

New *Zootoca vivipara* (Lichtenstein, 1823) haplogroup in the Carpathians

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The Eurasian common lizard, *Zootoca vivipara* (Lichtenstein, 1823), is the lizard with the largest distribution area on Earth (Guillaume et al. 1997). Its chromosome and genetic structure have been studied since the end of the 20th century and several overviews discussed the geographical distribution of the different chromosomal forms and haplogroups (Kupriyanova & Rudi 1990, Kupriyanova et al. 2006, Heulin et al. 1999, 2011, Odierna et al. 1993, 1998, Surget-Groba et al. 2001, 2006). In 2011 we started a systematic investigation of the phylogeography of the populations distributed in the Carpathian Basin, where the karyotype and genetic diversity of the species are considerably high (Crnobrnja-Isailovic & Aleksic 2004, Kupriyanova & Rudi 1990, Kupriyanova et al. 2006, Odierna et al. 1993, 1998, 2004, Surget-Groba et al. 2006). As a next step, after studying Hungarian populations (Velekei et al. 2014), the systematic investigation was continued in different lowland and mountain ranges in Romania.

Small tissue samples were collected (and stored in 95% ethanol) from 161 individuals from 18 locations during fieldwork in 2002-2003. Total DNA was extracted from the samples with Sigma GenElute Genomic DNA Kit, using manufacturer's protocol. A partial sequence (appr. 400 bp) of the protein encoding cytochrome *b* was selected for the phylogenetic analysis as target gene. The primers MVZ04 and MVZ05 (Smith and Patton, 1991) were used to amplify the cytochrome *b* region. The polymerase chain reaction (PCR) was performed with the following conditions: starting denaturation step of 3 min at 94 °C, followed by 35 cycles of 30 s denaturation at 95 °C, annealing at 50 °C for 60 s, extension at 72 °C for 30 s, and the final extension was conducted for 10 min at 72 °C. The alignment of the cytochrome *b* sequences was performed with ClustalX (Thompson et al., 1997) and corrected by eye. The recognized clades including several haplotypes were estimated in the MEGA software package (version 6.06, Tamura et al., 2013). The genetic relationship between the haplotypes was inferred by maximum likelihood analysis with the bootstrap method, Hasegawa-Kishino-Yano model parameter using 1000 replications.

Homologous sequences of two Lacertid species, *Podarcis muralis* (accession number: AY714980, Surget-Groba et al. 2006) and *Lacerta bilineata* (accession number: AY714981, Surget-Groba et al. 2006) were downloaded from GenBank and were used as outgroups. All sequences of the new haplotypes have been deposited in GenBank (GenBank accession numbers KP881723-KP881727).

The Carpathian Basin is an area with considerable diversity among *Z. vivipara* populations including four haplogroups from four clades (Crnobrnja-Isailovic & Aleksic 2004, Surget-Groba et al. 2006), but within the Carpathian Mountain range itself, the distribution pattern was relatively simple with the VB haplogroup present in the north in Poland and Slovakia and the VU haplogroup - in the middle and the south in Romania (Horváthova et al. 2013, Surget-Groba et al. 2006, data from this study) (Fig. 1.). In a recent review of the biogeography of the species Heulin et al. (2011) suggested a north-south corridor of the VB haplotype across eastern Hungary and western Romania connecting populations in the Balkans to the main distribution area in the north in Slovakia and Ukraine. Similar to the results of the earlier investigation of Velekei et al. (2014), this study did not find evidence for this hypothesis. On the other hand, in neighbouring regions south and southwest of Braşov (Făgăraş Mountains and Predeal) new haplotypes were detected. According to the maximum likelihood analysis they belong to a new, previously undescribed presumably ancient haplogroup with the closest relationship to the oviparous OS haplogroup (clade A) (from the previously described haplogroups (Fig. 2.)). This may strongly influence our present knowledge on the phylogeography of *Z. vivipara*. As the closely related *Z. v. carniolica* (clade A) represents a distinct evolutionary unit, with a presumably long-term evolutionary history of separation (Cornetti et al. 2014), the newly discovered Carpathian populations also occurring at high altitudes may also

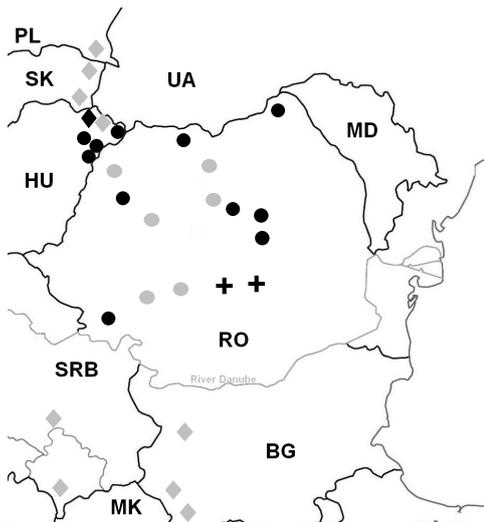


Figure 1. Genetic structure of the viviparous lizard (*Zootoca vivipara*) in the eastern part of the Carpathian Mountains and neighbouring areas (BG= Bulgaria, Hu= Hungary, MD= Moldova, MK= Macedonia, PL= Poland, RO= Romania, SK= Slovakia, SRB= Serbia, UA= Ukraine, ◆= VB haplogroup (Clade E, viviparous), ●= VU haplogroup (Clade D, viviparous), + = new haplogroup (reproduction mode unknown); black symbols are the results of this study).

need special conservation attention due to their genetic distinctness.

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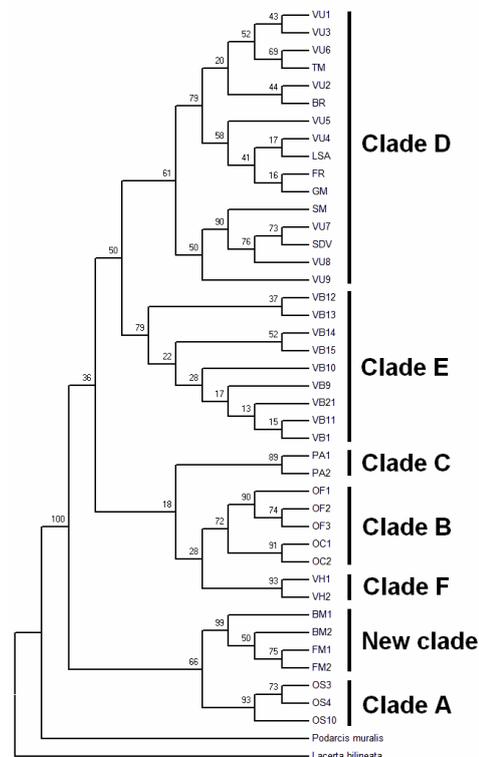


Figure 2. Maximum likelihood consensus tree of all previously described *Zootoca vivipara* clades and the results of this study (Letters indicate sampling locations as BR= Broscuti, FM= Făgăraș Mountains, FR= Frumoasa, LSA= Lacul Sfanta Ana, GM= Gurghiu Mountains, SM= Semic Mountains, TM= Tibles Mountains, BM= Bucegi Mountains, SDV= Stana de Vale).

References

- Cornetti, L., Menegon, M., Giovine, G., Heulin, B., Vernesi, C. (2014): Mitochondrial and Nuclear DNA Survey of *Zootoca vivipara* across the Eastern Italian Alps: Evolutionary Relationships, Historical Demography and Conservation Implications. PLOS ONE 9(1): e85912.
- Crnobrnja-Isailovic, J., Aleksic, I. (2004): Clutch size in two Central Balkan populations of European common lizard *Lacerta vivipara*. Biota 5: 5-10.
- Guillaume, C.P., Heulin, B., Bechkov, V. (1997): Biogeography of *Lacerta vivipara*: Reproductive mode and enzyme phenotypes in Bulgaria. Ecography 20: 240-246.
- Heulin, B., Surget-Groba, Y., Guiller, A., Guillaume, C. P., Deunff, J. (1999): Comparisons of mtDNA sequence (16S rRNA gene) between oviparous and viviparous strains of *Lacerta vivipara*: A preliminary study. Molecular Ecology 8: 1627-1631.
- Heulin, B., Surget-Groba, Y., Sinervo, B., Miles, D., Guiller, A. (2011): Dynamics of haplogroup frequencies and survival rates in a contact zone of two mtDNA lineages of the lizard *Lacerta vivipara*. Ecography 34: 436-447.
- Horváthova, T., Cooney, C.R., Fitz, P.S., Oksanen, T.A., Jelic, D., Ghira, I., Uller, T., Jandzik, D. (2013): Length of activity season drives geographic variation in body size of a widely distributed lizard. Ecology and Evolution 3(8): 2424-2442.
- Kupriyanova, L.A., Rudi E.R. (1990): Comparative karyological analysis of *Lacerta vivipara* (Lacertidae, Sauria) populations. Zoologicheski Zhurnal 69: 93-101.
- Kupriyanova, L.A., Mayer, W., Böhme, W. (2006): Karyotype diversity of the Eurasian lizard *Zootoca vivipara* (Jacquin, 1787) from Central Europe and the evolution of viviparity. Proceedings of the 13th Congress of the Societas Europaea Herpetologica pp. 67-72.
- Odierna, G., Kupriyanova, L.A., Capriglione, T., Olmo, E. (1993): Further data on sex chromosomes of Lacertidae and a hypothesis on their evolutionary trend. Amphibia-Reptilia 14: 1-11.
- Odierna G., Aprea G., Capriglione T., Arribas O. J., Kupriyanova L. A., Olmo E. (1998): Progressive differentiation of the W sex-chromosome between oviparous and viviparous populations of *Zootoca vivipara* (Reptilia, Lacertidae). Italian Journal of Zoology 65: 295-302.

- Odierna, G., Aprea, G., Capriglione, T., Puky, M. (2004): Chromosomal evidence for the double origin of viviparity in the European common lizard, *Lacerta (Zootoca) vivipara*. *Herpetological Journal* 14: 157-160.
- Smith, M.F., Patton, J.L. (1991): Variation in mitochondrial cytochrome b sequences in natural populations of South American akodontine rodents (Muridae: Sigmodontinae). *Molecular Biology and Evolution* 8: 85-103.
- Surget-Groba, Y., Heulin, B., Guillaume, C.P., Puky, M., Semenov, D., Orlova, V., Kupriyanova, L., Ghira, I., Smajda, B. (2006): Multiple origins of viviparity or reversal from viviparity to oviparity? The European common lizard (*Zootoca vivipara*, Lacertidae) and the evolution of parity. *Biological Journal of the Linnean Society* 87: 1-11.
- Surget-Groba, Y., Heulin, B., Guillaume, C.P., Thorpe, R. S., Kupriyanova, L., Vogrin, N., Maslak, R., Mazzotti, S., Venczel, M., Ghira, I., Odierna, G., Leontyeva, O., Monney, J. C., Smith, N. (2001): Intraspecific phylogeography of *Lacerta vivipara* and the evolution of viviparity. *Molecular Phylogenetics and Evolution* 18: 449-459.
- Tamura, K., Stecher, G., Peterson, D., Filipiński, A., Kumar, S. (2013): MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725-2729
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., Higgins, D. G. (1997): The clustal-x windows interface-flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.
- Velekei, B., Lakatos, F., Biró, P., Ács, É., Puky, M. (2014): The genetic structure of *Zootoca vivipara* (Lichtenstein, 1823) did not support the existence of a north - south corridor of the VB haplogroup in eastern Hungary. *North-Western Journal of Zoology* 10(1): 187-189.
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