The complete mitochondrial genome of the Xizang hot-spring snake, *Thermophis baileyi* Wall, 1907 (Reptilia, colubridae)

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**ABSTRACT**

The Xizang hot-spring snake, *Thermophis baileyi* (Reptilia, Colubridae), is a relict species of the family Colubridae, which is known only from Tibet, China. In this study, we report the complete mitochondrial genome of this species. The total length of the genome is 17,385 bp, and comprises 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and 2 control regions. The total base composition is 32.5% for A, 23.8% for T, 13.5% for G, and 30.2% for C. The phylogenetic tree with 13 protein-coding genes (PCGs) of *T. baileyi* together with 12 other closely related species belonging to the family Colubridae was built, in order to prove the validity of the mitogenome of *T. baileyi*.

*Thermophis baileyi*, is a relict species endemic to the Tibetan Plateau, achieve the world’s highest altitude distribution among all snakes, commonly restricted to the proximity of geothermal sites. Wall (1907) first described the Xizang hot-spring snake, a new snake from Tibet Autonomous Region (TAR), as *Tropidonotus* (=*Natrix*) *baileyi*. Malnate (1953) removed this species from the genus *Natrix* and designated it as the genotype of *Thermophis*, gen. nov., based on a study of the characters. In recent decades, the exploitation of geothermal energy resources has resulted in the environmental pollution and habitat destruction, and the wild populations of the Xizang hot-spring snakes have been facing severe threats. In 2010, *T. baileyi* was listed as ‘Vulnerable’ (IUCN, 2010). Up to now, this species was reported 29 distribution (PCR) with 13 pairs of primers described in He et al. (2010). The specimen of *T. baileyi* (Voucher number: HM060001) was sampled from Yangbajain, Tibet, China. It was deposited at the Museum of Huangshan University (HUM20160001). Total genomic DNA was extracted from the liver tissue of an adult Xizang hot-spring snake using Takara MiniBEST Universal Genomic DNA Extraction Kit (Takara, Dalian, China). The genomic DNA was amplified by polymerase chain reaction (PCR) with 13 pairs of primers described in He et al. (2010).

The mitogenome of *T. baileyi* comprises 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes (12S and 16S rRNA), and 2 control regions (D-loop). Nine of the 13 PCGs used ATG as the start codon, while ND1, ND2, and ND3 used ATA codon, and COX1 used GTG. The PCGs have five types of termination codon, including TAA for ND4L, ND5, and ATP8, AGG for ND1 and ND6, AGA for ND4, T— for ND1, ND2, COX2, COX3, ND3, and Cytb, TA- for ATP6. Twenty-two tRNA genes were interspersed in the mitochondrial genome and ranged in size from 57 to 75 bp. The 12S (927 bp) and 16S rRNA (1480 bp) genes are located between tRNA*Leu* and tRNA*Val* and separated by the trNA*Thr*. Two control regions found in the mitogenome of *T. baileyi* were 1097 and 1091 bp, respectively. The total length of the mitogenome (GenBank Accession No. KU196816) was 17,385 bp, and the base composition was 32.5% for A, 23.8% for T, 13.5% for G, and 30.2% for C.

In order to convince the mitochondrial DNA sequences obtained in this study, we used the 13 PCGs genes of mitogenome of *T. baileyi* and other 12 closely related species, belonging to the family Colubridae to construct the phylogenetic tree. These CDs sequences were concatenated as a supergene for each species. We constructed the ML tree (Figure 1) using the new supergenes in http://www.phylo.org/portal2/login!input.action. Likelihood settings from the best-fit model (GTR + I + G) selected by AIC in jModeltest 0.1 (–ln L = 66,969.5327). Rates = gamma, Shape = 0.4610, Pinvar = 0.3210. The phylogenetic analysis result was consistent with the previous research with high support. It indicated that our reported mitogenome sequences could meet the demands and explain some related evolution issues.

Up to now, the mitogenome in this study would be the last species of the *Thermophis* genus whose complete...
mitochondrial sequence could be available from GenBank, the other were *T. zhaoermii* (GQ166168) (He et al., 2010) and *T. shangrila* (KU174488) (Peng et al. Online). The mitogenome data in our study will be helpful to study the evolutionary relationships and genetic diversity of *T. baileyi*.

**Disclosure statement**

The authors have declared that no conflict of interest exists. The authors alone are responsible for the content and writing of the paper.

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**References**

Dorge T, Hofmann S, Wangdwei M, Duoje L, Solhoy T, Miehe G. 2007. The ecological specialist, *Thermophis baileyi* (Wall, 1907): new records, distribution and biogeographic conclusions. Br Herpetol Soc. 101:8–12.

He M, Feng J, Zhao EM. 2010. The complete mitochondrial genome of the Sichuan hot-spring keel-back (*Thermophis zhaoermii*; Serpentes: Colubridae) and a mitogenomic phylogeny of the snakes. Mitochondrial DNA. 21:8–18.

Hofmann S. 2012. Population genetic structure and geographic differentiation in the hot spring snake *Thermophis baileyi* (Serpentes, Colubridae): Indications for glacial refuges in southern-central Tibet. Mol Phylogenet Evol. 63:396–406.

Huang S, Liu SY, Guo P, Zhang YP, Zhao EM. 2009. What are the closest relatives of the hot-spring snakes (Colubridae, *Thermophis*), the relict species endemic to the Tibetan Plateau? Mol Phylogenet Evol. 51:438–446.

Malnate EV. 1953. The taxonomic status of the Tibetan colubrid snake *Natrix baileyi*. Copeia. 2:92–96.

Peng LF, Lu CH, Huang S, Guo P, YP Z. 2014. New Species of the Genus *Thermophis* (Serpentes: Colubridae) from Shangri-La, Northern Yunnan, China, with a proposal for an Eclectic rule for species delimitation. Asian Herpetol Res. 5:228–239.

Peng LF, Weng SY, Yang DC, Lu CH, Huang S. (Online). Complete mitochondrial genome of the Xianggelila Hot-spring snake, *Thermophis shangrila* (Reptilia, Colubridae). Mitochondrial DNA: Resources. 1: 536–537.

Wall F. 1907. Some new Asian snakes. J Bombay Nat Hist Soc. 17:612–618.

Zhao EM. 2006. Snakes of China. Hefei, China: Anhui Sciences and Technology Publishing House. p. 1–365 (In Chinese).