

The complete mitochondrial genome of *Toxotes chatareus* (Toxotes; Toxotidae; Carangaria) assembled by the next-generation sequencing data and phylogenetic analysis of Carangaria

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ABSTRACT

We present the complete mitochondrial genome of *Toxotes chatareus* yielded by the next-generation sequencing data in this study. The complete mitochondrial genome of *T. chatareus* has 16,543 bp and contained 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and a single control region (D-loop). The overall base composition was A 28.75%, C 29.80%, G 15.77%, T 25.68% and its gene arrangement was similar with other Carangaria mitochondrial genomes. Additionally, the phylogenetic relationships of 13 Carangaria species based on the complete mitochondrial genome was analyzed using the neighbor-joining method. The result showed *T. chatareus* was clustered with *L. lactarius* suggesting the close phylogenetic affinity they owned. Together, the complete mitochondrial genome of *T. chatareus* would be beneficial for the study of phylogenetic relationship, taxonomic classification and phylogeography of the Carangaria.

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Toxotes chatareus belongs to Toxotes; Toxotidae; Carangaria (Allen 1978, 2004; Simon et al. 2010; Schoch et al. 2020; Froese and Pauly 2021), is mainly distributed in the brackish water of mangrove-lined estuaries along the South Pacific and Indian Oceans, although it can also be found far upstream in fresh waters and more saline coastal waters (Allen 2001; Temple 2007; Temple et al. 2010). It is well known for its ability to shoot down insects with a jet of water squirted from the mouth (Elshoud and Koomen 1985; Simon et al. 2009, 2012). Several researches have focused on the mechanism of spitting (Timmermans 2001), visual capabilities (Temple et al. 2013) and brain atlas (Karoubi et al. 2016). Yet, little information about its genetic characteristics is available. In order to find new DNA markers for the future study of the phylogenetic relationship, taxonomic classification and phylogeography of the Carangaria, we assembled and acquired the complete mitogenome of *T. chatareus* by the next-generation sequencing (NGS) data.

The *T. chatareus* sample was acquired from Thailand (14°N; 100°E) and stored in a refrigerator of -80°C at School of Ecology and Environment, Northwestern Polytechnical University under the voucher number 20190815AP01. The species was identified based on morphologic features and *COI* gene (SequenceID: AP006806.1). The NGS data used in this

study was produced by our previous study (Lü et al. 2021) and could be acquired from the public database NCBI with the accession number SRX8345786. The short reads which were produced from the HiSeq platform were used to assemble the mitogenome by MitoZ software with default parameters (Meng et al. 2019). The MITOS Web Server (Bernt et al. 2013) and tRNAscan-SE Search Server (Chan & Lowe 2019) were used to predict and annotate the mitogenome. Whole mitogenome sequence of *T. chatareus* was 16,543 bp in length, containing 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and one control region (D-loop). The gene arrangement and base content were similar with other Carangaria species (Lv et al. 2016; Wang et al. 2016; Gan et al. 2017; Yang et al. 2019; Tabassum et al. 2020). Almost all the protein-coding genes were encoded by H-strand with the exception of *ND6* and eight tRNAs (Gln, Ala, Asn, Cys, Tyr, Ser, Glu, Pro) genes located on L-strand. The base composition was A 28.75%, C 29.80%, G 15.77%, T 25.68%. AT and GC contents were 54.43% and 45.57%, respectively. Twelve protein-coding genes started with an ATG initiation codon, while *COX1* used GTG as an initiation codon. For the termination codon, eight protein-coding genes (*ATP6*, *ATP8*, *COX1*, *COX3*, *ND1*, *ND2*, *ND4L*, and *ND5*) ended with TAA, two protein-coding genes (*ND3* and *ND6*) with TAG, and *COX2*, *CYTB* and *ND4* have an incomplete stop codon

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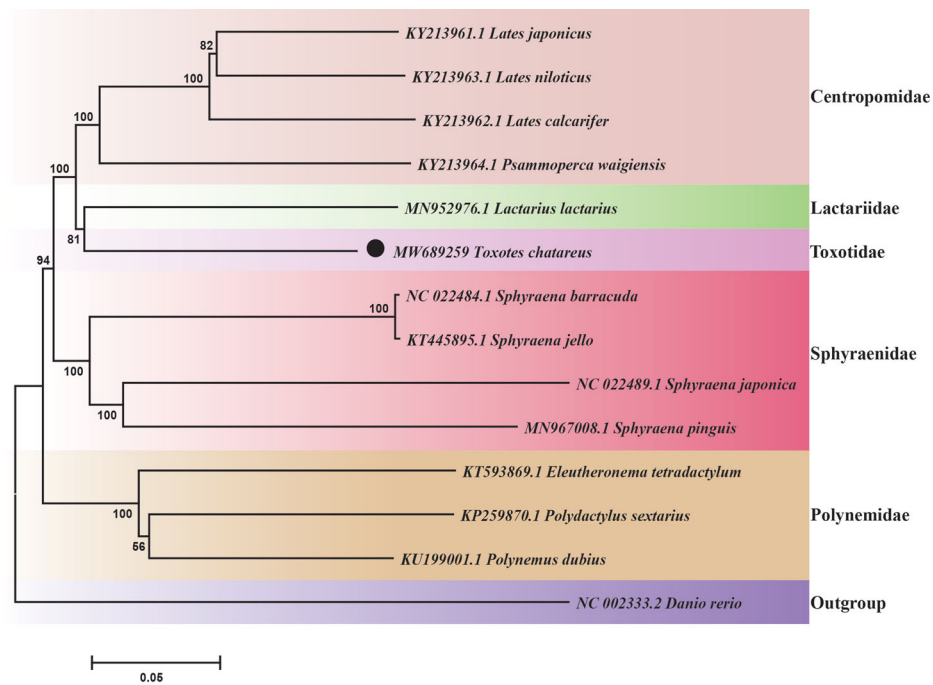


Figure 1. Neighbor-Joining tree of 13 Carangaria and one outgroup (*Danio rerio*) species based on the complete mitochondrial genome. The number at each node represents the bootstrap probability. The dark spot indicates the studied species.

T-. The 13 protein-coding genes were 11,433 bp in length, accounting for 69.11% of the whole mitogenome, which encodes 3,811 amino acids in total. The lengths of 12S rRNA located between tRNA^{Phe} and tRNA^{Val} and 16S rRNA located between tRNA^{Val} and tRNA^{Leu} were 959 bp and 1,697 bp, respectively. The control region (D-Loop) typically located between tRNA^{Pro} and tRNA^{Phe}, was 828 bp in length.

To further investigate the phylogenetic location of *T. chatareus* in Carangaria, the phylogenetic relationship was constructed in this study. Specifically, we first downloaded the mitochondrial genome of 13 species (including 12 Carangaria species and *Danio rerio*) in NCBI database. Then, the sequence alignment of these 14 species (including *T. chatareus*) was conducted by multiple sequence alignment program ClustalW (Thompson et al. 1994) in BioEdit software (Hall 1999). At last, the phylogenetic tree was constructed using the neighbor joining (NJ) method with 10,000 bootstrap replications using MEGA7 (Kumar et al. 2016). The result of the phylogeny shows that *T. chatareus* is clustered with the *L. lactarius* (Figure 1), which is belongs to Lactariidae, suggesting close phylogenetic relationship they owned. We expect that the information of the complete mitogenome of *T. chatareus* would be beneficial for the study of phylogenetic relationship, taxonomic classification and phylogeography of the Carangaria in the future.

Disclosure statement

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

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Data availability statement

The mitochondrial genome sequence data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> under the accession number MW689259. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA592745, SRR11793814, and SAMN13439313, respectively.

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