

Molecular data confirm recent fluctuations of northern border of dice snake (*Natrix tessellata*) range in Eastern Europe

The dice snake, *Natrix tessellata* is distributed from Italy over the Balkans, Near East, to China. It prefers aquatic or marshy habitats, including brackish water. ). In Eastern Europe, the northern border of continuous range of the species is between 49° N and 50° N latitude. However, there are some isolated populations more to the north, with the most well known of them in Galichya Gora Nature Reserve, the upper Don current, Lipetsk province, Russia.

In our study we used the cytochrome oxidase subunit 1 barcoding region to elucidate the phylogenetic relationship between previously uncovered *Natrix tessellata* populations from Eastern Europe and the Balkans. We were able to compare the concordance between topology of the phylogenetic tree based on the COI sequences and the phylogenetic tree based on the cytochrome b from the study of Guicking et al. (2009).

Samples were taken from animals caught in the field, which were safely released after the procedure. DNA was isolated from tissue samples of up to 5 mm of distal part of the tail. We also sampled tissues from specimens of museum collections.

We used in total 15 specimens of *N. tessellata*.

In order to obtain partial COI gene sequence, we used the degenerate primer pair V1F and V1R published by Smith et al (2008).

Out of the 15 specimens we have found 8 haplotypes provided with the following accession numbers: A (JN871603): Ro1 – Ro5, Ser1, Ser2; B (JN871604): Ser3; C

(JN871605): Ita; D (JN871606): Gr3, E (JN871607): Ukr1, Rus; F (JN871608): Ukr2; G (JN871609): Gr1; H (JN871610): Gr2 (Fig. 1).



The phylogenetic analyses of the *N. tessellata* sequences indicate 3 major clades. One group is formed by the Ukrainian and Russian sequences and another separate group by the Gr1 and Gr2 sequences, which are from the mainland of Greece. It was surprising that the third Greek sample (Gr3), from the island of Crete, which is spatially closer to the Greek mainland samples (Gr1 and Gr2) cluster together with distant samples from Romania, Italy and Serbia. . Consequently, the Gr1 and Gr2 COI haplotypes from our study probably represent the remnants of the first colonization and then survived isolated since the early Pliocene. Our northernmost and well isolated population of *N. tessellata* from Galichya Gora (Rus) seems to be the result from a very recent and fast colonization event, as the time of isolation was not long enough to gain any genetic divergence from the nearest Ukrainian sample.