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MITOGENOME ANNOUNCEMENT

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Next-generation sequencing yields complete mitochondrial genome assembly of peaceful betta fish, *Betta imbellis* (Teleostei: Osphronemidae)

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ABSTRACT

The complete mitochondrial genome (mitogenome) of the peaceful betta (*Betta imbellis*) was obtained using next-generation sequencing. The sample of *B. imbellis* was collected from its native habitat in Southern Thailand. The mitogenome sequence was 16,897 bp in length, containing 37 genes with identical order to most teleost mitogenomes. Overall nucleotide base composition of the complete mitogenome was determined as AT bias. Phylogenetic analysis of *B. imbellis* showed a closer relationship with bubble-nesting fighting fish. This annotated mitogenome reference can be utilized as a bioresource for phylogenetic studies to support betta conservation programs.

Southeast Asia (SEA) is a well-known biocultural hotspot of biodiversity and endemism encompassing a huge variety of betta fish (*Betta* spp.) belonging to the family Osphronemidae (Panijpan et al. 2014). The bubble-nesting species *Betta imbellis*, also known as the peaceful betta, is native to SEA and one of the most popular wild type betta fish for fishkeeping hobbyists (Kusrini et al. 2015). This betta fish inhabits still and sluggish waters including rice paddies, swamps, roadside ditches, streams, and ponds. Artificial selection activities for ornamental and aquarium trade purposes have resulted in inbreeding and outbreeding depression with an adverse effect on the genetic integrity of wild populations (Beer et al. 2019). Here, a complete mitochondrial genome (mitogenome) of *B. imbellis* collected from Nakhon Si Thammarat, Thailand (8.4325° N, 99.9599° E), was assembled, annotated, analyzed, and stored in the Thailand Natural History Museum (no. THM21221). Whole genomic DNA was extracted in accordance with the standard salting-out protocol (Supikamolseni et al. 2015), and next-generation sequencing was performed using an Illumina HiSeq platform at Vishuo Biomedical (Thailand) Ltd. (Bangkok, Thailand). Quality of Illumina reads was evaluated with FastQC software and the raw reads were trimmed to discard adapters using
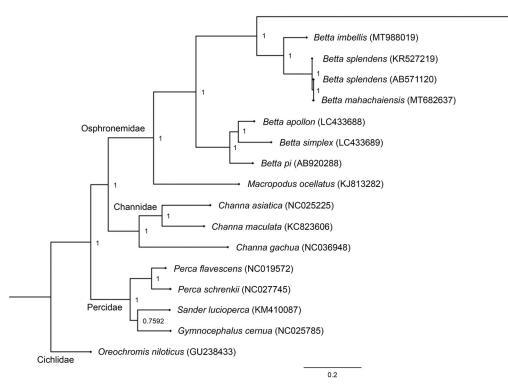
Trimmomatic (Bolger et al. 2014). The trimmed reads were subjected to alignments to isolate all mitogenome sequences by mapping whole genome Illumina reads against the complete mitogenome of *B. splendens* (AB571120), using bwa default parameters (Li and Durbin 2009). The mapped alignment was processed using Samtools (Li et al. 2009), and aligned reads were extracted using Bedtools (Quinlan and Hall 2010). Aligned reads with the mitogenome were then de novo assembled using Velvet (Velvet_1.1.07; kmer = 123) (Zerbino and Birney 2008). A total of 395,074 individual reads gave a mean coverage of more than 330X for the generated contigs. We then assembled a consensus scaffold of the complete mitogenome using the reference-based assembly approach in Geneious Prime (https://www.geneious.com/prime/), by mapping the Velvet contigs against the reference mitogenome of *B. splendens*. The assembled mitogenome was annotated in the MITOS WebServer (Bernt et al. 2013). Complete mitogenome sequences consisted of 16,897 bp for *B. imbellis* (GenBank Accession number: MT988019, SRA: SRR12614920, BioProject: PRJNA662470), containing 37 genes and a control region (CR). Gene arrangement patterns were identical to those of teleosts (Miya et al. 2013). Overall AT content values for the mitogenome were 61.9%. Average

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Figure 1. Phylogenetic relationships among 12 concatenated mitochondrial protein-coding genes, without *ND6* sequences of 17 mitochondrial genomes including *Oreochromis niloticus* 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.

nucleotide diversity among all *Betta* mitogenomes was determined at 24.56 ± 6.04%. Four conserved sequence blocks (CSB-D, CSB1, CSB2, and CSB3) in the CR of teleost mitogenomes were also present in *B. imbellis* (Lee and Kocher 1995; Prakhongcheep et al. 2018; Ponjarat et al. 2019; Singchat et al. 2020). Diverse numbers of tandem repeats were observed among *Betta* species (Song et al. 2016; Prakhongcheep et al. 2018; Ponjarat et al. 2019; Singchat et al. 2020), suggesting that the CR had large variation in different fighting fish species. A phylogenetic tree was constructed based on 12 concatenated protein-coding genes without *ND6* of 17 teleosts, using Bayesian inference with MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001). The group comprising *B. splendens*, *B. mahachaiensis*, *B. smaragdina*, and *B. imbellis* formed a monophyletic clade as bubble-nesting fighting fish, consistent with Ruber et al. (2004), Sriwattanarothai et al. (2010), and Singchat et al. (2020) (Figure 1). These complete mitogenomes comprise a reference annotated genome, and provide valuable information at the molecular level that can be utilized to sustain betta bioresources to improve conservation programs and commercial breeding management.

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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.

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Data availability statement

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