MITOCHONDRIAL DNA PART B: RESOURCES
2023, VOL. 8, NO. 7, 715–718
https://doi.org/10.1080/23802359.2023.2227744

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MITOGENOME ANNOUNCEMENT

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Complete mitochondrial genome and phylogenetic analysis of *Sineleotris saccharae* (Perciformes, Odontobutiae)

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ABSTRACT

The freshwater sleeper, *Sineleotris saccharae* Herre, 1940 is a member of the Odontobutiae family, widely distributed in southern China. In the present study, we determined the complete mitochondrial genome of *S. saccharae* for the first time and analyzed its evolutionary relationship. The complete mitochondrial genome of *S. saccharae* was 16,487 bp long, and had 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), 2 ribosomal RNA (rRNAs) and a control region (CR). The mitogenome of *S. saccharae* shared the same gene organization and orientation as other teleosts. According to phylogenetic research, *S. saccharae* was sister to *S. chalmersi* with high support value, providing the monophyly of the genus *Sineleotris*. These results will be helpful for understanding the systematics of the odontobutids.

ARTICLE HISTORY
Received 9 February 2023
Accepted 15 June 2023

KEYWORDS
Sineleotris saccharae; mitochondrial genome; odontobutiae; phylogenetic analysis

Introduction

Sineleotris saccharae originally described by Herre (1940), was native to Southern China (Figure 1). Based on morphological traits, the species was subsequently assigned to the genera *Philypnus* (Chen and Zheng 1985), *Hypseleotris* (Wu 1991), and *Sineleotris* (Wu and Zhong 2008). Chen et al. (2002) initially classified the genus *Sineleotris* as belonging to the Odontobutidae family, while Li et al. (2018) verified this classification using molecular data. Information about genetic characteristics of *S. saccharae*, including mitochondrial genome, genetic diversity, is still not available. In the present study, we determined mitogenome of *S. saccharae* for the first time and established the phylogenetic relationship of the odontobutids.

Figure 1. Specimen of *Sineleotris saccharae* was collected from the Fengshun County, Guangdong Province, China. The photo was taken by Huiwen Xiao on 11 October 2020.

Materials

Adult *S. saccharae* individuals were collected from Fengshun, Guangdong Province, China (23.817546°N, 116.287902E). A specimen and its DNA were deposited at the ichthyoligocal museum of Freshwater Fisheries Research Institute of Jiangsu Province, China (Dr Liqiang Zhong, e-mail: lqzhongffri@hotmail.com) under the voucher number JSFFRI-20008.

Methods

Total DNA was extracted with Qiagen Blood and Cell Midi Kit. The mitogenome was amplified using 20 pairs of *Odontobutis*-specific primers (Ma et al. 2015) and 30 sets of fish-universal primers (Miya and Nishida 1999). The gaps were filled with self-designed primers (supplemental Table S1). Using the same PCR primers, the PCR products were sequenced *via* an Applied Biosystems ABI 3730XL capillary sequencer.

After blasting in the GenBank, raw sequencing data were assembled to final mitogenome with manually inspecting. Then MitoFish was used to annotate and visualize it (Iwasaki et al. 2013). To analyze the phylogenetic position of *S. saccharae*, 13 PCGs from the closest thirteen fishes were downloaded according to blasting results in GenBank (Table 1, 12

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2023.2227744.

species of gobiiformes and an outgroup *Eleutheronema rhadinum*). Each of 13 PCGs was aligned separately using Clustal W with default settings and then concatenated to a single multiple sequence alignment. The substitution model mtREV + G + I were selected as the best model for analysis. The maximum likelihood (ML) analysis (Felsenstein 1985) was inferred on MEGA 11 (Tamura et al. 2021) with 1000 bootstrap replicates.

Table 1. Species and GenBank accession number of mitogenomes used in this study.

NO.	Species	Accession ID	References
1	*Sineleotris saccharae*	OP326576	This study
2	*Sineleotris chalmersi*	MH644035	Wang et al. 2019
3	*Rhyacichthys aspro*	AP004454	Miya et al. 2003
4	*Eleotris oxycephala*	KP713717	Xia et al. 2015
5	*Bostrychus sinensis*	JQ665462	Unpublished
6	*Oxyeleotris lineolata*	KP663727	Zang et al. 2016
7	*Oxyeleotris marmorata*	KF711995	Yang et al. 2016
8	*Hemieleotris latifasciata*	MF927495	Alda et al. 2017
9	*Ophiocara porocephala*	MW387001	Amin et al. 2021
10	*Perccottus glenii*	KC292213	Xue et al. 2013
11	*Eleotris picta*	MF927491	Alda et al. 2017
12	*Eleotris fusca*	KU674798	Unpublished
13	*Eleotris acanthopoma*	AP004455	Miya et al. 2003
14	*Eleutheronema rhadinum*	MW845829	Zhong et al. 2021

Results

The entire mitogenome of *S. saccharae* was 16,487 bp long, and had 13 PCGs, 22 tRNAs, 2 rRNAs and a CR (Figure 2). The mitogenome of *S. saccharae* shared the same gene organization and orientation as other teleosts (Table 2). The overall base composition was T 25.3%, C 30.0%, A 28.9%, and G 15.8%. Only one of the 13 PCGs, the ND6, was found to be encoded on the light strand (L-strand), with the other 12 being identified on the heavy strand (H-strand). The 850-bp-long CR has the highest A + T concentration (65.0%) in the entire mitogenome.

In the ML phylogenetic tree (Figure 3), *S. saccharae* was firstly clustered with *S. chalmersi* with high support value, then together with *Perccottus glenii* and *Rhyacichthys aspro* forming the *Gobioidei suorder* (bootstrap value >80). While the remaining nine sleepers clustered together forming the *Eleotroidei suorder*.

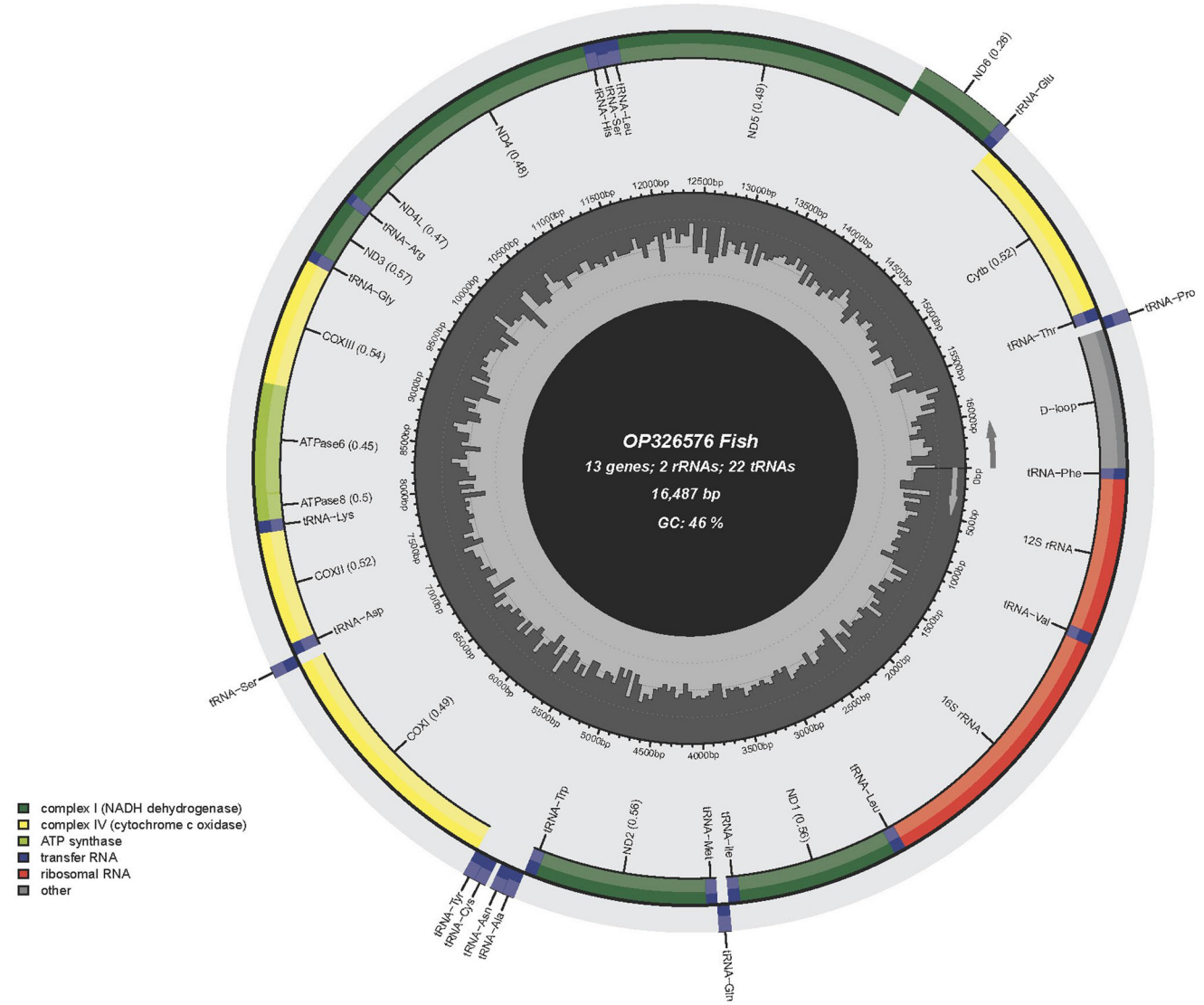

Figure 2. Gene map of the mitochondrial genome of *Sineleotris saccharae* (GenBank accession number: OP326576), with 13 protein coding genes, 22 tRNAs, 2 rRNAs, and a control region. Genes encoded on light strand and heavy-strand were shown inner and outside of the ring respectively.

Table 2. Organization of the mitogenome of *Sineleotris saccharae*.

Gene	Position From	To	Size (bp)	Codon Start	Stop[a]	Anti codon	Strand	Intergenic nucleotide (bp)[b]
tRNA- Phe	1	68	68			GAA	H	
12S rRNA	69	1024	956				H	0
tRNA-Val	1025	1096	72			TAC	H	0
16S rRNA	1097	2766	1680				H	0
tRNA-Leu	2767	2841	75			TAA	H	0
ND1	2842	3816	975	ATG	TAA		H	0
tRNA-Ile	3819	3888	70			GAT	H	2
tRNA-Gln	3888	3958	71			TTG	L	−1
tRNA-Met	3958	4026	69			CAT	H	−1
ND2	4027	5072	1046	ATG	TA-		H	0
tRNA-Trp	5073	5143	71			TCA	H	0
tRNA-Ala	5146	5214	69			TGC	L	2
tRNA-Asn	5216	5288	73			GTT	L	1
tRNA-Cys	5322	5389	68			GCA	L	33
tRNA-Tyr	5390	5460	71			GTA	L	0
CO I	5462	7015	1554	GTG	TAA		H	1
tRNA-Ser	7016	7082	67			TGA	L	0
tRNA-Asp	7085	7156	72			ATC	H	2
CO II	7160	7850	691	ATG	T-		H	4
tRNA-Lys	7851	7923	73			TTT	H	0
ATPase8	7925	8092	168	ATG	TAA		H	1
ATPase6	8083	8765	683	ATG	TA-		H	−10
CO III	8766	9550	785	ATG	TA-		H	0
tRNA-Gly	9551	9622	72			TCC	H	0
ND3	9623	9971	349	ATG	T-		H	0
tRNA-Arg	9972	10040	69			TCG	H	0
ND4L	10041	10337	297	ATG	TAA		H	0
ND4	10331	11711	1381	ATG	T-		H	−7
tRNA-His	11712	11780	69			GTG	H	0
tRNA-Ser	11781	11848	68			GCT	H	0
tRNA-Leu	11853	11925	73			TAG	H	4
ND5	11926	13764	1839	ATG	TAA		H	0
ND6	13761	14282	522	ATG	TAG		L	−4
tRNA-Glu	14283	14350	68			TTC	L	0
Cyt b	14356	15496	1141	ATG	T-		H	5
tRNA-Thr	15497	15568	72			TGT	H	0
tRNA-Pro	15568	15637	70			TGG	L	−1
Control region	15638	16487	850				H	0

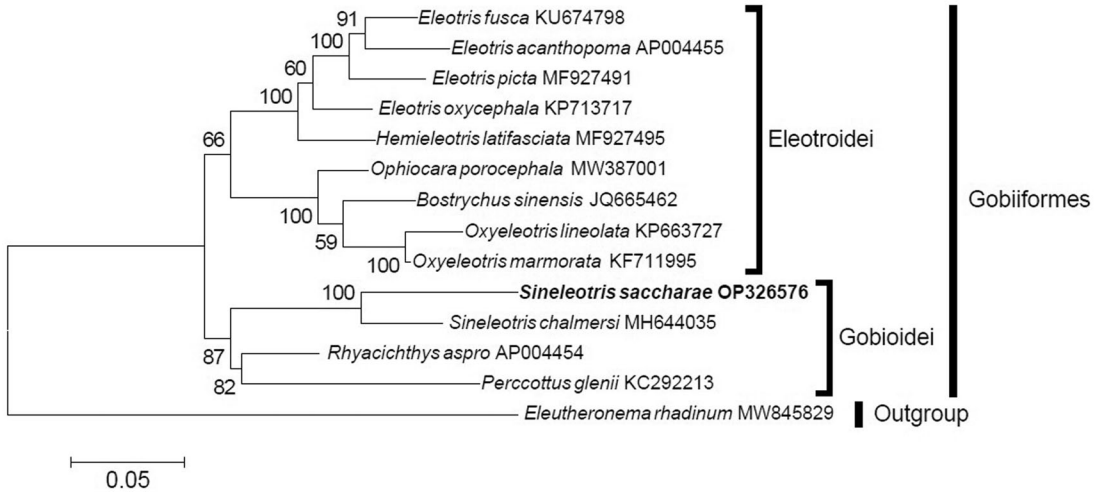

Figure 3. Maximum-likelihood (ML) phylogenetic tree was reconstructed based on the concatenated 13 protein-coding genes of *S. saccharae* and other 13 fishes. Accession numbers were indicated after the species names. Numbers at the nodes indicated bootstrap support values from 1000 replicates.

Discussion and conclusion

In this study, the entire mitogenome of *S. saccharae* was identified for the first time. It was similar to that of other teleosts in terms of gene organization, and composition (Miya et al. 2003). In the ML phylogenetic analysis, *S. saccharae* was sister to *S. chalmersi* with high support value, providing the monophyly of the genus *Sineleotris*. And all sleepers were placed into two well-supported suborder clusters, which was similar to those of previous studies (Zhong et al. 2018a,

2018b). These results will be essential to the species identification and systematics of the odontobutids in the future.

Acknowledgments

We are grateful to Shujie Liu, Binbin Zhan and Huiwen Xiao for help in field assistance and the species reference image.

Author contributions

ZLQ conceived this study; ZLY, LYS and CXH conducted the experiments, LDM and TSK analyzed the data; ZLY and WMH wrote the drafting of the paper; ZLQ revised it critically, and that all authors agree to be accountable for all aspects of the work.

Ethical approval

This study was approved by the animal care and Ethical Committee of Freshwater Fisheries Research Institute of Jiangsu Province.

Disclosure statement

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

Funding

The present work was supported by the Special Funds for Quality Management and Standardization of Jiangsu Province [2022-140]; and Freshwater Fishery Ecology and Resource Monitoring Program of Jiangsu Province [2022-SJ-061-02].

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Data availability statement

The mitochondrial genome sequence is available on GenBank of NCBI at www.ncbi.nlm.nih.gov with the accession number of OP326576.

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