Complete maternal mitochondrial genome of the freshwater mussel *Cuneopsis celtiformis* (Bivalvia: Unionidae)

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**ABSTRACT**

The genus *Cuneopsis* Simpson, 1900 comprises seven valid species, and *Cuneopsis celtiformis* (Heude, 1874) is the type species of this genus. Previous phylogenetic studies using complete mitochondrial genomes showed that *Cuneopsis* was not monophyletic, but the result was hampered by incomplete species sampling and lack of the type species of this genus. In this study, we collected *C. celtiformis* from the type locality and determined its complete maternal mitochondrial genome. This mitogenome is 15,922 bp in length and contains 14 protein-coding genes (including one F-orf), two rRNA genes, 22 tRNA genes, and 1 putative control region. Our mitochondrial phylogenomic analysis confirms that currently recognized genus *Cuneopsis* is polyphyletic, and *C. celtiformis* is the closest to *C. heudei* with high maximum likelihood bootstrap support value. Comprehensive sampling of all *Cuneopsis* species is needed for phylogenetic analysis to erect new genera in future studies.

The freshwater mussel genus *Cuneopsis* Simpson, 1900, distributed in Asia, comprises seven valid species. *Cuneopsis demangei* is currently only found in northern Vietnam (Do et al. 2018) and the remaining six species are endemic to China (i.e. *C. capitatus*, *C. heudei*, *C. celtiformis*, *C. pisciculus*, *C. rufescens*, and *C. kiangsiensis*) (Simpson 1900; Tchang and Li 1965). *Cuneopsis celtiformis* (Heude, 1874) is the type species of this genus, and is mainly distributed in Poyang Lake and Dongting Lake, as well as their connecting rivers (Liu et al. 1979). Although previous phylogenetic studies showed that *Cuneopsis* was not monophyletic (Wu et al. 2019; Lopes-Lima et al. 2020), the result needs to be further verified by comprehensive sampling of all *Cuneopsis* species, especially the inclusion of the complete mitogenome of the type species *C. celtiformis*.

Freshwater mussels have an unusual mode of mitochondrial DNA transmission named Doubly Uniparental Inheritance (DUI). There are two highly differentiated and gender-associated mitogenomes (i.e. maternal and paternal mitogenomes), which vary in sequence and in tissue localization (Breton et al. 2007). It has been demonstrated that complete mitogenomes can be effectively used in solving phylogenetic relationships at different taxonomic ranks of freshwater mussels (Lopes-Lima et al. 2017; Huang et al. 2018; Huang et al. 2019; Wu et al. 2020). In this study, we first determined and described the complete maternal mitogenome of *C. celtiformis*, providing useful information for mitogenome comparative analysis and taxonomy of *Cuneopsis*.

Specimens of *C. celtiformis* were collected from the Fuhe River (27°56′29″N, 116°26′51″E), Jiangxi Province, China, and were sexed by microscopic examination of gonadal tissues. Total genomic DNA was extracted from the adductor muscle of one live female sample using TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China). Subsequently, tissues were preserved in anhydrous ethanol, and the voucher specimen (number: 20-NCU-XPWU-CC01; contact Xiao-Chen Huang: xiaochenhuang@hotmail.com) was deposited in the Museum of Biology in Nanchang University. The mitogenome was sequenced by primer-walking strategy according to our previous studies (Huang et al. 2013; Wu et al. 2019). Partial *cox1*, *rrnL* and *nad1* genes were obtained using universal primers, and then used to design three sets of primers to amplify the complete mitogenome in three long fragments.

*SeqMan* program (DNASTAR) was used to check and assemble the mitogenome. All rRNA and tRNA genes were identified on the MITOS web server (Bernt et al. 2013). Protein coding genes (PCGs) were initially identified using the Open Reading Frame Finder (ORF Finder) implemented at the NCBI website (https://www.ncbi.nlm.nih.gov/orffinder/) with vertebrate mitochondrial genetic code. Gene Annotations were further adjusted by comparing BLAST searches with published congeneric mitogenomes.

We downloaded 31 published maternal mitogenomes from the subfamily Unioninae from GenBank, and used...
Sinosolenia oleivora, which is from the subfamily Gonideinae, as outgroup. A phylogenetic tree based on 12 PCGs (except atp8 and F-orf) and two rRNA genes was reconstructed under Maximum likelihood (ML) criteria with 10,000 ultrafast bootstrap replicates in IQ-TREE (Nguyen et al. 2015). We used ModelFinder (Kalyaanamoorthy et al. 2017) to select the best-fit partitioning schemes and models under greedy search with unlinked branch lengths.

The maternal mitogenome of C. celtiformis was 15,922 bp in length and deposited in GenBank under the accession number MW464617. The base composition was A (26.1%), T (38.4%), C (11.7%), and G (23.8%), with the A+T content of 64.5%. It was composed of 14 protein-coding genes (including one F-orf), 22 tRNA genes, 2 rRNA genes, and 1 putative control region (CR). The lengths of the 14 protein-coding genes, in descending order, were nad5, cox1, nad4, cob, nad2, nad1, cox3, atp6, cox2, nad6, nad3, nad4L, F-orf, and atp8. All 22 tRNA genes ranged from 61 to 70 bp, with typical secondary structure. Eleven of the 38 genes were encoded on the H strand, including cox1, cox2, cox3, nad3, nad4, nad4L, nad5, atp6, atp8, tRNA^A^, and tRNA^His^. The remaining genes were encoded on the L strand. The location of the F-orf (i.e. between ND2 and tRNA^Glu^) was the same as that of the other maternal mitogenomes in Unionidae (excluding Gonideinae) (Lopes-Lima et al. 2017).

Our phylogenetic tree had bootstrap values ≥95% for most nodes. This ML tree showed that C. celtiformis belonged to the subfamily Unioninae, and was the closest to C. heudei with a high node confidence value (BS = 100) (Figure 1). Schistodesmus and Nodularia were nested within the currently recognized genus Cuneopsis, respectively. The fact that not all species of Cuneopsis cluster together in the phylogenetic tree based on mitogenome data, which is in accordance with the previous study (Wu et al. 2019), confirmed that Cuneopsis is not monophyletic. Comprehensive sampling of all Cuneopsis species is needed for mitochondrial phylogenomics to erect new genera in future studies, together with morphological data.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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Figure 1. Maximum-likelihood tree of 33 freshwater mussels based on 12 PCGs (except atp8 and F-orf) and two rRNA genes of maternal mitogenomes. Sinosolenia oleivora (Gonideinae) was used as outgroup.
Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the Accession no. MW464617.

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