The complete mitochondrial genome of *Aspiorhynchus laticeps* and its phylogenetic analysis

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**Abstract**

The complete nucleotide sequence of the mitochondrial genome (mitogenome) of *Aspiorhynchus laticeps* was determined. The length of the complete mitochondrial DNA sequence of *A. laticeps* is 16,591 bp, which consists of 13 protein-coding genes, 22 transfer RNA (tRNA) genes, two ribosomal RNA (rRNA) genes, and a non-coding region 'D-loop'. Except for the D-loop, another non-coding region named replication origin of L-strand (OL) region was also found. According to the phylogenetic analysis, *A. laticeps* has a closer relationship with *Schizothorax*.

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**Keywords:** Mitochondrial genome, Aspiorhynchus laticeps, Phylogenetic analysis

**Introduction**

Mitochondrial DNA (mtDNA) is the only genetic material outside the nuclear DNA. It is characterized of small molecular weight, simple construction, fast evolutionary rate, maternal heredity and non-tissue specificity (Currie and Kocher, 1999). To date, the mtDNA is widely used in analyzing genetic relationship of animal or plant, population differentiation and colonial diversity (Knudsen et al., 2006; Lavoue et al., 2007; Morin et al., 2010; Stoneking and Soodyall, 1996).

*Aspiorhynchus laticeps* (Cypriniformes, Cyprinidae, Schizothorax subfamily) distributes only in Tarim River, China. This species is not only a Chinese specialty fish, but also included in the world's valuable species. It has a high economic and academic value (Bain and Zhang, 2001). However, due to the weak awareness of animal protection and overfishing, the population resources of *A. laticeps* have extremely declined, and are on the verge of extinction. Currently, there are a few studies on *A. laticeps*, which mainly...
focus on physiology, biochemistry, artificial propagation, resource survey and protection (Ren et al., 2007; Yi, 2001; Zhang et al., 2008). In this study, PCR amplification and DNA sequencing methods were used to determine the *A. laticeps* mitochondrial genome sequence. Structural and evolutionary analyses were also performed. The results will provide basic data for further studies such as population genetic diversity research of this species.

**Materials and methods**

*Sample source and DNA extraction*

*A. laticeps* samples were collected from Tarim River (Xinjiang, China). All specimens were preserved in 100% ethanol and stored at 4 °C before DNA extraction. Total genomic DNA was obtained by phenol–chloroform extraction from the fin tissue of *A. laticeps* and stored at −20 °C.

*Mitochondrial DNA amplification and sequencing*

As shown in Table 1, eight pairs of PCR primers were designed based on mtDNA sequences of *Schizothorax macropogon* (KC020113), *Schizothorax waltoni* (JX202592), *Schizothorax biddulphi* (JQ844133), *Carassius auratus* (JN105355), *Labeo rohita* (JN412817), *Cyprinus carpio* (JX188253), and *Squaliobarbus curriculus* (KC351187). PCR reactions were performed according to the reference protocol (Kong et al., 2009). The PCR products were firstly detected by visualization in a 1% agarose gel electrophoresis, and then sequenced by using DNA Sequencer (ABI 377) from BGI Inc. (Shenzhen, China).

*Sequence assembly and annotation*

The DNA fragment was preliminary analyzed by using Sequencing Analysis v3.4.1 (Applied Biosystems) and Seqman v5.05 (DNASTAR). DNA sequence alignment and fragment assembly were performed using ClustalX v1.81 (Thompson et al., 1997). Transfer RNAs were identified using tRNAscan-SE 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/) (Lowe and Eddy, 1997). The position of 13 protein-coding genes and two rRNA genes was identified by sequence homology analyses to other known Cypriniformes mitochondrial sequences in GenBank. Nucleotide composition was calculated by MEGA 4.0.

**Table 1**
The primer sequences.

| Name  | Sequences (5′–3′)                  |
|-------|-----------------------------------|
| YUM-F1| AGCCACACCCCCAAGGAAT               |
| YUM-R1| ACAGATAGAAAACGGTGCTGG             |
| YUM-F2| CTGGATGTGGATCAGAGACATC            |
| YUM-R2| AGTACGGATGTCGCCTGG               |
| YUM-F3| CCTACTAGAGGAGGAGGCC              |
| YUM-R3| CTTYCTAGYGGGCTCTTC               |
| YUM-F4| GGCAGGGNNTDTYTAAGYGAACA          |
| YUM-R4| TTTCCCTTTGCTTTAACACAAGA          |
| YUM-F5| TCYATCTAYTGAGGCTCA               |
| YUM-R5| GCACCAAGRCTTTTTGCTCTT           |
| YUM-F6| CATCCCGTGCTTACAGAACC            |
| YUM-R6| AGGRTAGGGCTACGGCGTT              |
| YUM-F7| ATYATYGAAGCCCTAAACACCTC         |
| YUM-R7| CTCCAAAGCCAGAATCTAAAA           |
| YUM-F8| AAGCATCGTCGTTGTAATCCGAAGA       |
| YUM-R8| TAACCCGGGTCGCTCGCAC             |
Phylogenetic analysis

The 13 protein-coding genes from 21 Cypriniformes and three Perciformes species were downloaded from GenBank (Table 3). All the sequences were concatenated and aligned using ClustalX 2.1. Phylogenetic analyses were performed using Neighbor-Joining (NJ) in MEGA 4.0 (Tamura et al., 2007) and Maximum Likelihood (ML) in PhyML 3.0 (Guindon et al., 2010). The bootstrap of NJ and ML was 1000.

Results and discussion

Genome structure of A. laticeps mtDNA

As indicated in Table 2, the length of the complete mitochondrial DNA sequence was 16,591 bp, consisting of 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a non-coding region ‘D-loop’ (GenBank accession number: KF564793). Except for the D-loop, another non-coding region named replication origin of
Table 3
List of taxa in the phylogenetic analyses with their GenBank accession numbers.

| Species                        | GenBank accession number | Reference            |
|--------------------------------|--------------------------|-----------------------|
| Niwaella delicata              | AP009308                 | Saitoh et al. (2010)  |
| Misgurnus nikolskyi            | AB242171                 | Saitoh et al. (2006)  |
| Pygocentrus nattereri          | AP012000                 | Nakatani et al. (2011)|
| Cohitis striata                | AB054125                 | Saitoh et al. (2003)  |
| Chalceus macrolepidotus        | AB054130                 | Saitoh et al. (2003)  |
| Hypophthalmichthys nobilis    | HM162839                 | Unpublished           |
| Ctenopharyngodon idella        | JQ231115                 | Unpublished           |
| Cyprinus carpio                | JX188254                 | Unpublished           |
| Xenocypris davidi              | KF039718                 | Unpublished           |
| Labeo calbasu                  | JQ231113                 | Unpublished           |
| Carassius auratus              | GQ303444                 | Komiyama et al. (2009)|
| Hypophthalmichthys molitrix   | EUJ15941                 | Unpublished           |
| Megalobrama amblycephala       | EU434747                 | Unpublished           |
| Mylopharyngodon piceus         | EU979305                 | Wang et al. (2012)    |
| Rhodeus ocellatus              | DQ026430                 | He et al. (2008)      |
| Acheilognathus macropterus     | EF483935                 | Unpublished           |
| Pseudorasbora parva            | JF802126                 | Unpublished           |
| Coreius heterodon              | JF906110                 | Xu et al. (2013)      |
| Schizothorax macropogon        | KC020113                 | Zhu et al. (2013)     |
| Spinibarbus denticulatus       | KC852197                 | Unpublished           |
| Lateolabrax japonicus          | JQ860199                 | Unpublished           |
| Schizothorax biddulphi         | JQ844133                 | Gong et al. (2012)    |
| Oreochromis niloticus          | GU370126                 | He et al. (2011)      |
| Pagrus major                   | AP002949                 | Unpublished           |

Fig. 1. The predicted structure of the ‘OL’ region.
L-strand (OL) region was also found. The “OL” region (CTTTTCCCGCCGCCTGCCTCAGTGAGGCGGGAA) is 33 bp in length and has the potential to fold into a stem-loop secondary structure (Fig. 1). The size, location and order of genes in *A. laticeps* mtDNA sequence were consistent with those of the other bony fishes.

### Non-coding region

The major non-coding sequence of the *A. laticeps* mitochondrial genome is D-loop, which is 940 bp in length. tRNAPro and tRNAPhe genes are at two ends of the D-loop, respectively. Previous reports demonstrated that the conserved sequence region exists at D-loop control region in vertebrate mitochondrial genome, which is the DNA polymerase and RNA polymerase binding site for transcription and replication of DNA (Foran et al., 1988; Shadel and Clayton, 1997). By analyzing the *A. laticeps* D-loop sequence, we found that its conserved sequence is

**Table 4**
The alignment of the D-loop with other carps.

| Species                  | Subject IDs | % identity | Alignment length | e value | Bit score |
|--------------------------|-------------|------------|------------------|---------|-----------|
| Procypris rabaudi        | gi|154818372| 91.55          | 852     | 0         | 1155      |
| Percocypris pingi        | gi|396580971| 89.38          | 923     | 0         | 1144      |
| Cyprinus carpio          | gi|168693379| 88.77          | 926     | 0         | 1112      |
| Puntius snyderi          | gi|429128434| 88.79          | 928     | 0         | 1110      |
| Schizothorax oconnori    | gi|459926856| 88.49          | 930     | 0         | 1098      |
| Schizothorax macropogon  | gi|428674415| 88.16          | 929     | 0         | 1081      |

![Table 4](image)

**Fig. 2.** The consensus phylogenetic relationship of *Aspiorhynchus laticeps* with the other species from Maximum Likelihood (ML) analyses. The numbers on the branches are bootstrap values for ML.
very similar to the sequences of conserved region in other carps (Table 4). Moreover, the position and size of the “OL” region of A. laticeps are also similar to those of other Cyprinids. For example, the length is 33 bp in S. macropogon, 32 bp in Hypophthalmichthys nobilis, and 33 bp in C. auratus. And their “OL” regions are all located between the tRNA-Asn and tRNA-Cys genes (Table 2).

tRNA and rRNA genes

The 12S and 16S rRNA genes in A. laticeps mtDNA were 958 bp and 1676 bp, respectively. Similar to other Cyprinidae, the rRNA gene composition was 34.4% A; 24.4% C; 21.2% G; 20.0% T (Wang et al., 2008). The sequence analysis revealed that there are 22 tRNA genes in A. laticeps mtDNA, which are dispersed in the mitochondrial genome and ranged from 69 to 76 bp in length (Table 2). Among these tRNA genes, tRNALeu, tRNAAln, tRNAGln, tRNACys, tRNATyr, tRNAGlu and tRNAPhe are located on the L chain and the others on the H chain. Except for tRNASer gene, all of the other tRNA genes can be folded into the typical cloverleaf structure.

Protein-coding genes

The size and positioning of 13 protein-coding genes in A. laticeps mtDNA are consistent with those of the other bony fishes. As shown in Table 2, all these protein coding gene sequences started with an ATG.

Fig. 3. The consensus phylogenetic relationship of Aspiorhynchus laticeps with the other species from Neighbor-Joining (NJ) analyses. The numbers on the branches are bootstrap values for NJ.
codon, except for CO1 gene (started with GTG). TNN stop codon is used in A. laticeps mitochondrial genome, in which TAA is the most commonly used. For instance, there are 5 genes (ND1, CO1, ND4L, ND5 and ND6) with TAA stop codon, while the other genes have the T and the secondary structure of the tRNA as a translation termination. ND2 and ND3 genes use the next tRNA to constitute a termination codon TAG (Table 2). ND6 gene is unique in the L chain gene. There are some that overlap between adjacent tRNA genes in the mitogenomes of the other bony fishes. For example, ATP8 and ATP6 shared with 7 bp, ND4L and ND4 shared with 7 bp and ND5 and ND6 shared with 4 bp in the grass carp and the black carp, and ATP8 and ATP6 shared with 9 bp, ND4L and ND4 shared with 7 bp and ND5 and ND6 shared with 4 bp in the Nile tilapia and Blue tilapia (He et al., 2011; Wang et al., 2008, 2012). Similarly, there are three overlapping structures in A. laticeps mtDNA (ATP8 and ATP6 shared with 7 bp; ND4L and ND4 shared with 7 bp; ND5 and ND6 shared with 4 bp).

**Phylogenetic analysis**

Phylogenetic trees were constructed by using the NJ and ML methods (Figs. 2 and 3). As indicated by the tree, different species from the same family clustered together (e.g. Cyprinidae), and the species from Perciformes formed a monophyletic group. Throughout the phylogenetic analysis, A. laticeps has a closer relationship to Schizothorax, but is distantly related to Xenocypris and Hypophthalmichthys which have the higher level of specialization. So far, there has been neither a good reference taxonomy nor a comprehensive phylogenetic study that encompasses the whole spectrum of cypriniform diversity because of a wide variety of Cypriniformes (Saitoh et al., 2006). Thus, the mitochondrial genome data and phylogenetic analysis of the A. laticeps can enrich the evolution research of Cypriniformes.

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