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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*.

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 (Curole 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

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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	AGCCACACCCCCAAGGGAAT
YUM-R1	ACAGATAGAAACTGACCTGG
YUM-F2	CTCGATGTTGGATCAGGACATC
YUM-R2	AGTAGATGGATGCYCGCTGG
YUM-F3	CCTTACTAGAYGGGAAGGCC
YUM-R3	CTTYCTAGYGAGGCGTCTTC
YUM-F4	GCCCAGGNGTDTTYTAYGGACA
YUM-R4	TTTCCYTGGGGTTTAACCAAGA
YUM-F5	TCYATCTAYTGATGAGGCTCA
YUM-R5	GCACCAAGRGTTTTTGGTTCCT
YUM-F6	CATCCRTTGGTCTTAGGAACC
YUM-R6	AGGRTTAGGGCTCAGGCGTT
YUM-F7	ATYATYGAAGCCCTAAACACCTC
YUM-R7	CTCCCAAAGCCAGAATTCTAAA
YUM-F8	AAGCATCGGTCTTGTAATCCGAAGA
YUM-R8	TAACCGCGGTGGCTGGCAC

Table 2
Characteristics of the mitochondrial genome of *Aspiorhynchus laticeps*.

Locus	Position number		Size (bp)	Anticodon	Strand[a]	Codon	
	Start	Stop				Start	Stop
tRNA-Phe	1	69	69	GAA	H		
12S rRNA	70	1027	958		H		
tRNA-Val	1028	1099	72	TAC	H		
16S rRNA	1100	2775	1676		H		
tRNA-Leu	2776	2851	76	TAA	H		
ND1	2852	3826	975		H	ATG	TAA
tRNA-Ile	3831	3902	72	GAT	H		
tRNA-Gln	3971	3901	71	TTG	L		
tRNA-Met	3974	4042	69	CAT	H		
ND2	4043	5087	1045		H	ATG	T
tRNA-Sec	5088	5158	71	TCA	H		
tRNA-Ala	5229	5161	69	TGC	L		
tRNA-Asn	5303	5231	73	GTT	L		
O_L	5304	5336	33				
tRNA-Cys	5403	5337	67	GCA	L		
tRNA-Tyr	5473	5403	71	GTA	L		
CO1	5475	7025	1551		H	GTG	TAA
tRNA-Ser	7096	7026	71	TGA	L		
tRNA-Asp	7100	7171	72	GTC	H		
CO2	7185	7875	691		H	ATG	T
tRNA-Lys	7876	7951	76	TTT	H		
ATP8	7953	8117	165		H	ATG	
ATP6	8111	8793	683		H	ATG	TA
CO3	8794	9578	785		H	ATG	TA
tRNA-Gly	9579	9650	72	TCC	H		
ND3	9651	9999	349		H	ATG	T
tRNA-Arg	10,000	10,069	70	TCG	H		
ND4L	10,070	10,366	297		H	ATG	TAA
ND4	10,360	11,740	1381		H	ATG	T
tRNA-His	11,741	11,809	69	GTG	H		
tRNA-Ser	11,810	11,878	69	AGC	H		
tRNA-Leu	11,879	11,951	73	TAG	H		
ND5	11,955	13,778	1824		H	ATG	TAA
ND6	14,296	13,775	522		L	ATG	TAA
tRNA-Glu	14,365	14,297	69	TTC	L		
CYTB	14,370	15,510	1141		H	ATG	T
tRNA-Thr	15,511	15,582	72	TGT	H		
tRNA-Pro	15,651	15,582	70	TGG	L		
D-loop	15,652	16,951	940				

[a] H and L indicate genes transcribed on the heavy and light strands, respectively.

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)
Cobitis 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	EU315941	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	JQ860109	Unpublished
Schizothorax biddulphi	JQ844133	Gong et al. (2012)
Oreochromis niloticus	GU370126	He et al. (2011)
Pagrus major	AP002949	Unpublished

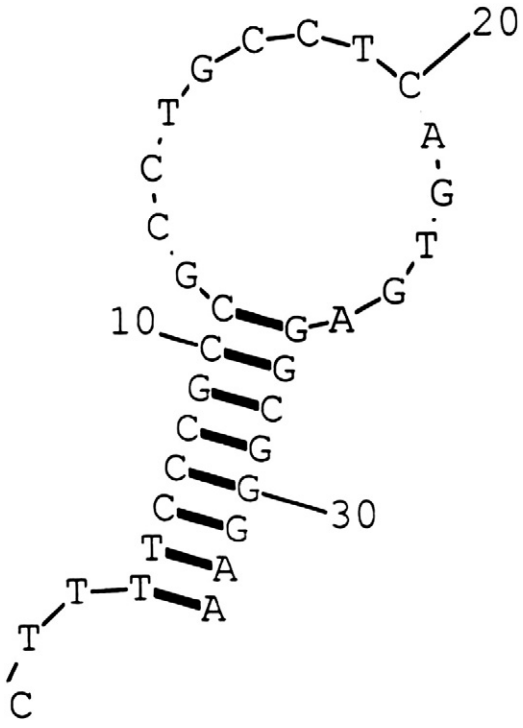

Fig. 1. The predicted structure of the 'OL' region.

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

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

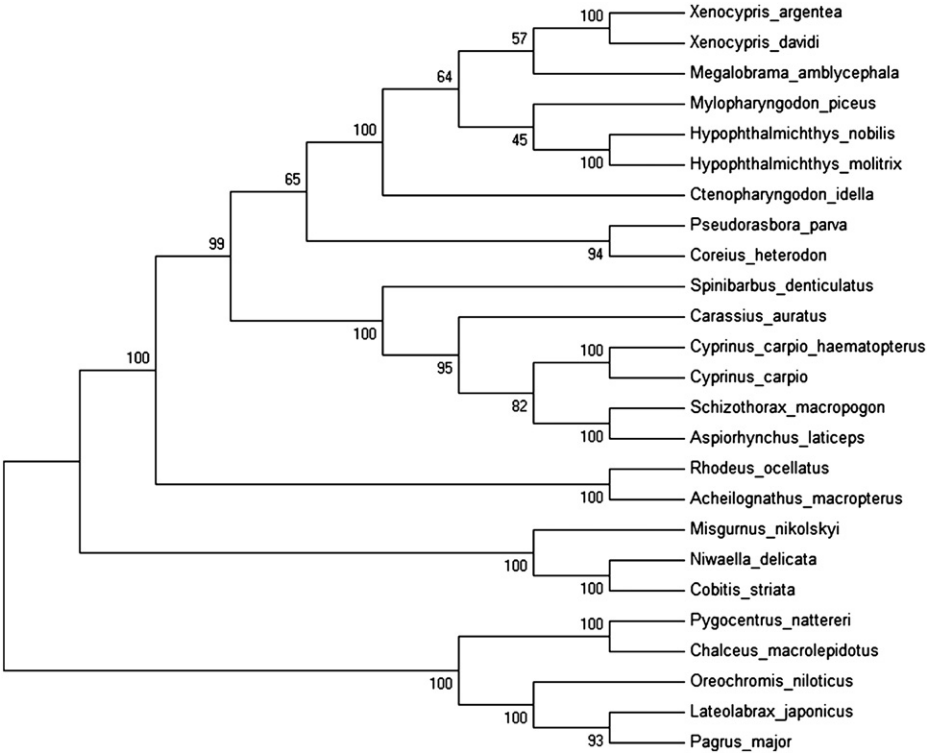

Fig. 2. The consensus phylogenetic relationship of *Aspiorhynchus laticeps* with the other species from Maximum Likelihood (ML) analyses. The numbers on the branches are bootstrap values for ML.

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, tRNAGln, tRNAAla, tRNAAsn, tRNACys, tRNATyr, tRNASer, tRNAGlu and tRNAPro 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

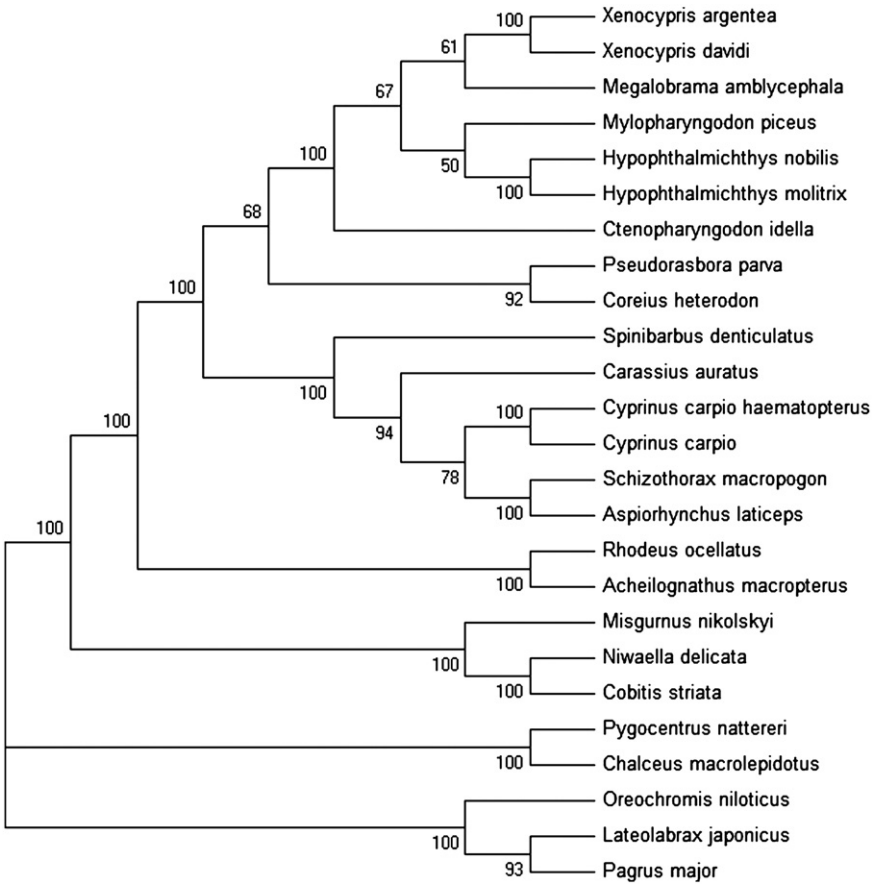

Fig. 3. The consensus phylogenetic relationship of *Aspiorhynchus laticeps* with the other species from Neighbor-Joining (NJ) analyses. The numbers on the branches are bootstrap values for NJ.

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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