The complete mitochondrial genome of Chinese minnow (*Rhynchocypris oxycephalus*) and its phylogenetic analyses

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**ABSTRACT**

The complete mitochondrial genome can provide novel insights into understanding the mechanism underlying mitogenome evolution. In the present study, the whole mitochondrial genome of *Rhynchocypris oxycephalus* was determined to 16608 bp (GenBank accession No: MW057563), including 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and one control region. The overall base composition was 28.62% A, 27.23% T, 26.31% C and 17.84% G, with a total A + T content of 55.85%. The Maximum Likelihood tree showed that the phylogenetic relationship is closer between *R. oxycephalus* and *Phoxinus oxycephalus jouyi* than the other species. The whole mitogenome of this species will be useful for the future animal evolutionary, phylogenetic relationship, and genomic studies in the genus *Phoxinus*.

Chinese minnow (*Rhynchocypris oxycephalus*) is a small cold-water fish, which is endemic to East Asia and generally inhabits stream headwaters (Jang et al. 2002; Bogutskaya et al. 2008). The life history characteristics of *R. oxycephalus* (such as low reproductive output, short life cycle, and low growth rate) and its ecological tolerance characteristics determine that *R. oxycephalus* is extremely sensitive and vulnerable to environmental changes or human interference, and their local populations are extremely vulnerable (Yu et al. 2013; Chu et al. 2015). Therefore, it is necessary to raise concern about long-term conservation of *R. oxycephalus*. Little is however known about the conservation status of *R. oxycephalus*. The classification status of this species and the phylogenetic relationship of genus *Phoxinus* was very complicated (Ito et al. 2002; Sasaki et al. 2007). To gain a better insight into its taxonomic relationship, the specimen was collected from Yongjiang River (121.63°E, 29.90°N), Ningbo City, Zhejiang Province, China, and they were deposited in Key Laboratory of Applied Marine Biotechnology, Ningbo University (Sample code is YG-150422). The entire genomic DNA was extracted from 30 to 50 mg of muscle tissue using the standard phenol-chloroform extracting method (Sambrook and Russell 2001), and preserved at −20 °C. Based on the conserved sequences of *P. semotilus* and *P. oxycephalus jouyi* (Miya et al. 2015; Yu et al. 2017), we designed 18 pairs of primers for polymerase chain reaction amplification. The PCR fragments were assembled by BioEdit version 7.2.5 software (Hall 1999) and then calculated the nucleotide base composition by MEGA6.0 (Tamura et al. 2013). To avoid assembling error, the complete mitochondrial sequence was aligned with its closely related species by BLAST. The annotated genomic sequence has been submitted to GenBank under the accession number MW057563.

In total, the complete mtDNA of *R. oxycephalus* was 16608 bp in length, and the content was consistent with the typical fishes’ mitochondrial genomes (Perna and Kocher 1995). It contained 13 PCGs, 22 tRNA genes, two rRNA genes, and one control region. Among the 37 genes, one PCGs (ND6) and eight tRNA genes (*tRNA^Gln^*, *tRNA^Aua^*, *tRNA^Asn^*, *tRNA^Cys^*, *tRNA^Trp^*, *tRNA^Ser^*(UGC), *tRNA^Glu^*, *tRNA^Pro^*) were on the light strand, and the remaining 28 genes were on the heavy strand. In 13 protein-coding genes, apart from COI utilizing GTG, the rest of the 12 protein-coding genes start with the same initiation codon ATG. The typical termination codons (TAA or TAG) were detected in 10 PCGs (TAA for *ND1, COI, ATP6, COX III, ND4L*, and *NDS* genes, TAG for *ATP8, ND3, ND4*, and *ND6* genes), and the remaining three genes (*ND2, COII, and Cyt b*) were ended by incomplete stop codons (T-). The length of all tRNAs ranged from 68 to 76 bp. The O1 region (L-strand replication origin) was located between *tRNA^Aua^* and *tRNA^Cys^*. The 12S and 16S rRNA genes are 954 bp and 1670 bp, respectively. The control region of 935 bp was located in *tRNA^Pro^* and *tRNA^Phe^* (Table 1).

To investigate the phylogenetic relationship among the genus *Phoxinus*, the mitochondrial genome sequences of seven currently available species of *Phoxinus* were
downloaded, including *P. oxycephalus jouyi* (AP011269.1), *P. semotilus* (NC_029341.1), *P. steindachneri* (NC_015357.1), *P. keumkang* (AP011363.1), *P. tumensis* (KC992395.1), *P. phoxinus* (AB671170.1), and *P. ujmonensis* (NC_023802.1), together with *Acrocheilus alutaceus* (NC_033927.1) as outgroup species.

The phylogenetic tree was constructed using Maximum Likelihood method based on complete mtDNA. Tree topology was evaluated by 1000 bootstrap replicates, and the tree had high bootstrap supporting values. The result indicated that the phylogenetic relationship is closer between *R. oxycephalus* and *P. oxycephalus jouyi* than the other species (Figure 1).

**Disclosure statement**

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

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**Data availability statement**

The data that support the findings of this study are available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov, reference number MW057563.

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### Table 1. Mitogenome characteristic of *Rhynchocypris oxycephalus*.

| Gene/element | Position | Length (bp) | Start codon | Stop codon | Anticodon | Strand |
|--------------|----------|-------------|-------------|------------|-----------|--------|
| tRNA^Phe    | 1-69     | 69          | GAA         | H          | -         | H      |
| 12S rRNA    | 70-1023  | 954         | -           | -          | -         | H      |
| tRNA^Met    | 1026-1097| 72          | TAC         | H          | -         | L      |
| ND1         | 1117-2786| 1670        | ATG         | TAA        | -         | H      |
| tRNA^Val    | 3844-3915| 72          | GAT         | H          | -         | L      |
| ND2         | 3914-3984| 71          | TTA         | H          | -         | L      |
| tRNA^Asp    | 3986-4054| 69          | CAT         | H          | -         | L      |
| ND3         | 4055-5101| 1046        | ATG         | T          | -         | H      |
| tRNA^Tyr    | 5100-5170| 70          | TCA         | H          | -         | H      |
| ND4         | 5172-5240| 69          | TGC         | H          | -         | L      |
| tRNA^Leu1   | 5242-5314| 73          | GTT         | H          | -         | L      |
| O2          | 5318-5347| 30          | -           | -          | -         | -      |
| tRNA^Gln    | 5348-5415| 68          | GCA         | L          | -         | L      |
| tRNA^Ala    | 5417-5487| 71          | GTA         | L          | -         | L      |
| COI         | 5489-7039| 1551        | GTG         | TAA        | -         | H      |
| tRNA^Val(UUCG) | 7040-7110 | 71          | TGA         | H          | -         | H      |
| tRNA^Asp    | 7114-7187| 74          | GTC         | H          | -         | H      |
| COII        | 7201-7891| 691         | ATG         | T          | -         | H      |
| tRNA^Leu(UUG) | 7892-7967 | 76          | TTT         | H          | -         | H      |
| ATPase8     | 7969-8133| 165         | ATG         | TAG        | -         | H      |
| ATPase6     | 8127-8810| 684         | ATG         | TAA        | -         | H      |
| COIII       | 8810-9594| 785         | ATG         | TAA        | -         | H      |
| tRNA^Leu2   | 9594-9664| 71          | TCC         | H          | -         | H      |
| ND3         | 9665-10015| 350        | ATG         | TAG        | -         | H      |
| tRNA^Glu    | 10014-10082| 69       | TCG         | H          | -         | H      |
| ND4L        | 10083-10379| 297      | ATG         | TAA        | -         | H      |
| ND4         | 10373-11751| 1379     | ATG         | TAG        | -         | H      |
| tRNA^Gl    | 11755-11823| 69       | GTG         | H          | -         | H      |
| tRNA^Val(UUCG) | 11824-11891 | 68  | GCT         | H          | -         | H      |
| tRNA^Leu(UAG) | 11893-11965| 73 | TAG         | H          | -         | H      |
| ND5         | 11966-13801| 1836     | ATG         | TAA        | -         | H      |
| ND6         | 13798-14319| 522      | ATG         | TAA        | -         | H      |
| tRNA^Glu    | 14320-14388| 69 | ATG         | TAG        | -         | L      |
| Cyt b       | 14391-15531| 1141    | TCC         | L          | -         | L      |
| tRNA^Asp    | 15532-15603| 72 | TGT         | H          | -         | H      |
| tRNA^Glu    | 15603-15673| 71 | TGG         | L          | -         | L      |
| D-loop      | 15674-16608| 935     | -           | -          | -         | -      |

*a* and *l* indicate heavy and light strands, respectively.

**Figure 1.** The Maximum Likelihood tree inferred from the complete mitogenomes of nine species.
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