

The complete mitochondrial DNA for the Fujian Bamboo-leaf Frog *Odorrana exiliversabilis* (Anura: Ranidae) by next-generation sequencing

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ABSTRACT

The complete mitochondrial DNA (mtDNA) for *Odorrana exiliversabilis* Li, Ye and Fei 2001 (Anura: Ranidae) was determined in this study. The length of complete mtDNA was 17,122 bp, including 13 PCGs (COI-III, ND1-6, ND4L, ATP6, ATP8 and CYTB), 25 tRNA genes, 2 rRNA genes, 2 non-coding regions of a L-strand replication origin and a control region. The overall base composition of the sequence is 28.27% T, 28.27% C, 28.52% A, and 14.94% G, with a total A+T content of 56.79%. The phylogenetic tree showed that *O. exiliversabilis* was the sister species of *O. tormota*, and formed a monophyletic clade with other *Odorrana* species. These data provide a powerful tool for evolutionary biology and population genetics of genus *Odorrana*.

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There are currently fifty-nine species of *Odorrana* (Anura: Ranidae) distributed in high-gradient streams of Japan, southern China and Indochina west to northeastern India, Myanmar and Thailand and south to through Malaya and Sumatra to Borneo (Frost 2020). Thirty-seven species of *Odorrana* are currently known in China, and twenty-six of which are endemic to China (AmphibiaChina 2020). The Fujian Bamboo-leaf Frog (*Odorrana exiliversabilis* Li, Ye and Fei 2001) is an endemic Chinese lotic frog, which is distributed in Southern Anhui, western Zhejiang, and Fujian Provinces of China (AmphibiaChina 2020). In GenBank, seven complete mitochondrial DNA (mtDNA) are available for *Odorrana* species, but not including *O. exiliversabilis*. Here, we present the complete mtDNA sequence of *O. exiliversabilis* via Illumina sequencing, and found that it had a close relationship with the other species from the genus *Odorrana*.

We collected the specimen (LSU20200716WY02) of *O. exiliversabilis* in Mount Wuyi National Park, Nanping, Fujian Province, China (N27.70342°, E117.65675°) in July 2020. Presently, the specimen was stored in the Laboratory of Amphibian Diversity Investigation (ADI) at Lishui University. Total DNA was extracted from muscle tissue of *O. exiliversabilis* using EasyPure Genomic DNA Kit (TransGen Biotech Co, Beijing, China). The mtDNA was sequenced by Illumina NovaSeq 6000 (Novogene Bioinformatics Technology Co. Ltd., Tianjin, China) for PE 2 × 150 BP sequencing. Raw sequence data (15.57 G) was deposited in NCBI's Sequence Read Archive (SRA accession: SRR12536539). The NOVO Plasty 3.7 was used to *de novo* assembled the clean data without sequencing adapters (Dierckxsens et al. 2017).

The complete mtDNA sequence (Genbank accession: MT934403) of *O. exiliversabilis* was 17,122 bp in length, which including 13 PCGs (COX1-3, ND1-6, ND4L, ATP6, ATP8 and CYTB), 25 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and 2 non-coding regions of a L-strand replication origin and a control region. The overall base composition of the sequence is 28.27% T, 28.27% C, 28.52% A, and 14.94% G, with a total A+T content of 56.79%. The number of tRNAs in *O. exiliversabilis* was great than in other *Odorrana* species (Su et al. 2007; Bu et al. 2016; Huang et al. 2016; Jin et al. 2020). There are two duplicate genes of tRNA^{Ala}, tRNA^{Asn}, and tRNA^{Tyr} in *O. exiliversabilis* mtDNA. All genes were encoded on the H-strand except ND6 and eleven tRNA genes (tRNA^{Pro}, tRNA^{Gln}, 2 tRNA^{Ala}, 2 tRNA^{Asn}, tRNA^{Cys}, 2 tRNA^{Tyr}, tRNA^{Ser}, and tRNA^{Glu}), which were encoded on the L-strand. Among the 13 PCGs, the ND5 was the longest (1,797 bp), while the ATP8 was the shortest (162 bp). Codon usage analysis of *O. exiliversabilis* showed that four kinds of start codons (ATA, ATG, ATT, and GTG) and six kinds of stop codons (AGA, AGG, TAA, TAG, TA, and T) were used. The 25 tRNA genes varied in size from 63 to 73 bp. The 12S rRNA (938 bp) and 16S rRNA genes (1,585 bp) are located between tRNA^{Phe} and tRNA^{Val} and between tRNA^{Val} and tRNA^{Leu}, respectively.

Phylogenetic analysis was inferred from available mtDNA of *O. exiliversabilis* and other 11 ranid species based on 13 PCGs with *Fejervarya multistriata* (Anura: Dicroglossidae) as the outgroup using Bayesian inference (BI) methods. The optimal substitution model (GTR+I+G) was implemented by MrModelTest 2.3 (Nylander 2004). We analyzed four

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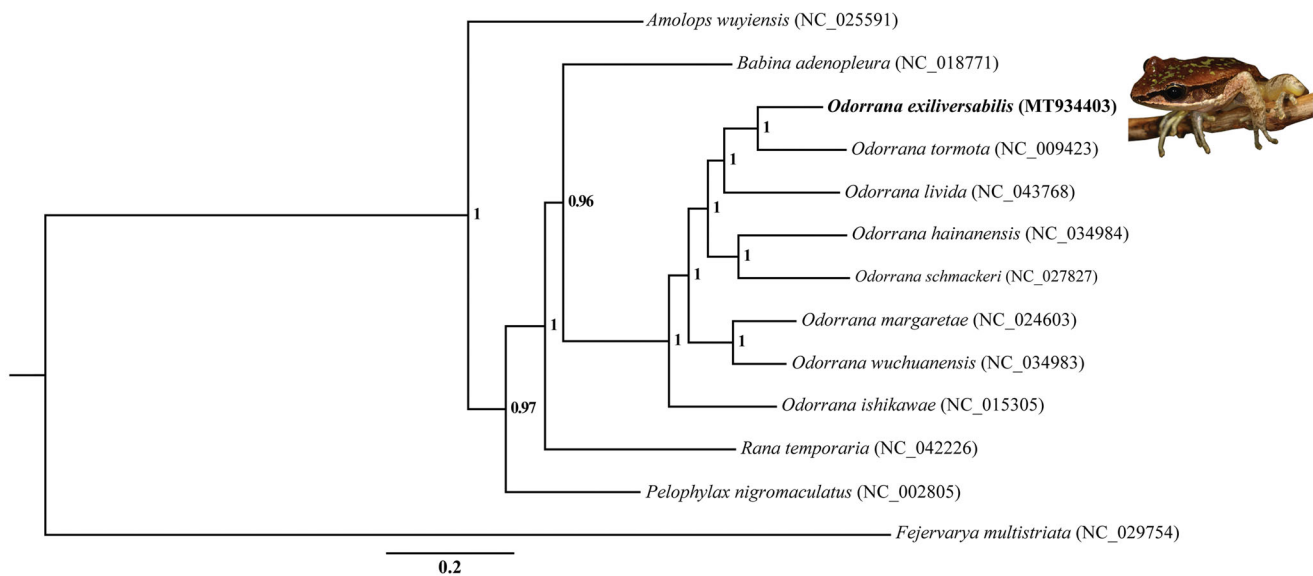


Figure 1. Phylogenetic tree (Bayesian inference) among the ranid species based on the mtDNA sequence of the 13 PCGs. Numbers beside the nodes are posterior probability. Samples sequenced in this study is highlighted in bold.

parallel runs of Markov Chain Monte Carlo (MCMC) for 1,000,000 generations, sampling every 1000 generations and discarded 1000 trees as burn-in. The phylogenetic tree showed that *O. exiliversabilis* was the sister species of *O. tormota*, and formed a monophyletic clade with other *Odorrana* species (Figure 1). *O. ishikawae* was a basal clade relative to others within *Odorrana*, which is similar to the results from Bu et al. (2016) and Jin et al. (2020). These data provide a powerful tool for evolutionary biology, population genetics of genus *Odorrana*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

Mitogenome data supporting this study is openly available in GenBank at: <https://www.ncbi.nlm.nih.gov/nuccore/MT934403>. The SRA accession number is <https://www.ncbi.nlm.nih.gov/sra/SRR12536539>.

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