The complete mitochondrial genome of the tree frog, *Polypedates braueri* (Anura, Rhacophoridae)

An Huang, Haijun Li, Hongdi Luo, Qingyong Ni, Yongfang Yao, Huai Li, Bo Zeng, Ying Li, Zhimin Wei and Mingwang Zhang

ABSTRACT

We determined the complete mitochondrial genome of the tree frog, *Polypedates braueri* using next generation sequencing (NGS) and Sanger sequencing. The mitogenome of *P. braueri* was 19,904 bp in length, which contained 12 protein-coding genes, 22 tRNAs, two rRNAs, and two control regions (D-Loop). A noncoding sequence (NC) was discovered between tRNA^{Lys} and ATP6 gene, as well as replaced the original position of ATP8 gene. The ND5 gene was found between the two control regions. More mitochondrial genomic information will contribute to revealing the phylogenetic relationships among species of the genus *Polypedates*.

The white-lipped tree frog, *Polypedates braueri* (Anura: Rhacophoridae), is widely distributed in south China (AmphibianChina 2019). Molecular studies of this species usually based on a single gene fragment to estimate the phylogenetic relationships and taxonomic status of tree frogs (Brown et al. 2010; Blair et al. 2013; Pan et al. 2013). Here, we sequenced the complete mitogenome of *P. braueri* to identify the gene arrangement and performed phylogenetic analyses to address higher level relationships within anura.

The specimen was collected from the Laoban hill (29°58′38.50″ N, 102°59′37.38″ E, Ya’an, Sichuan, China) and deposited in the College of Animal Sciences and Technology, Sichuan Agricultural University, China. DNA was extracted using Ezup type animal genomic DNA extraction kit (Sangon, Shanghai) followed the operation manual. Sequencing libraries were generated using the TruSeq DNA Sample Preparation Kit (Illumina, USA) and the Template Prep Kit (Pacific Biosciences, USA). The genome sequencing was performed by Personal Biotechnology Company (Shanghai, China) using the Pacific Biosciences platform and the Illumina Miseq platform. LA-PCR was used to amplify two large segments covering duplicated control regions where some reads were not assembled using next generation sequencing. The assembled mitogenome sequence was annotated using the MITOS web server under the genetic code for vertebrates (Bernt et al. 2013) and was manually compared with the mitochondrial genomes of Rhacophoridae species from GenBank.

The complete mitochondrial genome of *P. braueri* (GenBank Number: MK687567) was 19,904 bp in length and contained 12 protein-coding genes (ATP6, COI, COII, COIII, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, and Cytb), two ribosomal RNAs (12S and 16SrRNA), and 22 transfer RNAs genes. The base composition was A (29.7%), C (23.3%), G (15.1%) and T (31.9%) with an AT content of 61.6%. With the exception of ND6 and eight tRNAs, most genes were coded on the H-strand, which was consistent with other typical amphibian mitogenomes. Nine protein-coding genes were initiated by ATG, whereas six protein-coding genes (ND1, ATP6, COII, ND3, ND4, and Cytb) ended with an incomplete stop codon. The tRNA genes (tRNA^{Lys}, tRNA^{Ile}, tRNA^{Pro}, tRNA^{Sar}) arranged as “TLPF” gene cluster. Most tRNAs except tRNA^{Lys} and tRNA^{Ser} could be folded into the perfect cloverleaf secondary structure. A noncoding sequence (NC) was found between tRNA^{Lys} and ATP6 gene which may have replaced the original position of ATP8 as that of the hypothetical of the previous study (Zhang et al. 2005). Two control regions which located on both sides of ND5 were 1,753 bp and 2,757 bp in length, respectively.

Based on the concatenated nucleotide sequences of 12 protein-coding genes and two rRNAs, the phylogenetic relationships of the *P. braueri* and the other 13 frogs were reconstructed by MEGA6.0 using maximum-likelihood (ML) method with 1000 bootstrap replications (Tamura et al. 2013). *Polypedates braueri* and the other three Rhacophoridae species were recovered as monophyletic with strong bootstrap support.
value (Figure 1). Due to the molecular evidence inferred in this study is limited, more mitochondrial genomic information of other tree frogs is necessary in order to elucidate the evolutionary relationships within major lineages of Rhacophoridae.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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