Acta Herpetologica 13(2): 185-189, 2018
DOI: 10.13128/Acta_Herpetol-23394

The mitogenome of *Elaphe bimaculata* (Reptilia: Colubridae) has never been published: a case with the complete mitochondrial genome of *E. dione*

Evgeniy Simonov[1,2,*], Artem Lisachov[3], Natalia Oreshkova[1,4], Konstantin V. Krutovsky[1,5,6,7]

[1] *Laboratory of Forest Genomics, Genome Research and Education Center, Siberian Federal University, Krasnoyarsk 660036, Russia. *Corresponding author.* E-mail: ev.simonov@gmail.com
[2] *Laboratory of Biodiversity Monitoring, Tomsk State University, Tomsk 634050, Russia*
[3] *Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk 630090, Russia*
[4] *Laboratory of Forest Genetics and Selection, V.N. Sukachev Institute of Forest, Siberian Branch of Russian Academy of Sciences, Krasnoyarsk 660036, Russia*
[5] *Department of Forest Genetics and Forest Tree Breeding, Georg-August University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany*
[6] *Laboratory of Population Genetics, Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow 119991, Russia*
[7] *Department of Ecosystem Science and Management, Texas A&M University, College Station, TX 77843-2138, USA*

Submitted on: 2018, 14th June; revised: on 2018, 3rd August; accepted on: 2018, 3rd August
Editor: Uwe Fritz

Abstract. The steppes ratsnake, *Elaphe dione* (Pallas, 1773), is widely distributed across Eurasia, but the systematics and phylogeography of this species remain poorly studied. Sequencing of the full mitochondrial genome of this species provides a reference for its further study. Here, we report the full mitochondrial genome of an *E. dione* specimen from Krasnoyarsk Krai (East Siberia, Russia). We found that it is highly similar to the previously reported mitochondrial genome of the sister species, *E. bimaculata*. Both species misidentification by the authors of *E. bimaculata* mitogenome and the introgressive hybridization between these taxa can possibly explain this observation.

Keywords. Colubridae, *Elaphe*, mitogenome, phylogeny, Siberia.

Ratsnakes of the genus *Elaphe* make up a widely distributed colubrid group of 15 species (The Reptile Database: Uetz et al., 2018), which inhabits a range from Western Europe to the Russian Far East and China. Some closely related genera (often also referred to as "ratsnakes" or *Elaphe* sensu lato) such as *Pantherophis*, *Zamenis*, *Gonyosoma,* etc. inhabit zones with temperate, subtropical and tropical climate almost all over Eurasia and North America. Relatively few mitochondrial genomes of ratsnakes have been sequenced so far, excluding *E. anomala* (Liu and Zhao, 2015a), *E. bimaculata* (Yan et al., 2014), *E. carinata* (Ding et al., 2016), *E. davidi* (Xu et al., 2015), and *E. schrenckii* (Liu and Zhao, 2015b).

The steppes ratsnake, *Elaphe dione* (Pallas, 1773), is the most widespread species of the genus. It is present from Ukraine in the west to the shores of the Pacific Ocean in the east, and from the 56th degree of latitude in Russia in the north to Iran in the south (Schulz, 2013). Type locality of the species is "Gratscheffskoi outpost, near Semijarsk, upper Irtysh area, Semipalatinsk district", Kazakhstan [currently Grachi village, Beskaragay district of East Kazakhstan Region] (restricted by Mertens and Mueller, 1928). The systematics of this species remains controversial: so far, several subspecies have been described (such as *E. d. tenebrosa* Sobolevsky, 1929 and *E. d. czerskii* Vedmederya et al., 2009), but none of them

ISSN 1827-9635 (print)
ISSN 1827-9643 (online)

have been widely accepted. While the mitogenome of the steppes ratsnake has never been sequenced, it would provide an important resource for further studies in systematics and phylogeography of this widespread species. Therefore, we sequenced and annotated the complete mitochondrial genome of *E. dione* specimen and reconstructed the mitogenome phylogeny with other related species of the genus.

DNA was sampled via non-lethal buccal swabs from *E. dione* collected in Krasnoyarsk Krai, Russia (53.59°N 91.64°E) in June 2016, and extracted using standard proteinase K and phenol-chloroform methods (Sambrook et al., 1989). DNA quality and concentration were examined by electrophoresis in 1.5% agarose gel and Qubit fluorimeter, respectively (Thermo Fisher Scientific, USA). The DNA was fragmented using an ultrasonic Bioruptor Sonication System (Diagenode), and paired-end libraries were prepared using the TruSeq DNA LT Sample Prep Kit (Illumina) according to the TruSeq DNA Sample Preparation Guide. The quality control of the prepared library was carried out on the electrophoretic system Bioanalyzer 2100 (Agilent Technologies) using Agilent DNA 1000 Reagents (Agilent Technologies). The fragment size was approximately 400 bp (with insert size 260-280 bp). The library was sequenced on the MiSeq Illumina platform using the MiSeq Reagent Kit v3 (300-cycle, 2x150 bp) Illumina kit at the Laboratory of Forest Genomics, Siberian Federal University.

Read quality was assessed with FastQC 0.11.7 (Andrews, 2010). Adapter and quality trimming was performed using CLC Genomics Workbench (CLC bio, Aarhus, Denmark). To assemble the mitochondrial genome, reads were mapped to previously published mitogenomes of congeneric species: *E. bimaculata* (KM065513.1) and *E. schrenckii* (KP888955.1). Successfully mapped reads were merged into single consensus sequence representing mtDNA of *E. dione*. All aforementioned steps were also done with CLC Genomics Workbench.

The *E. dione* mitochondrial genome was annotated in the MITOS2 web server (http://mitos2.bioinf.uni-leipzig.de/index.py), manually checked and corrected for errors. Mitochondrial genomes of *E. anomala* (KP900218.1), *E. bimaculata* (KM065513.1), *E. carinata* (KU180459.1), *E. davidi* (KM401547.1), and *E. schrenckii* (KP888955.1) were obtained from GenBank to examine phylogenetic relationships between *E. dione* and related taxa basing on complete mtDNA sequences. Some members of closely related genera were used as outgroup: *Oocatochus rufodorsatus* (KC990020.1), *Orthriophis taeniurus* (KC990021.1), *Oreocryptophis porphyraceus* (GQ181130.1), *Pantherophis slowinskii* (DQ523162.1), and *Pituophis catenifer* (KU833245.1). A multiple sequence alignment was produced by Clustal Omega (Sievers et al., 2011) and trimmed with Gblocks (Talavera and Castresana, 2007); 95% (16,631) of the original 17,330 bp alignment remained after trimming. Maximum likelihood (ML) phylogenetic tree was inferred with IQ-TREE 1.6.1 (Nguyen et al., 2015) using the TIM2+F+I+G4 substitution model (selected within 286 tested models by ModelFinder; Kalyaanamoorthy et al., 2017) and 1,000 ultrafast bootstrap replicates (Hoang et al., 2018). The uncorrected genetic distances between species were calculated in MEGA7 (Kumar et al., 2016) with pairwise deletion of gaps/missing data.

In total, 2,132,080 Illumina paired-end reads were generated. We successfully retrieved 16,994 bp of sequence data of the *E. dione* mitochondrial genome with an average coverage of 15x (0.09% of all reads were mapped to mtDNA). No differences were found between the mtDNA sequences generated by mapping to *E. bimaculata* or *E. schrenckii* reference mitogenomes. The very small portions of the ND5 gene and the second D-loop region were not covered by the obtained reads. We estimated the length of the non-covered region was 178 bp. Thus, the full length of the *E. dione* mitochondrial DNA was around 17,172 bp with only ~1% not covered. The newly generated mitogenome is available under NCBI GenBank accession number MH460961.

The phylogenetic tree based on full mitochondrial genomes agreed with previous studies, placing members of genus *Elaphe* into a distinct monophyletic group (Utiger et al., 2002; Chen et al., 2010). The uncorrected genetic distance (*p*-distance) between the mitogenome of *E. dione* and the previously published mitogenome of *E. bimaculata* (KM065513.1) was 0.89% (for 16,989 aligned sites), while the mean distance between other *Elaphe* species is 10.1% (Table 1). Thus, the mitogenome of *E. dione* from Krasnoyarsk Krai was highly similar to the recently sequenced genome of its sister species *E. bimaculata*. The observed distance between the two genomes was too low even for closely related species and is rather at the intraspecific level.

The same result was reported by Hofmann et al. (2016), when they compared 12S, ND4, Cyt b, and COI sequences with this *E. bimaculata* mitogenome. However, the pronounced genetic difference between the two considered species has been shown previously (Utiger et al., 2002) and confirmed by Hofmann et al. (2016). To further clarify this situation, we extracted partial sequences of the 12S rRNA mitochondrial gene from mitogenomes of both species and compared them to the 12S sequences of *E. bimaculata* and *E. dione* available in GenBank. On the 12S gene tree (Fig. 1B), some *E. bimaculata* sequenc-

Table 1. Uncorrected genetic distances (%) between mitochondrial genomes of some species of the genera *Elaphe*, *Orthriophus*, *Oocatochus*, *Pituophis*, *Pantherophis*, and *Oreocryptophis*.

Species	1	2	3	4	5	6	7	8	9	10
1. Elaphe dione	-									
2. Elaphe bimaculata	**0.9**	-								
3. Elaphe schrenckii	**10.9**	**10.8**	-							
4. Elaphe carinata	**10.7**	**10.7**	**10.6**	-						
5. Elaphe anomala	**10.9**	**10.9**	**0.2**	**10.6**	-					
6. Elaphe davidi	**11.0**	**10.8**	**11.2**	**10.7**	**11.2**	-				
7. Orthriophis taeniurus	13.7	13.6	13.1	13.7	13.1	14.1	-			
8. Oocatochus rufodorsatus	13.7	13.6	13.6	14.1	13.6	14.5	13.6	-		
9. Pituophis catenifer	14.3	14.2	13.8	14.2	13.8	14.9	14.0	13.5	-	
10. Pantherophis slowinskii	14.3	14.3	14.0	14.3	14.0	14.8	14.0	13.4	9.9	-
11. Oreocryptophis porphyraceus	14.5	14.4	14.1	14.7	14.2	15.0	13.7	13.3	14.6	14.3

The distances between species of the genus *Elaphe* are highlighted in bold.

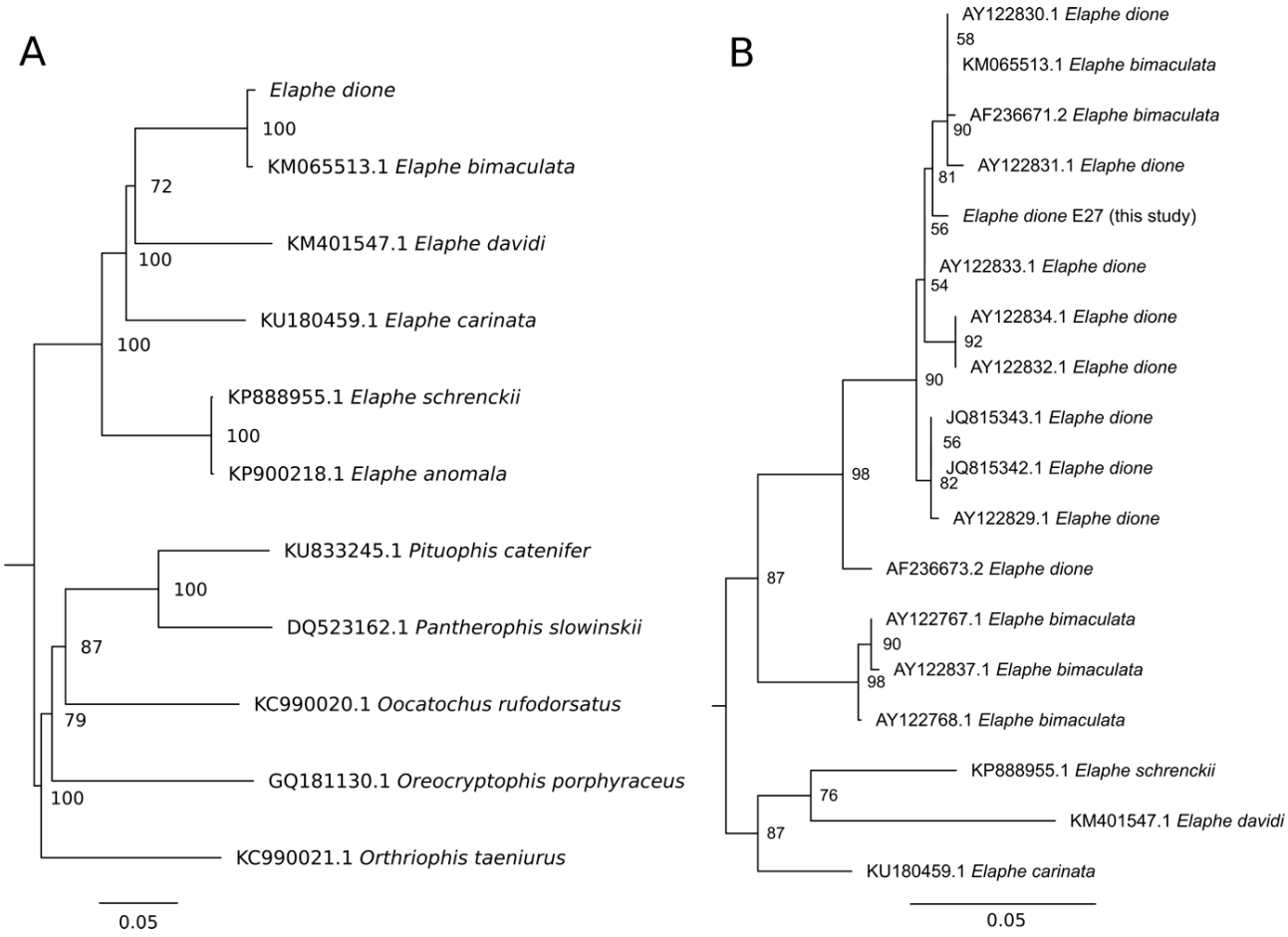

Fig. 1. (A) Maximum likelihood phylogenetic tree of the *Elaphe* sensu lato group based on full mitochondrial genomes; (B) maximum likelihood gene tree of *E. dione* and *E. bimaculata* based on 12S rRNA sequences. Bootstrap values above 50 are indicated.

es form a separate clade, distinct from the *E. dione* lineage. Two other *E. bimaculata* sequences (including the one extracted from the mitogenome) clearly fall into the *E. dione* cluster. The *E. dione* sample used in our work belongs to the *E. dione* clade. It is evident that the mitogenome of "*E. bimaculata*" sequenced by Yan et al. (2016) belongs to *E. dione*. The two species are very similar phenotypically, and their ranges overlap in China (Schulz, 2013; Wallach et al., 2014). Thus, species misidentification by the authors of *E. bimaculata* mitogenome could be a possible explanation. An alternative explanation is introgressive hybridization between the species. This phenomenon is well documented in animals (including reptiles) and results in bidirectional or unidirectional introgression of mtDNA (e.g. Plötner et al., 2008; Machado et al., 2014; Ermakov et al., 2015; Johnson et al., 2015). If hybridization between these two species indeed occurs in the area of their sympatry, an *E. bimaculata* specimen with introgressed mtDNA could be accidentally used for the mitogenome sequencing. Unfortunately, Yan et al. (2016) did not provide any information about geographical origin or other details of the specimen they used for mtDNA sequencing.

By this communication, we would like to not only provide a reliable mitogenome of *E. dione*, but also highlight the need for careful selection and documentation of specimens intended for full mitogenome/genome sequencing to avoid further confusion.

ACKNOWLEDGEMENTS

We thank Lengxob Yong (University of Exeter, UK) for English editing, and two reviewers for their valuable comments on the manuscript. The study is supported by "TSU competitiveness improvement programme" grant 8.1.19.2018. No animals were killed or removed from nature for this work. According to the national guidelines (Russia) no permit for animal handling or collection of non-invasive samples is necessary.

REFERENCES

Andrews, S. (2010): FastQC: a quality control tool for high throughput sequence data. Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc [accessed on 20 March 2018].

Ding, C., Zhou, B., Guo, H., Duan, Y., Wang, Z. (2016): Sequencing and analysis of mitochondrial genome of *Elaphe carinata* (Reptilia, Squamata, Colubridae). Mitochondrial DNA Part B **1**: 41-42.

Ermakov, O.A., Simonov, E., Surin, V.L., Titov, S.V, Brandler, O.V., Ivanova, N.V., Borisenko, A.V. (2015): Implications of hybridization, NUMTs, and overlooked diversity for DNA barcoding of Eurasian ground squirrels. PLOS ONE **10**: e0117201.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S. (2018): UFBoot2: Improving the ultrafast bootstrap approximation. Mol. Biol. Evol. **35**: 518-522.

Hofmann, S., Fritzsche, P., Miehe, G. (2016): A new record of *Elaphe dione* from high altitude in Western Sichuan reveals high intraspecific differentiation. Salamandra **52**: 273-277.

Johnson, B.B., White, T.A., Phillips, C.A., Zamudio, K.R. (2015): Asymmetric introgression in a spotted salamander hybrid zone. J. Hered. **106**: 608-617.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S. (2017): ModelFinder: Fast model selection for accurate phylogenetic estimates. Nat. Methods **14**: 587-589.

Kumar, S., Stecher, G., Tamura, K. (2016): MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. **33**: 1870-1874.

Liu, P., Zhao W.-G. (2015a): Sequencing and analysis of the complete mitochondrial genome of *Elaphe anomala* (Squamata Colubridae). Mitochond. DNA Part A **27**: 2742-2743.

Liu. P., Zhao, W.-G. (2015b): The complete mitochondrial genome of the Amur rat-snake *Elaphe schrenckii* (Squamata: Colubridae). Mitochond. DNA Part A **27**: 2529-2530.

Machado, T., Silva, V.X., Silva, M.J. de J. (2014): Phylogenetic relationships within *Bothrops neuwiedi* group (Serpentes, Squamata): geographically highly-structured lineages, evidence of introgressive hybridization and Neogene/Quaternary diversification. Mol. Phylogenet. Evol. **71**: 1-14.

Mertens, R., Müller, L. (1928): Liste der Amphibien und Reptilien Europas. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft **41**: 1-62.

Nguyen, L.-T., Schmidt, H.A., von Haeseler, A., Minh, B.Q. (2015): IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol. Biol. Evol. **32**: 268-274.

Plötner, J., Uzzell, T., Beerli, P., Spolsky, C., Ohst, T., Litvinchuk, S.N., Guex, G.D., Reyer, H.U., Hotz, H. (2008): Widespread unidirectional transfer of mitochondrial DNA: A case in western Palaearctic water frogs. J. Evol Biol. **21**: 668-681.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Schulz, K.-D. (2013): An annotated and illustrated checklist of Old World ratsnakes. In: Old World Ratsnakes, pp. 17-268. Schulz, K.-D. Ed, Bushmaster Publications, Berg.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J.D., Higgins, D.G. (2011): Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. **7**: 539.

Talavera, G., Castresana, J. (2007): Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. **56**: 564-577.

Uetz, P., Freed, P., Hošek, J. (eds.) (2018): The Reptile Database, http://www.reptile-database.org [accessed on 27 March 2018].

Utiger, U., Helfenberger, N., Schätti, B., Schmidt, C., Ruf, M., Ziswiler, V. (2002): Molecular systematics and phylogeny of Old and New World ratsnakes, *Elaphe* auct., and related genera (Reptilia, Squamata, Colubridae). Russ. J. Herpetol. **9**: 105-124.

Vedmederya, V., Zinenko O., Barabanov, A. (2009): An annotated type catalogue of amphibians and reptiles in the museum of nature at V. N. Karazin Kharkiv National University (Kharkiv, Ukraine). Russ. J. Herpetol **16**: 203-212

Wallach, V., Williams, K.L., Boundy J. (2014): Snakes of the World: A Catalogue of Living and Extinct Species. Taylor and Francis, CRC Press, Boca Raton.

Xu, C., Mu, Y., Kong, Q., Xie, G., Guo, Z., Zhao, S. (2015): Sequencing and analysis of the complete mitochondrial genome of *Elaphe davidi* (Squamata: Colubridae). Mitochondr. DNA Part A **27**: 2383-2384.

Yan, L., Geng, Z.-Z., Yan, P., & Wu, X.-B. (2016): The complete mitochondrial genome of *Elaphe bimaculata* (Reptilia, Serpentes, Colubridae). Mitochondr. DNA **27**: 1285-1286.