การศึกษาจีโนมไมโทคอนเดรียของปลากัดป่าภาคกลาง *Betta splendens*

Mitochondrial Genome Analysis of Siamese Fighting Fish *Betta splendens*

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บทคัดย่อ

งานวิจัยนี้เป็นรายงานฉบับแรกในการศึกษาจีโนมไมโทคอนเดรียของปลากัดป่าภาคกลาง ซึ่งเป็นตัวแทนของอันดับย่อย Anabantoidei โดยจีโนมไมโทคอนเดรียมีขนาด 16,982 คู่เบส ประกอบด้วย 13 ยีน ที่แปลรหัสเป็นโปรตีน, 2 ยีนอาร์อาร์เอ็นเอ, 1 control region และ 22 ยีนทีอาร์เอ็นเอ ผลการวิเคราะห์ยีนที่แปลรหัสเป็นโปรตีนทั้งหมดพบว่า ปลากัดป่าภาคกลางมีความหลากหลายของโคดอนตั้งต้น (start codon) มากกว่าปลาในกลุ่มปลาช่อน *Channa argus* และ *C. maculata* ซึ่งเป็นชนิดใกล้เคียงกันอยู่ในอันดับย่อย Channoidei ความหลากหลายของโคดอนตั้งต้นนี้อาจเกิดขึ้นในอันดับย่อย Anabantoidei หลังแยกจากบรรพบุรุษร่วมกับอันดับย่อย Channoidei นอกจากนี้การเปรียบเทียบความเหมือนกันของลำดับนิวคลีโอไทด์ใน 13 ยีน ที่แปลรหัสเป็นโปรตีน ระหว่างปลากัดป่าและปลาช่อนทั้ง 2 ชนิด พบว่ายีน *ATPase8* มีความเหมือนกันในระดับต่ำที่สุด ดังนั้น ยีน *ATPase8* สามารถนำมาใช้ในการแยกกลุ่มอันดับย่อย Anabantoidei และอันดับย่อย Channoidei ได้

ABSTRACT

This is the first report on complete mitochondrial genome (mt genome) of *Betta Splendens* in suborder Anabantoidei. The complete mt genome sequences were 16,982 base pair (bp) in length, which contained 13 protein-coding genes, 2 rRNA genes, a control region, and 22 tRNA genes. Analysis of all *B. Splendens* protein-coding genes exhibited more various types of start codon than those of *Channa argus* and *C. maculata* in suborder Channoidei, which were related species. These results suggest that the alteration of start codon type might occur in Anabantoidei after it diverges from common ancestor of suborder Channoidei. Moreover, comparison of sequence similarity between *B. splendens* and two *Channa* species with 13 protein coding gene revealed that *ATPase8* gene had the lowest similarity. This suggests that *ATPase8* gene could be used to identify Anabantoidei from Channoidei.

คำสำคัญ: ปลากัดป่าภาคกลาง, โคดอนตั้งต้น, จีโนมไมโทคอนเดรีย

Keywords: *Betta splendens*, start codon, mitochondrial genome

INTRODUCTION

Fighting fish in genus *Betta* (Anabantoidei) is divided into two clades: bubble nesting and mouthbrooding fighting fish. Approximately 30% of species within the genus exhibit bubble nesting. In Thailand, *Betta splendens* is the most iconic species, which are used to be breeding for ornamental fish and sport fighting. However, the species contamination might occur by hybridization between intrageneric *Betta* to develop various traits of fish. These issues need serious attention in the context of biodiversity conservation, and it therefore seems important to provide all DNA sequences of *B. splendens* to collect the authentic information and maintaining this ionic species.

Mitochondrial DNAs (mtDNAs) are abundant (multicopies) in a cell, intronless, and free from frequent DNA recombination with gene duplication/deletion. Because of these advantages, molecular evolutionists have frequently chosen orthologous sets of partial mtDNA sequences to reconstruct the phylogenetic relationships. Complete mitochondrial genome (mt genome) sequences provide valuable insights into a number of deep-level phylogenetic questions because of their information, such as genome content, gene order ,and the sequence of each genes (Brinkmann *et al*., 2004). However, the information of complete mtDNA genome remains unknown in *Betta* of Anabantoidei. Here, the complete mt genome of *B. splendens* was completely sequenced, and subsequently analyzed its genomic structure.

MATERIALS AND METHODS

A specimen of adults' *B. splendens* was collected from Pathumthani, Thailand. Whole genomic DNA was extracted from individual using a standard phenol/chloroform method, and used as a template for PCR. PCR primers for mt genome sequencing were taken from Mauro *et al* (2004), and/or designed with conserved sequence of teleost fishes using the alignment of sequence with ClustalW (http://www.ebi.ac.uk). Fifty nanogram of genomic DNA was taken in 20 µl of 1× reaction buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 5 pM specific primers and 0.5 U of *Taq* DNA polymerase (Invitrogen, California, USA), and PCR was performed in the following condition: an initial denaturation at 94°C for 3 min, following with 35 cycles of 94°C for 30s, 40–60°C for 40s and 72°C for 1 min 30s, and final extension at 72°C for 10 min. The PCR products were examined by electrophoresis on 1% agarose gel, and the nucleotide sequences of the DNA fragments determined using 1stBase DNA sequencing service (Seri Kembangan, Malaysia). The nucleotide sequence comparisons against the National Center for Biotechnology Information (NCBI) database were used to search using the blastx and the blastn program (http://blast.ncbi.nlm.nih.gov/ Blast.cgi). Genome assembly was conducted using CAP3 program (http://pbil.univ-lyon1.fr/cap3.php) and manual annotation. For identification of tRNA genes, the nucleotide sequences were used to search for regions, which can form characteristic secondary structures for mitochondrial tRNA genes using tRNA Scan-SE1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/).

RESULTS AND DISCUSSION

Complete mt genome of *B. splendens* is the first representative of suborder Anabantoidei. Genome size was 16,982 base pair (bp). The overall nucleotide base composition was: A 31.47%; C 23.83%; G 14.25% and T 30.45%. This suggests that *B. splendens* mt genome exhibited the lowest utilization of Guanine, which is affected in the term of codon usage. The genome contains 13 protein-coding genes, two rRNA genes, a control region, and 22 tRNA genes. Comparison of sequence similarity between *B. splendens* and *Channa argus* and *C. maculata* in suborder Channoidei, which closely related to suborder Anabantoidei (Ruber, 2009) revealed that *COIII* gene showed the maximum similarity between *B. splendens* and *C. maculata* (76.18%), *B. splendens* and *C. argus* (75.92%), whereas *ATPase8* gene showed the lowest identity (64.88%) between *B. splendens* and *C. maculata*, and *B. splendens* and *C. argus*. These results collectively suggest that *ATPase8* gene could be used to differentiate these two suborders (Anabantoidei and Channoidei)

Most of genes initiated with the standard codon (ATG), whereas *ND1*, *ND5*, *COI*, and *ATPase8* represented the alternative starting codon (ATT, ATA and GTG). The *COI* gene of *C. argus* and *C. maculata* was also start with GTG codon, while the remaining genes started with ATG. This suggests that start codon of *COI* gene was conserved between two suborders. By contrast, *B. splendens* had most genes ending with the TAA stop codon (*ND1*, *ATPase8*, *ATPase6*, *COIII*, *ND4L*, *ND5* and *ND6*), but *COI* gene end with AGG. Furthermore, the other genes have incomplete stop codons which are completed by the addition of 3'A residues to mRNA, either TA (*ND2*) or T (*COII*, *ND4*, *ND3* and *Cytb*), which were similar to *C. argus* (T: *COII, ND*3, *ND*4 and *Cytb*) and *C. maculata* (T: *COII*, *ND3* and *ND*4) (Wang *et al*, 2013). These results suggest that incomplete stop codon (T) was conserved at least three genes (*COII, ND*3, *ND*4) in the two suborders.

CONCLUSION

The structures of complete mt genome in *B. splendens* were highly similar to that of two *Channa* species in suborder Channoidei. However, there are several types of start codons in *B. splendens* which differed from those of *C. argus* and *C. maculata.* This suggests that the alteration of start codon might occur in Anabantoidei after it diverges from common ancestor of suborder Channoidei. Moreover, sequence similarity of *ATPase8* gene could be used to differentiate Anabantoidei from Channoidei

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