The complete mitochondrial genome sequence and phylogenetic position of *Sinocyclocheilus xiaotunensis* (Cypriniformes: Cyprinidae)

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**ABSTRACT**

The complete mitochondrial DNA genome of *Sinocyclocheilus xiaotunensis* was first reported by next-generation sequencing method. The entire length of mitochondrial genome is 16,588 bp and the nucleotide composition was made up of 32.3% A, 25.0% T, 27.2% C, and 15.5% G, indicating an A + T(57.3%) content is greater than C + G(42.7%). The mitogenome is a circular DNA molecule with a D-loop region and contains 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes and 13 protein-coding genes. To provide further info on the conserved sequence block observed in the control region of the mitochondrial genome. This info is critical for future application and determination of taxonomic status of this species.

**KEYWORDS**

Sinocyclocheilus xiaotunensis; Mitochondrial genome; Phylogeny

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Sinocyclocheilus (Cypriniformes: Cyprinidae) endemic to China and has been listed as endangered species in the IUCN Red List of Threatened Species. However, The fish was collected from Xiaotun Village, Zhenfeng County, Guizhou Province, China (25°50'42"N; 105°60'38"E) in Sancha River, a tributary of Beipanjiang River System and 1165 M above sea level. Through morphological analysis, it is different from *Sinocyclocheilus zhenfengensis* (Liu et al. 2018); through complete mitochondrial genome analysis, it is different from other species. So we named it *Sinocyclocheilus xiaotunensis*. In this study, we first reported the complete mitochondrial genome (mitogenome) of *S.xiaotunensis*, which could provide useful first-hand data for molecular phylogenetics and population genetics studies on this species and its closely related Sinocyclocheilus species. Voucher specimens (gzu202021204) were preserved in 100% ethanol and deposited at zoological museum of the School of Animal Science, Guizhou University.

In terms of morphology, we measured two categories: quantifiable traits and countable traits. In the statistics of the above-mentioned measured growth traits, in order to eliminate the influence on the reliability of the data caused by the size of the fish, we used the head shape measurement value divided by the head length, and the trunk shape measurement value divided by the body Length, the measured value of the tail shape divided by the length of the tail shank. Refer to Table 1 for detailed morphological data. Compared with Liu (2018) (*Sinocyclocheilus zhenfengensis*) collected in the Shuangrufeng Scenic Spot in Zhexiang Town, Zhenfeng County, among the measurable traits, eye diameter/head length, dorsal fin length/body length, pectoral fin length/body length, and pectoral fin length/body length all showed significant differences (\(p < 0.01\)), and the average values of *S.xiaotunensis* were 0.133, 0.184, 0.207 and 0.301, The average values of *S.zhenfengensis* were 0.291, 0.227, 0.247 and 0.337, respectively. In terms of countable traits, the number of dorsal fin spines and gill rakers of *S.xiaotunensis* was more than *S.zhenfengensis*. With the deepening of cave life, the eye diameter of the *S.xiaotunensis* is smaller than the *S.zhenfengensis*, which is related to the light intensity of the living environment (Langecker et al. 1995; Zhou et al. 2009). Not only that the dorsal and pectoral fins of *S.xiaotunensis* became shorter and the number of fin spines increased. This is an adaptive change to the living environment, and its balance function has been further adapted to evolve and it showed obvious difference from *S.zhenfengensis* in morphology.

The complete mitochondrial genome length of *S.xiaotunensis* was 16,588 bp (GenBank accession number MW574480). It consisted of 13 protein-coding genes, two rRNA genes, 22 tRNA genes and one D-loop region (Table 2; Figure 1). The overall base composition of the mitogenome is 32.3% for A, 27.2% for C, 15.5% for G and 25.0% for T. The percentage of G + C content is 42.7%. The gene arrangement and nucleotide composition of the mitogenome of *S.xiaotunensis* were similar to those of other Sinocyclocheilus species (Wu et al. 2010; Chen et al. 2017; Li et al. 2017; Xu et al. 2019). Most mitochondrial genes were encoded on the heavy strand (H-strand), except that the eight tRNA gene and ND6 genes were encoded on the light strand (L-strand). All 13 PCGs except for COI (with a GTG start codon) started with an ATG codon. Six PCGs ended with two types of complete stop codons, TAA (ND1, COI, ND4L, ND5 and ND6) and TAG (ATP8). The remaining PCGs ended with incomplete stop codon, including stop codon T–(ND2, COII, ND3, ND4 and...
Cytb) and TA-(ATP6 and COIII). The 22 tRNA genes have lengths ranging from 69 to 78 bp. The lengths of 12S and 16S rRNA genes were 955 bp and 1677 bp. The D-loop or control region was located between tRNA-Pro and tRNA-Phe genes with a length of 934 bp. The lengths of COI and Cytb genes were 1551 and 1441 bp, respectively.

To determine the phylogenetic position of *S.xiaotunensis*, phylogenetic analyses were conducted based on mitogenome sequences of 25 Sinocyclocheilus species and two outgroup species from GenBank by Neighbor-joining tree (NJ) methods (Tamura et al.2013) (Figure 2). The phylogenetic results showed that three clade were observed and *S. jii* was the most basal species among the Sinocyclocheilus species. Not only that *S.xiaotunensis* was independent with the bootstrap values 100% and had a close relationship with *S.altishoulderus* and *S.furcodorsalis*. In summary, the newly

| Table 1. Morphological proportion characters of *S. xiaotunensis*. |
|-----------------|--------------------|-----------------|-----------------|--------------------|--------------------|
| Character       | Holotype           | Range (mm)      | Character       | Holotype           | Range (mm)         |
| Number of samples | 10                |                 | Pectoral fin length | 17.67             | 16.79–18.34        |
| Body length     | 85.02             | 83.87–86.41     | Anal fin base length | 7.65              | 6.98–8.2          |
| Body depth      | 24.33             | 22.45–26.4      | Dorsal fin base length | 11.68             | 11.02–12.04       |
| Body width      | 14.03             | 12.94–15.03     | Caudal peduncle depth | 9.69              | 8.33–10.48        |
| Head length     | 25.31             | 23.89–26.86     | Caudal peduncle length | 15.33             | 14.36–17.09       |
| Snout length    | 9.54              | 8.4–10.14       | Caudal peduncle length | 15.33             | 14.36–17.09       |
| Percentage of standard length |
| Head height / Head length | 0.639 | Caudal fin rays | 19 |
| Head width / Head length | 0.493 | Gill rakers | 10–11 |
| Interorbital width / Head length | 0.292 | Lateral line scales | 40–42 |
| Eye diameter / Head length | 0.133 | Scale rows above lateral | 11–12 |
| Maxillary barbel length / Head length | 0.426 | Scale rows below lateral | 8–10 |
| Rictal barbel length / Head length | 0.461 | Pharyngeal teeth | 2.3,4–4.3,2 |
| Snout length / Head length | 0.376 | Dorsal fin rays | III,7–8 |
| Body depth / Body length | 0.286 | Anal fin rays | III,5–6 |
| Body width / Body length | 0.165 | Pectoral fin rays | I,14–15 |
| Caudal peduncle depth / Caudal peduncle length | 0.632 | Pelvic fin rays | I,7–8 |

Note: Roman symbol(I, III) indicates the number of fin spines.

| Table 2. Characterization and annotation of *S. xiaotunensis* mitogenome. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gene            | Strand | From | To | Length(bp) | Intergenic nucleotide | Anticodon | Codon |
| D-Loop          | H      | 1    | 934 | 934         | 0                 | GAA       |      |
| tRNA-phe        | H      | 935  | 1003 | 69         | 0                 | TAC       |      |
| 12S rRNA        | H      | 1004 | 1958 | 955        | 0                 | TAA       |      |
| tRNA-Val        | H      | 1959 | 2030 | 72         | 0                 | TAG       |      |
| 16S rRNA        | H      | 2031 | 3707 | 1677       | 0                 | TAA       |      |
| tRNA-Leu(UUR)   | H      | 3708 | 3785 | 78         | 0                 | TAA       |      |
| ND1             | H      | 3786 | 4760 | 975        | 3                 | GTG       |      |
| tRNA-Ile        | H      | 4764 | 4837 | 74         | −3                | CAT       |      |
| tRNA-Gln        | L      | 4835 | 4906 | 72         | 0                 | TAG       |      |
| tRNA-Met        | H      | 4907 | 4975 | 69         | 0                 | CAT       |      |
| ND2             | H      | 4976 | 6022 | 1047       | −2                | ATG       | T—   |
| tRNA-Trp        | H      | 6021 | 6093 | 73         | 1                 | TCA       |      |
| tRNA-Ala        | L      | 6095 | 6163 | 69         | 1                 | TGC       |      |
| tRNA-Asn        | L      | 6165 | 6237 | 73         | 3                 | GTT       |      |
| tRNA-Cys        | L      | 6269 | 6337 | 69         | −2                | GCA       |      |
| tRNA-Tyr        | L      | 6336 | 6406 | 71         | 1                 | GTA       |      |
| COI             | H      | 6408 | 7958 | 1551       | 0                 | GTG       | TAA  |
| tRNA-Ser(UCN)   | L      | 7959 | 8029 | 71         | 4                 | TGA       |      |
| tRNA-Asp        | H      | 8034 | 8105 | 72         | 12                | GTC       |      |
| COII            | H      | 8118 | 8808 | 691        | 1                 | ATG       | T—   |
| tRNA-Lys        | H      | 8810 | 8884 | 75         | −1                | TAG       |      |
| ATP8            | H      | 8886 | 9053 | 168        | −10               | ATG       | TAG  |
| ND3             | H      | 9044 | 9727 | 684        | −1                | ATG       | TAA  |
| tRNA-Gly        | H      | 9727 | 10512| 786        | −1                | ATG       | TAA  |
| ND5             | H      | 10512| 10583| 72         | 0                 | TCC       |      |
| ND3             | H      | 10583| 10934| 351        | −2                | ATG       | T—   |
| tRNA-Arg        | H      | 10933| 11002| 70         | 0                 | TGC       |      |
| ND4L            | H      | 11003| 11299| 297        | −7                | ATG       | TAA  |
| ND4             | H      | 11293| 12673| 1381       | 0                 | ATG       | T—   |
| tRNA-His        | H      | 12674| 12743| 70         | 0                 | GTG       |      |
| tRNA-Ser(AGY)   | H      | 12744| 12812| 69         | 1                 | GCT       |      |
| tRNA-Leu(UCN)   | H      | 12814| 12886| 73         | 3                 | TAG       |      |
| ND5             | H      | 12890| 14712| 1823       | −9                | ATG       | TAA  |
| ND6             | L      | 14704| 15231| 528        | 0                 | ATG       | TAA  |
| tRNA-Glu        | L      | 15232| 15300| 69         | 5                 | TTC       |      |
| Cytb            | H      | 15306| 16446| 1141       | 0                 | ATG       | T—   |
| tRNA-Thr        | H      | 16447| 16518| 72         | −2                | TGT       |      |
| tRNA-Pro        | L      | 16517| 16588| 72         | 0                 | TGG       |      |

Cytb) and TA-(ATP6 and COIII). The 22 tRNA genes have lengths ranging from 69 to 78 bp. The lengths of 12S and 16S rRNA genes were 955 bp and 1677 bp. The D-loop or control region was located between tRNA-Pro and tRNA-Phe genes with a length of 934 bp. The lengths of COI and Cytb genes were 1551 and 1441 bp, respectively.
Figure 1. Gene map of *Sinocyclocheilus xiaotunensis* mitogenome. All genes and control region are annotated.

Figure 2. Neighbor-joining phylogenetic tree of the *S. xiaotunensis* and other species based on the complete mitochondrial genome. Numbers on nodes indicate bootstrap support value, based on 1000 replicates.
obtained *S.xiaotunensis* extranuclear genomic resource would provide valuable molecular information fundamental to conservation and resource restoration studies on this cyprinid species.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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**Data availability statement**

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at [https://www.ncbi.nlm.nih.gov/](https://www.ncbi.nlm.nih.gov/) under the accession no MW574480.

**Museum collector**

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