

Underestimated diversity: A new species of the genus *Cuneopsis* (Bivalvia, Unionidae, Unioninae) from Henan, China

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Abstract

The global decline of freshwater mussels and their crucial ecological services highlights the necessity and urgency of developing and guiding conservation efforts for this group. Accurately delineating species and understanding their phylogeny are crucial to the core of species conservation. Here, we integrate shell morphology, soft-body anatomy, and molecular systematics to describe a new species of freshwater mussel from the Shi River in Xinyang City, Henan Province, China, i.e., *Cuneopsis celtiosimilis* **sp. nov.** Morphologically, this new species resembles its congeneric species *C. kiangsiensis* and *C. celtiformis* in shell size, shape, and sculpture. However, this new species can be distinguished from closely related congeners by the pseudocardinal teeth. For the new species, two pseudocardinal teeth of the left valve join together to form a continuous strip; the single pseudocardinal tooth on the right valve is more squashed and lower; the papillae of the incurrent aperture are shorter, forming a pyramidal shape and arranged in two rows. Molecularly, based on the mitochondrial barcoding gene cytochrome *c* oxidase subunit I (*COI*), the minimum genetic distance between the new species and other known congeners is 4.6% (*C. celtiosimilis* **sp. nov.** vs. *C. kiangsiensis*). Additionally, the complete mitogenome of *C. celtiosimilis* **sp. nov.** was also assembled and annotated. Based on the mitochondrial phylogenomic analyses, the results clarify the phylogenetic position of the new species and establish the most comprehensive phylogenetic relationship of the genus *Cuneopsis* to date, as follows: (((*C. demangei* + *C. heudei*) + *C. szechenyii*) + ((*C. kiangsiensis* + *C. celtiosimilis* **sp. nov.**) + *C. celtiformis*)) + *C. rufescens*). The discovery of this new taxon contributes to the existing knowledge on freshwater mussels in China, and a key to all known species of *Cuneopsis* is provided to aid the identification of species in this understudied genus.

Key Words

Cryptic species, *Cuneopsis*, freshwater mussels, integrative taxonomy, mitochondrial phylogenomics, Unioninae, Unionini

Introduction

Freshwater mussels (family Unionidae) have received substantial attention due to their unique parasitic life history (glochidium) (Lefevre and Curtis 1910; Wu et al. 1999; Barnhart et al. 2008), doubly uniparental mitochondrial inheritance (Zouros 2013; Guerra et al. 2017; Froufe et al. 2020), and imperiled status (Lopes-Lima et al. 2017; Gallardo et al. 2018; Böhm et al. 2021). Accurate species classification is essential for a comprehensive understanding of mussel biodiversity and the development of effective conservation measures (Lopes-Lima et al. 2018; Bolotov et al. 2020; Bolotov et al. 2022). Morphological

data play a crucial role in the diagnosis of species diversity (Liu 2016; Ma 2016; Hong 2016). However, relying solely on shell morphological characteristics for classification may pose problems due to the recognized high phenotypic plasticity in this group (Zieritz et al. 2010; Inoue et al. 2014; Wu et al. 2022a). The advancement and application of molecular technology and the integration of molecular phylogenetic evidence when delimiting species mitigates this problem and has fundamentally changed our ability to identify biodiversity (Konopleva et al. 2016; Bolotov et al. 2017; Bolotov et al. 2022).

Malacologists have long considered the freshwater mussel *Cuneopsis sensu lato* Simpson, 1900 to be a

distinct taxon, and clearly identified as belonging to the tribe Unionini within the subfamily Unioninae based on its distinctive wedge-shaped shell morphology (Liu et al. 1979; Graf and Cummings 2021). However, molecular systematics and the inclusion of more taxa in the group suggested that *Cuneopsis sensu lato* was polyphyletic (Bolotov et al. 2017; Wu et al. 2019; Dai et al. 2021). A recent mitogenomic analysis by Wu et al. (2022b) established the monophyly of *Cuneopsis sensu stricto* and proposed two new genera, *Arcuneopsis* Huang, Dai & Wu, 2022, and *Pseudocuneopsis* Huang, Dai, Chen & Wu, 2022. Recently, Wu et al. (2024a) supported the validity of *Cuneopsis szechenyii*, previously considered a synonym for *Cuneopsis heudei*, by integrating shell morphometry, soft-body anatomy, and molecular evidence. Currently, there are six extant species within this genus: *Cuneopsis celtiformis* (Heude, 1874), *Cuneopsis heudei* (Heude, 1874), *Cuneopsis kiangsiensis* Tchang & Li, 1965, *Cuneopsis rufescens* (Heude, 1874), *Cuneopsis szechenyii* (Neumayr, 1899), and *Cuneopsis demangei* Haas, 1929 (Wu et al. 2022b; MolluscaBase eds 2024). With the exception of *C. demangei*, which is distributed in southern China and northern Vietnam, the other five species are endemic to China and are mainly found within the Yangtze River basin, including Jiangxi, Fujian, Hunan, Hubei, Anhui, Jiangsu, and Zhejiang provinces (Liu et al. 1979; Graf and Cummings 2024). *C. kiangsiensis* and *C. rufescens* are currently only found in Jiangxi province, while other species have a wide distribution and co-occur to some extent (Zhang et al. 2013; Liu et al. 2020). In general, *Cuneopsis* species inhabit medium to large rivers and lakes, with a preference for gravel or sandy sediment (Liu et al. 1979).

Recently, while investigating the aquatic fauna of the Huai River basin in Henan Province, China, specimens with shell morphology similar to that of *C. kiangsiensis* and *C. celtiformis* were discovered (Fig. 1). By integrating shell morphometry, soft-body anatomy, and molecular evidence, we determined that this mussel is a cryptic new species. Subsequently, we constructed the most comprehensive phylogenetic relationship of *Cuneopsis* to elucidate the phylogenetic position of the newly described species based on mitochondrial genomics.

Materials and methods

Specimen sampling and morphological observations

The specimens with tissue were collected from the Shi River, Luoshan County, Xinyang City, Henan Province, China (32.2390°N, 114.4248°E) from August to October, 2024. Homemade mussel rakes (60 cm wide with a mesh size of 20 mm and rake tooth spacing of 15 mm) and a D-frame kick net (30 × 30 cm with a mesh size of 250 μm) were used to collect the specimens. The hand-held mussel rake was thrown into the water and slowly dragged to

the shore at a uniform speed in the river shallows (0.5–2.0 m deep). In water depths less than 0.5 m, we used the D-frame kick net for sampling. Finally, we found four target specimens in a sandy sediment habitat near the riverbank at a water depth of 0.4–0.8 m. The collected samples were placed into labeled bottles and brought back to the laboratory. In the laboratory, specimens were immediately subjected to anatomical observation of the soft body. The extracted muscle tissues were preserved in 95% ethanol for subsequent molecular analysis. In addition, we gathered *Cuneopsis celtiformis* and *Cuneopsis kiangsiensis* previously collected in Jiangxi Province to compare their shell morphology and anatomy. All specimens are stored as vouchers in the Zoological Museum of Shanxi Normal University. The collection information and voucher numbers are presented in Suppl. material 1: table S1.

Shell morphology and anatomical features were visually examined with the naked eye and under a stereoscopic microscope, including shell shape, umbo position and sculpture, shell surface sculpture, hinge structure, labial palps, muscle attachment, and incurrent and excurrent apertures (Figs 1, 2). The length, height, and width of the shell were accurately measured using a digital vernier caliper (± 0.02 mm).

DNA extraction, PCR sequencing, and mitogenome 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'-GGTCAACAAAYCATAARGATATTGG-3' and HCO700dy2 5'-TCAGGGTGACCAAAAAAYCA-3', ~680 bp) (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 °C for 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 (Suppl. material 1: table S1).

The sequencing and assembly of the mitochondrial genome follows methods found in Wu et al. (2023, 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

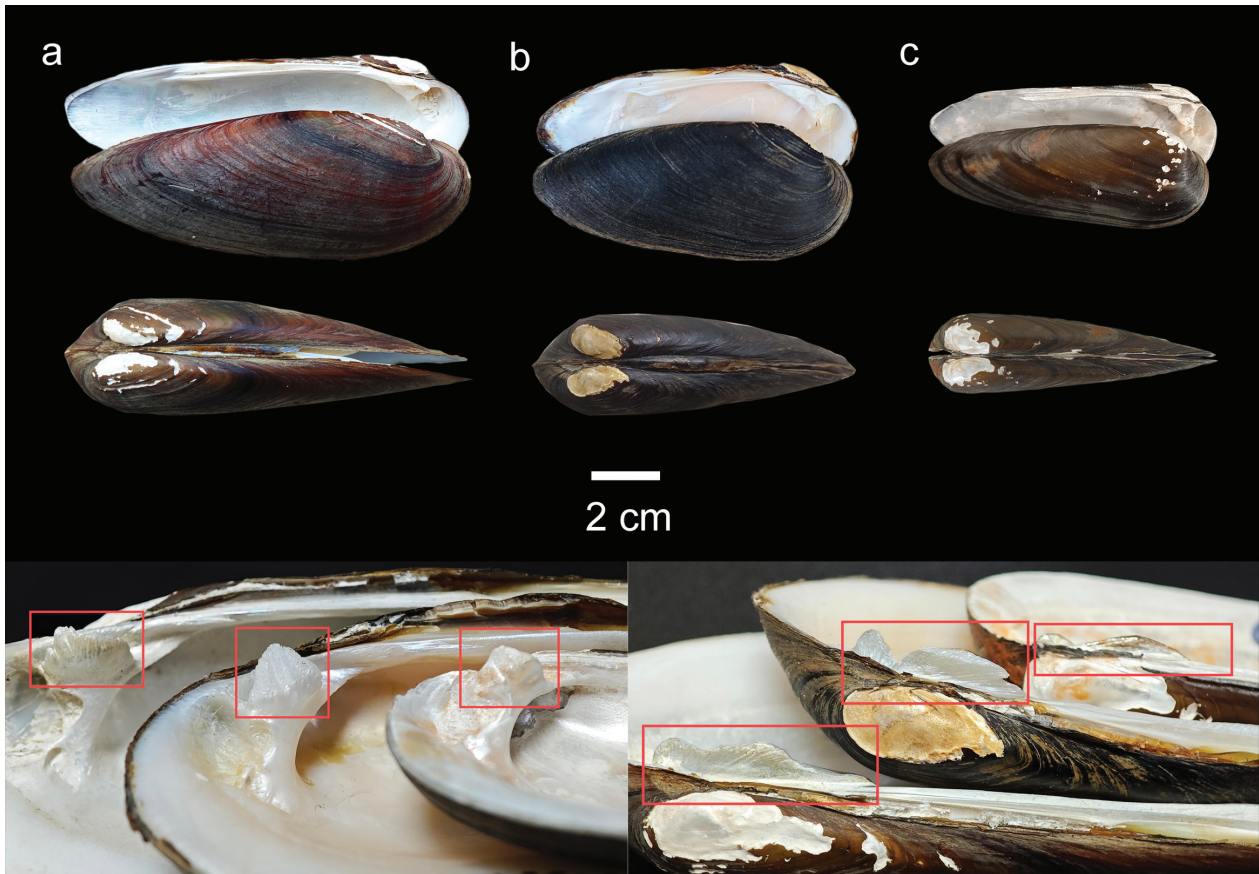


Figure 1. Shells and pseudocardinal teeth of *Cuneopsis celtiosimilis* sp. nov. (a), *Cuneopsis kiangsiensis* (b), and *Cuneopsis celtiformis* (c). Upper images show the inside of the left valve and the outside of the right valve for three species. The lower right image is the pseudocardinal teeth of the left valve; the lower left image is the pseudocardinal teeth of the right valve. The shells overlap from bottom to top: *C. celtiosimilis* sp. nov., *C. kiangsiensis*, and *C. celtiformis*.

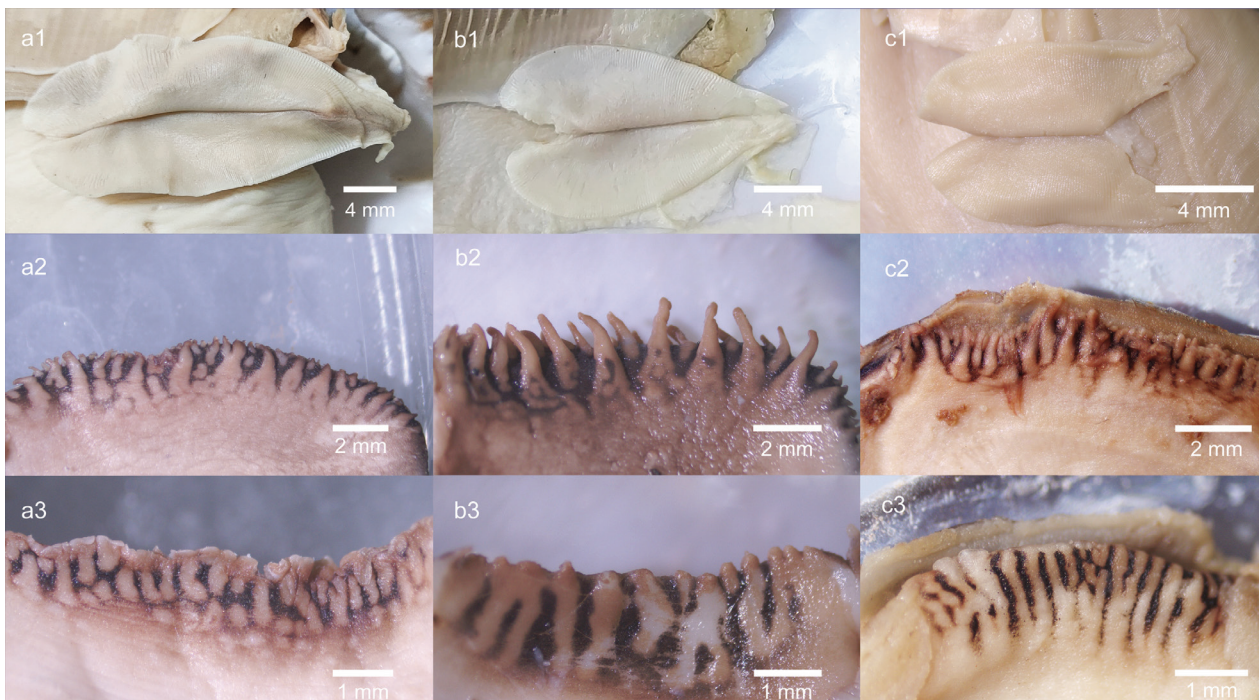


Figure 2. Anatomical features of *Cuneopsis celtiosimilis* sp. nov. (a), *Cuneopsis kiangsiensis* (b), and *Cuneopsis celtiformis* (c). a1–c1: labial palps, a2–c2: papillae in incurrent aperture, a3–c3: papillae in excurrent aperture.

approximately 4 Gb, 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 (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 and Canbäck 2008) was employed to verify the tRNA annotation results, while rRNA annotations were determined through alignment with closely related species. Finally, the complete mitochondrial genome was submitted to GenBank via BankIt (GenBank no. PQ463688).

Alignments, partitioning strategies, and model selection

Previous studies constructed the most comprehensive molecular datasets of the genus *Cuneopsis* to date (Wu et al. 2024a). Based on this, data generated in this study was incorporated with the dataset from Wu et al. (2024a) and separated into two datasets. First, a DNA barcoding (*COI*) dataset was prepared (Suppl. material 1: table S1). We downloaded 58 *COI* sequences of published *Cuneopsis* taxa, representing the six currently recognized valid species, and combined them with the four sequences generated in this study. Additionally, we also obtained *COI* sequences from its relative genera (i.e., *Middendorffinaia*, *Tchangsinaiia*, and *Pseudocuneopsis*), and *Lepidodesma languilati* (Heude, 1874) was selected as the outgroup. Second, a mitogenomic nucleotide dataset was compiled (containing 12 PCGs + 2 rRNA; *atp8* was excluded due to high sequence variation; Suppl. material 1: table S2). The dataset included 26 species from the subtribes Oxynaiina and Middendorffinaia in the tribe Unionini. Additionally, *Lepidodesma aligera* (Heude, 1877) and *L. languilati* in the tribe Lepidodesmini were chosen as outgroups.

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 v7.2 (Katoh and Standley 2013) with the L-INS-i algorithm. Ambiguous alignment areas were trimmed using 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, and the minimum length of the PCG block was set to three bp, with allowed gap position selected as none. The *COI* barcoding dataset had a fragment length of 522 bp after alignment and trimming. The mi-

togenomic dataset (12 PCGs + 2 rRNA) was concatenated using Phylosuite v1.2.3.

The built mitogenomic dataset was partitioned based on genes and codons using PartitionFinder (Lanfear et al. 2017) to select Bayesian inference (BI) analysis models for the partitioning schemes. ModelFinder (Kalyaanamoorthy et al. 2017) was employed in IQ-TREE (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 Suppl. material 1: table S3.

Pairwise genetic distances and phylogenetic analyses

The intraspecific and interspecific genetic distances were computed using the *COI* dataset with the uncorrected *p*-distance model in MEGA 7.0 (Kumar et al. 2016).

The IQ-TREE web server (<http://iqtree.cibiv.univie.ac.at/>) was used for maximum likelihood (ML) phylogenetic analyses of the *COI* and mitogenome datasets, employing the ultrafast bootstrap algorithm with 1000 repetitions (Minh et al. 2013). Bayesian inference (BI) phylogenetic analyses of the mitogenome dataset were conducted in MrBayes v2.01 (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 and Bork 2007).

Results

Systematics

Family Unionidae Rafinesque, 1820
Subfamily Unioninae Rafinesque, 1820
Tribe Unionini Rafinesque, 1820
Subtribe Oxynaiina Starobogatov, 1970

Genus *Cuneopsis* Simpson, 1900

Type species. *Cuneopsis celtiformis* (Heude, 1874).

***Cuneopsis celtiosimilis* Hou & Wu, sp. nov.**

<https://zoobank.org/37B48A53-B7B3-46D5-A4D5-4876C8F04660>

Fig. 1a; Suppl. material 2: fig. S1

Type material. *Holotype* (Fig. 1a) • SXNU_24100210 (length 119.1 mm, height 45.19 mm, width 32.74 mm); Luoshan County, Xinyang City, Henan Province, China. *Paratypes* (Suppl. material 2: fig. S1a, c, d) • three specimens, SXNU_24082201, SXNU_24100211, and SXNU_24100212. Same collection location as the holotype.

Morphological diagnosis. Shell long, wedge-shaped; the umbo situated at 1/6 of the shell length; the epidermis black-red; nacre blue-white; and the two pseudocardinal teeth on the left valve join together and form in a continuous strip (Fig. 1); right valve has one low, squashed, and irregular pseudocardinal tooth. The labial palps long elliptical (Fig. 2a1); the papillae of the incurrent aperture short, pyramidal shape, and arranged in two rows (Fig. 2a2); and the papillae at the edge of the excurrent aperture not prominent and tightly arranged in a row (Fig. 2a3).

Molecular diagnosis. The phylogenetic tree indicates that the new species forms a distinct lineage, and it is closely related to *Cuneopsis kiangsiensis* (Fig. 3). The genetic distance between the new species and its congeneric species shows that the smallest distance is observed with *Cuneopsis kiangsiensis* (4.6%) (Table 2).

Description. Shell thick, strong, both valves unequal. Shell long, wedge-shaped, slightly thickened, slightly inflated; anterior margin convex prominently; ventral margin arc-shaped; umbo located at 1/6 of the shell length; periostracum black-red; shell surface sculptured with fine concentric growth lines (Fig. 1; Table 1). Anterior adductor muscle attachment deep and rough; posterior adductor muscle attachment shallow and smooth; nacre blue-white; mantle muscle attachment obvious. Hinge well developed; left valve has two pseudocardinal teeth; anterior tooth small, and posterior tooth thick, both pseudocardinal teeth connect together and arranged in continuous strip; right valve has only one low, squashed, and irregular pseudocardinal tooth; left valve has two lateral teeth, and both arranged in a paralleled straight line; right valve has one lateral tooth

Table 1. Conchological and anatomical characters of *Cuneopsis celtiosimilis* sp. nov., *Cuneopsis kiangsiensis* and *Cuneopsis celtiformis*.

	<i>Cuneopsis celtiosimilis</i> sp. nov. (4 specimens)	<i>Cuneopsis kiangsiensis</i> (6 specimens)	<i>Cuneopsis celtiformis</i> (4 specimens)
Length	64.16–149.65 (mm)	94.77–115.53 (mm)	84.74–96.03 (mm)
Width	15.02–38.85 (mm)	27.20–34.77 (mm)	21.28–26.01 (mm)
Height	24.37–51.95 (mm)	42.77–50.50 (mm)	31.08–33.14 (mm)
Shell shape	Long wedge-shaped	Wedge-shaped	Lanceolate
Shell thickness	Thick	Thick	Moderately thick
Umbo position and sculpture	1/6 of shell length; umbo often eroded	1/5 of shell length; umbo often eroded	1/7 of shell length; umbo often eroded
Surface sculpture	Epidermis black-red; shell surface sculptured with fine concentric growth lines	Epidermis black-brown; lusterless; shell surface sculptured with fine concentric growth lines	Epidermis dark brown; shiny; shell surface sculptured with fine concentric growth lines
Nacre colour	Blue-white	Milky-white	White
Pseudocardinal tooth of the left valve	Anterior tooth small, posterior tooth thick, and both teeth join together	Anterior tooth small, posterior tooth strip shape, a deep gap between the two teeth	Anterior tooth small, posterior tooth slender triangle, shallow pit between the two teeth
Pseudocardinal tooth of the right valve	Only one; low and squashed	Only one; strong and triangular	Only one; thick and irregular
Lateral tooth	One tooth on the right valve, two teeth on the left valve nearly straight	One tooth on the right valve, two teeth on the left shell nearly straight	One tooth on the right valve, two teeth on the left shell nearly straight
Incurrent aperture	Papillae swelling at the base, shortly pyramidal overall and partially forked at the ends, arranged in two rows	Papillae slightly swelling at the base, elongated overall, sparsely arranged in three rows	Conical and tightly arranged in two rows
Excurrent aperture	Papillae tightly arranged in a row	Papillae sparsely arranged in a row	Papillae weak development and neatly arranged in a row
Labial palps	Medium-thick, long elliptical	Medium-thick, triangular	Slightly thick, irregular elliptical

Table 2. Intra- and interspecific genetic distances assessed using 1000 bootstrap replicates based on the uncorrected *p*-distance model in MEGA 7.0.

Taxon	Intraspecific distance	Interspecific distance					
		1	2	3	4	5	6
1. <i>Cuneopsis celtiosimilis</i> sp. nov.	0.000						
2. <i>Cuneopsis szechenyii</i>	0.003	0.101					
3. <i>Cuneopsis celtiformis</i>	0.003	0.062	0.090				
4. <i>Cuneopsis rufescens</i>	0.003	0.099	0.103	0.116			
5. <i>Cuneopsis heudei</i>	0.011	0.092	0.087	0.102	0.116		
6. <i>Cuneopsis demangei</i>	0.009	0.083	0.079	0.091	0.107	0.023	
7. <i>Cuneopsis kiangsiensis</i>	0.000	0.046	0.094	0.065	0.106	0.099	0.096

(Fig. 1; Table 1). Papillae in the incurrent aperture short, pyramidal shape, arranged in two rows; papillae in the excurrent aperture weakly developed, tightly arranged in one row; and the pigmentation of the incurrent and excurrent aperture significant; labial palps medium-thick, long elliptical (Fig. 2; Table 1).

Etymology. This newly discovered species exhibits morphological similarities with *Cuneopsis celtiformis*, which led us to incorporate this resemblance into its naming. Specifically, we used the word ‘celti’ (derived from ‘celtiformis’) with the middle ‘o’ connected to the Latinized word ‘similis’ (similar) to name this new species. For the common name, we recommend “Similar Wedged Mussel” (English) and “Jin Si Xie Bang” (近似楔蚌) (Chinese).

Distribution. Shi River, a tributary of Huai River, Luoshan County, Xinyang City, Henan Province, China.

Phylogenetic analyses

The ML tree based on the mitochondrial *COI* dataset suggests that the *C. celtiosimilis* sp. nov. sequences generated in this study form a monophyletic group and are well supported as a sister group to *C. kiangsiensis* (Bootstrap support (BS) = 85%; Fig. 3).

The complete mitochondrial genome of *Cuneopsis celtiosimilis* sp. nov. is 15,937 bp, which contains the typical 37 genes (13 PCGs, 2 rRNAs, and 22 tRNAs) (Fig. 4A). The A+T content of all four complete mitochondrial genomes is higher compared to the G+C content. The mitochondrial gene order is the same as that of the subfamily Unioninae (Froufe et al. 2020) (Fig. 4A). The heavy chain (H chain) encodes eleven genes (*cox1*, *cox2*, *cox3*, *nad3*, *nad4*, *nad4L*, *nad5*, *atp6*, *atp8*, *trnD*, and *trnH*),

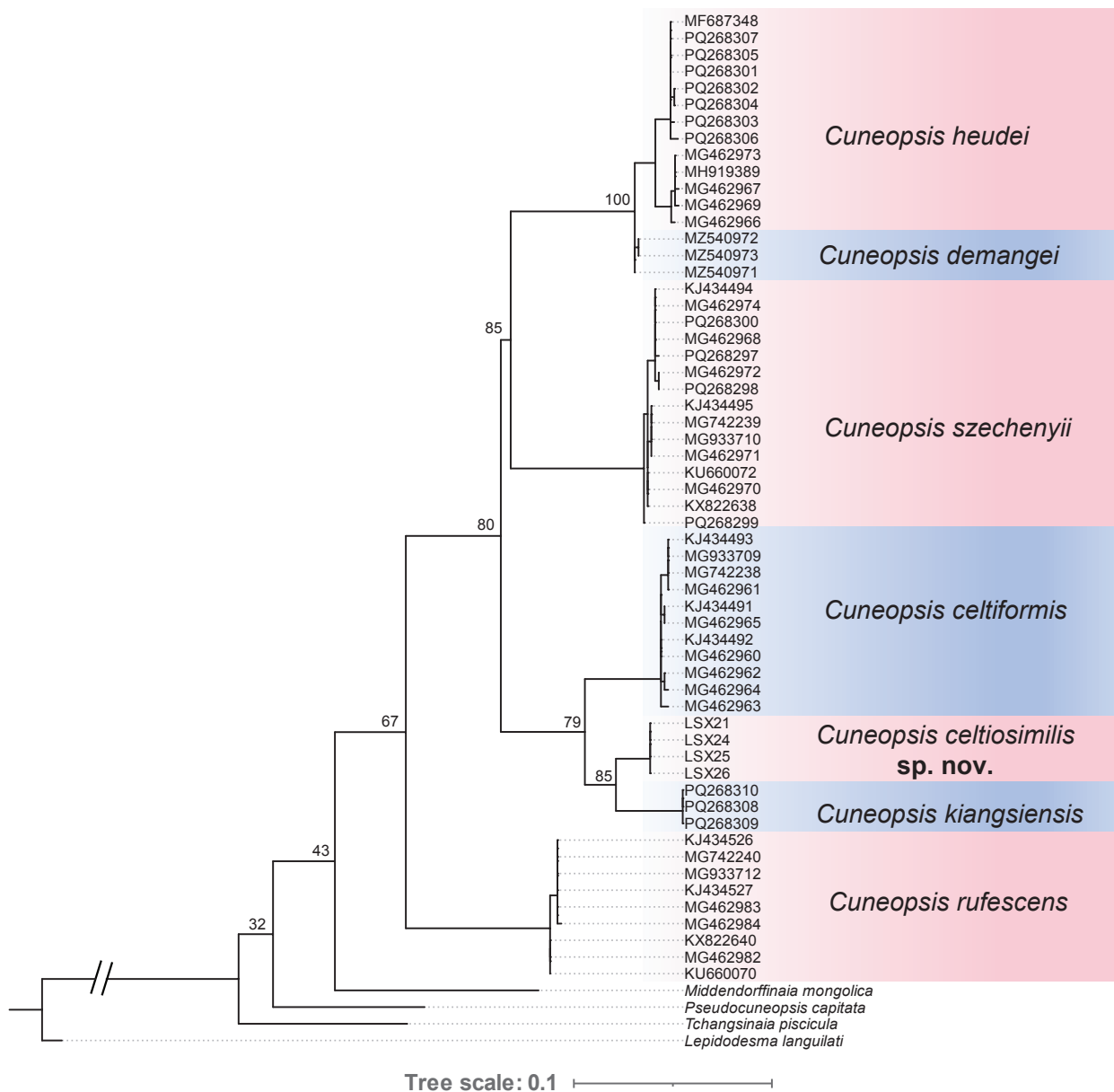


Figure 3. Maximum likelihood tree inferred from the *COI* dataset. Each branch is labeled with the GenBank accession numbers and specimen codes from this study. The bootstrap support values (BS) are indicated at the nodes.

while an additional twenty-six genes are located on the light chain (L chain).

In this study, we constructed phylogenetic trees using the complete mitogenome from 26 Unionini species and two outgroups. Both ML and BI trees produce identical topologies with high support on most nodes (BS > 90%, PP > 0.9; Fig. 4B). All genera within the subtribe Oxynaiina form a robust monophyletic clade (BS > 75%, PP = 1.0; Fig. 4B). Focusing on the genus *Cuneopsis*, both phylogenetic trees consistently support relationships at the species level as follows: ((((*C. demangei* + *C. heudei*) + *C. szechenyii*) + (*C. kiangsiensis* + *C. celtiosimilis* sp. nov.) + *C. celtiformis*)) + *C. rufescens*) (Fig. 4B).

Discussion

As a freshwater organism, mussels have a high plasticity in their shell morphology, which makes them well-adapted to various environmental conditions (Watters 1994; Whelan 2021; He et al. 2022). However, this adaptive trait poses taxonomic challenges due to the difficulty in accurately delimiting species based solely on shell characteristics (Bolotov et al. 2024; Cheng et al. 2024). As a result, malacologists have embarked on exploring more comprehensive and precise classification methodologies. In recent years, the advent of molecular techniques, particularly DNA barcoding technology, has revolutionized

the taxonomy of freshwater mussels (Keogh and Simons 2019; Dai et al. 2024; Wu et al. 2024b). This approach not only surpasses the limitations associated with morphological classification but also presents a novel avenue for uncovering cryptic species.

This study successfully identifies a new species, *Cuneopsis celtiosimilis* sp. nov., by integrating molecular evidence with shell morphological and soft-body anatomical features. The minimum genetic distance between the new species and its congener is calculated to be 4.6% with *C. kiangsiensis* (Table 2), which significantly exceeds the commonly accepted threshold of 3% for interspecies divergences (Hebert et al. 2003; Barrett and Hebert 2005). In the phylogenetic tree based on the *COI* dataset, four individuals of the new species form a separate clade and are sister to *C. kiangsiensis* (Fig. 3). The mitochondrial phylogenomics reveal a consistent topology within *Cuneopsis* and strong support for the new species being closely related to *C. kiangsiensis* and *C. celtiformis* (BS = 100%, PP = 1.00; Fig. 4B), which supports our initial identification hypothesis based on morphological characteristics.

In addition to molecular phylogenetic evidence, this study also performed a detailed comparative analysis of shell morphology and soft-body anatomy. Compared to *C. kiangsiensis* and *C. celtiformis*, the anterior margin of *C. celtiosimilis* is more prominent, and the ventral margin is curved. The left valve of the new species has two pseudocardinal teeth, and the small anterior tooth

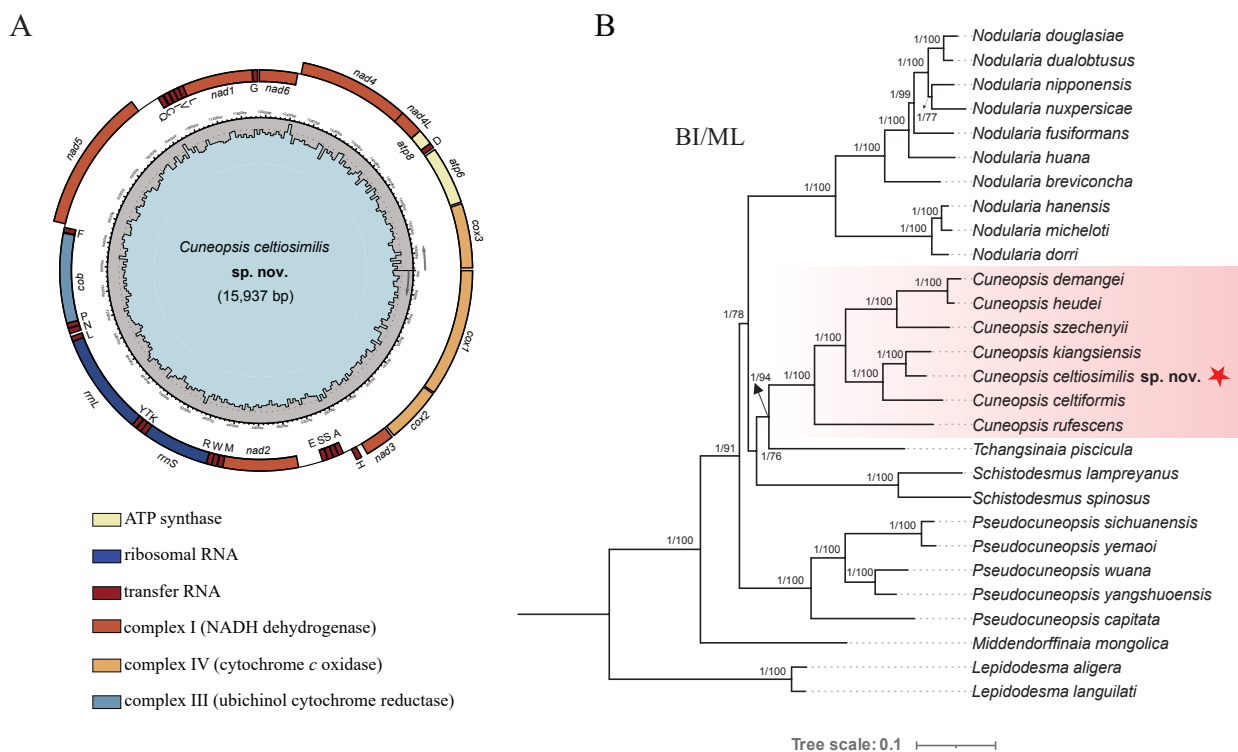


Figure 4. Analysis based on mitochondrial data. **A.** Gene map of the F-type mitochondrial genome of *Cuneopsis celtiosimilis* sp. nov. **B.** Phylogenetic trees inferred from Bayesian inference (BI) and maximum likelihood (ML) analyses. Support values above the branches are maximum likelihood bootstrap supports (BS) and Bayesian posterior probabilities (PP), respectively. The colored shaded clades represent the genus *Cuneopsis* taxa that are the focus of this study. The star symbolizes the sequences from this study.

is closely joined with the thick posterior tooth to form a long strip, while the two pseudocardinal teeth of the left valve of *C. kiangsiensis* and *C. celtiformis* are clearly separated (Table 1). And the right valve of the new species has a low, squashed, and irregular pseudocardinal tooth, compared with the higher and sharper pseudocardinal tooth in the right valve of *C. kiangsiensis* and *C. celtiformis* (Table 1). Additionally, the anatomical features of *C. celtiosimilis* are also different from those of *C. kiangsiensis* and *C. celtiformis*, especially the papillae in the incurrent aperture. Compared with *C. kiangsiensis* and *C. celtiformis*, the papillae are elongated, while those of *C. celtiosimilis* are shorter and less prominent, showing a pyramidal shape (Table 1). In summary, the results of this study not only enrich the species diversity of the genus but also establish a comprehensive phylogenetic relationship of *Cuneopsis* taxa based on mitochondrial genomics, providing a scientific foundation for further research on paleobiogeography and evolution in this group.

Freshwater mussels are currently one of the most endangered animal groups globally, with their extinction primarily attributed to a combination of anthropogenic influences and climate change (Gavriletea 2017; Lopes-Lima et al. 2017; Gallardo et al. 2018; Zieritz et al. 2018). Among these factors, human-induced destruction of natural habitats, including water pollution, river damming, sand mining, and dredging activities, plays a pivotal role in the decline of freshwater unionids and local extinctions (Bolotov et al. 2018; Brian and Aldridge 2019). In general, a smaller population size corresponds to increased vulnerability to extinction in the face of natural disasters and habitat destruction (Geist and Hawkins 2016). On this expedition, we discovered a remarkably low population density for *Cuneopsis celtiosimilis* sp. nov. Despite the extensive collection efforts, only four specimens with intact tissues were obtained. Our sampling method was unable to detect species inhabiting deeper water levels (> 2 m), which may also greatly affect their detection. Imperfect detection poses a major issue in freshwater mussel surveys due to the labor- and time-intensive nature of sampling in these environments. We acknowledge that additional specimens were not detected does not mean that they are not present. However, numerous individuals were found stranded on the shore with their shells, which may partially indicate the severity of their living conditions. We speculate that this may be due to a water quality issue; especially in the summertime, water levels can be low and air temperatures high, causing increased water temperature and decreased dissolved oxygen. Additionally, farmland fertilizers and industrialization contribute to water pollution, which can also cause periodic deaths. Our findings provide important insights into the diversity of this group of unionids for conservation efforts; however, the precise endangered status and conservation strategies remain uncertain due to limited available information, particularly regarding population trends, period of gravidity, and host fish uti-

lization. The lack of information not only impedes conservation efforts but also engenders passivity towards the threat of species extinction. Therefore, to enhance the efficacy of freshwater mussel protection, future research endeavors should be intensified to comprehend their distribution range, basic biology, and ecology. At the same time, public education and awareness regarding freshwater mussel conservation need to be enhanced in order to collaboratively construct a safer and more stable living environment for the protection of these essential members of the freshwater ecosystem.

Conclusion

Freshwater mussels are rapidly declining regionally and globally and are considered to be one of the most threatened animal groups (Lopes-Lima et al. 2018; Aldridge et al. 2023). Assessment and monitoring of species diversity are critical during times of ongoing biodiversity loss, and the discovery of new species is a fundamental first step. We integrate shell morphometry, soft-body anatomy, and molecular evidence to identify a new taxon from China, i.e., *Cuneopsis celtiosimilis* sp. nov. Based on mitochondrial phylogenetic analysis, we not only identify the phylogenetic location of the new species but also establish the most comprehensive phylogenetic relationship of the genus *Cuneopsis* to date. The discovery of this new taxon contributes to the existing knowledge on freshwater mussels in China. Further biological and ecological information needs to be collected to more comprehensively study its precise distribution, population dynamics, threat status, and conservation strategies.

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Supplementary material 1

Supplementary data

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Data type: xlsx

Explanation note: **table S1**. List of COI sequences used in this study, including the species, specimen codes, GenBank accession numbers, voucher specimen number and collecting locations. (*) Sequences from this study; **table S2**. Complete mitogenome sequences used in this study. (*) Sequences from this study; **table S3**. Partitioning strategies from ModerFinder and PartitionFinder for mitogenome dataset.

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Supplementary material 2

Supplementary image

Authors: Kaiyu Hou, Xianan Wang, Fang Nan, Ruiwen Wu
Data type: tif

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