

Complete mitochondrial genome of *Arcuatula japonica* (Mollusca: Mytilidae: Crenellinae) and its phylogenetic analysis

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ABSTRACT

Arcuatula japonica (Dunker, 1857) (Mollusca: Mytilidae: Crenellinae) is a marine mussel distributed primarily in the northwestern Pacific. To clarify its evolutionary position and evaluate the taxonomic status of Crenellinae, we sequenced and characterized the complete mitochondrial genome of *A. japonica*. The mitogenome is 16,198 bp in length and contains 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs), and 1 non-coding control region (CR), with an A + T bias (68.7%). Phylogenetic analysis based on 13 mitochondrial PCGs supports a sister relationship between *A. japonica* and *A. senhousia*. The mitochondrial gene arrangement is conserved between these two species. In contrast, gene order, including that of PCGs, is highly variable among species representing different genera currently assigned to Crenellinae. Our findings provide important mitogenomic resources for future phylogenomic, taxonomic, and evolutionary studies of Crenellinae.

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1. Introduction

The Mytilidae is a diverse group and phylogenetic classification above the genus level remains largely unresolved (Xu et al. 2023; Kartavtsev and Masalkova 2024; Tan and Tan 2024). *Arcuatula japonica* (Dunker, 1857), currently recognized as a valid species within the genus *Arcuatula* (Crenellinae, Mytilidae), is distributed mainly in the northwest Pacific. Along the coast of China, it has historically been recorded as *Musculus japonica* (Liu 2008). Nevertheless, morphological evidence supports the distinction between *Arcuatula* and *Musculus* as separate genera (Morton 2015; Audino et al. 2020). Phylogenetic analyses have further indicated close evolutionary relationships between *Arcuatula* and *Musculus* (Tan and Tan 2024). Now, there are 12 valid genera within Crenellinae, including *Arcuatula*, *Musculus*, *Arvella*, *Mytella*, *Perna*, et al. (MolluscaBase eds 2026). However, the monophyly and phylogenetic relationships of Crenellinae remain controversial, as the arrangement order of mitochondrial genomes belonging to the genera *Mytella*, *Perna*, and *Arcuatula* is highly inconsistent, reflecting extensive mitochondrial genome rearrangements within Mytilidae and suggesting that this subfamily should be divided into multiple subfamilies (Zhu 2025).

Mitogenomic gene rearrangements may provide additional evolutionary signals and improve phylogenetic inference at lower taxonomic levels (Xu et al. 2023). Within Crenellinae,

mitogenomes are currently available for only five species: *Arcuatula senhousia*, *Mytella strigata*, *Perna viridis*, *P. perna* and *P. canaliculus*. Here, we determined the complete mitochondrial genome of *A. japonica* and assessed its phylogenetic position within Crenellinae, with the aim of providing new insights into mitochondrial genome evolution and phylogenetic relationships within Mytilidae. It should be noted that Doubly Uniparental Inheritance (DUI) is a common mitochondrial inheritance pattern in mytilid species (Skibinski et al. 1994). It involves sex-linked mitochondrial inheritance, the F-type is present in somatic tissues and female gonads, while the M-type is usually found in male gonads and sperm. The mitogenome of *A. japonica* obtained in the present study was assembled from DNA extraction of somatic tissue. Thus should *A. japonica* be found to have DUI; the mitogenome we assemble is of the F-type based on the similarity of COI gene with the F-type mitogenome of *A. senhousia* (Figure S1).

2. Materials and methods

2.1. Sample collections

The specimen of *A. japonica* was collected from Qionghai, Hainan Province, China (110.62E, 19.23 N) (Figure 1). The specimen tissue was deposited at the Department of Marine Organism Taxonomy & Phylogeny, Qingdao Key Laboratory

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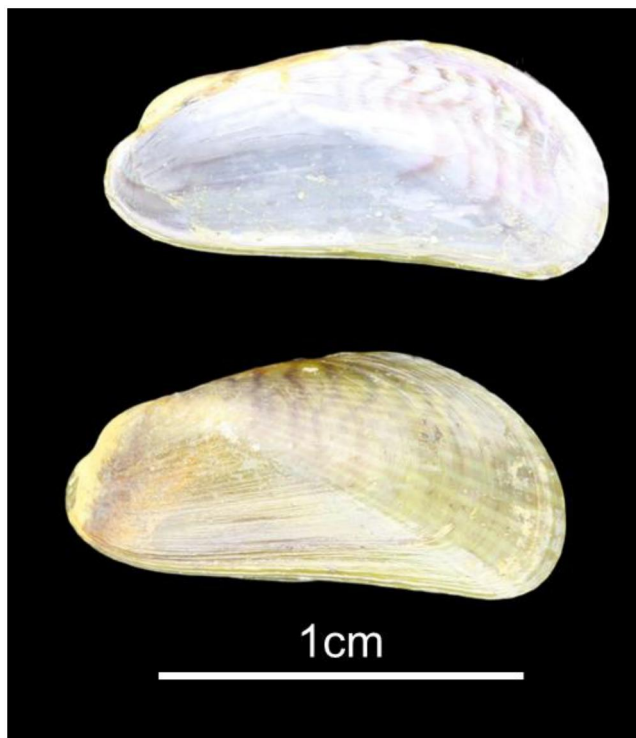


Figure 1. The photograph of *Arcuatula japonica*. Photo taken by the authors.

of Marine Biodiversity and Conservation, Institute of Oceanology, Chinese Academy of Sciences, under voucher number IOCAS-Myaj2025001 (contact Zhen Zhang, zhangzhen@qdio.ac.cn).

2.2. Sequencing and annotation

Total genomic DNA was extracted using the TIANamp Kit (DP324). Quantified DNA libraries were constructed and sequenced on the Illumina NovaSeq 6000 platform with paired-end 150 bp reads.

Raw sequencing reads were quality-filtered and adapter-trimmed using fastp v.0.23.2 (Chen et al. 2018). Filtered reads assembled into the complete mitochondrial genome using SPAdes v.3.15.2 (Prjibelski et al. 2020), and annotated using MITOS2 on the Galaxy platform (The Galaxy Community, 2024). tRNA genes and PCG boundaries were verified using ARWEN (Laslett and Canback 2008) and ORFfinder (<https://www.ncbi.nlm.nih.gov/orffinder>). Annotation results were adjusted refer to the mitochondrial genome of *A. senhousia* NC_014590 (Passamonti et al. 2011). *Atp8* gene in *A. japonica* was annotated by manually (Zhao et al. 2022). The graphical map was drawn with OGDRAW v.1.3.1 (Lohse et al. 2013).

2.3. Phylogenetic analysis

Phylogenetic analyses were conducted using PhyloSuite v.1.2.3 (Zhang et al. 2020). Nucleotide sequences of the 13 mitochondrial PCGs were extracted and aligned using MAFFT v.7.402 (Katoh and Standley 2013). Ambiguously aligned regions were removed using Gblocks v.0.91b (Talavera and Castresana 2007). The aligned PCGs were concatenated into a

supermatrix, and the dataset was partitioned by gene and codon position (charset *atp6* = 1-720; charset *atp8* = 721-867; charset *cox1* = 868-2484; charset *cox2* = 2485-3324; charset *cox3* = 3325-4320; charset *cytb* = 4321-5577; charset *nad1* = 5578-6573; charset *nad2* = 6574-7650; charset *nad3* = 7651-8034; charset *nad4L* = 8035-8313; charset *nad4* = 8314-9657; charset *nad5* = 9658-11487; charset *nad6* = 11488-12018).

The best-fit substitution model (TVM+F+I+G4: *atp6* + *atp8* + *nad2* + *nad3* + *nad4L* + *nad4* + *nad5* + *nad6*, K3Pu + F+R3: *cox1* + *cox2* + *cox3* + *cytb* + *nad1*) was selected using ModelFinder as implemented in IQ-TREE v.2.2.5 (Minh et al. 2020). Maximum-likelihood (ML) phylogenetic reconstruction was performed in IQ-TREE with 1,000 ultrafast bootstrap replicates. Phylogenetic tree was visualized and edited using FigTree v.1.4.3 (<https://tree.bio.ed.ac.uk/software/figtree/>).

3. Results

The complete mitochondrial genome of *A. japonica* was successfully sequenced (Figure 2). Length of the genome is 16,198 bp (GenBank Accession No. PZ247365), and the average read mapping depth was 47.38× (Figure S2). Total nucleotide composition was A (27.3%), T (41.4%), G (20.6%), and C (10.7%), showing a clear A + T bias (68.7%). The genome was characterized by a negative AT skew (-0.206) and a positive GC skew (0.318). Thirty-seven genes were identified, including 13 PCGs, 22 tRNAs, 2 rRNAs, and 1 non-coding control region (CR). All genes were encoded on the heavy strand. All PCGs used standard mitochondrial initiation codons, with *nad1* starting from GTG, and most genes terminated with TAA or TAG, except *nad5* with an incomplete stop codon.

The topology of the ML tree (Figure 3) showed that *A. japonica* and *A. senhousia* clustered into one clade. The gene arrangement of mitochondrial genes among Crenellinae species was shown in Figure S3. The result showed that, mitogenome of *A. japonica* shared the same gene order as that of *A. senhousia*.

4. Discussion and conclusion

In the present study, we sequenced and assembled a complete mitogenome of *A. japonica*. The mitogenome is 16,198 bp in length and includes 13 PCGs, 22 tRNAs, 2 rRNAs, and 1 CR, with a pronounced A + T bias (68.7%). This pattern is consistent with previously reported mitogenomes of Mytilidae (Lubośny et al. 2020; Zhao et al. 2022; Li et al. 2023; Xu et al. 2023), indicating relative conservation of mitochondrial genome architecture at genus level. The *atp8* gene was successfully annotated in the mitogenome of *A. japonica*, as well as in other published mytilid species, supporting the view that *atp8* may not actually be absent in Mytilidae (Zhao et al. 2022).

Phylogenetic reconstruction based on 13 mitochondrial PCGs supports a sister relationship between *A. japonica* and *A. senhousia*. Notably, these two *Arcuatula* species share an identical mitochondrial gene arrangement, indicating a high

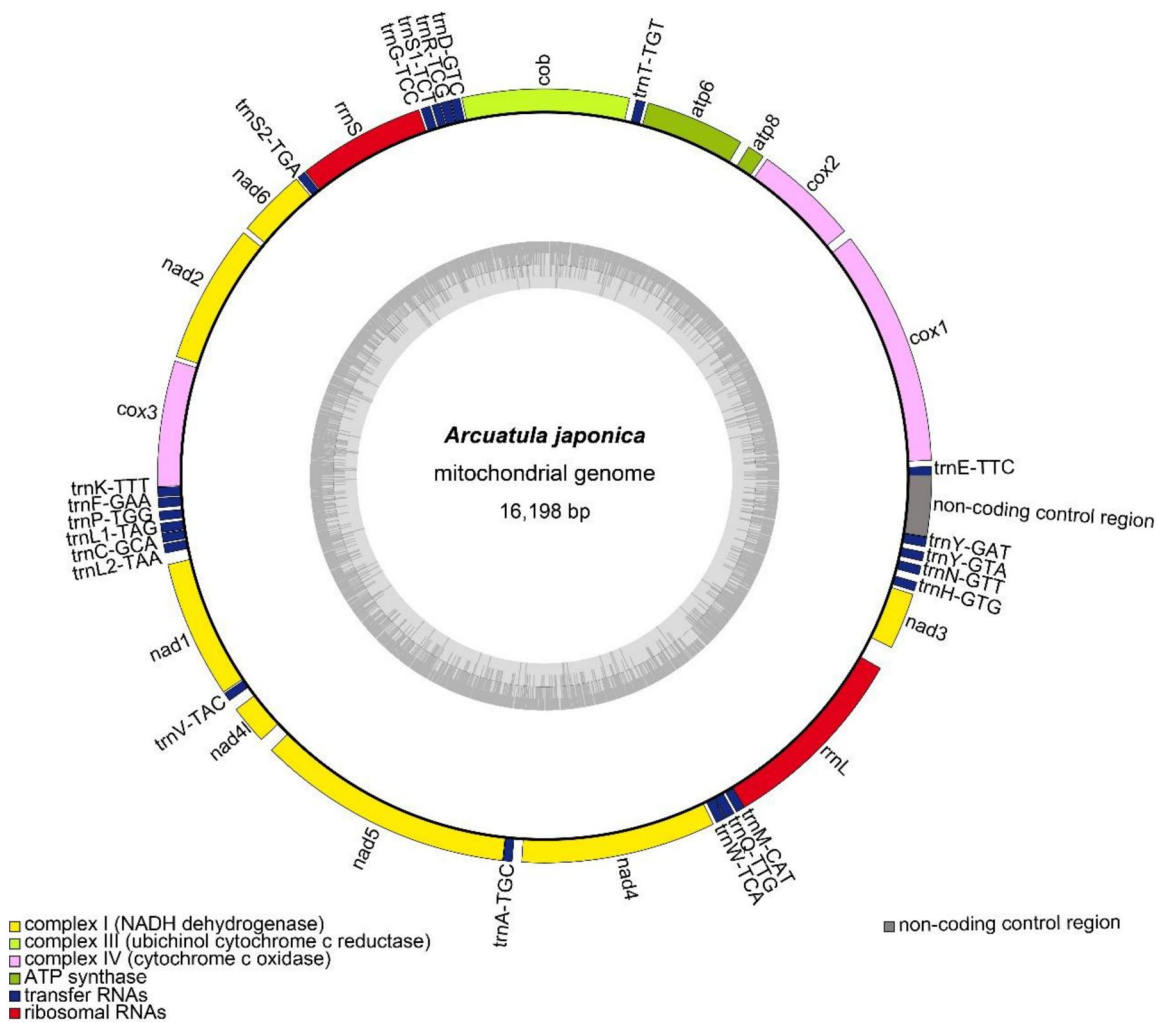


Figure 2. Circular map of the complete mitochondrial genome of *Arcuatea japonica*. Annotated genes are colored according to the functional categories. Genes on the outside are transcribed in the clockwise direction.

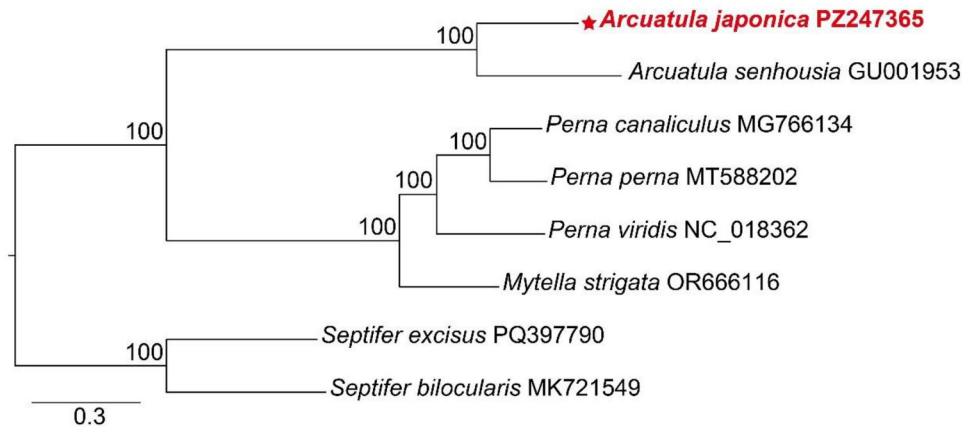


Figure 3. Maximum-likelihood (ML) phylogenetic tree for subfamily Crenellinae based on the nucleotide sequences of 13 PCGs. Star and red represent the complete mitogenome of *Arcuatula japonica* sequenced in the present study. The GenBank accession numbers of mitochondrial genomes used in this analysis are the following: *A. senhousia* GU001953 (Passamonti et al. 2011), *Mytella strigata* OR666116 (Yan et al. 2023), *Perna viridis* NC_018362 (Li et al. 2012), *P. perna* MT588202 (unpublished), *P. canaliculus* MG766134 (Ranjard et al. 2018), *Septifer excisus* PQ397790 (Zhu 2025), and *S. bilocularis* MK721549 (Lee et al. 2019).

degree of mitogenomic conservation within this genus. In contrast, variation in mitochondrial gene arrangements was observed among the sampled genera currently assigned to Crenellinae, particularly in the organization of PCGs and tRNA genes, consistent with previous studies (Lee et al. 2019; Xu et al. 2023). These differences highlight considerable

mitogenomic diversity within the sampled Crenellinae taxa. Broader taxonomic sampling and phylogenomic data from multiple independent loci will be necessary to determine whether the observed mitogenomic differences reflect lineage-specific evolution or deeper phylogenetic structure within the subfamily.

In the present study, a mitochondrial genome of *A. japonica* was obtained. Our results showed a similarity with the F-type mitogenome of the closely related species *A. senhousia*, which exhibits double uniparental inheritance. This evidence along with the fact that our sample came from somatic tissue, we infer that the sequenced mitogenome of *A. japonica* could be the F-type mitogenome; however, further work needs to be done to conclude that this species does in fact have two mitogenomes and the DUI pattern. Furthermore, broader mitogenome sampling across additional genera will be necessary to test the monophyly of Crenellinae. The newly generated mitogenome constitutes an important resource for future systematic and phylogenomic studies of Crenellinae.

Ethical approval

No ethical approval or special permits were required for this study, as the sampled species is not listed under CITES and was not collected from protected natural reserves. All sampling, handling, transport, and sequencing procedures complied with local legislation and relevant laboratory regulations for wild resource protection.

Author contributions

CRediT: **Zhao-Peng Ma**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Zhen Zhang**: Conceptualization, Funding acquisition, Methodology, Project administration, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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 number PZ247365. The associated BioProject, SRA and BioSample numbers are PRJNA1450845, SRR38011954, and SAMN57173007 respectively.

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