

Sequence and analysis of the complete mitochondrial genome of the Wuchuan Odorous Frog *Odorrana wuchuanensis* (Anura: Ranidae)

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ABSTRACT

The complete mitochondrial genome of the Wuchuan Odorous Frog was 18,256 bp in length including 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and a control region and was similar to that of typical vertebrates. The base composition was 27.89% A, 29.00% C, 15.34% G, and 27.78% T. All genes were encoded on the H-strand except *ND6* and eight tRNA genes (*tRNAPro*, *tRNAGln*, *tRNAAla*, *tRNAAsn*, *tRNACys*, *tRNATyr*, *tRNASer*, and *tRNAGlu*), which were encoded on the L-strand. The phylogenetic relationship of Anura based on complete mitochondrial genomes showed that *O. wuchuanensis* is closest to *O. margaretae* with strong support and the genetic distance between Ranidae, Dicroglossidae, and Rhacophoridae was closer than others.

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The wuchuan odorous frog (*Odorrana wuchuanensis*) belongs to the order Anura, the Ranidae and is an endemic species to China with a narrow distribution in karst area in southwestern China. The number of *O. wuchuanensis* is decreasing due to the increasing attenuation of habitat. In this study, we determined the complete mitochondrial DNA (mtDNA) sequence of *O. wuchuanensis* (Genbank accession: KU680791) for further evolutionary researches as well as for the protection of this species.

The toe-clip tissue of *O. wuchuanensis* were collected from a specimen (Sample No. LBML 5230) stored in MAO LAN national nature reserve (N25°09'~25°20', E107°52'~108°45') in Libo county, Guizhou province, China. Total genomic DNA was extracted with the TIANamp Genomic DNA Kit (Tiangen, Beijing, China) following the kit's instructions. The complete mitochondrial genome was amplified by polymerase chain reaction (PCR) with eight primers, of which primer L1 and Cytb is available from references (Rassmann 1997; Zhang et al. 2013) while primer ND2 and ND4 was designed based on the complete mtDNA sequence of *O. margaretae* (NC_024603) and the others were designed specifically for the rest long fragments of mtDNA amplified in long and accurate PCR (LA-PCR). All the PCR fragments were directly sequenced.

The complete sequence of the mitogenome of *O. wuchuanensis* is 18,256 bp in length with 55.66% A + T content. The overall nucleotide composition of this genome was 27.89% A, 29.00% C, 15.34% G, and 27.78% T, similar to typical vertebrate mtDNA. The genome contained 13 protein-coding

genes, 2 ribosomal RNA genes, 22 tRNA genes, and 1 control region. The total length of 22 tRNA genes was 1527 bp, ranging from 64 bp (*tRNACys*) to 74 bp (*tRNALeu(UUR)*). The total length of 13 protein-coding genes was 11,301 bp, of which all genes were encoded on the H-strand except *ND6*, as well as eight tRNA genes (*tRNAPro*, *tRNAGln*, *tRNAAla*, *tRNAAsn*, *tRNACys*, *tRNATyr*, *tRNASer*, *tRNAGlu*), which were encoded on the L-strand. The start codons were ATG for *ND1*, *COII*, *ATP8*, *COIII*, *ND3*, *ND4L*, *ND4*, *ND5*, *ND6*, and *CYTB* while ATT for *ND2*, GTG for *COI* and ATA for *ATP6*. *COI* and *ND6* terminated with AGG as stop codon; *ATP8*, *ND4L*, *ND4* with TAA; *ND5*, *CYTB* with TAG, and other six protein-coding genes end with an incomplete stop codon (a single stop nucleotide T). The two ribosomal RNA genes including 12SrRNA (1219 bp in length) and 16SrRNA (2874 bp in length) were commonly located between *tRNAPhe* and *tRNALeu(UUR)*. The control region was 2857 bp in length with 61.95% A + T content.

Within the complete mitochondrial genome of *O. wuchuanensis*, there were five reading frame overlaps (The *tRNAGln* and *tRNAMet* share two nucleotides; OL and *tRNACys* share three nucleotides; *COI* and *tRNASer* share nine nucleotides; *ATP8* and *ATP6* share four nucleotides; *ND4L* and *ND4* share seven nucleotides). Compared with the Green Odorous Frog (*O. margaretae*) (Chen et al. 2015) and Concave-eared Torrent Frog (*Amolops tormotus*) (Su et al. 2007), all of the start codons were similar except *ND1*, *ND2*, *COI*, and *ND3*. The stop codon was GTG for *ND1* in *O. margaretae*, while ATG in *O. wuchuanensis* and *A. tormotus*. The stop codon was ATG for *ND2* in *A. tormotus* while ATT in *O. wuchuanensis* and

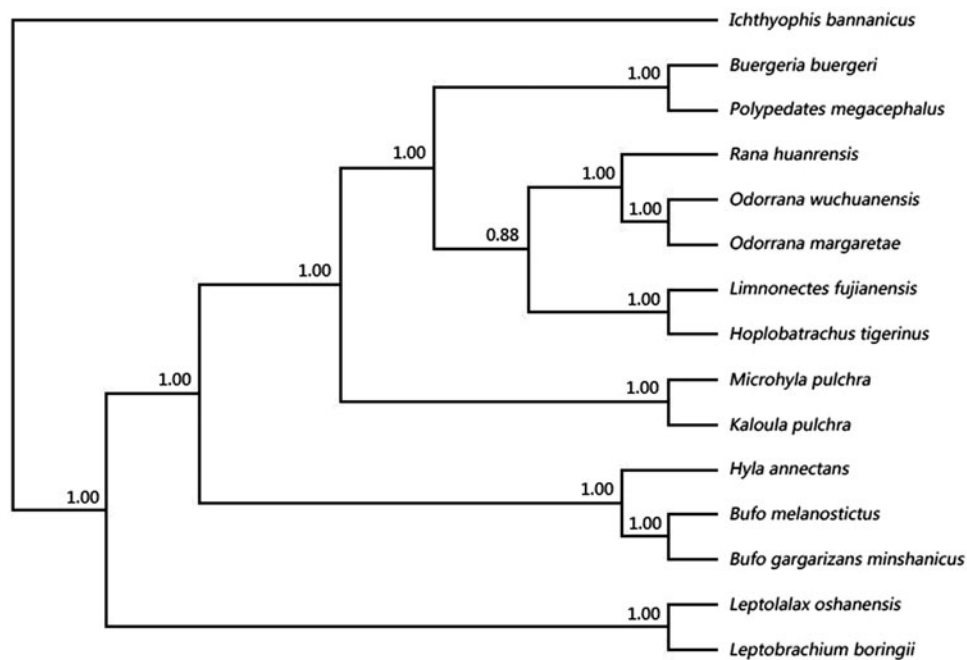


Figure 1. It is the 50% majority rule consensus tree from the Bayesian inference (BI) of complete mtDNAs. The values above branches represent Bayesian clade credibility values. The phylogenetic tree contains 14 species of Anura and *Ichthyophis bannanicus* (NC_006404) used as outgroup. Sequence data used in this study were the following: *Hoplobatrachus tigerinus* (AP011543), *Limnonectes fujianensis* (AY974191), *Bufo gargarizans minshanicus* (KM587710), *Bufo melanostictus* (AY458592), *Kaloula pulchra* (AY458595), *Microhyla pulchra* (KF798195), *L. boringii* (KJ630505), *L. oshanensis* (KC460337), *Buergeria buergeri* (AB127977), *Polypedates megacephalus* (AY458598), *Pelophylax nigromaculatus* (AB043889), *R. huanrensis* (NC_028521), *Hyla annectans* (KM271781), *O. wuchuanensis* (KU680791).

O. margaretae. The stop codon was ATG for *COI* in *A. tormotus* while GTG in *O. wuchuanensis* and *O. margaretae*. The stop codon was GTG for *ND3* in *O. margaretae* while ATG in *O. wuchuanensis* and *A. tormotus*.

The phylogenetic analysis of the Anura based on complete mitochondrial genomes was conducted with Bayesian inference (BI). The best-fitted substitution model was determined in MODELTEST v.3.7 (Posada & Crandall 1998) by Akaike Information Criterion (AIC). The BI was conducted in MrBayes 3.2 (Ronquist et al. 2012) with Markov Chain Monte Carlo (MCMC) running for 50,000,000 generations, and sampling frequency for 100. A consensus tree was calculated after omitting the first 25% trees as burn-in. The 50% majority rule consensus tree inferred from Bayesian analysis with high clade credibility values shows that the *Leptolalax oshanensis* and *Leptobrachium boringii* are grouped as the sister taxon to a clade comprising the others of Anura (Figure 1). Specially, *O. wuchuanensis* and *O. margaretae* are closest and they are grouped as the sister taxon to a clade comprising *Rana huanrensis*. Besides, the genetic distance between Ranidae, Dicroglossidae, and Rhacophoridae was closer than others in phylogenetic relationship as shown in Figure 1.

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Disclosure statement

The authors report no conflicts of interest. The authors are responsible for the content and alone writing of this paper.

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