ACADEMIA JOURNAL OF BIOLOGY 2024, 46(3): 131–137
DOI: 10.15625/2615-9023/20915

SHORT COMMUNICATION:

COMPLETE MITOCHONDRIAL GENOME OF VULNERABLE FIGHTING FISH *Betta coccina* (Actinoptergii: Perciformes: Osphronemidae) WITH PHYLOGENETIC CONSIDERATION

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Received 7 June 2024; accepted 12 September 2024

ABSTRACT

We reported the first complete mitochondrial genome of the Scarlet Fighting Fish, *Betta coccina* (Vierke, 1979), a species of concern due to its vulnerable status. Genome skimming was performed on a specimen collected from Johor, Malaysia to recover its mitochondrial genome. The assembled complete mitogenome is 16,502 bp in size, comprising the standard set of 13 protein-coding genes, 22 tRNAs, and two rRNAs, typical of most vertebrates' mitogenome. Phylogenetic analysis was conducted using the whole mitogenome of *B. coccina* and four publicly available mitogenomes of *Betta* spp. Our analysis revealed that the *B. coccina* currently occupied a basal position relative to other *Betta* spp. with maximal support value. Our results provide valuable insights for both evolutionary studies and genetic conservation efforts of *B. coccina* and its related species.

Keywords: Mitochondrial DNA, Betta coccina, phylogenetic, mitogenome.

Citation: Muhammad Fitri Yusof, Zarick Zainon, Muhamad Zudaidy Jaapar, Md. Ali Amatul-Samahah, Mohd Azrul Naim Mohamad, Adriana Batrisyia Mohd Faisal, Han Ming Gan, 2024. Complete mitochondrial genome of vulnerable fighting fish *Betta coccina* (Actinoptergii: Perciformes: Osphronemidae) with phylogenetic consideration. *Academia Journal of Biology*, 46(3): 131–137. https://doi.org/10.15625/2615-9023/20915

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INTRODUCTION

Mitochondrial DNA has been extensively used as a marker for understanding the genetic diversity, evolutionary relationships, and population dynamics of these endangered species (Osborne et al., 2020). In fishes, numerous effort has been done to sequencing the whole genome of mitochondrial DNA in fish species (Chen et al., 2022; Muhala et al., 2024; Zhang et al., 2023).

Betta coccina (Vierke, 1979) is a species of bubble-nesting fighting fish under the family Osphronemidae, order Perciformes. This species can only be found in peat swamp of Johor (Peninsular Malaysia), Riau and Jambi (Indonesia) (Fig. 1). They inhabit shallow water in peat swamp forest and like other fighting fishes under genus *Betta*, this species is paternal bubble nester (Linke, 1991; Tan & Ng, 2005). The species has been sought after as ornamental fish and exposed to habitat degradation in all their location (Tan & Ng, 2005), causing to be assigned under Vulnerable in the IUCN Redlist (IUCN, 2022).

Mitogenomes typically consist of a standard set of genes, including protein-coding genes, transfer RNA (tRNA) genes, and ribosomal RNA (rRNA) genes, arranged in a specific order and orientation (Dan et al., 2022). It provides valuable insights into the evolutionary relationships, genetic diversity, and phylogenetic analyses of various organisms. This particular DNA data provide a comprehensive understanding of the genetic diversity within populations, aiding in conservation efforts. By analyzing the complete mitogenome of various freshwater fish species, researchers able to gain insights into phylogenetics, population genetics, and conservation strategies (Chandra et al., 2024).

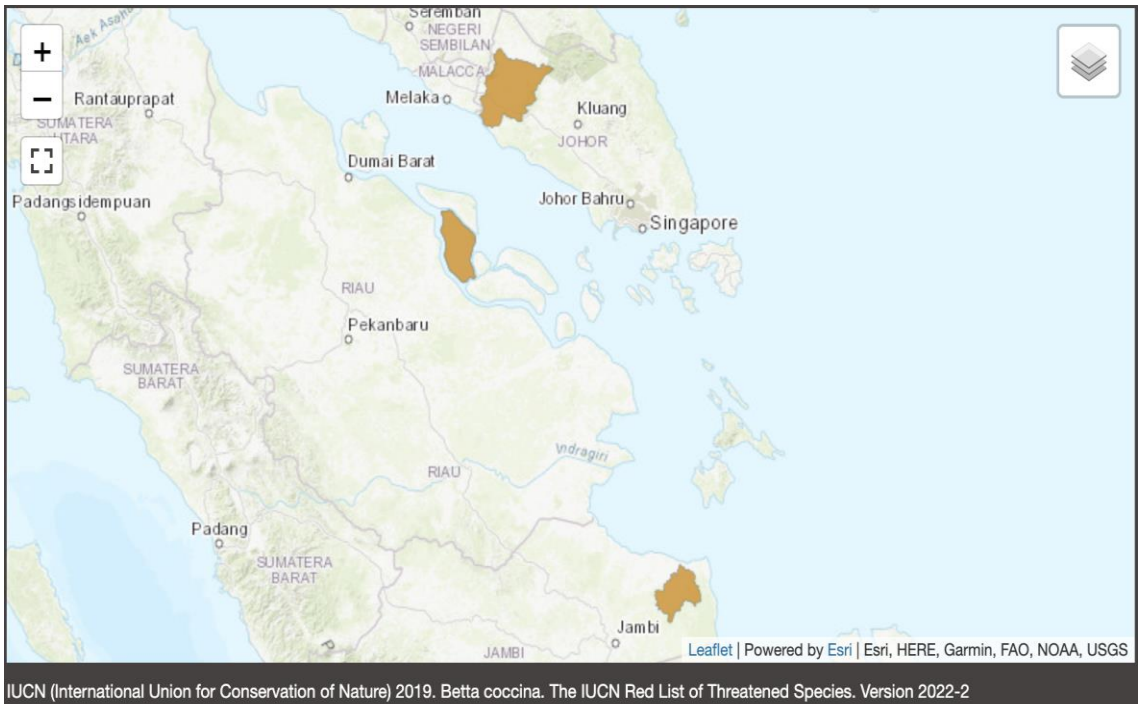

Figure 1. Location of *Betta coccina.* Source (IUCN Redlist)

The assembly of mitogenomes from a diverse range of freshwater fish specimens has provided valuable data for understanding the genetic diversity and relationships among different species, aiding in conservation efforts (Dziedzic et al., 2022; Dziedzic, 2023;

Dziedzic et al., 2023). So, the objective of this study is to sequence the whole genome of mitochondrial DNA from *B. coccina* and determine the taxonomic status of this species.

MATERIALS AND METHODS

A sample of *B. coccina* collected from Johor, Malaysia was preserved in 95% ethanol and deposited at (Marine Museum, Kulliyyah of Science) International Islamic University Malaysia. The total genomic DNA was extracted from 50 mg of dissected fish muscles using the G-spin Total DNA Extraction kit (iNtRON, country). Approximately 100 ng of gDNA was fragmented to 300 bp and used as the input for NEB Ultra II Illumina DNA library preparation (NEB, England). The constructed library was sequenced on an Illumina NovaSEQ6000 platform (Oxford, England), generating 1 gigabases of data (~ 3.3 million reads). The raw reads were trimmed with fastp default settings followed by mitogenome assembly and annotation using MITOz and MITOFISH 3.93 (Zhu et al., 2023), respectively. The mitogenome map was generated using what software. The evolutionary relationships between *B. coccina* and three other *Betta* species based on whole mitogenome data were explored using Phylosuite (Zhang et al., 2020). Briefly, two rRNA and 13 protein-coding genes from each mitogenome were identified from their Genbank annotations ((https://www.ncbi.nl-m.nih.gov/genbank/). Then, each gene was individually aligned and subsequently concatenated to be used as the input for maximum-likelihood tree construction using IQTree-2 (Minh et al., 2020). As an outgroup, mitogenomes from *Macropodus* sp. were used.

The phylogenetic analysis was conducted using Phylosuite, incorporating sequence alignment and subsequent phylogenetic tree reconstruction. The resulting CONTREE file was visualized on FigTree. Sequences were aligned using MAFFT with default parameters and subsequently concatenated for downstream analysis. The best-fit substitution model was GTR+F+I+G4, determined using ModelFinder, based on the Bayesian Information Criterion (BIC). The chosen model for phylogenetic reconstruction was implemented in MrBayes.

The phylogenetic tree was constructed using IQ-TREE, with default parameters. The analysis utilized the SH-aLRT branch test in ultrafast bootstrap mode. A total of 20,000 bootstrap replicates were performed, with a maximum of 1,000 iterations per replicate, and a minimum correlation coefficient of 0.9. The resultant tree in the CONTREE file was visualized on FigTree. Bootstrap probability values were indicated to highlight well-supported branches, providing a clear representation of the phylogenetic relationships.

RESULTS AND DISCUSSION

The entire mitochondrial genome of *B. coccina* is a circular molecule with a length of 16,502 bp, which consists of 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA)genes, 22 transport RNA (tRNA) genes, and a control region (D-loop) (Fig. 2), showing a typical arrangement of a fish mitogenome. The overall nucleotide composition is 27.9% A, 27.8% T, 27.7% C, and 16.6% G. MT-ND6 and eight tRNAs (tRNA-Glu, tRNA-Gln, tRNA-Aln, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Pro) are encoded on the L-strand, while the others are encoded on the H-strand. Two types of start codons (ATG, GTG) and five types of stop codons (TAA, TAG, TA-, AGA and AGG) were observed in this species. All protein-coding gene start with the start codon ATG except COI starts with GTG. Five protein coding genes were terminated with completed stop codons TAA or TAG; ND2, ND3, ND4L, ND5, ND6, COX3, and ATPase6 were terminated with in completed codon (TA-); and four protein coding genes (NAD4, COXI, COX2, Cytb) were terminated with (AGA, AGG) in which stop codons in metazoan mitochondrial genomes (Osawa et al., 1989).

The entire mitochondrial genome of *B. coccina* was submitted to GenBank with the accession numbers NC080232 and OQ784935.

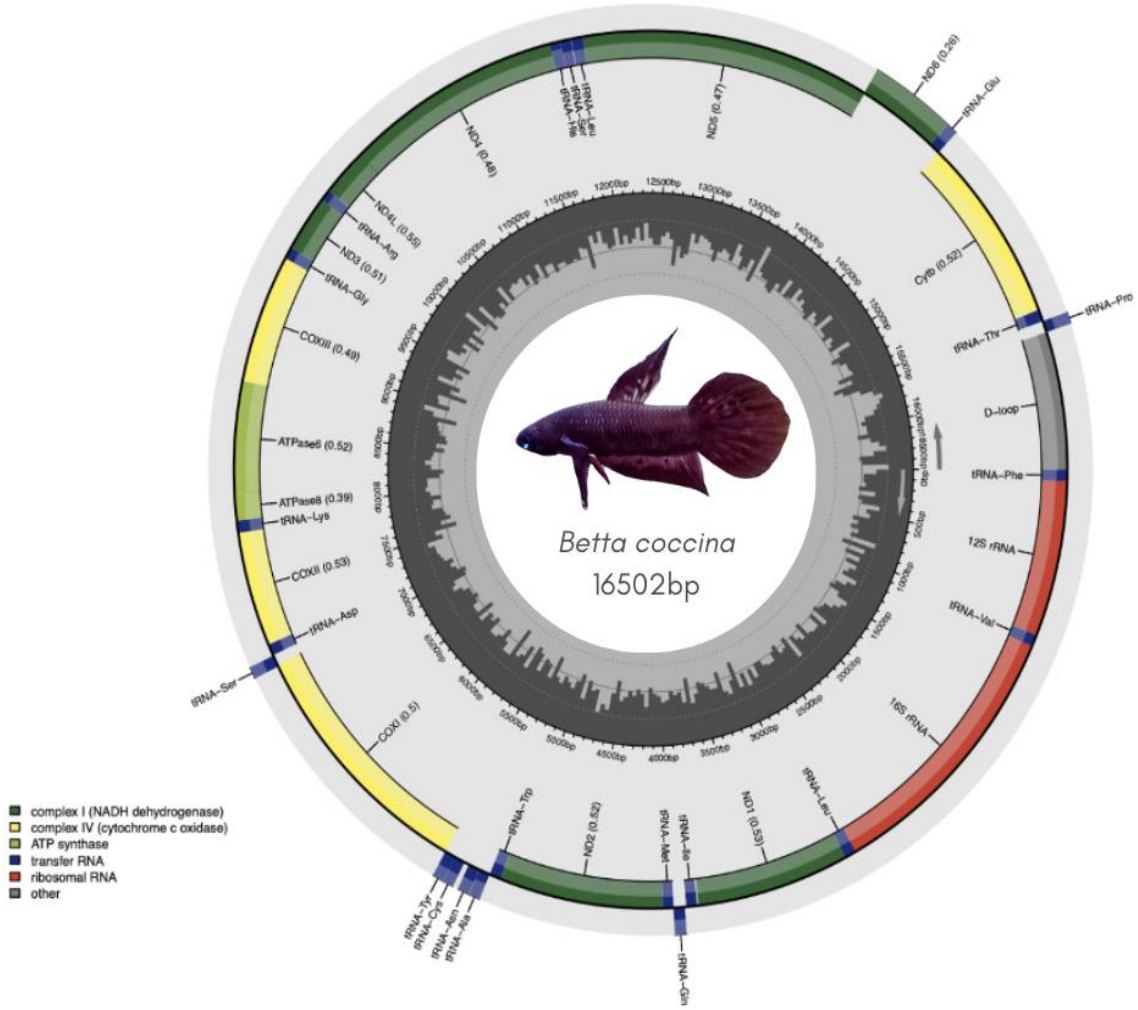

Figure 2. Mitogenome map of *Betta coccina*

From Figure 3, the basal position of *B. coccina* relative to other *Betta* spp. underscored its genetic divergence within the genus. All species were clustered to two main branches. The first branch contains all *Betta* species. *B. coccina* mitogenome (NC080232) was clustered together with *B. coccina* mitogenome (OQ784935), both samples from this study. They were more closely related to the mitogenomes of *Betta pi* and *Betta simplex*. The species *Betta mahachaiensis* and *Betta splendens* were also clustered in one sub-branch. The second branch contains species of the genus *Macropodus* including *Macropodus chinensis, Macropodus ocellatus, Macropodus erythropterus, Macropodus hongkongensis,* and *Macropodus opercularis*. Figure 3 shows the similarity of mitogenomes between two genera *Betta* and *Macropodus* which can help to have a better understanding of the evolutionary dynamics within this genus.

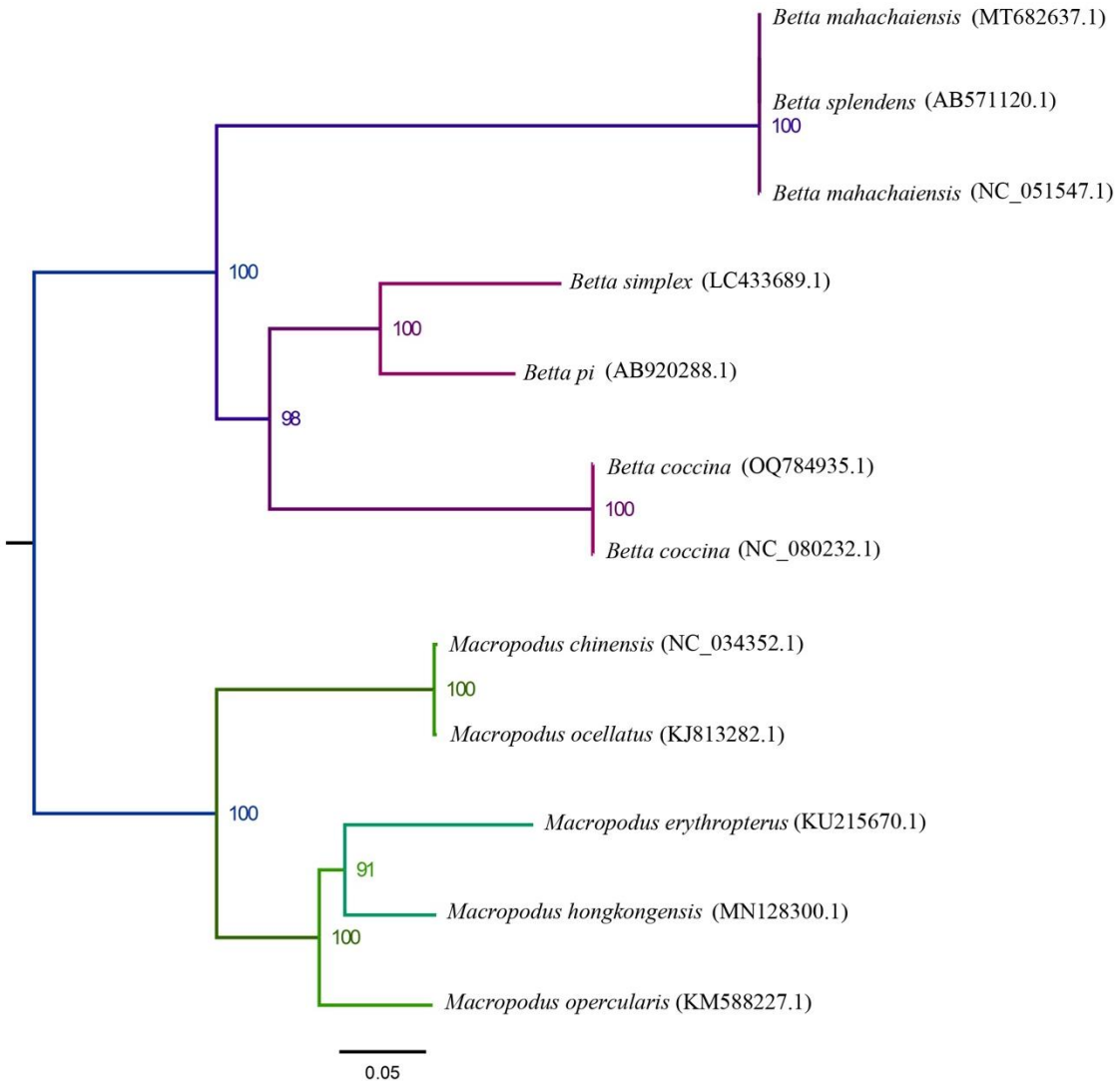

Figure 3. Phylogenetic evolutionary relationships of *Betta coccina* and other four *Betta* species. The tree was rooted with members from the genus *Macropodus* as the outgroup. Branch lengths represent the number of substitutions per site and node labels show the bootstrap supporting values

CONCLUSION

The assembled complete mitogenome is 16,502 bp including 13 protein-coding genes, 22 tRNAs, and two rRNAs, typical of most vertebrates mitogenome. Phylogenetic analysis was conducted using the whole mitogenome of *B. coccina* and four publicly available mitogenomes of *Betta* spp.

Ethic Statement: The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the International Islamic University of Malaysia.

Acknowledgements: The authors would like to thank Wild Betta Lover Society Malaysia for the assistance in sample collection. The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/, under accession no. NC_080232.1. The associated BioProject, Bio-Sample, and SRA accession

numbers are PRJNA938235, SAMN33427755, and SRS16896757, respectively.

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