Mitochondrial DNA, 2012; Early Online: 1–3

informa
healthcare

MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of the tiger tail seahorse, *Hippocampus comes* (Teleostei, Syngnathidae)

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(*Received 18 October 2012; accepted 26 October 2012*)

Abstract

The complete mitochondrial genome of the tiger tail seahorse was sequenced using a polymerase chain reaction-based method. The total length of mitochondrial DNA is 16,525 bp and includes 13 protein-coding genes, 2 ribosomal RNA, 22 transfer RNA genes, and a control region. The mitochondrial gene arrangement of the tiger tail seahorse is also matching the one observed in the most vertebrate creatures. Base composition of the genome is A (32.8%), T (29.8%), C (23.0%), and G (14.4%) with an A + T-rich hallmark as that of other vertebrate mitochondrial genomes.

Keywords: Hippocampus comes, *complete mitochondrial genome, tiger tail seahorse*

The seahorse genus *Hippocampus* (family Syngnathidae) comprises about 54 species of small- to medium-sized fishes which inhabit mainly in the coral reef areas (Froese and Pauly 2012). The discoveries of new species (Kuiter 2001, 2003; Lourie and Randall 2003; Lourie and Kuiter 2008; Gomon and Kuiter 2009; Randall and Lourie 2009; Foster and Gomon 2010) might suggest that the diversity is higher than expected. Because of the aquarium trade and medicine use in Asia countries, the populations have been over explored and all species of *Hippocampus* are treated in Class II in the categories of the International Union for Conservation of Nature (IUCN). The tiger tail seahorse (*Hippocampus comes*), which inhabits the South China Sea, is one of the target species for traditional medicine and the aquarium trade (Lourie et al. 2004; Morgan and Lourie 2006). The species is diagnosed by having a combination of following characters: trunk rings 11; tail rings 34–37 (modally 35–36); 2 trunk rings and 1 tail ring support the dorsal fin; 17–19 (18) dorsal-fin rays; 16–19 (17) pectoral-fin rays; 1 relatively low coronet with 5 knobs; 1 prominent nose spine; 2 cheek spines; whitish radiations around eye; and alternative black and white rings on the tail (Lourie et al. 1999, 2004; Morgan and Lourie 2006). The adults are found to be nocturnal with a small home range among corals and are suggested to be monogamous (Perante et al. 2002).

In this study we sequenced the complete mitochondrial genome of the tiger tail seahorse (GenBank accession number JX970937). The tiger tail seahorse specimen was purchased from the aquarium shop, Taipei City, Taiwan, and was deposited in the National Museum of Marine Biology & Aquarium, Taiwan, with the specimen number NMMB-P17187.

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ISSN 1940-1736 print/ISSN 1940-1744 online
DOI: 10.3109/19401736.2012.744983

Table I. Mitochondrial genome of the tiger tail seahorse, *H. comes*.

Gene	Position		Length (bp)	Codon		Intergenetic nucleotides*	Strand†
	From	To		Start	Stop[a]		
$tRNA^{Phe}$	1	71	71			–	H
12S rRNA	72	1010	939			0	H
$tRNA^{Val}$	1011	1083	73			0	H
16S rRNA	1084	2774	1691			0	H
$tRNA^{Leu(UUR)}$	2775	2848	74			0	H
ND1	2849	3823	975	ATG	TAA	0	H
$tRNA^{Ile}$	3825	3896	72			1	H
$tRNA^{Gln}$	3896	3966	71			−1	L
$tRNA^{Met}$	3968	4037	70			1	H
ND2	4038	5076	1039	ATG	T–	0	H
$tRNA^{Trp}$	5078	5147	70			1	H
$tRNA^{Ala}$	5149	5217	69			1	L
$tRNA^{Asn}$	5218	5290	73			0	L
O_L	5291	5326	36			0	–
$tRNA^{Cys}$	5327	5392	66			0	L
$tRNA^{Tyr}$	5393	5459	67			0	L
COI	5461	7014	1554	GTG	TAA	1	H
$tRNA^{Ser(UCN)}$	7016	7086	71			1	L
$tRNA^{Asp}$	7100	7167	68			13	H
COII	7172	7862	691	ATG	T–	4	H
$tRNA^{Lys}$	7863	7937	75			0	H
ATP8	7939	8106	168	ATG	TAA	1	H
ATP6	8097	8779	683	ATG	TA-	−10	H
COIII	8780	9563	784	ATG	T–	0	H
$tRNA^{Gly}$	9564	9633	70			0	H
ND3	9634	9982	349	ATG	T–	0	H
$tRNA^{Arg}$	9983	10,051	69			0	H
ND4L	10,052	10,348	297	ATG	TAA	0	H
ND4	10,342	11,722	1381	ATG	T–	−7	H
$tRNA^{His}$	11,723	11,791	69			0	H
$tRNA^{Ser(AGY)}$	11,792	11,859	68			0	H
$tRNA^{Leu(CUN)}$	11,862	11,934	73			2	H
ND5	11,935	13,770	1836	ATG	TAA	0	H
ND6	13,767	14,288	522	ATG	TAA	−4	L
$tRNA^{Glu}$	14,289	14,357	69			0	L
Cytb	14,362	15,502	1141	ATG	T–	4	H
$tRNA^{Thr}$	15,503	15,574	72			0	H
$tRNA^{Pro}$	15,574	15,644	71			−1	L
D-loop	15,645	16,525	881			0	–

* Numbers correspond to the nucleotides separating different genes. Negative numbers indicate overlapping nucleotides between contiguous genes; † H and L, respectively, denote heavy and light strands; [a]TA- and T– indicate incomplete stop codons.

The total DNA was extracted from a piece of fin tissue using a Quick Gene DNA tissue Kit S (Fujifilm, Tokyo, Japan). The mitochondrial genome was amplified with eight pairs of primers that were designed on the basis of the conserved regions of the mitochondrial genomes of *Hippocampus kuda* (NC_010272) and *Microphis brachyurus* (NC_010273). Polymerase chain reactions (PCRs) were carried out in a mixture with a final volume of 25 μl containing 10 ng template DNA, 5 μmol of each specific primer, 12.5 μl of Fast-Run™ Advanced *Taq* Master Mix (ProTech, Taipei, Taiwan), and distilled water. The thermal cycling began with one cycle at 94°C for 4 min; subsequently 35 cycles of denaturation at 94°C for 1 min, 50–60°C for 1 min, and 72°C for 1–5 min; and finally, a single extension step at 72°C for 10 min. PCR products were purified using a PCR DNA Fragments Extraction Kit (Geneaid, Taipei, Taiwan) and were sequenced on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster, CA).

The tiger tail seahorse had a similar mitochondrial genome structure to that of the most vertebrate animals, such as *Hippocampus trimaculatus*, and *Aphyocypris kikuchii* (Chang et al. 2012; Jang-Liaw et al. 2012). The complete mitochondrial genome of the tiger tail seahorse was 16,525 bp in size and consisted of 2 rRNA genes, 22 tRNA genes, 13 protein-coding genes, and a control region (D-loop) (Table I). The positions of all genes in the tiger tail seahorse mitochondrial genome were recognized by comparing with the homologous genes of other *Hippocampus* beings. The tiger tail seahorse mitochondrial genes, excluding eight tRNA genes

(tRNA^{Gln}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, tRNA$^{Ser(UCN)}$, tRNA^{Glu}, and tRNA^{Pro}) and *ND6* gene, were encoded on the heavy strand. The overall base composition of the entire genome is as follows: A (32.8%), T (29.8%), C (23.0%), and G (14.4%), that revealed an A + T (62.6%)-rich hallmark as that of other vertebrate mitochondrial genomes (e.g. Kawahara et al. 2008). In the mitochondrial RNA genes, the 2 rRNAs, 12S rRNA and 16S rRNA, located, respectively, between tRNA^{Phe} and tRNA^{Val} and between tRNA^{Val} and tRNA$^{Leu(UUR)}$, and the origin of L-strand replication (O_L) in tiger tail seahorse was between tRNA^{Asn} and tRNA^{Cys} within a cluster of five tRNAs (WANCY region, Table I) and had 36 bp in size. The tRNA genes ranged from 66–75 bp in size, and all of them, except for tRNA$^{Ser(AGY)}$, could fold into a distinctive cloverleaf secondary structure, which was estimated by the tRNAscan-SE v1.21 (Schattner et al. 2005). Among the mitochondrial protein coding genes, *ND5* was the longest (1836 bp), while *ATP8* was the shortest (168 bp). The usage of the start codon was mainly ATG in most of the mitochondrial protein-coding genes besides the *COI* gene employing the GTG; the usage of the stop codon was either complete, such as TAA or TAG, or incomplete, such as T– or TA-. The gene overlaps also could be observed between five pairs of the contiguous genes—tRNA^{Ile} and tRNA^{Gln} overlapped by 1 bp, *ATP8* and *ATP6* overlapped by 10 bp, *ND4L* and *ND4* overlapped by 7 bp, *ND5* and *ND6* overlapped by 4 bp, and tRNA^{Thr} and tRNA^{Pro} overlapped by 1 bp. Control region locating between tRNA^{Pro} and tRNA^{Phe} was 881 bp in size, and longer than it of the yellow seahorse (*H. kuda*, NC_010272) and three-spot seahorse (*H. trimaculatus*, JX682713).

Seahorses, which are materials for traditional Chinese medicine (TCM), were largely harvested from the wild habitats for satisfying the growing demand of the TCM market and, *H. comes*, consequently, have been officially listed under the Appendix II designation since 2004 by the Conservation on International Trade in Endangered Species (CITES). The data from this research can assist the future population genetic investigations, molecular taxonomy, and DNA barcoding in order to conserve seahorses and circumscribe the international trade of seahorse species which are on the margin of extinction.

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

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