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MITOGENOME ANNOUNCEMENT

Characterization of the complete mitochondrial genome of *Neolissochilus hendersoni* (Herre, 1940) (Cypriniformes: Cyprinidae)

Yongyao Guo[a], Yue Wang[a], Chunyan Yin[a], Jianhui Wang[a] and Ye Chen[b]

[a]College of Fisheries, Zhejiang Ocean University, Zhoushan, China; [b]College of Fisheries, Zhejiang Marine Fisheries Research Institute, Zhoushan, China

ABSTRACT

The differentiation between *Neolissochilus* and *Acrossocheilus* species based only on morphology is ambiguous; however, phylogenetic analysis using their mitogenome sequences provides conclusive results. Here, the phylogenetic position of *Neolissochilus hendersoni* (Herre, 1940) was determined using its mitogenome data. Total DNA from *N. hendersoni* was sequenced using the Illumina NovaSeq6000 platform, and annotation of mitochondrial genes was performed using MITOS2. Phylogenetic trees were constructed using the complete mitogenomes of 16 fish species. The mitogenome of *N. hendersoni* was found to be 16584 bp long, containing two ribosomal RNA genes, 13 protein-coding genes, 22 transfer RNA genes, and three non-coding control regions. The genome showed a slight A + T bias (A + *T* = 56.46%). Most PCGs were found to be located on the L-strand. Results of the phylogenetic analysis showed that *N. stracheyi* is closely related to *N. hendersoni*. Our results will help to clarify the phylogenetic relationship between *Neolissochilus* and *Acrossocheilus* species.

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The genus Neolissochilus Rainboth, 1985 (Cypriniformes: Cyprinidae) includes 34 species of freshwater fish (Singh et al. 2021), which are widespread across tropical regions of southern to southeastern Asia (Rainboth 1991; Khaironizam et al. 2015). The classification of species from the Cyprinidae family remains unclear (Chen 2013). In China, fish from the genus *Neolissochilus* are commonly known as 'new smooth-lipped fish' and their name has a meaning similar to that of *Acrossocheilus*, which means 'smooth-lipped fish' (Figure 1). The differentiation between *Neolissochilus* and *Acrossocheilus* species based only on morphology is ambiguous (Dasgupta 1988; Ambak and Jalal 2006; Sharma et al. 2019) (Table 1). Complete mitogenomes are being increasingly used to resolve ambiguity and provide accurate phylogenetic relationships between organisms. Preliminary studies have been performed on *Neolissochilus* species to analyze phylogenetics (Lalramliana et al. 2019; Gu et al. 2020; He et al. 2021); however, their phylogenetic analysis using mitogenome sequence has not been accomplished. In the present study, the complete mitochondrial genome of *Neolissochilus hendersoni* (Herre, 1940) was sequenced to analyze its phylogenetic position.

One specimen of *N. hendersoni* was collected from Daying River, Tengchong, Yunnan Province, China (24°36′36″ N, 97°49′12″ E) (Figure 2). Part of the specimen was used in this study and the remaining sample was stored in the Biological Specimen Room of Zhejiang Ocean University (www.zjou.edu.cn, Yong-YaoGuo, 353746433@qq.com), voucher numeral LJL20211120. Total DNA from *N. hendersoni* sample was extracted using the E.Z.N. A® tissue DNA kit (Qiagen, Manchester, UK). The Illumina NovaSeq6000 platform (Illumina, San Diego, CA) was used to construct DNA libraries and to perform sequencing following protocols used by Zhang et al. (2020). The raw sequencing data were filtered and 79,108,922 high-quality reads with a Q30% of 91.57% were obtained. The clean data were then assembled using Pilon v1.23. Gene annotation was performed using MITOS2 (http://mitos2.bioinf.uni-leipzig.de/index.py).

The complete mitogenome of *N. hendersoni* (GenBank accession no. OM202514) was found to be 16584 bp long. The Mitogenome contained two ribosomal RNA (12S rRNA and 16S rRNA) genes, 13 protein-coding genes (PCGs), 22 transfer

Figure 1. Photograph of *Neolissochilus hendersoni*.

CONTACT Ye Chen jimmycy85@163.com Zhejiang Marine Fisheries Research Institute, Zhoushan 316000, China

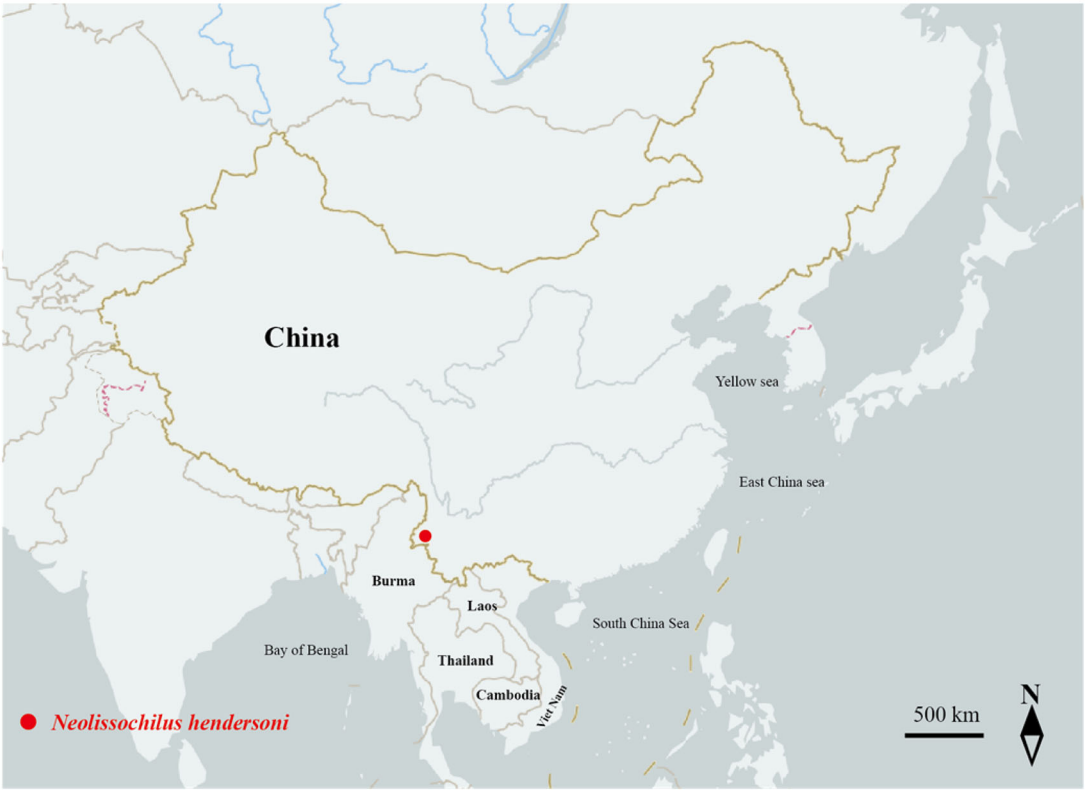

Figure 2. Collection site of *Neolissochilus hendersoni* in Daying River, Tengchong, Yunnan Province, China (24°36′36″ N, 97°49′12″ E).

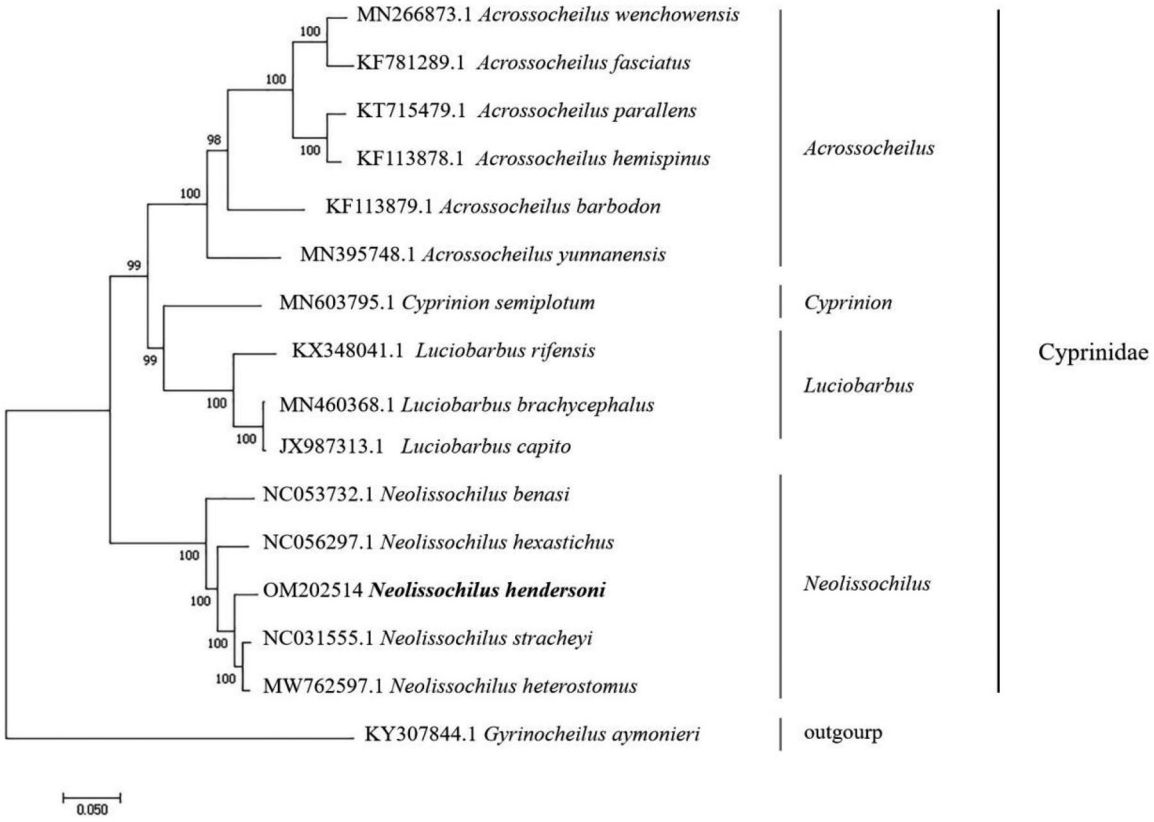

Figure 3. Phylogenetic analysis of *Neolissochilus hendersoni* based on the entire mtDNA genome sequences of 16 Cypriniformes available in GenBank. Numbers above the nodes indicate 1000 bootstrap values. Accession numbers are shown before species names.

Table 1. Difference in morphological features between *Neolissochilus* and *Acrossocheilus*.

Category	Morphological features
Neolissochilus	The lower lip has no fleshy leaves, the lower jaw develops a horny sheath, the forearm has few gill rakers, the nose is blunt, wide, and long, the body is flat and cylindrical, and the pharyngeal arch is short and thick.
Acrossocheilus	The lip has fleshy, upper and lower lips are connected at the corner of the mouth, there is a space between the two flaps of the lower lip, the front edge of the lower jaw is horny; two pairs of whiskers, the last dorsal fin does not branch The fin rays are ossified, and the posterior edge is smooth or serrated.

RNA (tRNAs) genes, and three non-coding control regions. The overall base composition of *N. hendersoni* was A 31.79%, T 24. 67%, G 15.78%, and C 27.76%. The sequence had a slight A + T bias with 56.46% A + T content. The maximum-likelihood (ML) phylogenetic tree was constructed using MEGA 7.0 with the GTR + G + I models (Xian et al. 2015) using the complete mitogenome of 16 fish species belonging to *Acorssocheilus*, *Cyprinion*, *Luciobarbus*, *Neolissochilus*, *Gyrinocheilus,* All complete mitogenome data are available for download from the Nucleotide at NCBI. As shown in the phylogenetic tree (Figure 3), *N. hendersoni* is closely related to *N. stracheyi.* Further, the analysis indicates that the genus *Neolissochilus* is nearest in origin to the genus *Luciobarbus* and is farther from the genus *Acrossocheilus* in *a* phylogenetic position. These data will help clarify the phylogenetic relationship between *Neolissochilus* and *Acrossocheilus* species.

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Ethical approval

This study was conducted following the guidelines and approval of the Institutional Animal Care and Use Committee at the Zhejiang Laboratory Animal Research Center and Zhejiang Ocean University. The approval number is 20210207.

Author contributions

Y.Guo and Y.C conceived the study. C.Yin and J.Wang collected the specimen. Y.Guo, Y.Wang, and C.Yin carried out the experiments and data analyses. Y.Guo wrote the manuscript with contributions from all authors. All authors approved the final manuscript and agreed to be accountable for all study aspects.

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/OM202514.1/) under accession no. OM202514. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA816692, SRR18336527, and SAMN26688729.

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