The complete mitochondrial genome of the freshwater fish Traccatichthys pulcher (Cypriniformes: Nemacheilidae) from China

Xiao Jiang Chen, Lin Song, and Wen Zhao Liu

ABSTRACT

This study aimed to sequence and annotate the complete mitochondrial DNA genome sequence of Traccatichthys pulcher. The mitochondrial genome comprised 16,583 bp, harboring 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and a control region. The whole genome contained T (25.8%), C (26.9%), A (31.4%), and G (15.9%), showing an obvious AT bias (57.2%). Based on the concatenated protein sequences of 13 PCGs, a phylogenetic tree was reconstructed by the maximum likelihood method, and the topology revealed the monophyly of Traccatichthys, and the gathering of T. pulcher and M. pulcher. The mitochondrial DNA of T. pulcher (MZ853162.1) and M. pulcher (NC_031581.1) were aligned by the BLAST 2 sequences tool, which showed 97% similarity.

Traccatichthys pulcher (Nichols & Pope, 1927) (Cypriniformes: Nemacheilidae) is of great ornamental value (Zheng et al. 2016), and often confused with Nemacheilus telenius (Pellegrin & chevey, 1936). The typical features that distinguish T. pulcher from morphology perspective are dorsal fin rays 3-10-11; anal fin rays 2-5; pectoral fins 1-9-13; ventral fin rays 1-7; gill harrow 11-12; short but high caudal fin; coneshaped head; three pairs of barbels; body covered by small round scales, no scale on the cheeks; green gray in the body, yellowish stomach; a prominent black spot in the middle of the origin of caudal fin; the distinct black submarginal streaks on dorsal and caudal fins (Pan et al. 1990). Nested-clade phylogeographic analysis based on nucleotide sequences of the mtDNA cytochrome b by Qiu et al. (2008) indicated that T. pulcher may have originated from the border area of Guangxi and Vietnam where the Dongzhong River, Beilun River and Fangcheng River are located. As the synonym name of T. pulcher, Micronemacheilus pulcher is invalid now. However, the organism of M. pulcher (NC_031581.1) was indicated to be T. telenius in GenBank, which confused whether this species should be T. telenius or T. pulcher. The sequence data of M. pulcher (KF765806.1) (Chen 2015) was unverified because it was not included in NCBI BLAST databases. This study sequenced the complete mitochondrial DNA genome of T. pulcher, and a phylogenetic analysis was accomplished with the available mitogenomes sequences among family Nemacheilidae.

Studies involving laboratory animals follow the ARRIVE guidelines (https://arriveguidelines.org/). The samples of T. pulcher (voucher number: ASTIH-21b0616d10) were collected from Beijiang River in Shaoguan City (24.7418N, 113.3758E), Guangdong Province of China by Lin Song in Jun 2021, and the samples were identified by morphological identification (Pan et al. 1990). The specimen were preserved in 95% ethanol and deposited at Aquatic Science and Technology Institution Herbarium of Jiangsu Agri-animal Husbandry Vocational College (https://www.jsahvc.edu.cn/, XJ Chen, 2007020030@jsahvc.edu.cn). Total genomic DNA was extracted from the muscle of T. pulcher using the Tguide cell/tissue genomic DNA Extraction Kit (Tiangen, Beijing, China) and stored in a deep freezer at −80°C. The extracted DNA was subjected to sample quality control, DNA library was subsequently constructed and amplified by PCR, followed by size selection and library quality check, and finally library pooling and sequencing were carried out on the Illumina Hiseq platform 2500 (Genesky Biotechnologies Inc. Shanghai, China). The next-generation sequencing raw data (3.18 GB) were assembled using MetaSPAdes 3.13.0 (Nurk et al. 2017; Yang et al. 2021), and the assembled mitochondrial genome sequences were annotated by MitoMaker 1.14 (Bernt et al. 2013) based on the reference sequence of Schistura incerta (MK361215), and the analyses were conducted using MetaSPAdes, MitoMaker with default parameters. The phylogenetic tree was based on MEGA–X software (Kumar et al. 2018).

The complete mitochondrial genome of T. pulcher comprised 16,583 bp, harboring 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and a control region (D-loop). The whole genome contained 25.8% T, 26.9% C, 31.4% A, and 15.9% G, showing an obvious AT bias (57.2%). The length of the 13 protein-coding genes ranged from 168 bp (ATP8) to 1,839 bp (ND5). Of the 13 PCGs, only the CO I gene...
started with GTG, the remaining 12 PCGs started with ATG as the initiation codon. As for stop codons, seven PCGs (ND1, CO I, ATP8, ATP6, ND4L, NDS, and ND6) used TAA, two genes (ND2 and ND3) used TAG. Four PCGs (CO II, CO III, ND4, and Cyt b) ended with an incomplete stop codon (T—–, TA—, TA—, and T——). T. pulcher contains 22 tRNAs with lengths ranging from 68 bp to 75 bp. In rRNAs, the shortest was 12S rRNA (954 bp) and the longest was 16S rRNA (1,636 bp).

To confirm the phylogeny of T. pulcher, the whole mitochondrial genomes of 17 fish species from 8 genera (Homatula, Schistura, Traccatichthys, Barbatula, Triplophysa, Claea, and Tarimichthys) were selected. Based on the concatenated amino acids sequence of 13 PCGs, the phylogenetic tree was reconstructed using the maximum likelihood method by MEGA X software, and model (mtREV + F + G + I) with the lowest BIC scores (Bayesian Information Criterion) was considered to describe the substitution pattern the best (Jones et al. 1992), with a bootstrap of 1000 replicates. The phylogenetic analysis showed that S. longa, S. reticulofasciata, and S. corica gathered together, and they formed a sister group with the clade (T. pulcher and M. pulcher) (Figure 1). The complete mitochondrial DNA sequences and concatenated nucleotides of 13 PCGs of T. pulcher (MZ853162.1) and M. pulcher (NC_031581.1) were aligned respectively using BLAST 2 sequences tool, which showed 97% similarity. (http://www.kinase.com/blast/wblast2.html), it is speculated that the two kinds of fish may be different species. Species name with GenBank accession number NC_031581.1 may be T. taeniatus, which needs more data for verification. The fundamental genetic data of T. pulcher in this study would be beneficial for further studies on population genetics and the evolution of the family Nemacheilidae.

**Ethical approval**

Experiments were approved by the Ethical Committee for Animal Experiments of Jiangsu Agri-animal Husbandry Vocational College and conducted following the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

**Author contributions**

Conception and design, XJ Chen and L Song; Data curation, WZ Liu and XJ Chen; Analysis and interpretation of the data, L Song and XJ Chen; Funding acquisition, XJ Chen; Writing – original draft, XJ Chen, L Song, and WZ Liu; Writing – review & editing, XJ Chen, L Song, and WZ Liu. All authors agree 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).

**Funding**

This work was supported by the 311 Talents Project in Taizhou City [2017III — 804]; the research project of Jiangsu Agri-animal Husbandry Vocational College [NSF2021ZR14] and [NSFHP202102].

**ORCID**

Xiao Jiang Chen http://orcid.org/0000-0002-0934-0330

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**Figure 1.** A phylogenetic tree was constructed for the genera Homatula, Schistura, Traccatichthys, Barbatula, Triplophysa, Claea, and Tarimichthys, with outgroup species, using the Maximum-likelihood (ML) method based on the connected protein sequences of 13 PCGs, with a bootstrap of 1000 replicates. GenBank accession numbers are given with species name, and the numbers at the nodes represent bootstrap values.
Data availability statement

The genome sequence data that support the findings of this study are openly available in NCBI at https://www.ncbi.nlm.nih.gov/nuccore/MZ853162, reference accession number MZ853162. The associated ‘BioProject’, ‘BioSample’, and ‘SRA’ numbers are PRJNA769998, SAMN22190292, and SRR16282473, respectively.

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