MITOCHONDRIAL DNA PART B: RESOURCES
2024, VOL. 9, NO. 10, 1418–1423
https://doi.org/10.1080/23802359.2024.2417975

Taylor & Francis
Taylor & Francis Group

MITOGENOME REPORT

OPEN ACCESS Check for updates

The complete mitochondrial genome and phylogenetic analysis for *Rhabdophis chiwen* (squamata: colubridae)

Jingyun Chen, Jingxue Luo, Ji Wang, Huina Song, Mingwen Duan and Guang-Xiang Zhu

College of Life Science, Sichuan Agricultural University, Ya'an, China

ABSTRACT

Rhabdophis chiwen is currently found so far in Sichuan Province, China, where it predominantly feeds on earthworms and firefly larvae. In this study, we sequenced and analyzed the mitochondrial genome of *R. chiwen*, which measured 17,646 bp in length and encompassed 37 genes along with two control regions. The base composition revealed percentages of 33.20% A, 25.94% T, 13.27% G, and 27.59% C. Phylogenetic analyses indicate that *R. chiwen* belongs to the family Colubridae and forms a sister branch with *R. tigrinus*. This study successfully obtained the first complete mitochondrial genome of *R. chiwen*, offering crucial genetic data for its evolutionary history and conservation.

ARTICLE HISTORY
Received 13 May 2024
Accepted 11 October 2024

KEYWORDS
Mitogenome; phylogeney; *Rhabdophis chiwen*

1. Introduction

The genus *Rhabdophis* was originally established by Fitzinger in 1843. However, it was not until 1960 that Malnate split *Natrix sensu lato* and reinstated the genus *Rhabdophis* as originally proposed by Fitzinger (Malnate 1960). Snakes in the *Rhabdophis* genus possess unique features, including nuchal and dorsal glands under the skin of their neck and back (Zhao 2006). Some species lack dorsal glands. These glands, which contain bufadienolides (BDs) (Yoshida et al. 2020), serve as defense organs (Mori et al. 2012, 2016). *Rhabdophis* species are primarily found in East and South Asia, with the most recent records listing 34 species of *Rhabdophis* in the Reptile Database (https://reptile-database.reptarium.cz/).

In 2020, Piao et al. classified species previously associated with *R. pentasupralabialis* in southwestern China as a new species named *R. chiwen* based on morphological and phylogenetic evidence (Piao et al. 2020). *R. chiwen* is currently only found in Sichuan Province, China, where it primarily feeds on earthworms and firefly larvae, often inhabiting agricultural fields and areas near water sources at altitudes of 1100–2200 meters (Piao et al. 2020). Research on this species is limited, focusing mainly on morphology and its phylogenetic relationships within the *R. nuchalis* group (Zhu et al. 2022; Liu et al. 2023; Yang et al. 2023). The mitogenomic data are limited even at the generic level for *Rhabdophis*.

In this study, we collected an adult female *R. chiwen*, extracted DNA from the muscle, and successfully assembled and annotated the first complete mitogenome of *R. chiwen*. This provides crucial molecular data for future phylogenetic studies of *R. chiwen*. Subsequently, we utilized existing mitogenome data from NCBI to construct a phylogenetic tree incorporating our assembly results, enabling us to analyze the phylogenetic relationships of *R. chiwen*.

2. Materials and methods

2.1. Sample collection

Previous studies have shown 6 ILs of *R. pentasupralabialis*, 7 ILs of *R. chiwen* and 8 ILs of *R. nuchalis* (Piao et al. 2020). In September 2022, we captured an adult female *R. chiwen* based on this one specific morphological trait in Xingou Village (latitude: 29.9303, longitude: 102.3882), Tianquan County, Ya'an City, Sichuan Province, China (Figure 1). Upon capture, we immediately brought the specimen back to the laboratory, where we collected its muscle and rapidly froze it in liquid nitrogen. The muscle was then stored in a refrigerator at −80 °C for subsequent DNA extraction. The specimen, catalogued as ZGX525, is currently preserved at the College of Life Sciences, Sichuan Agricultural University (URL, Guangxiang Zhu ZhuGX0711@163.com), located in Ya'an, Sichuan Province, China.

2.2. DNA extraction and sequencing

Total genomic DNA was extracted from the muscle using a DNeasy tissue kit (Qiagen, Beijing, China) following the manufacturer's protocols. After DNA isolation, 1 μg of purified DNA was fragmented to ∼500 bp using the Covaris M220 system.

CONTACT Mingwen Duan 2971054284@qq.com College of Life Science, Sichuan Agricultural University, Ya'an, China; Guang-Xiang Zhu ZhuGX0711@163.com College of Life Science, Sichuan Agricultural University, Ya'an, China

Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2024.2417975.

Table 1. List of species and GenBank accession numbers for the mitogenomes used for the phylogenetic analysis. Short lines represent no publications for this mitochondrial genome.

Species	Family	ID	Source
Rhabdophis chiwen	Colubridae	CW525	This study
Rhabdophis tigrinus	Colubridae	NC_030210.1	(Zhao et al. 2016)
Calamaria septentrionalis	Colubridae	NC_062677.1	—
Elaphe bimaculata	Colubridae	KM065513.1	(Yan et al. 2016)
Elaphe dione	Colubridae	NC_041068.1	(Simonov et al. 2018)
Elaphe schrenckii	Colubridae	NC_027605.1	(Liu and Zhao 2016)
Elaphe taeniurus	Colubridae	NC_025275.1	(Li et al. 2016)
Hebius craspedogaster	Colubridae	NC_070008.1	(Shan and Wang 2022)
Lycodon flavozonatus	Colubridae	NC_028730.1	(Mei et al. 2015)
Lycodon rufozonatus	Colubridae	NC_024559.1	(Qian et al. 2016)
Lycodon ruhstrati	Colubridae	NC_046046.1	(Gong et al. 2019)
Oligodon chinensis	Colubridae	MK347418.1	(Sun et al. 2019)
Opisthotropis latouchii	Colubridae	NC_046823.1	(Wang et al. 2019)
Orientocoluber spinalis	Colubridae	NC_049067.1	(Park et al. 2020)
Ptyas dhumnades	Colubridae	NC_028049.1	—
Ptyas major	Colubridae	NC_028048.1	(Sun et al. 2017)
Stichophanes ningshaanensis	Colubridae	NC_026083.1	—
Trimerodytes annularis	Colubridae	MW645347.1	—
Bungarus fasciatus	Elapidae	NC_011393.1	—
Naja atra	Elapidae	NC_011389.1	—
Naja kaouthia	Elapidae	LC431744.1	(Singchat et al. 2019)
Ophiophagus hannah	Elapidae	NC_011394.1	(Nian 2010)
Sinomicrurus macclellandi	Elapidae	NC_054255.1	(Yao Gong, Tang 2020)
Sinomicrurus peinani	Elapidae	MZ230594.1	—
Azemiops feae	Viperidae	NC_030781.1	—
Bothrops diporus	Viperidae	NC_039649.1	—
Bothrops jararaca	Viperidae	NC_030760.1	(Almeida et al. 2016)
Bothrops pubescens	Viperidae	NC_039648.1	—
Gloydius rubromaculatus	Viperidae	NC_064056.1	—
Gloydius shedaoensis	Viperidae	NC_029424.1	(Liu et al. 2016)
Gloydius strauchi	Viperidae	NC_036234.1	—
Lachesis muta	Viperidae	NC_081003.1	—
Macrovipera schweizeri	Viperidae	NC_044966.1	(Thanou and Kornilios 2018)
Trimeresurus sichuanensis	Viperidae	NC_029494.1	(Zhu et al. 2016)
Indotyphlops braminus	Typhlopidae	NC_010196.1	(Yan, Li, Zhou 2008)
Xerotyphlops vermicularis	Typhlopidae	NC_044967.1	(Kornilios 2019)
Anolis carolinensis	Dactyloidae	EU747728.2	(Castoe et al. 2008)

With this instrument, we constructed short-insert libraries according to the manufacturer's instructions. (TruSeq™ Nano DNA Sample Prep Kit, Illumina), and then sequenced on an Illumina NovaSeq 6000 platform (Modi et al. 2021) with 150 bp paired-end reads length.

2.3. Genome assembly and annotation

Prior to assembly, raw reads were filtered by Trimmomatic 0.39 (Bolger, Marc, Bjoern 2014). The mitogenome was reconstructed using a combination of *de novo* and reference-guided assemblies, and the following three steps were used to assemble the mitogenome. First, the filtered reads were assembled into contigs using MitoZ v2.3 (Meng et al. 2019), and potential mitochondrial contigs were extracted by aligning against the NCBI mitogenome database. Second, the potential mitochondrial contigs were aligned to the reference mitogenome (NC_030210.1 *R. tigrinus*) using BLAST v 2.8.1+, and aligned contigs (>80% query coverage) were ordered and connected manually according to the reference mitogenome. Finally, MUMmer 3.23 (Kurtz et al. 2004) was used to check whether these contigs were circular.

The mitochondrion genes were annotated using the online MITOS tool (Bernt et al. 2013), using default parameters to predict protein-coding genes (PCGs), transfer RNA (tRNA) genes and ribosome RNA (rRNA) genes. The position of each coding gene was determined using BLAST searches against reference mitogenome. Manual corrections of genes for start/stop codons were performed in SnapGene Viewer (available at snapgene.com) (Bmer) by referencing the reference mitogenome. The circular mitogenome map of *R. chiwen* was drawn using the OGDRAW (Stephan, Pascal, Ralph 2019)

2.4. Constructing a phylogenetic tree

First, we downloaded the complete mitogenome sequences of a total of 35 snake species from the families Typhlopidae, Viperidae, Elapidae, and Colubridae, as well as the complete mitogenome sequences of Anolis carolinensis as outgroups from the NCBI database (Table 1). Subsequently, 13 PCGs were concatenated for data partitioning and optimal model selection using PartitionFinder 2 to build a MrBayes phylogenetic tree using PhyloSuite v1.2.2 (Zhang et al. 2020). The Markov chain Monte Carlo (MCMC) method was employed, running for 10,000,000 generations with samples taken every 1,000 iterations and discarding the top 25% of the data.

3. Results

3.1. Characterization of the mitogenome of R. chiwen

We successfully obtained a complete circular mitogenome of *R. chiwen*, measuring 17,646 bp in length. The average depth of coverage of the whole mitogenome sequence was

Figure 1. Photograph of *Rhabdophis chiwen* (photo credit: Mingwen Duan, used with permission).

470.19× (Supplementary Figure S1). The base composition comprises 33.20% A, 25.94% T, 13.27% G, and 27.59% C. This mitogenome includes 13 protein-coding genes, with ND6 situated on the L chain and the remaining 12 genes on the H chain. The total length of the protein-coding genes is 11,283 bp, with an average gene length of 868 bp. Additionally, there are 22 tRNA genes, totaling 1,432 bp with an average length of 65 bp, along with a 12S s-rRNA of 926 bp and a l-rRNA of 1,451 bp. In addition to these 37 genes, there are two control regions (Figure 2), all of which align with the known mitogenomes of snakes.

3.2. Phylogenetic relationships of R. chiwen

In the phylogenetic tree, Typhlopidae, Viperidae, Elapidae, and Colubridae are observed to form distinct branches.

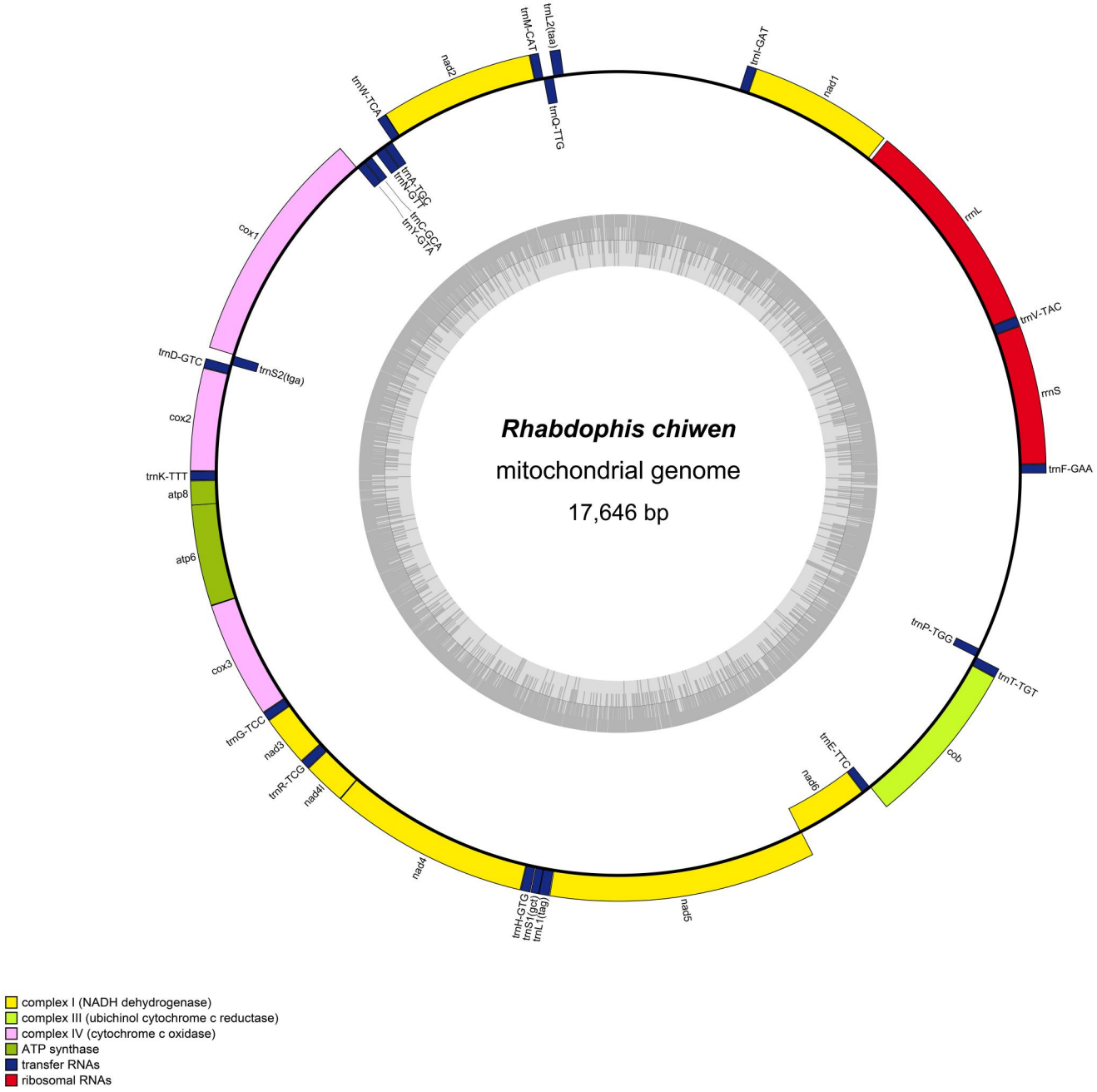

Figure 2. Mitogenomic map of the mitogenomes of *Rhabdophis chiwen*. Protein-coding, tRNA genes and rRNA genes are shown with standard abbreviations.

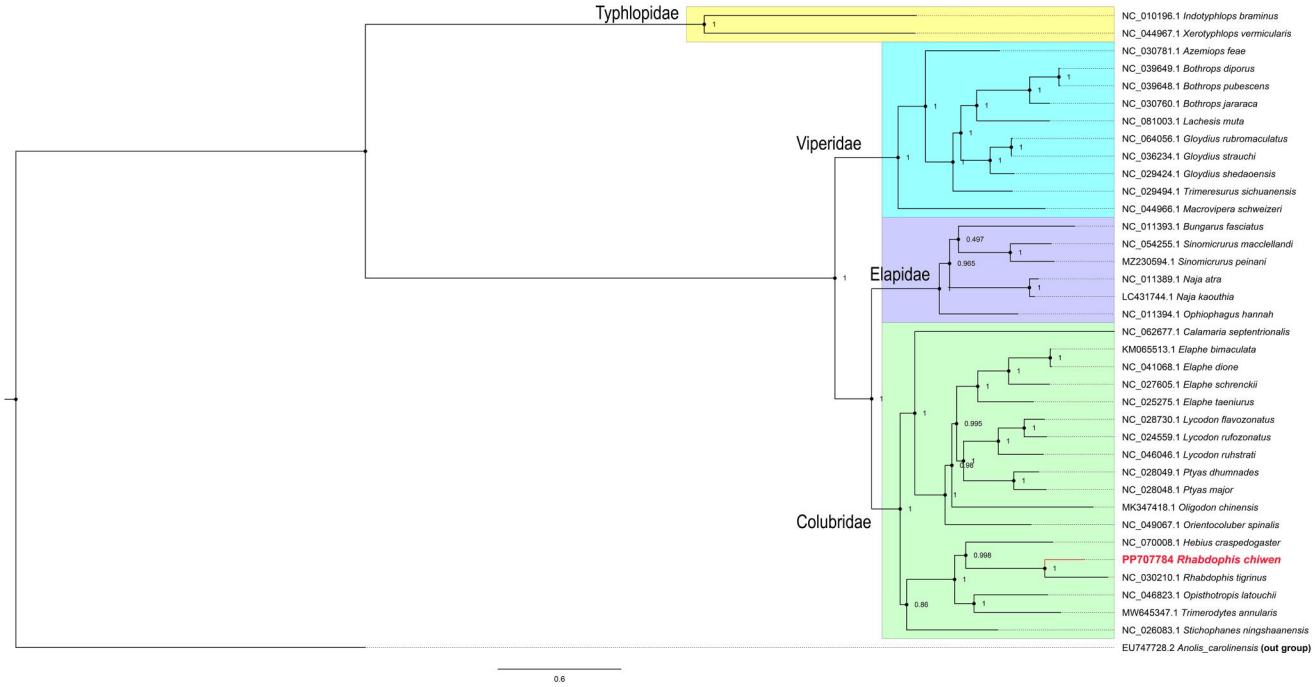

Figure 3. Phylogenetic tree was constructed using 13 protein-coding genes from 37 mitotic genome sequences, which including the newly sequenced *Rhabdophis chiwen*. The numbers displayed between branches represent the posterior probabilities derived from Bayesian inference (BI). Various colors are used to distinguish between different families. The GenBank accession numbers of all species are shown in the figure and the citations are in Table 1.

Typhlopidae diverges first, followed by Viperidae, with Elapidae and Colubridae diverging last to form a sister branch. *R. chiwen*, belonging to Natricinae, forms a sister branch with *R. tigrinus* (Figure 3). These results align with previous research on snake phylogeny (Peng et al. 2023).

4. Discussion and conclusions

In this study, we sequenced and assembled the first complete mitogenome of *R. chiwen*, which spans 17,646 bp and includes 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and two control regions. These findings align with the known characteristics of related species. Furthermore, the phylogenetic tree, constructed using the 13 protein-coding genes, places *R. chiwen* within the Natricinae, closely linked to *R. tigrinus*. This result corroborates earlier phylogenetic studies on the *Rhabdohips* genus (Zhu et al. 2022; Liu et al. 2023; Yang et al. 2023), affirming the accuracy and reliability of our mitogenome data. The mitogenome we have obtained offers valuable genetic insights for phylogenetic and biogeographic research on *R. chiwen*. Additional data, encompassing both morphology and molecular aspects, are essential for other members of the genus *Rhabdophis*, particularly the *R. nuchalis* group. These closely related lineages are crucial for elucidating species boundaries and exploring the potential cryptic diversity of *Rhabdophis* through an integrative taxonomic approach (Piao et al. 2020; Van Nguyen and David 2023).

Disclosure statement

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

Ethical approval

The animal use protocol listed below has been reviewed and approved by the Sichuan Agricultural University Animal Ethical and Welfare Committee (Approval No.20220125).

Author contributions

Jingyun Chen: Conceived and designed the experiments; data curation and analysis; writing (drafted the manuscript, review and editing). Jingxue Luo, Ji Wang, and Huina Song: Sample collection, data curation and analysis; writing (review and editing). Mingwen Duan: Conceived and designed the experiments; Sample collection, investigation; methodology; data curation and analysis; writing (drafted the manuscript, review and editing); supervision. Guangxiang Zhu: Conceived and designed the experiments; data curation; funding acquisition; resources; supervision; writing (drafted the manuscript, review and editing).

Funding

This work was supported by the National Natural Science Foundation of China under Grant NSFC 32270477.

Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under the accession PP707784. The associated BioProject, SRA, and BioSample numbers are PRJNA1102730, SRR28809585, and SAMN41028362 respectively.

References

Almeida DD, Kitajima JP, Nishiyama MY, Condomitti GW, de Oliveira UC, Setúbal JC, Junqueira-de-Azevedo ILM. 2016. The complete

mitochondrial genome of *Bothrops jararaca* (Reptilia, Serpentes, Viperidae). Mitochondrial DNA B Resour. 1(1):907–908. doi:10.1080/23802359.2016.1149783.

Bernt M, Bleidorn C, Braband A, Dambach J, Donath A, Fritzsch G, Golombek A, Hadrys H, Jühling F, Meusemann K, et al. 2013. A comprehensive analysis of bilaterian mitochondrial genomes and phylogeny. Mol Phylogenet Evol. 69(2):352–364. doi:10.1016/j.ympev.2013.05.002.

Bolger AM, Marc L, Bjoern U. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 30(15):2114–2120. doi:10.1093/bioinformatics/btu170.

Castoe TA, Jiang ZJ, Gu W, Wang ZO, Pollock DD. 2008. Adaptive evolution and functional redesign of core metabolic proteins in snakes. PLoS One. 3(5):e2201. doi:10.1371/journal.pone.0002201.

Gong Y-A, Peng L-F, Lu S-Q, Huang S. 2019. The complete mitochondrial genome of *Lycodon ruhstrati* (Serpentes: colubridae). Mitochondrial DNA B. 4(2):2165–2166. doi:10.1080/23802359.2019.1623730.

Kornilios P. 2019. The complete mitogenome of the Eurasian blindsnake *Xerotyphlops vermicularis* (Reptilia, Typhlopidae). Mitochondrial DNA Part B. 4(1):1990–1991. doi:10.1080/23802359.2019.1617080.

Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. Genome Biol. 5(2):R12. PubMed. doi:10.1186/gb-2004-5-2-r12.

Li E, Sun F, Zhang R, Chen J, Wu X. 2016. The complete mitochondrial genome of the striped-tailed rat-snake, *Orthriophis taeniurus* (Reptilia, Serpentes, Colubridae). Mitochondrial DNA A DNA Mapp Seq Anal. 27(1):599–600. doi:10.3109/19401736.2014.908364.

Liu Q, Lyu B, Xie X, et al. 2023. Genomic evidence sheds new light on phylogeny of *Rhabdophis nuchalis* (sensu lato) complex (Serpentes: natricidae). Mol Phylogenet Evol. 189:107893. doi:10.1016/j.ympev.2023.107893.

Liu P, Zhao WG. 2016. The complete mitochondrial genome of the Amur rat-snake *Elaphe schrenckii* (Squamata: colubridae). Mitochondrial DNA A DNA Mapp Seq Anal. 27(4):2529–2530. doi:10.3109/19401736.2015.1038789.

Liu Q, Zhu F, Wang X, Xiao R, Fang M, Sun L, Li P, Guo P. 2016. The complete mitochondrial genome sequence of *Gloydius shedaoensis* (Squamata: viperidae). Mitochondrial DNA A. 27(6):4679–4680. doi:10.3109/19401736.2015.1106499.

Malnate EV. 1960. Systematic division and evolution of the colubrid snake genus Natrix, with comments on the subfamily Natricinae. Proc Acad Nat Sci Phila. 112(3):41–71.

Mei D, Wu J, Qian L, et al. 2015. The complete mitochondrial genome of *Lycodon flavozonatum* and implications for Colubridae taxonomy. Mitochondrial DNA Part A. 27(5):3473–3474.

Meng G, Li Y, Yang C, Liu S. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 47(11):e63.

Modi A, Vai S, Caramelli D, et al. 2021. The Illumina Sequencing Protocol and the NovaSeq 6000 System. New York, NY: Springer US, 15–42.

Mori A, Burghardt GM, Savitzky AH, Roberts KA, Hutchinson DA, Goris RC. 2012. Nuchal glands: a novel defensive system in snakes. Chemoecology. 22(3):187–198. doi:10.1007/s00049-011-0086-2.

Mori A, Jono T, Takeuchi H, Ding L, de Silva A, Mahaulpatha D, Tang Y. 2016. Morphology of the nucho-dorsal glands and related defensive displays in three species of Asian natricine snakes. J Zool. 300(1):18–26. doi:10.1111/jzo.12357.

Nian C. 2010. Sequencing and analysis of the complete mitochondrial genome of the King Cobra, *Ophiophagus hannah* (Serpents: elapidae). Hereditas (Beijing). 32(7):719–725.

Park J, Park I-K, Ra N-Y, Min S-H, Park D. 2020. Complete mitochondrial genome of the slender racer (*Orientocoluber spinalis* Peters, 1866; Squamata, Colubridae). Mitochondrial DNA Part B. 5(3):2693–2694. doi:10.1080/23802359.2020.1787257.

Peng C, Wu D-D, Ren J-L, Peng Z-L, Ma Z, Wu W, Lv Y, Wang Z, Deng C, Jiang K, et al. 2023. Large-scale snake genome analyses provide insights into vertebrate development. Cell. 186(14):2959–2976.e22. doi:10.1016/j.cell.2023.05.030.

Piao Y, Chen Z, Wu Y, et al. 2020. A New Species of the Genus *Rhabdophis Fitzinger*, 1843 (Squamata: colubridae) in Southwestern Sichuan, China. Asian Herpetol Res. 11(2):95–111.

Qian L, Zhang C, Huang X, Pan T, Wang H, Zhang B. 2016. Mitochondrial genome of *Dinodon rufozonatum* (Squamata: colubridae: dinodon). Mitochondrial DNA A DNA Mapp Seq Anal. 27(2):970–971. doi:10.3109/19401736.2014.926510.

Shan S, Wang Y. 2022. Complete mitochondrial genomes of *Boiga kraepelini* and *Hebius craspedogaster* (Reptilia, Squamata, Colubridae) and their phylogenetic implications. Zookeys. 1124(2):191–206. doi:10.3897/zookeys.1124.87861.

Simonov E, Lisachov A, Oreshkova NV, et al. 2018. The mitogenome of *Elaphe bimaculata* (Reptilia: colubridae) has never been published: a case with the complete mitochondrial genome of E. dione. Acta Herpetol. 13(2):185–189.

Singchat W, Areesirisuk P, Sillapaprayoon S, Muangmai N, Baicharoen S, Suntrarachun S, Chanhome L, Peyachoknagul S, Srikulnath K. 2019. Complete mitochondrial genome of Siamese cobra (*Naja kaouthia*) determined using next-generation sequencing. Mitochondrial DNA Part B. 4(1):577–578. doi:10.1080/23802359.2018.1558123.

Stephan G, Pascal L, Ralph B. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. Nucl Acids Res. (W1):W59–W64.

Sun H, Li E, Sun L, Yan P, Xue H, Zhang F, Wu X. 2017. The complete mitochondrial genome of the greater green snake *Cyclophiops major* (Reptilia, Serpentes, Colubridae). Mitochondrial DNA B Resour. 2(1):309–310. doi:10.1080/23802359.2017.1331326.

Sun Z, Zhou Z, Tu F, Qin Y, Peng L, Huang X, Ma J. 2019. Next-generation sequencing of the mitochondrial genome of *Oligodon chinensis* (Squamata: colubridae) with a pylogenetic analysis of Colubridae. Mitochondrial DNA Part B. 4(1):1116–1117. doi:10.1080/23802359.2019.1586489.

Thanou E, Kornilios P. 2018. Next-generation sequencing yields the complete mitochondrial genome of the endangered Milos viper *Macrovipera schweizeri* (Reptilia, Viperidae). Mitochondrial DNA B Resour. 3(2):1250–1251. doi:10.1080/23802359.2018.1532348.

Van Nguyen T, David P. 2023. On the occurrence of *Rhabdophis leonardi* (Wall, 1923) in Vietnam and Laos, with preliminary comments on the taxonomy and distribution of the complex of *Rhabdophis nuchalis* (Squamata: serpentes: natricidae). RJH. 30(4):224–236. doi:10.30906/1026-2296-2023-30-4-224-236.

Wang Y, Liu P, Li H, Shao C. 2019. The complete mitochondrial genome of *Opisthotropis latouchii* (Squamata: colubridae). Mitochondrial DNA Part B Resources. 4(1):1437–1438. doi:10.1080/23802359.2019.1598816.

Yan L, Geng Z-Z, Yan P, Wu X-B. 2016. The complete mitochondrial genome of *Elaphe bimaculate* (Reptilia, Serpentes, Colubridae). Mitochondrial DNA A DNA Mapp Seq Anal. 27(2):1285–1286. doi:10.3109/19401736.2014.945558.

Yang S-J, Savitzky AH, Gower DJ, Deepak V, Mori A, Khot R, Shi J-S, Ding L, Hou M, Xu H-Y, et al. 2023. Identity of the holotype and type locality of *Rhabdophis leonardi* (Wall, 1923) (Colubridae: natricinae), with notes on the morphology and natural history of the species in southwestern China. Ecol Evol. 13(5):e10032. doi:10.1002/ece3.10032.

Yan J, Li H, Zhou K. 2008. Evolution of the mitochondrial genome in snakes: gene rearrangements and phylogenetic relationships. BMC Genom. 9(1):569–569. doi:10.1186/1471-2164-9-569.

Yao LZ, Gong YA, Tang XS. 2020. Complete mitochondrial genome of the MacClelland's Coral Snake *Sinomicrurus macclellandi* (Reinhardt, 1844) (Serpentes: elapidae). Mitochondrial DNA Part B. 5(3):2582–2584. doi:10.1080/23802359.2020.1781571.

Yoshida T, Ujiie R, Savitzky AH, et al. 2020. Dramatic dietary shift maintains sequestered toxins in chemically defended snakes. Proceedings of the National Academy of Sciences. 117(11):5964–5969. doi:10.1073/pnas.1919065117.

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT. 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary

phylogenetics studies. Mol Ecol Resour. 20(1):348–355. doi:10.1111/1755-0998.13096.

Zhao EM. 2006. Snakes of China. Hefei, Anhui: Anhui Science and Technology Publishing House.

Zhao D, Liu H, Zhao W-G, Liu P. 2016. The complete mitochondrial DNA sequence and the phylogenetic position of *Rhabdophis tigrinus* (Reptilia: squamata). Mitochondrial DNA B Resour. 1(1):216–217. doi:10.1080/23802359.2016.1155092.

Zhu F, Liu Q, Zhong G, Xiao R, Fang M, Guo P. 2016. Complete mitochondrial genome of *Sinovipera sichuanensis* (Reptilia: squamata: viperidae). Mitochondrial DNA, Part A. 27(5):3666–3667. doi:10.3109/19401736.2015.1079846.

Zhu G, Yang S, Savitzky AH, Cheng Y, Mori A, Ding L, Rao D, Wang Q. 2022. Cryptic diversity and phylogeography of the *Rhabdophis nuchalis* group (Squamata: colubridae). Mol Phylogenet Evol. 166:107325. doi:10.1016/j.ympev.2021.107325.