
















Complete mitochondrial genome of Mekong fighting fish, *Betta smaragdina* (Teleostei: Osphronemidae)

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ABSTRACT

Mekong fighting fish (*Betta smaragdina*) are found in Northeast Thailand. A complete mitochondrial genome (mitogenome) of *B. smaragdina* was assembled and annotated. Mitogenome sequences were 16,372 bp in length, with slight AT bias (59.8%), containing 37 genes with identical order to most teleost mitogenomes. Phylogenetic analysis of *B. smaragdina* showed closer relationship with *B. splendens* and *B. mahachaiensis* as the bubble-nesting group, compared to the mouthbrooder group (*B. apollon*, *B. simplex*, and *B. pi*). Results will allow the creation of a reference annotated genome that can be utilized to sustain biodiversity and eco-management of betta bioresources to improve conservation programs.

ARTICLE HISTORY



Received 14 September 2020
Accepted 24 January 2021

KEYWORDS

Fighting fish; mitogenome; bioresource; bubble-nesting group

Southeast Asia, including the mainland and numerous islands, has a hot and humid climate that supports high biodiversity and bioresources. Fighting fish or betta (*Betta* spp.) were studied for their fighting and ornamental attributes. Long-term artificial crossbreeding among different species has honed their aggressive behavior and body features, resulting in many novel inbred lines (Witte and Schmidt 1992; Ramos and Gonçalves 2019). However, artificial selection has resulted in inbreeding and outbreeding depression between different betta species. The biodiversity of betta is being lost more rapidly now than at any time in the past several million years, with the invasion of hybrids introduced into the wild leading to genetic admixture (Beer et al. 2019). This is a very serious problem in the context of local conservation and indigenous species/lines. Genetic profiles for each local species must be resolved as a matter of urgency. One such species is the bubble-nesting Mekong fighting fish (*Betta smaragdina*) which is found in Northeast Thailand. However, the lack of a reference mitochondrial genome (mitogenome) has, to date, limited understanding of the genetic basis of aggression in this species. A sample of *B. smaragdina* was collected from Dong Luang, Mukdahan

Province, Thailand (16.7966°N, 104.6238°E) and then stored in the Thailand Natural History Museum (no. THM21222). Whole genomic DNA was extracted in accordance with the standard salting-out protocol (Supikamolseini et al. 2015), and next-generation sequencing was performed using an Illumina HiSeq platform at Vishuo Biomedical (Thailand) Ltd. (Bangkok, Thailand). The quality of Illumina reads was evaluated with FastQC and the raw reads were trimmed to discard adapters using Trimmomatic software (Bolger et al. 2014). The filtered Illumina paired end reads were then assembled using a mitochondrial genome toolkit, MitoZ version_2.4-alpha (Meng et al. 2019) and processed with multiple modules including raw data pretreatment, de novo assembly, candidate mitochondrial sequences searching, and mitogenome annotation. A phylogenetic tree was constructed using Bayesian inference with MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001). The Markov chain Monte Carlo process was used to run four chains simultaneously for 1 million generations. After the log-likelihood value plateaued, a sampling procedure was performed every 100 generations to obtain 10,000 trees, and a majority-rule consensus tree with average branch lengths was provided.

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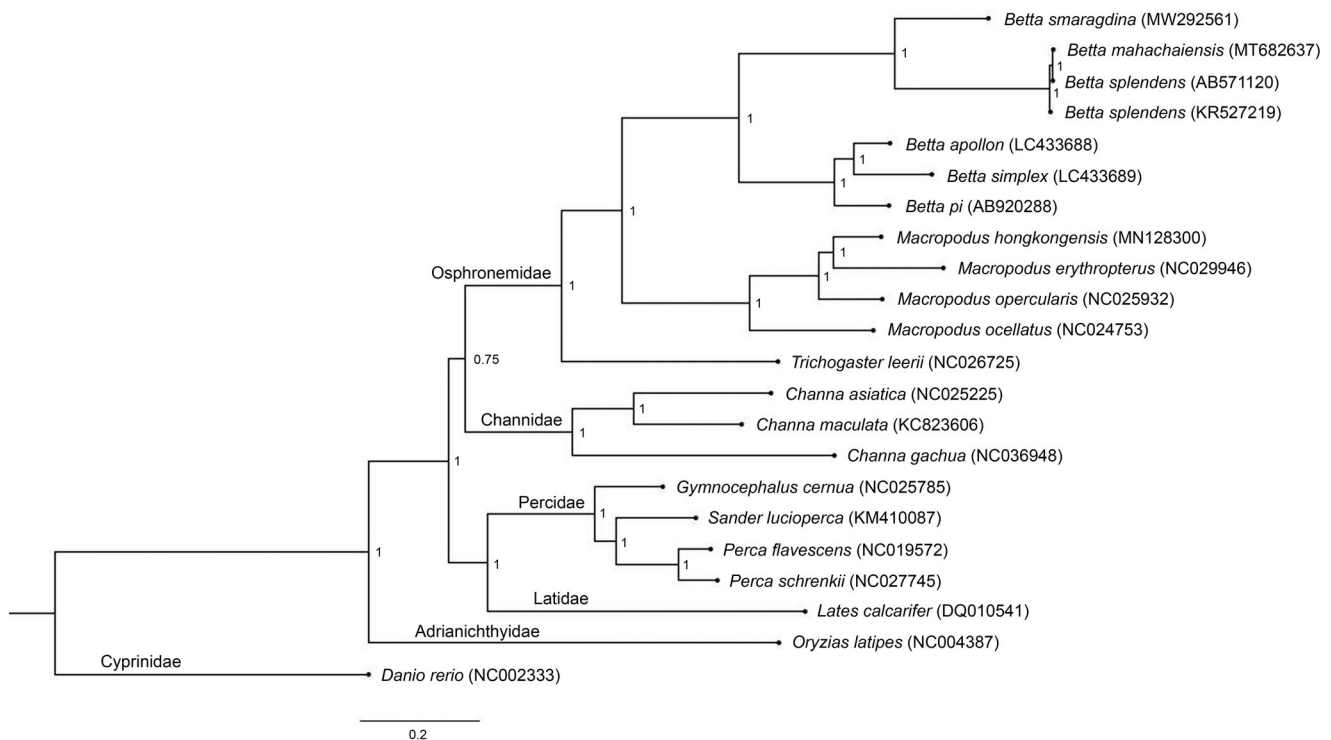


Figure 1. Phylogenetic relationships among 12 concatenated mitochondrial protein-coding genes, without *ND6* sequences of 22 mitochondrial genomes, including *Danio rerio* as the outgroup using Bayesian inference analysis. The complete mitochondrial genome sequence was downloaded from GenBank. Accession numbers are indicated in parentheses after the scientific names of each species. Support values at each node are Bayesian posterior probabilities, while branch lengths represent the number of nucleotide substitutions per site.

A total of 118,164 individual reads gave an average coverage of around 170X for the generated contigs. Complete mitogenome sequences consisted of 16,372 bp (GenBank accession number: MW292561, SRA: SRR12599606, BioProject: PRJNA661175), containing 37 genes and a control region (CR), which indicated a conserved pattern of mitochondrial genome architecture in *Betta* species. Bioinformatic analysis of whole mtgenome comparisons for a total of 54 teleost species revealed a similar pattern of gene arrangement (Miya et al. 2013). Overall AT content for the mitogenome was 59.8%. Average nucleotide diversity among seven *Betta* mitogenomes was determined at $17.493 \pm 2.453\%$. Four conserved blocks including CSB-D, CSB1, CSB2, and CSB3 were characterized in *B. smaragdina*. Previous findings suggest that these blocks within the CR region remain conserved across the mtgenomes of teleost lineages (Lee and Kocher 1995; Prakhongcheep et al. 2017; Ponjarat et al. 2019; Ahmad et al. 2020; Singchat et al. 2020). We annotated the mitogenome for repeats identification using RepeatMasker, version-4.1.1 (Smit et al. 2015) and found a total of six repeat elements. Tandem repeats have also been found previously in the mtgenomes of several *Betta* species including *B. pi* (AB920288) and *B. splendens* (AB571120 and KR527219) (Song et al. 2016; Prakhongcheep et al. 2017; Ponjarat et al. 2019; Ahmad et al. 2020; Singchat et al. 2020), suggesting that the CR had large variation in different fighting fish species. Twenty-two teleosts including seven betta mitogenomes were compared using CLUSTALW based on 12 concatenated protein-coding genes without *ND6*, and a phylogenetic tree was constructed using Bayesian inference with MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001). The close group

comprising *B. splendens*, *B. mahachaiensis*, and *B. smaragdina* formed a monophyletic clade, consistent with Sriwattanarothai et al. (2010) as the bubble-nesting group, whereas the three remaining bettas (*B. apollon*, *B. simplex*, and *B. pi*) were classified to the mouthbrooder group (Ruber et al. 2004) (Figure 1).

Acknowledgements

The authors would like to thank Sunchai Makchai (Thailand Natural History Museum) for advice on sample preparation. We are also indebted to Vishuo Biomedical (Thailand) Ltd. for excellent service collaboration. The Center for Agricultural Biotechnology (CAB) at Kasetsart University Kamphaeng Saen Campus provided support with server analysis services, and the Faculty of Science and the Faculty of Forestry at Kasetsart University provided the research facilities. The National Biobank of Thailand (NBT) under the National Science and Technology Development Agency (NSTDA), Thailand supported this study through the use of a high-performance computer. We also thank the Plakad Association, Thailand for providing information concerning *Betta* spp. in Thailand.

Disclosure statement









The authors report no conflicts of interest and are entirely responsible for the contents of this article. Animal care and all experimental procedures were approved by the Animal Experiment Committee, Kasetsart University, Thailand (approval no. ACKU63-SCI-007) and conducted in accordance with the Regulations on Animal Experiments at Kasetsart University.

Funding

This research was financially supported by a grant from the National Research Council of Thailand [NRCT/16/2563] awarded to NL, SFA, WS,

AS, TP, KJ, JB, TP, TP, NA, NS, SD, NM, PD, and KS, the Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University awarded to KS, the Thailand Research Fund [Nos. RSA6180075, PHD6010014, and PHD6010082] through a grant awarded to KS, WS, and AS, the Science Achievement Scholarship of Thailand [No. 5917400296] through a grant from the Office of the Higher Education Commission, Thailand awarded to NL, the Capacity Building of KU Students on Internationalization Program (KUCSI) awarded to NL and KS, a grant from the Graduate Scholarship Program of the Graduate School, Kasetsart University, Thailand awarded to TP and KS, and a Postdoctoral Researcher award at Kasetsart University awarded to SFA and KS.

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Data availability statement

Data supporting the study findings are available in GenBank through the NCBI at <https://www.ncbi.nlm.nih.gov>. Isolated mitogenome reads were deposited at NCBI SRA database (accession ID: SRR12599606), and the assembled mitogenome sequences are available in GenBank (accession ID: MW292561).

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