

## Correspondence

A new record of *Elaphe dione* from high altitude in Western Sichuan reveals high intraspecific differentiationSYLVIA HOFMANN<sup>1</sup>, PETER FRITZSCHE<sup>2</sup> & GEORG MIEHE<sup>3</sup><sup>1</sup>Helmholtz Centre for Environmental Research, 04318 Leipzig, Germany<sup>2</sup>Martin-Luther University, Institute of Zoology, 06118 Halle/Saale, Germany<sup>3</sup>Philipps-Universität Marburg, Faculty of Geography, 35032 Marburg, Germany

Corresponding author: SYLVIA HOFMANN, e-mail: s.hofmann@zoologie.uni-halle.de

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The genus *Elaphe* is represented by the following eleven taxa in Eurasia (SCHULZ 2013): *E. anomala* (BOULENGER, 1916), *E. bimaculata* SCHMIDT, 1925, *E. carinata* (GÜNTHER, 1864), *E. climacophora* (BOIE, 1826), *E. davidi* (SAUVAGE, 1884), *E. dione* (PALLAS, 1773), *E. quadrivirgata* (BOIE, 1826), *E. quatuorlineata* (LACÉPÈDE, 1789), *E. sauromates* (PALLAS, 1811), *E. schrenckii* (STRAUCH, 1873), and *E. zoigeensis* HUANG, DING, BURBRINK, YANG, HUANG, LING, CHEN & ZHANG, 2012.

While most *Elaphe* species in China can be easily distinguished from each other, the Dione's ratsnake, *E. dione*, can be confused with the twin-spotted ratsnake, *E. bimaculata*, because they closely resemble each other in their coloration and scalation. However, *E. dione* and *E. bimaculata* are parametric and treated as valid species due to differences in colour pattern (SCHULZ 1996) and genetic distances based on 12S and CO1 gene sequences (UTIGER et al. 2002).

Dione's ratsnake is the most widespread snake of the genus *Elaphe*, occurring in Afghanistan, Armenia, Azerbaijan, China, Georgia, Iran, Kazakhstan, the Kyrgyz Republic, Mongolia, North and South Korea, Russia, Tadjikistan, Turkmenistan, Turkey, Ukraine, and Uzbekistan (SCHULZ 2013). Even though *E. dione* has adapted to steppe habitats, it has a broad ecological range that permits it to inhabit a variety of habitats (SCHULZ 1996). In China, the species has been recorded from the following provinces and municipalities: Anhui, Beijing, Gansu, Hebei, Heilongjiang, Henan, Hubei, Jiangsu, Jilin, Liaoning, Nei Mongol, Ningxia, Qinghai, Shaanxi, Shandong, Shanxi, Tianjin, and Xinjiang (LU et al. 1999, MACEY et al. 1988, SCHULZ 1996, WALLACH et al. 2014). Reports from southern China, e.g., from Kunshan and Shanghai, might be based on misidentifications of *E. bimaculata* (SCHULZ 1996). The species has been also

reported from Sichuan, e.g., from the Tangjiahe National Nature Reserve (CHEN et al. 1999), located at the north-eastern edge of Sichuan, from the Yalong and Dadu River (SCHULZ 1996), both flowing through the western part of Sichuan and from the Yele Nature Reserve at the south-western edge of the Sichuan Basin (HU 2005) (Fig. 1).

Here we report a new record of Dione's ratsnake from a high-altitude area of Garzê Tibetan Autonomous Prefecture, Sichuan, and provide genetic data on species identity. We found two adult snakes and a complete exuvia at 3,520 m a.s.l. (31°41' N 99°44' E, WGS84; Fig. 1A) in the north of the Chola Shan, near the Yalong River Valley on 7 June 2014. The snakes were basking in the afternoon between stones in a ruderal, vegetated area close to a small Tibetan village (Fig. 1B). No voucher specimens were collected. The dry exuvia was preserved in a standard 50 ml plastic centrifuge tube.

Basic measurements of length and weight were recorded in the field using a measuring tape and a spring balance to the nearest 1 cm and 1 g, respectively. Abbreviations are as follows: SVL (snout–vent length, measured from the tip of the snout to the anterior margin of the cloaca); TaL (Tail length, measured from the posterior tip of the anal plate to the posterior terminus of the tail button); and TL (total length, SVL+TaL). In addition, several scale counts were recorded following SCHULZ (1996): VEN (ventrals); SC (subcaudals); DSR (number of dorsal scale rows [1/2/3], counted at the level of one head length behind the head, at midbody [i.e., at the level of the ventral plate corresponding to half the total number of ventrals], and at one head length before the vent); IL (infralabials); SL (supralabials); P (postoculars). Bilateral scale counts were given as left/right. The two snakes were also buccal-swabbed using a PG-100 Performagene™

kit in order to validate the species taxonomic identity by means of DNA-barcoding. DNA samples were extracted from the swabs with the PG-AC4 Performagene™ reagent package according to the manufacturer's instructions. For DNA extraction from the skin sample, we used a peqGOLD MicroSpin Tissue DNA Kit (Peqlab Biotechnologie GmbH, Germany). DNA quantity and quality was assessed using the Epoch Microplate Spectrophotometer (BioTek, USA). DNA concentration in the buccal extracts was 23.93 and 38.28 ng/μl, respectively, and 7.63 ng/μl in the skin extract. These values are within the lower range of DNA concentrations usually obtained from tissue samples, but similar or even higher than concentrations obtained using other non-invasive approaches in vertebrates (e.g., faeces or hairs; BALL et al. 2007, MORIN et al. 2001). The three DNA samples have been deposited in the Natural History Museum Erfurt (SH1406T0, SH1406T1, SH1406T2).

We obtained partial sequences of the mitochondrial 12S rRNA subunit, nadh dehydrogenase subunit 4 (nd4), cytochrome b (cyt-b), and cytochrome oxidase subunit 1 (co1) genes and the nuclear oocyte maturation factor Mos c-mos from the three samples. We used the primers

L1091 and H1478 (KOCHER et al. 1989) to amplify the 12S region, LEU and nd4 (AREVALO et al. 1994, FORSTNER et al. 1995) for the nd4 fragment, L14724 (IRWIN et al. 1991) and H15547b (KELLY et al. 2003) for the cyt-b fragment, co1deg1 and co1bdeg (UTIGER et al. 2002) for co1, and S77 and S78 (LAWSON et al. 2005) for the c-mos region, respectively. Amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced in both directions with the same amplification primers by Eurofins-MWG-Operon (Ebersberg, Germany). Sequences of eight (nd4, cyt-b, c-mos) or eleven (12S, co1) *Elaphe* species were derived from GenBank (Supplementary table 1) and aligned with sequences from our samples using BioEdit 7.09.0 (HALL 1999). Protein-coding gene fragments (nd4, cyt-b, co1, c-mos) were translated into amino acids and no stop codons were observed. No heterozygous position was present in the c-mos sequences. To assess the evolutionary distance between these species and our samples we calculated uncorrected p-distances among haplotypes of the four partial genes in Mega 6.06 (TAMURA et al. 2013), using the pairwise deletion option and considering both transitions and transversions. New sequences generated

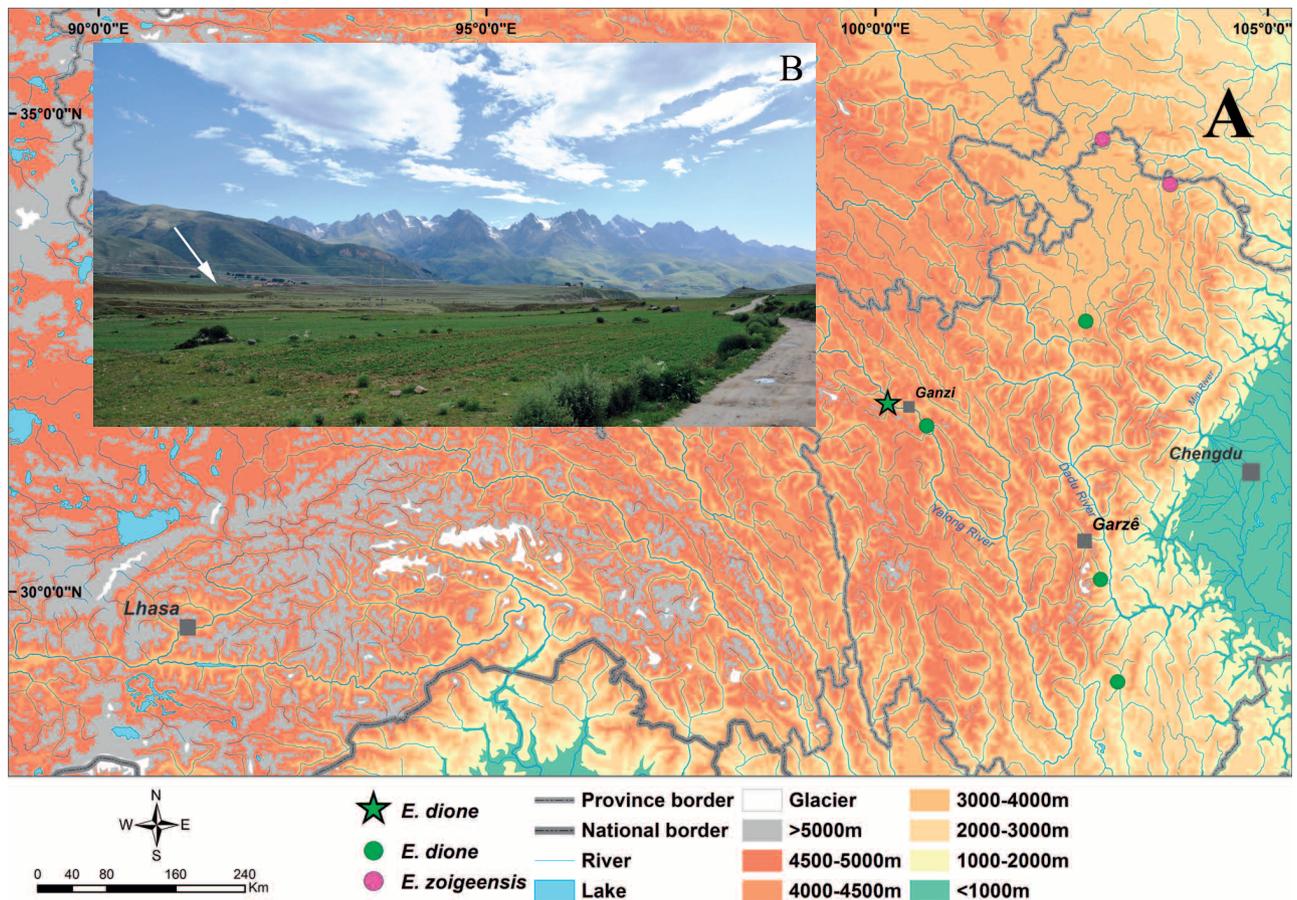


Figure 1. Sampling site and habitat of *Elaphe dione* in Garzê Prefecture, Sichuan. A) The record site of *E. dione* north of the Chola Shan is indicated by a star (map after MIEHE et al. 2001). Green dots represent reports of *E. dione* according to SCHULZ (1996) and HU (2005). The type locality and another record of *E. zoigeensis* have been also included (HUANG et al. 2012, SCHULZ 2013). B) The white arrow points to the location where we found specimens of *E. dione*. Photo by S. HOFMANN.

Table 1. Measurements and scale counts of *E. dione* from Sichuan.

	Adult individual (sample SH1406T1)	Adult individual (sample SH1406T2)	Exuvia (sample SH1406T0)
Weight (g)	184.0	159.0	–
SVL (mm)	740.0	680.0	850.0
TaL (mm)	140.0	140.0	200.0
TL (mm)	880.0	820.0	1050.0
VEN	not counted	not counted	188
SC	58	58	62
DSR	not counted	not counted	25-22-19
IL	10/10	11/10	not counted
SL (eye contact)	8/8 (2/2)	9/8 (2/2)	8/8 (2/2)
P	3/3	2/2	2/3

in this study were deposited in GenBank (Supplementary table 1, accession codes KP091848, KP091851, KP091850, KP091853, KP115289, KP115290).

Morphological characteristics and measurements are summarized in Table 1. These data, accompanied with features of coloration and pattern (Figs. 2A–E), fit in with those reported for *E. dione* (SCHULZ 1996). Since detailed descriptions of *E. dione* have been published frequently in the past, we omit the descriptions of the colouration and body/headshapes of the two individuals.

Uncorrected p-distances ranged from ~4.1–11.5% for 12s, 10.9–15.5% for nd4, 9.8–13.7% for cyt-b, 8.2–15.0% for co1 (Supplementary tables 2 and 3), and between 0.2% and 1.1% for c-mos (data not shown) between species. Intraspecific divergence ranged from 0.0–2.1% for 12s, 0.0–2.3% for co1, or was lower than 4.0% (nd4) and 3.0% (cyt-b), respectively (Supplementary tables 2 and 3). The close relationship between *E. dione* and *E. bimaculata* is highlighted by the generally low levels of genetic distance between them compared to the p-distances between other *Elaphe* species. However, the extremely low divergences between sequences of *E. bimaculata* that were derived from the complete mitochondrial genome data (GenBank accession codes NC\_024743; YAN et al. 2014) and all other sequences of *E. dione* used in our study suggest that the former are based on sample misidentification and were probably derived from *E. dione* specimens too. There is also striking molecular evidence that *E. anomala* and *E. schrenckii*, formerly classified as subspecies *E. schrenckii anomala* (BOULENGER, 1916), might be conspecific (Supplementary table 2). Moreover, the sequence divergences between the *E. sauromates* specimens are of similar magnitude compared to the interspecific distances between *E. sauromates* and *E. quatuorlineata*, emphasizing the need for further studies of the taxonomic status of this species complex (Supplementary table 2). Our results also support that the recently described *E. zoigeensis* represents a (phylogenetically) distinct species. Although its morphological characters seem very similar to those reported for *E. dione*, the genetic distances between *E. zoigeensis* and all other *Elaphe*

species warrant its species status under an evolutionary species concept (DE QUEIROZ 2007). The low levels of 12s rRNA gene sequence divergence between our sample and the *E. bimaculata* and even the *E. carinata* specimens (Supplementary table 2: no. [2] to [4] and [7], respectively) are difficult to interpret, in particular because these specimens were purchased through the pet trade without locality data. In contrast, with respect to the co1 data, the sequence distances between these specimens and our samples were within the range of interspecific divergence. Interestingly, we observed higher p-distance values between sequences of *E. dione* and our samples for all mt-markers and particularly for co1 (5.1–5.8%; Supplementary table 2) and nd4 (3.7%; Supplementary table 3). Remarkably, the co1-differences between *E. dione* samples from locations as far apart as Korea and Ukraine (Supplementary table 2 no. [12] and [14], respectively) are smaller compared to those observed between our samples and the Ukrainian sample. Overall, however, at least for co1, the p-distance values between *E. dione* and our samples are substantially lower compared

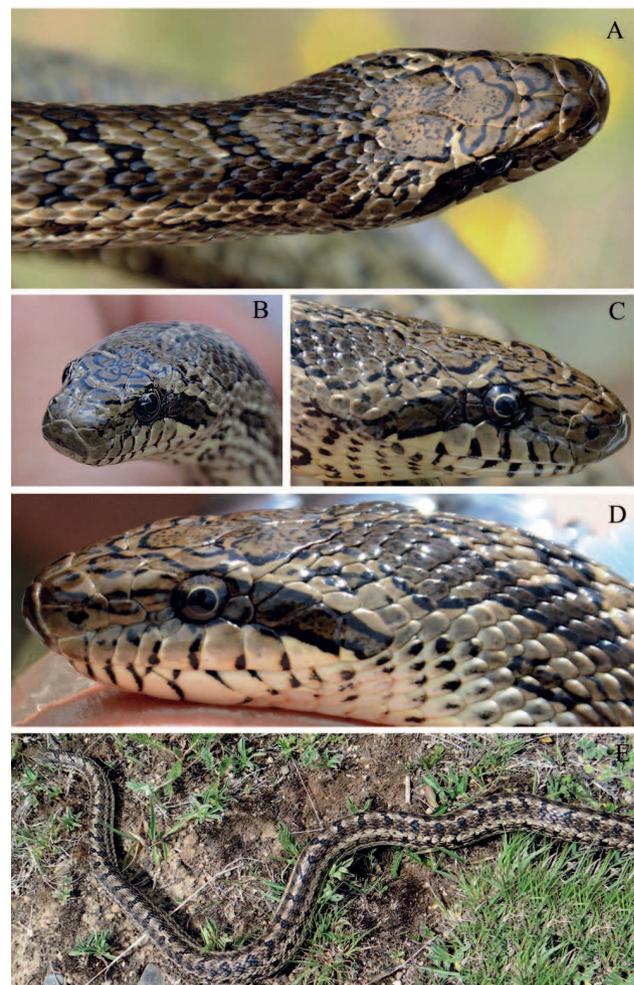


Figure 2. Head of *Elaphe dione*. A) Dorsal; B) frontal; C) right lateral; and D) left lateral views; E) back of the body.

to the mean distances between groups of species (8.2% to 13.8%; excluding the misidentified *E. bimaculata* and grouping *E. anomala* and *E. schrenckii* together; data not shown). Furthermore, we found no evidence of nDNA divergence between our sample and *E. dione* (c-mos, p-distance 0.0%, data not shown).

The area from which our samples originated belongs to the Hengduan Shan region that consists of a series of north–south aligned, highly rugged mountain chains alternating with deep gorges and river valleys, resulting in a complex topography, heterogeneous climate, and ecological niches. During the Quaternary glaciations, the Hengduan Shan formed a mosaic of temporarily isolated refuges, contributing to the currently observed exceptional intra- and interspecific diversity and a high level of endemism across western Sichuan and northeastern Yunnan (GAO et al. 2007, LEI et al. 2007, LÓPEZ-PUJOL et al. 2011, HUANG et al. 2013, WEN et al. 2014, ZHANG et al. 2009). Today, that mountain area is recognized as a unique biodiversity hotspot, emphasising its biogeographical significance (BOUFFORD & VAN DYCK 1999, MYERS et al. 2000, BOUFFORD et al. 2004). It is reasonable to assume that the population from which we drew our samples is somewhat geographically isolated from other populations, which may result in regional molecular differences due to restricted gene flow. Moreover, since mtDNA is haploid, maternally transmitted and, thus, has a smaller effective population size than does nuclear DNA, it is susceptible to stronger genetic drift, resulting in increased genetic divergence. The higher p-distances in mtDNA and the lack of differentiation in nDNA between our samples and *E. dione* are consistent with substantial intraspecific mtDNA sequence divergence due to genetic drift triggered by isolation and a potentially small population size, rather than (cryptic) species- or subspecies-level differentiation.

Our sample site of *E. dione* in Garzê confirms the presence of the species in Sichuan, in the area north of the Chola Shan. There are a few previous reports of the species from areas around that site (reviewed in SCHULZ 1996, Hu 2005), indicating that the species is more common in Western Sichuan than would be expected. Although *E. dione* has been reported to occur at altitudes of up to 3,500 m (BANNIKOV et al. 1977), our observation of the species at 3,520 m might be the first validated record for its occurrence at such high altitudes. The only known species from similar altitudes in Sichuan is *Thermophis zhaoermi*, which might be sympatric with *E. dione* at some locations. Given the ecological plasticity of the species, the presence of *E. dione* in areas farther south (e.g., in mountainous regions in northwestern Yunnan) is conceivable and should be investigated in the future.

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#### References

- AREVALO, E., S. K. DAVIS & J. W. SITES (1994): Mitochondrial-DNA sequence divergence and phylogenetic relationships among 8 chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. – *Systematic Biology*, **43**: 387–418.
- BALL, M. C., R. PITHER, M. MANSEAU, J. CLARK, S. D. PETERSEN, S. KINGSTON, N. MORRILL & P. WILSON (2007): Characterization of target nuclear DNA from faeces reduces technical issues associated with the assumptions of low quality and quantity template. – *Conservation Genetics*, **8**: 577–586.
- BANNIKOV, A. G., I. S. DAREVSKY, V. G. ISHCHENKO, A. K. RUSTAMOV & N. N. SHCHERBAK (1977): Guide to the USSR amphibian and reptile fauna. – Prosveshchenie, Moscow.
- BOUFFORD, D. E., P. P. VAN DIJK & L. ZHI (2004): Mountains of Southwest China. – pp. 159–164 in: MITTERMEIER, R. A., P. ROBLES GIL, M. HOFFMANN, J. PILGRIM, T. BROOKS, C. G. MITTERMEIER, J. LAMOREU & G. A. B. DA FONSECA (eds): Hotspots revisited – Cemex, Mexico City.
- BOUFFORD, D. E. & P. P. VAN DYCK (1999): South-central China – pp. 338–351 in: MITTERMEIER, R. A., N. MYERS & C. G. MITTERMEIER (eds): Hotspots: Earth's biologically richest and most endangered terrestrial ecoregions – Cemex, Mexico City.
- CHEN, L.-M., Z.-F. GAO, W.-F. OU, W.-L. CHEN & W.-H. MA (1999): Surveys of amphibians and reptiles in Tangjiahe Nature Reserve, Sichuan Province. – *Sichuan Journal of Zoology*, **18**: 132–134.
- DE QUEIROZ, K. (2007): Species concepts and species delimitation. – *Systematic Biology*, **56**: 879–886.
- FORSTNER, M. R. J., S. K. DAVIS & E. AREVALO (1995): Support for the hypothesis of anguimorph ancestry for the suborder Serpentes from phylogenetic analysis of mitochondrial DNA sequences. – *Molecular Phylogenetics and Evolution*, **4**: 93–102.
- GAO, L. M., M. MOLLER, X. M. ZHANG, M. L. HOLLINGSWORTH, J. LIU, R. R. MILL, M. GIBBY & D. Z. LI (2007): High variation and strong phylogeographic pattern among cpDNA haplotypes in *Taxus wallichiana* (Taxaceae) in China and North Vietnam. – *Molecular Ecology*, **16**: 4684–4698.
- HALL, T. A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – *Nucleic Acids Symposium Series*, **41**: 95–98.
- HU, J.-C. (2005): A Report of the Comprehensive Survey on Yele Nature Reserve in Sichuan, China. – Sichuan Science and Technical Press, Chengdu.
- HUANG, D.-H., Q.-Q. LI, C.-J. ZHOU, S.-D. ZHOU & X.-J. HE (2013): Intraspecific differentiation of *Allium wallichii* (Amaryllidaceae) inferred from chloroplast DNA and internal transcribed spacer fragments. – *Journal of Systematics and Evolution*, **52**: 341–354.
- HUANG, S., L. DING, F. T. BURBRINK, J. YANG, J. HUANG, C. LING, X. CHEN & Y. ZHANG (2012): A new species of the genus *Elaphe* (Squamata: Colubridae) from Zoige County, Sichuan, China. – *Asian Herpetological Research*, **3**: 38–45.

- IRWIN, D. M., T. D. KOCHER & A. C. WILSON (1991): Evolution of the cytochrome b gene of mammals. – *Journal of Molecular Evolution*, **32**: 128–144.
- KELLY, C. M., N. P. BARKER & M. H. VILLET (2003): Phylogenetics of advanced snakes (Caenophidia) based on four mitochondrial genes. – *Systematic Biology*, **52**: 439–459.
- KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PAABO, F. X. VILLABLANCA & A. C. WILSON (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. – *Proceedings of the National Academy of Sciences USA*, **86**: 6196–6200.
- LAWSON, R., J. B. SLOWINSKI, B. I. CROTHER & F. T. BURBRINK (2005): Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. – *Molecular Phylogenetics and Evolution*, **37**: 581–601.
- LEI, F.-M., G.-A. WEI, H.-F. ZHAO, Z.-H. YIN & J.-L. LU (2007): China subregional avian endemism and biodiversity conservation. – *Biodiversity and Conservation*, **16**: 1119–1130.
- LING, C., S. Y. LIU, S. HUANG, F. T. BURBRINK, P. GUO, Z. Y. SUN & J. ZHAO (2010): Phylogenetic analyses reveal a unique species of *Elaphe* (Serpentes, Colubridae) new to science. – *Asian Herpetological Research*, **1**: 90–96.
- LÓPEZ-PUJOL, J., F.-M. ZHANG, H.-Q. SUN, T.-S. YING & S. GE (2011): Centres of plant endemism in China: places for survival or for speciation? – *Journal of Biogeography*, **38**: 1267–1280.
- LU, Y., WANG X. & LI P. (1999): Diversity of amphibians and reptiles in Shandong Province of China. – *Sichuan Journal of Zoology*, **18**: 128–129.
- MACEY, J. R., T. J. PAPPENFUSS & E. ZHAO (1988): The snakes of Ningxia Hui Autonomous Region as an indication of a herpetofaunal corridor. – *Chinese Herpetological Research*, **2**: 4–5.
- MIEHE, G., M. WINIGER, J. BÖHNER & Y. ZHANG (2001): The climatic diagram map of Highasia. Purpose and concepts. – *Erdkunde*, **55**: 94–97.
- MORIN, P. A., K. E. CHAMBERS, C. BOESCH & L. VIGILANT (2001): Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). – *Molecular Ecology*, **10**: 1835–1844.
- MYERS, N., R. A. MITTERMEIER, C. G. MITTERMEIER, G. A. DA FONSECA & J. KENT (2000): Biodiversity hotspots for conservation priorities. – *Nature*, **403**: 853–858.
- SCHULZ, K.-D. (1996): A monograph of the colubrid snakes of the genus *Elaphe* Fitzinger. – Koeltz Scientific Books, Havlickuv, Czech Republic.
- SCHULZ, K.-D. (2013): An annotated and illustrated checklist of Old World ratsnakes – pp. 17–268 in: SCHULZ, K.-D. (ed.): Old World Ratsnakes. – Bushmaster Publications, Berg, Switzerland.
- TAMURA, K., G. STECHER, D. PETERSON, A. FILIPSKI & S. KUMAR (2013): MEGA6: Molecular evolutionary genetics analysis version 6.0. – *Molecular Biology and Evolution*, **30**: 2725–2729.
- UTIGER, U., N. HELFENBERGER, B. SCHÄTTI, C. SCHMIDT, M. RUF & V. ZISWILER (2002): Molecular systematics and phylogeny of Old and New World ratsnakes, *Elaphe* auct., and related genera (Reptilia, Squamata, Colubridae). – *Russian Journal of Herpetology*, **9**: 105–124.
- WALLACH, V., K. L. WILLIAMS & J. BOUNDY (2014): Snakes of the world. – CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA.
- WEN, J., J.-Q. ZHANG, Z.-L. NIE, Y. ZHONG & H. SUN (2014): Evolutionary diversifications of plants on the Qinghai-Tibetan Plateau. – *Frontiers in Genetics*, **5**: 4.
- YAN, L., Z. Z. GENG, P. YAN & X. B. WU (2014): The complete mitochondrial genome of *Elaphe bimaculata* (Reptilia, Serpentes, Colubridae). – *Mitochondrial DNA*, **4**: 1–2.
- ZHANG, D.-C., Y.-H. ZHANG, D. E. BOUFFORD & H. SUN (2009): Elevational patterns of species richness and endemism for some important taxa in the Hengduan Mountains, southwestern China. – *Biodiversity and Conservation*, **18**: 699–716.

### Supplementary material

Additional information is available in the online version of this article at <http://www.salamandra-journal.com>

Supplementary table 1. Sequences for the genus *Elaphe* retrieved from GenBank and used in the present study.

Supplementary table 2. Uncorrected p-distances of partial 12s and co1 genes from 11 *Elaphe* species.

Supplementary table 3. Uncorrected p-distances of the partial cyt-b gene and partial nd4 gene from 8 *Elaphe* species.

**Supplementary table 1.** Sequences for the genus *Elaphe* retrieved from GenBank and used in the present study. \* Tissue collection of N. HELFENBERGER (UTIGER et al. 2002); \*\* YAN et al. (2014); \* The three samples yielded identical sequences for the respective partial genes except for co1 (variation for a single base pair at position 317 that could be A or G).

Taxon	Voucher/ isolate No.	Origin	GenBank accession codes				
			12S	nd4	cyt-b	co1	c-mos
<i>Elaphe anomala</i>	(SH577*)	China	AY122803			AY122719	
<i>E. bimaculata</i>	(SH1164*)	(pet trade)	AY122767			AY122683	
<i>E. bimaculata</i>	(SH1420*)	(pet trade)	AY122768			AY122684	
<i>E. bimaculata</i>	(SH1438*)	(pet trade)	AY122837			AY122753	
<i>E. bimaculata</i>	No voucher	unknown		DQ902283	DQ902104		DQ902062
<i>E. bimaculata</i>	(complete genome)**	unknown	NC_024743	NC_024743	NC_024743	NC_024743	
<i>E. carinata</i>	(SH1177*)	(pet trade)	AY122838			AY122754	
<i>E. carinata</i>	(SH575*)	(pet trade)	AY122839			AY122755	
<i>E. carinata</i>	LSUMZ 37012	China		DQ902284	DQ902133		DQ902063
<i>E. climacophora</i>	(SH528*)	Japan	AY122770			AY122686	
<i>E. climacophora</i>	CAS 163993	Japan		DQ902285	DQ902105		DQ902064
<i>E. davidi</i>	(SH576*)	China	AY122775			AY122691	
<i>E. dione</i>	LSUMZ 45799	Russia		DQ902287	DQ902107		DQ902066
<i>E. dione</i>	(SH2166*)	Kazakhstan	AY122832			AY122748	
<i>E. dione</i>	(SH1389*)	Kazakhstan	AY122834			AY122750	
<i>E. dione</i>	(SH1152*)	Korea	AY122829			AY122745	
<i>E. dione</i>	(SH1155*)	Russia	AY122830			AY122746	
<i>E. dione</i>	(SH545*)	Ukraine	AY122831			AY122747	
<i>E. dione</i>	(SH952*)	China	AY122833			AY122749	
<i>E. dione</i> (3 samples)#	No voucher, SH1406T0	this study	KP091848	KP091851	KP091850	KP115289	KP091853
<i>E. dione</i>	No voucher, SH1406T2	this study				KP115290	
<i>E. quadrivirgata</i>	No voucher	Japan		DQ902300	DQ902120		DQ902078
<i>E. quadrivirgata</i>	(SH551*)	Honshu, Japan	AY122793			AY122709	
<i>E. quadrivirgata</i>	(SH470*)	Honshu, Japan	AY122794			AY122710	
<i>E. quatuorlineata</i>	LSUMZ 40626	Turkey		AY487067	AY486931		AY486955
<i>E. quatuorlineata</i>	(SH1112*)	Italy	AY122796			AY122712	
<i>E. quatuorlineata</i>	(SH975*)	unknown	AY122798			AY122714	
<i>E. sauromates</i>	(SH972*)	Turkey	AY122795			AY122711	
<i>E. sauromates</i>	(SH556*)	Turkey	AY122797			AY122713	
<i>E. schrenkii</i>	No voucher	unknown		DQ902302	DQ902124		DQ902082
<i>E. schrenkii</i>	(SH857*)	(pet trade)	AY122804			AY122720	
<i>E. zoigeensis</i>	isolate ZJ200801-02	China	HQ330522	HQ330526	HQ330524		
<i>E. zoigeensis</i>	isolate ZJ200803	China	HQ330523	HQ330527	HQ330525		
<i>E. zoigeensis</i>	HS2010014	China				JF510474	
<i>E. zoigeensis</i>	HS2010016	China				JF510475	

**Supplementary table 2.** Uncorrected p-distances of partial 12S (346 bp; lower left) and co1 genes (513 bp; upper right) for 25 sequences from 11 *Elaphie* species; species names are followed by GenBank accession codes. Sequence divergences between our samples and *E. dione* are highlighted in bold. Conspecific distances are shadowed in grey. \* The small distances between one of the sequences of *E. bimaculata* [5] (YAN et al. 2014) and *E. dione* [10]–[15] (JEONG et al. 2013, URTIGER et al. 2002) suggest that the sequences were derived from conspecific individuals. Also, the distances of 0.3% (12S) and 0.0% (co1) between *E. anomala* [1] and *E. schrenckii* [23] suggest they are conspecific (HUANG et al. 2012, LING et al. 2010, URTIGER et al. 2002). \*\* *E. saurimates* was previously known as *E. quatuorlineata saurimates* (PALLAS 1814). Distances of 3.8% (12S) and 7.6% (co1) between the *E. saurimates* individuals [21] vs [22] and the interspecific distances of similar magnitudes between *E. saurimates* and *E. quatuorlineata* [19]/[20] emphasize the need for further studies of this species (complex) taxonomic status. # High distance values between *E. dione* [10–15] and our samples [16] indicate genetic differentiation due to genetic drift over the reasonably large spatial scale rather than different (cryptic) species.

	[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]
[1] <i>E. anomala</i> AY122803/AY122719	12.9	13.1	12.5	11.5	10.3	10.7	12.5	12.1	11.5	11.5	11.9	11.3	11.5	11.5	12.7	8.2	8.2	11.7	12.5	11.9	12.3	0.0*	12.7	12.7	
[2] <i>E. bimaculata</i> AY122767/AY122683	5.0	1.4	0.4	9.2	12.3	12.5	13.5	11.9	9.6	9.6	9.4	9.0	9.2	9.4	8.8	10.9	10.9	12.9	13.1	13.5	13.8	12.9	13.5	13.5	
[3] <i>E. bimaculata</i> AY122768/AY122684	5.3	0.3	1.8	8.8	12.5	12.3	13.1	10.7	9.6	9.6	9.4	8.6	8.8	8.6	8.8	11.5	11.5	13.1	12.9	13.5	14.0	13.1	13.5	13.5	
[4] <i>E. bimaculata</i> AY122837/AY122753	5.3	0.3	0.6	9.2	12.7	12.9	13.8	12.1	9.6	9.6	9.7	9.0	9.2	9.4	9.2	11.3	11.3	12.5	13.1	13.1	13.8	12.5	13.5	13.5	
[5] <i>E. bimaculata</i> NC024743	4.7	5.0	5.3	5.3	10.5	11.1	10.9	10.5	1.0*	1.0*	1.6*	0.2*	0.4*	1.2*	4.9	10.5	10.5	12.3	12.3	11.9	10.9	11.5	13.1	13.1	
[6] <i>E. carinata</i> AY122838/AY122754	4.1	5.3	5.0	5.6	5.3	2.5	11.9	11.9	11.3	11.3	10.3	10.5	11.7	11.3	8.6	8.6	11.9	11.9	12.9	11.1	10.3	14.4	14.0	14.0	
[7] <i>E. carinata</i> AY122839/AY122755	4.1	4.7	4.4	5.0	4.7	0.6	12.1	11.5	11.9	11.9	10.9	11.1	12.3	10.7	8.6	8.6	12.1	12.1	13.5	11.3	10.7	13.5	13.1	13.1	
[8] <i>E. climacophora</i> AY122770/AY122686	5.9	6.8	6.5	6.8	6.5	5.9	5.3	11.3	11.5	11.5	11.3	10.7	10.9	11.3	11.3	11.9	11.9	15.0	15.0	12.9	12.5	12.5	14.0	13.6	
[9] <i>E. davidi</i> AY122775/AY122691	5.9	7.6	7.4	7.9	6.7	5.0	4.4	5.6	10.9	10.9	11.1	10.3	10.5	10.3	11.3	9.9	9.9	13.3	13.1	13.1	13.1	12.1	13.3	12.9	
[10] <i>E. dione</i> AY122832/AY122748	4.7	4.4	4.7	4.7	1.5*	4.7	4.7	6.5	7.3	0.0	1.9	1.2	1.4	2.1	5.8*	10.7	10.7	12.9	12.9	11.9	10.7	11.5	12.9	12.9	
[11] <i>E. dione</i> AY122834/AY122750	4.7	4.4	4.7	4.7	1.5*	4.7	4.7	6.5	7.3	0.0	1.9	1.2	1.4	2.1	5.8*	10.7	10.7	12.9	12.9	11.9	10.7	11.5	12.9	12.9	
[12] <i>E. dione</i> AY122829/AY122745	6.2	5.0	5.3	5.3	1.5*	5.3	4.7	7.3	7.0	1.8	1.8	1.8	1.9	2.3	5.3*	10.5	10.5	12.7	13.1	12.3	11.7	11.9	13.5	13.5	
[13] <i>E. dione</i> AY122830/AY122746	4.7	5.0	5.3	5.3	0.0*	5.3	4.7	6.5	6.7	1.5	1.5	1.5	0.2	1.4	5.1*	10.3	10.3	12.5	12.5	11.7	11.1	11.3	12.9	12.9	
[14] <i>E. dione</i> AY122831/AY122747	4.4	5.6	5.9	5.9	0.6*	5.9	5.3	6.5	7.0	2.1	2.1	2.1	0.6	1.6	5.3*	10.5	10.5	12.7	12.7	11.9	11.3	11.5	13.1	13.1	
[15] <i>E. dione</i> AY122833/AY122749	5.3	4.4	4.7	4.7	0.6*	5.3	4.7	6.5	7.3	0.9	0.9	0.9	0.6	0.0	5.3*	10.9	10.9	12.3	12.3	12.3	11.3	11.5	13.1	13.1	
[16] <i>E. dione</i> (this study) KP091848/KP115289	5.3	3.5	3.2	3.8	2.6	4.4	3.8	5.3	6.5	1.8	1.8	2.3	2.6*	3.2*	2.1	9.6	9.6	11.3	11.7	10.9	10.3	12.7	13.5	13.5	
[17] <i>E. quadrivirg.</i> AY122793/AY122709	5.0	6.2	6.5	6.2	7.0	6.2	5.6	5.9	6.5	7.3	7.3	7.9	7.0	6.7	7.3	6.5	0.0	11.5	11.5	12.1	10.9	8.2	11.5	11.5	
[18] <i>E. quadrivirg.</i> AY122794/AY122710	5.0	6.2	6.5	6.2	7.0	6.2	5.6	5.9	6.5	7.3	7.3	7.9	7.0	6.7	7.3	6.5	0.0	11.5	11.5	12.1	10.9	8.2	11.5	11.5	
[19] <i>E. quatuorlin.</i> AY122796/AY122712	5.9	6.5	6.8	6.2	7.3	7.3	6.7	7.3	9.1	7.3	7.3	7.9	7.3	7.0	7.3	7.9	7.9	0.8	8.4	8.8	11.7	13.5	13.5	13.5	
[20] <i>E. quatuorlin.</i> AY122798/AY122714	5.6	6.2	6.5	5.9	7.0	7.0	6.5	7.0	8.8	7.0	7.0	7.6	7.0	6.7	6.7	7.0	7.6	0.3	9.2	8.8	12.5	13.5	13.5	13.5	
[21] <i>E. saurimates</i> AY122795/AY122711	5.6	7.1	7.4	6.8	7.3	8.5	7.9	7.9	9.1	7.9	7.9	8.5	7.3	7.0	7.6	7.9	7.9	2.9	3.2	7.6**	11.9	13.1	13.1	13.1	
[22] <i>E. saurimates</i> AY122797/AY122713	6.8	8.5	8.8	8.2	9.4	10.0	9.4	9.4	9.7	10.0	10.0	10.6	9.4	9.1	9.7	10.0	9.4	9.4	3.8	4.1	3.8**	12.3	13.1	13.5	
[23] <i>E. schrenckii</i> AY122804/AY122720	0.3*	5.3	5.6	5.6	5.0	4.4	4.4	5.6	5.6	5.0	5.0	6.5	5.0	4.7	5.6	5.6	4.7	4.7	5.6	5.3	6.5	6.5	12.7	12.7	
[24] <i>E. zoigeensis</i> HQ330522/JF510474	7.7	8.0	8.0	7.7	9.1	7.9	8.2	9.1	10.9	8.5	8.5	9.4	9.1	9.4	9.1	7.6	9.1	9.1	9.7	9.4	10.0	11.5	8.0	0.4	
[25] <i>E. zoigeensis</i> HQ330523/JF510475	6.8	7.1	7.1	6.8	8.2	7.1	7.4	8.2	10.0	7.6	7.6	8.5	8.2	8.5	8.2	6.8	8.2	8.2	8.8	8.5	9.1	10.6	7.1	0.9	

**Supplementary table 3.** Uncorrected p-distances of the partial cyt-b gene (779 bp; lower left) and partial nd4 gene (682 bp; upper right) for 11 sequences from 8 *Elaphe* species; species name and GenBank accession code. The p-distance between our samples and *E. dione* is highlighted in bold. Conspecific distances are shadowed in grey. \*[2] might be conspecific with *E. dione* [5] and our samples [6] or may indicate subspecies level; see also the annotations to Supplementary table 2. \*\* Higher distance values between *E. dione* [5] and our samples [6] indicate, again, differentiation due to genetic drift within the species' range.

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]
[1] <i>E. bimaculata</i> DQ902104/DQ902283		10.9	12.5	12.9	11.1	11.4	13.6	12.2	14.1	13.5	13.6
[2] <i>E. bimaculata</i> NC_024743	10.0		14.5	13.3	1.2*	3.4*	13.2	13.7	14.8	14.2	14.4
[3] <i>E. carinata</i> DQ902133/DQ902284	12.0	11.3		11.9	14.2	13.9	11.0	12.5	12.3	12.6	12.8
[4] <i>E. climacophora</i> DQ902105/DQ902285	11.3	11.4	10.7		13.0	14.1	12.2	12.5	12.9	13.6	13.8
[5] <i>E. dione</i> DQ902107/DQ902287	10.2	0.4*	11.4	11.6		3.7**	12.9	13.4	14.8	13.6	13.8
[6] <i>E. dione</i> (this study) KP091850/KP091851	9.8	2.6*	12.1	11.9	2.4		13.2	13.1	15.5	15.0	15.1
[7] <i>E. quadrivirgata</i> DQ902120/DQ902300	12.7	11.3	11.2	10.8	11.4	11.8		13.5	12.8	12.8	12.9
[8] <i>E. quatuorlineata</i> AY486931/AY487067	11.8	10.7	12.5	10.8	10.8	11.7	12.3		14.0	12.8	13.0
[9] <i>E. schrenckii</i> DQ902124/DQ902302	11.8	11.7	13.0	11.3	11.8	11.4	11.3	11.7		13.6	13.8
[10] <i>E. zoigeensis</i> HQ330524/HQ330526	12.5	10.9	13.7	11.6	11.0	11.4	12.8	11.3	12.3		0.1
[11] <i>E. zoigeensis</i> HQ330525/HQ330527	12.5	10.9	13.7	11.6	11.0	11.4	12.8	11.3	12.3	0.0	