

MITOGENOME ANNOUNCEMENT

Mitochondrial genome of longheaded eagle ray *Aetobatus flagellum* (Chondrichthyes: Myliobatidae)Jie Zhang^{1*}, Baojuan Yang^{2*}, Atsuko Yamaguchi³, Keisuke Furumitsu³ and Baowei Zhang²¹Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, ²School of Life Science, Anhui University, Hefei, China and ³Faculty of Fisheries, Nagasaki University, Nagasaki, Japan**Abstract**

The complete mitochondrial genome sequence of the *Aetobatus flagellum* is 20,201 bp long and consists of 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and 1 control region (CR). The base composition of the genome is 30.9% A, 28.2% T, 27.1% C and 13.8% G. Comparing mtDNA of elasmobranchs submitted in NCBI, our study not only identified the longest mitochondrial genome with 4490 bp CR in *A. flagellum*, but also strongly revealed that records in the northwest Pacific may belong to a separate species from those distributed in Indonesia.

Keywords*Aetobatus flagellum*, genome, mitochondrion**History**

Received 11 September 2013

Accepted 21 September 2013

Published online 7 January 2014

Aetobatus, one of the genera of the family, are benthopelagic batoids comprising three valid species of eagle rays in the family Myliobatidae (Oh et al., 2006). *Aetobatus flagellum* has been distributed disjunctly to Pakistan, India, Indonesia, southern China, Korea, southern Japan and possibly in the eastern Atlantic, where it is primarily found on the inner continental shelf (Compagno, 1999). Considering the limitations of life history parameters in myliobatid rays (including low fecundity) and very high level of fishing pressure in inshore regions, *A. flagellum* has been categorized as an endangered species (EN) by IUCN (White, 2006).

In this study, the complete mtDNA of *A. flagellum* was first amplified and sequenced by polymerase chain reaction (PCR) with 15 primer pairs. Nucleotide sequences were deposited in GenBank (Accession Number KF482070). The complete mitochondrial genome of *A. flagellum* is 20,201 bp, containing 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes and 1 CR. The nucleotide composition is 30.9% A, 27.1% C, 13.8% G and 28.2% T, and this composition shows a bias for A+T. The mitochondrial genes from *A. flagellum* are overlapped in a total of 27 bp at six locations and interleaved with a total of 224 bp intergenic spacers at 22 locations (Table 1).

Thirteen protein-coding genes for 3799 amino acids were identified. The longest gene is *ND5* with 1833 bp and the shortest is *ATP8* with only 168 bp. Eleven of the 13 protein-coding genes require ATG as the start codon, whereas *COI* and *ND6* utilize

GTG and TTA, respectively. The *COI* gene has GTG instead of ATG as a start codon, as numerous fish do in mitochondrial genomes (Miya & Nishida, 1999). Eight use TAA as stop codons. *ND6* ends with CAT; *COI* ends with TAG; and *CO2*, *ND3* and *ND4* use T as an incomplete stop codon. The 22 tRNA genes range in size from 75 bp in tRNA^{Leu} to 68 bp in tRNA^{Cys}, tRNA^{Trp} and tRNA^{Tyr}. The 12S and 16S rRNA genes are 952 and 1642 bp, respectively, and are located between the tRNA^{Phe} and tRNA^{Leu} genes and separated by the tRNA^{Val} gene. This species has the longest mitochondrial genome with 4490 bp CR, which lies between the tRNA^{Pro} and tRNA^{Phe} genes.

Mitochondrial genome sequences are very useful in determining the phylogenetic relationships among individuals, populations and even species (Irwin et al., 1991; Kocher et al., 1989). Studies on *A. narinari* have revealed that deep molecular differences are found among various geographic populations in this circum globally distributed species (Vincent et al., 2009). Through the comparison with the mtDNA sequences deposited in NCBI, the Chinese individuals used in this study share common COI or Cyt *b* sequences with those collected in Japan and Korea (Nos. FJ8122051, FJ8122041), but quite different from the *ND2* gene of the individual from Java, Indonesia (No. JQ518839). This revealed the high possibility of the existence of cryptic species in *Aetobatus* and this taxonomic clarity represents a key element to the urgent conservation of elasmobranchs in the northwest Pacific.

*These authors contributed equally to this work.

Table 1. Characteristics of the *A. flagellum* mitochondrial DNA genome.

Gene name	Coding strand	Start position	End position	Intergenic nucleotides	Overlapping nucleotides	Size (bp)	No. of codons	Start codon	Stop codon
<i>tRNA^{Phe}</i>	H	1	69			69			
<i>12S rRNA</i>	H	72	1023	2		952			
<i>tRNA^{Val}</i>	H	1032	1103	8		72			
<i>16S rRNA</i>	H	1178	2748	74	3	11,642			
<i>tRNA^{Leu}</i>	H	2808	2882	59		75			
<i>ND1</i>	H	2883	3857			975	324	ATG	TAA
<i>tRNA^{Ile}</i>	H	3859	3926	1		70			
<i>tRNA^{Gln}</i>	L	3928	3999	1		72			
<i>tRNA^{Met}</i>	H	4000	4069			71			
<i>ND2</i>	H	4070	5116		2	1047	348	ATG	TAA
<i>tRNA^{Trp}</i>	H	5117	5184			68			
<i>tRNA^{Ala}</i>	L	5186	5254	1		69			
<i>tRNA^{Asn}</i>	L	5256	5328	1		73			
<i>tRNA^{Cys}</i>	L	5362	5429	33		68			
<i>tRNA^{Tyr}</i>	H	5435	5502	5		68			
<i>CO1</i>	H	5509	7062	6		1554	517	GTG	TAG
<i>tRNA^{Ser}</i>	L	7067	7137	4		71			
<i>tRNA^{Asp}</i>	H	7138	7208			71			
<i>CO2</i>	H	7212	7902	3		691	230	ATG	T -
<i>tRNA^{Lys}</i>	H	7903	7976			74			
<i>ATP8</i>	H	7978	8145	1		168	55	ATG	TAA
<i>ATP6</i>	H	8136	8819		10	684	227	ATG	TAA
<i>CO3</i>	H	8819	9604		1	786	261	ATG	TAA
<i>tRNA^{Gly}</i>	H	9613	9684	8		72			
<i>ND3</i>	H	9686	10,034	1		349	116	ATG	T -
<i>tRNA^{Arg}</i>	H	10,035	10,108			74			
<i>ND4L</i>	H	10,109	10,405			297	98	ATG	TAA
<i>ND4</i>	H	10,399	11,779		7	1381	460	ATG	T -
<i>tRNA^{His}</i>	H	11,780	11,848			69			
<i>tRNA^{Ser}</i>	H	11,850	11,918	1		69			
<i>tRNA^{Leu}</i>	H	11,921	11,992	2		72			
<i>ND5</i>	H	11,993	13,825			1833	610	ATG	TAA
<i>ND6</i>	L	13,822	14,343		4	522	173	TTA	CAT
<i>tRNA^{Glu}</i>	L	14,345	14,413	1		69			
<i>Cyt b</i>	H	14,416	15,558	2		1143	380	ATG	TAA
<i>tRNA^{Thr}</i>	H	15,565	15,638	6		74			
<i>tRNA^{Pro}</i>	L	15,643	15,711	4		69			
D-loop	H	15,712	20,201			4490			

Acknowledgements

The authors are grateful to Dr. Nong Zhang, Ms. Hui Wang, Xia Luo, Mr. Tao Pan and Xin Huang for their help with laboratory work and specimen collection.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing style of the article. This study was funded by the National Natural Science Foundation of China (Nos. 30970321 and 31272287), the JSPS Invitation Fellowship Program for Research in Japan and Graduate Student Academic Innovation Research Project of Anhui University (01001770-10117700141).

References

Compagno JV. (1999). The biology of elasmobranch. Baltimore: The Johns Hopkins University Press.

- Irwin DM, Kocher TD, Wilson AC. (1991). Evolution of the cytochrome b gene of mammals. *J Mol Evol* 32:128–44.
- Kocher TD, Thoms WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, Wilson AC. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc Natl Acad Sci USA* 86:6196–200.
- Miya M, Nishida M. (1999). Organization of the mitochondrial genome of a deep-sea fish, *Gonostoma gracile* (Teleostei: Stomiiformes): First example of transfer RNA gene rearrangements in bony fishes. *Mar Biotechnol* 1:416–26.
- Oh J, Kim S, Kim C-G, Soh HY, Jeong D, Lee YH. (2006). The first record of long headed eagle ray, *Aetobatus flagellum* (Pisces: Myliobatidae) from Korea. *Ocean Sci J* 41:53–7.
- Vincent PR, Marcy H, Wayne W, Mahmood SS. (2009). Species delineation and evolutionary history of the globally distributed spotted eagle ray (*Aetobatus narinari*). *J Hered* 100:273–83.
- White WT. (2006). *Aetobatus flagellum*. IUCN Red list of Threatened Species. Switzerland: Gland.