heavy chain (H chain) encoded eleven genes (*cox1*, *cox2*, *cox3*, *nad3*, *nad4*, *nad4L*, *nad5*, *atp6*, *atp8*, *trnD* and *trnH*), while additional twenty-six genes were located on

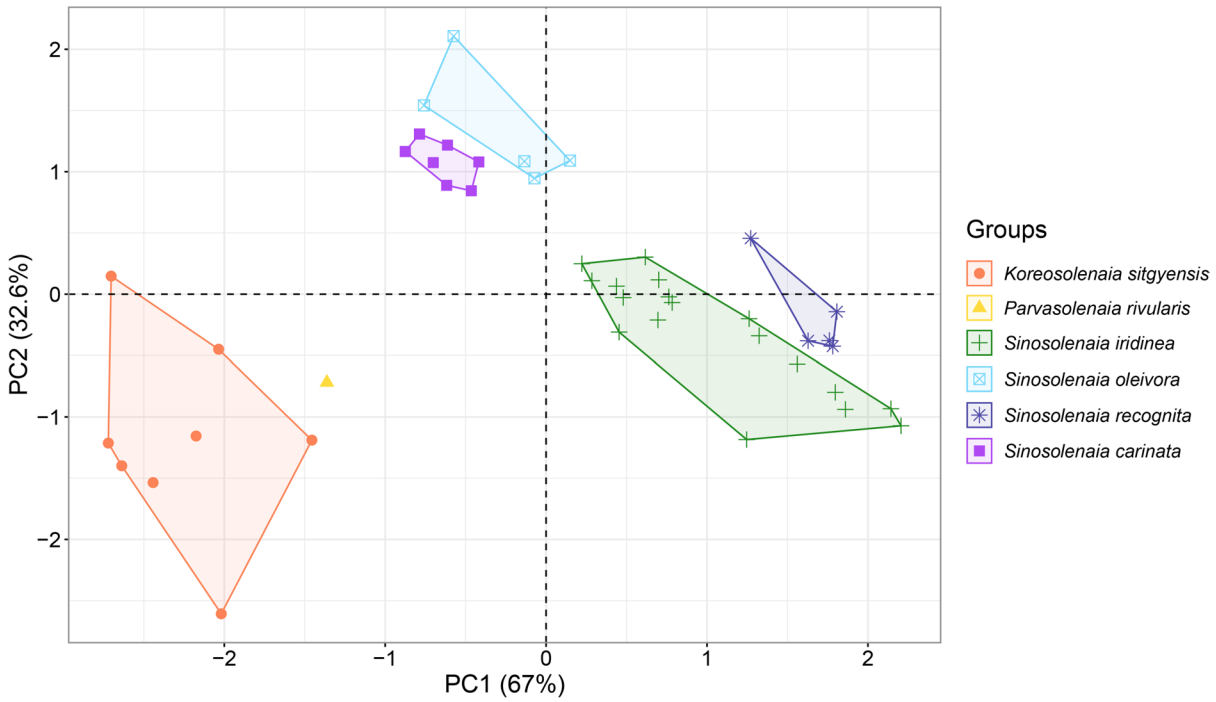

Fig. 4 Principal component analysis (PCA) based on the data of (H1-H2)/W, (H1-H2)/L, and W/L

Table 2 Mitochondrial genome structural characterization of *Koreosolenaia sitgyensis*, *Parvasolenaia rivularis*, *Sinosolenaia recognita*, *Sinosolenaia oleivora*, *Sinosolenaia iridinea*, and *Sinosolenaia carinata*

	K. sitgyensis	***P. rivularis***	***S. iridinea***	***S. oleivora***	***S. recognita***	***S. carinata***
Total (bp)	16,045	16,607	16,368	16,392	16,312	16,716
AT%	60.52	57.85	60.78	59.93	61.64	60.89
ATskew	0.22	0.25	0.21	0.22	0.2	0.22
GCskew	−0.34	−0.37	−0.38	−0.37	−0.37	−0.39
No. of gene intervals	28	28	31	24	24	25
No. of gene overlaps	4	4	2	1	3	2
Size range of gene overlap	1 ~ 20	1 ~ 22	2 ~ 10	8	1 ~ 9	1 ~ 8
CR	509	994	778	789	689	1049
rrnS	840	849	844	843	842	857
rrnL	1326	1288	1286	1287	1289	1296
atp6	702 (ATG/TAG)	708 (ATG/TAG)	708 (ATG/TAG)	708 (ATG/TAG)	708 (ATG/TAG)	708 (ATG/TAG)
atp8	192 (GTG/TAA)	192 (GTG/TAG)	198 (ATG/TAA)	198 (ATG/TAG)	198 (ATG/TAA)	198 (GTG/TAG)
cox1	1545 (TTG/TAA)	1536 (TTG/TAG)	1545 (TTG/TAG)	1545 (TTG/TAA)	1548 (ATA/TAG)	1545 (TTG/TAG)
cox2	681 (ATG/TAA)	681 (ATG/TAA)	681 (ATG/TAA)	681 (ATG/TAA)	681 (ATG/TAA)	681 (ATG/TAA)
cox3	780 (ATG/TAG)	780 (ATG/TAG)	780 (ATG/TAG)	780 (ATG/TAA)	780 (ATG/TAA)	780 (ATG/TAA)
cob	1137 (ATA/TAA)	1137 (ATA/TAA)	1137 (ATA/TAA)	1149 (ATC/TAG)	1158 (ATT/TAA)	1161 (ATC/TAG)
nad1	885 (ATT/TAA)	885 (ATT/TAG)	894 (ATT/TAA)	897 (ATC/TAA)	897 (ATC/TAA)	897 (ATC/TAA)
nad2	963 (ATG/TAA)	963 (ATG/TAA)	963 (ATG/TAA)	963 (ATG/TAA)	963 (ATG/TAA)	963 (ATG/TAA)
nad3	357 (ATG/TAG)	357 (ATG/TAG)	345 (ATG/TAG)	357 (ATG/TAG)	357 (ATG/TAA)	357 (ATG/TAG)
nad4	1332 (ATG/TAA)	1347 (ATT/TAA)	1332 (ATA/TAA)	1350 (ATT/TAG)	1350 (ATT/TAA)	1350 (ATT/TAG)
nad4L	297 (ATG/TAG)	297 (ATG/TAG)	297 (ATG/TAG)	297 (ATG/TAG)	297 (ATG/TAG)	297 (ATG/TAG)
nad5	1734 (GTG/TAA)	1728 (ATT/TAG)	1728 (ATT/TAG)	1734 (GTG/TAG)	1734 (ATG/TAG)	1734 (ATG/TAA)
nad6	489 (ATT/TAA)	492 (ATT/TAA)	489 (ATA/TAA)	489 (ATT/TAA)	489 (ATT/TAG)	489 (ATC/TAA)

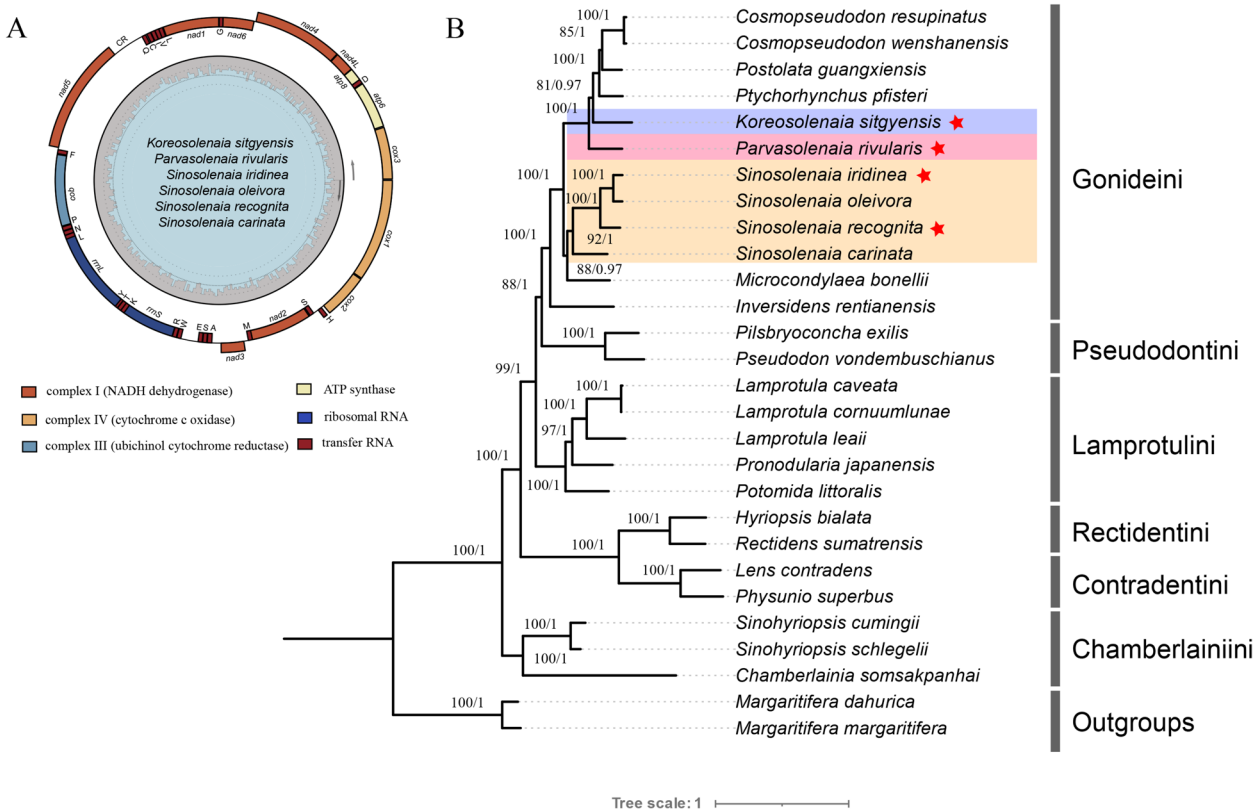

Fig. 5 Analysis based on mitochondrial data. **A**: Gene map of the F-type mitochondrial genome of *Koreosolenaia sitgyensis*, *Parvasolenaia rivularis*, *Sinosolenaia iridinea*, *Sinosolenaia oleivora*, *Sinosolenaia recognita*, *Sinosolenaia carinata*. **B**: Phylogenetic trees inferred from Bayesian Inference (BI) and Maximum Likelihood (ML) analyses. Support values above the branches are maximum likelihood bootstrap support values and Bayesian posterior probabilities, respectively. The colored shaded clades represent three genera that are the focus of this study. Pentagrams symbolize the sequences from this study

the light chain (L chain) (Table S7), which was consistent with the mitochondrial genomes of other Gonideinae species [38, 39].

Most protein-coding genes (PCGs) in the six mitochondrial genomes started with the typical start codon ATN (ATA, ATC, ATG, ATT). However, exception made for *Sinosolenaia recognita*, which used ATA as the start codon for *cox1*, the other five species used TTG as the start codon for *cox1*. Additionally, GTG was found to be used as the start codon for some PCGs (e.g., *nad5* in *Koreosolenaia sitgyensis* and *Sinosolenaia oleivora*; *atp8* in *Koreosolenaia sitgyensis*, *Parvasolenaia rivularis*, and *Sinosolenaia carinata*). Furthermore, all PCGs had the typical TAR (TAA, TAG) as the stop codon (Table 2; Table S7). For all six mitochondrial genomes, the A + T content of PCGs (57.01% ~ 60.69%) was slightly lower than that of the whole genome and also exhibited positive AT skew (0.23 ~ 0.29) as well as negative GC skew (−0.38 ~ −0.43).

In the six mitochondrial genomes, the most frequently utilized amino acids were Val, Gly, and Leu (UUN), while the least commonly used were Arg, Cys, and Gln (Fig. 6A). By calculating the relative synonymous codon usage (RSCU), it was found that the codon usage bias in the six mitochondrial genomes was generally similar (Fig. 6B). The preferred amino acid coding codons mostly have A or T in the third position (Fig. 6B).

tRNA, rRNA, and CR structure

The six mitochondrial genomes contained a total of 22 predicted tRNA genes. The length of tRNAs ranged from 52 to 74 bp, with the smallest being tRNA-Ala in *Parvasolenaia rivularis* and the largest being tRNA-Phe, tRNA-Pro and tRNA-Ala in *Koreosolenaia sitgyensis*, *Sinosolenaia iridinea* and *Sinosolenaia carinata*, respectively.

Most tRNAs adopted a characteristic cloverleaf secondary structure, encompassing distinct regions such as discriminator nucleotide, acceptor stem, TψC arm, variable loop, anticodon arm, and DHU arm (Fig. 7A). However, all six mitochondrial genomes showed a large loop

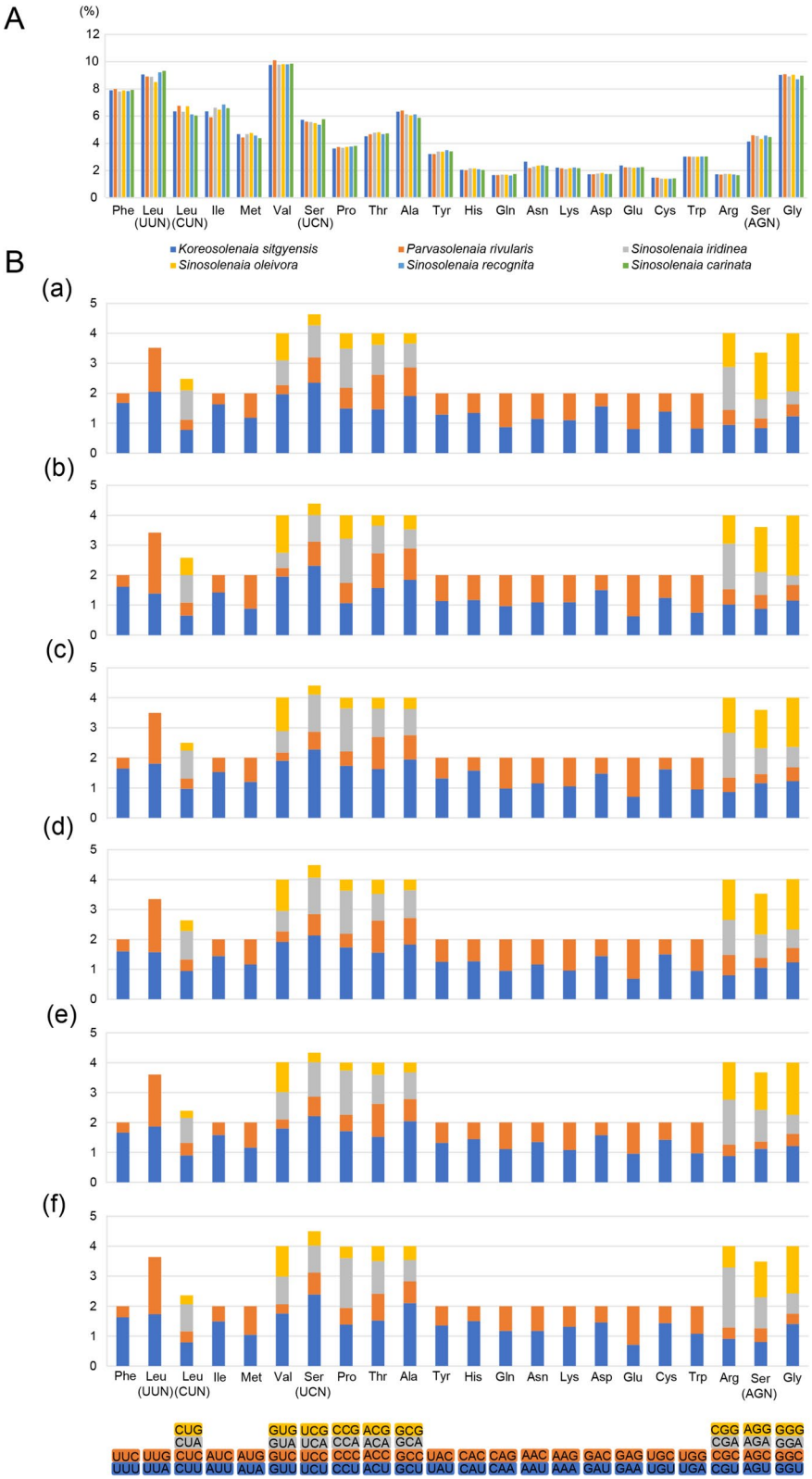

Fig. 6 The amino acid usage (**A**) and relative synonymous codon usage (**B**) of Protein-coding genes (PCGs). (a): *Koreosolenaia sitgyensis*; (b): *Parvasolenaia rivularis*; (c): *Sinosolenaia iridinea*; (d): *Sinosolenaia oleivora*; (e): *Sinosolenaia recognita*; (f): *Sinosolenaia carinata*

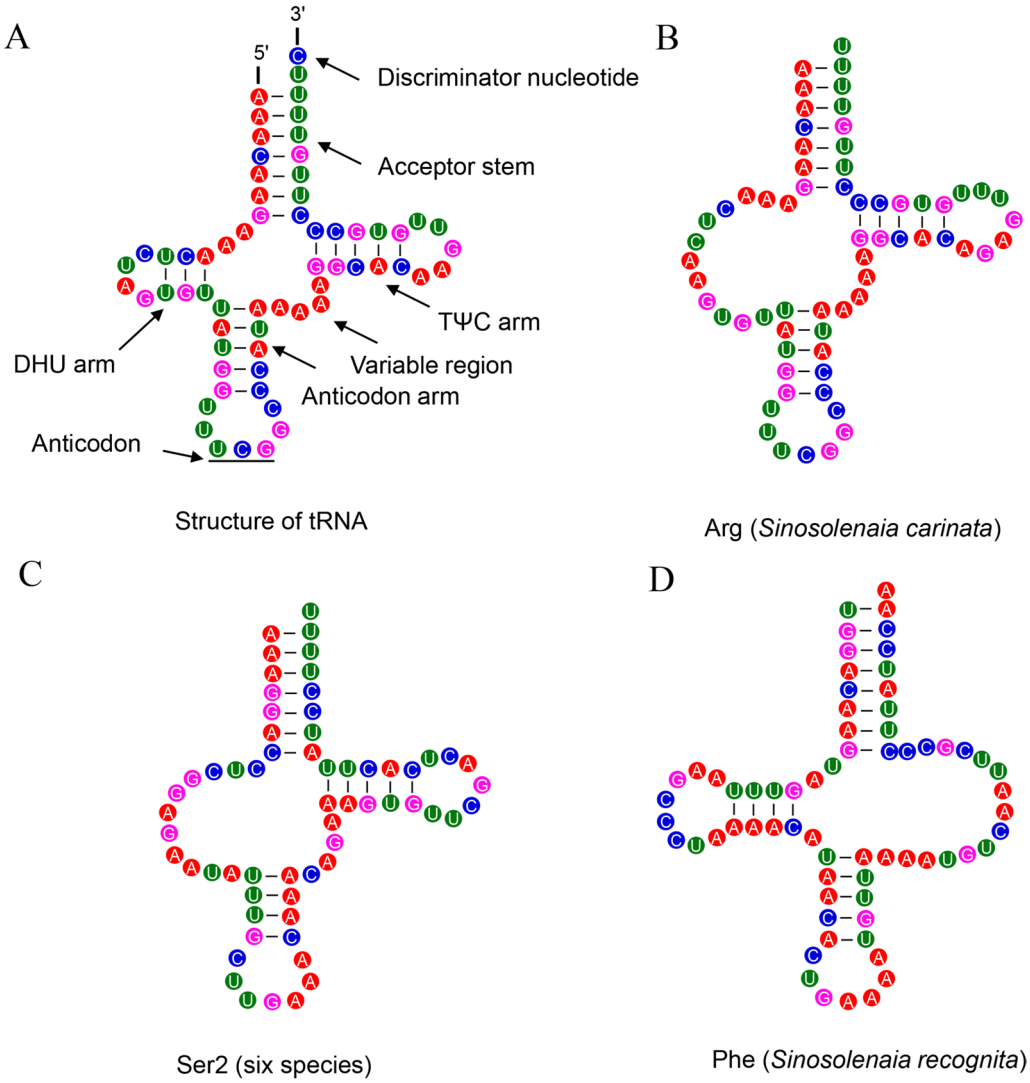

Fig. 7 Predicted secondary structure for tRNAs. **A**: Standard secondary cloverleaf structure; **B**, **C**, **D**: abnormal secondary structure (**B**: Arg in *Sinosolenaia carinata*; **C**: Ser2 in six species; **D**: Phe in *Sinosolenaia recognita*)

in the DHU arm for the tRNA-Ser2 instead of the typical stem-loop structure (Fig. 7C). In addition, the tRNA-Arg in *Sinosolenaia carinata* lacked DHU arm (Fig. 7B), and the tRNA-Phe in *Sinosolenaia recognita* lacked the TΨC arm (Fig. 7D).

The two rRNA genes (*rrnS* and *rrnL*) of the six mitochondrial genomes were both encoded on the L chain. The *rrnL* was located between tRNA-Tyr and tRNA-Leu1, while *rrnS* was located between tRNA-Arg and tRNA-Lys. Among the six mitochondrial genomes, the lengths of *rrnS* and *rrnL* genes ranged from 840 to 857 bp and 1286 to 1326 bp, respectively (Table 2). Both rRNA genes showed a preference for (A + T) bases.

The control region (CR) was the largest non-coding segment of the mitochondrial genome, exhibiting the highest degree of nucleotide composition asymmetry and sequence length variation. In this study, the CRs of six mitogenomes were consistently located between ND5 and tRNA-Gln, with lengths ranging from 509 to 1049 bp. They displayed a higher A + T content compared to G + C. Furthermore, the CRs exhibited a variety of structural elements, such as stem-loop structures and tandem repeat sequences (Fig. S3).

Mitochondrial phylogenomics

In this study, we used mitochondrial genomes from 26 Gonideinae species to construct a consistent topology using ML and BI analyses. The monophyly of all tribes within Gonideinae was strongly supported by 100% maximum likelihood bootstrap supports (BS) and Bayesian

posterior probabilities (PP) in most nodes, and their relationships at the tribal level were consistent with previous studies (Fig. 5B) [38–41, 44].

Focusing on the tribe Gonideini, BI and ML trees consistently supported genus-level relationships as follows: (*Inversidens* + ((*Microcondylaea* + *Sinosolenaia*) + (*Parvasolenaia* + (*Koreosolenaia* + (*Ptychorhynchus* + (*Postolata* + *Cosmopseudodon*)))))) (BS > 80%, PP > 0.9; Fig. 5B). In the genus *Sinosolenaia*, BI and ML yielded consistent and strongly supported phylogenetic relationships as follows: (*S. carinata* + (*S. recognita* + (*S. iridinea* + *S. oleivora*))) (BS > 90%, PP = 1; Fig. 5B).

Discussion

Comparative morphology and species distribution

A robust taxonomy is crucial for inferring biological characteristics from closely related species, comprehending their evolutionary history, and effectively prioritizing conservation efforts [90–92]. Combining morphological and molecular data, we re-examined the three recently separated genera from *Solenaia sensu lato*, namely *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* [35–37]. *Koreosolenaia* and *Parvasolenaia* can be easily distinguished from *Sinosolenaia* by shell shape and sculpture (Fig. 1). Compared to *Parvasolenaia rivularis*, *Koreosolenaia sitgyensis* had wider margins on the anterior margin and posterior margin. *Sinosolenaia carinata* could be distinguished from other congeneric species (*S. oleivora*, *S. iridinea*, *S. recognita*) by the prominent keel-like ridges on its dorsal ridge (Fig. 1). However, the shell features of *S. oleivora*, *S. iridinea*, and *S. recognita* were very similar, making it difficult to distinguish them based solely on shell characteristics (Fig. 1). Capturing changes in shell height, length, and width is crucial for accurately identifying distinctive features for unionids [93]. During the examination of specimens, we observed visible differences in the ratio of the anterior and posterior margins among different species. Therefore, we quantified three indicators ((H1-H2)/L, W/L, and (H1-H2)/W3) for PCA analysis. The results indicated that there was no overlap among *S. oleivora*, *S. iridinea*, and *S. recognita* with statistically significant differences (Fig. 4; $p < 0.05$). Due to the taxonomic challenges posed by the considerable variability of shells, it has been acknowledged that considering soft-body characteristics is equally imperative [38, 39, 57, 94, 95]. By observing and comparing the soft-body morphology, we identified distinct variations in the papillae on excurrent apertures among *S. oleivora*, *S. iridinea*, and *S. recognita*, which can be considered as species diagnostic characteristics (Fig. 3). Furthermore, our findings discovered that *Sinosolenaia* species exhibited small cysts closely interconnected on both the dorsal surface of apertures, potentially representing a unique feature specific to the genus *Sinosolenaia* (Fig. 3).

The comprehension of species distribution is a fundamental requirement for comprehending their spatial patterns and paleobiogeography. The currently available distribution data suggest that *Koreosolenaia sitgyensis* is endemic to Korea, while Chinese *Sinosolenaia recognita* is found in the Yangtze River Basin, encompassing Jiangxi, Hunan, Hubei, Anhui, and Jiangsu [27, 35–37]. In this study, we conducted a comprehensive survey and sampling throughout China, revealing the presence of *S. recognita* in Nanwan Lake, Henan Province. Additionally, we also collected *K. sitgyensis* from the Yalu River, Liaoning Province. These findings significantly expand the known distribution range of these species, providing a solid foundation for subsequent assessments regarding their endangerment status and conservation strategies.

Mitochondrial genome structure and phylogenetic relationships

At present, three gene arrangement patterns have been found in the family Unionidae [43, 96]. We successfully assembled the complete mitochondrial genomes of *Parvasolenaia rivularis*, *Koreosolenaia sitgyensis*, *Sinosolenaia iridinea*, and *Sinosolenaia recognita*. Our findings indicated that their gene arrangements align with those observed in *Sinosolenaia carinata* and *Sinosolenaia oleivora*, while also sharing the gene arrangement with the majority of species within Gonideinae.

The six complete mitogenomes exhibited a high A + T content in genomes, protein-coding genes, tRNAs, rRNAs, and CRs. Upon analyzing the protein-coding genes, we observed that nucleotide A or T was predominantly favored in the third codon position for most amino acids (Fig. 6). This phenomenon is also prevalent among other taxa [97–100]. The majority of tRNAs adopt a typical cloverleaf secondary structure. However, in the DHU domain, tRNA-Ser2 of six species exhibited an atypical large ring structure (Fig. 7C). Interestingly, the absence of the stem in tRNA-Ser is commonly observed in other metazoan mitochondrial genomes [99–104], although the reasons for this absence remain unclear. This study also observed that the CR of the six complete mitochondrial genomes contained some structural elements, such as stem-loop structures and tandem repeat sequences (Fig. S3). Clayton et al. [105] believed that these stem-loop structures play a role in initiating replication within animal mitochondria.

Recent molecular phylogenetic studies showed that the multi-locus phylogeny yielded inconsistent results at the generic level [36, 38–40, 47]. This inconsistent phylogenetic relationship may be attributed to the limited number of polymorphic sites and its non-neutral

nature, which might not provide sufficient information for resolving deep nodes. However, the mitochondrial genome is widely acknowledged for its remarkable capacity to elucidate both shallow and deep relationships in freshwater mussels [35, 44, 106–109]. Therefore, based on the complete mitochondrial genome, we constructed a robust phylogenetic framework focusing on the subfamily Gonideinae in Unionidae. Both BI and ML trees strongly supported that *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia* belonged to the tribe Gonideini, with the following phylogenetic relationships: (*Inversidens* + ((*Microcondylaea* + (*Sinosolenaia carinata* + (*Sinosolenaia recognita* + (*Sinosolenaia iridinea* + *Sinosolenaia oleivora*)))) + (*Parvasolenaia* + (*Koreosolenaia* + (*Ptychorhynchus* + (*Postolata* + *Cosmopseudodon*)))))).

Threats and conservation implications

It is imperative to evaluate and monitor species diversity in the era of ongoing biodiversity loss. A scientific comprehension of both species diversity and phylogenetic diversity serves as the foundation for species conservation efforts [40, 88, 110, 111]. Here, we integrated morphology, morphometrics, and mitochondrial genomics to conduct a comprehensive study on *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia*, elucidating their distinctive classification features and phylogenetic relationships, while also supplementing new information regarding the geographic distribution. These findings establish a scientific groundwork for subsequent species threat assessment and conservation endeavors.

The *Sinosolenaia* group is an important local source of aquatic food with fresh and tender meat [15]; however, due to overconsumption, habitat degradation, alteration, and fragmentation caused by human activities, the distribution range of *Sinosolenaia* species is becoming increasingly narrow and population numbers are rapidly declining [17, 24, 25, 112–114]. In recent years, the Chinese government has implemented a series of policies for the protection of aquatic animals, including a 10-year fishing ban in the Yangtze River and an updated list of wildlife species under national key protection. These measures have significantly improved the quality of habitat for these groups. The acquisition of fundamental knowledge regarding biological diversity, taxonomy, evolutionary relationships, essential biological and ecological characteristics, as well as habitat requirements of freshwater bivalves, is imperative for conducting effective conservation assessments, planning, and implementation [3]. Based on comprehensive research conducted by conchologists on its reproductive characteristics, habitat, population distribution, and genetic diversity, the government has officially designated *Sinosolenaia carinata* as a nationally protected secondary keystone species in 2021. However, there is still a significant lack of basic biological and ecological information for most species of *Koreosolenaia*, *Parvasolenaia*, and *Sinosolenaia*, which hinders the assessment of their endangerment status. Therefore, we urgently call for more colleagues to participate in a detailed study of these groups in order to understand their genetic diversity and population structure, as well as the microhabitat, behavioral characteristics, and host fish.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-11164-7.

Supplementary Material 1: Table S1. Collection information of specimens in the present study. Supplementary Table S2. List of sequences used in COI phylogenetic analyses. Bold fonts in red represent the sequence from this study. Supplementary Table S3. List of sequences used in mitochondrial phylogenomic analyses. (*) the sequence from this study. Supplementary Table S4. Partitioning strategies from ModelFinder and PartitionFinder for mitogenome dataset. Supplementary Table S5. Morphological dataset for principal coordinates analysis. Variables used for morphological analysis are three quantitative ones: (H1-H2)/L, W/L, (H1-H2)/W. Supplementary Table S6. Adjusted *p*-values (< 0.05 in bold) obtained by Tukey's pairwise posthoc-tests following ANOVAs testing for significant differences between species in PC1 (upper right) and PC2 (lower left) values, respectively. Supplementary Table S7. Annotated files of the four mitochondrial genomes sequenced in this study.

Supplementary Material 2: Fig. S1. The definition of morphometric characteristics and soft-body anatomy. Abbreviations: H1 and H2, shell height; L, shell length; W, width; aam, anterior adductor muscle; pam, posterior adductor muscle; exa, excurrent aperture; ia, incurrent aperture; f, foot; ig, inner gill; og, outer gill; lp, labial palps; m, mantle.

Supplementary Material 3: Fig. S2. Anatomical features of labial palps and anal opening. A: *Koreosolenaia sitgyensis*; B: *Parvasolenaia rivularis*; C: *Sinosolenaia iridinea*; D: *Sinosolenaia oleivora*; E: *Sinosolenaia recognita*; 1: labial palps; 2: anal opening.

Supplementary Material 4: Fig. S3. Secondary structure of the control region. A: *Koreosolenaia sitgyensis*; B: *Parvasolenaia rivularis*; C: *Sinosolenaia iridinea*; D: *Sinosolenaia oleivora*; E: *Sinosolenaia recognita*; F: *Sinosolenaia carinata*.

Acknowledgements

We are grateful to Editor Saylee Sarolkar for her dedication to this manuscript. We would also like to express our gratitude to the two anonymous reviewers and Editor Arsalan Emami-Khoyi for their positive and constructive comments on the manuscript.

Authors' contributions

WR conceived the study; LXJ and HX procured the samples; ZL, JJ and LXY analyzed and interpreted the morphological and genomic data. ZL and WR drafted the article. All authors reviewed the manuscript.

Funding

This work was funded by the National Natural Science Foundation of China (No. 32200370), the Basic Research Program of Shanxi Province, China (No. 20210302124253), and the Research Project Supported by Shanxi Scholarship Council of China (2024–088).

Data availability

All COI and mitogenome sequences used in this study are available on NCBI's GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). Accession numbers for all sequences can be found in Table S2-S3.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]School of Life Science, Shanxi Normal University, Taiyuan 030031, China. [2]Shandong Fisheries Development and Resources Conservation Center, Jinan 250000, China. [3]Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources in Mountainous Areas, School of Life Sciences, Jiaying University, Meizhou 514015, China.

Received: 27 July 2024 Accepted: 17 December 2024
Published online: 24 December 2024

References

1. Dudgeon D, Arthington AH, Gessner MO, Kawabata Z, Knowler D, Lévêque C, et al. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol Rev. 2006;81(2):163–82. https://doi.org/10.1017/S1464793105006950.
2. Strayer DL, Dudgeon D. Freshwater biodiversity conservation: recent progress and future challenges. J N Am Benthol Soc. 2010;29(1):344–58. https://doi.org/10.1899/08-171.1.
3. Lopes-Lima M, Burlakova LE, Karatayev AY, Mehler K, Seddon M, Sousa R. Conservation of freshwater bivalves at the global scale: Diversity, threats and research needs. Hydrobiologia. 2018;810:1–14. https://doi.org/10.1007/s10750-017-3486-7.
4. Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PTJ, et al. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol Rev Camb Philos Soc. 2019;94(3):849–73. https://doi.org/10.1111/brv.12480.
5. Wächtler K, Dreher-Mansur MC, Richter T. Larval Types and Early Postlarval Biology in Naiads (Unionoida). In: Bauer, G., Wächtler, K. (eds) Ecology and Evolution of the Freshwater Mussels Unionoida. Ecological Studies, vol 145. Springer, Berlin, Heidelberg. 2001;93–125. https://doi.org/10.1007/978-3-642-56869-5_6.
6. Barnhart MC, Haag WR, Roston WN. Adaptations to host infection and larval parasitism in Unionoida. J N Am Benthol Soc. 2008;27(2):370–94. https://doi.org/10.1899/07-093.1.
7. Modesto V, Ilarri MI, Souza AT, Lopes-Lima M, Douda K, Clavero M, Sousa R. Fish and mussels: Importance of fish for freshwater mussel conservation. Fish Fish. 2018;19(2):244–59. https://doi.org/10.1111/faf.12252.
8. Liu XJ, Liu YY, Wu RW, Zanatta DT, Lopes-Lima M, Gonçalves DV, et al. Systematics, distribution, biology, and conservation of freshwater mussels (Bivalvia: Unionida) in China. Aquat Conserv. 2022;32(5):859–95. https://doi.org/10.1002/aqc.3799.
9. Graf DL, Cummings KS. A 'big data' approach to global freshwater mussel diversity (Bivalvia: Unionoida), with an updated checklist of genera and species. J Mollus Stud. 2021;87(1):eyaa034. https://doi.org/10.1093/mollus/eyaa034.
10. Vaughn CC, Nichols SJ, Spooner DE. Community and foodweb ecology of freshwater mussels. J N Am Benthol Soc. 2008;27(2):409–23. https://doi.org/10.1899/07-058.1.
11. Smith TA, Meyer ES. Freshwater Mussel (Bivalvia: Unionidae) Distributions and Habitat Relationships in the Navigational Pools of the Allegheny River. Pennsylvania Northeast Nat. 2010;17(4):541–64. https://doi.org/10.1656/045.017.0403.
12. Pandolfo TJ, Kwak T, Cope W, Heise RJ, Nichols RB, Pacifici K. Species traits and catchment-scale habitat factors influence the occurrence of freshwater mussel populations and assemblages. Freshwater Biol. 2016;61:1671–84. https://doi.org/10.1111/FWB.12807.
13. Nakano M, Takakura KI, Morii K, Urabe M. Unionid mussel composition and ditch environments in floodplain and alluvial fan geomorphic types: a case study of a Lake Biwa river basin. Limnology (Tokyo). 2016;18:41–9. https://doi.org/10.1007/s10201-016-0486-7.
14. Haag WR. North American freshwater mussels: Natural history, ecology, and conservation. Cambridge: Cambridge University Press; 2012. https://doi.org/10.1017/CBO9781139048217.
15. Liu YY, Zhang WZ, Wang YX. Economic Fauna of China: Freshwater Mollusks. Beijing: Science Press; 1979.
16. Savazzi E, Yao PY. Some morphological adaptations in freshwater bivalves. Lethaia. 1992;25(2):195–209. https://doi.org/10.1111/j.1502-3931.1992.tb01384.x.
17. Cao YL, Liu XJ, Wu RW, Xue TT, Li L, Zhou CH, et al. Conservation of the endangered freshwater mussel *Solenaia carinata* (Bivalvia, Unionidae) in China. Nat Conserv. 2018;26:33–53. https://doi.org/10.3897/natureconservation.26.25334.
18. Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark S, et al. The global decline of nonmarine mollusks. Bioscience. 2004;54(4):321–30. https://doi.org/10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2.
19. Downing JA, Van Meter P, Woolnough DA. Suspects and evidence: A review of the causes of extirpation and decline in freshwater mussels. Anim Biodiv Conserv. 2010;33(2):151–185. https://doi.org/10.32800/abc.2010.33.0151.
20. Liu XJ, Yang X, Zanatta DT, Lopes-Lima M, Bogan AE, Zieritz A, et al. Conservation status assessment and a new method for establishing conservation priorities for freshwater mussels (Bivalvia: Unionida) in the middle and lower reaches of the Yangtze River drainage. Aquat Conserv. 2020;30(5):1000–11. https://doi.org/10.1002/aqc.3298.
21. De Queiroz K. Species concepts and species delimitation. Syst Biol. 2007;56(6):879–86. https://doi.org/10.1080/10635150701701083.
22. Haag WR, Williams JD. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia. 2014;735:45–60. https://doi.org/10.1007/s10750-013-1524-7.
23. Taylor CA, DiStefano RJ, Larson ER, Stoeckel J. Towards a cohesive strategy for the conservation of the United States' diverse and highly endemic crayfish fauna. Hydrobiologia. 2019;846:39–58. https://doi.org/10.1007/s10750-019-04066-3.
24. Huang XC, Rong J, Liu Y, Zhang MH, Wan Y, Ouyang S, et al. The complete maternally and paternally inherited mitochondrial genomes of the endangered freshwater mussel *Solenaia carinatus* (Bivalvia: Unionidae) and implications for Unionidae taxonomy. PLoS ONE. 2013;8(12):e84352. https://doi.org/10.1371/journal.pone.0084352.
25. Wang YN, Zhang GR, Wei KJ, Gardner JPA. Reproductive traits of the threatened freshwater mussel *Solenaia oleivora* (Bivalvia: Unionidae) from the middle Yangtze River. J Mollus Stud. 2015;81(4):522–6. https://doi.org/10.1093/mollus/eyv016.
26. Conrad TA. Notes on Recent and fossil shells, with descriptions of new genera. American Journal of Conchology. 1869;4:246–9.
27. MolluscaBase eds (2024) MolluscaBase. http://www.molluscabase.org.
28. Simpson CT. Synopsis of the naiades, or pearly fresh-water mussels. Proc US Natl Mus. 1900;22:501–1044. https://doi.org/10.5479/si.00963801.22-1205.501.
29. Simpson CT. A descriptive catalogue of the naiades, or pearly fresh-water mussels. Parts I-III. Bryant Walker, Detroit, Michigan; 1914. https://doi.org/10.5962/bhl.title.10910.
30. Haas, F. Superfamilia Unionacea (pp. 1–663). Das Tierreich; 1969. https://doi.org/10.1515/9783111721569.
31. Deein G, Unakornsawat Y, Rattanadaeng P, Sutcharit C, Kongim B, Panha S. A new Species of *Solenaia* from Thailand (Bivalvia: Unionidae: Ambleminae). Natural History Journal of Chulalongkorn University. 2004;3(2):53–8.
32. Graf DL, Cummings KS. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). J Mollus Stud. 2007;73(4):291–314. https://doi.org/10.1093/mollus/eym029.
33. He J, Zhuang Z. The Freshwater Bivalves of China. ConchBooks; 2013.
34. Pfeiffer JM, Graf DL, Cummings KS, Page LM. Taxonomic revision of a radiation of Southeast Asian freshwater mussels (Unionidae: Gonideinae: Contradentini+Rectidentini). Invertebr Syst. 2021;35:394–470. https://doi.org/10.1071/IS20044.

35. Huang XC, Su JH, Ouyang JX, Ouyang S, Zhou CH, Wu XP. Towards a global phylogeny of freshwater mussels (Bivalivia: Unionida): Species delimitation of Chinese taxa, mitochondrial phylogenomics, and diversification patterns. Mol Phylogenet Evol. 2019;130:45–59. https://doi.org/10.1016/j.ympev.2018.09.019.
36. Lopes-Lima M, Hattori A, Kondo T, Hee Lee J, Ki Kim S, Shirai A, et al. Freshwater mussels (Bivalvia: Unionidae) from the rising sun (Far East Asia): phylogeny, systematics, and distribution. Mol Phylogenet Evol. 2020;146: 106755. https://doi.org/10.1016/j.ympev.2020.106755.
37. Bolotov IN, Kondakov AV, Konopleva ES, Vikhrev IV. A new genus of ultra-elongate freshwater mussels from Vietnam and eastern China (Bivalvia: Unionidae). Ecol. Montenegrina. 2021;39:1–6. https://doi.org/10.37828/em.2021.39.1.
38. Dai YT, Huang XC, Wu CX, Chen ZM, Guo L, Shu FY, et al. Multilocus and mitogenomic phylogenetic analyses reveal a new genus and species of freshwater mussel (Bivalvia: Unionidae) from Guangxi. China Invertebr Syst. 2023;37(2):152–66. https://doi.org/10.1071/IS22048.
39. Wu RW, Zhang LP, Liu LL, Jia JL, Liu XJ. Unraveling the phylogenetic relationships and taxonomic status of a puzzling freshwater mussel genus *Inversidens* (Bivalvia, Unionidae) through multilocus phylogeny and mitochondrial phylogenomics. J Zool Syst Evol Res. 2024;1499508:1–18. https://doi.org/10.1155/2024/1499508.
40. Wu RW, Liu LL, Zhang LP, Bogan AE, Niu GY, Jin DD, et al. Taxonomic revision of two species in the genus *Ptychorhynchus* Simpson, 1900 (Bivalvia: Unionidae: Gonideinae), with description of a new species. Invertebr Syst. 2024;38:IS24014. https://doi.org/10.1071/IS24014.
41. Pfeiffer JM, Breinholt JW, Page LM. Unioverse: a phylogenetic resource for reconstructing the evolution of freshwater mussels (Bivalvia, Unionoida). Mol Phylogenet Evol. 2019;137:114–26. https://doi.org/10.1016/j.ympev.2019.02.016.
42. Lopes-Lima M, Froufe E, Do VT, Ghamizi M, Mock KE, Kebapći Ü, et al. Phylogeny of the most species-rich freshwater bivalve family (Bivalvia: Unionida: Unionidae): Defining modern subfamilies and tribes. Mol Phylogenet Evol. 2017;106:174–91. https://doi.org/10.1016/j.ympev.2016.08.021.
43. Froufe E, Bolotov I, Aldridge DC, Bogan AE, Breton S, Gan HM, et al. Mesozoic mitogenome rearrangements and freshwater mussel (Bivalvia: Unionoidea) macroevolution. Heredity (Edinb). 2020;124(1):182–96. https://doi.org/10.1038/s41437-019-0242-y.
44. Zieritz A, Froufe E, Bolotov IN, Gonçalves DV, Aldridge DC, Bogan AE, et al. Mitogenomic phylogeny and fossil-calibrated mutation rates for all F- and M-type mtDNA genes of the largest freshwater mussel family, the Unionidae (Bivalvia). Zool J Linn Soc. 2021;193(3):1088–107. https://doi.org/10.1093/zoolinnean/zlaa153.
45. Heude MP. Conchyliologie Fluviatile de la Provoince de Nanking (3): pls. 17–24. 1877.
46. Heude MP. Diagnoses molluscoum in fluminibus provinciæ Nankingensis collectorum. Journal de Conchyliologie. 1874;22:112–8.
47. Liu LL, Zhang LP, Hou KY, Ning LY, Wu RW. Addition to the known diversity of Chinese freshwater mussels: integrative description of a new species of *Postolata* Dai et al., 2023 (Bivalvia, Unionidae, Gonideinae). Zoosyst Evol. 2024;100(3):769–778. https://doi.org/10.3897/zse.100.126069.
48. Zieritz A, Aldridge DC. Identification of ecophenotypic trends within three European freshwater mussel species (Bivalvia: Unionoida) using traditional and modern morphometric techniques. Biol J Linn Soc. 2009;98(4):814–25. https://doi.org/10.1111/j.1095-8312.2009.01329.x.
49. Prié V, Puillandre N, Bouchet P, Bad taxonomy can kill: molecular reevaluation of Unio mancus Lamarck,. (Bivalvia: Unionidae) and its accepted subspecies. Knowl Manag Aquat Ecosyst. 1819;2012:405. https://doi.org/10.1051/kmae/2012014.
50. Inoue K, Hayes DM, Harris JL, Christian AD. Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (Bivalvia: Unionidae). Ecol Evol. 2013;3(8):2670–83. https://doi.org/10.1002/ece3.649.
51. Inoue K, Mcqueen AL, Harris JL, Berg DJ. Molecular phylogenetics and morphological variation reveal recent speciation in freshwater mussels of the genera *Arcidens* and *Arkansia* (Bivalvia: Unionidae). Biol J Linn Soc. 2014;112(3):535–45. https://doi.org/10.1111/bij.12282.
52. Klishko OK, Lopes-Lima M, Bogan AE, Matafonov DV, Froufe E. Morphological and molecular analyses of Anodontinae species (Bivalvia, Unionidae) of Lake Baikal and Transbaikalia. PLoS ONE. 2018;13(4): e0194944. https://doi.org/10.1371/journal.pone.0194944.
53. Wu RW, Liu XJ, Guo L, Zhou CH, Ouyang S, Wu XP. DNA barcoding, multilocus phylogeny, and morphometry reveal phenotypic plasticity in the Chinese freshwater mussel *Lamprotula caveata* (Bivalvia: Unionidae). Ecol Evol. 2022;12(7): e9035. https://doi.org/10.1002/ece3.9035.
54. Zieritz A, Bogan AE, Froufe E, Klishko O, Kondo T, Kovitvadhi U, et al. Diversity, biogeography and conservation of freshwater mussels (Bivalvia: Unionida) in East and Southeast Asia. Hydrobiologia. 2018;810(1):29–44. https://doi.org/10.1007/s10750-017-3104-8.
55. Liu LL, Zhang LP, Jin DD, Wang HT, Liu XJ, Wu RW. Molecular and morphological evidence reveals a hidden new taxon in the endemic genus *Pseudocuneopsis* (Bivalvia, Unionidae) from China. ZooKeys. 2023;1179:219–29. https://doi.org/10.3897/zookeys.1179.109817.
56. Wu RW, Liu LL, Zhang LP, Jia JL, Wu XP, Liu XJ. New species of the genus *Pseudocuneopsis* Huang, Dai, Chen & Wu, 2022 (Bivalvia, Unionidae) from Guangxi Province. China ZooKeys. 2023;1166:261–70. https://doi.org/10.3897/zookeys.1166.104150.
57. Wu RW, Liu LL, Zhang LP, Jin DD, Wu XP, Liu XJ, et al. Molecular phylogeny and comparative morphology reveal the species validity and systematic position of *Lepidodesma* (Bivalvia: Unionidae). Org Divers Evol. 2023;1–16. https://doi.org/10.1007/s13127-023-00627-6.
58. Wu RW, Liu YT, Wang S, Liu XJ, Zanatta DT, Roe KJ, et al. Testing the utility of DNA barcodes and a preliminary phylogenetic framework for Chinese freshwater mussels (Bivalvia: Unionidae) from the middle and lower Yangtze River. PLoS ONE. 2018;13(8): e0200956. https://doi.org/10.1371/journal.pone.0200956.
59. Guo L. 'Unionida of China.' (Straits Publishing); 2022.
60. Zouros E. Biparental inheritance through uniparental transmission: The doubly uniparental inheritance (DUI) of mitochondrial DNA. Evol Biol. 2013;40:1–31. https://doi.org/10.1007/s11692-012-9195-2.
61. Guerra D, Plazzi F, Stewart DT, Bogan AE, Hoeh WR, Breton S. Evolution of sex-dependent mtDNA transmission in freshwater mussels (Bivalvia: Unionida). Sci Rep. 2017;7(1):1551. https://doi.org/10.1038/s41598-017-01708-1.
62. Capt C, Renaut S, Ghiselli F, Milani L, Johnson NA, Sietman BE, Stewart DT, Breton S. Deciphering the Link between Doubly Uniparental Inheritance of mtDNA and Sex Determination in Bivalves: Clues from Comparative Transcriptomics. Genome Biol Evol. 2018;10(2):577–90. https://doi.org/10.1093/gbe/evy019.
63. Walker JM, Bogan AE, Bonfiglio EA, Campbell DC, Christian AD, Curole JP, et al. Primers for amplifying the hypervariable, male-transmitted COII-COI junction region in amblemine freshwater mussels (Bivalvia: Unionoidea: Ambleminae). Mol Ecol Notes. 2007;7:489–91. https://doi.org/10.1111/j.1471-8286.2006.01630.x.
64. Chen SF, Zhou YQ, Chen YR, Gu J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34(17):i884–90. https://doi.org/10.1093/bioinformatics/bty560.
65. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28(12):1647–9. https://doi.org/10.1093/bioinformatics/bts199.
66. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, et al. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 2013;69(2):313–9. https://doi.org/10.1016/j.ympev.2012.08.023.
67. Laslett D, Canbäck B. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics. 2008;24(2):172–5. https://doi.org/10.1093/bioinformatics/btm573.
68. Zheng S, Poczai P, Hyvönen J, Tang J, Amiryousefi A. Chloroplot: An Online Program for the Versatile Plotting of Organelle Genomes. Front Genet. 2020;11: 576124. https://doi.org/10.3389/fgene.2020.576124.
69. SeqMan NGen®. Version 17.5. DNASTAR, Inc. Madison, WI.
70. Perna NT, Kocher TD. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. J Mol Evol. 1995;41(3):353–8. https://doi.org/10.1007/BF00186547.
71. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016;33(7):1870–4. https://doi.org/10.1093/molbev/msw054.
72. Mathews DH, Disney MD, Childs JL, Schroeder SJ, Zuker M, Turner DH. Incorporating chemical modification constraints into a dynamic

programming algorithm for prediction of RNA secondary structure. Proc Natl Acad Sci U S A. 2004;101(19):7287–92. https://doi.org/10.1073/pnas.0401799101.
73. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32(5):1792–7. https://doi.org/10.1093/nar/gkh340.
74. Zhang D, Gao FL, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol Ecol Resour. 2020;20(1):348–55. https://doi.org/10.1111/1755-0998.13096.
75. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30(4):772–80. https://doi.org/10.1093/molbev/mst010.
76. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol. 2000;17(4):540–52. https://doi.org/10.1093/oxfordjournals.molbev.a026334.
77. Minh BQ, Nguyen MA, von Haeseler A. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol. 2013;30(5):1188–95. https://doi.org/10.1093/molbev/mst024.
78. Xia XH, Xie Z, Salemi M, Chen L, Wang Y. An index of substitution saturation and its application. Mol Phylogenet Evol. 2003;26(1):1–7. https://doi.org/10.1016/s1055-7903(02)00326-3.
79. Xia XH, Lemey P. "Assessing substitution saturation with DAMBE," in The Phylogenetic Handbook: a Practical Approach to DNA and Protein Phylogeny, ed. L. Philippe (London: Cambridge University Press), 2009;615–630. https://doi.org/10.1017/cbo9780511819049.022.
80. Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. Mol Biol Evol. 2017;34(3):772–3. https://doi.org/10.1093/molbev/msw260.
81. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017;14(6):587–9. https://doi.org/10.1038/nmeth.4285.
82. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 2012;61(3):539–42. https://doi.org/10.1093/sysbio/sys029.
83. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. IQ-TREE 2: New models and efficient methods for phylogenetic Inference in the genomic era. Mol Biol Evol. 2020;37(5):1530–4. https://doi.org/10.1093/molbev/msaa015.
84. Letunic I, Bork P. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics (Oxford, England). 2007;23(1):127–8. https://doi.org/10.1093/bioinformatics/btl529.
85. Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G, Wu S, Wang Y. SRplot: A free online platform for data visualization and graphing. PLoS ONE. 2023;18(11): e0294236. https://doi.org/10.1371/journal.pone.0294236.
86. IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp.
87. Midway S, Robertson M, Flinn S, Kaller M. Comparing multiple comparisons: practical guidance for choosing the best multiple comparisons test. PeerJ. 2020;8: e10387. https://doi.org/10.7717/peerj.10387.
88. Dai YT, Chen ZG, Hu CL, Ning PF, Ouyang S, Huang XC, Wu XP. Taxonomic reassessment of *Scabies* (Bivalvia: Unionidae) species in China based on multilocus and mitogenomic phylogenetic analyses. Invertebr Syst. 2024;38(6). https://doi.org/10.1071/IS24020.
89. Shu FY, Wu XP. Comparative anatomy of the anus of 20 unionid species (Bivalvia: unionidae) and its systematic significance. Transactions of Oceanology and Limnology. 2005;2:29–33.
90. Barraclough TG, Nee S. Phylogenetics and speciation. Trends Ecol Evol. 2001;16(7):391–9. https://doi.org/10.1016/S0169-5347(01)02161-9.
91. Mace GM. The role of taxonomy in species conservation. Philos Trans R Soc Lond B Biol Sci. 2004;359(1444):711–9. https://doi.org/10.1098/rstb.2003.1454.
92. Johnson NA, Smith CH, Pfeiffer JM, Randklev CR, Williams JD, Austin JD. Integrative taxonomy resolves taxonomic uncertainty for freshwater mussels being considered for protection under the U.S. Endangered Species Act. Sci Rep. 2018;8(1):15892. https://doi.org/10.1038/s41598-018-33806-z.
93. Pieri AM, Inoue K, Johnson NA, Smith CH, Harris JL, Robertson C, Randklev CR. Molecular and morphometric analyses reveal cryptic diversity within freshwater mussels (Bivalvia: Unionidae) of the western gulf coastal drainages of the USA. Biol J Linn Soc. 2018;124(2):261–77. https://doi.org/10.1093/biolinnean/bly046.
94. Klishko OK, Lopes-Lima M, Froufe E, Bogan AE. Are *Cristaria herculea* (Middendorff, 1847) and *Cristaria plicata* (Leach, 1815) (Bivalvia, Unionidae) separate species? Zookeys. 2014;438:1–15. https://doi.org/10.3897/zookeys.438.7493.
95. Klishko OK, Lopes-Lima M, Froufe E, Bogan AE, Vasiliev L, Yanovich LN. Taxonomic reassessment of the freshwater mussel genus Unio (Bivalvia: Unionidae) in Russia and Ukraine based on morphological and molecular data. Zootaxa. 2017;4286:93–112. https://doi.org/10.11646/zootaxa.4286.1.4.
96. Wu RW, An JM, Chen RR, Ouyang S, Wu XP. Taxonomy, phylogeny and evolution of freshwater mussels (Unionoida: Unionoidea) in China revealed by multilocus phylogenetic analyses and mitochondrial phylogenomics. J Lake Sci. 2021;33(6):1788–1804. https://doi.org/10.18307/2021.0615.
97. Yang HR, Zhang JE, Xia J, Yang JZ, Guo J, Deng ZX, Luo MZ. Comparative Characterization of the Complete Mitochondrial Genomes of the Three Apple Snails (Gastropoda: Ampullariidae) and the Phylogenetic Analyses. Int J Mol Sci. 2018;19(11):3646. https://doi.org/10.3390/ijms19113646.
98. Xu W, Ding JY, Lin SP, Xu RF, Liu HY. Comparative mitogenomes of three species in *Moenkhausia*: Rare irregular gene rearrangement within Characidae. Int J Biol Macromol. 2021;183:1079–86. https://doi.org/10.1016/j.ijbiomac.2021.05.049.
99. Yu P, Zhou L, Yang WT, Miao LJ, Li Z, Zhang XJ, et al. Comparative mitogenome analyses uncover mitogenome features and phylogenetic implications of the subfamily Cobitinae. BMC Genomics. 2021;22(1):50. https://doi.org/10.1186/s12864-020-07360-w.
100. Mendivil A, Ramírez R, Morin J, Ramirez JL, Sicha-Ramirez R, Britzke R, Rivera F, Ampuero A, Oliveros N, Congrains C. Comparative Mitogenome Analysis of Two Native Apple Snail Species (Ampullariidae, *Pomacea*) from Peruvian Amazon. Genes. 2023;14(9):1769. https://doi.org/10.3390/genes14091769.
101. Zhao Q, Wang J, Wang MQ, Cai B, Zhang HF, Wei JF. Complete Mitochondrial Genome of *Dinorhynchus dybowskyi* (Hemiptera: Pentatomidae: Asopinae) and Phylogenetic Analysis of Pentatomomorpha Species. J Insect Sci. 2018;18(2):44. https://doi.org/10.1093/jisesa/iey031.
102. Yang Q, Liu S, Song F, Liu GF, Yu XP. Comparative mitogenome analysis on species of four apple snails (Ampullariidae: *Pomacea*). Int J Biol Macromol. 2018;118:525–33. https://doi.org/10.1016/j.ijbiomac.2018.06.092.
103. Guo Q, Liu L, Huang W, Sang W, Chen X, Wang X. Characterization of the complete mitogenome of *Trachylophus sinensis* (Coleoptera: Cerambycidae: Cerambycinae), the type species of *Trachylophus* and its phylogenetic implications. J Asia Pac Entomo. 2022;25(4):101977–86. https://doi.org/10.1016/j.aspen.2022.101977.
104. Li H, Li J. Comparative analysis of four complete mitogenomes from hoverfly genus *Eristalinus* with phylogenetic implications. Sci Rep. 2022;12(1):4164. https://doi.org/10.1038/s41598-022-08172-6.
105. Clayton DA. Replication and transcription of vertebrate mitochondrial DNA. Annu Rev Cell Biol. 1991;7:453–78. https://doi.org/10.1146/annurev.cb.07.110191.002321.
106. Lopes-Lima M, Fonseca MM, Aldridge DC, Bogan AE, Gan HM, Ghamizi M, et al. The first Margaritiferidae male (M-type) mitogenome: mitochondrial gene order as a potential character for determining higher-order phylogeny within Unionida (Bivalvia). J Mollus Stud. 2017;83:249–52. https://doi.org/10.1093/mollus/eyx009.
107. Zhou CH, Huang XC, Ouyang S, Ouyang JX, Wu XP. Characterization of the complete maternal mitochondrial genome of *Ptychorhynchus pfisteri* (Bivalvia: Unionidae: Gonideinae). Conserv Genet Resour. 2017;9:233–5. https://doi.org/10.1007/s12686-016-0657-1.
108. Huang XC, Wu RW, An CT, Xie GL, Su JH, Ouyang S, Zhou CH, Wu XP. Reclassification of *Lamprotula rochechouartii* as *Margaritifera rochechouartii* comb. nov (Bivalvia: Margaritiferidae) revealed by time-calibrated multi-locus phylogenetic analyses and mitochondrial phylogenomics of Unionoida. Mol Phylogenet Evol. 2018;120:297–306. https://doi.org/10.1016/j.ympev.2017.12.017.

109. Wu XP, Dai YT, Yin N, Shu FY, Chen ZG, Guo L, Zhou CH, Ouyang S, Huang XC. Mitogenomic phylogeny resolves *Cuneopsis* (Bivalvia: Unionidae) as polyphyletic: The description of two new genera and a new species. Zool Scr. 2022;51(2):173–84. https://doi.org/10.1111/zsc.12527.
110. Dai YT, Chen ZM, Peng KJ, Ouyang S, Huang XC, Wu XP. Revisiting the genus *Pseudocuneopsis* (Bivalvia, Unionidae): Morphology, mitochondrial phylogenomics, and the description of a new species. Zool Scr. 2024;53:323–37. https://doi.org/10.1111/zsc.12647.
111. Wu RW, Liu LL, Zhang LP, Liu XJ, Hu ZK, Jin DD, et al. Diversity, morphology, and phylogeny of freshwater mussels of the genus *Nodularia* (Bivalvia: Unionidae) from China, with descriptions of four new species. Zool Scr. 2024;00:1–20. https://doi.org/10.1111/zsc.12677.
112. Shu FY, Wang HJ, Pan BZ, Liu XQ, Wang HZ. Assessment of species status of mollusca in the mid-lower Yangtze lakes. Acta Hydrobiol Sin. 2009;33:1051–8. https://doi.org/10.3724/SP.J.1035.2009.61051.
113. Xiong LF, Ouyang S, Wu XP. Fauna and standing crop of freshwater mussels in Poyang Lake. Chin J Oceanol Limnol. 2012;30:124–35. https://doi.org/10.1007/s00343-012-1070-6.
114. Xu Y, Zhang GR, Guo SS, Guo XB, Wei K, Ge T. Isolation and characterization of fifteen polymorphic microsatellite loci in the threatened freshwater mussel *Solenaia oleivora* (Bivalvia: Unionidae). Biochem Syst Ecol. 2013;47:104–7. https://doi.org/10.1016/j.bse.2012.11.009.

Publisher's Note