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MITOGENOME REPORT

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Complete mitochondrial genome sequence and phylogenetic analysis of the hybrid flat fish *Platichthys stellatus* (♀) × *Platichthys bicoloratus* (♂)

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ABSTRACT

We report the complete mitochondrial genome of the hybrid flounder *Platichthys stellatus* (♀) × *Platichthys bicoloratus* (♂). The mitochondrial genome contained **13** protein-coding genes, 2 ribosomal RNA (rRNA) genes, **22** transfer RNA (tRNA) genes, and 1 control region (D-loop), for a total length of 16,874 bp. The nucleotide composition of the heavy strand was 29.15% C, 26.99% A, 26.14% T, and 17.71% G. A maximum-likelihood phylogenetic analysis showed that the hybrid flat fish was a member of the same clade as *P. stellatus* (maternal inheritance). Our findings add to the extant data on the subfamily Pleuronecidae and provide insight into their molecular phylogeny and taxonomy.

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Pleuronecidea; *Platichthys stellatus*; *Platichthys bicoloratus*; flounder; mithogenome; hybridization

Introduction

Flat fishes were formerly categorized in the Pleuronectidae (Jordan and Goss 1889), but Norman (1934) classified them into five families. In nature, hybridization between species occurs frequently as a result of changes in habitat (Purdom 1992). Starry flounder (*Platichthys stellatus*) is a benthic fish belonging to the Pleuronectidae in the order Pleuronectiformes and is mainly distributed in the East Sea of Korea. It is a large species widely distributed in low-temperature areas from central Japan, the Russian Maritime Province, the Sea of Okhotsk, the Bering Sea, and the Gulf of California and is characterized by a high body height and thick body width (Lim et al. 2007). Stone flounder (*Platichthys bicoloratus*) is distributed in China and Japan, as well as the coast of Korea, and has been the subject of numerous studies on ecology and resource management worldwide because of its industrial value (Mori 1986; Kimoto et al. 1991). These two species spawn at similar times of year and hybrids thereof are frequently found in nature (Fujio 1977; Kosaka 1980). Flounder hybrids typically have characteristics intermediate to those of the parent species (Kim et al. 1996; Park et al. 2003; Garrett 2005), and the eye position may vary depending on environmental factors (Bergstrom 2007; Yamashita et al. 2014). In this study, we evaluated the complete mitochondrial genome of *Platichthys stellatus* (♀) × *Platichthys bicoloratus* (♂), which was discovered in Korea.

Materials and methods

Sample collection

A specimen of *P. stellatus* (♀) × *P. bicoloratus* (♂) (Figure 1) was caught off the coast of Pohang-si Gyeongsangbuk-do, Republic of Korea, on January 15, 2023 (35°58′35.26″ N 129°34′40.53″E), and the specimen was preserved in 99% ethanol and deposited at the specimen storage facility of Soonchunhyang University (Prof. I.-C. Bang, incbang@gmail.com) under voucher number SCU-26326.

Figure 1. Photograph of *P. stellatus* (♀) × *P. bicoloratus* (♂) taken by in-Gug Baek. It has dorsal, anal, and caudal fins yellow-brown or orange in color with seven, five, and four dark bars, respectively, as in *P. stellatus* (Pallas 1787). It has longitudinal rows of bony plates on the ocular side, as does *P. bicoloratus* (Basilewsky 1855).

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Table 1. Sequence characteristics of *P. stellatus* (♀) × *P. bicoloratus* (♂) mitochondrial genome.

Genes	Strand	Start/stop	Size (bp)	Intergenic spacer* (bp)	Codon start/stop	GC_ercent
tRNA-*phe*	H	1/68	68	0		47%
12S rRNA	H	69/1017	949	0		48%
tRNA-*Val*	H	1018/1090	73	0		52%
16S rRNA	H	1091/2805	1715	0		46%
tRNA-*Leu*	H	2806/2879	74	0		53%
ND1	H	2880/3854	975	5	ATA/TAG	49%
tRNA-*Ile*	H	3860/3930	71	−1		49%
tRNA-*Gln*	L	3930/4000	71	−1		45%
tRNA-*Met*	H	4000/4068	69	0		46%
ND2	H	4069/5113	1045	0	ATG/T-	49%
tRNA-*Trp*	H	5114/5185	72	0		51%
tRNA-*Ala*	L	5187/5255	69	1		42%
tRNA-*Asn*	L	5257/5329	73	1		44%
tRNA-*Cys*	H	5368/5432	65	38		48%
tRNA-*Tyr*	L	5433/5500	68	1		50%
*CO*I	H	5502/7061	1560	0	GTG/TAA	46%
tRNA-*Ser*	L	7062/7132	71	14		48%
tRNA-*Asp*	H	7147/7217	71	6		42%
*CO*II	H	7224/7914	691	0	ATG/T-	46%
tRNA-*Lys*	H	7915/7987	73	1		44%
ATP8	H	7989/8156	168	−10	ATG/TAA	41%
ATP6	H	8147/8830	684	−1	ATG/TAA	47%
*CO*III	H	8830/9615	786	0	ATG/TAA	48%
tRNA-*Gly*	H	9616/9686	71	0		37%
ND3	H	9687/10035	349	1	ATG/T-	49%
tRNA-*Arg*	H	10037/10105	69	−1		38%
ND4L	H	10105/10401	297	−7	ATG/TAA	53%
ND4	H	10395/11775	1381	1	ATG/T-	48%
tRNA-*His*	H	11776/11845	70	0		31%
tRNA-*Ser*	H	11846/11912	67	4		60%
tRNA-*Leu*	H	11917/11989	73	0		45%
ND5	H	11990/13828	1839	−4	ATG/TAA	46%
ND6	L	13825/14346	522	0	ATG/TAG	48%
tRNA-*Glu*	L	14347/14415	69	4		41%
Cytb	H	14420/15560	1141	0	ATG/T-	47%
tRNA-*Thr*	H	15561/15633	73	0		55%
tRNA-*Pro*	L	15634/15704	71	0		39%
D-loop	H	15705/16874	1170	0		42%

DNA extraction, sequencing, assembly, and annotation

Genomic DNA (gDNA) was extracted from the dorsal fin using the HiGene™ Genomic DNA Prep Kit (BioFact, Daejeon, Republic of Korea), and a genomic library for next-generation sequencing (NGS) extracted gDNA was stored at the specimen storage facility of Soonchunhyang University. The complete mitochondrial genome was analyzed according to the MGISEQ-2000 protocol (MGI Tech Co. Ltd., Shenzhen, China) with 150 bp paired-end reads, and a contig sequence was assembled using the default option in the *de nove* assembler of CLC Genomics Workbench 20.04(CLC, Aarhus, Denmark). The circular form of the mitochondrial genome was confirmed by mapping the clean data onto a contig sequence using Geneious R11 (Kearse et al. 2012) (Figure S1). Annotation of the mitochondrial genomes was performed using MitoFish webserver v. 3.90 (http://mitofish.aori.utokyo.ac.jp/; Zhu et al. 2023). The *P. stellatus* (♀) × *P. bicoloratus* (♂) sequence has been registered in GenBank at the National Center for Biotechnology Information (GenBank accession no. OQ656297).

Phylogenetic analysis

The sequences were downloaded from GenBank and used to construct a phylogenetic tree, with *Okameiei kenojei* (NC_007173.1) as the outgroup and the sequences of related Pleuronectidae species were used as references (Chae et al. 2023). Sequence alignment was performed using ClustalW in MEGA 11 (Tamura et al. 2021). The best-fit substitution model (GRT + I + G) was selected using jModelTest v. 2.1.4 (Posada 2008) based on the Akaike information criterion. A maximum-likelihood phylogenetic tree was generated with 1000 replications using MEGA v. 11 (Tamura et al. 2021).

Results

Mitogenomic characterization

The complete mitochondrial genome (GenBank accession no. OQ656297) contained 13 protein-coding genes, 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, and 1 control region (D-loop), for a total length of 16,874 bp. The gene distribution for *P. stellatus* (♀) × *K. bicoloratus* (♂) was identical to those of typical vertebrates (Miya et al. 2001; Wang et al. 2008). The nucleotide composition of the light strand in descending order was 29.15% C, 26.99% A, 26.14% T, and 17.71% G. The G + C content (46.86%) was lower than that of A + T (53.14%). Among the genes, *ND6* and seven tRNA (*Gln*, *Ala*, *Asn*, *Tyr*, *Ser*, *Glu*, and *Pro*) genes were positioned on the L-strand. Most of the coding sequences started with ATG, although COXI began with GTG. Six of the protein-coding genes (*COXI*, *ATP8*, *ATP6*, *COXIII*, *ND4L*, and *ND5*) stopped with TAA, and two with TAG (*ND1*, *ND6*). The *ND2*, *COXII*, *ND3*,

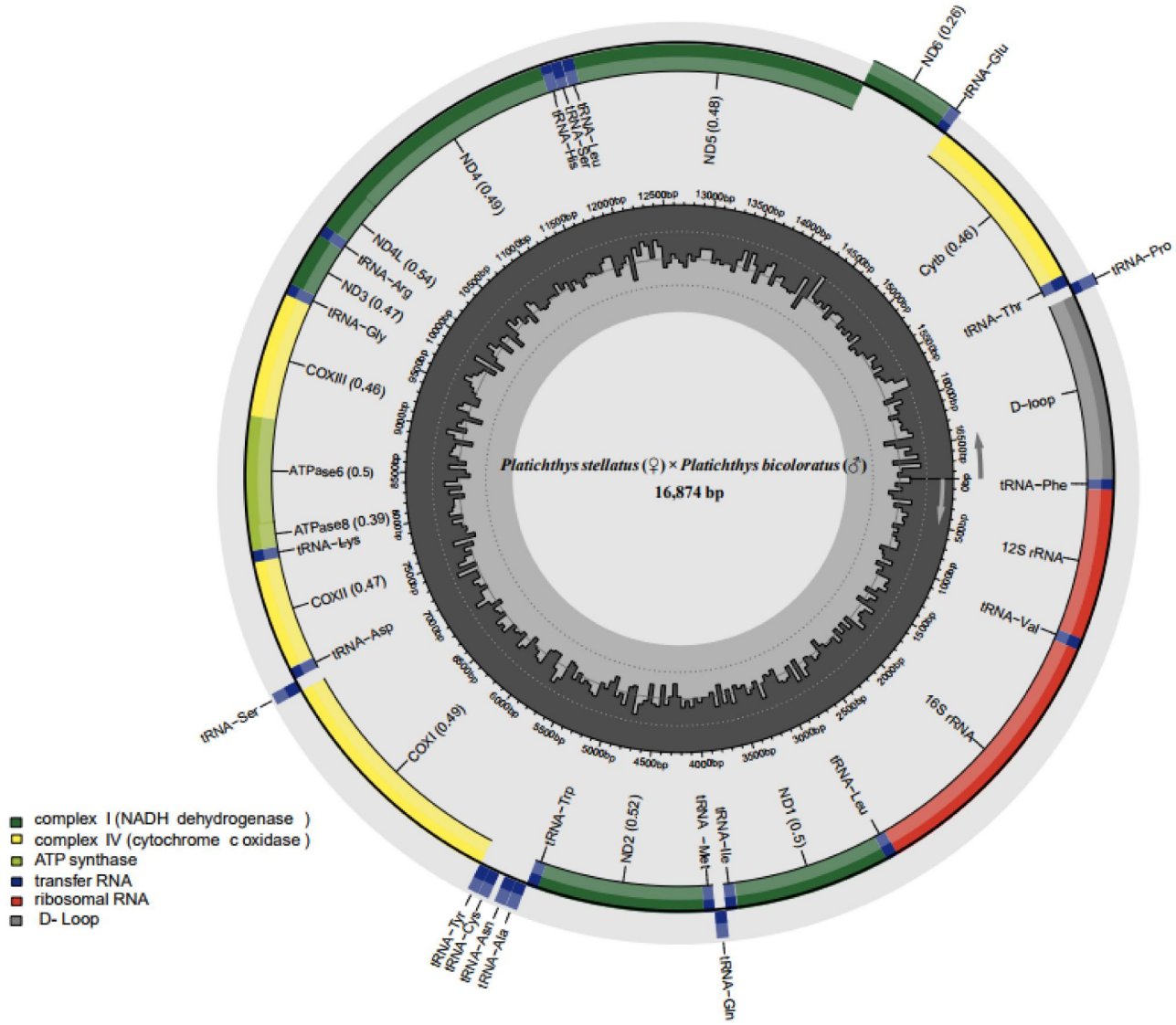

Figure 2. Gene map of the mitochondrial genome of *P. stellatus* (♀) × *P. bicoloratus* (♂). Genes encoded on the light and heavy strands are shown on the inner and outer sides of the ring, respectively.

ND4, CYTb were terminated with the truncated condons T-. The 22 transfer RNA genes ranged from 65 to 74 bp in length. The 12S ribosomal RNA gene was located between transfer RNA-Phe and transfer RNA-Val and was 959 bp in length. The 16S ribosomal RNA gene was positioned between transfer RNA-Val and transfer RNA-Leu and was 1715 bp in length. The control region (D-loop) was 1170 bp in length and was located between tRNA-Pro and tRNA-Phe (Table 1; Figure 2). A molecular phylogenetic tree was constructed using the complete mitochondrial genomes of 18 members of Pleuronectidae as well as *P. stellatus* (♀) × *P. bicoloratus* (♂).

Phylogenetic analysis

A Pleuronectidae phylogenetic tree prepared using the maximum-likelihood (ML) method indicated it to be a member of the same genetic clade as the maternal species, *P. stellatus*, based on the high bootstrap value (100), in accordance with maternal inheritance of mitochondrial DNA in eukaryotes and similar to other hybrid fishes (Sato and Sato 2012) (Figure 3).

Discussion and conclusion

This study is the first to identify the complete mitochondrial genome of *P. stellatus* (♀) × *P. bicoloratus* (♂) using NGS technology. The circle mitochondrial genome was 16,874 bp in length, containing 37 genes, including 13 PCGs, 22 tRNAs and two rRNAs. The results show that *Parophrys vetulus* (OL806591), excluding maternal and paternal lines, are closely related. The mitochondrial genome data from this study will be useful for studies on hybrid identification in the Pleuronectidae family, biodiversity monitoring and conservation, DNA barcording, and population genetics.

Ethical approval

The sample used in this study was a dead body of fish, and as per the animal experimental ethics of the Republic of Korea (standard operating guideline; IACUC – Institutional Animal Care and Use Committee, Book no. 11-1543061-000457-01, effective Dec. 2020) does not need any approval from an ethics committee. The data collection was conducted in accordance with the policies of the international Union for Conservation of Nature (IUCN) pertaining to research on species at risk of extinction (refer

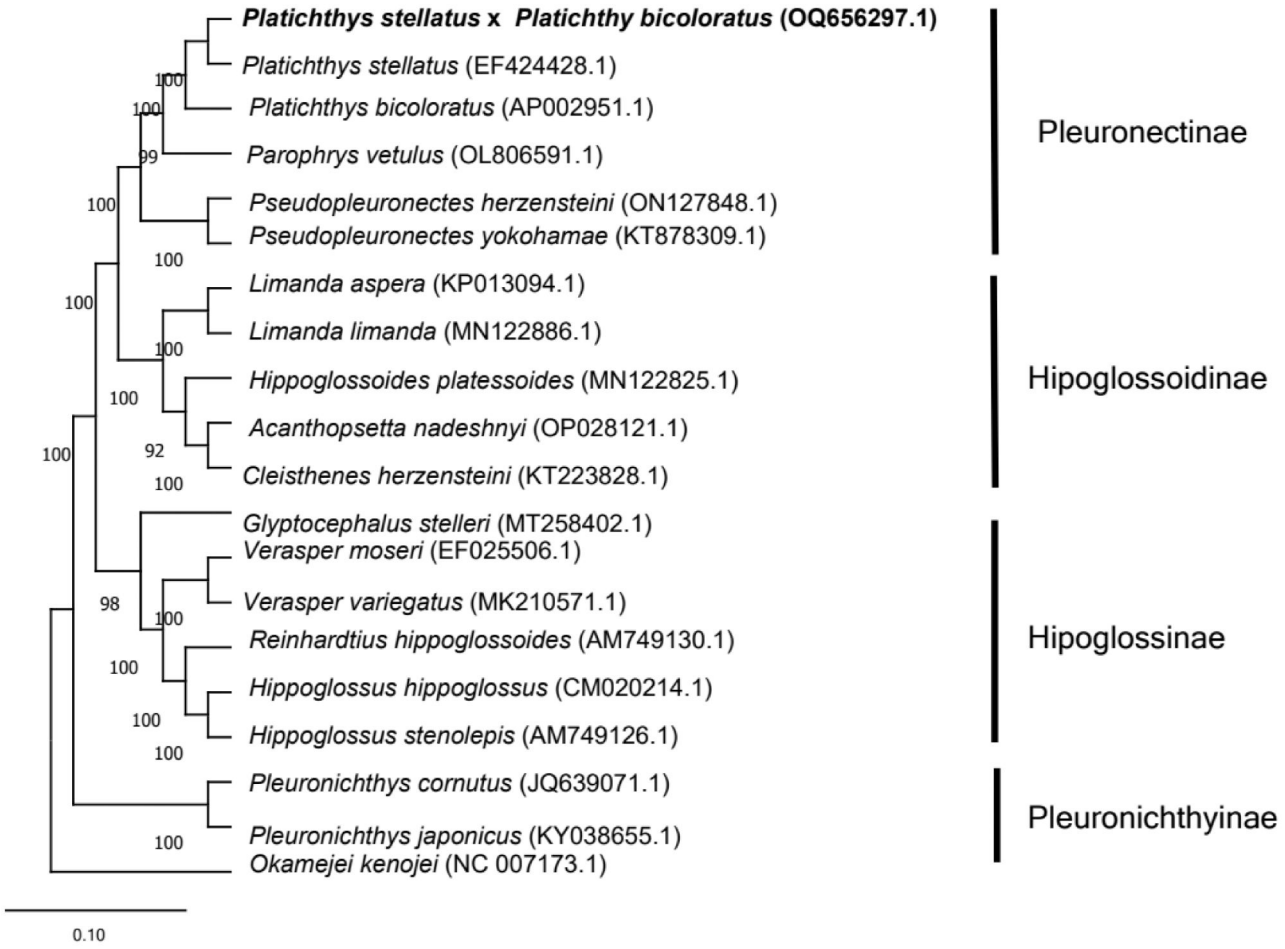

Figure 3. Maximum-likelihood phylogenetic tree of *P. stellatus* (♀) × *P. bicoloratus* (♂) with 12 members of the Pleuronectidae group. Numbers at nodes are bootstrap probabilities. GenBank accession numbers are provided after the scientific names, and follows: *A. nadeshnyi* (OP028121; Chae et al. 2023), *C. herzensteini* (KT223828; Bo et al. 2016), *G. stelleri* (MT258402; Kim and Jang 2022), *H. platessoides* (MN122825; Mjelle et al. 2008), *H. hippoglossus* (CM020214; Mjelle et al. 2008), *H. stenolepis* (AM749126; Mjelle et al. 2008), *L. aspera* (KP013094; Song et al. 2017), *L. limanda* (MN122886; Zheng et al. 2016), *O. kenojei* (outgroup; NC007173; Kim et al. 2005), *P. vetulus* (OL806591; Zheng et al. 2016), *P. bicoloratus* (AP002951; Miya et al. 2001), *P. stellatus* (EF424428; Shi et al. 2013), *P. stellatus*(♀) × *P. bicoloratus* (♂) (OQ656297; this study), *P. cornutus* (JQ639071; Shi et al. 2013), *P. japonicas* (KY038655; Song et al. 2017), *P. herzensteini* (ON127848; Chae et al. 2022), *P. yokohamae* (KT878309; Zheng et al. 2016), *R. hippoglossoides* (AM749130; Mjelle et al. 2008), *V. moseri* (EF025506; He et al. 2008), *V. variegatus* (MK210571; Lim et al. 2019).

to the Guidelines for appropriate uses of IUCN Red List data). Additionally, the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora were also adhered to. The species used in this study are not endangered fish in Republic of Korea and the sampling site is not located in any protected area.

Authors' contributions

In-Gug Baek, Yong Hwi Kim, and In-Chul Bang conceived the original idea. In-Gug Baek carried out the experiments. In-Gug Baek wrote the manuscript with support from Youg Hwi Kim, Ho-seop Han, Duc Tam Huynh and In-Chul Bang. Ho-seop Han and Duc Tam Huynh analyzed the experimental data. All authors have agreed to be accountable for all aspects of the work.

Disclosure statement

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (http://www.ncbi.nlm.nih.gov/) under the accession no. OQ656297. The associated BioProject, SRA and Bio-Sample numbers are PRJNA1023879, SRP465213 and SAMN 37674712 respectively.

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