

UNIVERSITY OF WEST HUNGARY

SUMMARY OF PhD THESIS

**PHYLOGEOGRAPHICAL STUDY OF THE COMMON LIZARD, *ZOOTOCA VIVIPARA*
(LICHTENSTEIN, 1823) IN THE CARPATHIAN BASIN**

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1. Introduction

The intensive research of the viviparous lizard (*Zootoca vivipara* LICHTENSTEIN, 1823) is due to its reproduction mode. The species is one of the only three known lizard species, within which it can be distinguished egg-laying and also viviparous populations.

According to the previous researches the species is genetically very diverse in Central Europe. Nevertheless, most of these studies only paid little attention to this area, giving rise to several important questions remain open, and the phylogeographic distribution map of the species is remained vague in many respects.

The depth exploration of the topic of the following objectives:

I. Enlarge the knowledge on the distribution area of the species - finding new populations

II. New phylogeographic distribution maps of the species using molecular genetic methods, in particular in the following areas:

1. The "F" clade (VH haplogroup) South Transdanubia "corridor"
2. The "E" clade (VB haplogroup) Balkan "corridor"
3. Search for an ancient Eastern (egg-laying) - previously unknown - clade

2. Materials and methods

Small tissue samples from the tail were collected from over 161 individuals from 18 locations collected during fieldwork in the Carpathian Basin between 2002-2012 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. 429 bp) of the protein encoding cytochrome b and a partial sequence (appr. 480, according to the haplotype) of the non-protein coding 16s rRNA were 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, and the primers 16sar-L and 16sbr-H (Palumbi et al. 2002) were used to amplify the 16s RNA 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 sequences was performed with ClustalX (Thompson et al., 1997) and corrected by eye. The recognized clades including several haplotypes were estimated in BEAST (version 1.8.2, Drummond et al. 2012) and

MEGA software packages (version 6.06, Tamura et al., 2013). The genetic relationship between the haplotypes was inferred by Bayesian analysis with Hasegawa-Kishino-Yano model, posterior distributions for each parameter were obtained using a Monte Carlo Markov Chain (MCMC), which was run for 100 million generations, and sampled every 10000 generations, and 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).

3. Summary and thesis

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).

Between 2002 and 2012 we made a systematic investigation of the phylogeography of 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.

Thesis 1: *The author clarified the knowledge of the distribution area of the species in Hungary.*

In the Duna-Tisza köze, further south of the previous sampling, near Kiskőrös found new, previously unknown populations.

The populations near Sényő and Fülöp in eastern Hungary were previously unknown, the second one is the southernmost known population in eastern Hungary.

Thesis 2: *There is no connection in the the "F" clade between the haplotypes VH of Eastern Austria and the Duna-Tisza köze.*

In South West Hungary we did not find any new population, which is consistent with previous knowledge. In a previous study Heulin et al (2011) suggested a west-east corridor connection between the haplotypes of these areas, which thus refuted.

Thesis 3: *there is no corridor in the clade "E" within eastern Hungary and western Romania, that connects the populations of the Balkans and Western / Central Europe*

In a recent review on the biogeography of the species Heulin et al. (2011) also 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. Thus it can be stated that the populations of the Balkan are isolated from the main distribution area

Thesis 4: *The "D" clade is genetically most diverse in the Carpathian Basin within the entire distribution area*

Two new haplotype was found in the semeic and Țibleș Mountains. The already known 7 haplotype in Transilvania rose up to 9. In addition to the Carpathians, both north and east there are only two known haplotypes.

Thesis 5: *Identification of another ancient clade*

We identified a new clade in the Southern Carpathians in Fagaras Mountains and in Bucegi Mountains. All of the phylogeographic analyzes in both genes of the new clade were

supported by a high "posterior" probability and "bootstrap" values. The "A" clade known as *Zootoca vivipara carniolica* represents a subspecies level status (Mayer et al., 2000). This newly discovered clade had appr. 1.7 million years common and 3.4 million years separated evolution history with the subspecies *carniolica*.

Thesis 6: *The author outlined new evolutionary scenarios with the determination of the divergence time of the individual clades*

The ancient "A" and the new clade branched off to other clades of about 5.1 million years ago. The divergence time of these two clades is around 3.4 million years. The divergence time of the other clades is between 3 and 4.16 million years: clades "B", "C" and "F" branched off the "D" and "E" clade approx. 4.16 million years ago, the latter branched apart about 3 million years ago.

Based on these results, it is likely that viviparity already formed in the Pliocene not during the Pleistocene.

4. List of publications related to the dissertation

Short notes

1. Velekei B., Lakatos F., Covaciu-Marcov S. D., Sas-Kovács I., Puky M. (2015): New *Zootoca vivipara* haplogroup in the Carpathians. North-Western Journal of Zoology 11: 366-368. (IF: 0,869)
2. Velekei B., Lakatos F., Bíró P., Ács É., Puky M. (2014): 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. North-Western Journal of Zoology 10: 187-189. (IF: 0,869)

Conference posters

1. Puky M., Tóth V., Lakatos F., Tóth M., Mester B., Bíró P., Ács É., Velekei B. (2012): Phylogeographic pattern, parasite load and fluctuating asymmetry of *Zootoca vivipara*. in Hungary World Congress of Herpetology, Vancouver, Canada, 8-14 August 2012.

2. Velekei B., Tóth V., Lakatos F., Bíró P., Ács É., Puky M. (2012): Phylogeographic pattern of *Zootoca vivipara* in Hungary ConGRESS, Utilization of genetic approaches for effective conservation of endangered species. Regional Workshop, Debrecen, Hungary. March 14-16, 2012.
3. Velekei B., Tóth V., Lakatos F., Bíró P., Ács É., Puky M. (2012): Az eleven szülő gyík filogeográfia mintázata Magyarországon. III. Herpetológiai Előadókörös, 2012 március 27., Budapest
4. Velekei B., Tóth V., Lakatos F., Bíró P., Ács É., Puky M. (2012): Magyarországi eleven szülő gyík (*Zootoca vivipara* LICHTENSTEIN, 1832) populációk közötti különbségek mitokondriális DNS szakaszok alapján. IX. Magyar Ökológus Konferencia, Keszthely, 2012. szeptember 5-7.

List of publications not closely related to the dissertation

Conference proceedings

1. Velekei B. (2012): A sárgahasú unka (*Bombina variegata* LINNEAUS, 1758) kutatása a Soproni-hegységben In: Lakatos F., Szabó Z. (szerk.) Kari Tudományos Konferencia Kiadvány: Nyugat-magyarországi Egyetem, Erdőmérnöki Kar. 315 p. Konferencia helye, ideje: Sopron, Magyarország, 2011.10.05. Sopron: NyME, 2012. pp. 280-281.
2. Velekei B. (2011): Új adatok Sopron és környékének herpetofaunájához In: Lakatos F., Polgár A., Kerényi-Nagy V. (szerk.) Nyugat-magyarországi Egyetem, Tudományos Doktorandusz Konferencia, Konferencia-kötet. 258 p. Konferencia helye, ideje: Sopron, Magyarország, 2011.04.13. Sopron: Nyugat-magyarországi Egyetem, 2011. pp. 167-172.
3. Velekei B. (2005): A fekete gólya (*Ciconia nigra*) szigetközi előfordulása In: Tamás E. Anna (szerk.) Élet a Duna-ártéren: Ember a természetben. 166 p. Konferencia helye, ideje: Érsekcsanád, Magyarország, 2005.09.23-2005.09.24. Baja: Baja Ifjúsági Természetvédelmi Egyesület, 2005. pp. 47-49.

Conference abstract

1. Velekei B. (2011): Új adatok Sopron és környékének herpetofaunájához In: Lakatos F., Polgár A., Kerényi-Nagy V. (szerk.) Nyugat-magyarországi Egyetem, Tudományos

Doktorandusz Konferencia, Konferencia-kötet. 258 p. Konferencia helye, ideje: Sopron, Magyarország, 2011.04.13. Sopron: Nyugat-magyarországi Egyetem, 2011. p. 48.

Book chapter

1. Dankovics R., Mille J., Velekei B. (2015): Kétéltűek. In Haraszthy L. (szerk.): Az Élőhely Irányelvben szereplő állatfajok elterjedési atlasza Vas, Zala és Somogy megye Natura 2000 területein. Somogy Természetvédelmi Szervezet, pp 37. – in press –
2. Rendes N., Velekei B. (2014): Kétéltűek, hüllők és a holtfa. In Batha D., Puskás L. (szerk.): A holtfa. Silva Naturalis vol. 5. pp. 121.