

The complete mitochondrial genome of *Rana wuyiensis* (Anura, Ranidae) and its phylogenetic analysis

Yan-Mei Wang^{a,b}, Hui-Guang Zhang^c, Bin Cai^c, Guo-Hua Ding^b and Yong Zhang^a

^aCollege of Biology and the Environment, Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing, China; ^bCollege of Ecology, Laboratory of Amphibian Diversity Investigation, Lishui University, Lishui, China; ^cResearch and Monitoring Center, Wuyishan National Park, Wuyishan, China

ABSTRACT

The complete mitochondrial genome (mitogenome) of *Rana wuyiensis* (Anura, Ranidae) was sequenced and annotated. The circular complete mitogenome was 17,779 bp in length (OL467321) and constituted of 37 genes including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs), and a D-loop region. The result of Bayesian phylogenetic analysis indicated that *R. wuyiensis* exhibits a close relationship with *R. johnsi*. *R. wuyiensis* was recently discovered and therefore holds a lot of potential for future research studies. Our study added a new mitochondrial data and provided valuable reference material to the taxonomy of *Rana*.

ARTICLE HISTORY

Received 23 December 2021
Accepted 7 April 2022

KEYWORDS

Ranidae; *Rana wuyiensis*; mitogenome; phylogenetic relationship

The brown frog genus *Rana* sensu lato Linnaeus, 1758 (Anura, Ranidae Batsch, 1796) is broadly distributed across Eurasia, Indochina, and North America (Frost 2021). The sub-genus *Rana*, which is currently well recognized, contains 41 known species. Among them, 26 species occur in China (Wu et al. 2021). Recently, a new *Rana* species, *Rana wuyiensis*, from Wuyi Mountain of Fujian Province in China was described (Wu et al. 2021). Here, we sequenced the complete mitochondrial genome (mitogenome) of *R. wuyiensis* and determine the systematic relationship to other *Rana* species.

The specimen of *R. wuyiensis* (species voucher: LSU202109001WYS01) was collected at an altitude of 923 m from Wuyishan National Park, Fujian Province, China (27.7291N, 117.6964E) on 5 September 2021. The experimental procedures followed in this study complied with the animal welfare and research laws in China and were approved by the Ethics Committee of Lishui University (permit no. AREC-LSU202109001DGH). The collected specimen was stored in 75% ethanol and deposited at the Museum of Laboratory of Amphibian Diversity Investigation (contact person: Guo-Hua Ding, E-mail: guowoding@lsu.edu.cn) at Lishui University. The experimental procedures used in this study complied with the current laws related to animal welfare and research in China and were specifically approved by the Animal Research Ethics Committee of Lishui University (no. ARECLSU20210905001). Total genomic DNA was extracted from the leg muscle using EasyPure Genomic DNA Kit (TransGen Biotech Co., Beijing, China). The sequencing library was produced and loaded on the Illumina HiSeq 6000 platform for paired 150 bp sequencing. The raw sequence data of species were deposited in NCBI's Sequence Read Archive

(SRA accession: SRR16914143). NOVO Plasty 3.7 was used to assemble the clean data without sequencing adapters (Dierckxsens et al. 2017). Genome annotation was performed with Mitoz annotation module (Meng et al. 2019).

The circular complete mitogenome of *R. wuyiensis* (GenBank accession no. OL467321) was 17,779 bp in length, with 28.0% of A, 28.5% of T, 14.2% of G, and 29.3% of C. The higher value of A + T content (56.5%) compared to G + C content (43.5%) indicated that there was a slight A + T bias in *R. wuyiensis*. In total, 38 genes were predicted, which are 13 protein-coding genes (PCGs) containing COX1-3, ND1-6, ND4L, ATP6, ATP8, and CYTB, 22 transfer RNAs (tRNAs), two ribosomal RNAs (12 rRNA and 16 rRNA), and a D-loop. The D-loop (2245 bp) was found between CYTB and tRNA^{Leu}. The total length of the 13 PCGs was 11,294 bp. Nine of them (COX2, COX3, ND3, ND4, ND5, ND6, ATP6, ATP8, and CYTB) started with ATG as a start codon, two PCGs (ND1, COX1) started with ATA as a start codon. Furthermore, three typical stop codons were found: TAG, AGG, TAA, and meanwhile the incomplete stop codons (TA and T) were also present in the species' mitogenome.

In order to determine the taxonomic status of *R. wuyiensis*, we constructed the phylogenetic tree using Bayesian's inference method by MrBayes 3.2.7 (Ronquist and Huelsenbeck 2003) based on concatenation sequence, including 13 PCGs and two rRNAs. The final alignment consisted of 16 sequences of genus *Rana* sensu lato (*R. amurensis*, *R. chaochiaoensis*, *R. chensinensis*, *R. dabieshanensis*, *R. draytonii*, *R. dybowskii*, *R. huanrenensis*, *R. johnsi*, *R. kukunoris*, *R. kunyuensis*, *R. omeimontis*, *R. pyrenaica*, *R. temporaria*, *R. uenoi*, *R. wuyiensis*, and *R. zhenhaiensis*), and *Lithobates catesbeianus* was selected as the out-group. The optimal substitution model (GTR + I + G) was

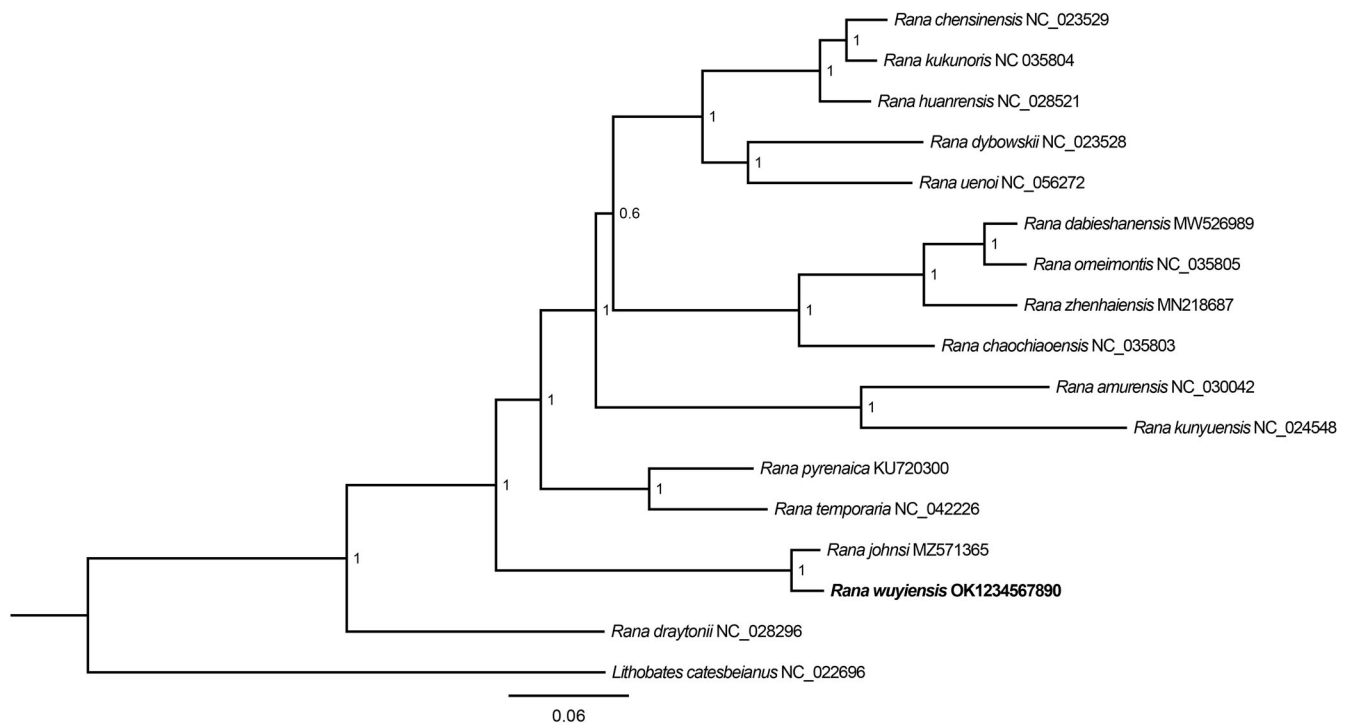


Figure 1. Phylogenetic tree of *Rana wuyiensis* and other related species based on the concatenation sequence including 13 PCGs and two rRNAs from mitogenome. Numbers at the nodes represent Bayesian posterior probabilities. Sample sequenced in the present study is highlighted in bold.

implemented via jModelTest (Darriba et al. 2012). Four parallel runs of Markov Chain Monte Carlo (MCMC) were analyzed for 1,000,000 generations, sampling every 1000 generations and discarded 1000 trees as burn-in. The results of phylogenetic relationship showed that *R. wuyiensis* and *R. johnsi* were close related, and *R. draytonii* was a basal clade relative to others within *Rana sensu lato* (Figure 1). Further, we analyzed the genetic divergence between *R. wuyiensis* and other *Rana* species based on the 13 PCGs and two rRNA using MEGA 5.05. As noted, the *p*-distance between *R. wuyiensis* and *R. johnsi* was 2.8%, while those between *R. wuyiensis* and other 14 *Rana* species were all more than 14%. The new complete mitogenome of *R. wuyiensis* in this study will contribute to the further research of molecular evolution of the genus *Rana sensu lato*.

Authors contributions

Y.M.W., G.H.D., and Y.Z. wrote the paper. G.H.D. and Y.Z. designed and supervised the study. H.G.Z. and B.C. captured and maintained animals. Y.M.W. and G.H.D. collected data for the study. G.H.D. did the analysis and prepared the figure. All authors read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Project of Amphibian and Reptile Biodiversity Survey in National Park of Wuyi Mountain [[3500]BYZB[CS]2020001].

Data availability statement

The mitogenome data supporting this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> under the accession no. OL467321. The associated SRA, BioSample, and BioProject accession nos. are SRR16914143, SAMN23039770, and PRJNA636742, respectively.

References

- Batsch AJGK. 1796. Umriß der gesammten Naturgeschichte: ein Auszug aus den frühern Handbüchern des Verfassers für seine Vorlesungen. Gabler; p. 287.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods*. 9(8):772.
- Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Res*. 45(4):e18.
- Frost DR. 2021. Amphibian species of the world: an online reference. Version 6.0. New York: American Museum of Natural History; [accessed 2021 Nov 12]. <http://research.amnh.org/herpetology/amphibia/index.html>.
- Linnaeus C. 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. 10th ed. Vol. 1. Stockholm: L. Salvii.
- Meng GL, Li YY, Yang CT, Liu SL. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. *Nucleic Acids Res*. 47(11):e63.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*. 19(12):1572–1574.
- Wu Y, Shi S, Zhang H, Chen W, Cai B, Hoang VC, Wu J, Wang B. 2021. A new species of the genus *Rana sensu lato* Linnaeus, 1758 (Anura, Ranidae) from Wuyi Mountain, Fujian Province, China. *Zookeys*. 1065: 101–124.