



Characterization of the complete mitochondrial genome of the Chong'an Moustache Toad, *Leptobrachium liui* (Pope, 1947) with a phylogenetic analysis of Megophryidae

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ABSTRACT

The Chong'an Mustache Toad, *Leptobrachium liui* (Pope, 1947) is a Chinese endemic species, inhabiting the mountain streams with rich vegetation in southeastern China. The first complete mitochondrial genome (mitogenome) of *L. liui* was assembled using the data of whole-genome sequencing. The size of the complete mitogenome for *L. liui* was 17,190 bp, which included 13 *PCGs*, 23 *tRNAs* with two concatenated *tRNA^{Met}* genes, 2 *rRNAs*, a non-coding region, and a *D-loop*. The Bayesian tree shows that *L. liui* was positioned near *L. leishanense* within the genus *Leptobrachium*.

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The genus of *Leptobrachium* Tschudi, 1838 currently contained 37 species distributed in Southern China and India to islands of the Sunda Shelf and the Philippines (Frost 2020). Previously, five *Leptobrachium* species with spines on the upper lip in adult males were classified as *Vibrissaphora* Liu, 1945 (Fei and Ye 2016), but phylogeny showed that *Vibrissaphora* was not a subgenus and placed within the genus of *Leptobrachium* (Matsui et al. 2010). At present, only the complete mitochondrial genomes (mitogenomes) of *Leptobrachium leishanensis* (Liang et al. 2016) and *Leptobrachium boringii* (Li et al. 2019) could be download from the Genbank database. Therefore, more complete mitogenomes of this genus need to be obtained for phylogenetic studies. The Chong'an Mustache Toad, *Leptobrachium liui* (Pope, 1947) is a Chinese endemic species, which mainly inhabits in the mountain streams with rich vegetation in southeastern China (Fujian, Jiangxi, and Zhejiang) (Frost 2020). Here, we firstly reported the complete mitogenome of *L. liui*, representing the third mitogenome from the genus *Leptobrachium*.

The specimens of *L. liui* were captured from the Jiulongshan National Nature Reserve (N28.371988°, E111.898758°) in Suichang County, Zhejiang Province, eastern China. Then these samples were preserved in 90% ethanol at the Museum of Laboratory of Amphibian Diversity Investigation (contact person: Guo-Hua Ding,

E-mail: guwodong@lsu.edu.cn), Lishui University, China. We randomly selected an adult sample (species voucher: LSU20191108JLS001) with the ethanol removed and finished the DNA extraction using the EasyPure genomic DNA kit (TransGen Biotech Co, Beijing, China). Preparation and sequencing of the whole genomic DNA library were finished in Novogene Bioinformatics Technology Co. Ltd. (Tianjin, China). Genomic DNA was fragmented with Covaris to an average insert size of 350 bp and sequenced on the Illumina NovaSeq 6000 platform for paired-end 150 bp. By using NOVO Plasty 3.7 (Dierckxsens et al. 2017), we assembled the mitogenome of *L. liui* from the data of whole-genome sequencing. Then The position and direction of 13 protein-coding genes (*PCGs*), 2 *rRNA* (16S and 12S) genes, 23 *tRNA* genes, a non-coding region (*NCR*) of an L-strand replication origin, and a *D-loop* were predicted by *MITOS WebServer* (Matthias et al. 2013) and *tRNA-scan* (Chan and Lowe 2019).

The length of complete mitogenome of *L. liui* was 17,190 bp (Genbank accession: MW429348) with 28.1% A, 32.6% T, 14.9% G, and 24.4% C, the higher value of A + T content (60.7%) compare to G + C content (39.3%), indicating there was slight A + T bias in *L. liui*. The arrangement pattern and transcription directions of mitogenome of *L. liui* were identical to previous studies in the genus *Leptobrachium* (Liang et al. 2016; Li et al. 2019). Two *tRNA^{Met}* genes (*tRNA^{Met}1* and *tRNA^{Met}2*) were derived from tandem

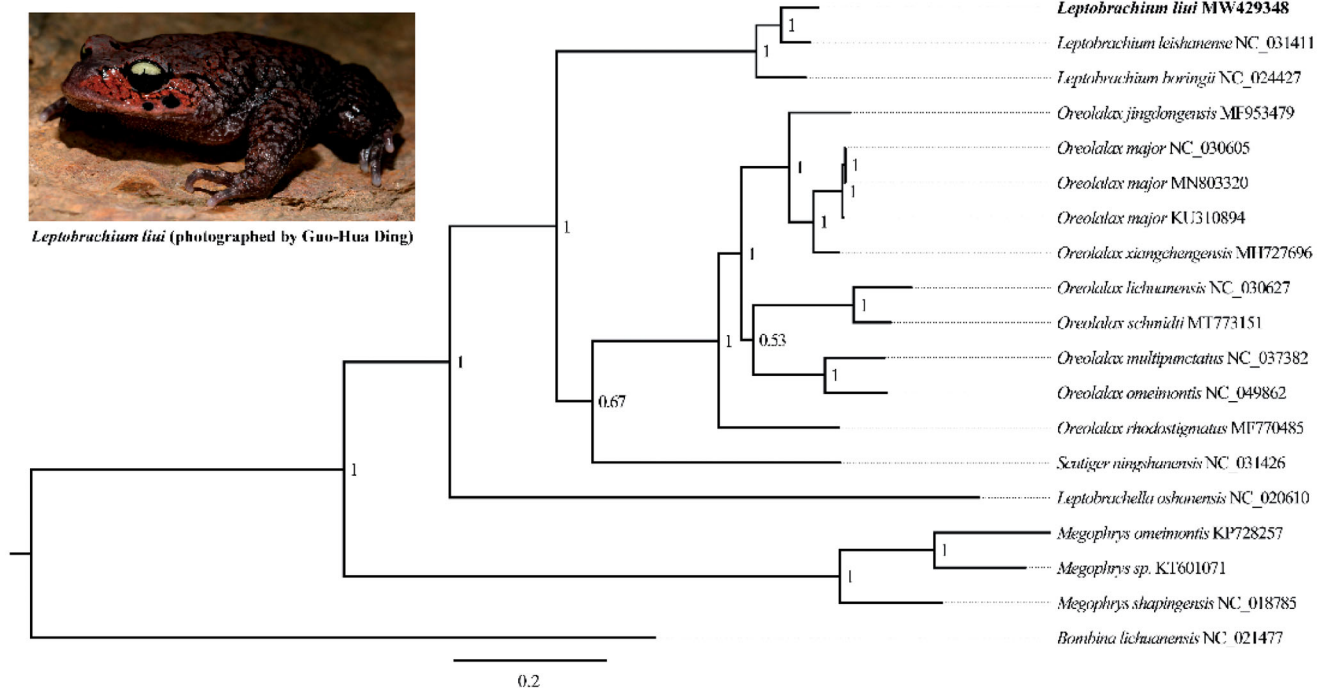


Figure 1. Bayesian tree (BI) tree showing the phylogenetic position of *L. liui* among Megophryidae species based on 13 concatenated mitochondrial *PCGs*. Bayesian posterior probabilities were showed at the nodes. Sample sequenced in this study is highlighted in bold.

duplication in this mitogenome. The *D-loop* (1492 bp) was found between *tRNA^{Trp}* and *tRNA^{Phe}*, and the *NCR* (28 bp) is found between *tRNA^{Asn}* and *tRNA^{Cys}*. The total length of the 13 *PCGs* was 11,394 bp. Nine of them (*ND1*, *ND2*, *COII*, *ATP6*, *COIII*, *ND4L*, *ND5*, *ND6*, and *CYTb*) started with ATG as a start codon, while *COI*, *ATP8*, *ND3*, and *ND4* started with GTG. Two *PCGs* (*ND5* and *ND6*) end with AGG, four *PCGs* (*ND2*, *COI*, *ATP8*, and *ND4*) used completed stop codon TAA, whereas the other seven *PCGs* ended with an incompleting stop codon (T in *COII*, *COIII*, *ND3*, *ND4*, and *CYTb* and TA in *ND1* and *ATP6*).

Bayesian inference tree was constructed with concatenated 13 *PCGs* on MrBayes v3.2.2 setting 4 independent chains and executing 1,000,000 generations based on the published complete mitogenome sequences of the family Megophryidae. *Bombina lichuanensis* (Anura: Bombinatoridae) was chosen as an outgroup. As shown in Figure 1, *L. liui* was positioned near *L. leishanense* within the genus *Leptobrachium*. The *p*-distances of the *CYTb* gene between *L. liui* and the other two *Leptobrachium* species were both more than 7.98% by MEGA 5.05. In addition, the genus *Megophrys* was a basal clade relative to others within The family Megophryidae, which is similar to previous results (Pyrone and Wiens 2011; Liang et al. 2016). The complete mitogenome of *L. liui* in this study will be an important genetic resource to the studies of conservation and restoration of *L. liui*. Furthermore, it will play an important role in understanding the molecular evolution of the family Megophryidae.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The mitogenome data supporting this study are openly available in GenBank at (<https://www.ncbi.nlm.nih.gov/nuccore/MW429348>). Reference number (Accession number: MW429348). BioSample and SRA accession numbers are (<https://www.ncbi.nlm.nih.gov/biosample/SAMN17205596>), (<https://www.ncbi.nlm.nih.gov/sra/SRR13345317>), respectively.

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