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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of the three-spot seahorse, *Hippocampus trimaculatus* (Teleostei, Syngnathidae)

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Abstract
The complete mitochondrial genome of the three-spot seahorse was sequenced using a polymerase chain reaction-based method. The total length of mitochondrial DNA is 16,535 bp and includes 13 protein-coding genes, 2 ribosomal RNA genes, 22 transfer RNA genes, and a control region. The mitochondrial gene order of the three-spot seahorse also conforms to the distinctive vertebrate mitochondrial gene order. The base composition of the genome is A (32.7%), T (29.3%), C (23.4%), and G (14.6%) with an A + T-rich hallmark as that of other vertebrate mitochondrial genomes.

Keywords: Hippocampus trimaculatus, *complete mitochondrial genome, three-spot seahorse*

The family Syngnathidae comprises seahorses and pipefishes, including 52 genera and about 232 species. They were placed in the suborder Syngnathoidei with other families, including Pegasidae (seamoths), Solenostomidae (ghost pipefish), Aulostomidae (trumpet fish), Fistulariidae (cornetfishes), Macroranphosidae (snipefishes), and Centriscidae (shrimpfishes) of the order Gasterosteiformes (Nelson 2006). Within Syngnathidae, the genus *Hippocampus* contains 54 valid seahorse species (Froese and Pauly 2012). Seahorse and pipefishes were traded for traditional medicine, dried curios, and pets (Lourie et al. 2004). Because of their decline in wild population, the Conservation on International Trade in Endangered Species (CITES 2012) lists seahorse as threatened species in international trade in Appendix II. The IUCN currently treat one was endanger, and the remains were vulnerable or data deficient (IUCN 2012). Recent researches of seahorses and pipefishes included biogeography (Lourie and Vincent 2004), evolution (Wilson and Orr 2011), conservation and their behavior (Mobley et al. 2011), and aquaculture (Koldewey and Martin-Smith 2010). Nevertheless, the evolutionary relationships within Syngnathidae and among higher groups remain poorly known (Wilson and Orr 2011). The benefit of using complete mitochondrial genome sequences has been analyzed for higher taxonomic groups (Miya et al. 2001, 2003, 2005). However, none of any Syngnathid sequence was included. For this species, the newly added complete mitogenome sequence will provide useful information in resolving higher level relationships and on conservation genetics. Several synonyms of *Hippocampus trimaculatus* Leach, 1814 were used in formal literatures; they were listed as follows: *Hippocampus mannulus* Cantor, 1850; *Hippocampus kampylotrachelos* Bleeker, 1854; *Hippocampus manadensis* Bleeker, 1856; *Hippocampus planifrons* Peters, 1877; *Hippocampus dahlia* Ogilby, 1908; and *Hippocampus takakurae* Tanaka, 1916 (Lourie et al. 2004).

In this study, we sequenced the complete mitochondrial genome of the three-spot seahorse

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Table I. Mitochondrial genome of the three-spot seahorse *H. trimaculatus*.

Gene	Position		Length (bp)	Codon		Intergenetic nucleotides*	Strand†
	From	To		Start	Stop		
$tRNA^{Phe}$	1	71	71			–	H
12S rRNA	72	1009	938			0	H
$tRNA^{Val}$	1010	1082	73			0	H
16S rRNA	1083	2788	1706			0	H
$tRNA^{Leu(UUR)}$	2789	2861	73			0	H
ND1	2862	3836	975	ATG	TAG	0	H
$tRNA^{Ile}$	3838	3909	72			1	H
$tRNA^{Gln}$	3909	3979	71			−1	L
$tRNA^{Met}$	3981	4050	70			1	H
ND2	4051	5089	1039	ATG	T–	0	H
$tRNA^{Trp}$	5090	5161	72			0	H
$tRNA^{Ala}$	5163	5231	69			1	L
$tRNA^{Asn}$	5233	5305	73			1	L
$tRNA^{Cys}$	5342	5407	66			36	L
$tRNA^{Tyr}$	5408	5474	67			0	L
COI	5476	7029	1554	GTG	TAA	1	H
$tRNA^{Ser(UCN)}$	7030	7100	71			0	L
$tRNA^{Asp}$	7113	7180	68			12	H
COII	7185	7875	691	ATG	T–	4	H
$tRNA^{Lys}$	7876	7951	76			0	H
ATP8	7952	8119	168	ATG	TAA	0	H
ATP6	8110	8792	683	ATG	TA-	−10	H
COIII	8793	9576	784	ATG	T–	0	H
$tRNA^{Gly}$	9577	9646	70			0	H
ND3	9647	9995	349	ATG	T–	0	H
$tRNA^{Arg}$	9996	10,064	69			0	H
ND4L	10,065	10,361	297	ATG	TAA	0	H
ND4	10,355	11,735	1381	ATG	T–	−7	H
$tRNA^{His}$	11,736	11,804	69			0	H
$tRNA^{Ser(AGY)}$	11,805	11,872	68			0	H
$tRNA^{Leu(CUN)}$	11,875	11,947	73			2	H
ND5	11,948	13,783	1836	ATG	TAA	0	H
ND6	13,780	14,301	522	ATG	TAA	−4	L
$tRNA^{Glu}$	14,302	14,370	69			0	L
Cytb	14,375	15,515	1141	ATG	T–	4	H
$tRNA^{Thr}$	15,516	15,588	73			0	H
$tRNA^{Pro}$	15,588	15,657	70			−1	L
D-loop	15,658	16,535	878			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.

(GenBank accession number JX682713). The three-spot seahorse specimen was collected from Daxi Fishery Harbor, Yilan County, Taiwan and was deposited in the Biodiversity Research Center, Academia Sinica, Taiwan with the specimen number ASIZP0072960. 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 12 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 of template DNA, 5 μmol of each specific primer, 12.5 μl of Fast-Run™ PCR PreMix (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 1–3 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, USA).

The three-spot seahorse had a similar mitochondrial genome to that of the typical vertebrate. The complete mitochondrial genome of the three-spot seahorse was 16,535 bp in size, and had a similar mitochondrial gene order to that of typical vertebrate mitochondrial genomes, which contained 2 ribosomal RNA (*rRNA*) genes, 22 transfer RNA (*tRNA*) genes, 13 protein-coding genes, and a control region

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(D-loop) (Table I). Most of the three-spot 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 (32.7%), T (29.3%), C (23.4%), and G (14.6%), which revealed a A + T (62%)-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 RNA*, located, respectively, between *tRNA*^{Phe} and *tRNA*^{Val} and between *tRNA*^{Val} and *tRNA*$^{Leu(UUR)}$ and all the *tRNA*s ranging from 66 to 76 bp in size could fold into a distinctive cloverleaf structure. Among the mitochondrial protein-coding genes, the *ND5* was the longest (1836 bp), whereas the *ATP8* was the shortest (168 bp). The usage of the start codon was mainly ATG in the most of 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 could also 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 located between *tRNA*^{Pro} and *tRNA*^{Phe} was 878 bp in size, and slightly shorter than that of the yellow seahorse (*H. kuda*, NC_010272).

Seahorses, one of the most appealing of the 300 known species in the family Syngnathidae, are famous for their distinctive appearances (Wilson and Orr 2011) and feature in the traditional medicine as well as aquarium display and curiosities. All seahorses have been officially listed under the Appendix II designation since 2004 by the CITES on the grounds that many seahorse populations face the habitat destruction and overfishing pressure. The result of this study can give an impulse to the population genetic researches and molecular forensic in the future.

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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