


Complete mitochondrial genome of *Devario kakhienensis* (Cypriniformes: Danionidae: Danioninae): genome characterization and phylogenetic consideration

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ABSTRACT

Complete mitochondrial genome and phylogenetic analysis of *Devario kakhienensis*, endemic minnow from southwest China, for the first time was presented. It was determined to be a 16,777 bp long circular molecule and the genome organization was consistent with that of Danioninae species published previously. Based on PCGs, the maximum likelihood phylogenetic analysis supported the close genetic relationship between *D. kakhienensis* and *Devario interruptus*. These data would contribute toward the genetic resource enrichment, and provide a valuable framework for future research in completely resolving phylogenetic relationships with the family Danionidae.

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Devario kakhienensis (Anderson 1879) is a fish grouped under the subfamily Danioninae in the Danionidae family, distributed in western Yunnan, Irrawaddy drainage, China. *D. kakhienensis* has most recently been assessed for the IUCN Red List of Threatened Species in 2010 and is listed as Data Deficient based on its high value to fisheries. This species combines morphological characters typical of the genus *Devario* and is often misidentified as *Devario aequipinnatus* due to their high morphological similarities by having almost equally wide, complete three stripes and three interspaces (Fang 1997), which accounts for the urgency to unravel the taxonomy and phylogeny of *D. kakhienensis*. Mitogenome has been successfully applied to resolve the phylogenetic relationships among various groups at different taxonomic ranks (He et al. 2008; Inoue et al. 2010). This study is the first to characterize the mitogenome of *D. kakhienensis* and evaluate its taxonomic position in Danionidae fish.

We obtained the specimen from Nujian River, Longling area, Yunnan Province, China (24°19'46.04" N, 98°95'47.19"E). The specimen was preserved in 95% ethanol and deposited at Aquatic Science and Technology Institution Herbarium under voucher number ASTIH-21b1108d32 (<https://www.jsahvc.edu.cn/>). The person in charge of the collection: Lin SONG, email: tianxinlinger@126.com). After being transported to Shanghai Genesky Biotechnologies Inc., total genomic DNA was extracted from muscle using Tguide Cell/tissue genomic DNA Extraction Kit (OSR-M401) (Tiangen, Beijing, China), followed by DNA sample quality control, DNA library construction, PCR amplification, size selection, library quality check, and library pooling. Qualified PCR products were sequenced on Illumina HiSeq 4000 Sequencing platform

(Illumina, CA, USA). The complete mitogenome was obtained by sequence assembly on the software MetaSPAdes (Nurk et al. 2017), and the annotation process was executed on MitoMaker software (Bernt et al. 2013).

The size of the complete mitogenome was 16,777 bp (GenBank Accession No. OM732332), encompassing a typical structure of 13 protein-coding genes (PCGs), and two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and a control region (D-loop). Overall base composition appeared as follows: A (33.1%), T (27.2%), G (15.2%), and C (24.5%), demonstrating that the mitochondrial genome was biased toward AT (60.3%) rather than GC (39.7%), which was similar to the mitogenome of *Trigonopoma pauciperforatum* (Chung et al. 2020). The gene order and composition were identical to the typical arrangement in other teleost fishes. H-strand accommodated most of the PCGs and tRNA genes, the exceptional nine genes included *ND6* and eight tRNA genes (*tRNA^{Gln}*, *tRNA^{Ala}*, *tRNA^{Asn}*, *tRNA^{Cys}*, *tRNA^{Tyr}*, *tRNA^{Ser(UCN)}*, *tRNA^{Glu}*, and *tRNA^{Pro}*) which were encoded on the L-strand.

Within the mitogenome of *D. kakhienensis*, a complete set of 22 tRNAs individually ranged in size from 66 to 74 bp, while the noncoding control region located between *tRNA^{Phe}* and *tRNA^{Pro}* was 578 bp. Moreover, the large ribosomal gene (16S) was 1653 bp long and was located between *tRNA^{Val}* and *tRNA^{Leu(UUR)}*; the small (12S) was 955 bp long and was located between *tRNA^{Phe}* and *tRNA^{Val}*. Among the protein-coding genes, the length varied from 165 bp (*ATP8*) to 1812 bp (*ND5*), 12 PCGs conventionally utilized ATG as the start codon except for *COI* which was found exclusively using GTG instead. 3 PCGs (*COII*, *ND3*, and *Cytb*) ended with a single nucleotide T, as *ND4* setting incomplete TA as the stop

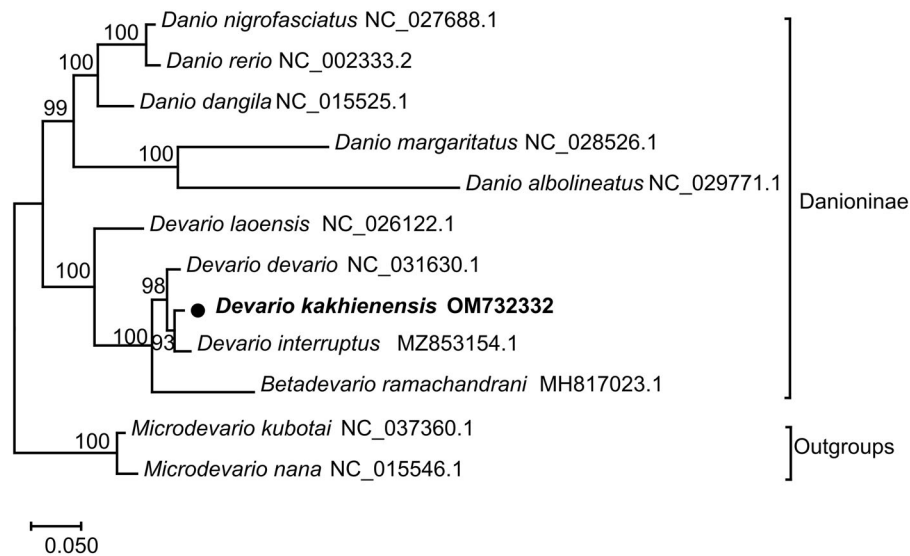


Figure 1. Maximum-likelihood phylogenetic tree reconstructed using concatenated mitochondrial protein-coding genes of *D. kakhienensis* and other 11 fishes. Accession numbers were indicated after the species names. The tree topology was evaluated by 1000 bootstrap replicates. Bootstrap values at the nodes correspond to the support values for ML methods.

codon. 6 PCGs (*ND1*, *COI*, *ATP6*, *COIII*, *ND4L*, and *ND5*) were terminated with canonical TAA, while 3 PCGs (*ND2*, *ATP8*, and *ND6*) ended with complete TAG. Sequence analyses displayed that overlaps between tRNAs were $tRNA^{Ile}-tRNA^{Gln}$, $tRNA^{Ser(AGY)}-tRNA^{Leu(CUN)}$, and the overlaps were also found between protein-coding genes (*ATP8-ATP6*, *ATP6-COIII*, *ND4L-ND4*, *ND5-ND6*).

A model test was performed based on the concatenated 11 PCG sequences of closely related species of the Danioninae subfamily available publicly from NCBI GenBank, the best suited JTT + G + F model was employed via the Maximum Likelihood method in MEGA X software (Kumar et al. 2018) with a bootstrap of 1000 replicates to construct the phylogenetic tree, *Microdevario kubotai* and *Microdevario nana* were selected as the outgroups. The topology of the phylogenetic tree supported that *D. kakhienensis* was most closely related to *Devario interruptus*, and it was a basal species in the clade comprising *Devario* and *Betadevario*, showing congruent phylogenetic relationships to previous studies (Pramod et al. 2010). This clade was sister to the tribe of *Danio*. The phylogenetic relationship clearly illustrated that the genus *Devario* was not monophyletic, *D. kakhienensis*, *D. interruptus*, and *D. devario* were found to be closely related to *Betadevario ramachandrani* (Norén & Kullander 2018) (Figure 1). In conclusion, our results might serve as enrichment toward the complete mitochondrial genome count of the family Danionidae in terms of evolution and conservation genetics.

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Ethical approval

Experiments were performed in accordance with the recommendations of the Ethics Committee for Animal Experiments of Jiangsu Agri-animal

Husbandry Vocational College. These policies were enacted according to the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

Author contributions

Lin Song and Xiao Jiang Chen make substantial contributions to the conception or design of the work, and drafting the paper, and Final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; Quan Wang and Sai Zhang were involved in the acquisition, analysis and interpretation of the data; the drafting of the paper, and the final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The contributions are ranked in order.

Disclosure statement

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

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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 reference number OM732332.

The associated “BioProject”, “Bio-Sample” and “SRA” numbers are PRJNA816570, SAMN26686137, and SRR18335089 respectively.

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