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Characteristic and phylogenetic analyses of mitochondrial genome for *Rhinogobius filamentosus* (Teleostei: Gobiidae: Gobionellinae), an endemic species in China

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ABSTRACT

This study sequenced and annotated the complete mitochondrial genome sequence of *Rhinogobius filamentosus*, an endemic species in China. The complete mitochondrial genome was 16,510 base pairs long and contained 13 protein-coding genes (PCGs, 11,414 bp), 22 tRNA genes (1,555 bp), two rRNA genes (2,615 bp), two non-coding regions (D-loop: control region displacement loop, 478 bp; OL: origin of L-strand replication, 30 bp). The overall base composition of the genome was estimated to be T (25.4%), C (30.2%), A (27.6%) and G (16.7%), showing an AT bias (53%). Phylogenetic analyses revealed that the genus *Rhinogobius* included two clades. For one clade, *R. filamentosus* clustered with *R. duospilus*, and they formed a sister-group relationship with other ten *Rhinogobius* species. This work would be essential in revealing the evolutionary relationships in Gobionellinae.

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1. Introduction

Freshwater Gobionellinae fish Rhinogobius filamentosus (Teleostei: Gobiidae) is a small benthic fish (60–80 mm) of great ornamental development value (Zheng et al. 2016; Liu et al. 2021). *Ctenogobius filamentosus* was treated as the synonym of R. filamentosus in the past. It is an endemic species from China mainly distributed in the Lijiang River, Xijiang River and Beijiang River, inhabiting the tributaries of rivers and streams. R. filamentosus can be distinguished from other *Rhinogobius* fish by dorsal fin rays VI, I-8-9; anal fin rays I-8; pectoral fins 16-17; ventral fin rays I-5; caudal fins 2 + 18 + 2; vertical scales 30–33; horizontal scales series 8–10; predorsal scales 8–11; gill rakes 5–8; body with 5–6 dark transverse stripes; reticular fine lines on the back of the head; fine stripes extending to the ventral surface on the cheeks; a large spot on the fin membrane between the spines of the first and third fins of the first dorsal fin for male (Wu and Zhong 2008) (Figure 1). Although some studies on the complete mitochondrial genome in Rhinogobius fishes have been reported (Zhong et al. 2018; Chen et al. 2019; Zhang and Shen 2019), there were still no relevant reports on the genetic characteristics and phylogenetic position of *R. filamentosus*. This research aimed to sequence the complete mitogenome of R. filamentosus, provide fundamental molecular data for evolution, and determine its phylogenetic placement in the genus *Rhinogobius*. Therefore, this study is of great significance.

2. Materials and methods

2.1. Sample collection and preservation

Samples of R. filamentosus were collected by hand net from the Lijiang River, Guilin city, Guangxi province, China (24°40′46.95″ N, 110°36′25.91″E) in Jun 2021, which was permitted by Jiangsu Agri-animal Husbandry Vocational College (granted # NSF2021ZR14). Studies involving laboratory animals followed the ARRIVE guidelines (https://arriveguidelines.org/). The specimens were euthanized: they were anesthetized with 0.2 ml/l eugenol solution first, then fixed in 75% ethanol, and finally transferred to 95% ethanol for long-term storage. All specimens were deposited at Aquatic Science and Technology Institution Herbarium of Jiangsu Agri-animal Husbandry Vocational College (https://www.jsahvc.edu.cn/, Chen XiaoJiang, 2007020030@jsahvc.edu.cn, voucher number: ASTIH- 21B1108D19). According to the morphological characteristics of R. filamentosus described by Wu and Zhong (2008), identification of the species including all counts and measurements was taken from samples preserved in 95% ethanol.

2.2. DNA extraction and sequencing

DNA was extracted from the muscle of R. filamentosus using the Tguide cell/tissue genomic DNA Extraction Kit (OSR-M401) (Tiangen, Beijing, China) and stored in a deep freezer at −80 °C. The extracted DNA was subjected to sample

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Figure 1. *Rhinogobius filamentosus*. The specimen is from the Lijiang River, Guilin city, Guangxi province, China. Photographs by Xiao Jiang CHEN on Jun 10, 2021.

quality control, subsequently, the DNA library was constructed and amplified by PCR, followed by size selection and library quality check (The concentration of DNA sample detected by NanoDrop 2000 (Thermo Fisher Scientific, USA) was not less than 20 ng/μL, the total amount was not less than 100 ng, and OD260/OD280 = 1.8–2.2. The main band of genomic DNA detected by agarose gel electrophoresis was clearly visible, and there was no obvious degradation dispersion). Finally, library pooling and sequencing were carried out on Illumina Hiseq platform 2500 (Genesky Biotechnologies Inc. Shanghai, China). The next-generation sequencing raw data (2.39 GB) were assembled using MetaSPAdes 3.13.0 (Nurk et al. 2017) with *Rhinogobius duospilus* MH127918 as

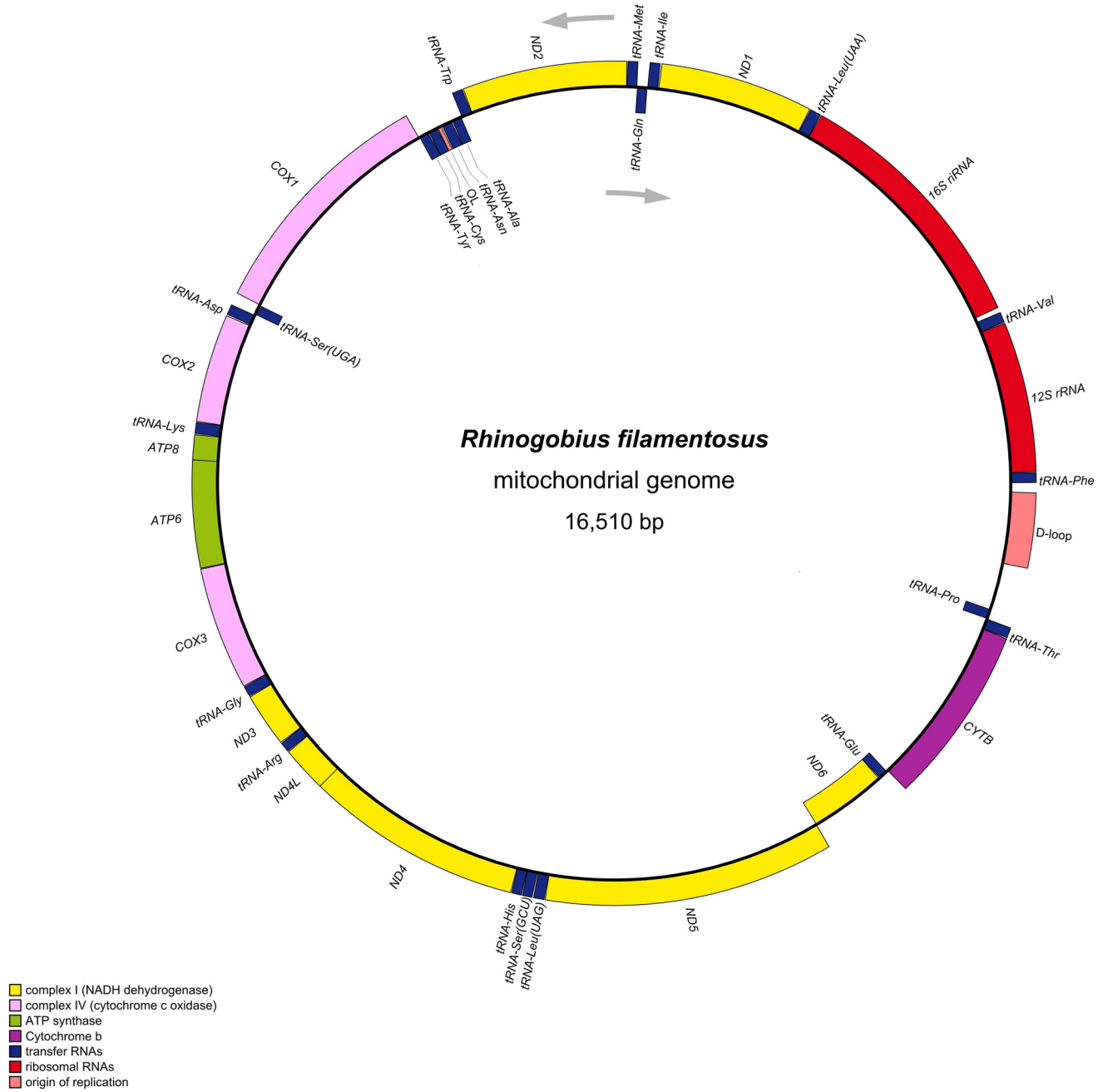

Figure 2. The complete mitochondrial genome map of *Rhinogobius filamentosus* (GenBank accession no.OM678440), consists of 13 PCGs, 22 tRNAs, two rRNAs, the origin of L-strand replication (OL) and the control region (D-loop). The arrows represent the direction of transcription, H-strand is located in the outer ring and the L-strand is located in the inner ring.

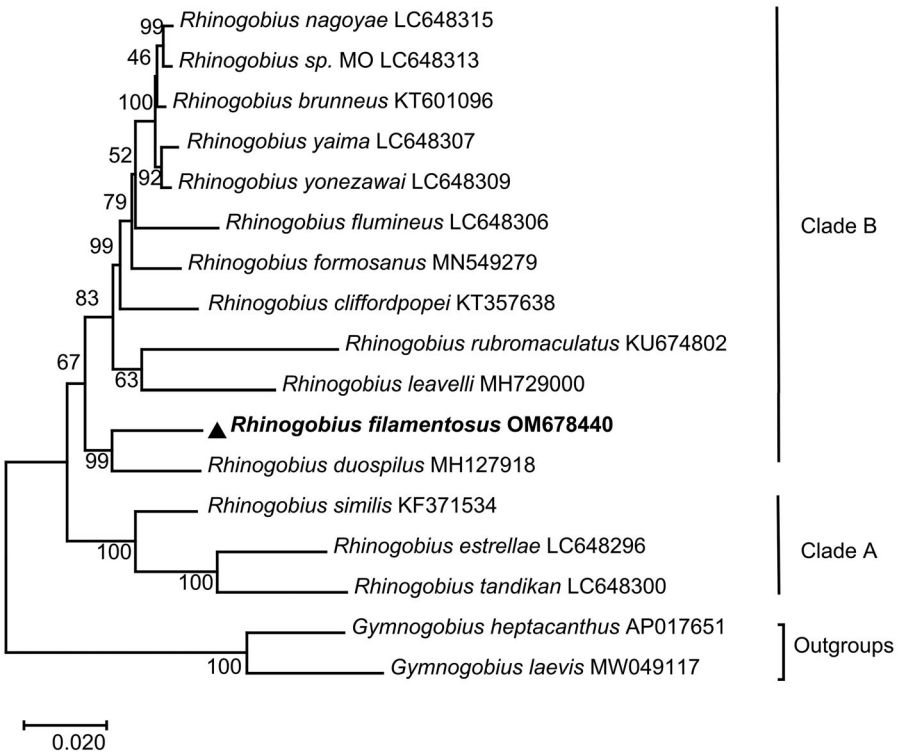

Figure 3. A phylogenetic tree was constructed for the genera Rhinogobius and *Gymnogobius*, using the Maximum-likelihood (ML) method based on the protein-coding regions of their mitogenomes, with a bootstrap of 1000 replicates. *Gymnogobius heptacanthus* and *Gymnogobius laevis* were used as outgroups to root the tree. Accession numbers were given with species names, and the numbers at the nodes represented bootstrap values.

reference (Tan et al. 2020), and then the assembled mitochondrial genome sequences were annotated by MitoMaker 1.14 (Bernt et al. 2013) with default parameters. OGDRAW (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) was used to draw the genome maps (Greiner et al. 2019).

2.3. Phylogenetic analysis

To confirm the phylogeny of *R. filamentosus*, all 17 mitochondrial genomes from genera *Rhinogobius* and *Gymnogobius* were obtained from Genbank. The phylogenetic tree was reconstructed using the Maximum-likelihood (ML) method based on the concatenated amino acids sequences of 13 PCGs, with a bootstrap of 1000 replicates. *Gymnogobius heptacanthus* (Song et al. 2016) and *Gymnogobius laevis* (Peng et al. 2022) were used as outgroups to root the tree. JTT + G + I + F was selected as the optimal evolutionary model for phylogenetic analysis due to the lowest Bayesian information standard scores (BIC) (Jones et al. 1992). MEGA X was used for alignments, analyses, model calculation, and phylogeny reconstruction (Kumar et al. 2018).

3. Results and discussion

3.1. The mitochondrial genome of rhinogobius filamentosus

The complete mitochondrial genome of *R. filamentosus* was 16,510 bp in length, consisting of 13 protein-coding genes (PCGs, 11,414 bp), 22 tRNAs (1,555 bp), two rRNAs (2,615 bp), and two non-coding regions (D-loop: control region displacement loop, 478 bp; OL: origin of L-strand replication, 30 bp). Among the 37 genes, 28 genes were encoded on the H-strand while *tRNA-Asn, tRNA-Ala*, *tRNA-Gln*, *tRNA-Pro*, *tRNA-Glu*, *ND6*, *tRNA-Ser(UGA)*, *tRNA-Tyr*, and *tRNA-Cys* were encoded on L-strand (Figure 2). The overall base composition of the genome was estimated to be T (25.4%); C (30.2%); A (27.6%); and G (16.7%), with an AT bias (53%). The length of the 13 protein-coding genes ranged from 165 bp (*ATP8*) to 1,821 bp (*ND5*). All protein-coding genes started with ATG as an initiation codon except for *COX1*, which started with GTG. As for stop codons, seven PCGs (*ND1*, *ND2*, *COX1*, *ATP8*, *ATP6*, *ND4L*, and *ND5*) used TAA, two genes (*ND3* and *ND6*) used TAG. Four PCGs (*COX2*, *COX3*, *ND4*, and *CYTB*) ended with an incomplete stop codon (T or TA). The length of 22 tRNAs was in the range of 66–76 bp. As for rRNA, the *12S rRNA* was 957 bp in length and the *16S rRNA* was 1,658 bp.

3.2. Phylogenetic analysis

The phylogenetic analysis showed that the genus Rhinogobius included two clades (Figure 3), Clade A combined *R. similis* (Suzuki et al. 2015), *R. estrellae* (Maeda et al. 2021), and *R. tandikan* (Maeda et al. 2021), while *R. filamentosus*, *R. duospilus* (Tan et al. 2020), and other ten species (*R. nagoyae* (Maeda et al. 2021), *R. sp.* MO (Maeda et al. 2021), *R. brunneus* (Maeda et al. 2021), *R. yaima* (Maeda et al. 2021), *R. yonezawai* (Maeda et al. 2021), *R. flumineus* (Maeda et al. 2021), *R. formosanus* (Yang et al. 2020), *R. cliffordpopei* (Wang et al. 2019), *R. leavelli* (Zhang and Shen 2019), *R. rubromaculatus*) formed Clade B. Furthermore, *R. filamentosus* clustered with *R. duospilus*, and they formed a sister-group relationship with the other ten *Rhinogobius* species. The topological

structure of Rhinogobius in this paper was similar to that of Song et al (Song et al. 2022). This study provided information on the complete mitochondrial genome of *R. filamentosus* for the first time, and confirmed its phylogenetic position in genus *Rhinogobius*, which would be beneficial for further studies on population genetics and biodiversity.

4. Conclusions

The complete mitochondrial genome of *Rhinogobius filamentosus* had been firstly sequenced and annotated on the Illumina HiSeq platform using high-throughput sequencing technology. The assembly circular mitogenome was 16,510 bp long. The genetic data has been submitted to NCBI with the accession number OM678440, and the phylogenetic analysis results strongly supported that *R. filamentosus* was clustered with *R. duospilus*. The mitochondrial genomic data of *R. filamentosus* would be essential for further genetic studies such as phylogenetic relationship investigations, genetic taxonomy, DNA barcode development, and so on. In the future, we will supplement more mitochondrial genomes of other goby fishes.

Ethical approval

Experiments were approved by the Ethical Committee for Animal Experiments of Jiangsu Agri-animal Husbandry Vocational College and conducted following the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

Author contributions

Conception and design, Chen XJ and Song L; Data curation, Song L, Wang Q, Liu WZ, and Chen XJ; Analysis and interpretation of the data, Song L, Wang Q, and Chen XJ; Funding acquisition, Chen XJ and Wang Q; Writing – original draft, Chen XJ, Song L, Wang Q, and Liu WZ; Writing – review & editing, Chen XJ, Song L, Liu WZ, and Wang Q. All authors agree 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.

Disclosure statement

The authors declare no potential conflict of interest.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession no.OM678440. The associated "BioProject", "Bio-Sample" and "SRA" numbers are PRJNA808168, SAMN26030913, and SRR18131290, respectively. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA808168.

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