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A new ovoviviparous rat snake species of the genus *Elaphe* (Squamata: Colubridae) from western Sichuan, China

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Abstract

The genus *Elaphe* is a highly diverse snake group with wide distribution in Eurasia. Here, based on morphological comparisons and molecular data, we describe a new species of this genus from western Sichuan, China. Bayesian inference and maximum-likelihood analyses of two mitochondrial DNA fragments (CO1 and 12S) showed that the new taxon differs from its congeners (CO1-based *p*-distance ⩾ 4.1%). Morphologically and ecologically, the new species can be diagnosed from other species by a combination of the following characters: **1)** medium body size, < 780 mm in total length; **2)** midbody dorsal scales in 23 rows generally, not keeled; **3)** ventrals 169–180 and subcaudal pairs 56–63; **4)** first preocular docked with frontal; **5)** internasal scales approximately trapezoidal; **6)** length of gap between internasals more than 3/5 that between prefrontal; **7)** length of tip of snout to frontal slightly larger than length of frontal; **8)** ovoviviparous. Currently, the new species is known only from the Sichuan and Shaanxi provinces in China. This new species brings the total number of species in the genus *Elaphe* to 18.

Keywords

Snake, new species, morphology, mitochondrial DNA, taxonomy, Hengduan Mountains

Introduction

The Hengduan Mountains are part of the Qinghai-Tibet Plateau and are at the junction of the second and first steps of China's terrain. This range contains a series of north-south parallel mountains in the western Sichuan and Yunnan provinces as well as the eastern Tibet Autonomous Region. The Hengduan Mountains cover an area of approximately 50 km^2, with an average altitude of more than 4 000 m. Due to the unique geographical position and variable climate, organisms distributed in the region have evolved highly unique adaptations. In recent years, an increasing number of new snake species have been discovered in this region, including *Thermophis zhaoermii* (Guo et al. 2008), *Gloydius rubromaculatus* (Shi et al. 2017), *Hebius yanbianensis* (Liu et al. 2018), *Trimerodytes yapingi* (Guo et al. 2019), and *Gloydius huangi* (Wang et al. 2019), indicating that current species diversity is likely underestimated.

The genus *Elaphe* Fitzinger in Wagler, 1833 (i.e., rat snakes) was originally erected based on the species *Coluber quatuorlineatus* Lacépède, 1789. The genus currently contains 16 species distributed throughout Eurasia (Helfenberger 2001; Zhao 2006; Huang et al. 2012; Jablonski et al. 2019; Uetz et al. 2021), including 12 in China: i.e., *E. anomala* (Boulenger, 1916), *E. bimaculata* Schmidt, 1925, *E. cantoris* (Boulenger, 1894), *E. carinata* (Günther, 1864), *E. davidi* (Sauvage, 1884), *E. dione* (Pallas, 1773), *E. hodgsoni* (Günther, 1860), *E. moellendorffi* (Boettger, 1886), *E. schrenckii* (Strauch, 1873), *E. taeniura* (Cope, 1861), *E. zoigeensis* Huang et al., 2012, and *E. xiphodonta* Qi et al., 2021, three of which are endemic (i.e., *E. bimaculata*, *E. xiphodonta*, and *E. zoigeensis*) (Wang et al. 2020; Qi et al. 2021). Rat

snakes are highly adaptable to the environment and are found in varied habitats, including at high elevation (average 3 500 m) and in dry cold climates. All currently recognized species are considered oviparous (Uetz et al. 2021).

In 2020, two snake specimens were collected in Ganzi County, Sichuan Province, China (Figure 1). They were identified as members of *Elaphe* based on phenotypical characters. However, subsequent molecular phylogenetics, morphological comparison, and reproduction model analysis indicated that these specimens differed from all known congeners. Thus, the Ganzi specimens were considered to represent an undescribed taxon, which we describe herein.

Materials and Methods

Molecular phylogeny

Six samples of the genus *Elaphe* were collected, sequenced, and analyzed (Table 1). Two were unidentified species from Sichuan, China, and four were identified as *E. dione*. The collection information, including elevation, latitude, and longitude, were recorded by GPS. All specimens and tissue samples were deposited at Yibin University (YBU).

Total DNA was extracted using a Tissue DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). Two mitochondrial gene fragments, i.e., cytochrome c oxidase subunit 1 (CO1) and mitochondrial 12S ribosomal RNA (12S), were amplified using primers Chmf4/Chmr4 (Che et al. 2012) and12S268/12S916 (Utiger 2002), respectively. The double-stranded products

were sequenced by a commercial company (Sangon, Chengdu, China). Sequences were edited manually using SeqMan in Lasergene v15.1 (DNASTAR, Inc., Madison, Wis.), aligned in MEGA v7 using the ClustalW algorithm with default parameters (Thompson et al. 2003; Kumar et al. 2016), and checked by eye for ambiguous alignments. We translated the protein-coding gene (CO1) into an amino acid sequence using MEGA v7 to evaluate sequence quality (Kumar et al. 2016). The newly generated sequences were submitted to GenBank, and composed the dataset (Table 1).

We downloaded 88 sequences from 18 *Elaphe* species from GenBank, with *Euprepiophis mandarinus* used as the outgroup (Qi et al. 2021). Details on sample information are shown in Table 1.

Bayesian inference (BI) and maximum-likelihood (ML) analyses were executed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) and IQ-tree v1.6.12 (Lam-Tung 2015) respectively based on the combined CO1 and 12S dataset. Prior to analysis, best-fit nucleotide substitution models were chosen using PartitionFinder v2.1.1 under Akaike information criteria (Lanfear et al. 2017). For BI, all searches consisted of three heated chains and a single cold chain. Three independent iterations were performed, each comprised of two runs of 1×10^7 generations, with sampling every 1 000 generations. The parameter estimates were plotted against the generations and the first 25% were discarded as burn-in. ML analysis was performed with 1 000 fast bootstrap repeats.

Uncorrected *p*-distances based on the partial CO1 fragment were calculated using MEGA v7 (Kumar et al. 2016).

Morphological comparison

In total, 37 specimens, including three unidentified specimens and 34 specimens from *E. dione*, were examined morphologically (Appendix I). All specimens examined are preserved at Yibin University (YBU) or the Chengdu Institute of Biology, Chinese Academy of Sciences (CIB, CAS).

Snout-vent length (SVL, tip of snout to vent) and tail length (TaL) were measured with a tape measure to the nearest 1 mm. Symmetrical head characters were measured on both sides and given in left/right order. Number of dorsal scale rows is given at two head lengths posterior to head, at midbody (corresponding to half total ventral number), and at two head lengths anterior to anus. Ventral scales were counted according to Dowling (1951). Coloration patterns were based on preserved specimens. Abbreviations of body characters include: **DS:** dorsal scale rows, counted at two head length behind head, at midbody, and at two head length before vent; **Sup:** supralabials; **In:** infralabials; **Pr:** preoculars; **Pt:** postoculars; **Vs:** ventral scales; **Sc:** subcaudals; **IgL**: length of gap between internasals; **PgL:** length of gap between prefrontal; **FL:** maximal length of frontal; **FAW:** anterior width of frontal; **FPW:** posterior width of frontal; **RFL:** distance between anterior frontal and rostral; **PrF:** preocular touching frontal or not.

For comparison with other species, we used data from previous studies (Boulenger 1894; Che et al. 2020; Hofmann et al. 2016; Jablonski et al. 2019; Huang 2012; Qi et al. 2021; Shi et al. 2019; Stejneger 1907; Wen and Ji 1997; Zhao 2006).

Reproductive observations

In August 2020, we collected two pregnant female snakes in Toba Township, Ganzi County, Ganzi Tibetan Autonomous Prefecture, Sichuan, China. They were bred in the laboratory and their reproduction was observed and recorded.

Results

Phylogenetic analyses

The final alignment of mitochondrial gene fragment consisted of 941 nucleotide base pairs (bp) (CO1: 513 bp, 12S: 428 bp). The best-fit model for each coding position in both sequences is listed in Table 2. The BI and ML results were generally identical in topology, with only slight disagreement in some nodal support (Figure 2). Both trees indicated that all putative species of *Elaphe* formed a highly supported lineage (1.00 posterior probability (PP) in BI and 100% bootstrap support (BS) in ML) (Figure 2). The two unidentified specimens from Sichuan were nested within the genus and formed a clade with three specimens previously identified as *E. dione* with high support (1.00 PP and 96% BS) and were sister to a clade including the other recognized specimens of *E. dione*.

The uncorrected P-distance of CO1 gene (513bp) for 31 sequences from 18 species of *Elaphe*, its congeners was 4.1%-13.0% and conspecific divergence was 0.2%-0.8% (Table 3).

Morphology comparison

The general external morphology and body coloration pattern of the two unidentified specimens from Sichuan were typical of the genus *Elaphe*. The specimens showed high similarity to *E. dione* and shared several features, e.g., midbody scales in 21 rows, postoculars 2, and paired subcaudals (Zhao et al. 1998). However, the unidentified specimens also possessed some unique characters, distinct from all other named species of *Elaphe* (see below).

Reproduction model

On 26 August 2020, female G20821 (SVL 74.8 mm, weight 100.07 g) gave birth to seven neonates in Baiyu County, Sichuan, China, all of which were wrapped in an egg membrane (Figure 3). On 5 September 2020, five neonates broke through the oolemma, while the other two neonates died. On 8 September 2020, female G20822 (YBU20780, SVL 69.6 mm, weight 114.91 g) gave birth to three neonates and laid two eggs the following day at Yibin Key Laboratory of Zoological Diversity and Ecological Conservation, Yibin University. The surface of the eggs was pale yellow, but no embryos were observed inside the eggs (Figure 3).

The surviving neonates were fed individually in a transparent incubator at the captive breeding laboratory, and their growth was observed and recorded (Table 4).

Based on molecular phylogenetic analyses, morphological comparisons, and the

reproduction model, the two specimens from Sichuan, China, were considered to represent an unidentified species, as described herein.

Taxonomy

Elaphe ganziensis sp. nov.

(Figures 3–6)

Holotype. YBU20780, adult female collected at 9:30 am on 21 August 2020, at an elevation of 3 342 m in Ganzi County, Sichuan Province, China (N 31°33′37″, E 100°5′6″). The specimen was deposited at the Yibin Key Laboratory of Zoological Diversity and Ecological Conservation Captive Breeding Laboratory at Yibin University.

Paratypes. CIB9230, adult female from Kangding County, Sichuan; CIB92289, adult male from Hongyuan County, Sichuan. Both specimens were deposited at the Chengdu Institute of Biology, Chinese Academy of Sciences.

Etymology. The specific name refers to the type locality of the new species, Ganzi County, Sichuan, China.

Diagnosis. 1) medium body size, less than 780 mm in total length; **2)** midbody dorsal scales in 23 rows generally, not keeled; **3)** ventrals 169–180 and subcaudal 56–63 pairs; **4)** first preocular docked with frontal; **5)** internasal scales approximately trapezoidal, length of gap between internasals more than 3/5 that between prefrontal; **6)** length of tip of snout to

frontal slightly larger than length of frontal; **7)** length of tip of snout to frontal slightly larger than length of frontal; **8)** ovoviviparous.

Description of holotype. Adult female, snout-vent length 587 mm, tail length 118 mm.

Head distinct from neck. Head approximately ellipse-shaped in dorsal view, 22.76 mm long, 10.77 mm in wide. Rostral scale visible from above. Loreal 1, not entering eye. Preoculars 2, postoculars 2, and temporals 2+3/2+4. Supralabials 9/8, 6^{th} and 5^{th} (left), 5^{th} and 4^{th} (right) extending to bottom of orbit, $8^{th}/7^{th}$ largest. Infralabials 11/10, first four or five touching chin shields. Two paired chin shields, parallelogram shaped.

Dorsal scales 23-23-19 rows, not keeled, glossy (Figure 7). Reduction of dorsal scales from 23 to 21 rows at ventral position of 96 and from 21 to 19 rows at ventral position of 106. Ventrals 180; anal divided, subcaudal pairs 59.

Zigzagging irregular brown stripe extending from top of head to nape; dark stripe extending from eye to corner of mouth. Head below milky white without spots normally. Body light brown with dark brown transverse bands on back of body; three dark, longitudinal stripes, two of which extend onto tail; abdomen milky white with scattered dark spots.

Infraspecific morphological variation. Based on several other specimens, certain intraspecific morphological variations, including body measurement and scale number, were found in the new species. Detailed morphological comparisons of the *Elaphe ganziensis* **sp. nov.** specimens are listed in Appendix II.

Comparisons. *Elaphe ganziensis* **sp. nov.** can be differentiated from all known congeners by its ovoviviparous reproduction model and midbody dorsal scales not keeled (Table 5).

Elaphe ganziensis **sp. nov.** can be distinguished from *E. anomala*, *E. cantoris*, *E. carinata*, *E. climacophora*, *E. davidi*, *E. hodgsoni*, *E. moellendorffi*, *E. quadrivirgata*, *E. quatuorlineata*, *E. sauromates*, *E. schrenckii*, and *E. taeniura* by smaller body size (< 1 000 mm vs. > 1 000 mm).

Elaphe ganziensis **sp. nov.** differs from *E. cantoris*, *E. dione*, *E. moellendorffi*, *E. quadrivirgata*, *E. quatuorlineata*, *E. sauromates*, *E. urartica*, *E. zoigeensis*, and *E. xiphodonta* by number of midbody dorsal scales (23 rows vs. 19, 21, or 25 rows).

Elaphe ganziensis **sp. nov.** differs from *E. anomala*, *E. cantoris*, *E. climacophora*, *E. hodgsoni*, *E. moellendorffi*, *E. quadrivirgata*, *E. sauromates*, *E. taeniura*, *E. zoigeensis*, and *E. xiphodonta* by having fewer ventrals (< 190 vs. > 190). The new species can be distinguished from *E. cantoris*, *E. carinata*, *E. climacophora*, *E. hodgsoni*, *E. moellendorffi*, *E. quadrivirgata*, *E. taeniura*, *E. zoigeensis*, and *E. xiphodonta* by having fewer subcaudals (56–63 vs. 67–121).

Elaphe ganziensis **sp. nov.** is very similar to *E. dione* in morphology and genetics. However, in addition to the characters mentioned above, these species can be differentiated based on the following characters: **1)** first preocular docked with frontal and internasal scale approximately trapezoidal in shape in *Elaphe ganziensis* **sp. nov.** (vs. not docked with

frontal and internasal scale approximately triangular in shape *E. dione*) (Figure 8); **2)** ratio of length of gap between internasals to that between prefrontals (IgL/PgL) 64.7%–73.6% in *Elaphe ganziensis* **sp. nov.** (vs. 27.7%–55.6% in *E. dione*); and **3)** ratio of length between rostral and anterior frontal (RFL/FL) 114.8%–117.6% in *Elaphe ganziensis* **sp. nov.** (vs. 64.2%–104.0% in *E. dione*) (Appendix II).

Distribution. This species is currently known only from the Sichuan (Ganzi, Hongyuan, and Kangding) and Shaanxi provinces in China.

Natural history. The specimens were found between 9:30 am and 10:30 am on grass between the road and hill at an elevation > 3 000 m (Figure 9). The area has a plateau mountain climate characterized by a short spring and summer and long autumn and winter.

The new species is ovoviviparous, and gives birth from August to September, with 3–7 neonates each time. Newborns weigh 4.05–6.81 g (body mass) and are 19.20–23.70 cm in total body length (Table 4). No information on diet is available.

Discussion

Oviparity and ovoviviparity are two reproduction models in snakes, though most colubrid snake species are oviparous (Zhao 2006). As far as we know, all previously described species of *Elaphe* are oviparous (Zhao 2006; Uetz et al. 2021). In this study, based on genetics, morphology, and ecology, we described a new species with unique reproduction from the Hengduan Mountains and adjoining regions. Morphologically, the new species is

very similar to *E. dione*, and both species are close in genetic distance. However, *Elaphe ganziensis* **sp. nov.** differs from *E. dione* based on its ovoviviparous reproduction and several morphological characters.

Elaphe dione has been recorded from China, Russia, Korea, and Central Asia (Uetz 2021). Previously collected specimens from western Sichuan were designated as *E. dione* (Zhao et al. 1998, 2003; Zhao 2006; Hofmann 2016), although Huang et al. (2012) described the Ruoergai population as *E. zoigeensis*.

However, Qi et al. (2021) questioned the *E. dione* designation of previously collected specimens from Ningshan County, Shaanxi province, and surrounding areas, and proposed a re-examination of these specimens. Our description of a new species (*Elaphe ganziensis* **sp. nov.**) from the Hengduan Mountains and surrounding regions supports the speculation of Qi et al. (2021).

Our description of *Elaphe ganziensis* **sp. nov.** brings the total number of species within the genus of *Elaphe* to 18.

Acknowledgements

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Figure Legends

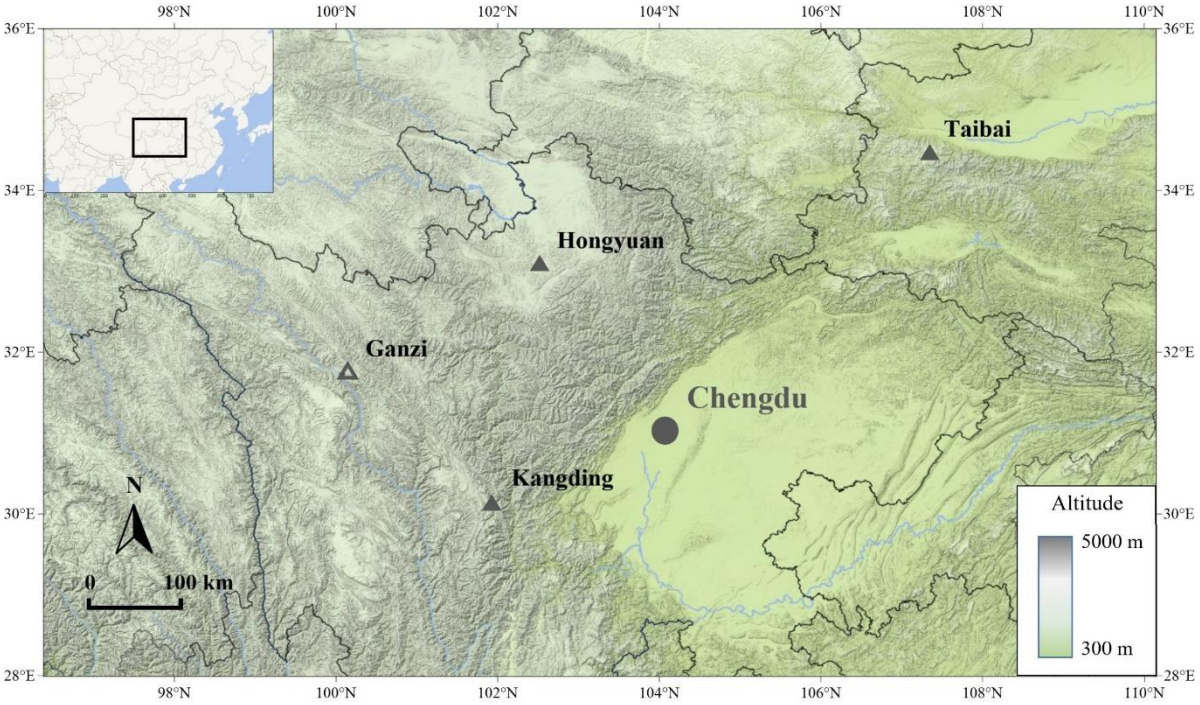

Figure 1. Map showing the type locality (empty triangle) and other known localities (filled triangles) of *Elaphe ganziensis* **sp. nov**

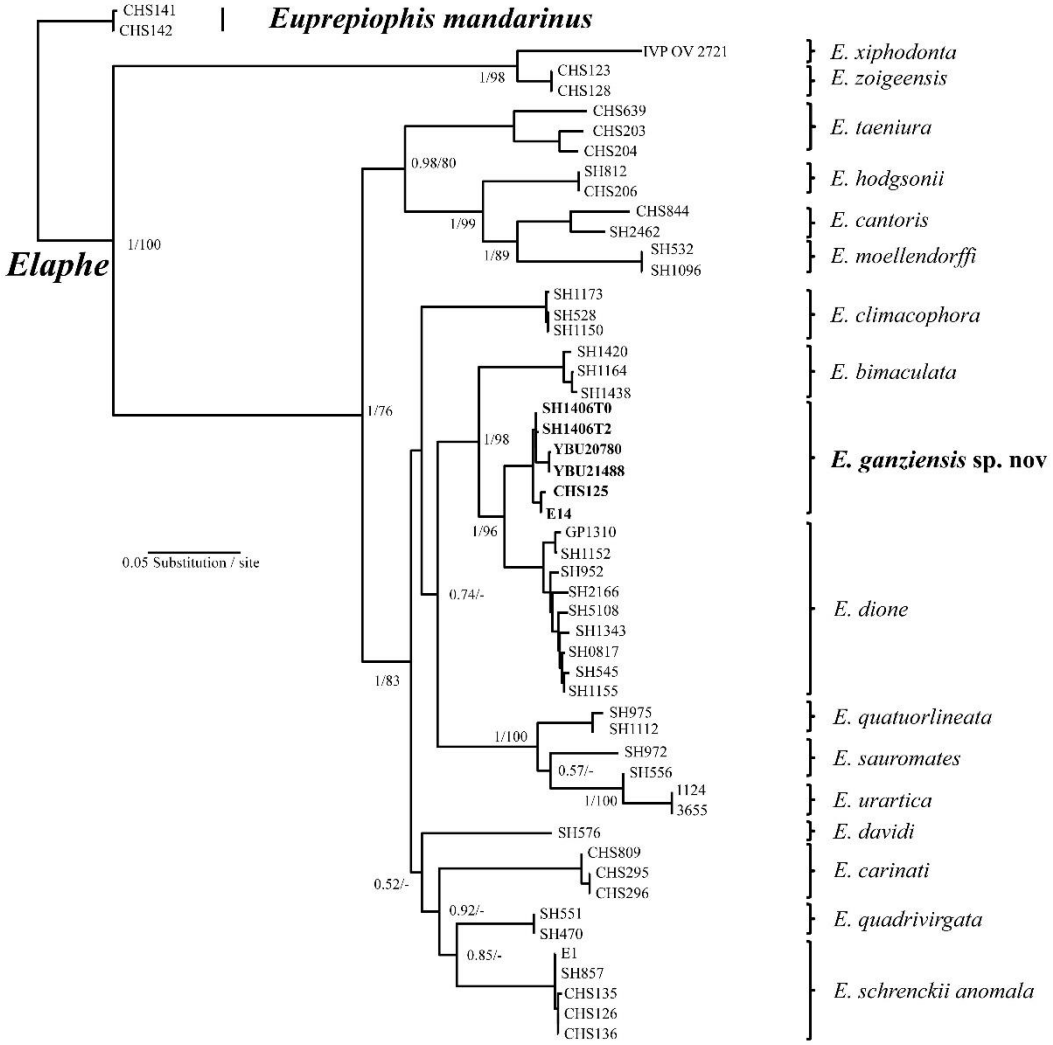

Figure 2. Bayesian phylogenetic tree of *Elaphe* species (rat snakes) based on CO1 and 12S gene sequences. Posterior probabilities (PP) from Bayesian inference (> 50%) and bootstrap support (BS) from maximum-likelihood analysis (> 50) are given adjacent to respective nodes for major nodes.

Figure 3. Neonates and juveniles of *Elaphe ganziensis* **sp. nov. A:** Seven neonates with egg membrane; **B:** Neonates without membranes; **C:** Juveniles. Photos by Yang-Mei Zeng (A & B) and Peng Guo (C).

Figure 4. General view of holotype of *Elaphe ganziensis* **sp. nov.** (YBU20780) in life. Photo by Peng Guo.

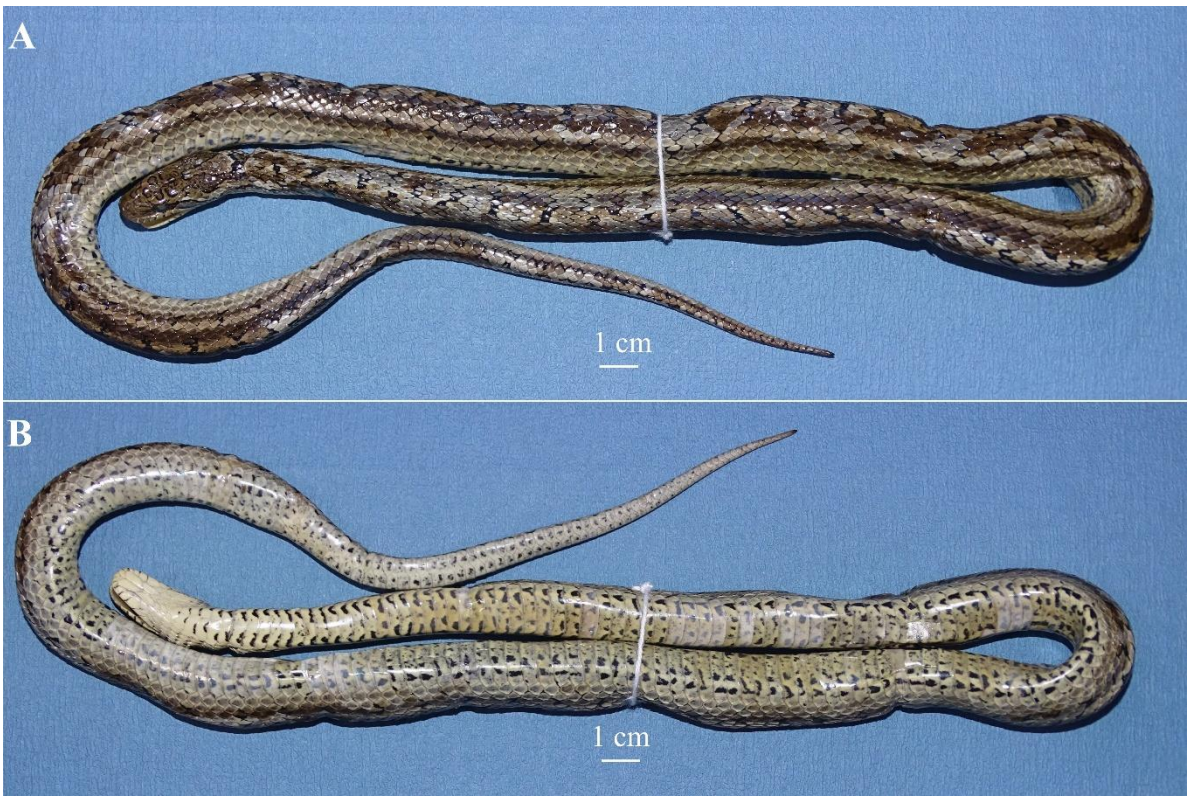

Figure 5. Dorsal (top) and ventral (bottom) views of holotype of *Elaphe ganziensis* **sp. nov.** (YBU20780) in preservative.

Figure 6. Dorsal (A), ventral (B), and lateral (C) views of head of holotype of *Elaphe*

ganziensis **sp. nov.** in preservative.

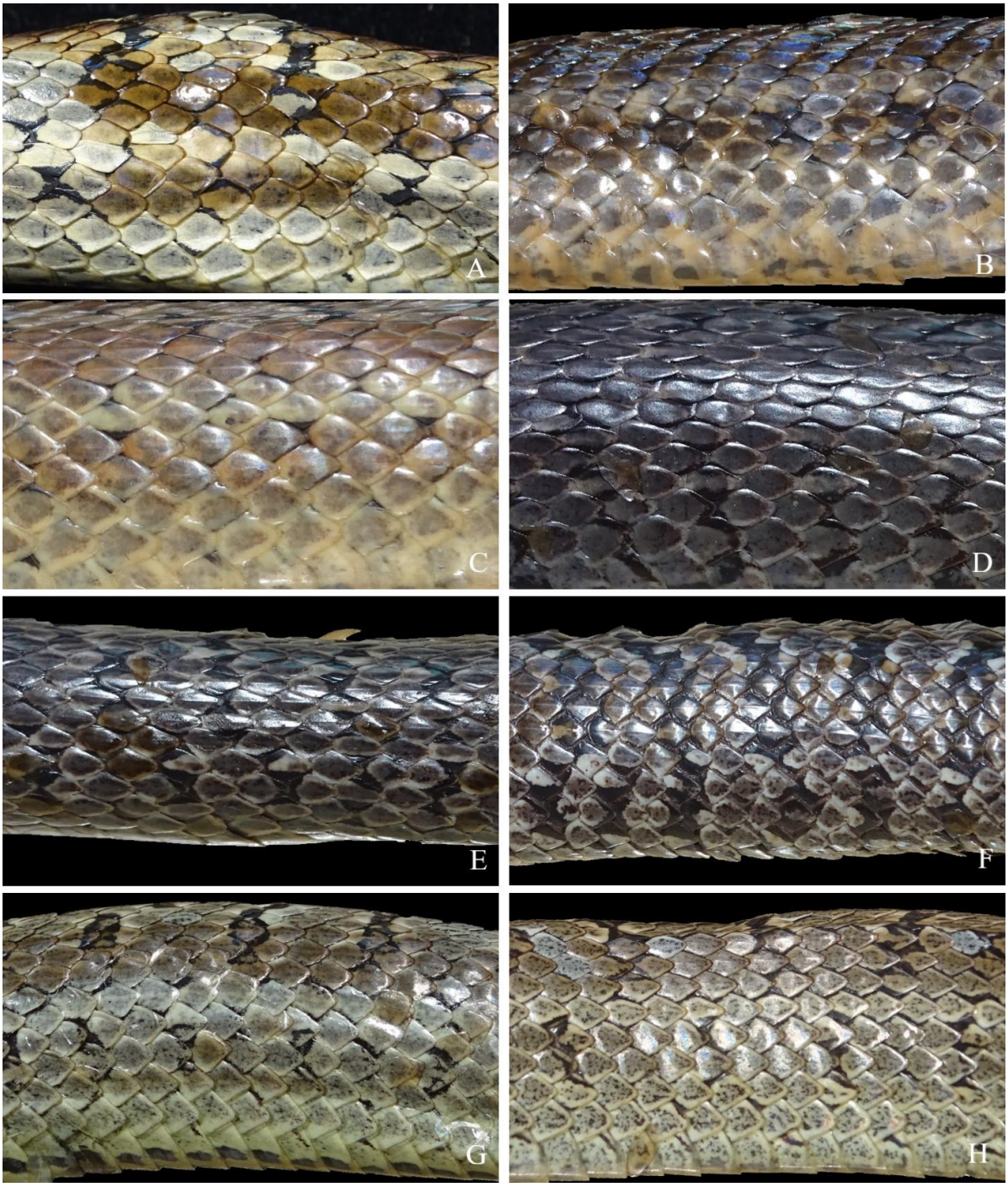

Figure 7. Dorsal views of midbody of *Elaphe ganziensis* **sp. nov.** and *Elaphe dione*. **A & B:** *Elaphe ganziensis* **sp. nov.**, YBU20780 (A) and CIB92289 (B) from Sichuan, China; **C-H:** *Elaphe dione* from Xinjiang, China (C, CIB9191), Jilin, China (D, CIB9214), Heilongjiang,

China (E, CIB9217), Liaoning, China (F, CIB9218), Inner Mongolia, China (G, CIB95381), and Russia (H, CIB95382).

Figure 8. Dorsal view of head of *Elaphe ganziensis* **sp. nov.** and *Elaphe dione*: **A & B:** *Elaphe ganziensis* **sp. nov.**, YBU20780 (A) and CIB92289 (B) from Sichuan, China; **C-F:** *Elaphe dione* from Jilin, China (C, YBU10032), Xinjiang, China (D, CIB9191), Inner Mongolia, China (E, CIB95381), Russia (F, CIB95382).

Figure 9. Habitat of *Elaphe ganziensis* **sp. nov.** in Ganzi County, Sichuan, China.

Table 1. Detailed information for samples of molecular study. The newly sequenced samples were indicated by bold.

Voucher number	Species	Location	GenBank accession no.	
			CO1	12S rRNA
YBU20780	*Elaphe ganziensis* **sp. nov**	Ganzi, Sichuan, China	OK576873	OK585068
YBU21488	*Elaphe ganziensis* **sp. nov**	Ganzi, Sichuan, China	OK576874	OK585069
SH1406T0	*Elaphe ganziensis* **sp. nov**	Ganzi, Sichuan, China	KP115289	KP091848
SH1406T2	*Elaphe ganziensis* **sp. nov**	Ganzi, Sichuan, China	KP115290	N/A
CHS125	*Elaphe ganziensis* **sp. nov**	Taibai, Shaanxi, China	MK064631	MK065363
E14	*Elaphe ganziensis* **sp. nov**	N/A	JQ712877	JQ712873
GP0817	*Elaphe dione*	Shangzhi, Heilongjiang, China	OK576869	OK585064
YBU10028	*Elaphe dione*	Jian, Jilin, China	OK576870	OK585065
GP1343	*Elaphe dione*	Hengren, Liaoning, China	OK576871	OK585066
YBU17279	*Elaphe dione*	Altay, Xinjiang, China	OK576872	OK585067
SH952	*Elaphe dione*	Shaanxi, China	AY122749	AY122833
SH2166	*Elaphe dione*	Lake Alakol, Kasachstan	AY122748	AY122832
SH1155	*Elaphe dione*	Lazo, Primorye, Russia	AY122746	AY122830
SH545	*Elaphe dione*	Ukraine	AY122747	AY122831
SH1152	*Elaphe dione*	Wonju, South Korea	AY122745	AY122829
SH1164	*Elaphe bimaculata*	N/A	AY122683	AY122767
SH1420	*Elaphe bimaculata*	N/A	AY122684	AY122768
SH1438	*Elaphe bimaculata*	N/A	AY122753	AY122837
CHS123	*Elaphe zoigeensis*	Zoige, Sichuan, China	MK064630	MK065362
CHS128	*Elaphe zoigeensis*	Zoige, Sichuan, China	MK064633	MK065365
E1	*Elaphe anomala*	N/A	JQ712878	JQ712875
CHS126	*Elaphe anomala*	Huangshan, Anhui, China	MK064632	MK065364
CHS295	*Elaphe carinata*	Huangshan, Anhui, China	MK064721	MK065464
CHS296	*Elaphe carinata*	Shaoguan, Guangdong, China	MK064722	MK065465
CHS809	*Elaphe carinata*	Qixiling, Jiangxi, China	MK064894	MK065670
SH528	*Elaphe climacophora*	Central Honshu, Japan	AY122686	AY122770
SH1150	*Elaphe climacophora*	Kuriles, Russia	AY122687	AY122771
SH1173	*Elaphe climacophora*	Gifu Prefecture, Honshu, Japan	AY122688	AY122772
SH576	*Elaphe davidi*	Shaanxi, China	AY122691	AY122775
SH551	*Elaphe quadrivirgata*	Gifu Prefecture, Honshu, Japan	AY122709	AY122793
SH470	*Elaphe quadrivirgata*	Central Honshu, Japan	AY122710	AY122794
SH975	*Elaphe quatuorlineata*	N/A	AY122714	AY122798
SH1112	*Elaphe quatuorlineata*	Rome, Italy	AY122712	AY122796
SH556	*Elaphe sauromates*	Mt. Ararat, Turkey	AY122713	AY122797
SH972	*Elaphe sauromates*	Selcuk, Turkey	AY122711	AY122795

Table 1. Continue

CHS135	*Elaphe schrenckii*	Changbai, Jilin, China	MK064638	MK065370
CHS136	*Elaphe schrenckii*	Huangshan, Anhui, China	MK064639	MK065371
SH857	*Elaphe schrenckii*	N/A	AY122720	AY122804
CHS203	*Elaphe taeniura*	Recaokao, Sichuan, China	MK064678	MK065413
CHS204	*Elaphe taeniura*	Qingling, Zhouzhi, Xi'an, China	MK064679	MK065414
CHS639	*Elaphe taeniura*	Heishiding, Guangdong, China	MK064790	MK065542
CHS844	*Elaphe cantoris*	Pailong, Tibet, China	MK064913	MK065692
SH2462	*Elaphe cantoris*	Nepal	AY122685	AY122769
SH812	*Elaphe hodgsonii*	Katmandu, Nepal	AY122694	AY122778
CHS206	*Elaphe hodgsonii*	Jilong, Tibet, China	MK064680	MK065415
SH532	*Elaphe moellendorffi*	N/A	AY122703	AY122787
SH1096	*Elaphe moellendorffi*	N/A	AY122702	AY122786
1124	*Elaphe urartica*	Kısıklı, Süphan Mts., Bitlis, Turkey	MK640299	N/A
3655	*Elaphe urartica*	Guzdak, Qobustan, Azerbaijan	MK640269	N/A
IVPP OV 2721	*Elaphe xiphodonta*	N/A	MK191765	N/A
CHS141	*Euprepiophis mandarinus*	HuangShan, Anhui, China	MK064643	MK065374
CHS142	*Euprepiophis mandarinus*	HuangShan, Anhui, China	MK064644	MK065375

TABLE 3. The best–fit models for each coding position of both sequences selected by PartitionFinder–2.1.1.

Fragments	Coding position	Model
CO1	Pos1	TRN+G
	Pos2	GTR+G
	Pos3	GTR+I+G
12s	Pos1, Post2, Pos3	GTR+I+G

TABLE 2. Uncorrected *P*-distances for 31 sequences from 18 species of *Elaphe*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1 GP6620 *E. ganziensis* **sp. nov**	0.0																														
2 GP8540 *E. ganziensis* **sp. nov**	0.0	0.0																													
3 SH1406T0 *E. ganziensis* **sp. nov**	0.0	0.0	0.0																												
4 SH1406T2 *E. ganziensis* **sp. nov**	0.2	0.2	0.2	0.0																											
5 CHS125 *E. ganziensis* **sp. nov**	0.6	0.6	0.6	0.8	0.0																										
6 E14 *E. ganziensis*	0.6	0.6	0.6	0.8	0.0	0.0																									
7 GP0817 *E. dione*	4.1	4.1	4.1	4.3	4.3	4.3	0.0																								
8 GP1310 *E. dione*	4.5	4.5	4.5	4.7	4.7	4.7	1.6	0.0																							
9 GP1343 *E. dione*	4.7	4.7	4.7	4.9	4.9	4.9	1.0	1.4	0.0																						
10 GP5108 *E. dione*	4.3	4.3	4.3	4.5	4.5	4.5	0.6	1.8	1.2	0.0																					
11 SH952 *E. dione*	4.3	4.3	4.3	4.5	4.5	4.5	0.6	1.8	1.2	0.8	0.0																				
12 SH2166 *E. dione*	5.0	5.0	5.0	5.2	5.2	5.2	1.2	1.8	1.8	1.4	1.4	0.0																			
13 SH545 *E. dione*	4.3	4.3	4.3	4.5	4.5	4.5	0.2	1.8	1.2	0.8	0.8	1.4	0.0																		
14 SH1155 *E. dione*	4.1	4.1	4.1	4.3	4.3	4.3	0.0	1.6	1.0	0.6	0.6	1.2	0.2	0.0																	
15 SH1152 *E. dione*	4.3	4.3	4.3	4.5	4.5	4.5	1.4	0.2	1.2	1.6	1.6	1.6	1.6	1.4	0.0																
16 SH1438 *E. bimaculata*	6.8	6.8	6.8	6.5	7.0	7.0	6.3	7.2	6.5	7.0	7.0	7.0	6.5	6.3	7.0	0.0															
17 CHS128 *E. zoigeensis*	10.2	10.2	10.2	10.4	10.4	10.4	9.4	9.4	9.1	9.9	9.6	9.4	9.6	9.4	9.6	9.7	0.0														
18 CHS126 *E. anomala*	11.3	11.3	11.3	11.6	10.7	10.7	9.0	9.5	9.7	9.0	9.2	9.3	9.2	9.0	9.7	9.4	8.8	0.0													
19 CHS296 *E. carinata*	10.0	10.0	10.0	9.7	9.7	9.7	9.2	9.2	10.4	9.2	9.9	10.2	9.4	9.2	9.4	9.4	10.7	7.2	0.0												
20 CHS204 *E. taeniura*	10.0	10.0	10.0	9.7	10.2	10.2	10.7	11.2	11.0	11.0	11.5	10.8	10.5	10.7	11.5	10.3	10.1	10.2	10.2	0.0											
21 SH528 *E. climacophora*	9.2	9.2	9.2	9.0	9.2	9.2	9.0	9.3	10.0	9.3	9.3	10.1	9.3	9.0	9.5	11.4	10.9	10.8	9.3	9.7	0.0										
22 SH576 *E. davidi*	8.9	8.9	8.9	9.2	9.2	9.2	7.7	8.0	8.0	8.0	7.5	8.4	8.0	7.7	8.2	9.4	8.7	8.6	8.9	9.7	8.6	0.0									
23 SH470 *E. quadrivirgata*	8.7	8.7	8.7	8.5	8.4	8.4	9.2	8.7	9.4	9.7	9.4	9.7	9.4	9.2	8.9	8.2	8.2	5.8	6.2	8.2	10.0	7.6	0.0								
24 SH1112 *E. quatuorlineata*	9.2	9.2	9.2	9.4	8.9	8.9	9.7	9.7	9.4	9.4	9.9	10.3	9.9	9.7	9.9	8.4	10.7	8.7	9.6	9.5	12.4	9.4	8.1	0.0							
25 SH972 *E. sauromates*	8.6	8.6	8.6	8.9	8.8	8.8	8.7	9.1	9.4	8.9	9.4	9.0	8.9	8.7	9.4	9.4	9.7	9.0	10.5	9.5	9.8	9.9	10.2	5.8	0.0						
26 CHS136 *E. schrenckii*	11.3	11.3	11.3	11.6	10.7	10.7	9.0	9.5	9.7	9.0	9.2	9.3	9.2	9.0	9.7	9.4	8.8	0.0	7.2	10.2	10.8	8.6	5.8	8.7	9.0	0.0					
27 CHS844 *E. cantoris*	12.3	12.3	12.3	12.0	12.2	12.2	12.1	11.8	12.6	12.6	12.9	11.6	12.3	12.1	12.1	12.5	11.3	12.2	12.2	10.4	13.0	13.0	12.1	12.3	12.8	12.2	0.0				
28 SH812 *E. hodgsonii*	11.1	11.1	11.1	11.4	11.1	11.1	12.2	12.7	12.5	12.8	13.0	12.2	12.5	12.2	13.0	11.0	9.0	11.6	11.6	8.7	11.7	9.7	9.0	10.6	11.3	11.6	9.1	0.0			
29 SH1096 *E. moellendorffi*	11.3	11.3	11.3	11.6	11.4	11.4	11.2	12.0	12.2	12.0	11.4	11.2	11.4	11.2	12.2	10.7	9.8	11.1	11.9	10.6	10.8	11.6	11.3	10.9	11.1	11.1	9.2	8.2	0.0		
30 6584 *E. urartica*	8.1	8.1	8.1	7.9	7.8	7.8	8.1	8.1	8.8	8.2	8.4	7.7	8.4	8.1	8.4	10.1	9.4	9.4	8.7	10.4	9.2	9.1	8.4	6.4	5.8	9.4	11.3	10.2	10.5	0.0	
31 IVPP OV 2721 *E. xiphodonta*	10.5	10.5	10.5	10.3	10.2	10.2	10.2	10.2	10.5	10.8	10.5	10.3	10.5	10.2	10.5	10.8	5.6	8.1	9.9	9.4	10.5	9.7	7.2	11.2	10.5	8.1	9.5	9.2	9.8	9.4	0.0

Table 4. Data on neonates' growth of *Elaphe ganziensis* **sp. nov**.

	2020/09/08				2021/08/08			
Neonate No.	**Weight/g**	**SVL/cm**	**TaL/cm**	**HL/mm**	**Weight/g**	**SVL/cm**	**TaL/cm**	**HL/mm**
G20821-A	4.61	17.90	3.90	10.86	7.46	24.60	4.50	12.20
G20821-B	4.55	18.70	3.60	10.72	9.07	27.10	5.30	12.50
G20821-C	4.05	16.10	3.10	10.71	7.93	24.70	4.80	12.82
G20821-D	4.10	17.80	3.50	10.81	9.45	25.60	4.90	13.24
G20821-E	4.58	17.40	4.20	10.80	NA	NA	NA	NA
Average	4.38 (n=5)	17.58 (n=5)	3.66 (n=5)	10.78 (n=5)	8.48 (n=4)	25.50 (n=4)	4.88 (n=4)	12.69 (n=4)
G20822-A	6.77	19.10	3.90	11.07	19.71	34.50	6.70	15.88
G20822-B	6.34	18.30	4.70	11.17	9.85	27.80	6.20	12.62
G20822-C	6.81	19.10	4.60	10.95	15.05	29.80	7.20	14.86
Average	6.64 (n=3)	18.83 (n=3)	4.40 (n=3)	11.06 (n=3)	11.51 (n=3)	26.47 (n=3)	5.50 (n=3)	13.18 (n=3)

SVL: tip of snout to vent; TaL: tail length; HL: head length.

TABLE 5. Diagnostic characters separating all 18 species of the *Elaphe*, with distinguishing characters. marked in bold. *: Middle dorsal rows keeled or not.

Species	maximum SVL (mm)	DSR	MDK*	Ve	Sc	Reproduction
Elaphe ganziensis **sp. nov.**	651	23-23−19	No keeled	169-180	56-63	viviparity
Elaphe anomala	**1925**	23 (21–25)-23 (19–23)-19 (17–19)	Keeled	**203-225**	45–77	oviparity
Elaphe bimaculata	760	23 (23–25)-23 (21–25)-19 (21)	Keeled	170-209	61–81	oviparity
Elaphe cantoris	**1158**	19 (20, 21)-19 (21–23)-17	Keeled	**226–239**	**78–87**	oviparity
Elaphe carinata	**1810**	23 (21–25)-23 (21–25)-19 (17)	Keeled	186-227	**69–102**	oviparity
Elaphe climacophora	**>1500**	NA-23 (25)-NA	Keeled	**222-236**	**97–116**	oviparity
Elaphe davidi	**1227**	25 (22–27)-23 (22–25)-19 (17–21)	Keeled	155-183	53–72	oviparity
Elaphe dione	893	25 (21–27)-25 (21–27)-19 (17–21)	Keeled	168-206	51–84	oviparity
Elaphe hodgsoni	**1190**	23 (21–25)- 23 (21–25)-17	Keeled	**228-247**	**72–92**	oviparity
Elaphe moellendorffi	**1602**	25 (23–27)-27 (5)-19 (21)	Keeled	**270–278**	**92–102**	oviparity
Elaphe quadrivirgata	**>1000**	NA-19-NA	Keeled	**195–215**	**70–96**	oviparity
Elaphe quatuorlineata	**>2000**	**25-25 (23–27)-19**	Keeled	187–234	56–90	oviparity
Elaphe sauromates	**1250**	25 (21–27)-**25 (23, 24)**-19 (18–21)	Keeled	**199–222**	61–79	oviparity
Elaphe schrenckii	**1335**	23 (21)-23 (21)-19	Keeled	**208–224**	57–75	oviparity
Elaphe taeniura	**1962**	25 (23)-23 (21, 25)-19 (17)	Keeled	**223–261**	**73–121**	oviparity
Elaphe urartica	**970**	25 (23, 24)- **25 (23, 24)**-19 (18)	Keeled	154–211	60–74	oviparity
Elaphe zoigeensis	722	**21-19(21)-17**	Keeled	**202–212**	**68–79**	oviparity
Elaphe xiphodonta	785	**21-21-17**	Keeled	**202-204**	**67-68**	oviparity

Appendix I. Table 1. Information and morphological characters of specimens were examined and recorded morphologically.

Museum voucher	Taxon	Locatlty	Sex	SVL	TaL	IgL	PgL	FL	FAW	FPW	RFL	DS	Sup	In	Pr	Vs	Sc	Reference
CIB92289	*E. ganziensis* **sp. nov**	Ganzi, Sichuan, China	M	513	125	2.06	2.80	5.56	4.50	2.60	6.54	23-23-19	8/8	11/11	3/3	169	63	This study
CIB9230	*E. ganziensis* **sp. nov**	Ganzi, Sichuan, China	F	651	129	2.20	3.40	5.32	4.12	2.64	6.14	23-23-19	8/8	11/10	2/2	174	56	This study
YBU20780	*E. ganziensis* **sp. nov**	Ganzi, Sichuan, China	F	587	118	1.73	2.50	5.83	4.86	2.48	5.28	23-23-19	9/8	11/10	2/2	180	59	This study
CIB78022	*E. dione*	Tacheng, Xinjiang, China	M	713	152	1.30	3.58	7.16	5.36	3.12	6.86	23-23-19	8/8	11/11	2/2	156	61	This study
CIB78023	*E. dione*	Tacheng, Xinjiang, China	F	211	46	0.80	1.88	4.32	3.10	2.00	3.40	23-23-19	9/8	13/11	2/2	177	55	This study
CIB83614	*E. dione*	Mudanjiang, Heilongjiang, China	F	694	133	1.54	3.06	6.48	4.10	3.12	5.46	23-25-19	8/8	11/11	2/2	193	55+?	This study
CIB9191	*E. dione*	Habahe, Xinjiang, China	M	671	161	1.50	3.20	7.46	4.92	2.45	6.18	23-24-19	8/8	12/11	2/2	187	68	This study
CIB9192	*E. dione*	Habahe, Xinjiang, China	F	704	156	1.22	3.24	6.84	4.76	2.80	5.47	25-25-19	8/9	11/11	2/2	201	64	This study
CIB9193	*E. dione*	Habahe, Xinjiang, China	F	627	126	0.90	2.92	6.34	4.40	2.58	5.10	25-25-19	8/8	N/A	2/2	200	59	This study
CIB9196	*E. dione*	Habahe, Xinjiang, China	F	712	147	1.00	3.10	6.92	4.70	3.54	6.20	25-25-19	8/8	N/A \	2/2	204	63	This study
CIB9197	*E. dione*	Menyuan, Qinghai, China	F	594	127	1.40	2.70	5.50	4.26	2.50	5.40	25-23-19	9/8	10/9	2/2	180	60	This study
CIB9200	*E. dione*	Qinghai, China	F	908	164	1.78	3.90	7.48	5.90	3.70	6.44	25-27-21	9/8	10/11	2/2	202	58+?	This study
CIB9201	*E. dione*	Qinghai, China	M	741	174	1.76	3.42	7.26	5.00	2.90	6.14	25-27-21	8/8	11/11	2/2	182	70	This study
CIB9214	*E. dione*	Jilin, China	F	564	127	1.34	3.30	6.29	3.89	2.74	5.10	23-23-19	8/8	10/10	2/2	190	63	This study
CIB9215	*E. dione*	Jilin, China	F	571	132	2.00	3.60	6.58	4.62	2.60	6.38	23-23-19	8/8	10/10	2/2	190	59+?	This study
CIB9216	*E. dione*	Panshi, Jilin, China	F	572	122	1.10	3.32	5.84	3.88	2.40	5.66	23-23-19	8/8	10/11	2/2	189	63	This study
CIB9217	*E. dione*	Heilongjiang, China	F	583	126	1.62	3.10	6.10	4.06	2.34	5.90	25-23-19	8/8	10/11	2/2	189	54	This study
CIB9218	*E. dione*	Benxi, Liaoning, China	F	762	141	1.28	2.50	6.40	4.08	2.10	6.10	25-23-19	8/8	10/10	2/2	193	60	This study
CIB9219	*E. dione*	Benxi, Liaoning, China	M	545	139	1.18	2.76	5.92	4.00	2.10	5.10	23-25-19	8/8	12/11	2/2	191	63	This study
CIB9220	*E. dione*	Benxi, Liaoning, China	M	551	138	1.12	2.86	5.00	4.10	2.10	4.90	24-25-19	8/8	10/10	2/2	185	74	This study
CIB9221	*E. dione*	Benxi, Liaoning, China	M	595	143	1.28	3.30	5.86	3.80	2.60	5.32	23-25-19	8/8	11/11	2/2	190	65	This study
CIB9222	*E. dione*	Dandong, Liaoning, China	M	455	114	1.30	2.50	5.40	3.70	1.88	5.08	23-23-19	8/8	11/11	2/2	181	64	This study
CIB9223	*E. dione*	Nilka, Xinjiang, China	F	492	103	0.78	2.82	5.50	4.06	2.14	4.86	25-25-19	8/9	11/11	2/2	190	59	This study
CIB9225	*E. dione*	Nilka, Xinjiang, China	M	564	133	0.92	3.24	7.16	4.74	3.08	5.80	25-25-19	8/8	12/11	2/2	185	67	This study
CIB9226	*E. dione*	Nilka, Xinjiang, China	M	357	95	0.66	2.30	5.20	4.10	2.36	3.34	23-23-19	9/8	12/12	2/2	183	65	This study
CIB9227	*E. dione*	Nilka, Xinjiang, China	M	508	128	0.86	2.92	5.86	4.48	2.66	4.94	25-23-19	8/8	12/12	3/2	185	67	This study
CIB9231	*E. dione*	Dandong, Liaoning, China	F	581	115	1.56	3.48	6.18	4.22	2.46	6.16	23-25-19	8/8	10/10	2/2	195	56	This study
CIB9232	*E. dione*	Dandong, Liaoning, China	M	454	122	1.18	2.36	5.90	3.50	2.50	4.86	25-25-19	8/8	12/11	2/2	182	70	This study
CIB95381	*E. dione*	Inner Mongolia, China	M	425	124	1.06	2.46	5.40	3.58	2.00	4.53	23-23-19	8/8	10/11	2/2	173	56+?	This study

Appendix I. (Continue)

CIB95832	*E. dione*	Russia	M	452	112	1.00	2.73	5.40	4.20	2.46	4.75	23-24-19	8/8	11/12	2/2	183	68	This study
YBU10025	*E. dione*	Tonghua, Jinlin, China	F	741	146	1.50	3.28	6.80	4.90	2.62	5.97	23-23-19	8/9	11/11	2/2	197	64	This study
YBU10027	*E. dione*	Tonghua, Jinlin, China	F	617	126	1.60	3.84	6.54	4.24	2.89	5.70	23-23-19	8/8	10/10	2/2	191	60	This study
YBU10028	*E. dione*	Tonghua, Jinlin, China	F	692	144	1.48	2.64	6.76	4.50	2.94	5.90	23-23-19	8/8	11/11	2/2	194	67	This study
YBU10031	*E. dione*	Helong, Jinlin, China	M	771	153	1.84	3.42	7.24	4.76	3.36	5.30	23-23-19	8/8	11/10	2/2	183	67	This study
YBU10032	*E. dione*	Tonghua, Jinlin, China	F	603	121	1.44	3.44	6.44	4.46	2.76	6.70	23-23-19	8/8	10/10	2/2	190	58	This study
YBU17275	*E. dione*	Altay, Xinjiang, China	F	195	44	0.74	1.80	4.18	3.00	1.76	3.70	23-25-19	8/8	10/10	2/2	184	67	This study
YBU17279	*E. dione*	Altay, Xinjiang, China	F	202	43	0.76	2.40	4.22	2.58	1.98	3.74	25-25-19	8/8	11/10	2/2	190	67	This study

SVL: snout-vent length, TaL tail length; IgL: The length of the gap between the internasals; PgL: the length of the gap between the prefrontal; FL: the length of frontal; FAW: the anterior width of frontal; FPW: the posterior width of frontal; RFL: the length of rostral to frontal; DS: dorsal scales; Sup: supralabials; In: infralabials; Pr: preocular; Vs: ventral scales; Sc: subcaudal scales; M: male; F: female.

Appendix II. Table 2. Morphological comparisons between *E. ganziensis* **sp. nov** and *E. dione*.

Species	*E. ganziensis*	*E. dione*						
	Sichuan	Heilongjiang	Xinjiang	Liaoling	Qinghai	Jilin	Inner Mongolia	Russia
Sample size	3	2	8	7	3	8	1	1
TaL/SVL	18.9-24.4%	19.2-21.6%	20.1-25.2%	18.5-26.9%	18.1-23.5%	19.7-23.1%	29%	25%
IgL/PgL	**64.7-73.6%**	50.3-52.3%	27.7-46.9%	33.8-52.0%	45.6-51.9%	33.1-55.6%	43%	37%
FAW/FL	77.4-84.3%	63.3-66.6%	61.1-78.8%	59.3-82.0%	68.9-78.9%	61.8-72.1%	66%	78%
FPW/FAW	57.8-64.1%	57.6-76.1%	49.8-76.7%	50.8-71.4%	58.0-62.7%	53.5-70.6%	56%	59%
RFL/FL	**114.8-117.6%**	84.3-96.7%	64.2-89.6%	82.4-98.4%	84.6-98.2%	73.2-104.0%	84%	88%
PrF (Left/Right)	Butted/Butted	Non/Non	Non/Non	Non/Non	Non/Non	Non/Non	Non/Non	Non/Non
Keeled	only near the cloaca	6-19 rows of dorsal scales	6-19 rows of dorsal scales	6-19 rows of dorsal scales	6-19 rows of dorsal scales	6-19 rows of dorsal scales	6-19 rows of dorsal scales	6-19 rows of dorsal scales