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Complete Nucleotide Sequence of Mitochondrial Genome of Silver Carp, *Hypophthalmichthys molitrix*

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ABSTRACT

Silver carp (*Hypophthalmichthys molitrix)* of the family Cyprinidae, the largest family of the freshwater fishes, is one of the most important fish species in the world. The complete nucleotide sequence of the mitochondrial genome for silver carp was determined by overlapping and accurate polymorphism chain reaction. The genome of the silver carp is 16620 bp in size, containing 13 protein-coding genes (ND1-6, ND4L, COI-III, ATPase8, ATPase6, Cytb), 2 ribosomal RNAs (12S rRAN and 16S rRNA), 22 transfer RNAs (tRNAs) and a putative control region (D-loop). The codon usage also follows the typical vertebrate pattern (ATG) except for an unusual GTG start codon for the COI gene. The complete sequences of silver carp mitochondrial genome has been deposited in GenBank with accession No. EU315941 or NC_010156.

Keywords: genome composition, mtDNA, protein coding, tRNA

INTRODUCTION

The silver carp, *Hypophthalmichthys molitrix*, family Cyprinidae, genus *Hypophthalmichthys*, is native to eastern China and Siberia, and a wide alien species in the Europe, North America and South Asia, the most economically important freshwater species in the world. Its production reached 3.52 million tons in China and 4.15 million tons in the world in 2005 (FAO 2005).

Although the silver carp is one of the most valuable aquatic animals, relatively few scientific papers have been published for the species in genetic characteristics, comparing with it in biological characteristics. From late of 20th century, a few molecular genetics reports on this carp appeared using PCR-RFLP of mtDNA (Lu *et al.* 1997; Zhang *et al.* 2002), RAPD (Zhang *et al.* 2001) and microsatellite (Zhu *et al.* 2007). mtDNA is a double-strand circular molecule, approximately 16-18 kb in size in vertebrates, and has been widely used in the study on phylogenetic relationship and genetic structure at various taxonomic levels of aquatic animals (Chang *et al*. 1994; Cho *et al.* 2007; Mahidol *et al*. 2007; Moriya *et al.* 2007; Azuma *et al*. 2008), and the complete mtDNA genomes have been determined for approximately 220 fish species, but the complete mtDNA sequence of the silver carp has not yet determined. In this paper, the complete mitochondrial genome sequence of the silver carp is first reported. The aim is to provide the more sequence information for studies not only on population genetic structure of silver carp, but also on phylogenetic relationships of the Cyprinidae in future.

MATERIALS AND METHODS

Sample collection and DNA extraction

Three individuals with body weight of 400-500 g were collected in the lower stream of the Yangtze River in 2005. A small piece of caudal-fin clip from each individual was taken and stored in 95% ethanol. Whole genomic DNA including mtDNA and nDNA was extracted using a proteinase K and phenol-chloroform procedure. The quantity and quality of the extracted DNA were estimated on 1% agarose gels stained with ethidium bromide (EB).

Primer design, PCR amplification and sequencing

According to the alignment of the reported complete mtDNA sequences of common carp, *Cyprinus carpio* (Chang *et al*. 1994: GenBank accession No. NC_001606; Mabuchi *et al.* 2006: Gen Bank accession No. AP009047), crucian carp, *Carasius auratus* (Murakami *et al.* 1998: GenBank accession No. NC_002079) and tinca, *Tinca tinca* (Saitoh *et al.* 2006: GenBank accession No. AB218686), 18 pairs of primers were designed to amplify the complete mtDNA sequence of the silver carps (**Table 1**).

The polymerase chain reaction (PCR) was performed in an Eppendorf Thermal Cycler in a reaction mixture of 50 μl containing 2 μl DNA polymerase (Tiangen products, China), 5 μl PCR buffer (Tiangen products, China), 2 μl template DNA (50 ng/μl), 2 μl dNTP (0.4 mM), 4 μl primers (0.2 μM each), and 35 μl distilled water. The amplification conditions were 94°C for 5 min. This was followed by 30 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, and a final extension was done at 72°C for 10 min. The verification of successful PCR amplification was assessed by agarose gel electrophoresis.

All amplified products were purified using a 3S Spin PCR Product Purification Kit (Biocolor Inc., China) following the supplier's instructions. The purified products then were directly sequenced on an Applied Biosystems ABI 3730 capillary sequencer using the same PCR primers.

Sequence assembly and gene identification

DNA sequences were first used BLAST searches on Genbank, then edited and assembled using BioEdit software (Hall 1998) and checked by eyes. Finally, the complete mtDNA of the silver carp was obtained.

tRNA genes were identified using the tRNAscan-SE 1.21 (Lowe and Eddy 1997), using the default search mode and specifying mitochondrial/chloroplast DNA as the source and using the vertebrate mitochondrial genetic code for tRNA structure prediction. Protein and ribosomal RNA genes were determined by sequence similar comparing with mtDNA genomes of common carp, crucian carp and tinca. The 5′ end of protein-coding genes were

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Table 1 PCR and sequencing primers for silver carp.

Forward	Sequences (5′ to 3′)	Reverse	Sequences (5′ to 3′)
SA0	CATGCCGAGCATTCTTTT	SB0	GAGACTTGCATGTGTAAG
SA1	CAAAGCATAGCACTGAAGATGC	SB1	TTTTGACAGGGGAGAGTGA
SA2	CCA GCC TAT ATA CCG CCG T	SB2	CTA TCA CCA GGT TCG GTA GG
SA3	TTAGCCAGTACACCCAAGCA	SB3	AAAGACAAGTGATTGCGCT
SA4	AAGGAACTCGGCAAACACAA	SB4	TGG TGC TCA TAA GGT TAT GG
SA5	CCATCCACATCATCCCCA	SB5	ATT GGC GGA GGA GGG ACT TT
SA6	CCCTACCAATTGCACTAGCA	SB6	GTTTGTAGGATCGAGGCCTT
SA7	AGACCAAAAGCCTTCAAAGC	SB7	TGTGGCTAATCAGCTAAA
SA8	TCCGCAACAATAATTATCGC	SB8	TGGGACTGCGTCCATTTTTA
SA9	CACCCAACGCAACTAGGT(A)TT	SB9	GGCTTGCAAATTGGTCGAA
SA10	CAAATGAACCAACCCCAGTA	SB10	TGCAATTGTGAAGGGTGCTT
SA11	CTTCACAATTGCAGAT(C)GGG(A)G	SB11	AGCTG(A)AAATGTACGGGTGTC
SA12	AGAATGAGCAGAATAGGGA	SB12	TTCGTTCATAGGCTGTGTT
SA13	ATCAATTTGCCTCCGACA	SB13	GATTTGTTGAATTTCTCAGG
SA14	ATGATGACAT(C)GGACGA(G)GCAG	SB14	GCGGCTGATTGT(A)CCTAGA(G)GT
SA15	AGCAGCCCTCA(C)TA(C)GTAACAA	SB15:	TTGAATAACAACGGTGGTTC
SA16	CTTGCTCAGACTTTAACCGA	SB16	GTTTAGAATTCTGGCTTTGG
SA17	GGTCTTGTAATCCGAAGATC	SB17	GGG GTT TGA CAA GGA TAA

Table 2 Organization and profile of the mitochondrial DNA of silver carp.

	Position number		Size	Codon		Intergenic	Strand
	Start	Stop	(bp)	Start	Stop	nucleotide	
$tRNA^{Phe}$	1	69	69				H
12SrRNA	70	1031	962			0	H
$tRNA^{Val}$	1032	1103	72			0	H
16S rRNA	1104	2794	1691			0	H
$tRNA^{Leu(UUR)}$	2795	2870	76			1	H
ND1	2872	3846	975	ATG	TAA	4	H
$tRNA^{Ile}$	3851	3922	72			-2	H
$tRNA^{Gln}$	3921	3991	71			1	L
$tRNA^{Met}$	3993	4061	69			0	H
ND2	4062	5108	1047	ATG	TAG	-2	H
$tRNA^{Trp}$	5107	5177	71			1	H
$tRNA^{Ala}$	5179	5247	69			1	L
$tRNA^{Asn}$	5249	5321	73			32	L
$tRNA^{Cys}$	5354	5421	68			-3	L
$tRNA^{Tyr}$	5423	5493	71			1	L
CO I	5495	7045	1551	GTG	TAA		H
$tRNA^{Ser(UCN)}$	7046	7116	71			3	L
$tRNA^{Asp}$	7120	7193	74			13	H
CO II	7207	7897	691	ATG	T++	1	H
$tRNA^{Lys}$	7898	7973	76			1	H
ATPase8	7975	8142	168	ATG	TAA	-10	H
ATPase6	8133	8816	684	ATG	TAA	-1	H
CO III	8816	9601	786	ATG	TAA	-1	H
$tRNA^{Gly}$	9601	9673	73			0	H
ND3	9674	10024	351	ATG	TAG	-2	H
$tRNA^{Arg}$	10023	10091	69			-2	H
ND4L	10092	10388	297	ATG	TAA	-8	H
ND4	10382	11764	1383	ATG	TAA	-1	H
$tRNA^{His}$	11764	11832	69			0	H
$tRNA^{Ser(AGN)}$	11833	11901	69			1	H
$tRNA^{Leu(CUN)}$	11903	11975	73			0	H
ND5	11976	13811	1836	ATG	TAA	-4	H
ND6	13808	14329	522	ATG	TAA	0	L
$tRNA^{Glu}$	14330	14398	69			4	L
Cytb	14403	15542	1141	ATG	T++	1	H
$tRNA^{Thr}$	15544	15615	72			-1	H
$tRNA^{Pro}$	15615	15684	70			0	L
D-loop	15685	16620	936			0	H

inferred to be at the first start codon (ATG, GTG, TTG and GTT) and the 3′ end termini were inferred to be at the stop codon (TAA, TAG, AGA and AGG), In some genes, T or TA nucleotide adjacent to the beginning of a downstream gene were designated as the truncated codon and assumed to be completed by polyadenylation after transcript cleavage (Ojala *et al.* 1980).

RESULTS AND DISCUSSION

Genome organization and composition

The organization of mitochondrial genome in the silver carp is shown in **Fig. 1**. The arrangement order of the silver carp mtDNA genes is consistent with other Cyprinidae fishes, such as common carp (Chang *et al.* 1994; Mabuchi *et al.* 2006), crucian carp (Murakami *et al.* 1998; Guo *et al.* 2007), and tinca (Saitoh *et al.* 2006). Meanwhile, some nucleotides

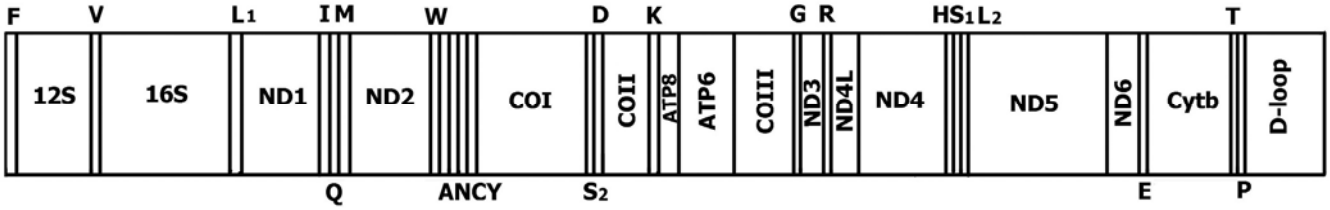

Fig. 1 The mitochondrial genome of silver carp. H-strand is designated on the upper side and L-strand on the lower side of the molecule. 12S and 16S, genes of the 12S and 16S ribosomal RNA; ND1-6 and 4 L, nicotinamide adenine dinucleotide dehydrogenase subunits 1-6 and 4 L; COI-III, cytochrome *c* oxidase subunits I-III; ATP6 and ATP8, ATPase subunits 6 and 8; cyt*b*, cytochrome *b*; CR, control region; tRNA are designated by single-letter amino acid codes except leucine and serine are labeled as L_1($tRNA^{Leu(UUR)}$), L_2 ($tRNA^{Leu(CUN)}$), S_1($tRNA^{Ser(AGN)}$) and S_2($tRNA^{Ser(UCN)}$).

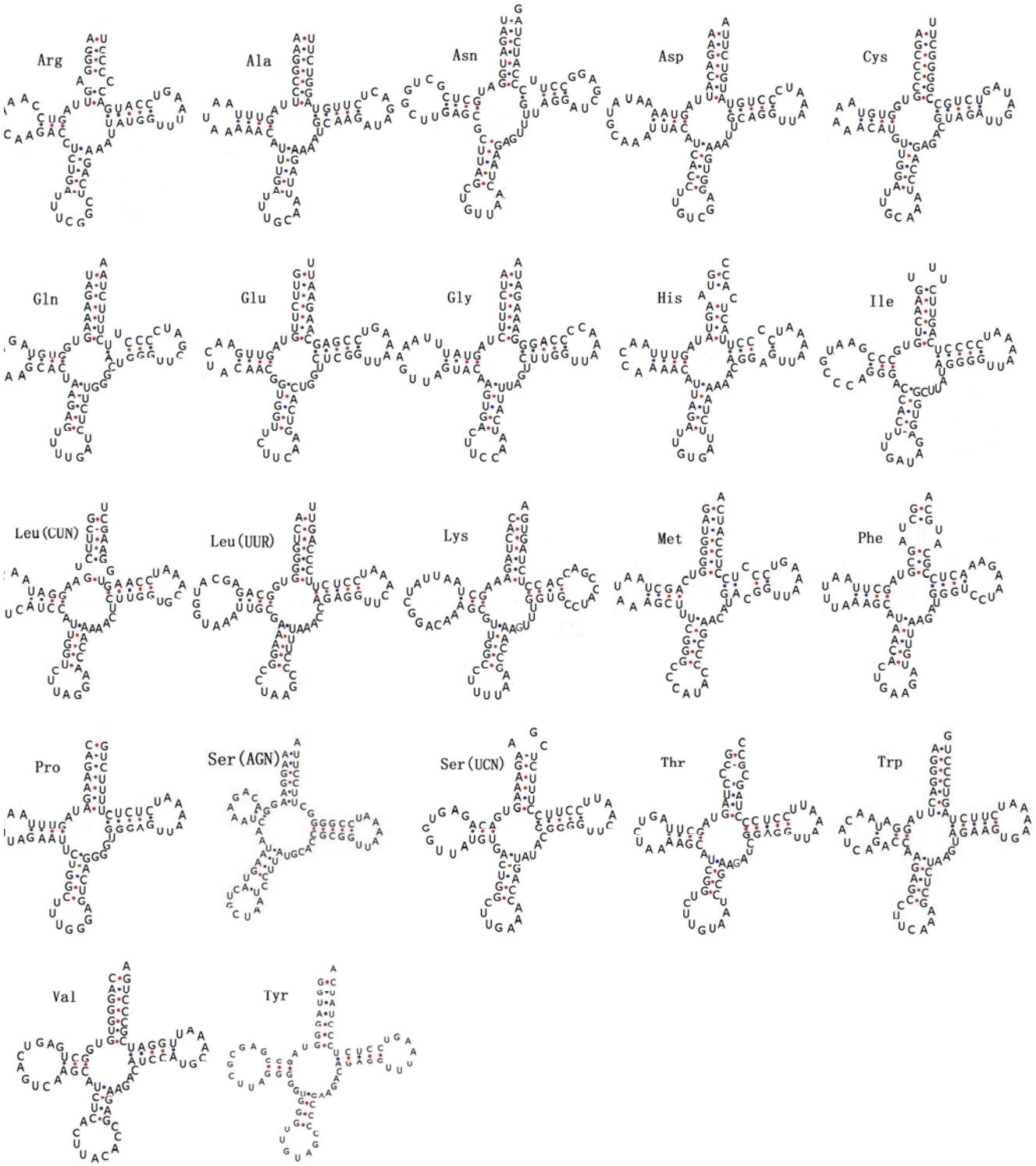

Fig. 2 The putative secondary structure of 22 tRNAs in the mitochondrial genome of silver carp.

overlaps in adjacent genes were also observed as the other fishes (**Table 2**). The complete sequences of silver carp mitochondrial genome was deposited in GenBank with accession No. EU315941 or NC_010156.

Protein-coding genes

As with other Cyprinidae fishes, the mtDNA protein-coding genes of the silver carp showed a regular start codon ATG except for the COI gene, which starts with GTG (**Table 2**). The ends of the open reading frames (ORFs) for protein-coding genes are signaled with TAA (ND1, COI, ATPase8, ATPase6, COIII, ND4L, ND5 and ND6), TAG (ND2), and incomplete stop codon T- (COII, Cytb). The incomplete stop codons are quite common among mtDNA genes in other fish (Peng *et al*. 2006; Kartavtsev *et al*. 2007). 12 protein-coding genes are encoded by the H-strand of the mtDNA, only ND6 is encoded by the L-strand (**Table 2**). Three cases of reading-frame overlap on the same strand are observed in silver carp mtDNA. The ATP8 and ATP6 genes share 10 nucleotides, ATP6 and COIII genes share 1 nucleotide, ND4 and ND4L genes share 7 nucleotides. In addition, the ND5 and ND6 genes, which are located in the H and L strands respectively, have an overlap of 4 nucleotides.

Non-coding sequence

The major non-coding sequence in mtDNA is called the control region, also known as the D-loop. As occurs in some other Cyprinidae fishes, the control region (D-loop) of silver carp is located between $tRAN^{Pro}$ and $tRAN^{Phe}$, and is 936 bp long.

In silver carp, a small non-coding region, a putative origin of light strand replication (O_L), is located in a cluster of $tRAN^{Trp}$ - $tRAN^{Ala}$ - $tRAN^{Asn}$ - $tRAN^{Cys}$ - $tRAN^{Tyr}$ region (WANCY region) and comprised with 42 nucleotides in length.

Ribosomal and transfer RNA genes

In most fishes, genes encoding rRNA are typically located between $tRNA^{Phe}$ and $tRAN^{Phe(UUR)}$, 12SrRNA is located between $tRNA^{Phe}$ and $tRNA^{Val}$, and 16SrRNA is located between $tRNA^{Val}$ and $tRAN^{Phe(UUR)}$.

Twenty-two tRNA genes are interspersed by the rRNA and protein-encoding genes and their length range from 68 to 76 bp. The secondary structure of 22 tRNA genes was a typical clover-leaf structure by using the tRNAscan-SE 1.21 (**Fig. 2**).

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