

The genetic structure of *Zootoca vivipara* (Lichtenstein, 1823) populations did not support the existence of a north - south corridor of the VB haplogroup in eastern Hungary

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Received: 04. December 2013 / Accepted: 04. February 2014 / Available online: 15. February 2014 / Printed: June 2014

Abstract. The Eurasian common lizard, *Zootoca vivipara* (Lichtenstein, 1823) has seven haplogroups. A north-south corridor of the VB haplogroup across eastern Hungary and western Romania has recently been suggested. We tested this hypothesis by sequencing a fragment (appr. 400 bp) of the protein encoding cytochrome *b* gene of the mtDNA. All four previously known haplogroups were detected in the country but along the eastern border with Romania only the VU haplogroup was found. As such, either the proposed corridor is narrower and lies further to the east, in Romania, or the haplogroup distribution area is discontinuous between eastern Slovakia - western Ukraine and the Balkan.

Key words: *Zootoca vivipara*, distribution, haplogroup, Hungary.

The Eurasian common lizard, *Zootoca vivipara* (Lichtenstein, 1823), is the lizard with the largest distribution area on Earth (Guillaume et al. 1997). Its genetical structure has been studied for more than a decade, and several articles have discussed the geographical distribution of the different haplogroups (Heulin et al. 1999, Surget-Groba et al. 2001, 2006). The VU haplogroup can be found from Central Europe through Siberia to Japan, VB from the British Isles to Scandinavia and Poland and also in the Balkan. The PA and VH haplogroups are Central Europeans; the oviparous OS populations live in the Alps and the Western Balkan. These molecular differences also correlate with chromosomal forms as it was described e.g. for the VU and the VB haplogroups (Kupriyanova et al. 2005). Four haplogroups are known in Hungary, PA in the north-western, VH in the central, VU and VB in the northeastern part of the country (Surget-Groba et al. 2006). In a recent review on the biogeography of the species Heulin et al. (2011) suggested a north-south corridor of the VB haplogroup across eastern Hungary and western Romania connecting populations in the Balkan to the main distribution area in the north in Slovakia and Ukraine. As a first step to investigate this question, we studied populations along the Hungarian side of the proposed corridor to test whether this corridor exists in Hungary and if it does, where exactly these populations can be found.

We collected small tissue samples of the tail from 50 individuals in nine locations (1-5 new samples per site and previously collected material, if available) from the whole country and stored in 95% ethanol. 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 MEGA software package (version 5.0, Tamura et al., 2011). The genetic relationship between the haplotypes of new localities in eastern Hungary was inferred by maximum likelihood analysis with bootstrap method, Kimura 2 parameter using 1000 replications (Fig. 1).

The genetic diversity of *Z. vivipara* populations can be extremely low in some areas. Within the range of the VU haplogroup, for example, a single nucleotide difference was revealed between Belorussian and Japanese populations (Takeuchi et al. 2013). In other areas, such as the Carpathian Basin (Fig. 2), there is considerable diversity among the populations (Crnobrnja-Isailovic & Aleksic 2004, Horváthova et al. 2013, Surget-Groba et al. 2006). The study of Hungarian *Z. vivipara* populations

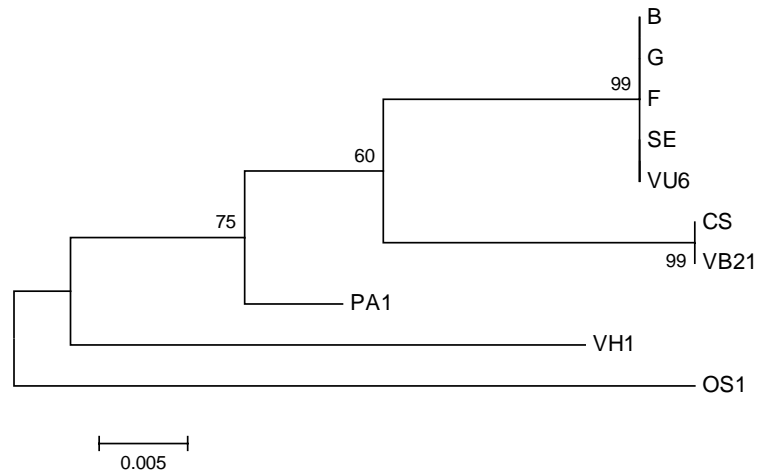


Figure 1. Maximum Likelihood consensus tree of five haplotypes (OS1, VH1, PA1, VB21, VU6) previously identified in the Carpathian Basin including the results of this study (Letters indicate the sampling locations as B= Bátorliget, G= Garbolc, F= Fülöp, SE= Sényő, CS= Csaroda). Bootstrap values are indicated at the branches.)

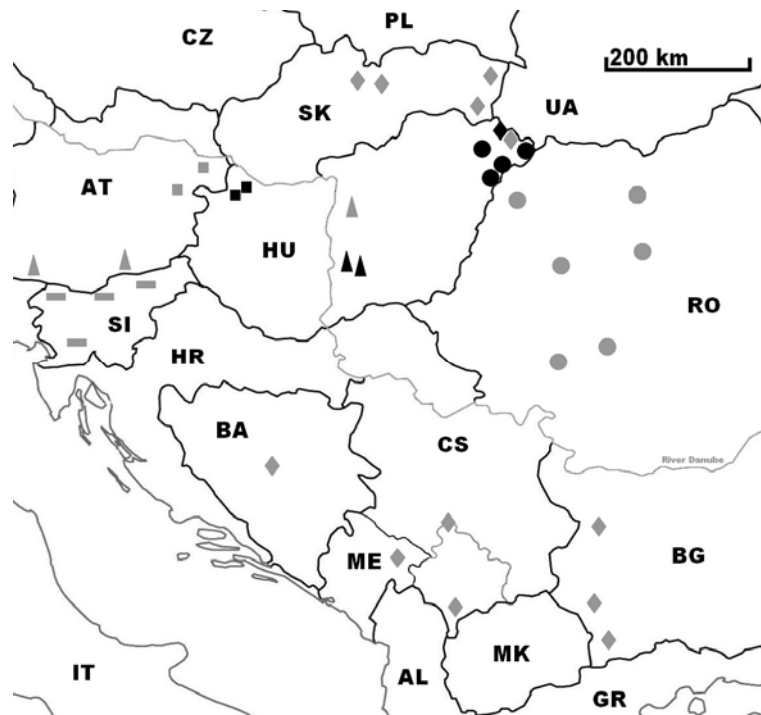


Figure 2. Genetic structure of the viviparous lizard (*Zootoca vivipara*) in the Carpathian Basin and the Balkan (Al= Albania, At= Austria, Ba= Bosnia-Herzegovina, Bg= Bulgaria, Cs= Serbia, Cz= Czech Republic, Gr= Greece, Hr= Croatia, Hu= Hungary, It= Italy, Me= Montenegro, Mk= Macedonia, Pl= Poland, Ro= Romania, Si= Slovenia, Sk= Slovakia, Ua=Ukraine, ◆= VB haplogroup, ●= VU haplogroup, ■= PA haplogroup, ▲= VH haplogroup, —= OS haplogroup, black symbols are the results of this study, grey symbols represents localities from Horváthová et al. 2013, Surget-Groba et al. 2001, 2006).

including all major distribution areas in Hungary (Puky et al. 2005) revealed the presence of four haplogroups (Surget-Groba et al. 2006). In the region of the proposed corridor *Z. vivipara* populations have a patchy distribution in Hungary (Puky et al. 2004, 2005) as well as in neighbouring areas in Romania, where they are limited by climatic conditions and are often under threat (Covaci-Markov et al. 2008). Though there were populations with VB haplotypes north to the River Tisza (Genbank accession no.: KJ396237) in East-Hungary among the localities (Fig. 2), all populations we studied along the Hungarian - Romanian border (Genbank accession no.: KJ396236, KJ396238-KJ396240) belonged to the VU6 haplotype (Fig. 1 & 2). Contact zones among haplogroups are sometimes found in areas with no apparent geographic barriers (Milá et al. 2013) also in lowlands and differences in the survival between mtDNA haplogroups might change their actual position. As such, either the proposed corridor is narrower and lies further to the east, in Romania, or the haplogroup distribution area is discontinuous between eastern Slovakia - western Ukraine and the Balkan.

Acknowledgements. We thank Tímea Mechura, Mónika Szalai, Béla Mester and Mihály Tóth for participating in the fieldwork in eastern Hungary and Karl Jarvis for improving the English text. We also thank the editor and three reviewers for their constructive comments, which helped us to improve the manuscript. This work was funded by the Hungarian National Science Foundation (KTIÁ-OTKA CNK 80140).

References

- Covaci-Markov, S.D., Cicort-Lucaciu, A.S., Ferenti, S., David, A. (2008): The distribution of lowland *Zootoca vivipara* populations in North-Western Romania. *North-Western Journal of Zoology* 4(1): 72-78.
- 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.
- Horváthová, T., Cooney, C.R., Fitze, 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., Odierna, G., Capriglione, T., Olmo, E., Aprea, G. (2005): Chromosomal changes and form-formation, subspeciation in the wideranged Euroasian species *Zootoca vivipara* (evolution, biogeography). In: Ananjeva N., Tsinenko, O. (eds.): *Herpetologica Petropolitana. Proc. of the 12th Ord. Gen.Meeting Soc. Eur. Herpetol.*, August 12 - 16, 2003, St. Petersburg, Russian Journal of Herpetology 12(Suppl.): 47-52.
- Milá, B., Surget-Groba, Y., Heulin, B., Gosá, A., Fitze, P. S. (2013): Multilocus phylogeography of the common lizard *Zootoca vivipara* at the Ibero-Pyrenean suture zone reveals lowland barriers and high-elevation introgression. *BMC Evolutionary Biology* 13:192.
- Puky, M., Hajdu, Á., Surget-Groba, Y., Heulin, B., Odierna, G. (2004): Fajvédelmi programok létjogosultsága és feladatai Magyarországon: az elevenesülő gyík (*Zootoca vivipara* Mayer & Bischoff, 1996) vizsgálatának eredményei és tanulságai. *Természetvédelmi Közlemények* 11: 411-418.
- Puky, M., Schád, P., Szövényi, G. (2005): Magyarország herpetológiai atlasza/Herpetological atlas of Hungary. Varangy Akciócsoport Egyesület, Budapest. pp. 207.
- 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.
- Takeuchi, H., Takeuchi, M., Hikida, T. (2013): Extremely low genetic diversity in the Japanese population of *Zootoca vivipara* (Squamata: Lacertidae) revealed by mitochondrial DNA. *Current Herpetology* 32(1): 66-70.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011): MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28(10): 2731-2739.
- 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.