

SUPPORTING INFORMATION

What makes a hot-spring habitat “hot” for the hot-spring snake: Distributional data and niche modelling for the genus *Thermophis* (Serpentes, Colubridae)

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Text S1: Details on site visits.

Each site visit lasted up to 300 minutes, depending upon the size of the area and site conditions (including weather). All sites were surveyed by three to four people in a systematic fashion by searching of all likely microhabitats. Shorelines, basking places, and shelters were searched for adults, juveniles and skins while walking slowly along parallel lines, turning stones and rocks, and looking under shrubs, under woody and rocky debris. Some sites were visited more than twice to increase the probability of detection. Residents were asked about the presence of snakes in the respective area. In particular, the details given by local Tibetans had turned out to be a reliable source of information on species presence. Field determination of species based on morphological characters proposed in the original description of *T. baileyi* (Wall 1907) and *T. zhaoermii* (Guo et al. 2009).

Table S1: Hot-spring localities surveyed for *Thermophis zhaoermii*. Water parameters were measured at hot-spring sites 1–16 (see Figure S1 for geographic reference, below); localities 17–22 were surveyed for snakes only.

Locality	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Snakes occur
1 (Dêqên, Xiagei), Yunnan	27°46'	99°51'	3,440	uncertain ¹
2 (Lamaya1), Sichuan	29°45'	99°48'	3,620	-
3 (Lamaya2), Sichuan	29°50'	99°46'	3,810	-
4 (Zhara Lhatse1), Sichuan	30°22'	101°40'	4,050	-
5 (Zhara Lhatse2), Sichuan	30°23'	101°40'	4,050	-
6 (Longpucun), Sichuan	30°57'	101°14'	3,370	-
7 (Gyawa1), Sichuan	29°47'	100°24'	3,750	uncertain ¹
8 (Qimei), Sichuan	31°08'	101°10'	3,920	-
9 (Garzê), Sichuan	31°40'	99°43'	3,460	uncertain ¹
10 (Cuo'axiang), Sichuan	31°47'	99°23'	3,830	-
11 (Amula), Sichuan	31°51'	99°14'	3,960	-
12 (Cogsum), Sichuan	29°41'	100°23'	3,660	n=12 ² , n=2 our survey
13 (Litang), Sichuan	29°60'	100°13'	3,920	n=3 ³
14 (Batang), Sichuan	30°16'	99°27'	3,930	n=5
15 (Cuopu1), Sichuan	30°24'	99°22'	3,600	n=1
16 (Cuopo2), Sichuan	30°28'	99°31'	4,090	n=1
17 (Riwa), Sichuan	28°41'	100°16'	3,560	-
18 (Rubachacka), Sichuan	29°1'	100°19'	3,650	-
19 (Gyawa2), Sichuan	29°48'	100°25'	3,710	-
20 (Kangding), Sichuan	29°57'	101°57'	3,030	-
21 (Mugeco Lake), Sichuan	30°11'	101°52'	3,380	-
22 (Litang, Yongjun), Sichuan	29°59'	100°13'	3,942	n=2
Shangri-La (<i>T. shangrila</i>), Yunnan	27°28'	99°29'	3,362	n=3 ⁴
Tianshengqiao (<i>T. shangrila</i>), Yunnan	27°48'	99°49'	—	n=1 ⁵

¹At these sites, we observed snakes only from long distances in difficult mountainous terrain and could not verify them morphologically; ²(Guo et al. 2008, Peng et al. 2014); ³(Hofmann 2012); ⁴according to (Peng et al. 2014), not shown in Fig. 1 due to spatial uncertainty, for details see text; ⁵Li et al. 2018, shown in Fig. 1.

Table S2: Estimates of relative contributions of the environmental variables to the Maxent model based on subsample (left) and bootstrap algorithm (right).

Variable	Percent contribution	Permutation importance	Variable	Percent contribution	Permutation importance
>45-100°C	58.2	7.8	>45-100°C	55.6	19.3
BIO19	14.7	9.8	BIO19	14.3	31
<35°C	6.7	3.1	>100°C	7.6	3.2
>100°C	6.6	1.1	>35-45°C	7.5	5.8
>35-45°C	6.2	1.9	<35°C	7.4	6.2
BIO 1	3.7	74.6	BIO1	3.3	26.5
BIO 2	2.1	0.6	BIO15	2.1	1.5
LC	0.8	0	BIO2	1.2	1.5
BIO15	0.5	0.9	LC	0.4	0.7
BIO4	0.4	0	BIO3	0.4	4
BIO3	0.1	0.2	hydro	0.1	0.1
RN	0	0	BIO4	0.1	0

Table S3: Spearman correlation coefficients (SCE) of microhabitat characteristics.

The following variables showed pairwise correlation $> |0.5|$.

<i>Var1</i>	<i>Var2</i>	<i>SCE</i>
ALT	PO4	0.572
COND	TDS	0.608
COND	SAL	0.600
COND	KH	0.651
TDS	SAL	0.997
TDS	NO3	0.530
TDS	KH	0.968
TDS	AA	0.926
pH	OX	0.572
SAL	NO3	0.519
SAL	KH	0.959
SAL	AA	0.916
NO3	KH	0.601
NO3	AA	0.563
KH	AA	0.883

Correlation coefficients $> |0.7|$ were considered indicative of high inter-feature correlation.

Therefore, TDS, SAL, AA (NH₃/NH₄) were excluded from the final model.

Table S4: Analysis of deviance table (generalized linear model). Species presence was treated as a dichotomous response variable. Variables' regression coefficients were incrementally tested for being non-zero by using a likelihood ratio test comparing the model of the previous model selection step to the one additionally including the newly introduced variable MRD = distances of a hot-spring site to the major river (riverine corridor); TEMP = hot-spring water temperature. Please note that a residual deviance of zero (last line) is indicative of over-fitting of the model to the data, caused by our very small data set. Df: degrees of freedom.

Variable	Df	Deviance	Residual Df	Residual.Deviance	p-values
NULL			12	17.323	
MRD	1	1.930	11	15.393	1.647×10^{-01}
TEMP	1	15.39	10	0.000	8.731×10^{-05}

Table S5: Summary statistics of variables in the model for “absence” localities of *Thermophilis zhaoermii*. ALT = altitude; SRD = distances (in m) of a hot-spring site to the nearest small (seasonal) river; TRIB = distances (in km) of a hot-spring site to the next merging point with the superordinate river (tributary flow path of SRD); MRD = distances (in km) of a hot-spring site to the major river (riverine corridor); TEMP = hot-spring water temperature; COND = hot-spring water conductivity; pH = measure of acidity or alkalinity; ORP = oxidation reduction potential of the hot-spring water; OX = dissolved oxygen; NO3, PO4, SO4 = ionic concentrations in hot-spring water; CH = carbonate hardness of hot-spring water; BIO19 = precipitation of coldest quarter at hot-spring locality.

	ALT	SRD	TRIB	MRD	TEMP	COND	pH
Minimum	3365	0.00	0.00	38.00	38.00	510	6.700
1st Quantile	3761	5.00	0.00	75.38	42.33	1052	6.975
Median	3872	27.50	3.25	88.30	46.05	1354	7.050
Mean	3826	66.25	6.00	124.01	46.71	1345	7.100
3rd Quantile	3986	48.75	13.00	159.50	47.73	1554	7.225
Maximum	4050	350.00	15.50	255.00	62.00	2500	7.600
	ORP	OX	NO3	PO4	SO4	KH	BIO19
Minimum	-40.0	0.40	0	0.50	15.00	9.00	5.00
1st Quantile	60.0	1.40	0	0.50	15.00	23.25	10.25
Median	179.0	1.90	0	1.50	15.00	33.00	12.00
Mean	159.0	1.85	0	1.31	23.75	33.25	12.50
3rd Quantile	269.8	2.23	0	2.00	15.00	42.50	15.75
Maximum	303.0	3.50	0	2.00	85.00	64.00	19.00

Table S6: Summary statistics of variables for “presence” localities of *Thermophilis zhaoermii*. For abbreviations see Table S3. Mean values of variables that remained in the model are in bold.

	ALT	SRD	TRIB	MRD	TEMP	COND	pH
Minimum	3597	6.0	0.0	3.80	41.0	284	6.80
1st Quantile	3660	10.0	0.0	4.00	44.0	1150	6.90
Median	3924	25.0	0.0	105.00	60.0	1160	7.90
Mean	3837	28.2	3.4	69.56	55.6	1359	7.86
3rd Quantile	3925	30.0	0.0	110.00	63.0	1490	8.70
Maximum	4080	70.0	17.0	125.00	70.0	2710	9.00
	ORP	OX	NO3	PO4	SO4	CH	BIO19
Minimum	34.0	1.00	0.0	0.5	15	21.0	5.0
1st Quantile	150.0	1.10	0.0	0.5	15	33.0	6.0
Median	209.0	1.20	0.0	0.5	15	49.0	7.0
Mean	184.8	1.98	9.5	0.7	28	50.6	6.6
3rd Quantile	258.0	2.50	7.5	1.0	15	70.0	7.0
Maximum	273.0	4.10	40.0	1.0	80	80.0	8.0

Figure S1: Known records of *Thermophis zhaoermii* and *T. shangrila* and sites surveyed for *Thermophis*. Details to the locality numbers are listed in Table S1.

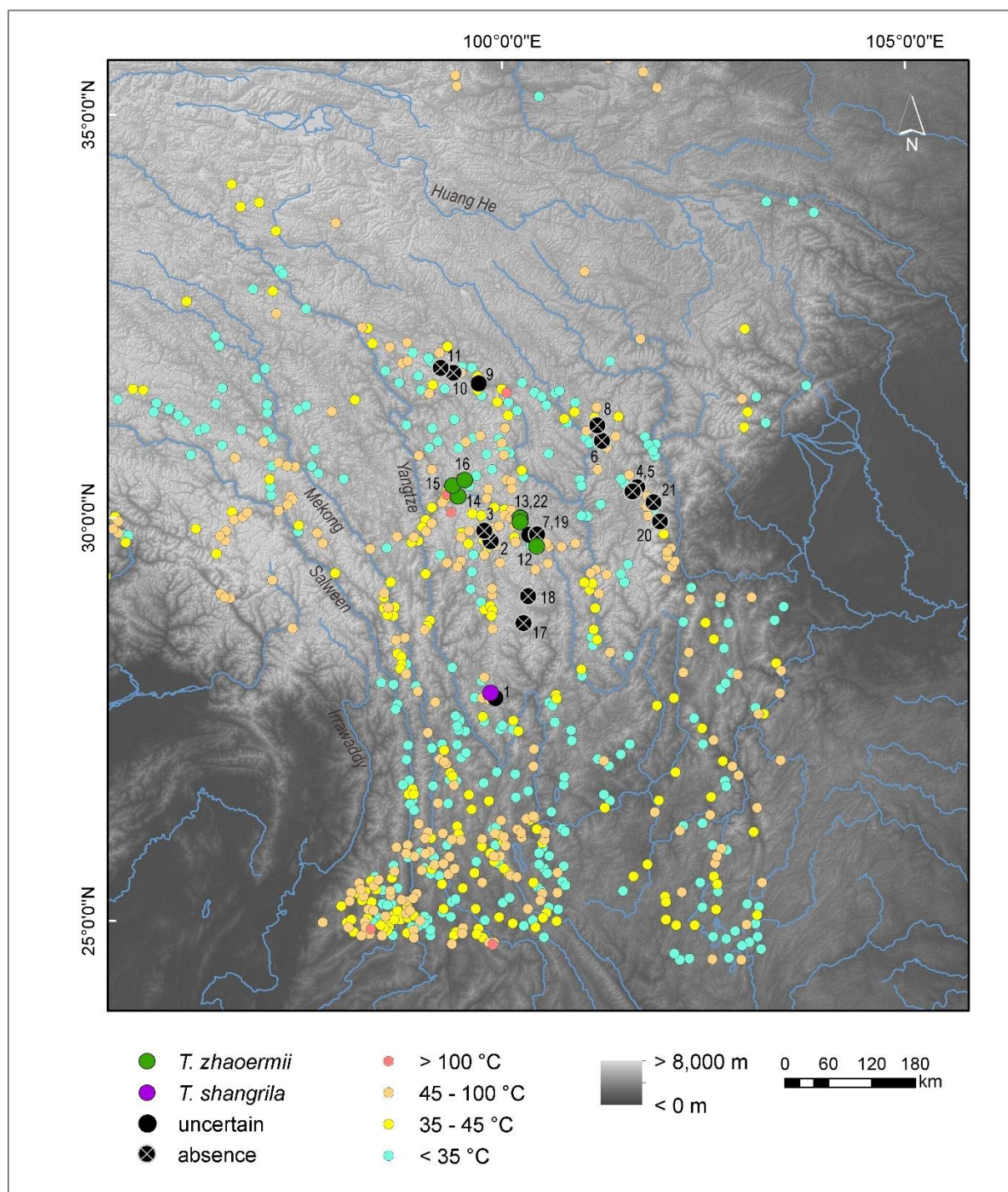


Figure S2: Climate heterogeneity in the distribution area of *Thermophilis* species. Shown are (A) the results of the Principal Components (PC) Analysis, and (B) the variation in climate as a measure of heterogeneity based on the six bioclimatic variables (BIO1-4, BIO15, BIO19) used in the Maxent models. Layers were generated with SDMtoolbox v.2.4 (Brown 2014) implemented in ArcGIS 10.8. The PCA figure depicts climate space: the more similar the colours the more similar values. Climate heterogeneity is weighted by the variation represented in each PC; warm colours depict areas of high climatic heterogeneity.

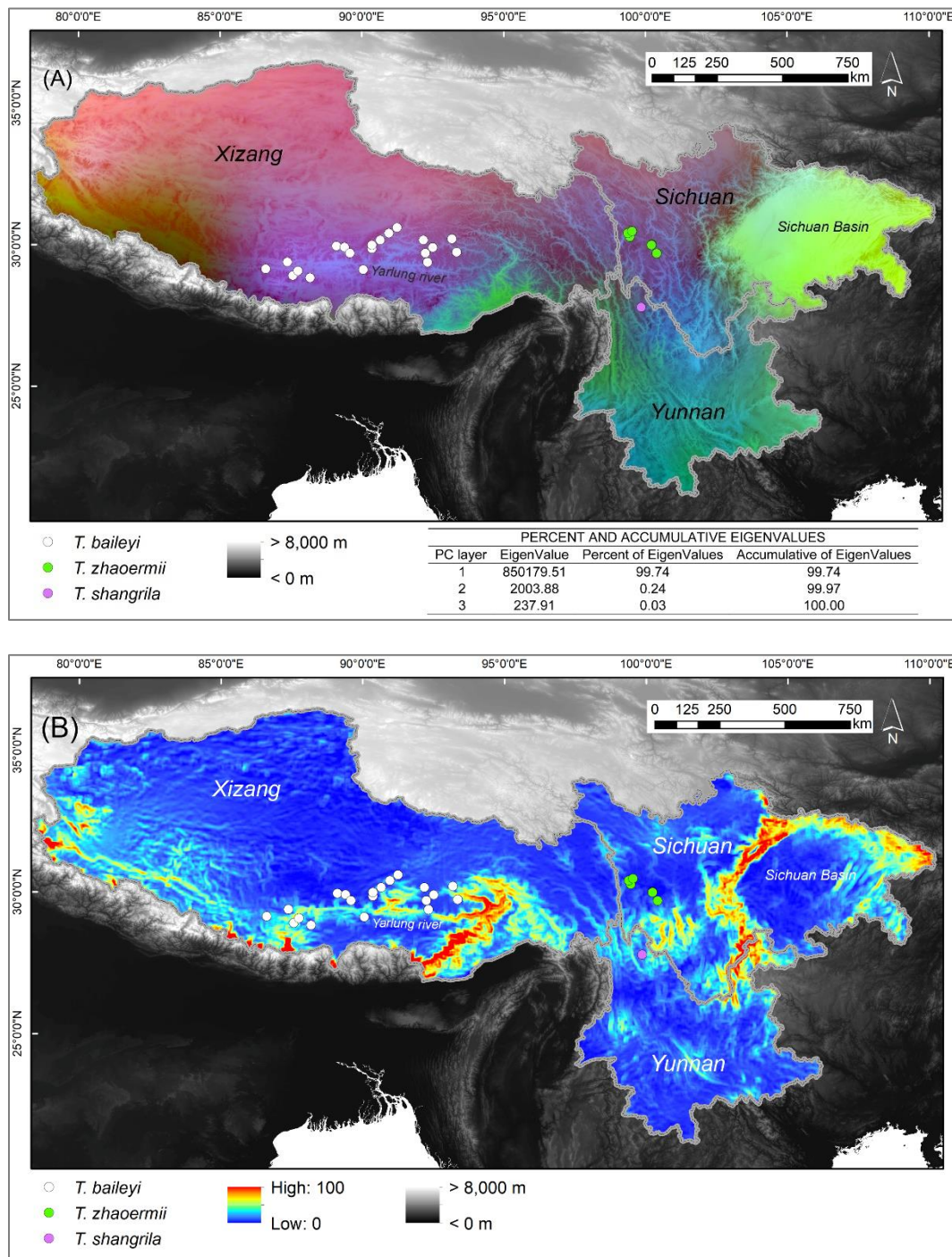


Figure S3: Diagnostic plots for the Maxent model of *Thermophilis* based on bootstrap algorithm and 100 replicates. Panel (A) depicts the Maxent receiver operator characteristic (ROC) curve, (B) shows the omission rate and predicted area as a function of the cumulative threshold, averaged over the replicate runs, (C) represents the results of the jackknife test of variable importance, and (D) shows the Maxent predictions of suitable habitat for *Thermophilis* (point-wise mean of the 100 output grids).

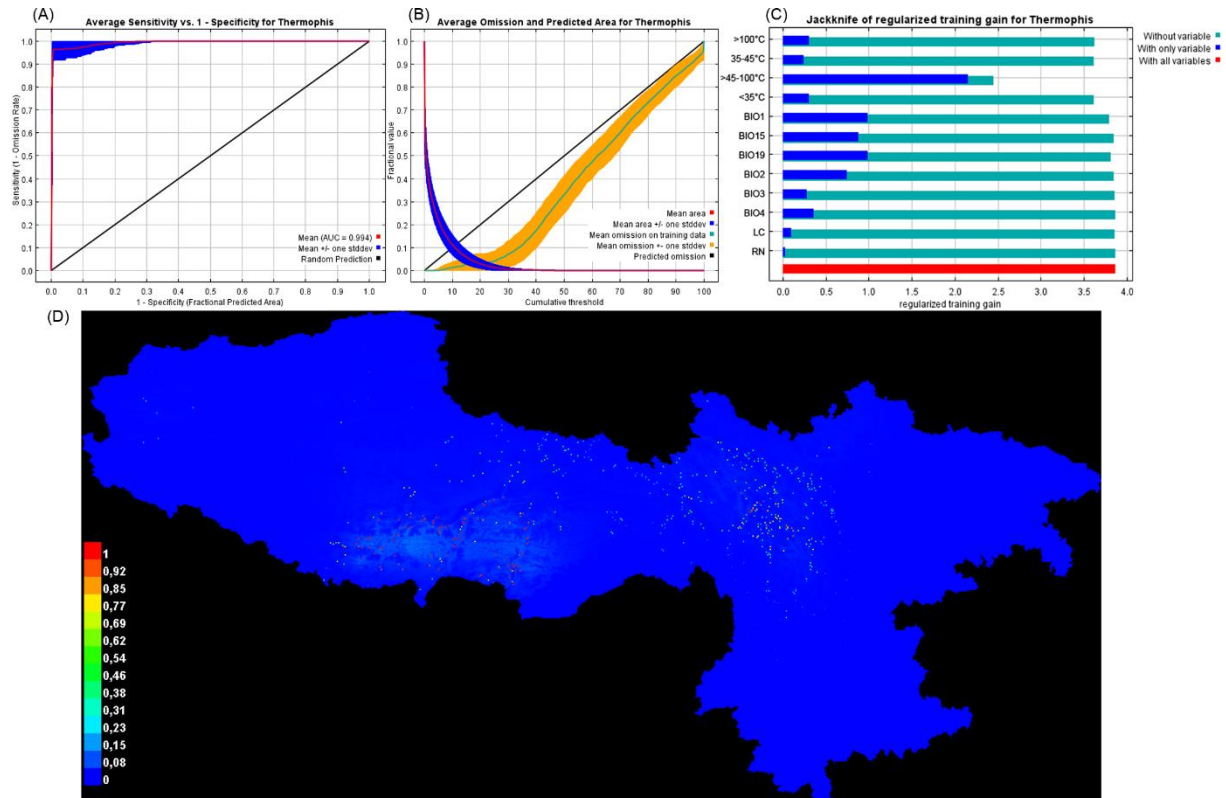
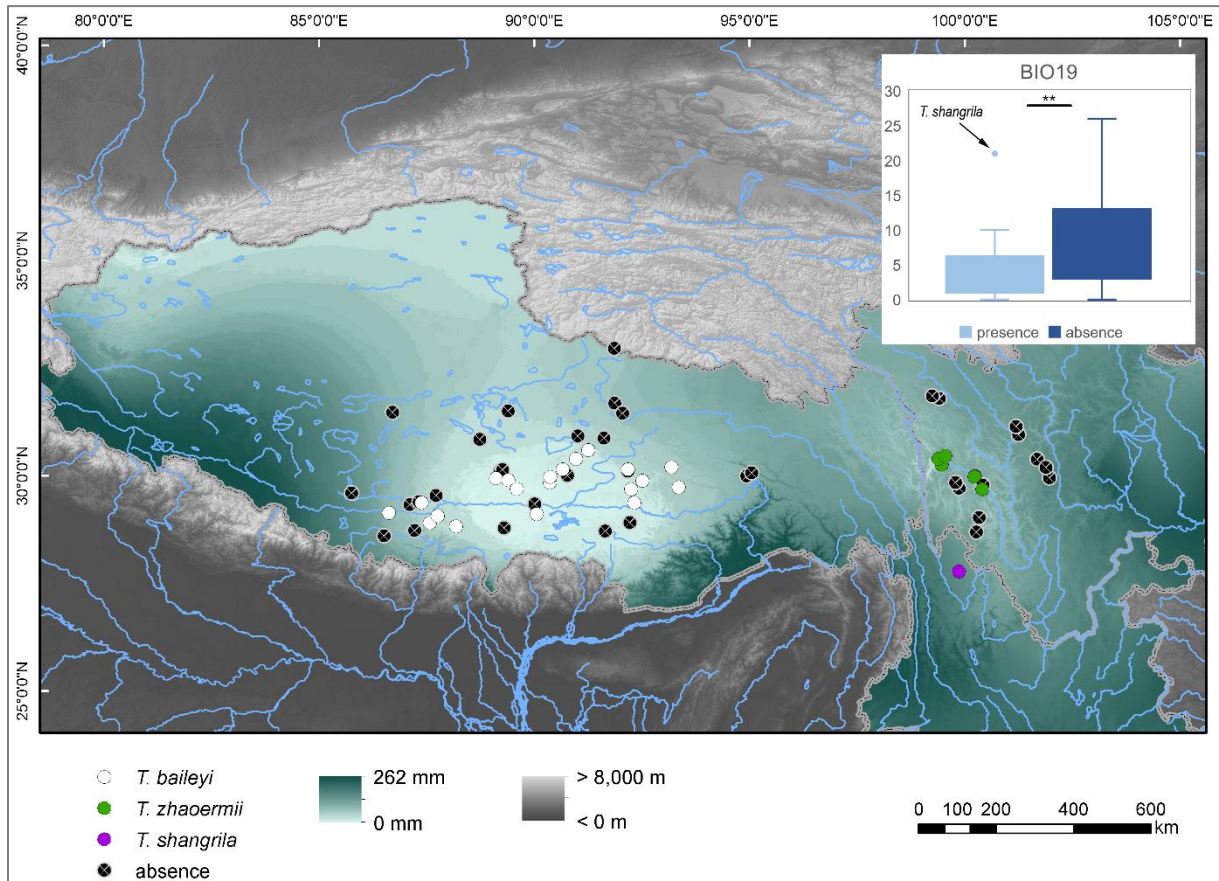


Figure S4: Precipitation of the coldest quarter of the year (BIO19) in the modelled area.

The small panel inside shows the differences between the two groups (presence vs. absence of *Thermophis*), tested by the Wilcoxon rank sum test with continuity correction ($W = 288.5$, $p\text{-value} = 0.007$). The outlier among the presence data refers to the locality of *Thermophis shangrila* (marked by an arrow).



References

- Brown JL (2014) SDMtoolbox: a python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *Methods in Ecology and Evolution* 5:694–700.
- Guo P, Liu SY, Feng JC, Miao HE (2008) The description of a new species of *Thermophis* (Serpentes: Colubridae). *Sichuan Journal of Zoology* 27:321.
- Guo P, Liu SY, Huang S, He M, Sun ZY, Feng JC, Zhao EM (2009) Morphological variation in *Thermophis* Malnate (Serpentes: Colubridae), with an expanded description of *T. zhaoermii*. *Zootaxa*:51–60.
- 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. *Molecular Phylogenetics and Evolution* 63:396–406.
- Li J-T, et al. (2018) Comparative genomic investigation of high-elevation adaptation in ectothermic snakes. *Proc Natl Acad Sci USA* 115:8406–8411.
- Peng L, Lu C, Huang S, Guo P, Zhang Y (2014) A 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 Herpetological Research* 5:228–239.
- Wall F (1907) Some new Asian snakes. *Journal of Bombay Natural History Society* 17:612-618.