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An integrative taxonomic reassessment of the Chinese endemic genus *Acuticosta* (Bivalvia: Unionidae), with the description of a new genus

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Abstract

Traditional species classification and identification based solely on shell morphology encounter significant challenges in freshwater mussels due to morphological variation and convergence. As a freshwater mussel genus endemic to China, *Acuticosta* (Bivalvia: Unionidae) has not been systematically revised due to the lack of molecular data. Moreover, the considerable phenotypic variation among individuals further complicates species taxonomy. This study conducted the most comprehensive sampling of *Acuticosta* to date in China, and the preliminary analyses challenged the monophyly of this genus. Integrating mitochondrial genomics, shell morphology, and soft-body anatomical evidence, we proposed a new genus, *Pseudoacuticosta* **gen. nov.**, and supported the reclassification of three species originally belonging to *Acuticosta*: *Pseudoacuticosta retiaria* **gen. & comb. nov.**, *Inversidens trisulcata* **comb. nov.**, and *Inversidens sichuanica* **comb. nov.** Meanwhile, we identified various phenotypes that complicate species identification in *Acuticosta chinensis* and *Acuticosta ovata*, and provided updated DNA barcode data. This study highlights the potential utility of soft-tissue anatomical characteristics for genus-level classification within this taxonomic group. Furthermore, our findings underscore that integrative taxonomy, based on molecular phylogenetics, can effectively resolve issues arising from phenotypic variation and historical classification ambiguities.

Keywords

Acuticosta – anatomy – China – endemic species – mitochondrial phylogenomics – morphology – taxonomic revision – Unionidae

Introduction

Biodiversity constitutes the foundation of life on Earth, and accurate classification is the first step in the conservation of threatened species. Freshwater mussels of the family Unionidae are the most species-rich group within the order Unionida (Graf & Cummings, 2007; Bogan & Roe, 2008; Lopes-Lima et al., 2017a). This family is generally recognized as comprising five subfamilies: Ambleminae, Unioninae, Gonideinae, Parreysiinae, and Modellnaiinae, which together include more than 800 species (Huang et al., 2019; Graf & Cummings, see https://musselpdb.org/). Unionids are widely distributed across most freshwater habitats worldwide, with the highest species diversity found in East Asia and North America (Bogan, 2008). As an important part of East Asia, China's unique paleogeographic

environment and intricate water systems have created diverse aquatic ecosystems and habitat heterogeneity, giving rise to an important biodiversity hotspot for freshwater mussels (Zieritz et al., 2018; Liu et al., 2022). This fauna has garnered increasing attention from both the scientific community and the general public due to its significant ecological, ornamental, and economic value, as well as its vulnerable conservation status (Lydeard et al., 2004; Vaughn et al., 2008; Haag & Williams, 2014; Lopes-Lima et al., 2017b; Ferreira-Rodríguez et al., 2019).

Traditionally, taxonomic studies of freshwater mussels have primarily relied on the morphological characteristics of shells (Heude, 1874; Simpson, 1900, 1914; Haas, 1969; Liu et al., 1979; He & Zhuang, 2013). However, it is widely recognized that shell morphology in freshwater bivalves often exhibits considerable phenotypic plasticity and convergence, which poses challenges to traditional classification approaches and may result in misclassification of species as well as inaccurate estimations of species diversity (Zieritz & Aldridge, 2009; Inoue et al., 2013, 2014; Wu et al., 2022a). Fortunately, phylogenetic data, as a critical source of information in modern systematics research, provide valuable insights into taxonomy and evolutionary relationships (Bolotov et al., 2020, 2023; Zieritz et al., 2021; Wu et al., 2023). Over the past decade, the integration of morphological, anatomical, and molecular phylogenetic data has significantly improved biodiversity assessments and taxonomic revisions in freshwater mussels (Keogh & Simons, 2019; Sano et al., 2022; Bogan et al., 2023; Whelan et al., 2023).

The genus *Acuticosta*, endemic to China, was established by Simpson (1900) with *Unio chinensis* Lea, 1868 as the type species. Based on the morphological characteristics of the shells, such as shape, sculpture, size and color, *Acuticosta* is currently considered to include five species, namely *Acuticosta chinensis* (Lea, 1868), *A. ovata* (Simpson, 1900), *A. retiaria* (Heude, 1883), *A. sichuanica* Zeng & Liu, 1989 and *A. trisulcata* (Heude, 1883), which are widely distributed in the Yangtze River Basin (Liu et al., 1979; Liu et al., 2020, 2022). In recent years, with the development and application of molecular technology, the taxonomic status of the genus *Acuticosta*, which belongs to the tribe Acuticostini Starobogatov, 1967 in the subfamily Unioninae based on the model species *A. chinensis*, has been recognized (Huang et al., 2019; Lopes-Lima et al., 2020). However, due to the lack of molecular data for other species, their validity and taxonomic position remain controversial and unconfirmed.

Graf & Cummings (2021), based on shell morphology, considered *A. chinensis* to be a synonym of *A. retiaria*. Huang et al. (2019) suggested that *A. chinensis* and *A. ovata* belong to the same species using DNA barcoding data (mitochondrial *CO1* gene fragment). However, Wu et al. (1999a) conducted a comparative analysis of the glochidia morphology between *A. chinensis* and *A. ovata*, revealing significant morphological differences between the two species. Traditional morphology holds that the morphological characteristics of the glochidia are an important basis for the classification of unionids (Simpson, 1900, 1914; Haas, 1969; Wu et al., 1999b; Haag, 2012). We speculate that the above-mentioned different results may be caused by two reasons. On the one hand, it is due to species misidentification caused by individual variation or convergence in

the morphological identification of shells in the wild; on the other hand, the characteristics of the glochidia are not only of limited significance in classification at the genus level (Wu et al., 2021), but may also be inapplicable to species-level identification. *A. retiaria* and *A. trisulcata* have been accepted in this classification since Haas (1914) revised the genus-level classification based on shell morphological characteristics. *A. sichuanica*, which shares similar shell morphological features with *A. trisulcata*, is also accepted under the current classification. However, due to the lack of molecular data for *A. retiaria*, *A. trisulcata*, and *A. sichuanica*, their taxonomic positions have not been reviewed. Therefore, modern molecular methods are needed to re-evaluate species validity and phylogenetic relationships.

At present, research and field investigations on *Acuticosta* in China are mainly confined to the Poyang Lake Basin in Jiangxi Province (Ouyang et al., 2015; Wu et al., 2018; Huang et al., 2019), with less sampling conducted in the upper reaches of the Yangtze River, the Yellow River Basin, and other river basins in Southwest China. Meanwhile, during our sampling process, we found that different populations of *Acuticosta* show significant differences in shell shape, sculpture, and color. However, due to the lack of molecular evidence, it is impossible to determine whether these variations are intraspecific adaptations or indicative of cryptic species. Therefore, the imbalance in regional surveys has hindered a comprehensive understanding of the diversity of this genus, thereby impeding a thorough understanding of its phylogeny and evolution.

In this study, we conducted the most extensive species sampling in China to date, covering 11 provinces and collecting over 500 specimens of the genus *Acuticosta*. We integrated molecular phylogenetics, shell morphology, and soft-body anatomy to achieve the following objectives: (1) to evaluate and identify the validity and diversity of *Acuticosta* species; and (2) to clarify the taxonomic positions and phylogenetic relationships of species. The research outcomes not only establish a comprehensive and accurate classification framework for the genus *Acuticosta*, benefiting subsequent species conservation and ecological monitoring efforts, but also enhance our understanding of the origin and diversification of this group, providing an essential classification basis for future biogeographic studies.

Materials and methods

Taxon sampling, morphological and anatomical observations

From 2020 to 2025, we collected a total of over 500 *Acuticosta* spp. specimens from 11 provinces in China, including Zhejiang (ZJ), Hunan (HUN), Anhui (AH), Guangxi (GX), Jiangxi (JX), Hubei (HB), Chongqing (CQ), Henan (HEN), Guangdong (GD), Fujian (FJ), and Jiangsu (JS) (Fig. 1). Of these, 160 specimens with tissue were selected for subsequent DNA extraction. Detailed information regarding the collection sites and specimen data is provided in Table 1. All specimens have been deposited as voucher specimens at the Museum of Zoology, Shanxi Normal University (SXNU), China.

Detailed conchological and anatomical features of the collected specimens were examined through naked-eye observation and with a stereoscopic microscope

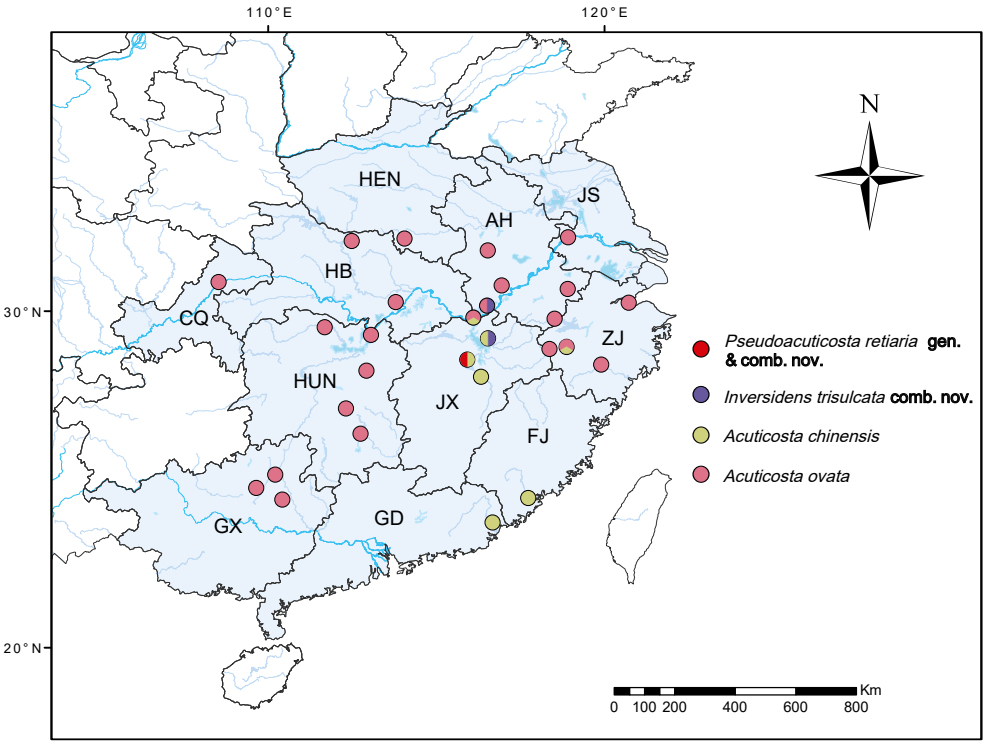

FIGURE 1 Map of collection sites. Coloured circles represent the collection sites for each target species. The background layer represents the recorded species distribution. Capital abbreviations denote Chinese provinces as follows: Hunan (HUN), Hubei (HB), Henan (HEN), Hebei (HB), Jiangxi (JX), Jiangsu (JS), Anhui (AH), Zhejiang (ZJ), Guangdong (GD), Guangxi (GX), and Chongqing (CQ).

(SZX10, Olympus), including shell shape, umbo position, surface sculpture, hinge structure, muscle scar attachment, labial palps, and papillae in the incurrent and excurrent apertures. Based on the MUSSEL Project website (Graf & Cummings, see https://musselpdb.org/) and published literature (Lea, 1868; Heude, 1883; Simpson, 1900; Liu et al., 1979; Zeng & Liu, 1989; He & Zhang, 2013), morphological identification revealed four species of the genus *Acuticosta*, namely *A. chinensis* (Lea, 1868), *A. ovata* (Simpson, 1900), *A. trisulcata* (Heude, 1883), and *A. retiaria* (Heude, 1883) (Fig. 2). Additionally, several specimens were difficult to identify morphologically (Fig. S1-S2), and we conduct molecular identification on them subsequently.

DNA extraction, PCR sequencing, and mitogenomes assembly

According to the manufacturer's instructions, a small piece of foot tissue was dissected for DNA extraction using the TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China). Polymerase chain reaction (PCR) amplification of the mitochondrial *COI* gene was performed using a primer pair consisting of (LCO22me2 5′-GGTCAACAAAY CATAARGATATTGG-3′ and HCO700dy2 5′-TCAGGGTGACCAAAAAAYCA-3′, ~680 bp)

TABLE 1 Collection information in this study

Species	Type locality	Collecting location	Voucher code	Specimen code
Acuticosta ovata	Xuancheng City and Chizhou City, Anhui Province	Dayang River, Lishui City, Zhejiang Province	SXNU_23091401	ZL01
			SXNU_23091402	ZL02
			SXNU_23091403	ZL03
			SXNU_23091404	ZL04
			SXNU_23091405	ZL05
			SXNU_23091406	ZL06
			SXNU_23091408	ZL08
			SXNU_23091409	ZL09
			SXNU_23091410	ZL10
			SXNU_23091411	ZL11
			SXNU_23091412	ZL12
			SXNU_23091413	ZL13
			SXNU_23091414	ZL14
			SXNU_23091415	ZL15
		Dongting Lake, Yueyang City, Hunan Province	SXNU_23032612	DTH12
			SXNU_23032614	DTH14
			SXNU_24091807	QZ02
		Qinhuai River, Nanjing City, Jiangsu Province	SXNU_23072001	HS01
			SXNU_24072605	HS10
			SXNU_24072606	HS11
		Haichao River, Lishui City, Zhejiang Province	SXNU_24042005	LS09
			SXNU_24042006	LS10
			SXNU_24042007	LS11
		Qujiang River, Quzhou City, Zhejiang Province	SXNU_23040113	ZJ13
			SXNU_23040116	ZJ16
			SXNU_23040117	ZJ17
			SXNU_23040118	ZJ18
			SXNU_23040119	ZJ19
			SXNU_23040120	ZJ20
		Xiao'an River, Lishui City, Zhejiang Province	SXNU_24032301	ZJL01
			SXNU_24032302	ZJL02
			SXNU_24032303	ZJL03
			SXNU_24032304	ZJL04
			SXNU_24032305	ZJL05
			SXNU_24032306	ZJL06

TABLE 1 Collection information in this study (*cont.*)

Species	Type locality	Collecting location	Voucher code	Specimen code
			SXNU_24032307	ZJL07
			SXNU_24032308	ZJL08
			SXNU_24032309	ZJL09
		Xin'anjiang, Huangshan	SXNU_24072602	HS07
		City, Anhui Province	SXNU_24072603	HS08
			SXNU_24072609	HS14
			SXNU_24072610	HS15
		Shuiyang River,	SXNU_25021513	CS13
		Xuancheng City, Anhui	SXNU_22110203	JJ1
		Province	SXNU_22110202	JJ2
			SXNU_22110201	WX01
		Tiansha River, Anqing	SXNU_24110822	AQ08
		City, Anhui Province		
		Lijiang River, Guilin	SXNU_23032530	XL06
		City, Guangxi Zhuang		
		Autonomous Region		
		Yulong River, Guilin	SXNU_22121138	BS09
		City, Guangxi Zhuang	SXNU_22121145	BS11
		Autonomous Region		
		Yueshan River, Guilin	SXNU_22121123	BS23
		City, Guangxi Zhuang		
		Autonomous Region		
		Poyang Lake, Nanchang	SXNU_18071305	JC5
		City, Jiangxi Province	SXNU_18071306	JC6
			SXNU_18071313	JC13
			SXNU_18071330	JC30
			SXNU_18071331	JC31
			SXNU_18092104	JC4
		Jinjiang River,	SXNU_22110421	XJ21
		Xiangixiang City,	SXNU_22110422	XJ22
		Hunan Province	SXNU_22110423	XJ23
			SXNU_22110424	XJ24
		Lishui River, Changde	SXNU_25041126	JSS26
		City, Hunan Province		
			SXNU_25041127	JSS27
			SXNU_25041133	JSS33
			SXNU_25041137	JSS37
			SXNU_25041138	JSS38

TABLE 1 Collection information in this study (*cont.*)

Species	Type locality	Collecting location	Voucher code	Specimen code
			SXNU_25041145	JSS45
			SXNU_25041146	JSS46
			SXNU_25041147	JSS47
			SXNU_25041148	JSS48
		Lei River, Hengyang City, Hunan Province	SXNU_24091803	LLY03
		Zhengshui River, Hengyang City, Hunan Province	SXNU_24120904	SH04
			SXNU_24120905	SH05
			SXNU_24120906	SH06
			SXNU_24120907	SH07
		Pi River, Lu'an City, Anhui Province	SXNU_24091808	LA07
		Longrao Stream, Quzhou City, Zhejiang Province	SXNU_22121137	BS08
			SXNU_22121139	BS10
			SXNU_23030520	LJ20
			SXNU_23032531	XL07
			SXNU_23032532	XL08
			SXNU_25021512	CS12
			SXNU_25021514	CS14
			SXNU_25021516	CS16
		Cao'e River, Shaoxing City, Zhejiang Province	SXNU_23091604	ZSX01
			SXNU_23091605	ZSX02
			SXNU_23091606	ZSX03
		Han River, Wuhan City, Hubei Province	SXNU_23050721	WH01
			SXNU_23050722	WH02
		Shahe River, Xiangyang City, Hubei Province	SXNU_25050401	ZYS01
			SXNU_25050402	ZYS02
		Nan River, Kaizhou, Chongqing	SXNU_24091809	KZ01
		Nanwan Lake, Xinyang City, Henan Province	SXNU_22121807	XY07
			SXNU_22121808	XY08
			SXNU_22121809	XY09
			SXNU_22121810	XY10
			SXNU_22121811	XY11
			SXNU_22121812	XY12
			SXNU_22121813	XY13
			SXNU_22121814	XY14

TABLE 1 Collection information in this study (*cont.*)

Species	Type locality	Collecting location	Voucher code	Specimen code
			SXNU_22121815	XY15
			SXNU_22121816	XY16
Acuticosta chinensis	Hong Kong, China	Fuhe River, Fuzhou City, Jiangxi Province	SXNU_24101011	LC11
			SXNU_24101012	LC12
			SXNU_24101013	LC13
			SXNU_24101014	LC14
			SXNU_24101015	LC15
		Fuhe River, Nanchang City, Jiangxi Province	SXNU_24110808	XT08
			SXNU_24110809	XT09
			SXNU_24110810	XT10
		Hanjiang River, Chaozhou City, Guangdong Province	SXNU_23011004	CZH04
			SXNU_23011005	CZH05
			SXNU_23011006	CZH06
			SXNU_23011010	CZH10
			SXNU_23011011	CZH11
		Ninety-Nine Bays, Zhangzhou City, Fujian Province	SXNU_24060808	ZZ08
		Poyang Lake, Nanchang City, Jiangxi Province	SXNU_18071303	JC3
			SXNU_18071307	JC7
			SXNU_18071309	JC9
			SXNU_18071310	JC10
			SXNU_18071312	JC12
			SXNU_18071314	JC14
			SXNU_18071316	JC16
			SXNU_18071317	JC17
			SXNU_18071321	JC21
			SXNU_18071322	JC22
			SXNU_18071323	JC23
			SXNU_18071327	JC27
			SXNU_18071328	JC28
			SXNU_18071329	JC29
			SXNU_18071332	JC32
			SXNU_18071937	JC37
			SXNU_18071942	JC42
			SXNU_18071943	JC43

TABLE 1 Collection information in this study (*cont.*)

Species	Type locality	Collecting location	Voucher code	Specimen code
			SXNU_18071944	JC44
			SXNU_18071945	JC45
			SXNU_18080136	JC36
			SXNU_18080139	JC39
			SXNU_18092138	JC38
		Qujiang River, Quzhou City, Zhejiang Province	SXNU_24091806	QZ01
		Rao River, Shangrao City, Jiangxi Province	SXNU_24082613	SR13
			SXNU_24081501	SR27
		Yudai River, Nanchang City, Jiangxi Province	SXNU_24082801	JNC01
			SXNU_24082802	JNC02
			SXNU_24082803	JNC03
			SXNU_24082804	JNC04
			SXNU_24082805	JNC05
			SXNU_24082806	JNC06
			SXNU_24082807	JNC07
Pseudo-acuticosta retiaria **gen. & comb. nov.**	Xuancheng City and Chizhou City, Anhui Province	Fuhe River, Nanchang City, Jiangxi Province	SXNU_20241001	DTHS01
			SXNU_20241002	DTHS02
			SXNU_20241003	DTHS03
			SXNU_20241004	DTHS04
			SXNU_20241005	DTHS05
			SXNU_20241006	DTHS06
Inversidens trisulcata **comb. nov.**	Poyang County, Shangrao City, Jiangxi Province and Dongzhi County, Chizhou City, Anhui Province	Rao River, Shangrao City, Jiangxi Province	SXNU_24082610	SR10
			SXNU_24082611	SR11
			SXNU_24082614	SR14
			SXNU_24082615	SR15
			SXNU_24082616	SR16
		Xin'anjiang, Huangshan City, Anhui Province	SXNU_25112702	XA02
			SXNU_25112703	XA03

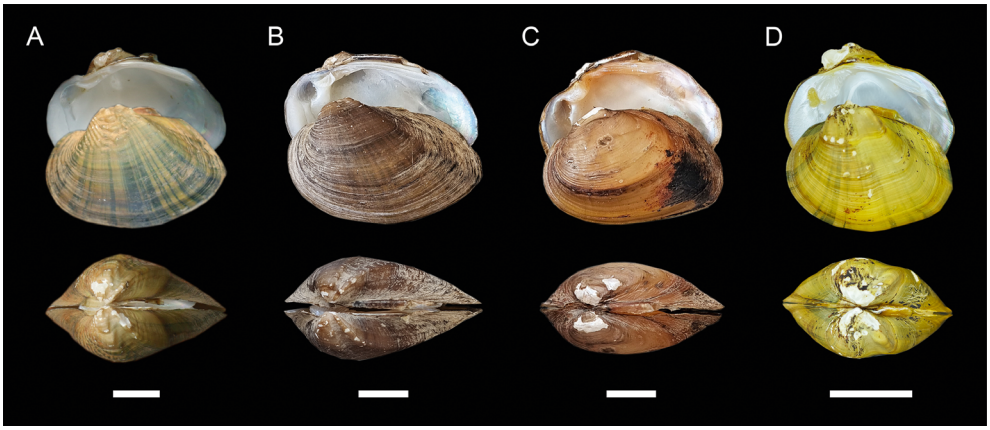

FIGURE 2 Shells of *Acuticosta chinensis* (A. voucher code: 01-ACF-2018), *Acuticosta ovata* (B. voucher code: SXNU_25041148), *Inversidens trisulcata* **comb. nov.** (C. voucher code: SXNU_24082611), and *Pseudoacuticosta retiaria* **gen. & comb. nov.** (D. voucher code: SXNU_20241005). For each species, 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; the shell umbo view is composed of both left and right valves. Scale = 1 cm.

(Walker et al., 2007). The PCR conditions followed the TaKaRa Ex TAQ polymerase manufacturer's protocol (TaKaRa Bio, Inc., Kusatsu, Shiga, Japan), with an initial denaturation step at 98°C for 10 s, followed by 35 cycles of amplification consisting of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°Cfor 1 minute. The final extension was performed at 72°C for 7 minutes. The amplified PCR products were visually examined on agarose gel electrophoresis (TAE, 1.5% gel), and purified and sequenced by Sangon Biotech (Shanghai). All obtained sequences were deposited in GenBank (Supplementary Table S1).

The sequencing and assembly of the mitochondrial genome follows methods found in Wu et al. (2024a, 2024b). Genomic DNA quality was assessed by agarose gel electrophoresis, and approved samples were submitted to Novogene Co., Ltd. (China) for library construction and sequencing. The sequencing procedure was performed on an Illumina Novaseq 6000 platform in accordance with the manufacturer's instructions (Illumina, Inc., San Diego, CA, USA). The resulting data comprises approximately ~ 4×10^9 bp, consisting of paired-end reads with a length of 150 bp. The raw data was filtered to obtain clean reads, which were then de novo assembled using the CLC Genomic Workbench (Qiagen, Hilden, Germany). The mitogenome sequence was identified from the resulting contigs using BLAST (http://blast.ncbi.nlm.nih.gov/) and then merged into a complete mitogenome using Geneious v.11 (Biomatters, Auckland, New Zealand). The complete mitogenome was annotated using MITOS WebServer (see http://mitos.bioinf.uni-leipzig.de/; Bernt et al., 2013). The NCBI ORF finder (http://www.ncbi.nlm.nih.gov/orffinder/) and nucleotide BLAST (Blastn)

analysis were used to determine the accuracy of protein-coding gene annotation. ARWEN (http://130.235.244.92/ARWEN/index.html; Laslett & Canbäck, 2008) was employed to verify the tRNA annotation results, while rRNA annotations were determined through alignment with closely related species. Finally, the four complete mitochondrial genome were submitted to GenBank via BankIt (Genbank no. PX277030–PX277032, PX644763; Supplementary Table S2).

Molecular dataset generation, alignment, partitioning strategies, and model selection

To achieve the objectives of this study, we constructed two molecular datasets: (1) A DNA barcoding (*COI*) dataset (Supplementary Table S1). We downloaded a total of 26 *COI* sequences for all available *Acuticosta* taxa in GenBank and combined them with the 160 sequences generated in this study. To reduce redundancy and identify potential conflicts in species identification, we used DnaSP (ver. 6.0, http://www.ub.edu/dnasp/; Rozas et al., 2017) to screen for haplotypes among the 186 *COI* sequences of *Acuticosta* taxa generated in this study. As a result, a total of 66 haplotypes were identified. Additionally, we downloaded sequences from seven species in the subfamily Unioninae, three species in the subfamily Gonideinae, and one species in the subfamily Ambleminae from GenBank to serve as ingroup taxa. Two species from the family Margaritiferidae, *Margaritifera margaritifera* (Linnaeus, 1758) and *Gibbosula rochechouartii* (Heude, 1875), were selected as outgroup taxa. Finally, the DNA barcode dataset was constructed by combining the identified haplotypes with the downloaded sequences. (2) A mitogenome dataset containing 12 protein-coding genes (PCGs) and 2 rRNA genes (*atp8* was excluded due to high sequence variation; Supplementary Table S2). In comparison to limited molecular markers, mitochondrial genomes are known for providing significantly more informative characters for phylogenetics and have proven highly effective in resolving both shallow and deep relationships in freshwater mussels (Zieritz et al., 2021, Wu et al., 2022b). To clarify the classification status and phylogenetic relationships within the *Acuticosta* taxa, we sequenced and assembled the complete mitochondrial genomes of four species for the first time: *Pseudoacuticosta retiaria* **gen. & comb. nov.**, *Acuticosta ovata*, *Inversidens trisulcata* **comb. nov.** and *Huizhou xietianxiangi* Chen, Chen, Xiang, He & Wu, 2025. In addition, we retrieved mitochondrial genome sequences of 63 species from the subfamily Unioninae and 29 species from the Gonideinae subfamily from GenBank. *Margaritifera margaritifera* and *Gibbosula rochechuartii* were also selected as outgroup taxa. Consequently, a comprehensive mitochondrial genome dataset containing a total of 98 complete sequences was established.

Protein-coding genes (PCGs) were aligned using the invertebrate mitochondrial codon models implemented by the built-in MACSE in PhyloSuite v1.2.3 (Zhang et al., 2020). Ribosomal genes (*12S rRNA* and *16S rRNA*) were aligned using MAFFT (ver. 7.2, see https://mafft.cbrc.jp/alignment/software/; Katoh & Standley, 2013) with the L-INS-i algorithm. Ambiguous alignment areas were trimmed using Gblocks (ver. 0.91b, see http://phylogeny.lirmm.fr

/phylo_cgi/one_task.cgi?task_type=gblocks; Castresana, 2000), the parameter ribosomal gene block with a minimum length was set to two base pairs (bp), allowed gap position was selected with half; the minimum length of PCG block was set to three bp, allowed gap position was selected none. The *COI* barcoding dataset had a fragment length of 552 bp after alignment and trimming. The mitogenomic dataset (12 PCGS + 2 rRNA) was concatenated using Phylosuite v1.2.3, resulting in a total of 11,913 bp.

The mitogenomic dataset was partitioned based on genes and codons using PartitionFinder (ver. 2.1.1, see https://github.com/brettc/partitionfinder; Lanfear et al., 2017) to select Bayesian inference (BI) and BEAST analysis models for the partitioning schemes. ModelFinder (see http://www.iqtree.org/ModelFinder/; Kalyaanamoorthy et al., 2017) was employed in IQ-TREE (ver. 2.4.0, see http://iqtree.cibiv.univie.ac.at/; Minh et al., 2020) to determine the maximum likelihood (ML) analysis models. The selection of best-fit models was based on the corrected Akaike Information Criterion (AICc). Substitution models assigned to each partition by PartitionFinder and ModelFinder were listed in Supplementary Table S3.

Phylogenetic analyses

Based on the *COI* dataset, Neighbour-joining (NJ) analysis and inter- and intra-specific distance calculations were conducted using the uncorrected p-distance model in MEGA 7.0 (Kumar et al., 2016) with 1000 bootstrap replicates (Minh et al., 2013).

The IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) was used for Maximum Likelihood (ML) analyses based on the mitogenome dataset, employing the ultrafast bootstrap algorithm with 1000 replicates (Minh et al., 2013). Bayesian inference (BI) analyses of the mitogenome dataset were conducted in MrBayes ver. 2.01 (Ronquist et al., 2012), using models generated in PartitionFinder (Lanfear et al., 2017). Four independent Markov Chain Monte Carlo (MCMC) chains were simultaneously run for ten million generations, with sampling conducted every 1000 generations and a burn-in of 25%. The process terminated when the average standard deviation of splitting frequency dropped below 0.01. Finally, the constructed phylogenetic trees were utilized in online iTOL (https://itol.embl.de/itol.cgi) for editing and visualization (Letunic & Bork, 2007).

Results

Species delimitation

Based on the barcoding dataset, the NJ tree shows that the genus *Acuticosta* forms four independent monophyletic branches, representing *A. chinensis*, *A. ovata*, *A. retiaria*, and *A. trisulcata*, which are consistent with morphological identification (Fig. 3).

A. chinensis and *A. ovata* exhibit a shallow divergence and form a sister relationship (bootstrap support (BS) = 100%). The calculation results of the intra- and interspecific genetic distances based on the uncorrected *p*-distance model show that the interspecific genetic distance between *A. chinensis* and *A. ovata* is 3.9%, and the intra-specific genetic distances are 1.0%

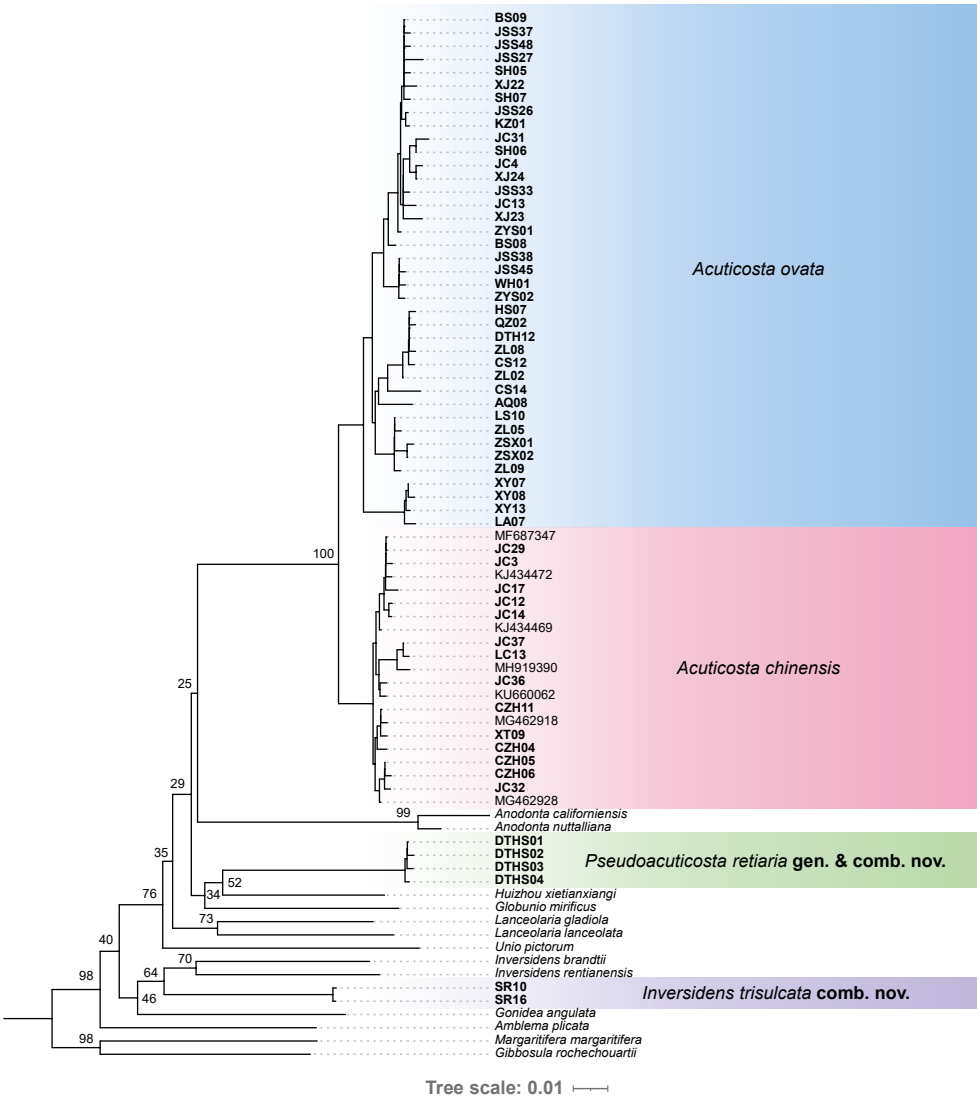

FIGURE 3 Neighbour-joining tree inferred from COI sequences based on the uncorrected *p*-distance model. Specimens collected in this study were shown in bold. The numbers on the branches represent the support values.

and 1.9%, respectively. The molecular identification results help clarify the morphological identification of the problematic individuals. *A. chinensis* (Fig. S1) and *A. ovata* (Fig. S2) exhibit considerable intraspecific morphological variation. Among the *Acuticosta* species, shell characteristics such as shape, sculpture patterns, and color are found to be unstable and are therefore unsuitable as diagnostic criteria for morphological species identification.

We noticed that the MUSSELp database has associated molecular data, including *COI* barcodes, for the listed

species. However, upon verification, it was found that some of the associated sequences have incorrect identifications. For instance, sequences MG462926, MG462927, MG462928, KJ434477, and KJ434478 were originally identified as *A. ovata* in GenBank, but based on the NJ tree constructed in this study, they cluster with *A. chinensis*. This result indicates that the species identification information for these sequences in GenBank is incorrect. Such errors may reflect the inherent difficulty in identifying both species.

Surprisingly, *A. retiaria* and *Huizhou xietianxiangi* are recovered as sister group (BS = 52%; Fig. 3). *A. trisulcata* is found to be sister to the genus *Inversidens* – *I. brandtii* and *I. rentianensis* (BS = 64%; Fig. 3). Although the bootstrap values in the NJ analysis are relatively low, the topological relationships suggest the need to reevaluate the phylogenetic placement of both *A. trisulcata* and *A. retiaria*. Here, we integrate mitochondrial genomic data with morphological characteristics to further investigate.

Mitochondrial genome structure

In this study, the maternally inherited mitochondrial genomes of *A. retiaria*, *A. ovata*, *A. trisulcata*, and *Huizhou xietianxiangi* were newly sequenced and determined, with lengths ranging from 15,653 bp to 16,047 bp.

All genomes contained all 37 genes commonly found in animal mtDNAs (Wolstenholme, 1992): 13 protein-coding genes (PCGs), 22 tRNAs (including 2 *trnL* and 2 *trnS*) and 2 rRNAs (Fig. 4A). The gene arrangement pattern of *A. retiaria*, *A. ovata*, and *H. xietianxiangi* was consistent with that observed in species of the subfamily Unioninae, whereas that of *A. trisulcata* aligned with species in the subfamily Gonideinae (except for *Chamberlainia somsakpanhai*) (Froufe et al., 2020, Wu et al., 2021). The gene arrangement pattern in unionid mussels followed the typical arrangement, with 11 genes (*trnH*, *cox2*, *cox1*, *cox3*, *atp6*, *trnD*, *atp8*, *nad4l*, *nad4*, *nad5* and *nad3*) located on the heavy strand and the remaining 26 genes encoded on the light strand (Fig. 4A). An (A + T) nucleotide bias has been commonly reported in unionid mitogenomes (Soroka, 2010), and the newly sequenced genomes exhibited this same characteristic.

Phylogenies based on the whole mitochondrial genome

The data matrix used for mitochondrial phylogenomics consisted of 11,913 bp and encompassed a total of 7,042 variable sites and 6,267 parsimony-informative sites. ML and BI trees based on the mitogenome dataset yielded an identical topology and were statistically well-supported by 100% bootstrap support (BS) values and posterior probabilities (PP) in most nodes (Fig. 4B).

Both the BI and ML trees support the monophyly of the subfamilies Unioninae and Gonideinae in the family Unionidae. Additionally, the five tribes within the Unioninae and the six tribes within the Gonideinae each form monophyletic groups. The specific phylogenetic relationships are as follows: (I) Unioninae: ((Unionini + (Anodontini + (Lanceolariini + Acuticostini))) + Lepi-dodesmini) (PP ≥ 0.81, BS ≥ 73%); (II) Gonideinae: ((((Gonideini + Pseudodontini) + Lamprotulini) + (Rectidentini + Contra-den-tini)) +

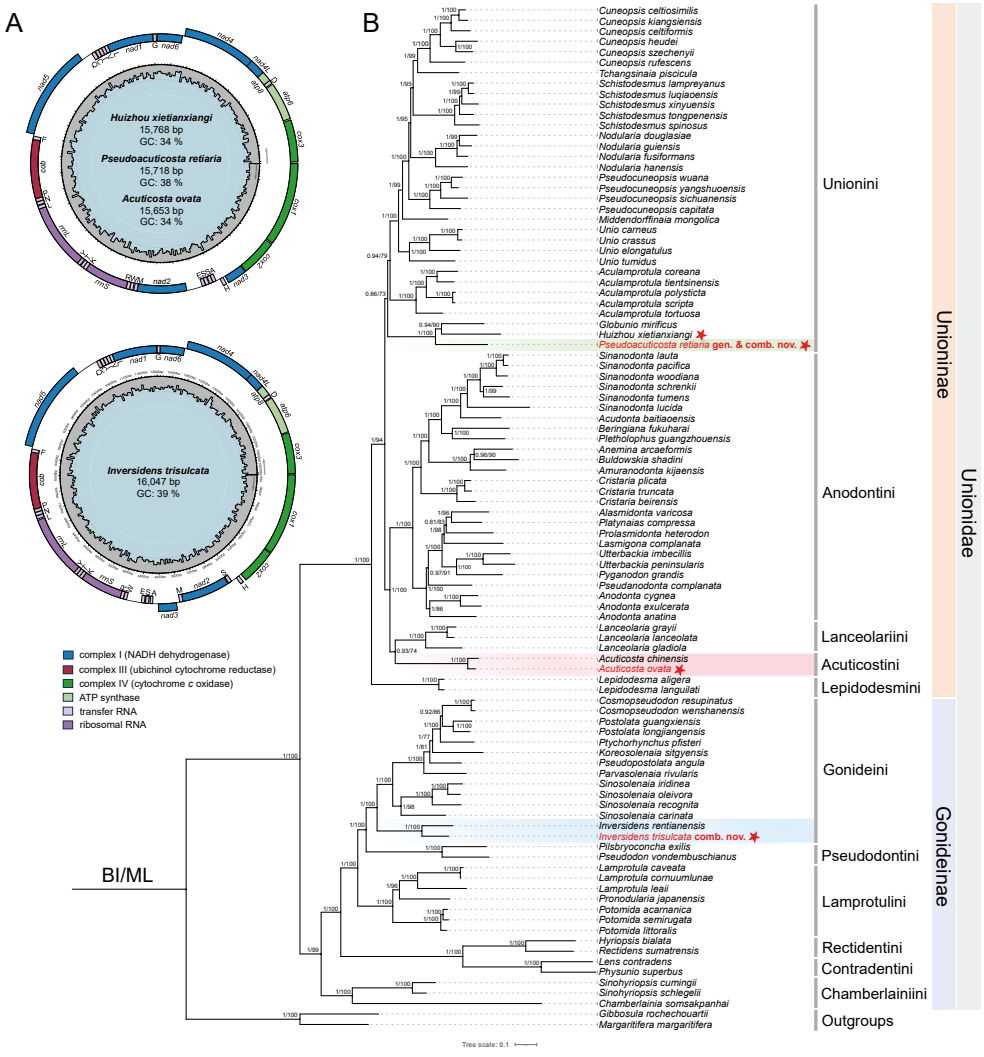

FIGURE 4 Analysis based on mitochondrial data. A. Gene map of the F-type mitochondrial genome of *Acuticosta ovata*, *Pseudoacuticosta retiaria* **gen. & comb. nov.**, *Inversidens trisulcata* **comb. nov.**, and *Huizhou xietianxiangi*. B. Phylogenetic trees inferred from Bayesian Inference (BI) and 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.

Chamberlainiini) (PP ≥ 0.92, BS ≥ 77%) (Fig. 4B).

Mitochondrial phylogenomic analyses confirm that the genus *Acuticosta* is paraphyletic (Fig. 4B). Focusing on the subfamily Unioninae, *A. ovata* and *A. chinensis* within the tribe Acuticostini form a sister relationship with maximum support (PP = 1.0, BS = 100%; Fig. 4B). However, *A. retiaria* is sister to (*Globunio mirificus* + *Huizhou xietianxiangi*) with strong support in the tribe Unionini (PP = 1.0, BS = 100%). Within the subfamily Gonideinae, *A. trisulcata* firmly forms a sister group with *Inversidens rentianensis* (PP = 1.0, BS = 100%; Fig. 4B), and constitutes the basal lineage of the tribe Gonideini.

Mitochondrial phylogenomics provides strong support for revising the taxonomic positions of *A. retiaria* and *A. trisulcata*. We support the transfer of *A. retiaria* to a new genus, i.e., *Pseudoacuticosta* **gen. nov.**, within the tribe Unionini, resulting in the new combination *Pseudoacuticosta retiaria* **gen. & comb. nov.** Similarly, *A. trisulcata* is transferred to the genus *Inversidens* within the tribe Gonideini, establishing the new combination *Inversidens trisulcata* **comb. nov.**

Soft-body anatomical features

We further examined the soft-body anatomical structures (including the labial palps and papillae in the incurrent and excurrent apertures) of the following species: *A. chinensis*, *A. ovata*, *Pseudoacuticosta retiaria* **gen. & comb. nov.**, *Inversidens trisulcata* **comb. nov.** and *I. rentianensis*.

The anatomical structures of *A. chinensis* and *A. ovata* are broadly similar. The papillae of the incurrent aperture for both species are slender and conical, arranged irregularly in two to three rows (Fig. 5A1, B1). The papillae of the excurrent aperture are short in *A. chinensis* but long and slender in *A. ovata*, and both exhibit dark pigment streaks (Fig. 5A1, B1). The labial palps in both species are of a long elliptical shape (Fig. 5A2, B2).

Pseudoacuticosta retiaria differ from the two species of *Acuticosta*. The papillae of the incurrent aperture in *P. retiaria* are nearly cylindrical and arranged in two neat rows; the papillae of the excurrent aperture are undeveloped (Fig. 5C1). The labial palps of *P. retiaria* are approximately equilateral triangular (Fig. 5C2).

In contrast to *Acuticosta* and *Pseudoacuticosta* mentioned previously, the incurrent aperture papillae of both *I. trisulcata* **comb. nov.** and *I. rentianensis* are characterized by their short, blunt, and thick morphology, and are arranged irregularly in a single, densely clustered row (Fig. 5D1, E1). Moreover, the excurrent aperture papillae in both species are undeveloped and display distinct pigmentation (Fig. 5D1, E1). The labial palps in both species exhibit an irregularly oval shape (Fig. 5D2, E2).

Systematics

Family Unionidae Rafinesque, 1820

Subfamily Unioninae Rafinesque, 1820

Tribe Unionini Rafinesque, 1820

Genus *Pseudoacuticosta* Wu, Wang, Nan, Cheng & An, gen. nov.

https://zoobank.org/E126FDF7-8C61-4EB6-90B7-1ECC5A973D76

Type species. *Pseudoacuticosta retiaria* (Heude, 1883) **gen. & comb. nov.**

Type locality: Xuancheng City and Chizhou City, Anhui Province

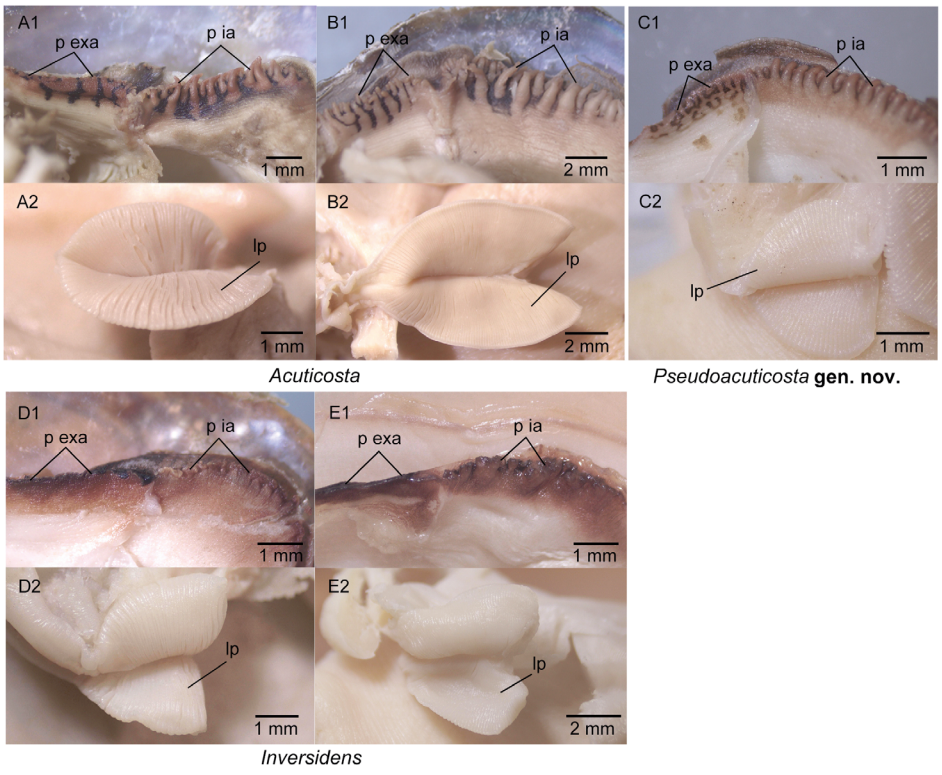

FIGURE 5 Anatomical features of *Acuticosta chinensis* (A1-A2), *Acuticosta ovata* (B1-B2), *Pseudoacuticosta retiaria* **gen. & comb. nov.** (C1-C2), *Inversidens trisulcata* **comb. nov.** (D1-D2), and *Inversidens rentianensis* (E1-E2). Abbreviations: lp, labial palps; p ia, papillae in incurrent aperture; p exa, papillae in excurrent aperture.

Etymology: The name of this new genus is compiled using the words "*Pseudo*" and "*Acuticosta*". For the common name of *Pseudoacuticosta retiaria*, we recommend "Warrior Pseudo-acute Ridge Mussel" (English) and "Yong Shi Wei Jian Ji Bang" (勇士伪尖嵴蚌) (Chinese).

Diagnosis: Shell small, ovate to rounded-trigonal, and distinctly inflated. Surface with fine growth lines. Posterior margin noticeably swollen, forming a broadly rounded contour. Periostracum yellow, often with faint greenish bands.

Description: Shell small, ovate to rounded-trigonal, of medium thickness and distinctly inflated; umbo often eroded; periostracum smooth, yellow to yellow-green with fine growth lines; anterior margin rounded, ventral margin arched and slightly expanded, dorsal margin straight or gently sloping, posterior margin swollen and broadly rounded; incurrent papillae nearly cylindrical in two rows, excurrent papillae weakly developed with pigmentation; labial palps elongate and nearly equilateral-triangular; hinge well developed with two pseudocardinal and two lateral teeth in the left valve, and one robust pseudocardinal and one lateral tooth in the right valve; nacre silvery white.

Distribution: The middle and lower reaches of the Yangtze River Basin, China, including Xuancheng and Chizhou in Anhui Province, and the Fuhe River, Jiangxi Province, China.

Comments: This genus was established to accommodate *Pseudoacuticosta retiaria*. Although its shell shape is homologous to that of *Acuticosta*, evidence from anatomical morphology and molecular systematics indicates the uniqueness of this genus (Figs. 3, 5). As a monotypic genus, *Globunio* Dai, Chen, Huang & Wu, 2025 and *Huizhou* Chen, Chen, Xiang, He & Wu have recently been discovered and described (Dai et al., 2025; Chen et al., 2025). Morphologically, the new genus *Pseudoacuticosta* differs significantly from *Globunio*, which has a diagnostic long – spherical shell, and from *Huizhou*, which has a zigzag sculpture covering its entire surface. Additionally, phylogenetically, the new genus has a well – supported lineage with a considerable genetic distance from the other two genera (>10% for CO1), and the mitochondrial phylogeny also supports its status as an independent lineage (Fig. 4). This combination of obvious morphological discontinuity, significant genetic differences, and well phylogenetic relationships provides compelling evidence for its separation at the genus level.

Discussion

Taxonomic revision of the genus Acuticosta

The long-term absence of molecular data has hindered our understanding of the systematics of the genus *Acuticosta*. In this study, our molecular evidence indicates that the genus *Acuticosta* is paraphyletic. Recent studies have shown that mitochondrial genomics has become an important tool for resolving polyphyly in the Unionidae (Wu et al., 2022b; Wu et al., 2024a). Based on mitochondrial genomic data and anatomical features, we strongly advocate for the reclassification of *A. retiaria* and *A. trisulcata* as *Pseudoacuticosta retiaria* **gen. & comb. nov.** and *Inversidens trisulcata* **comb. nov.** Lopes-Lima et al. (2017a) demonstrated that an examination of morphological and anatomical traits commonly employed in traditional phylogenetic analyses within the family Unionidae reveals no single morphological, anatomical, or behavioral characteristic that is diagnostic at the subfamily level, with only a limited number providing utility at the tribal level. Wu et al. (2021) discovered that traits previously considered reliable for high-level taxonomic classification, such as shell shape, glochidium shape, and marsupium anatomy, are not sufficient to serve as diagnostic characters for classification at the genus level or higher within the Unionidae. However, it is worth noting that the soft part anatomy examined in this study – particularly the papillae in the incurrent apertures – provides compelling evidence supporting the taxonomic revisions of *Inversidens trisulcata* and *Pseudoacuticosta retiaria*. This finding suggests that within the family Unionidae, specific features of the soft anatomy can serve as diagnostic characters for distinguishing taxonomic units at various levels. We advocate for increased research attention on soft part anatomical features, as their correlation with precise

phylogenetic relationships has significant implications for elucidating the adaptive evolution within Unionidae.

Zeng & Liu (1989) noted that *A. sichuanica* is morphologically similar to *I. trisulcata*, particularly in terms of the concentric circular grooves on the shell surface and the conical pseudocardinal tooth. However, due to slight differences in shell morphology and geographic distribution, it is still classified as a distinct species. Although *A. sichuanica* was not collected during this sampling, due to its close morphological affinity to *I. trisulcata*, we provisionally reclassify it into the genus *Inversidens* as *I. sichuanica* **comb. nov.**

Phenotypic plasticity of shell form

In the classification of freshwater bivalves, a major challenge arises from the high variability in shell morphology, as well as morphological differences among individuals within the same group due to age-related variations (Zieritz et al., 2011; Inoue et al., 2013, 2014; Dai et al., 2024). Unionids possess a distinctive life cycle characterized by a parasitic larval stage known as glochidia, which depends on specific host fish species (Kat, 1984; Wächtler et al., 2001). The variability in host fish habitats provides diverse environmental conditions for juvenile mussels. Variations in environmental and hydrological factors, such as substrate type and flow velocity, contribute to the plasticity of shell morphology. Furthermore, female unionids can fertilize their eggs using sperm from multiple males that is transported by water currents, which may result in offspring with considerable genetic diversity and potentially contribute to phenotypic plasticity (Kat, 1984). Recent studies addressing the influence of environmental, ontogenetic, and genetic factors on shell shape variation suggest that environmental variables are the primary drivers of intraspecific shell morphological variation in unionid species, while genetic differentiation plays a relatively minor role (Keogh et al., 2025; Sánchez González et al., 2025; Tomilova et al., 2025).

The target unionid groups in this study display considerable variability in shell morphology, particularly *A. chinensis* and *A. ovata* (Figs S1, S2). Both species occupy diverse habitats, including rivers, lakes, and streams, with substrates varying from silt to sand and gravel, which directly drive adaptive changes in shell morphology. Additionally, age-related differences further contribute to morphological variation within populations, as juveniles and adults may exhibit markedly distinct shell characteristics. Wu et al. (1999a) conducted a comparative morphological study on the glochidia of the Unionid species and found that the larvae of *A. chinensis* and *A. ovata* exhibit distinguishable differences in features such as the curvature of the shell hooks and the shell surface patterns, thereby providing a basis for taxonomic classification. However, the morphology of the glochidia is constrained by their developmental stage, which necessitates the incorporation of adult morphological characteristics for a comprehensive evaluation. This dependency, in turn, complicates the practical application of species identification. Based on molecular data, this study confirms that *A. chinensis* and *A. ovata* are distinct species, expands their documented phenotypic variation, and presents an updated DNA barcode dataset (Table S1). The revised

phenotypic-genotypic reference database not only provides a foundation for the classification of fossil species in paleontological studies but also serves as a crucial taxonomic resource for future regional biodiversity assessments.

Conservation implications

Freshwater mussels are among the most threatened bivalve species, increasingly vulnerable to human-induced habitat alterations and extreme climatic events (Lydeard et al., 2004; Lopes-Lima et al., 2014, 2017b; Aldridge et al., 2023). These pressures have contributed to widespread population declines, which in turn compromise both short-term and long-term ecological functions within freshwater systems. Accurate identification and precise taxonomic classification of freshwater mussel species are therefore essential to inform effective conservation strategies and management practices. This study integrates morphological and molecular approaches to revise the classification of *Pseudoacuticosta retiaria* **gen. & comb. nov.**, *Inversidens trisulcata* **comb. nov.**, and *I. sichuanica* **comb. nov.**, which were previously assigned to the genus *Acuticosta*. It also clarifies the species boundaries and examines multiple ecophenotypes within *A. chinensis* and *A. ovata*. The taxonomic investigation of the genus *Acuticosta* underscores the complementary roles of morphological analyses and molecular techniques. Environmentally induced phenotypic variation, cryptic species complexes, and historical taxonomic ambiguities are effectively addressed through DNA barcoding and phylogenetic analysis.

Although our research has provided important insights into the conservation of these groups, particularly regarding taxonomy and distribution, the limited availability of information on these species means that their endangered status and appropriate conservation strategies remain poorly understood. The IUCN Red List categorizes *A. chinensis* and *A. ovata* as Least Concern, whereas *P. retiaria*, *I. trisulcata*, and *I. sichuanica* have not yet been assessed (IUCN, 2011a, 2011b; Graf & Cummings, see https://musselpdb.org/). In our field sampling, we observed that the population of *A. ovata* is significantly greater than that of other species. *I. trisulcata* and *P. retiaria* have long been classified as rare species, which partly explains the historical lack of molecular data for these taxa. Therefore, we recommend further in-depth studies on these species to improve our understanding of their biological and ecological traits, geographic distributions, potential threats, and habitat preferences. Such research will provide essential foundational information for the effective implementation of comprehensive conservation assessments, strategies, and targeted interventions in the future.

Conclusion

We integrated morphological, soft-body anatomical, and mitochondrial genomic data to revise the classification of the Chinese endemic genus *Acuticosta* (Bivalvia: Unionidae), resulting in the reclassification of its three species as *Pseudoacuticosta retiaria* **gen. & comb. nov.**, *Inversidens trisulcata* **comb. nov.**, and *I. sichuanica* **comb. nov.** Moreover, through extensive sampling across China, we identified multiple ecophenotypes that complicate the

accurate identification of *A. chinensis* and *A. ovata*, and we presented an updated DNA barcode dataset. Our findings provide a case study demonstrating that integrative taxonomy based on molecular phylogenetics can effectively resolve species classification challenges in freshwater mussels, underscoring the need for molecular investigations of other Chinese unionid species that may exhibit similar taxonomic uncertainties.

Supplementary material

Supplementary material can be found under the tab 'Suppl material' on the article landing page.

Supplementary Table S1. List of COI sequences used in this study, including the species, specimen codes, voucher code, GenBank accession numbers and haplotypes.

Supplementary Table S2. List of sequences used in mitochondrial phylogenomic analyses and corresponding GenBank numbers.

Supplementary Table S3 Partitioning schemes and best-fit models identified from PartitionFinder and ModelFinder for the whole mitogenome dataset.

Supplementary Figure S1. Various ecophenotypes in *Acuticosta chinensis*. A. SXNU_24082803, B. Personal: Xiao-Ping Wu:01-ACF-2018, C. SXNU_24110809, D. SXNU_23011005, E. SXNU_24101015, F. SXNU_24110810. Scale = 2 cm.

Supplementary Figure S2. Various ecophenotypes in *Acuticosta ovata*. A. SXNU_25041148; B. SXNU_25041133; C. SXNU_22110423; D. SXNU_23032533; E. SXNU_24091807; F. SXNU_25050402; G. SXNU_22110203; H. SXNU_23040116; I. SXNU_22121815; J. SXNU_25041145. Scale = 2 cm.

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