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MITOGENOME ANNOUNCEMENT



# The complete mitochondrial genome of *Neolissochilus soroides* (Duncker, 1904) (Cypriniformes: Cyprinidae)

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**ABSTRACT**

In this paper, we first report the complete mitochondrial genome of *Neolissochilus soroides*. The main purpose of this study was to determine the mitochondrial genome and phylogenetic status of *N. soroides*. The length mitogenome was 16584 bp, containing 2 ribosomal RNA genes, 13 protein-coding genes, 22 transfer RNA genes, and 3 non-coding control regions. The genome showed a slight A + T bias (A + T = 56.47%). 12 genes (*ND1*, *COX2*, *ATP6*, *ND4L*, *ND5*, *ND6*, *ND2*, *ATP8*, *ND3*, *ND4*, *Cytb*, *COX3*) start with ATG codon, besides one gene (*COX1*) start with GTG codon. Six genes (*ND1*, *COX1*, *ATP6*, *ND4L*, *ND5*, *ND6*) end with a TAA codon, 3 genes (*ND2*, *ATP8*, *ND3*) end with a TAG codon, and four genes (*COX2*, *ND4*, *Cytb*, *COX3*) end with the TA or T codon. The phylogenetic analysis showed that *N. soroides* was closely related to *N. hendersoni*. The mitogenome could have important implications for phylogeny, population genetics, and conservation of the *N. soroides*.

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*Neolissochilus soroides*; mitochondrial genome; phylogeny

## Introduction

*Neolissochilus soroides* (Duncker, 1904) is a freshwater ornamental carp that lives in fast-flowing rivers and streams with high levels of dissolved oxygen. It is mainly distributed in Southeast Asia (Rainboth, 1996) and had also been reported in China (Tan & Lim, 2004). In the past, *Tor* (now placed in *Neolissochilus*) *soso* was treated as the synonym name of *N. soroides*. Due to the loss of the holotype, the classification of *T. soso* requires additional material (Scharpf, 2015). And the lateral-line scales which are the main morphological features of *N. soroides* are 20–24, rarely 20; no black line on the lateral-line scales; the ventral fin has fin hooks in orange, and the scales are yellowish brown (Figure 1) (Khaironizam, Akaria-Ismail, & Armbruster, 2015). In addition to numerical analysis methods for morphological traits, molecular phylogenetic analysis, which assesses biological evolution based on biomolecular sequence differences, is increasingly being applied. Yang et al. (2015) used sequences from the mitochondrial gene (*Cytb*) to analyze the phylogenetic relationships of *N. soroides* in Cypriniformes. Nevertheless, until now, the complete mitochondrial genome of *N. soroides* could not be retrieved in NCBI. In this paper, we first reported the complete mitochondrial genome of *N. soroides,* and analyzed the main structural information and the phylogenetic relationships, which could provide useful information for studying genetic diversity and phylogeny conservation.

## Materials and methods

### *Sample collection and preservation*

The fish sample of this study was collected from Daying River, Tengchong City, Yunnan Province, China (24°36′36″ N, 97°49′12″ E). The specimen was euthanized by snap-frozen in liquid nitrogen and then transferred by stored in dry ice to the laboratory of Zhejiang Marine Fisheries Research Institute (http://www.zjhys.cn, Ye Chen, jimmycy85@163.com) with the voucher number HZ20211021. The *N. soroides* fish sample was identified by morphometric measurements and meristic counts using calipers and a dissecting microscope, based on the morphological characteristics described by Khaironizam


**Figure 1.** *Neolissochilus soroides* was collected from Daying River, Tengchong city, Yunnan province, China (24°36′36″ N, 97°49′12″ E) (photo by Ye Chen).

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et al. (2015). Then the muscle sample from the back of the *N. soroides* was dissected and preserved in 95% ethanol for total genomic DNA extraction.

### *Mitochondrial genome sequencing*

Total genomic DNA was extracted following the protocol described by Wei et al. (2022). After the sample genomic DNA had been tested for sequencing compliance, the DNA was sheared off by physical means (ultrasound). Then, the interrupted DNA was purified to construct a sequencing library, and the steps were as follows: (1) DNA end repair, 3′ end addition A, sequencing junction ligation. (2) Using Agarose gelation to recover the target fragments by gel electrophoresis. (3) Target fragment amplification by PCR. (4) Building sequencing libraries. They were then sequenced by Illumina NovaSeq6000 platform (Illumina, San Diego, CA).

### *Assembly, annotation, and analysis*

Getorganells splicing software (https://github.com/Kinggerm/GetOrganelle) was used to splice the sequenced reads in multiple iterations, and the initial assembly results were obtained. Then, the clean reads were compared back to the mitochondrial genome sequence, and the bases were corrected using Pilon v1.23 (Walker et al. 2014) and annotated using MITOS2 (http://mitos2.bioinf.uni-leipzig.de/index.py) (Ren et al. 2020). The mitochondrial circle mapping was done by OGDRAW (https://chlorobox.mpimp-golm.mpg.de/O).

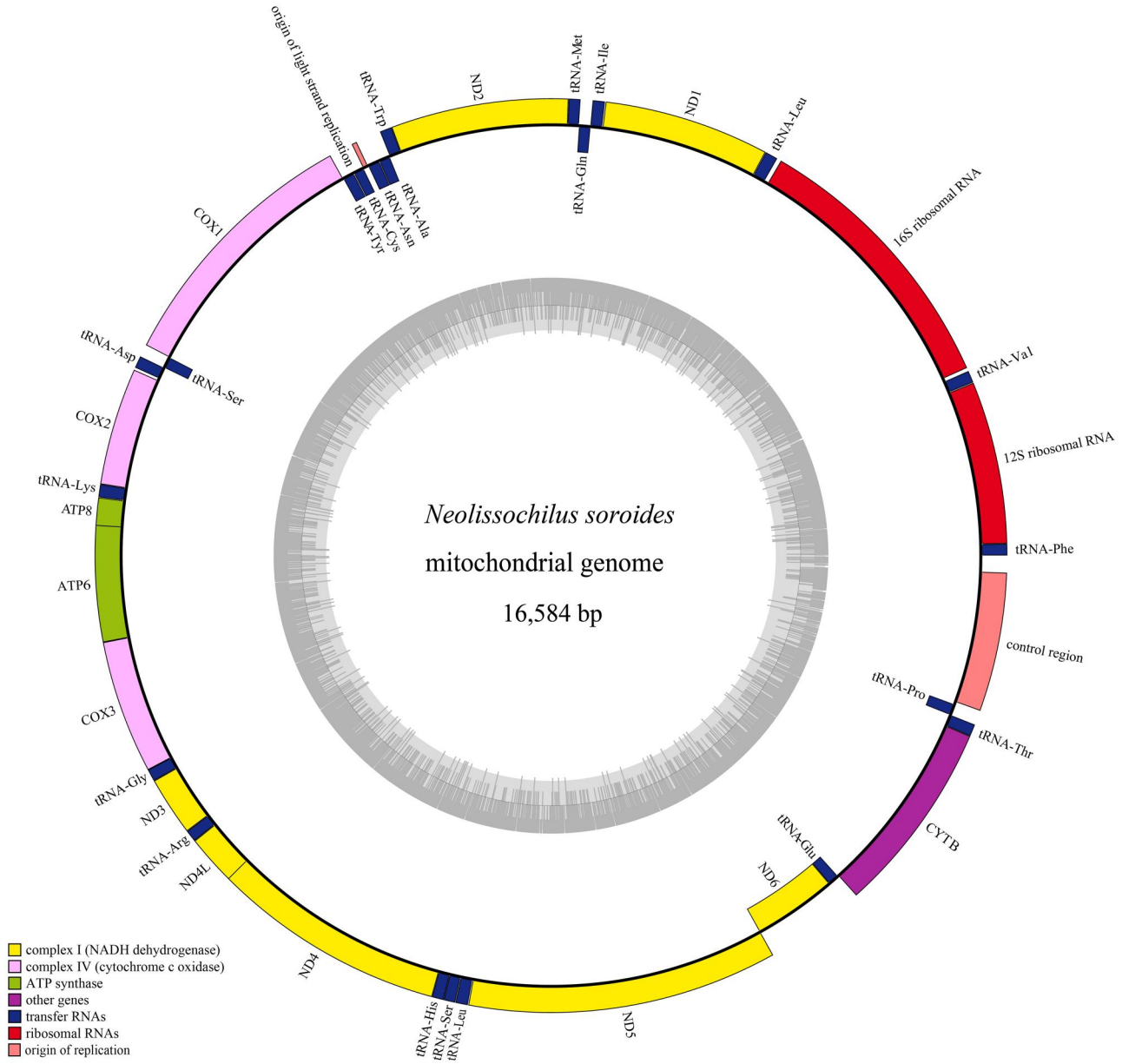


**Figure 2.** Mitochondrial genome map of *Neolissochilus soroides*. H-strand is located in the outer ring and L-strand is located in the inner ring. The gene consists of 13 protein-coding genes, two rRNA genes, 22 tRNA genes and three non-coding control regions. Among them, yellow: complex I (NADH dehydrogenase); light green: complex III (ubiquinol cytochrome c reductase); pink: complex IV (cytochrome c oxidase); dark green: ATP synthase; blue: transfer RNAs; red: ribosomal RNAs.

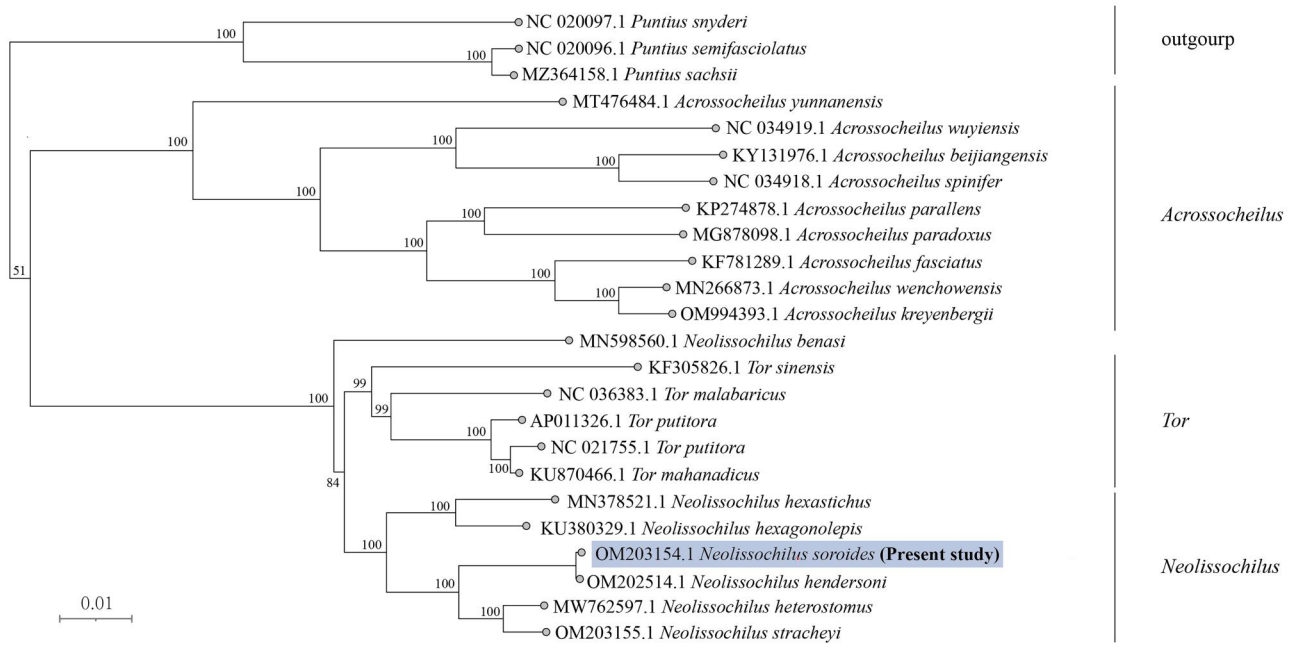


**Figure 3.** Phylogenetic analysis of *N. soroides* based on the entire mtDNA genome sequences of 24 Cypriniformes available in GenBank. Numbers above the nodes indicate 1000 bootstrap values. Accession numbers are shown before species names. The following sequences were used: *Neolissochilus hendersoni* OM202514.1 (Guo et al. 2023), *Neolissochilus stracheyi* OM203155.1 (Wei, Z. et al. 2022), *Neolissochilus heterostomus* MW762597.1 (He et al. 2021), *Neolissochilus hexagonolepis* KU380329.1 (Zhou et al. 2016), *Neolissochilus hexastichus voucher* MN378521.1 (Singh et al. 2021), *Tor sinensis* KF305826.1 (Huang et al. 2015), *Tor malabaricus* NC 036383.1 (Chandhini et al. 2019), *Tor putitora* AP011326.1 (Sati et al. 2014), *Tor mosal mahanadicus* KU870466.1 (Sarma et al. 2022), *Tor putitora* NC 021755.1 (Sati, J. et al. 2014), *Neolissochilus benasi* MN598560.1 (Gu et al. 2020), *Acrossocheilus yunnanensis* MT476484.1 (Chen et al. 2022), *Acrossocheilus spinifer* NC 034918.1 (Zhao et al. 2022), *Acrossocheilus beijiangensis* KY131976.1 (Liu et al. 2018), *Acrossocheilus wuyiensis* NC 034919.1 (Yuan et al. 2017), *Acrossocheilus paradoxus* MG878098.1 (Ju et al. 2018), *Acrossocheilus parallens* KP274878.1 (Xie et al. 2016), *Acrossocheilus fasciatus* KF781289.1 (Cheng et al. 2015), *Acrossocheilus kreyenbergii* OM994393.1 (Zhou et al. 2023), *Acrossocheilus wenchowensis* MN266873.1 (Pan et al. 2019), *Puntius snyderi* NC 020097.1 (Jang-Liaw et al. 2013), *Puntius sachsii* MZ364158.1 (Sun et al. 2023), *Puntius semifasciolatus* NC 020096.1 (Sun et al. 2023), *Puntius chalakkudiensis* NC 018566.1 (Khare et al. 2014).

GDraw.html). In order to explore the phylogenetic position of *N. soroides*, we used the whole mitochondrial data from the nucleotide and performed multiple sequence alignments. After multiple sequence alignments, a phylogenetic tree by MEGA 7.0 (Kumar et al. 2016) was generated, using maximum likelihood (ML) to performe phylogenetic analysis, and generated 1000 bootstraps. The phylogenetic trees in this study include 24 fish species belonging to *Neolissochilus*, *Tor*, *Acorssocheilus*, and *Puntius* (as an outgroup).

## Results

### Genomic analysis results

The genome of *N. soroides* was assembled correctly (Figure S1, supplementary material). The entire mitochondrial genome of *N. soroides* (GenBank accession no. OM202554) had a length of 16,584 bp. The overall base composition of the mitogenome was 31.8% for A, 27.76% for C, 15.78% for G and 24.67% for T. The percentage of A + T content was 56.47%. The gene consists of 13 protein-coding genes (*COX1*, *COX2*, *ATP8*, *ATP6*, *COX3*, *ND3*, *ND1*, *ND5*, *ND4*, *ND4L*, *ND6*, *Cytb*, *ND2*), two rRNA genes (*12S rRNA*, *16S rRNA*), 22 tRNA genes and three non-coding control regions (Figure 2). Most of the sequence elements were located on the heavy strand, except one protein-coding gene and eight tRNA (*ND6* and *Gln*, *Ala*, *Asn*, *Cys*, *Tyr*, *Ser*, *Glu*, *Pro*). The three non-coding controls were 32, 45, and 816 bp long respectively. Among 13 protein-coding genes,12 genes (*ND1*, *COX2*, *ATP6*, *ND4L*, *ND5*, *ND6*, *ND2*, *ATP8*, *ND3*, *ND4*, *Cytb*, *COX3*) were started with ATG codon, besides one gene (*COX1*) was started with GTG codon. And six genes (*ND1*, *COX1*, *ATP6*, *ND4L*, *ND5*, *ND6*) ended in the TAA codon, three genes (*ND2*, *ATP8*, *ND3*) ended in the TAG codon, and four genes (*COX2*, *ND4*, *Cytb*, *COX3*) ended in the TA or T codon.

### Phylogenetic analysis

The phylogenetic position of *N. soroides* in subfamily *Neolissochilus* was shown in Figure 3, and *N. soroides* was closely related to *N. hendersoni* (OM202514.1). The phylogenetic analysis showed that *N. soroides* was clustered together with *N. hendersoni*.

## Discussion and conclusion

Three non-coding regions include an L-strand replication origin and two control regions, which were different from *Neolissochilus heterostomus* (He et al. 2021), and similar with *Garra motuoensis* (Gong et al. 2022).

The phylogenetics indicates that *N. soroides* belonged to *Neolissochilus* and not to *Tor,* which was the same as Khaironizam et al. (2015). Phylogenetic tree in this paper showed that *Neolissochilus and Tor* and *Acrossocheilus* have been reasonably identified by morphology. However, *N. benasi* (MN598560.1) did not belong to the genus *Neolissochilus,* which was not consistent with the study of Gu et al. (2020). In this regard, we hypothesize that the

inconsistent results may be because the reliability (numbers on branches) of *N. benasi* phylogenetic trees in the study of Gu et al. (2020) was not high enough.

The complete mitochondrial genome of *N. soroides* was sequenced and annotated using high-throughput sequencing technology. The basic genetic data provided in this paper could provide a reference for further research on the genetics and evolutionary study of *N. soroides*.

## Author contributions statement

Ye Chen and Yongyao Guo designed the experiment and wrote the original manuscript. Yongyao Guo, Zhenzhu Wei and Xiaoli Zhao provided sample collection and analyzed the data. All authors approved the final manuscript and agreed to be accountable for all aspects of the study.

## Ethics statement

All animal protocols have been reviewed and approved by the Ethics Review Committee for Experimental Animal Welfare of Zhejiang Ocean University. The approval number is 20210504.

## Disclosure statement

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

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## Data availability statement

The genome sequence data supporting this study's findings are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/nuccore/OM203154.1/) under accession no. OM203154. The associated BioProject, SRA, and Bio-Sample Numbers are PRJNA817098, SRR18356122, and SAMN26747790.

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