Supplementary Materials
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Population genomic analysis
Samples used for genome re-sequencing were collected from seven Sichuan hot-spring snakes (*T. zhaoermii*) in 2014–2019. A short paired-end insert library (300–500 bp) was constructed using standard protocols provided by Illumina, and whole-genome sequencing was employed using the NovaSeq 6000 system to produce 15-fold coverage of the hot-spring snake genome (~1.76 Gb). All raw data were submitted to the Genome Sequence Archive (https://ngdc.cncb.ac.cn) in the China National GeneBank Database (CNGBdb) under Bioproject accession No. PRJCA007353. The raw data were filtered by removing adaptor sequences, polymerase chain reaction (PCR) duplicates, and low-quality reads using Trimmomatic (v.0.32) (Bolger et al., 2014). Clean reads were then mapped to the genome of *Thermophis baileyi* (assembly DSBC_Tbai_1.0) using BWA-MEM (v.0.7.17) (Li and Durbin, 2009) with default parameters. SAMtools (v.0.1.18) was used for sorting and removing PCR duplicates (Li, 2011; Li et al., 2009). To minimize false-positive SNP calls around indels, local realignment around indels was performed using the Genome Analysis Tool Kit (v4.0.11) (Brouard et al., 2019). The whole-genome diploid consensus sequence of one individual was generated by bcftools (v.1.9) (Li, 2011). The results were then used in the PSMC model (Li and Durbin, 2011) with the following parameters: -N25 -t15 -r5 -b -p "4+25 * 2+4+6". One hundred bootstraps were performed to assess variation in the inferred effective population size (*Ne*) trajectories. Generation time and neutral mutation rate were set to 3 years and 5e-09, and were used to convert population sizes and scale time into real sizes and time (Li et al., 2018).

The vcf files were extracted from previous bam files with bcftools (Li, 2011) and filtered using vcftools (v0.1.17) with the parameter "--maf 0.1 --max-missing 0.75 --minQ 30 –minDP 4".

Average theta pi was calculated using vcftools (v0.1.17) (Danecek et al., 2011) with the sliding windows method. Window size was set to 1 000 bp. Theta w (Watterson estimator) was calculated based on the formula:

$$\frac{S}{L * \sum_{i=1}^{2n-1} 1/n}$$

Where S is the variable site in a window, L is the window size, and n is the sample size. Recent demographic history was inferred using genetic optimization for *Ne* estimation (GONE) (Santiago et al., 2020).

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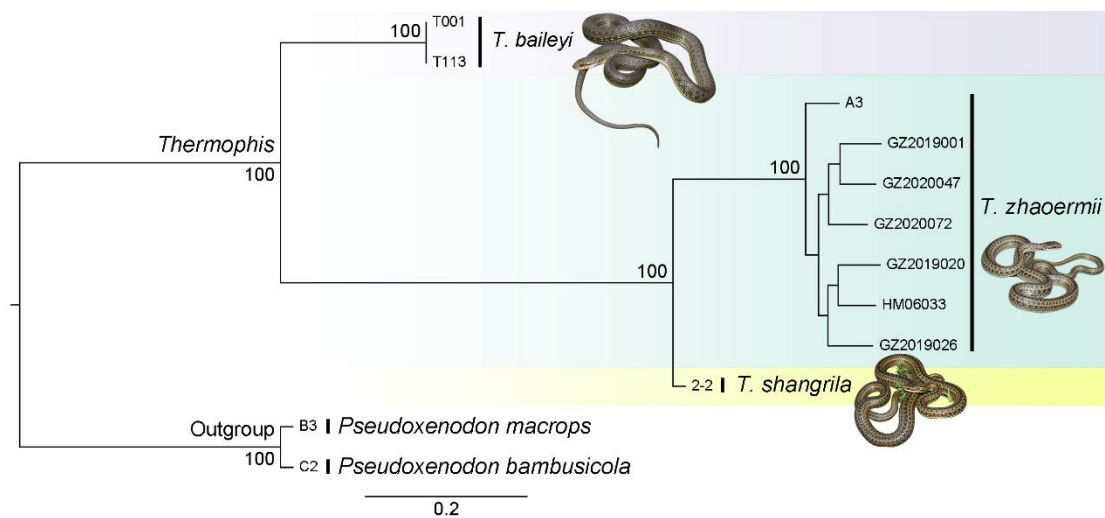
Supplementary Figure S1. Maximum-likelihood (ML) phylogenetic tree estimated from genome-level SNP data, depicting phylogenetic relationships of *Thermophis* members. Photographs by Jin-Long Ren.

Supplementary Table S1. Sample locations and SNP genetic diversity of *T. zhaoermii* used in this study.

Sample ID	locality	homogenous sites	heterogenous sites	heterogenous ratio (×10⁻³)	θπ (×10⁻³)	θω (×10⁻³)	Tv/Ts	SNPden
A3	Litang, Sichuan	1 544 048 253	2 610 502	1.68	2.20	2.20	2.3987	6.4735
GZ2019001	Xinlong, Sichuan	1 493 376 298	2 626 129	1.75	2.37	2.37	2.4820	6.2464
GZ2019020	Xiangcheng, Sichuan	1 493 013 655	2 549 347	1.70	2.32	2.32	2.4633	6.2346
GZ2019026	Litang, Sichuan	1 525 201 927	2 679 594	1.75	2.41	2.41	2.4369	6.2795
GZ2020047	Baiyu, Sichuan	1 544 543 587	2 535 022	1.66	2.30	2.30	2.4424	6.4375
GZ2020072	Batang, Sichuan	1 541 379 964	2 573 145	2.08	2.34	2.34	2.4199	6.3906
HM06033	Litang, Sichuan	1 539 142 567	3 216 802	1.63	2.69	2.69	2.4344	6.3641
All samples	western Sichuan, China	1 525 815 179	2 684 363	1.75	1.91	1.95	2.4682	8.7802

Supplementary Table S2. Information on samples used in phylogenetic analysis.

Species	Sample ID	Locality	Source
T. zhaoermii	A3	Litang, Sichuan	Li et al., (2018)
T. zhaoermii	GZ2019001	Xinlong, Sichuan	This study
T. zhaoermii	GZ2019020	Xiangcheng, Sichuan	This study
T. zhaoermii	GZ2019026	Litang, Sichuan	This study
T. zhaoermii	GZ2020047	Baiyu, Sichuan	This study
T. zhaoermii	GZ2020072	Batang, Sichuan	This study
T. zhaoermii	HM06033	Litang, Sichuan	Li et al., (2018)
T. baileyi	1hao	Xizang	Li et al., (2018)
T. baileyi	1-13	Xizang	Li et al., (2018)
T. shangrila	2-2	Shangri-La, Yunnan	Li et al., (2018)
Pseudoxenodon macrops	B3	-	Li et al., (2018)
Pseudoxenodon bambusicola	C2	-	Li et al., (2018)