การวิเคราะห์และแก้ไขไมโทคอนเดรียลจีโนมของ *Rachycentron canadum* (Teleostei, Perciformes, Rachycentridae) ใหม่

Reanalysis and revision of the complete mitochondrial genome of *Rachycentron canadum* (Teleostei, Perciformes, Rachycentridae)

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บทคัดย่อ

ไมโทคอนเดรียลจีโนมของปลาช่อนทะเล (*Rachycentron canadum*) ได้ถูกวิเคราะห์และแก้ไขใหม่อีกครั้ง จีโนมมีความยาว 18,008 bp ประกอบด้วยยีนที่ถอดและแปลรหัสให้โปรตีน 13 ยีน ยีน *ribosomal RNA* (*rRNA*) 2 ยีน ยีน *translation RNA* (*tRNA*) 22 ยีน และส่วนของ control region (d-loop) การเรียงลำดับในจีโนมเหมือนกันกับที่พบในไมโทคอนเดรียลจีโนมของสัตว์ที่มีกระดูกสันหลังส่วนใหญ่ องค์ประกอบของนิวคลีโอไทด์เบสคือ A 30.14% C 25.22% G 15.80% และ T 28.84% ส่วนของ control region อุดมไปด้วย A + T และมีชุดนิวคลีโอไทด์ซ้ำ ๆ ได้แก่ TATATACATGG TATATGCACAA และ TATATGCACGG การศึกษาพบว่าไมโทคอนเดรียลจีโนมนี้แตกต่างจากจีโนมที่ตีพิมพ์ก่อนหน้านี้ในสองส่วนคือ ส่วน control region ไปจนถึงยีน *12S* และส่วนของยีน *ND5* ไปจนถึงยีน $tRNA^{Glu}$ นอกจากนี้ลำดับเบสของยีน *12S* ยังแตกต่างจากที่มีตีพิมพ์ในฐานข้อมูล การวิเคราะห์สายสัมพันธ์เชิงวิวัฒนาการพบว่าความแตกต่างดังกล่าวอาจเกิดจากความผิดพลาดในการเรียงลำดับสายดีเอ็นเอ หรือในการระบุชนิดพันธุ์ของตัวอย่างในการศึกษาก่อนหน้านี้

ABSTRACT

The complete mitochondrial genome of cobia, *Rachycentron canadum*, was reanalyzed and revised. The genome is 18,008 bp in length, containing 13 protein-coding genes, 2 *ribosomal RNA* (*rRNA*) genes, 22 *translation RNA (tRNA)* genes, and a control region (D-loop). The gene arrangement is identical to that observed in most vertebrates. Base composition on the heavy strand is 30.14% A, 25.22% C, 15.80% G, and 28.84% T. The D-loop region exhibits an A+T rich pattern, containing short tandem repeats of TATATACATGG, TATATGCACAA, and TATATGCACGG. The mitochondrial genome studied differs from the previously published genome in two segments; the control region to *12S*, and *ND5* to $tRNA^{Glu}$. The *12S* sequence also differs from those published in the databases. Phylogeny analyses revealed that the differences could be due to errors in sequence assembly and/or species identification of the previous studies.

คำสำคัญ: *Rachycentron canadum*, ปลาช่อนทะเล, ไมโทคอนเดรียลจีโนม

Keywords: *Rachycentron canadum*, cobia, mitochondrial genome

INTRODUCTION

Rachycentron canadum (cobia) is a single species of the family Rachycentridae. It is recognized as a commercially important species for aquaculture in many regions around the world, especially in the Asia Pacific region (FAO 2013). Cobia is also a pelagic fish, which is found to migrate over a long distance and hitchhike following other large marine animals. We interested to study 13 peptide encoding genes which are essential for oxidative phosphorylation — the process that produces a high energy compound during aerobic cell respiration in the cobia mitochondrial genome.

MATERIALS AND METHODS

Cobia sample was collected from the Andaman Sea. Genomic DNA was extracted from fin tissue using E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek Inc.). The mitochondrial genome was PCR amplified using 11 primer pairs, which were designed based on Miya et al. (2010) and the multiple alignment of carangids mitochondrial genomes (AB108498, AP003091, AP003092, AB517556, AB517557, AB517558, AB517559, AP001444, AP004445). The sequences were obtained by a primer walking technique using a total of 31 primers.

RESULTS AND DISCUSSION

The complete mitochondrial genome of cobia (GenBank accession number KC782769) is 16,008 bp in length containing 13 protein-coding genes, 2 *rRNA*s, 22 *tRNA*s, and 1 control region (D-loop). Most genes encode on the H-strand, except for *ND6* and eight *tRNA*s which encode on the L-strand (Table 1). The gene arrangement of the cobia mitochondrial genome is similar to that of other teleost mitochondrial genomes (Miya et al. 2003). However, the overall nucleotide composition of the H-strand slightly deviates from 25%, with 30.14% A, 25.22% C, 15.80% G, and 28.84% T, similar to what found in several teleosts (Ye et al. 2013; Jang-Liaw et al. 2013; Li et al. 2013; Tang et al. 2013).

Table 1 Characteristics of the mitochondrial genome of *R. canadum*.

Gene	Position		Start/ Anticodon	Strand	Gene	Position		Start/ Anticodon	Strand
	From	To				From	To		
tRNA^{Phe}	1	68	GAA	H	*tRNA*^{Lys}	7914	7988	TTT	H
12S rRNA	69	1021		H	*ATP8*	7992	8159	ATA	H
tRNA^{Val}	1021	1093	TAC	H	*ATP6*	8150	8833	ATG	H
16S rRNA	1095	2817		H	*COIII*	8833	9618	ATG	H
tRNA$^{Leu(UUR)}$	2816	2889	TAA	H	*tRNA*^{Gly}	9618	9689	TCC	H
ND1	2890	3864	ATG	H	*ND3*	9691	10041	ATG	H
tRNA^{Ile}	3885	3956	GAT	H	*tRNA*^{Arg}	10040	10108	TCG	H
tRNA^{Gln}	3956	4026	TTG	L	*ND4L*	10109	10405	ATG	H
tRNA^{Met}	4026	4094	CAT	H	*ND4*	10399	11779	ATG	H
ND2	4095	5139	ATG	H	*tRNA*^{His}	11780	11848	GTG	H
tRNA^{Trp}	5140	5211	TCA	H	*tRNA*$^{Ser(AGY)}$	11850	11917	GCT	H
tRNA^{Ala}	5213	5281	TGC	L	*tRNA*$^{Leu(CUN)}$	11920	11992	TAG	H
tRNA^{Asn}	5283	5355	GTT	L	*ND5*	11993	13831	ATG	H
O_L	5358	5382		L	*ND6*	13828	14349	ATG	L
tRNA^{Cys}	5383	5450	GCA	L	*tRNA*^{Glu}	14350	14417	TTC	L
tRNA^{Tyr}	5455	5524	GTA	L	*CYTB*	14423	15563	ATG	H
COI	5526	7073	GTG	H	*tRNA*^{Thr}	15564	15636	TGT	H
tRNA$^{Ser(UCN)}$	7076	7146	TGA	L	*tRNA*^{Pro}	15636	15706	TGG	L
tRNA^{Asp}	7149	7218	GTC	H	Control Region	15707	18008		H
COII	7223	7913	ATG	H					

The start codon of all protein-coding genes is ATN, except for that of *COI* is GTG. Seven genes show typical stop codons (TAA or TAG) while the other four genes possess incomplete stop codons with a terminal T, which is commonly found in teleost mitochondrial genomes (Tang et al. 2013). All but two *tRNA* genes ($tRNA^{Ser(AGY)}$ and $tRNA^{Glu}$) showed typical cloverleaf secondary structures predicted by tRNAscan-SE 1.21 (Schattner et al. 2005): $tRNA^{Ser(AGY)}$ and $tRNA^{Glu}$ were verified based on sequence similarity using DOGMA (Wyman et al. 2004). The origin of L-strand replication (O_L), which is 25 bp in length, is located within the WANCY region. The D-loop contains three types of short tandem repeats (TATATACATGG, TATATGCACAA, and TATATGCACGG), exhibiting an A+T rich pattern.

The mitochondrial genome studied differs from the previous published genome (FJ154956) in two segments; the control region to the first 437 bp of *12S*, and the last 468 bp of *ND5* to $tRNA^{Glu}$. Additionally, the sequence of *12S* also differs from other cobia *12S* previously published in the database (AF311947, FJ374798) with 17.9 — 20.0% nucleotide dissimilarity. The phylogenetic analyses of *12S* revealed a well bootstrap supported (99.6%) cluster between the cobia sequence obtained from this study and the sequences of its closely related species, *Coryphaenidae*, (FJ374794, FJ374797), showing 9.7% nucleotide divergence. The previously published sequences, on the other hand, were grouped with the species outgroup of the suborder Carangoidei: FJ154956 and AF311947 were grouped with *Mugil curema* (Mugiliformes, Mugilidae; JF911710, with 90.1% nucleotide identity), and FJ374798 was grouped with *Acanthocybium solandri* (Perciformes, Scombridae; DQ854648, with 100% nucleotide identity). The analyses indicated errors in sequence assembly and/or species identification of the previous studies.

CONCLUSION

Cobia mitochondrial genome is 18,008 bp in size. It consists of 13 peptide encoding genes which are essential for oxidative phosphorylation — the process that produces a high energy compound during aerobic cell respiration. Gene organization in cobia mitochondrial genome is generally the same as what is found in other vertebrates. However, cobia's ability to swim fast suggests that these organisms may have experienced selective pressures to increase aerobic capacity or metabolism. This information may have advantage to examine the possibility of positive selection on mitochondrial evolution of cobia and relative species.

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