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MITOGENOME ANNOUNCEMENT

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Characterization of the complete mitochondrial genome and phylogenetic analysis of *Opsarius pulchellus* (Cypriniformes, Danionidae, Chedrinae)

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ABSTRACT

This study aimed to sequence and annotate the complete mitochondrial DNA genome sequence of *Opsarius pulchellus*. Complete mitochondrial genome is a circular molecule of 16,552 bp in length, including 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and a displacement loop region. Proportion of nucleotides in mitochondrial genome is 26.8% T, 26.9% C, 27.6% A, and 18.8% G, with an AT bias of 54.4%. Maximum-likelihood phylogenetic tree was reconstructed using the connected protein sequences of 13 PCGs of *O. pulchellus* and other 18 fishes. Result of phylogenetic analysis supports a close relationship between *O. pulchellus* and *Barilius bendelisis* (Hamilton, 1807). Fundamental genetic data of *O. pulchellus* would be beneficial for further studies on genetic diversity and phylogeny.

Opsarius pulchellus (Smith, 1931) belongs to the subfamily Chedrinae (family: Danionidae). It is widely distributed in Cambodia; the Yunnan Province of China (Lancang River system and Yuanjiang River system); Lao People's Democratic Republic; Thailand and Viet Nam. Habitat degradation caused by deforestation and agricultural pollution is the main potential threat to *O. pulchellus* (Vidthayanon 2012). The synonyms names of *O. pulchellus* such as *Barilius pulchellus*, *Barilius Pellegrini* and *Daniops macropterus* are not valid. At present, the research on *O. pulchellus* is limited to morphological characteristics (Kottelat 1998; Beamish et al. 2006; Tejavej 2012). This study is the first to report the complete mitochondrial genome of *O. pulchellus* using the next-generation sequencing method, which will be beneficial for future studies on population genetics and evolution of the subfamily Chedrinae.

Samples were collected from Xishuangbanna, Yunnan province, China (21°98′75.2″N, 100°81′43.28″E). *Opsarius pulchellus* was identified by consulting FishBase Database, Fish Database in Inland Waters of China, and Native Ornamental Fish of China Illustrated Book (Chu and Chen 1989; Zheng et al. 1991; Zheng et al. 2016). Samples were anesthetized with MS-222 before the experiment. Some specimens were fixed with 10% formaldehyde and deposited at Aquatic Science and Technology Institution Herbarium (voucher ASTIH-21b0616d06, https://www.jsahvc.edu.cn/, XJ CHEN, cq_cxj@126.com). Genomic DNA was extracted from muscle tissues by Tguide cell/tissue genomic DNA Extraction Kit (Tiangen, Beijing, China) and then sequenced on the Illumina HiSeq platform. MetaSPAdes (Nurk et al. 2017) was used to

assemble the sequence and MitoMaker (Bernt et al. 2013) for gene annotation. The animal experiments were approved by the Ethical Committee for Animal Experiments of Jiangsu Agri-animal Husbandry Vocational College and conducted following the Guidelines for Experimental Animals of the Ministry of Science and Technology (Beijing, China).

The complete mitochondrial genome of *O. pulchellus* is 16,552 bp in length, including 13 proteins, 22 tRNAs, two rRNAs, and one displacement loop region (D-loop). The D-loop is located between *tRNA^{Phe}* and *tRNA^{Pro}* with 932 bp in size. The proportion of nucleotides in mitochondrial genome is T (26.8%), C (26.9%), A (27.6%), G (18.8%), with an AT bias of 54.4%. Among the 37 genes, 27 genes are encoded on the H-strand while *tRNA^{Phe}*, *tRNA^{Gln}*, *tRNA^{Ala}*, *tRNA^{Asn}*, *tRNA^{Cys}*, *tRNA^{Tyr}*, *tRNA$^{Ser(UCN)}$*, *tRNA^{Glu}*, *tRNA^{Pro}*, and *ND6* are encoded on L-strand. The characteristics of gene overlap and segregation are also observed in the mitochondrial genome of *O. pulchellus*. The interval length of 15 genes is 117 bp, ranging from 1 to 35 bp. The overlap length of 11 genes is 42 bp, with overlapping base numbers between 1 and 19 bp (Table 1).

Among 13 protein-coding genes (PCGs), the longest gene is *ND5* (1812 bp) and the shortest gene is *ATP8* (165 bp). Two genes (*CO I* and *ND3*) take GTG and ATA as the starting codon, respectively. The other 11 genes take ATG as the starting codon. Seven genes (*ND1*, *CO I*, *Atp8*, *Atp6*, *COIII*, *ND4L*, and *ND6*) take TAA as complete termination codon while three genes (*ND2*, *ND3*, and *ND5*) end with TAG, respectively. Three genes (*CO II*, *ND4*, and *Cyt b*) end with an

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Table 1. Characteristics of the mitochondrial genome of *Opsarius pulchellus*.

Gene	Position		Nucleotide size (bp)	Amino acid	Space (+) overlap (−)	Codon		Strand
	from	to				Initial	Terminal	
tRNA^Phe	16,547	63	68		0			L
12SrRNA	64	1016	953		0			H
tRNA^Val	1018	1089	72		1			H
16SrRNA	1108	2768	1661		18			H
tRNA^Leu(UUR)	2768	2842	75		−1			H
ND1	2847	3818	972	323	4	ATG	TAA	H
tRNA^Ile	3819	3890	72		0			H
tRNA^Gln	3889	3959	71		−2			L
tRNA^Met	3962	4030	69		2			H
ND2	4031	5077	1047	348	0	ATG	TAG	H
tRNA^Trp	5076	5145	70		−2			H
tRNA^Ala	5147	5214	68		1			L
tRNA^Asn	5215	5287	73		0			L
tRNA^Cys	5323	5388	66		35			L
tRNA^Tyr	5388	5457	70		−1			L
CO I	5459	7009	1551	516	1	GTG	TAA	H
tRNA^Ser(UCN)	7010	7080	71		0			L
tRNA^Asp	7084	7153	70		3			H
CO II	7168	7858	691	230	14	ATG	T	H
tRNA^Lys	7859	7934	76		0			H
ATPase8	7936	8100	165	54	1	ATG	TAA	H
ATPase6	8082	8777	696	231	−19	ATG	TAA	H
CO III	8777	9562	786	261	−1	ATG	TAA	H
tRNA^Gly	9562	9632	71		−1			H
ND3	9633	9983	351	116	0	ATA	TAG	H
tRNA^Arg	9982	10,051	70		−2			H
ND4L	10,052	10,348	297	98	0	ATG	TAA	H
ND4	10,342	11,719	1378	459	−7	ATG	T	H
tRNA^His	11,724	11,793	70		4			H
tRNA^Ser(AGY)	11,794	11,862	69		0			H
tRNA^Leu(CUN)	11,864	11,936	73		1			H
ND5	11,938	13,749	1812	603	1	ATG	TAG	H
ND6	13,746	14,240	495	164	−4	ATG	TAA	L
tRNA^Glu	14,268	14,335	68		27			L
Cyt b	14,340	15,480	1141	380	4	ATG	T	H
tRNA^Thr	15,481	15,552	72		0			H
tRNA^Pro	15,551	15,620	70		−2			L
D-loop	15,621	16,552	932		0			−

incomplete termination codon (T). The length of tRNA is ranging from 66 to 76 bp. The length of 12S rRNA and 16S rRNA is 953 bp and 1661 bp, respectively.

The phylogenetic relationships of *O. pulchellus* (MZ853157.1) were reconstructed using the maximum-likelihood (ML) method with 1000 bootstrap replicates based on concatenated amino acids and nucleotides of 13 PCGs of 19 fishes, *Amblypharyngodon mola* and *Rasbora tornieri* were used as outgroups. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018), and the General Reversible Mitochondrial (mtREV) + Gamma distributed with invariant sites (G + I) were used as the best model (Adachi and Hasegawa 1996). The phylogenetic tree was analyzed based on amino acids and nucleotides sequences of 13 PCGs, both analyses were run with very similar topology.

The result of the phylogenetic analysis showed that *O. pulchellus* was close to *B. bendelisis* (Figure 1). Presently, the studies recording *O. pulchellus* were limited, and we believed that the fundamental genetic data could be beneficial for further studies on genetic diversity and phylogeny.

Authors' contributions

Xiao Jiang Chen made 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; Lin Song made substantial contributions to the design of the work, and drafting the work and analysis and final approval of the version to be published; and agreement to be accountable for all aspects of the work; Wen Zhao Liu and Peng Gao 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.

Disclosure statement

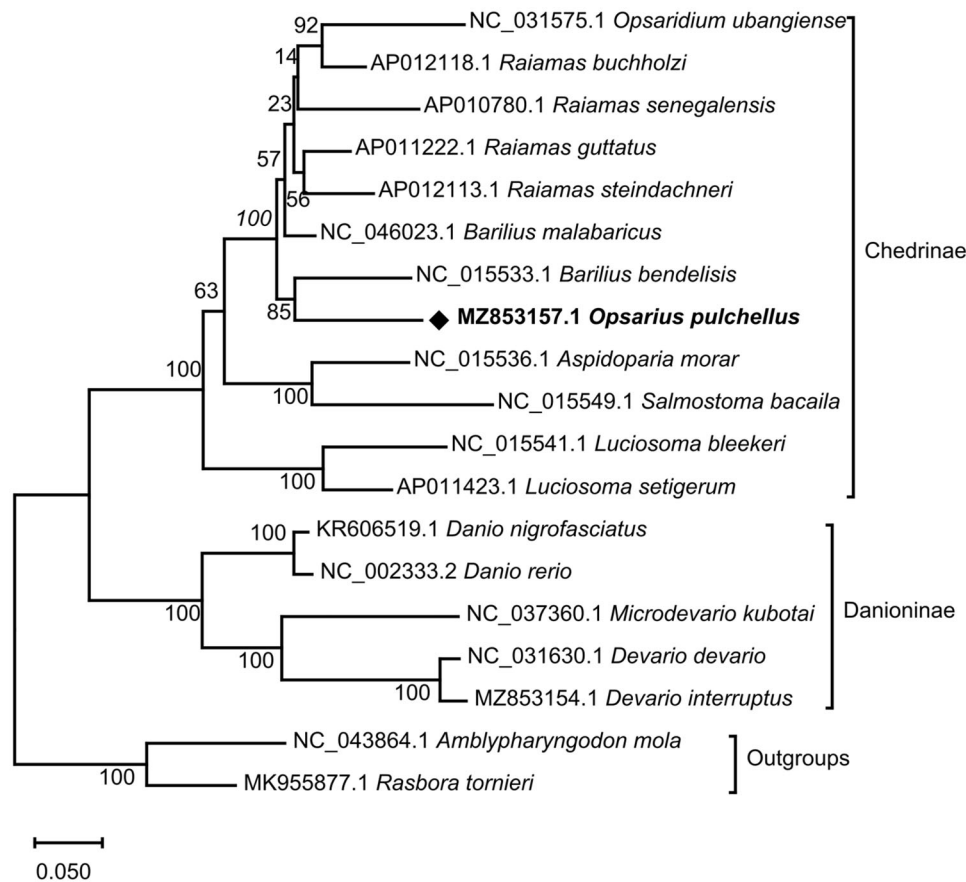No potential conflict of interest was reported by the author(s).

Figure 1. Phylogenetic tree reconstructed using the connected protein sequences of 13 PCGs of *O. pulchellus* together with other 18 fishes based on maximum-likelihood (ML), tree topology was evaluated by 1000 bootstrap replicates. *Amblypharyngodon mola* and *Rasbora tornieri* were used as outgroups. The species names are followed by their GenBank accession numbers.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/, accession number MZ853157.1

The associated BioProject, SRA, and Bio-Sample numbers are PRJNA769989, SRR16282297, and SAMN22187389, respectively.

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