


Complete mitochondrial genome of the Red Keelback (*Pseudagkistrodon rudis* Boulenger, 1906)

Yi-Nan Zhou^a, Chuan-Ying Zhang^b, Zheng-Hua Zhang^c, Jun-Jie Zhong^a and Guo-Hua Ding^a 

^aLaboratory of Amphibian Diversity Investigation, College of Ecology, Lishui University, Lishui, Zhejiang, China; ^bDevelopment Center of Suichang Ecological Forestry, Suichang, China; ^cKey Laboratory of Biosafety, Nanjing Institute of Environmental Sciences, Nanjing, China

ABSTRACT

The Red Keelback (*Pseudagkistrodon rudis* Boulenger, 1906) is widely distributed in the southern of China. The complete mitochondrial genome (mitogenome) of *P. rudis* was determined for the first time by using next-generation sequencing. The size of assembled mitogenome for *P. rudis* was 19,150 bp, which included 13 protein coding genes (PCGs), 22 tRNAs, two rRNAs and two control regions (D-loop1 and D-loop2). The Bayesian tree showed that *P. rudis* and *Rhabdophis tigrinus* have a closed relationship. These results can provide data for phylogeny and molecular classification of the genus.

ARTICLE HISTORY

Received 31 December 2020
Accepted 24 April 2021

KEYWORDS

Colubridae;
Pseudagkistrodon rudis;
mitogenome; phylogeny



The Red Keelback (*Pseudagkistrodon rudis* Boulenger, 1906) belongs to the snake family of Colubridae, which is an aggressive but harmless snake, and widely distribution in the southern of China (Uetz et al. 2020). The genus *Macropisthodon* includes two branches, the branch represented by *P. rudis* is an independent monotypic genus and *P. rudis* is a Chinese endemic species, therefore this species in China use *Pseudoagkistrodon* as genus name instead of *Macropisthodon* (Takeuchi et al. 2018). And *P. rudis* was included in the 'National Protected List of Terrestrial Wild Animals with Good Benefits or Important Economic and Scientific Values' issued by the State Forestry Administration of China on 1 August 2000. Mitochondrial genome (mitogenome) is widely used in phylogenetic and evolutionary studies in these days (Crimi and Rigolio 2008). It is the first time to study the complete mitochondrial genome of *P. rudis*.

The specimen of *P. rudis* (species voucher: LSU202007 22WF01) was collected at Xiayangxiang (N27.87354294°, E119.82021360°) from Wencheng County, Wenzhou City, Zhejiang Province, China. The collected specimen was stored in 90% 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. Whole genomic DNA was extracted from muscle tissue using the EasyPure genomic DNA kit (TransGen Biotech Co., Beijing, China). The whole genome sequencing (WGS) was implemented by Illumina NovaSeq 6000 platform (Novogene Bioinformatics Technology Co. Ltd., Tianjin, China) for paired-end 150 bp. We used NOVO Plasty 3.7 (Dierckxsens et al. 2017) to assemble the mitogenome referencing random partial mitogenome (about 200 bp) of a snake from family

Colubridae based on the WGS data, and then used MITOS WebServer (Bernt et al. 2013) to annotate and locate the genes.

The complete mitogenome of *P. rudis* (GenBank accession no. MW327508) is 19,150 bp in length, and contains 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNA), two ribosomal RNA genes (rRNA) and two control regions (D-loop1 and D-loop2). In these 37 mitochondrial genes, most of them were in the positive strand, except *ND6*, *tRNA^{Gln}*, *tRNA^{Ala}*, *tRNA^{Asn}*, *tRNA^{Cys}*, *tRNA^{Tyr}*, *tRNA^{Ser}*, *tRNA^{Glu}*, and *tRNA^{Pro}*, which were in the minus chain. Among the 13 PCGs, the longest gene is *ND5* (1782 bp), while the shortest is *ATP8* (165 bp). The 12S rRNA (922 bp) was located between *tRNA^{Phe}* and *tRNA^{Val}*, and the 16S rRNA (1448 bp) was located between *tRNA^{Val}* and *ND1*. D-loop1 (1926 bp) was located between *tRNA^{Leu}* and *tRNA^{Leu}*; D-loop2 (2164 bp) was located between *tRNA^{Pro}* and *tRNA^{Phe}*. ATA and ATG are the start codons of PCGs; only three PCGs (*ND1*, *ND3*, *COI*) use ATA; the others use ATG. There are six types of termination codons, include T (*ND1*, *COIII* and *Cytb*); TA (*ATP6*); TAG (*ND2* and *ND6*); AGA (*COI*); TAA (*COII*, *ATP8*, *ND3*, and *ND4L*); AGG (*ND4* and *ND5*).

In the study, we used PhyloSuite v1.2.1 (Zhang et al. 2020) to assemble the 13 PCGs (11,319 bp) in mitogenomes of 18 snakes from the family of Colubridae and Elapidae. Based on the assembled PCGs the phylogenetic relationship were constructed by MrBayes v3.2.2. (Ronquist and Huelsenbeck 2003) (Figure 1). *Naja atra* (Squamata: Elapidae), which is relatively close to their ancestors, was selected as the outgroup. GTR+I+G as the best-fit substitution model was obtained using MrModelTest 2.3 (Nylander 2004). It appeared that *P. rudis* and *Rhabdophis tigrinus* have a closed relationship, and

CONTACT Guo-Hua Ding  guowoding@lsu.edu.cn  Laboratory of Amphibian Diversity Investigation, College of Ecology, Lishui University, Lishui 323000, Zhejiang, China

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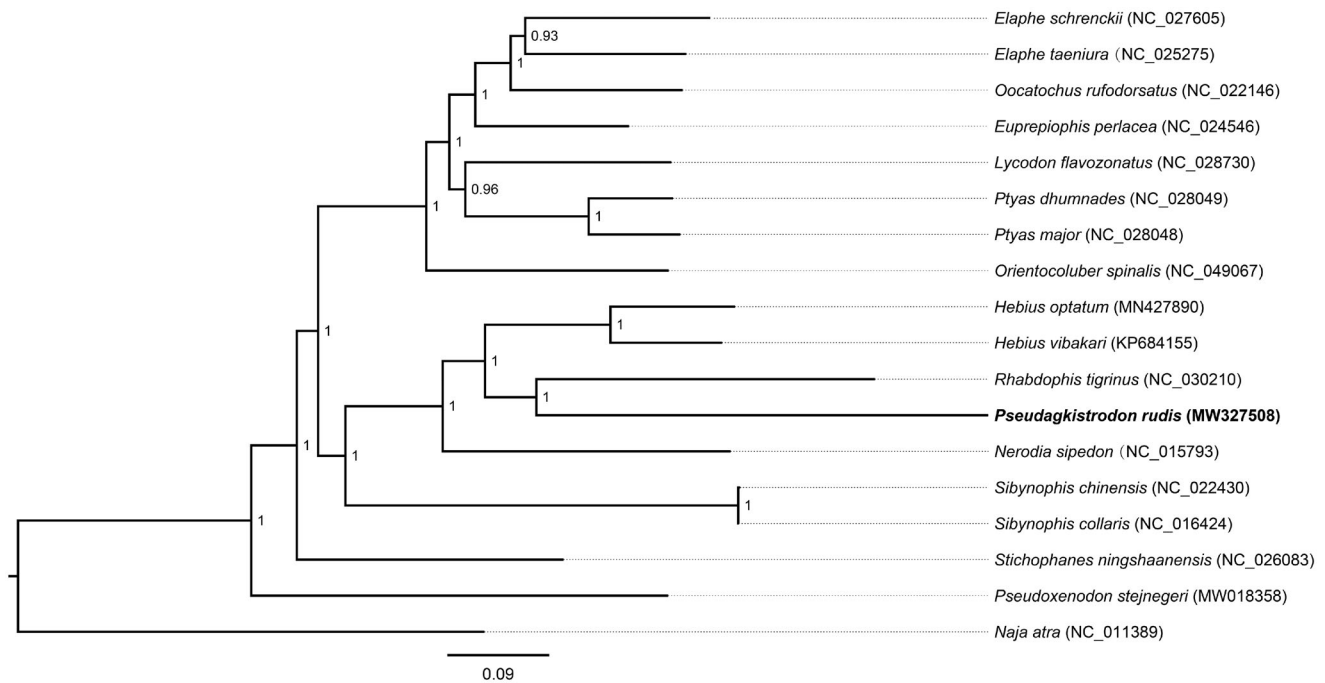


Figure 1. Bayesian tree of 18 snakes based on 13 concatenated mitochondrial protein-coding genes. Numbers at the nodes represent posterior probabilities.

both species were closed with *Hebius* species. It is similar to a previous study (Pyron et al. 2013). These results can provide data for phylogeny and molecular classification of the genus.

Disclosure statement

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

Funding

This work was supported by Public Welfare Technology Application Research Project of Lishui City [2020GYX06], Biodiversity Survey, Observation and Assessment Program of Ministry of Ecology and Environment of China, A Cooperative Project between Suichang Country and University [2020HZ17], and The Program of Innovation and Entrepreneurship Training of Undergraduates [2020-S45].

ORCID

Guo-Hua Ding  <http://orcid.org/0000-0002-2484-5855>

Data availability statement

The mitogenome data supporting this study are openly available in GenBank at (<https://www.ncbi.nlm.nih.gov/nuccore/MW327508>). Reference number (Accession no. MW327508). BioSample and SRA accession

numbers are <https://www.ncbi.nlm.nih.gov/biosample/SAMN17167287> and <https://www.ncbi.nlm.nih.gov/sra/SRR13309610>, respectively.

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