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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome of *Rhinogobius maculagenys* (Gobiidae:Gobionellinae)

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ABSTRACT

The complete mitochondrial genome of *Rhinogobius maculagenys* Wu et al., (Perciformes,Gobiidae) was sequenced and annotated in this study. The circular mitogenome is 16,500 bp long and consists of 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes and two main non-coding regions (a putative control region and an L-strand replication origin). The overall base composition is 27.5% A, 25.5% T, 16.9% G, and 30.1% C. The gene order and composition are similar to those of other Gobionellinae species. Phylogenetic analysis revealed that *R. maculagenys* is closely related to *Rhinogobius* shennongensis in both the maximum likelihood tree and the Bayesian inference tree. The complete mitogenome of R. maculagenys will serve as a valuable resource for future studies on evolution, taxonomy, and genetic conservation of *Rhinogobius*.

1. Introduction

Of the freshwater gobies, the genus Rhinogobius (Gill, 1859) is a dominant group of benthic fishes in most drainages of East Asia (Chen and Shao 1996; Chen et al. 1999; Chen and Miller 2014). In southern and southeastern mainland China, most species of Rhinogobius are non-diadromous and landlocked (Takahashi and Yanagisawa 1999; Huang and Chen 2007). *Rhinogobius maculagenys* Wu et al. (2018) was first discovered in the upper reaches of the Xiangjiang River in Lanshan Country, a county located southeast of mainland China (Wu et al. 2018). *R. maculagenys* can be distinguished from all congeners by a combination of the following features: dorsal fins VI, I/7-9; anal fin I/6-8; pectoral fins 16; longitudinal scales series 32–34; transverse scales series 9–13; predorsal scale series 0; vertebral count 27; pore $\omega1$ missing; head and body yellowish brown; cheek and opercle yellowish brown with over 30 small orange spots (Wu et al. 2018). Currently, there is no information on the mitogenome of this species in the public nucleotide database. This study aims to present the complete mitochondrial genome, which will contribute new data for the reconstruction of the *Rhinogobius* phylogeny.

2. Materials and methods

2.1. Materials

The specimen in this study was collected from Wanshan District, Tongren City, Guizhou province, China (27.7167°N;

108.8350°E). The morphological measurements were conducted following the methods described by Miller (Miller 1988) and Suzuki (Suzuki et al. 2017). Reference images were captured using a Canon camera (Figure 1). The collection of fish specimens adhered to the Aquatic Wildlife Protection Regulations of the People's Republic of China. Specimens were initially fixed in 75% ethanol and later transferred to 95% ethanol for long-term storage. A voucher specimen has been deposited at Mianyang Academy of Agricultural Sciences under the voucher number GY1 (contact Jia Hu: hujia1021@hotmail.com).

2.2. Methods

Total genomic DNA was extracted from the muscle tissue of *R. maculagenys* using a Tissue DNA Kit (Qiagen, Germany) following the manufacturer's protocol. Library pooling and sequencing (PE150 Illumina Hiseq platform 2500) were performed by GeneSky Biotechnologies Inc. Shanghai, China. The depth of coverage is shown in Supplementary Figure S1. The raw data was filtered using fastp v0.36 software (Chen et al. 2018) for quality control (QC). The clean reads were then assembled using metaSPAdes v3.13.0 (Nurk et al.2017) with multiple kmers used to find assemblies with the highest N50 values. Contigs of interest were selected by conducting customized command-line blastn analyses, using sequences from *Rhinogobius leavelli* (GenBank accession: MH729000) as the query (Zhang and Shen 2019). The inner gaps within

each scaffold were filled using GapFiller v1.11 (Boetzer and Pirovano 2012). The assembled sequences were annotated using MitoMaker 1.14 (Bernt et al. 2013) with default parameters. Maps of the organellar genomes were generated using CGView (Grant and Stothard 2008).

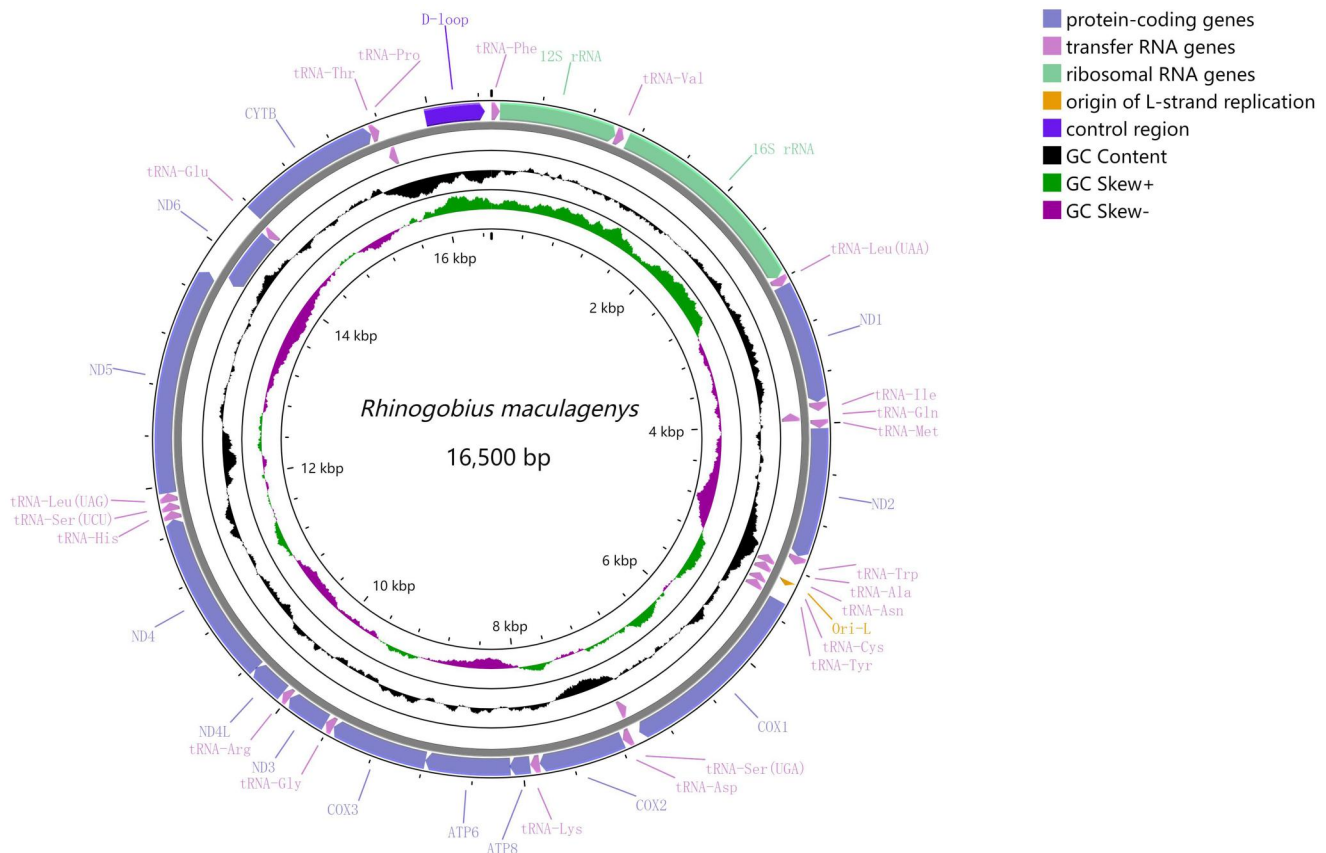We utilized Blast to filter 12 complete mitochondrial sequences from the same genus in the NCBI database, with a minimum of 88% identity. Those sequences were then used to construct phylogenetic trees, with *Pseudogobius taijiangensis* (KM624630) serving as the outgroup. The alignment of sequences was performed using ClustalW in MEGA 11 (Tamura et al. 2021). Intraspecies genetic distance was calculated using MEGA 11 (Tamura et al. 2021). The best fit substitution model (GTR + G) was selected using jModelTest 2.1.10 (Darriba et al.2012) based on the Akaike information criterion (AIC). Maximum-likelihood phylogeny was generated with 1000 replications using MEGA 11(Tamura et al. 2021). Bayesian inference phylogenies were inferred using MrBayes3.2.7 (Ronquist et al. 2012) with a mixed-model approach (two parallel runs, 1,000,000 generations), in which the initial 25% of the sampled data were discarded as burn-in. Tree information was visualized using FigTree ver.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. Mitogenomic characterization

The complete mitogenome of *R. maculagenys* is 16,500 bp in length (GenBank accession number OK545540). It consists of 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, a putative control region and an L-strand replication origin (OL) (Figure 2). Structurally, it is very similar to other Gobionellinae mitogenomes. Most of these genes

Figure 1. The specimen of *Rhinogobius maculagenys* from the Wanshan District, Tongren City, Guizhou province, China(27.7167°N;108.8350°E). the main identifiable morphological features are the first dorsal fin □, the second dorsal finⅠ/7; anal-finsⅠ/7; pectoral fin 16; longitudinal scales series 32; transverse scales series 9; predorsal scale series 0; vertebral count 27; pore ω1 missing (Wu Q 2018). (Photo by Jie Mei).

Figure 2. Mitochondrial map of *Rhinogobius maculagenys*. Circular maps were drawn with CGView (Grant and Stothard 2008). The arrows indicate the orientation of gene transcription. Protein-coding genes are shown as blue arrows, rRNA genes as green arrows, tRNA genes as purple arrows and putative control region(D-loop) as dark blue. The GC content was plotted using a black sliding window, as the deviation from the average GC content of the entire sequence. GC-skew was plotted as the deviation from the average GC-skew of the entire sequence, with an average value of −0.28. The window width was set to 500 bp and the step size was set to 1 bp. The inner cycle indicates the location of the genes in the mt genome.
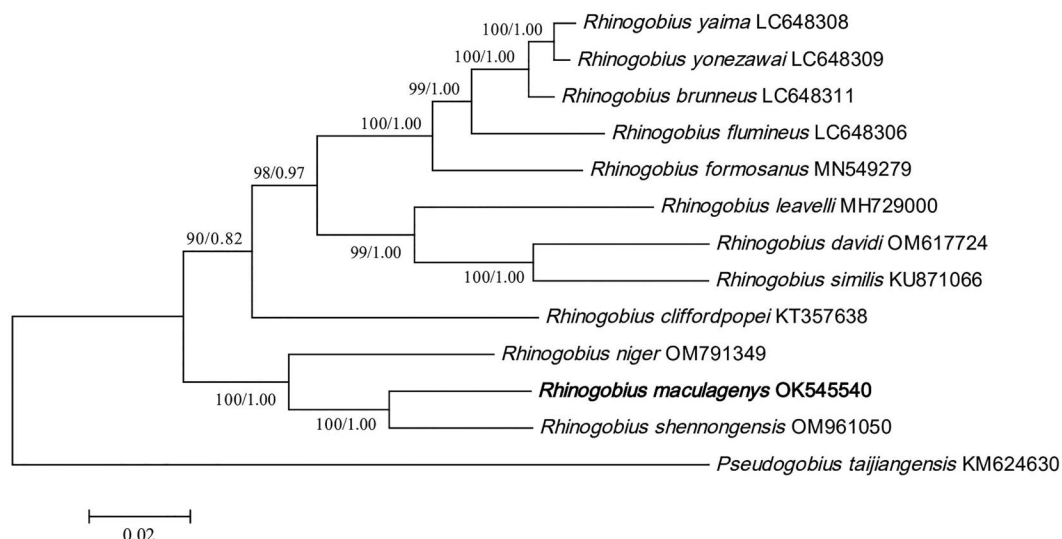
Figure 3. A Phylogenetic tree of thirteen species was constructed based on complete mitogenome using maximum-likelihood method and Bayesian inference. *Pseudogobius taijiangensis* was used as outgroups. Numbers near nodes indicate maximum-likelihood bootstrap percentages (BP) and Bayesian posterior probabilities (BPP), given as BP/BPP. The estimates of branch lengths from ML methods. The following sequences were used: *Rhinogobius maculagenys* OK545540, *Rhinogobius yaima* LC648308 (Maeda et al. 2021), *Rhinogobius yonezawai* LC648309 (Maeda et al. 2021), *Rhinogobius brunneus* LC648311 (Maeda et al. 2021), *Rhinogobius flumineus* LC648306 (Maeda et al. 2021), *Rhinogobius formosanus* MN549279, *Rhinogobius davidi* OM617724 (Song et al.2023), *Rhinogobius leavelli* MH729000 (Zhang and Shen 2019), *Rhinogobius cliffordpopei* KT357638 (Wang et al. 2019), *Rhinogobius niger* OM791349, *Rhinogobius shennongensis* OM961050, *Rhinogobius similis* KU871066, *Pseudogobius taijiangensis* KM624630.

are encoded by the H-strand, except for the *ND6* gene and eight tRNA genes. The overall base composition is 27.5% A, 25.5% T, 30.1% C and 16.9% G. All protein-coding genes use the initiation codon ATG except for the *COX1* gene, which begins with GTG. Additionally, most protein-coding genes use TAA or TAG as stop codons, while *COX3* uses an incomplete stop codon TA. *ND4*, *COX2* and *CYTB* use an incomplete stop codon T. The 13 protein-coding genes encode 3787 amino acids, with leucine being the most frequently used amino acid (17.6%), while cysteine is the least used (0.7%). The two rRNA genes (12S and 16S) are separated by *tRNA-Val*, located between *tRNA-Phe* and *tRNA-Leu*. The 22 tRNA genes are interspersed between rRNAs and protein-coding genes, with sizes ranging from 66 bp (*tRNA-Cys*) to 76 bp (*tRNA-Lys*). The 32 bp OL is located between the *tRNA-Asn* and *tRNA-Cys* genes. The putative control region is 481 bases long and located between *tRNA-Pro* and *tRNA-Phe*.

3.2. Phylogenetic analysis

The phylogenies reconstructed by Bayesian and ML methods were topologically identical (Figure 3). Our analyses revealed that both trees showed that *R. maculagenys* shared the closest mitochondrial genome relationship with *R. shennongensis*, and together they formed a clade with *Rhinogobius niger*. The genetic distance between *R. maculagenys* and the other 11 *Rhinogobius* species ranged from 0.0567 to 0.1319.

4. Discussion and conclusion

The circular mitogenome of *R. maculagenys* is 16,500 bp in length. The gene order and composition are similar to those of other Gobionellinae species (Wang et al. 2019; Tan et al. 2020; Yang et al. 2020; Song et al. 2022). When compared

with sequences deposited in the GenBank, the control region in the *Rhinogobius* genus was found to be 843 bp in *R.duospilus* (MH127918) and 529 bp in *R.szechuanensis* (OM617727). The composition and arrangement of the control region varied considerably within this genus. Phylogenetic analyses of *R. maculagenys* reveal a close relationship with *R.shennongensis*, which is consistent with the findings of Song (Song et al.2023). However, it is important to note that changes in taxonomic sampling can potentially impact the relationships of species in the phylogenetic tree. Therefore, further studies involving extensive taxon sampling are necessary to accurately verify the phylogenetic relationships among the *Rhinogobius* genus. Our findings significantly contribute to understanding the genetic diversity and evolution of *Rhinogobius* and provide valuable insights for future studies in this field.

Authors' contribution

JH and YL made substantial contributions to the design of this work, drafting of the paper and final approval of the version to be published. JL, HW and ZX analyzed the data; CC, XC and ZW conducted DNA experiment; All authors agreed to be accountable for all aspects of this work.

Ethical approval

Sample collection protocols were approved by the Ethics Committee for Animal Experiments of Mianyang Academy of Agricultural Sciences (approval number: MAAS 2021001). These policies were enacted according to the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

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Data availability statement

The data that support the findings of this study are openly available in Genebank at http://www.ncbi.nlm.nih.gov/, under the accession number OK545540. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA944416, SRR23852506 and SAMN33748151, respectively.

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