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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of *Mesogobio lachneri* (Cypriniformes: Gobionidae) from Northeast Asia

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ABSTRACT

Although *Mesogobio lachneri* is the type species of the genus *Mesogobio*, its systematic position and status have remained unresolved to date. In this study, for the first time, we report the complete mitochondrial genome of *M. lachneri* using Sanger sequencing. It is a circular genome with a length of 16,602 bp, comprising 22 tRNAs, 13 protein-coding genes (PCGs), two rRNAs, and one non-coding control region. Our phylogenetic analysis reveals that *M. lachneri* is the close relative of the genus *Gobio*, indicating that *Mesogobio* may be a valid genus.

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1. Introduction

The freshwater fish genus *Mesogobio* is placed into the order Cypriniformes, the family Gobionidae, and the sub-family Gobioninae (Tan and Armbruster 2018). The genus comprises two species *Mesogobio lachneri* Bănărescu & Nalbant 1973 and *Mesogobio tumenensis* Chang 1980 which are known to be distributed in Northeast Asia (Xie 2007). The results of two prior studies on the molecular phylogeny of the Gobioninae have indicated that *M. tumensis* should be moved into the *Gobio*, as *G. tumensis* (Yang et al. 2006; Tang et al. 2011). However, the type species (*M. lachneri*) of the genus *Mesogobio* has lacked molecular data until now. Hence, the systematic position and status of *M. lachneri* have remained unresolved. *M. lachneri* is a small benthic and rheophilic species with the body length less than 14 cm (Figure 1). It prefers pebbly or sandy substrate environments, and is endemic to the Yalu River (the boundary river between North Korea and China) drainage (Yue 1998; Xie 2007). *M. lachneri* differs from other gobionid species, in having the following six morphological characters: a naked breast, absence of sub-lobes on the lower lip, absence of mental barbels, two rows of teeth, lips with developed papillae, and scale rows above lateral lines 5.5 (Yue 1998).

In the present study, the complete mitochondrial genome of *M. lachneri* is obtained for the first time, which may be used to clarify the phylogenetic position of the genus *Mesogobio* within the family Gobionidae in the future. The mitogenome is also useful as a reference sequence for molecular species identification, as well as for further research on the molecular evolution of the family Gobionidae.

2. Materials

M. lachneri was collected from the Yalu River in the Linjiang City, Jilin Province, China (41.8087°N, 126.9211°E), and deposited at the Zoological Museum of Fudan University (Cuizhang Fu, czfu@fudan.edu.cn) under the voucher number FDZM-MeLLJ 20170922-01.

3. Methods

We extracted genomic DNA from muscle tissues following a high-salt protocol (Miller et al. 1988). The mitochondrial genome fragments were amplified using 13 primer pairs following a protocol previously described in our lab (Chai and Fu 2020; PCR gel image in Figure S1 of Appendix I). For the present study, we designed the forward primer of Primer9 (MeLND4F: 5′-TAGCCAGCCAAAAYCACAT-3′), as well as the reverse primers of Primer8 (MeLND4R: 5′-TAAAATCTGRTG

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Figure 1. Female *Mesogobio lachneri*. The specimen from the Linjiang City, Jilin Province, China. The photograph by Cuizhang Fu on 22 September 2017.

GGCCGG-3′) and Primer10 (MeLND5R: 5′-AAACGRCTTGCCT GRGGAAG-3′). The remaining primers were adopted from Chai and Fu (2020) (Table S1 in Appendix I). The PCR products were sequenced by the Sanger method using the ABI 3730 platform (Applied Biosystems, Foster City, USA; raw sequencing results in Appendix II).

The contiguous and overlapping segments of *M. lachneri* were assembled with default parameters, using Sequencher 5.4 (Gene Codes, Ann Arbor, MI) on the basis of the reference genome of *Gobio macrocephalus* (MT632636; Yi and Fu 2020). MitoAnnotator was selected as the method for genome annotation (http://mitofish.aori.u-tokyo.ac.jp/annotation/input.html; Iwasaki et al. 2013). The CGView Server was used to generate the genome map (Grant and Stothard 2008).

Tang et al. (2011) have suggested that the genus *Gobio* is a close relative of the *Mesogobio*. Due to the fact that no other mitochondrial genome of fish belonging to the genus *Mesogobio* is available in GenBank, the mitochondrial genomes of five *Gobio* species (*G. gobio*, AB239596, Saitoh et al. 2006; *G. acutipinnatus*, MT632635, Yi and Fu 2020; *G. macrocephalus*, MT632636, Yi and Fu 2020; *G. cynocephalus*, KU314700, Li et al. 2018; and *G. coriparoides*, MN864250, Ge et al. 2020) and one *Romanogobio* species (*R. ciscaucasicus*, AP011259, Iwasaki et al. 2013) were downloaded from GenBank. The 13 protein-coding genes (PCGs) were used in the phylogenetic analyses, and *R. ciscaucasicus* was chosen as the outgroup taxon (Tang et al. 2011). For phylogenetic analyses, the data were partitioned by gene. The maximum-likelihood analysis was conducted using 1000 ultra-fast bootstrap replicates, and the best nucleotide substitution models were selected using the '-MF-mtree' module- implemented in IQ-TREE v2.1.3 (Minh et al. 2020). Bayesian's analysis was performed using two replicates of 50 million iterations, and the best models were automatically searched using the bModelTest module, implemented in BEAST v2.6.7 (Bouckaert and Drummond 2017).

4. Results

The mitochondrial genome of *M. lachneri* (OL678457) assembled from PCR products, presented as a circular structure with length of 16,602 bp, including a total of 37 genes (22 tRNA genes, 13 PCGs, and two rRNA genes) and one control region (Figure 2). The overall base composition was composed of 30.1% A, 26.4% T, 17.1% G, and 26.5% C. For the 13 PCGs, two types of starting codons (ATG and GTG), and four kinds of stop codons (TAA, TAG, TA-, and T-) were observed (Table 1). Same patterns for codon use, are common in published mitochondrial genomes of the subfamily Gobioninae (Tong and Fu 2019; Zhang and Fu 2019; Fu and Fu 2020; Yi and Fu 2020). Reconstructed phylogenetic relationships between mitogenomes show that *M. lachneri* is the close relative of the genus *Gobio* with strong bootstrap support (100%, Figure 3).

Table 1. Characteristics of the mitochondrial genome of *M. lachneri*.

Element	From	To	Length (bp)	Start codon	Stop codon
tRNA^{Phe}	1	69	69		
12S rRNA	70	1028	959		
tRNA^{Val}	1029	1100	72		
16S rRNA	1101	2791	1691		
tRNA^{Leu}	2792	2867	76		
ND1	2869	3843	975	ATG	TAG
tRNA^{Ile}	3848	3919	72		
tRNA^{Gln}	3918	3988	71		
tRNA^{Met}	3990	4058	69		
ND2	4059	5103	1045	ATG	T-
tRNA^{Trp}	5104	5173	70		
tRNA^{Ala}	5177	5245	69		
tRNA^{Asn}	5247	5319	73		
tRNA^{Cys}	5350	5417	68		
tRNA^{Tyr}	5419	5489	71		
COI	5491	7041	1551	GTG	TAA
tRNA^{Ser}	7042	7112	71		
tRNA^{Asp}	7116	7187	72		
COII	7201	7891	691	ATG	T-
tRNA^{Lys}	7892	7967	76		
ATPase8	7969	8133	165	ATG	TAA
ATPase6	8127	8810	684	ATG	TAA
COIII	8810	9593	784	ATG	T-
tRNA^{Gly}	9594	9664	71		
ND3	9665	10,014	350	ATG	TA-
tRNA^{Arg}	10,015	10,084	70		
ND4L	10,085	10,381	297	ATG	TAA
ND4	10,375	11,756	1382	ATG	TA-
tRNA^{His}	11,757	11,825	69		
tRNA^{Ser}	11,826	11,894	69		
tRNA^{Leu}	11,896	11,968	73		
ND5	11,969	13,804	1836	ATG	TAG
ND6	13,801	14,322	522	ATG	TAG
tRNA^{Glu}	14,323	14,391	69		
Cytb	14,396	15,536	1141	ATG	T-
tRNA^{Thr}	15,537	15,608	72		
tRNA^{Pro}	15,608	15,677	70		
D-loop	15,678	16,602	925		

5. Discussion and conclusions

The systematic position and status of the genus *Mesogobio* have remained unresolved to date, due to a lack of molecular data for the type species *M. lachneri* (Tang et al. 2011). In the present study, we determine the complete mitochondrial genome of *M. lachneri* for the first time. Our phylogenetic analysis reveals that *M. lachneri* has a close relationship with the genus *Gobio*, indicating that the genus *Mesogobio* may be a valid genus. The provided mitogenome of *M. lachneri* is expected to be useful for molecular species delimitation and for assessment of the molecular evolution of the family Gobionidae in the future.

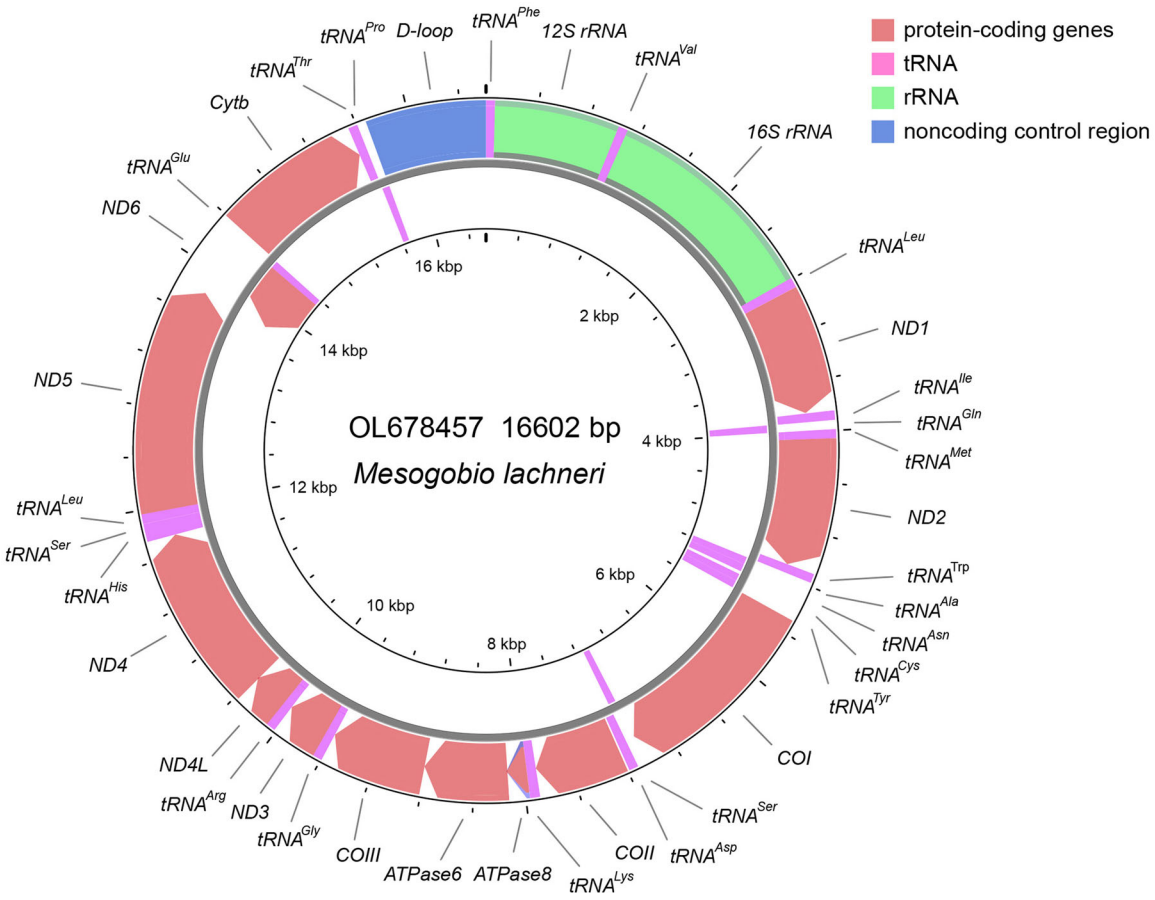

Figure 2. Mitochondrial genome map of *M. lachneri*.

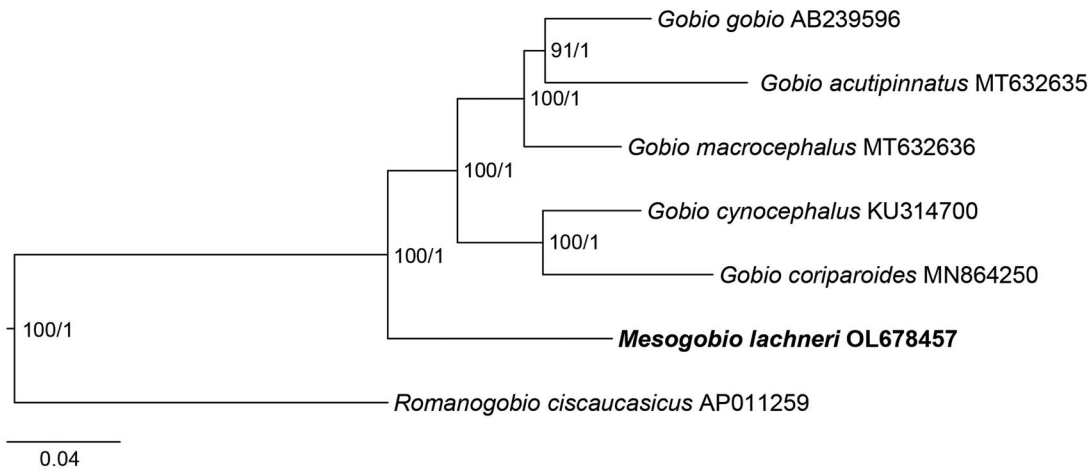

Figure 3. Phylogenetic reconstruction of *M. lachneri* and its close relatives based on 13 mitochondrial protein-coding genes. Numbers near the nodes indicate bootstrap support and posterior probability.

Ethics statement

The sample of this study did not involve endangered or protected animals, and the experiment followed Laboratory animal—Guideline for ethical review of animal welfare of the National Standard of the People's Republic of China (GB/T 35892-2018).

Author contributions

FCZ conceived this study; TW and NXM conducted the experiments, and analyzed the data; TW wrote the drafting of the paper; FCZ and NXM revised it critically, and that all authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The new genome in this study is openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/nuccore/OL678457. Raw Sanger sequencing data are provided in supplementary material.

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