The complete mitochondrial genome of *Rana johnsi* (Smith, 2009) (Anura: Ranidae) and its phylogeny

Qing-Ping Chen\textsuperscript{a,b}, Lian Wu\textsuperscript{c}, Shu-Sheng Zhang\textsuperscript{c}, Lei-Lei Liu\textsuperscript{c}, Wan-Ting Jin\textsuperscript{a}, Jia-Yong Zhang\textsuperscript{a,d} \textsuperscript{b}, Yong-Pu Zhang\textsuperscript{a,d} and Dan-Na Yu\textsuperscript{a,d}

\textsuperscript{a}College of Chemistry and Life Science, Zhejiang Normal University, Jinhua, PR China; \textsuperscript{b}College of Life and Environmental Science, Wenzhou University, Wenzhou, PR China; \textsuperscript{c}The Management Center of Wuyanling, National Natural Reserve in Zhejiang, Wenzhou, PR China; \textsuperscript{d}Key Lab of Wildlife Biotechnology, Conservation and Utilization of Zhejiang Province, Zhejiang Normal University, Jinhua, PR China

**ABSTRACT**

*Rana johnsi* (Smith 2009) firstly considered as the member of genus *Pseudorana*, has been moved into the genus *Rana*. In this study, we sequenced the complete mitochondrial (mt) genome of *R. johnsi* using the Sanger method. The circular mt genome was 17,873 bp in length and contains 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, two ribosomal RNA genes, and one control region. The overall nucleotide composition in majority-strand was 28% A, 29% T, 29% C, and 14% G. We discussed the phylogenetic relationship of *R. johnsi* in genus *Rana* using ML and BI analyses based on 13 PCGs. Excluding the clade of subgenus Lithobates, *Rana draytonii* was the basal clade to all other *Rana* species, which included *R. johnsi* as the basal clade. The monophyly of genus *Rana* was supported, whereas *Pseudorana* was failed to support.

The phylogenetic relationships of *Rana* were involved in a heated discussion using mitochondrial (mt) genomes (Dubois 1992; Frost et al. 2006; Che et al. 2007). *Rana johnsi* (Smith 2009) (Anura: Ranidae), called as John’s groove-toed frog (Fei et al. 1999), was distributed in China, Vietnam, Laos, Thailand, and Cambodia (Frost 2021). Whereas *R. johnsi* was belonging to genus *Pseudorana* according to the taxonomy of Fei et al. (2010). But now it is considered as genus *Rana* species. The mitogenome of *R. johnsi* has not been reported to date. So, in this study, we sequenced and analyzed the mt genome of *R. johnsi* to discuss its phylogenetic relationship.

The frog sample (No. GJX20180723-1) collected from Jinxiu, Guangxi Province, China (24.14°N, 110.18°E) was identified by JY Zhang and stored at −40°C in the Animal Specimen Museum, College of Life Sciences and Chemistry, Zhejiang Normal University, China. Total genomic DNA (No. YNGZW0723) was extracted from leg muscle using an Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech Company, Shanghai, China) and stored in the Zhang’s lab (http://sky.zjnu.edu.cn/2019/0319/c4853a284409/page.htm, DN Yu, email: ydn@zjnu.cn). The mt genome was amplified by polymerase chain reaction (PCR) with universal primers according to Zhang et al. (2013). The specific primers were designed based on the amplified fragments by Primer Premier version 5.0 (Primer Biosoft International, Palo Alto, CA) to amplify the remaining gaps between sequences. The obtained whole mt genome was deposited in the NCBI with accession number MZ571365.

The complete mt genome of *R. johnsi* in majority-strand was 17,873 bp in length with negative AT-skew and GC-skew, which were −0.006 and −0.349, respectively. It encoded 37 genes including 13 protein-coding genes (PCGs), 22 transfer RNAs, two ribosomal RNAs genes, and one control region. The total length of the PCGs was 11,295 bp. The start codons of PCGs were ATG (in COX2, ATP8, ATP6, COX3, ND3, ND4, ND5, ND6, and Cytb), ATA (in ND1 and COX1), ATT (in ND2) and GTG (in ND4L). The stop codons of PCGs were TAA (in ND4L and Cytb), TAG (in ND2 and ATP8), AGA (in COX2, ND5, and ND6), AGG (in COX1), and the incomplete stop codon T (in ND1, ATP6, COX3, ND3, and ND4). The longest spacer region was 246 bp and it located between ND5 and ND6, which was also found in *Rana cf. chensinensis* (Li et al. 2016a). The gene arrangement was identical to the gene order pattern of *Rana* (Huang et al. 2019; Jiang et al. 2020; Suk et al. 2021). The overall nucleotide composition of A, T, C, and G in majority-strand was 28%, 29%, 29%, and 14%, respectively.

To explore the phylogenetic relationship of *R. johnsi*, a total of 28 mitogenomes were analyzed, including 23 mt genomes of *Rana* downloaded from NCBI (Lin et al. 2014; Ni et al. 2016; Li et al. 2016a, 2016b; Liu et al. 2017; Chen 2018; Yang et al. 2018; Huang et al. 2019; Jiang et al. 2020; Wang et al. 2020; Suk et al. 2021; Xiong et al. 2021) and four mt genomes of *Pseudorana*.
The phylogenetic relationships of the other species were similar to the results of Chen (2018), Wang et al. (2020), and Suk et al. (2021). According to the importance of mt taxonomy and phylogenetic inference, our study provided a correct phylogenetic relationship of *R. johnsi*. The new mt genomes of *Rana* can give us a further understanding of phylogenetic relationships within *Rana*.

Figure 1. Phylogenetic tree of the relationships among 28 species of Ranidae including *Rana johnsi* based on the nucleotide dataset of the 13 mitochondrial protein-coding genes. Numbers around the nodes are the posterior probabilities of ML (left) and the bootstrap values of BI (right). The GenBank numbers and tribe of all species are shown in the figure.

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

Funding
This work was supported by the College Students’ Innovation and Entrepreneurship Project of China (201910345030 and 202010345026) and the College Students’ Innovation and Entrepreneurship Project of Zhejiang Province (202010345R128). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ORCID
Jia-Yong Zhang http://orcid.org/0000-0002-7679-2548
Dan-Na Yu http://orcid.org/0000-0002-9870-1926

Data availability statement
The genome sequence data that support the findings of this study are openly available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/nucleotide) under the accession no. MZ571365.

References
Che J, Pang JF, Zhao H, Wu GF, Zhao ZM, Zhang YP. 2007. Phylogeny of Raninae (Anura: Ranidae) inferred from mitochondrial and nuclear sequences. Mol Phylogenet Evol. 43(1):1–13.

Chen JJ. 2018. The complete mitochondrial genome of common terrestrial frog (*Rana temporaria*). Mitochondrial DNA B Resour. 3(2):978–979.

Dubois A. 1992. Notes sur la classification des Ranidae (Amphibiens Anoures). Bull Mens Soc Linn Lyon. 61(10):305–352.

Fei L, Ye CY, Huang YZ, Liu MY. 1999. Atlas of amphibians of China. Zhengzhou, China: Henan Science and Technology Press.
Fei L, Ye CY, Jiang JP. 2010. Phylogenetic systematics of Ranidae. Herpetol Sin. 12:1–43.

Fouquette JMJ, Dubois A. 2014. A checklist of North American amphibians and reptiles: the United States and Canada. Bloomingtom (IN): Xlibris Corporation.

Frost DR. 2021. Amphibian species of the world: an online reference. Version 6.1 (date of access). Electronic database. New York (NY): American Museum of Natural History. https://amphibiansoftheworld.amnh.org/index.php.

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, Sá ROD, Channing A, Wilkinson M, Donnellan SC, et al. 2006. The amphibian tree of life. Bull Am Museum Nat Hist. 297:1–370.

Huang MY, Zhao Q, Wu YQ. 2019. Characterization of the complete mitochondrial genome of Zhenhai brown frog Rana zhenhaiensis (Anura: Ranidae). Mitochondrial DNA B Resour. 4(2):3204–3205.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17(8):754–755.

Jiang LC, Zhang M, Deng L, Xu ZW, Shi HY, Jia XD, Lai ZL, Ruan QP, Chen W. 2020. Characteristics of the mitochondrial genome of Rana omeimontis and related species in Ranidae: gene rearrangements and phylogenetic relationships. Ecol Evol. 10(23):12817–12837.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33(7):1870–1874.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol. 29(6):1695–1701.

Li J, Lei GC, Fu CZ. 2016a. Complete mitochondrial genomes of two brown frogs, Rana dybowski and Rana cf. chensinensis (Anura: Ranidae). Mitochondrial DNA Part A. 27(1):155–156.

Li J, Yin W, Xia R, Lei GC, Fu CZ. 2016b. Complete mitochondrial genome of a brown frog, Rana kunyuenis (Anura: Ranidae). Mitochondrial DNA Part A. 27(1):34–35.

Lin YB, Tao BF, Fang XD, Wang TT, Zhang JY. 2014. The complete mitochondrial genome of Lithobates catesbeianus (Anura: Ranidae). Mitochondrial DNA. 25(6):447–448.

Liu P, Wang H, Zhao WG. 2017. Sequencing and analysis of the complete mitochondrial genome of Rana amurensis (Anura: Ranidae). Mitochondrial DNA B Resour. 22(2):424–425.

Ni NN, Yu DN, Storey KB, Zheng RQ, Zhang JY. 2016. The complete mitochondrial genome of Lithobates sylvaticus (Anura: Ranidae). Mitochondrial DNA A DNA Mapp Seq Anal. 27(4):2460–2461.

Pyron RA, Wiens JJ. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Mol Phylogenet Evol. 61(2):543–583.

Schwarz G. 1978. Estimating the dimension of a model. Ann Statist. 6(2):461–464.

Smith MA. 2009. New or little-known reptiles and batrachians from southern Annam (Indo-China). Proc Zool Soc Lond. 91(2):423–440.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 22(21):2688–2690.

Suk HY, Jeon JY, Kim DY, Cha S, Min MS. 2021. The complete mitochondrial genome information of Rana uenoi (Amphibia, Anura, Ranidae) and the phylogenetic implication. Mitochondrial DNA B Resour. 6(2):689–690.

Wang JF, Li ZZ, Gao H, Liu ZS, Teng LW. 2020. The complete mitochondrial genome of the Rana kukunoris (Anura: Ranidae) from Inner Mongolia, China. Mitochondrial DNA B Resour. 5(1):586–587.

Xiong G, Jiang H, Wang XQ, Kang L, Liu L, Zhou XW, Wang P, Yang T. 2021. The complete mitochondrial genome of the Rana sanghensis Shen and its phylogenetic analyses. Mitochondrial DNA B Resour. 6(2):329–330.

Yang JD, Yu JJ, Liu JB, Zhou M, Li B, Ouyang B. 2018. Three new Ranidae mitogenomes and the evolution of mitochondrial gene rearrangements among Ranidae species. Asian Herpetol Res. 9(02):85–98.

Zhang JY, Zhang LP, Yu DN, Storey KB, Zheng RQ. 2018. Complete mitochondrial genomes of Nanorana talihangnica and N. yunnanensis (Anura: Dicroglossidae) with novel gene arrangements and phylogenetic relationship of Dicroglossidae. BMC Evol Biol. 18(1):26.

Zhang P, Liang D, Mao RL, Hillis DM, Wake DB, Cannatella DC. 2013. Efficient sequencing of anuran mtDNAs and a mitogenomic exploration of the phylogeny and evolution of frogs. Mol Biol Evol. 30(8):1899–1915.