



The complete mitochondrial genome of *Fejervarya kawamurai* (Anura: Dicroglossidae) and its phylogeny

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ABSTRACT

The mitochondrial genome of *Fejervarya kawamurai* is a circular molecule of 17,650 bp in length, containing 13 protein-coding genes, two rRNA genes, 23 tRNA genes (including an extra tRNA-Met), and the control region. The AT content of the whole genome is 56.9%. In Bayesian inference (BI) and Maximum likelihood (ML) analyses, we found that *F. kawamurai* is a sister clade to *F. multistriata* and *F. limnocharis*. The monophyly of *Fejervarya*, *Quasipaa*, *Nanorana* was well supported (1.00 in BI and 100% in ML).

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Fejervarya kawamurai was described as a new species of dicroglossid frog and a member of the *Fejervarya limnocharis* complex from western Honshu, Japan mainland by Djong et al. (2011). It is distributed broadly across parts of Japan, Taiwan, and China (Frost 2018). There is much controversy about the classification of *F. kawamurai* and the closely related *F. limnocharis* and *F. multistriata* (Djong et al. 2011; Huang and Tu 2016). The complete mitochondrial genomes of *F. limnocharis* and *F. multistriata* were sequenced but the comparable genome of *F. kawamurai* was unknown. Hence, we sequenced the mitochondrial genome of *F. kawamurai* to discuss the relationship within the *Fejervarya limnocharis* complex.

Samples of *F. kawamurai* were collected from Zunyi city, Guizhou province, China (27°42'30", 106°55'12'). Whole genomic DNA was extracted from the hind leg muscle. The frog's samples and DNA samples were stored at the College of Chemistry and Life Science, Zhejiang Normal University, China. DNA fragments were amplified using 15 pairs of highly conserved primers for mitochondrial genes which were designed according to the method of Liu et al. (2005) and Huang and Tu (2016). All PCR procedures were performed using an Arktik Thermal Cycler (Thermal Scientific, Shanghai, China). The PCR products were sequenced by Sangon Biotech Company (Shanghai, China).

The complete mitochondrial genome of *F. kawamurai* is 17,650 bp in length, containing 13 protein-coding genes, two rRNA genes, 23 tRNA genes (an extra tRNA-Met is

present), and the control region. The AT content of the complete mtDNA is 56.9%. Most protein-coding genes begin with ATG as the start codon, except for ND5 gene with GTC, ND2 gene with ATT, COI gene with ATA, and ND3 gene with GTG. The ND6 gene is terminated with AGG as the stop codon, whereas ND1, ND2, ND5, COI, COII, ATP6, and COIII genes end with an incomplete stop codon (T-) and the other protein-coding genes end with TAA.

Bayesian inference (BI) and maximum likelihood (ML) trees were constructed using the 13 protein-coding genes of 25 species (Liu et al. 2005; Ren et al. 2009; Zhang et al. 2009; Zhou et al. 2009; Alam et al. 2010; Chen et al. 2011; Yu et al. 2012; Shan et al. 2014; Yu et al. 2015; Chen et al. 2015a,b; Jiang et al. 2016; Kiran et al. 2017), including *Occidozyga martensii* (Li et al. 2014) as outgroup to confirm the phylogenetic position of *F. kawamurai* in Dicroglossidae (Figure 1). To select conserved regions of the putative nucleotide sequences, each alignment was analyzed with the program Gblocks 0.91 b (Castresana 2000) using default settings. BI analysis and ML analysis was performed by MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) and RaxML HPC (Stamatakis et al. 2008), respectively. In both the BI and ML trees, we found that *F. kawamurai* is a sister clade to (*F. multistriata* + *F. limnocharis*). The monophyly of *Fejervarya*, *Quasipaa*, *Nanorana* was well supported (1.00 in BI and 100% in ML) as also reported in other recent studies (Zhang et al. 2009; Cai et al. 2018).

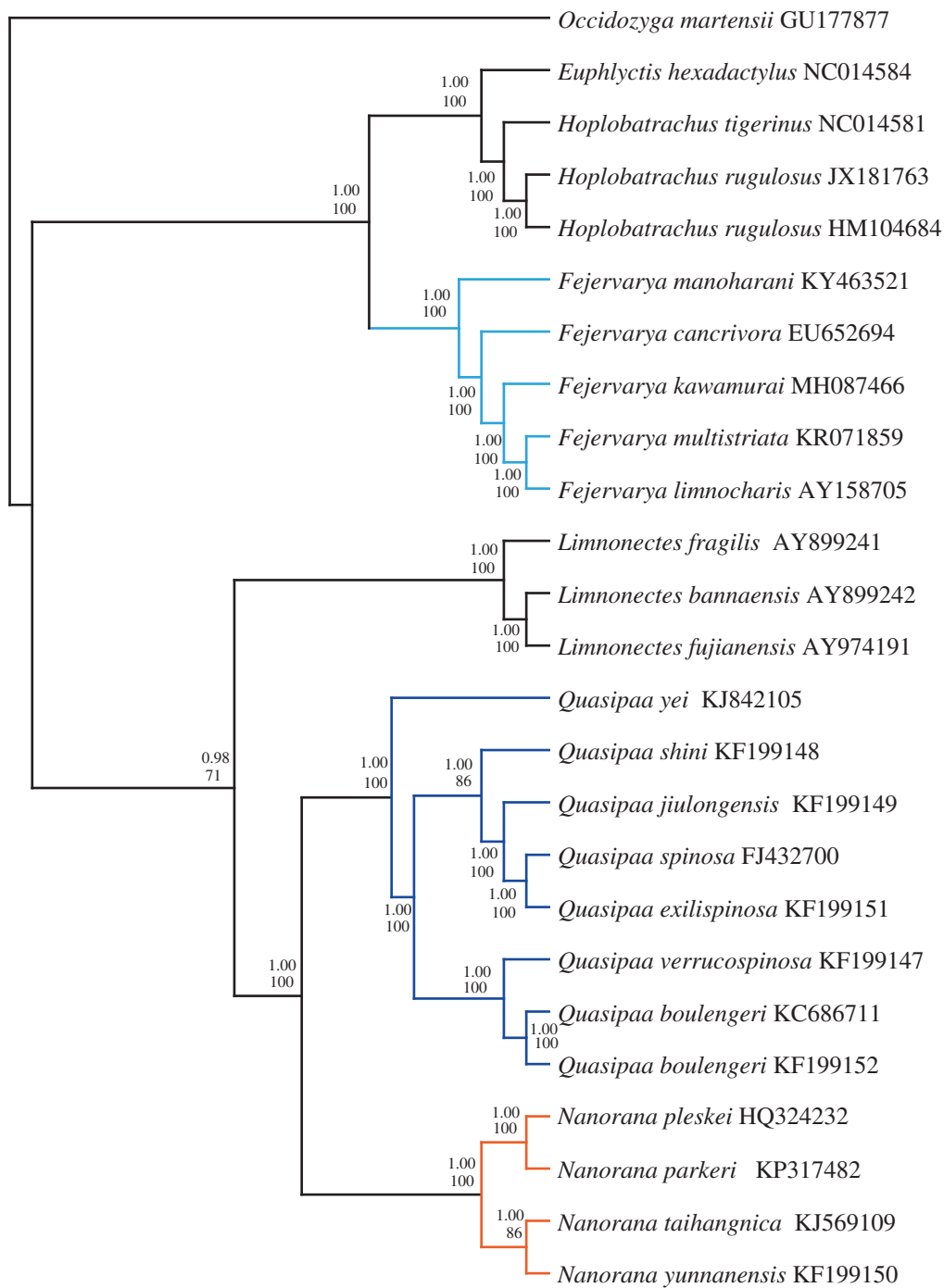


Figure 1. Phylogenetic relationships among 25 species of Dicroglossidae based on 13 protein-coding genes using nucleotide datasets. The tree was rooted with *Occidozyga martensii* as the out-group. Numbers above the nodes are the posterior probabilities of BI on top and the bootstrap values of ML on the bottom.

Nucleotide sequence accession number

The complete mitochondrial genome of *F. kawamurai* has been assigned the GenBank accession number MH087466.



Disclosure statement

The authors declare no conflict of interest.

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