

The complete mitogenome of the *Mugil cephalus* (Mugiliformes: Mugilidae) from the Yellow Sea, China

Haitao Li^a, Wei Chen^{a,b,c}, Yanmei Huang^a, Lei Gao^{a,d}, Chengzhong Yang^a and Yuanjun Zhao^a

^aChongqing Key Laboratory of Animal Biology, College of Life Sciences, Chongqing Normal University, Chongqing, P.R. China; ^bHubei Key Laboratory of Tumor Microenvironment and Immunotherapy, Medical College, China Three Gorges University, Yichang, Hubei, P.R. China; ^cThe Institute of Infection and Inflammation, Medical College, China Three Gorges University, Yichang, Hubei, P.R. China; ^dChongqing Institute of Metrology and Quality Inspection, Chongqing, P.R. China

ABSTRACT

Mugil cephalus Linnaeus, 1758 is a teleost fish widely distributed in coastal waters that plays an important role in commercial fisheries. In the present study, the complete mitogenome of *M. cephalus* from the Yellow Sea, China, was sequenced using Illumina Novaseq sequencing. The mitogenome of the *M. cephalus* was 16,744 bases in length (GenBank accession No. ON262567) including 2 rRNA genes, 22 tRNA genes, 13 protein-coding genes and a D-loop control region. The overall base composition of the genome was 28.2% A, 29.5% C, 26.9% T, and 15.4% G. The analysis of genetic similarity and phylogenetic relationship of *M. cephalus* from different geographic regions of the world indicated that the species from the Yellow Sea was most similar to NWP1 which is one of the three cryptic species of *M. cephalus* in Northwestern Pacific.

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1. Introduction

Flathead mullet *Mugil cephalus* Linnaeus, 1758 (Mugiliformes: Mugilidae) is an ecologically important species with a wide distribution, and it is also the main economic income for some regions of the world (Durand et al. 2012). Recent studies suggest that there might be three cryptic species (NWP1, NWP2, and NWP3) of *M. cephalus* in the Northwestern Pacific (Shen et al. 2011; Durand et al. 2012; Whitfield et al. 2012; Shen et al. 2016). However, currently, only four complete mitogenomes of *M. cephalus* from limited regions have been reported: NWP1 (GenBank: AP002930; Miya et al. 2001), NWP2 (GenBank: KM368340), NWP3 (GenBank: KP861225; Shen et al. 2011) and Eastern Australia (hereafter called from EA; GenBank: KP018403; Shen et al. 2016). Despite the ecological and economical importance of *M. cephalus* (Harrison and Howes 1991), the genetic resources and evolutionary relationships of the species from different geographic regions remain largely unknown. In the present study, we provided a new mitogenome of *M. cephalus* from the Yellow Sea, China (hereafter called from YS) and conducted a genetic comparison and phylogenetic analysis of the species from different geographic regions. The study will provide a valuable genetic resource for future evolution study of *M. cephalus*.

2. Materials and methods


2.1. Sample collection and DNA extraction

A tail muscle sample was taken from a dead mature individual of *M. cephalus* (Figure 1) collected from the coastal waters of Qingdao (120°22'44"E, 36°18'0"N) in 2019. The specimen was deposited at the collection center of Chongqing Key Laboratory of Animal Biology (<https://www.cqnu.edu.cn/>, Haitao Li, cqnuhaitao@126.com) under voucher number MG-qd-201908. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) following the manufacturer's protocol.

2.2. Genome sequencing, assembly, and annotation

The complete mitogenome of *M. cephalus* was sequenced using the Illumina Novaseq 6000 platform as paired-end 2 × 150 base reads. A total of 76.8G read bases of filtered data in 255,983,845 reads were assembled using Getorganelle (v1.7.7.0; Jin et al. 2020). The mitochondrial genome was annotated using MitoFish (<http://mitofish.aori.u-tokyo.ac.jp/>; Wataru et al. 2013) and then further checked using an existing annotated mitogenome of *M. cephalus* (Shen et al. 2016). The mitochondrial genome map of *M. cephalus* was constructed using MitoFish. The mitogenome read coverage

CONTACT Chengzhong Yang  drczyang@126.com  Chongqing Key Laboratory of Animal Biology, College of Life Sciences, Chongqing Normal University, Chongqing 401331, P.R. China; Yuanjun Zhao  zhaoyuanjuncqnu@126.com  Chongqing Key Laboratory of Animal Biology, College of Life Sciences, Chongqing Normal University, Chongqing 401331, P.R. China.

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Figure 1. Species image of *Mugil cephalus*. The dead mature individual was collected by Lei Gao from the Coast of Qingdao (120°22'44" E, 36°18'0" N) in 2019 which body's length was about 20 cm, fully possessed the characteristics of *M. cephalus*. This photo was taken in Qingdao in 2019, by Lei Gao with the author's approval for use.

depth map is shown in [Supplementary Figure S1](#). The raw data, final genome assembly sequence, and annotated information were deposited in the National Center for Biotechnology Information (NCBI), with the accession number SRR21493539.

2.3. Genetic similarity analysis

The genetic similarities were calculated from complete mitochondrial DNA sequences and entire amino acid sequences (which were estimated from the nucleotide sequences) among *M. cephalus* from five localities (EA, NWP1, NWP2, NWP3, and YS) using the EMBOSS Needle.suite with the algorithm of Needleman–Wunsch (<https://www.ebi.ac.uk/Tools/psa/>; Rice et al. 2000).

2.4. Phylogenetic analysis

The dataset for phylogenetic tree reconstruction was composed of 13 of the concatenated protein-coding genes of *M. cephalus* and 23 other related species. Multiple alignments of the sequences of the dataset were conducted by Clustal X (Thompson et al. 1997) under the setting of the default parameters. MrBayes 3.1.2 (Ronquist et al. 2012) was employed to infer the phylogenetic tree, and the best fitting model (GTR+I+G) was selected by Modeltest 3.7 under the Akaike information criterion. Four independent Markov chain Monte Carlo ran simultaneously for 10 million generations, sampling one tree per 200 generations, and the first 25% of the samples were discarded as the burn-in. Chain convergence and parameter mixing were checked by Tracer v1.3

(Rambaut and Drummond 2005) based on the average standard deviation of split frequencies (0.004733).

3. Results

3.1. Characteristics of the mitogenomes

The complete mitogenome of *M. cephalus* was 16,744 bp (GenBank accession No. ON262567) with a base composition of 28.2% A, 29.5% C, 26.9% T, and 15.4% G. The genome possessed 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and one control region ([Figure 2](#)). Among them, ND6 and eight tRNA genes (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu, and tRNA-Pro) were encoded on light strand, and the remaining genes were encoded on heavy strand ([Figure 2](#)). The gene numbers and the order of the mitogenome were consistent with other vertebrates (Yang et al. 2013; Sun and Xu 2018; Zhang et al. 2019). All protein-coding genes used ATG as a start codon except for COI which started with GTG. There were three stop codon types for genes: TAA type for ND1, COI, ATPase8, ATPase6, ND4L, and ND5; TAG type for ND6 and, T – type for ND2, COII, COIII, ND3, ND4, and CYTB.

3.2. Similarity among five mitogenomes

The similarities of the complete mitochondrial nucleotide sequences and amino acid sequences of *M. cephalus* from five localities were 94.0%–99.4% and 98.1%–99.9%, respectively. The highest similarities (99.4% for nucleotide and 99.9% for amino acid) of *M. cephalus* were found between YS and NWP1 ([Table 1](#)).

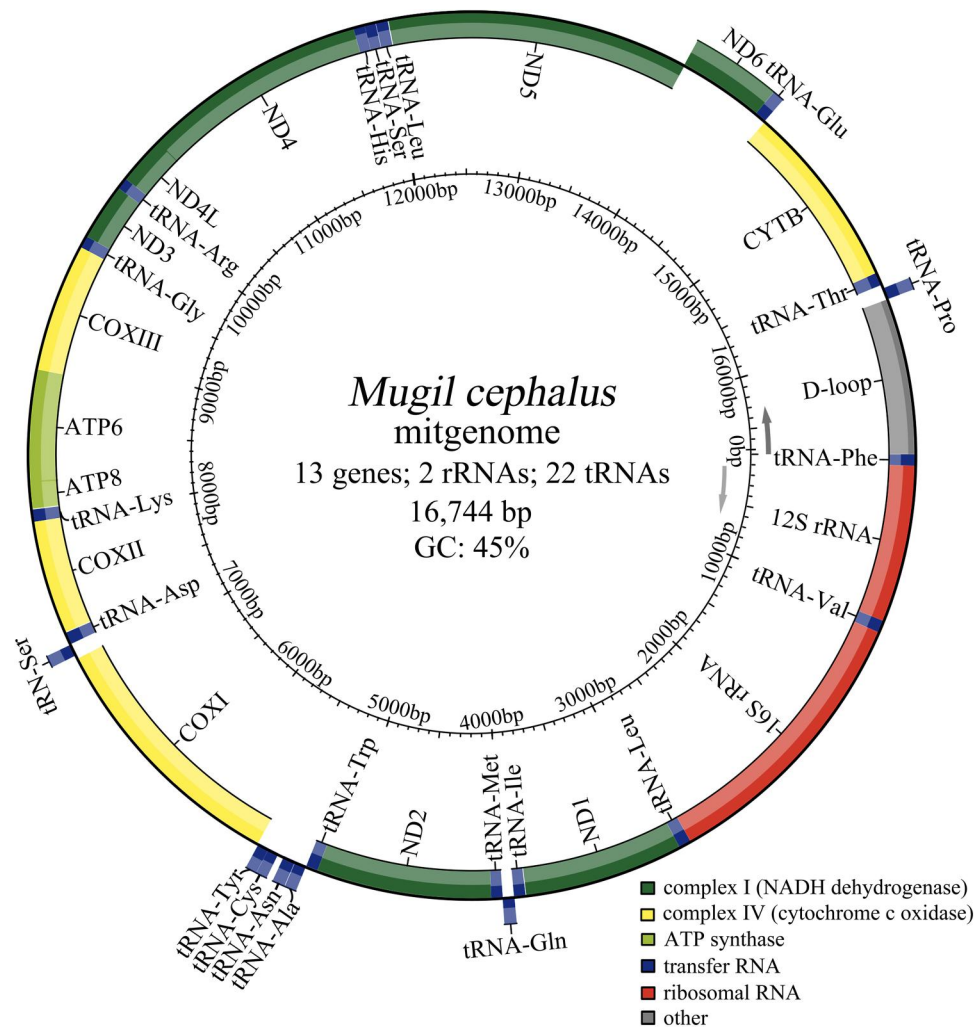


Figure 2. The circular-mapping mitochondrial genome of *Mugil cephalus* ON262567 prepared using the MitoFish web server. Genes inside the circle are transcribed clockwise, whereas those outside are transcribed counterclockwise.

Table 1. Sequence similarities of mitochondrial DNA among five localities of *Mugil cephalus*.

Region	Similarity (%)									
	YS vs. NWP1	YS vs. NWP2	YS vs. NWP3	YS vs. EA	NWP1 vs. NWP2	NWP1 vs. EA	NWP1 vs. NWP3	EA vs. NWP2	NWP2 vs. NWP3	EA vs. NWP3
ND1	100 (99.7)	95.0 (97.8)	94.2 (96.9)	95.3 (98.4)	95.1 (98.1)	95.4 (98.8)	94.9 (96.9)	95.8 (98.8)	95 (96.9)	94.6 (96.9)
ND2	99.2 (99.2)	91.1 (95.5)	90.4 (95.8)	91.5 (94.9)	90.3 (94.4)	90.8 (94.4)	90.0 (94.7)	95.0 (97.7)	93.6 (98.3)	93.4 (96.9)
12S rRNA	100	98.4	97.5	97.8	98.4	97.8	97.5	98.7	98.4	98
16S rRNA	99.9	98.7	97.4	98.1	98.7	98.1	97.4	98.4	97.6	97.6
COI	100 (100)	97.4 (100)	95.9 (99.6)	97.5 (99.8)	97.4 (100)	97.5 (100)	95.9 (99.6)	98.3 (100)	96.6 (99.6)	96.9 (99.6)
COII	100 (100)	98.3 (100)	96.5 (99.6)	97.8 (100)	98.3 (100)	97.8 (100)	96.4 (99.6)	99.1 (100)	97.0 (99.6)	96.2 (99.6)
ATP8	100 (100)	97.6 (98.2)	95.2 (100)	97.6 (98.2)	97.6 (100)	97.6 (100)	95.2 (100)	97.6 (100)	95.2 (100)	100 (100)
ATP6	99.9 (100)	95.3 (99.6)	94.7 (99.6)	95.2 (99.6)	95.2 (100)	95.0 (100)	94.6 (99.6)	95.6 (100)	94.9 (99.6)	95.2 (99.6)
COIII	100 (100)	96.9 (100)	96.8 (100)	97.2 (99.6)	96.9 (99.9)	97.2 (99.6)	96.8 (100)	96.8 (100)	96.8 (100)	96.8 (99.6)
ND3	100 (100)	92.3 (99.1)	91.7 (98.3)	94.0 (99.1)	92.3 (99.1)	94.0 (99.1)	91.7 (98.3)	92.8 (98.3)	91.4 (97.4)	93.7 (98.3)
ND4L	99.9 (100)	96.0 (99.0)	94.3 (95.9)	95.3 (99.0)	94.4 (100)	95.4 (100)	94.3 (95.9)	97.3 (100)	92.3 (95.9)	92.3 (95.9)
ND4	100 (100)	95.7 (98.7)	94.9 (98.7)	96.4 (98.5)	95.7 (98.3)	96.4 (98.5)	94.9 (98.7)	96.0 (99.1)	94.8 (98.6)	95.1 (98.9)
ND5	99.9 (100)	95.3 (99.7)	92.9 (98.4)	100 (97.6)	95.4 (99.8)	95.6 (99.5)	93.0 (98.5)	95.9 (99.3)	93.4 (98.4)	94.6 (98.4)
ND6	100 (100)	94.1 (94.8)	89.8 (94.8)	87.9 (92.1)	94.1 (95.4)	88.1 (88.4)	89.7 (94.8)	87.9 (90.8)	89.0 (93.7)	87.9 (87.9)
Cytb	99.9 (100)	94.6 (99.7)	93.6 (98.9)	95.2 (100)	94.6 (99.7)	95.3 (99.2)	93.7 (98.9)	95.3 (98.9)	93.3 (98.7)	93.4 (98.7)
D-loop	91.6	75.0	61.9	70.1	74.1	64.8	64.9	68.9	64.3	58.0
Total	99.4 (99.9)	95.0 (99.0)	94.0 (98.3)	94.5 (98.5)	94.9 (98.9)	94.1 (98.5)	93.9 (98.3)	95.2 (98.9)	94.6 (98.5)	93.8 (98.1)

Values in parentheses indicate amino acid similarity. YS: *M. cephalus* from the Yellow Sea (GenBank: ON262567); NWP1: *M. cephalus* from Northwestern Pacific1 (GenBank: AP002930); NWP2: *M. cephalus* from Northwestern Pacific2 (GenBank: KM368340); NWP3: *M. cephalus* from Northwestern Pacific3 (GenBank: KM861225); EA: *M. cephalus* from East Australia (GenBank: KP018403).

3.3. Phylogenetic analysis

Phylogenetic analysis showed that all the *M. cephalus* from different localities clustered together and were sisters to

M. curema (Figure 3). Within the clade of *M. cephalus*, the YS and NWP1 were formed a clade sister to NWP3, and then to EA, and then to NWP2, successively.

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