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GEOGRAPHIC DIFFERENTIATION IN NEWTS (*Triturus*) OF EASTERN EUROPE: GENOME SIZE, ALLOZYMES, AND MORPHOLOGY

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Eight species of the genus *Triturus* inhabit eastern Europe. The geographic variation in salamanders of this vast territory was poorly studied. The goal of present paper was to evaluate such variation, evidenced by DNA flow cytometry, allozymes and standard morphological treatments.

The amount of DNA per nucleus (genome size) was measured by flow cytometry (Borkin et al., 2001; Litvinchuk et al., 2004). The details of the allozyme and morphological techniques have been published previously (Litvinchuk et al., 1994; Litvinchuk and Borkin, 2000, 2003).

The *Triturus cristatus* superspecies is a large group of European salamandrids consisting of seven species and subspecies (Arntzen, 2003; Table 1). The study of allozymes, genome size and morphology allowed us (Litvinchuk et al., 1997, 1999; Litvinchuk, 1998) to reveal quite narrow hybrid zone between *T. cristatus* and *T. dobrogicus* in the Ukrainian Transcarpathians (Fig. 1). Therefore, our data supported that these former subspecies of *T. cristatus* should be elevated to full distinct species in the framework of the *T. cristatus* superspecies (Arntzen, 2003). Currently we recognized two subspecies of the Danube newt (Litvinchuk and Borkin, 2000). Most part of the species range, including lowland of Ukrainian Transcarpathians, is inhabited by *T. dobrogicus macrosomus*, as well as Danube and Dnepr river deltas — by *T. d. dobrogicus* (Litvinchuk and Borkin, 2002). Two other members of the *T. cristatus* superspecies were also splitted in two subspecies, namely *T. carnifex carnifex* and *T. c. macedonicus* (Kalezić et al.,

1997), as well as *T. karelinii karelinii* and *T. k. arntzeni* (Litvinchuk et al., 1999).

The genome size variation in the *Triturus cristatus* superspecies ($n = 836$) was polymodal (Table 1). Differences in the nuclear DNA content between *T. cristatus* and *T. dobrogicus* (both subspecies included) were small. Another group was formed by two subspecies of *T. carnifex* and the Balkan subspecies *T. karelinii arntzeni* (Litvinchuk et al., 1999). The group with the largest genome size contained *T. k. karelinii* only. According to genome size data, in the Caucasus, the subspecies consists of two geographically separate groups of populations. These are the western group (the northwestern Caucasus) and the eastern group (Dagestan, Georgia, and Azerbaijan).

The allozyme analysis ($n = 265$; Litvinchuk et al., 1994, 1999; Litvinchuk, 1998) showed obvious between-

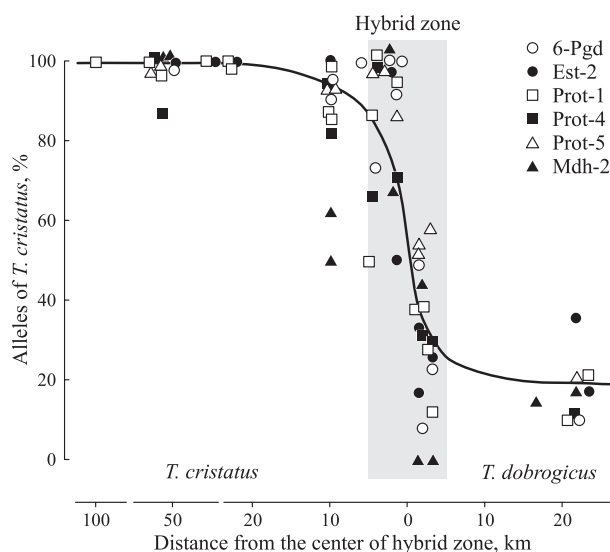


Fig. 1. The distribution of *T. cristatus* alleles in samples from the hybrid zone between *T. cristatus* and *T. dobrogicus* in the Ukrainian Transcarpathians.

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TABLE 1. Genome Size Variation (in picograms) in East European Newts of the genus *Triturus*

Taxon	Locality	N_{samples}	N_{spec}	Mean \pm SD	Range
<i>Triturus alpestris</i>					
<i>alpestris alpestris</i>	Maloe Opol'e Eminence (Lvov Oblast', Ukraine)	2	16	59.7 \pm 0.5	59.0 – 61.1
	Ukrainian Carpathians	2	12	59.8 \pm 0.6	58.7 – 60.7
	Romanian Bihor Mts.	1	1	60.0	
	Montenegro	1	2	60.1	59.9 – 60.3
<i>alpestris montenegrinus</i>	Montenegro	1	36	59.5 \pm 0.4	58.3 – 60.6
<i>alpestris serdarus</i>	Montenegro	1	6	59.5 \pm 0.1	59.4 – 59.6
<i>Triturus cristatus</i> superspecies					
<i>carnifex carnifex</i>	Northeastern Italy	2	3	58.2 \pm 0.0	58.2 – 58.3
<i>carnifex macedonicus</i>	Montenegro	2	11	58.3 \pm 0.6	57.5 – 59.3
<i>cristatus</i>	Eastern Europe	51	423	55.5 \pm 0.5	54.2 – 57.1
<i>dobrogicus dobrogicus</i>	Danube River Delta	3	78	56.3 \pm 0.5	55.0 – 57.6
	Dnepr River Delta	1	1	56.6	
<i>dobrogicus macrosomus</i>	Danube River Basin	11	177	56.2 \pm 0.4	55.1 – 57.4
<i>karelinii arntzeni</i>	Serbia	2	14	58.8 \pm 0.5	58.2 – 59.7
<i>karelinii karelinii</i>	European Istanbul (Halkali)	1	2	62.6	62.4 – 62.8
	Northwestern Anatolia	1	2	64.0	63.7 – 64.4
	The Crimea	4	68	62.7 \pm 0.5	60.8 – 63.5
	Northwestern Caucasus	6	36	62.4 \pm 0.4	61.6 – 63.1
	Dagestan	1	8	63.4 \pm 0.2	63.3 – 63.8
	Eastern Georgia	1	1	63.6	
	Southeastern Azerbaijan	2	12	63.5 \pm 0.3	63.0 – 63.9
<i>Triturus vulgaris</i> group					
<i>montandoni</i>	Maloe Opol'e Eminence (Lvov Oblast', Ukraine)	3	36	59.7 \pm 0.5	58.6 – 60.6
	Ukrainian and Romanian Carpathians	4	14	59.7 \pm 0.8	58.2 – 60.8
	Gutyi Mts. (Zakarpatskaya Oblast', Ukraine)	1	9	59.2 \pm 0.7	58.5 – 60.1
<i>vulgaris ampelensis</i>	Romanian Transylvania	2	34	58.6 \pm 0.6	57.1 – 60.1
<i>vulgaris ampelensis?</i>	Romanian Bihor Mts. (Cimpeni)	1	10	57.0 \pm 0.2	56.7 – 57.3
<i>vulgaris graecus</i>	Montenegro	1	11	62.3 \pm 1.0	60.7 – 63.5
<i>vulgaris kosswigi?</i>	European Istanbul (Halkali)	1	34	58.1 \pm 0.4	57.1 – 58.8
<i>vulgaris lantzi</i>	Abkhazia	3	15	63.6 \pm 0.5	62.9 – 64.7
	Northwestern Caucasus	7	39	62.6 \pm 0.5	61.8 – 63.6
<i>vulgaris schmidtlerorum</i>	Northwestern Anatolia	1	6	59.7 \pm 0.4	59.1 – 60.3
<i>vulgaris vulgaris</i>	Eastern Europe	63	430	56.7 \pm 0.6	54.6 – 59.4
<i>Triturus ophryticus</i>					
<i>ophryticus</i> ssp.	Northwestern Anatolia	1	4	63.1 \pm 0.6	62.5 – 63.7
<i>ophryticus ophryticus</i>	The Caucasus	7	20	67.6 \pm 0.7	66.8 – 68.8

N_{samples} and N_{spec} are numbers of sample locations and specimens studied, respectively.

population differences within two species only: *T. cristatus* and *T. karelinii* (Fig. 2). The treatment of standard morphometrical characters and trunk vertebrae count allowed us to identify all four species of the complex, and even both subspecies of *T. dobrogicus* (Litvinchuk et al., 1999; Litvinchuk and Borkin, 2000).

The second large group of newts in eastern Europe contained two species, namely: *T. vulgaris* with several currently recognized subspecies and *T. montandoni*. The latter two species plus *T. helveticus* comprise the *T. vulgaris* group. The smooth newt has the widest distribution in

eastern Europe. The genome size variation in this species was polymodal ($n = 638$). We recognized four groups composed by various subspecies (Table 1). The smallest amount of the nuclear DNA was characteristic to *T. v. vulgaris* and morphologically intermedian between *T. v. vulgaris* and *T. v. ampelensis* population from Romanian Bihor Mountains. The Transylvanian *T. v. ampelensis* and the sample from European part of Istanbul provided the second group. The latter sample was morphologically intermedian between *T. v. vulgaris* and *T. v. kosswigi*. The Anatolian subspecies *T. v. schmidtlerorum* formed the

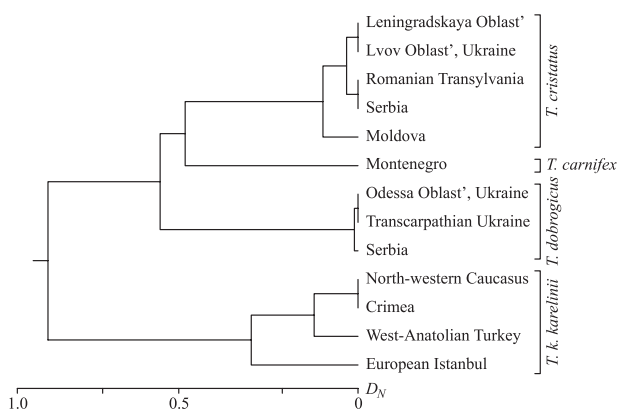


Fig. 2. UPGMA dendrogram for the *Triturus cristatus* superspecies based upon Nei's (1972) genetic distance.

third group. Finally, the Balkan subspecies *T. v. graecus* and the Caucasian newt *T. v. lantzi* had the largest genome size within the superspecies. Abkhazian populations of the latter subspecies had slightly larger amount of nuclear DNA in comparison with newts of the northwestern Caucasus (Krasnodar and Stavropol' Krai's).

The study of allozyme variation in the smooth newt (18 loci, $n = 197$) showed that the levels of differences between three subspecies were quite different (Fig. 3). Two European subspecies *T. v. vulgaris* and *T. v. ampelensis* ($D_{\text{Nei}'72} = 0.030$) were much closer each other than both to the Caucasian *T. v. lantzi* ($D_{\text{Nei}'72} = 0.174$). The analysis of standard morphological characters in the smooth newt ($n = 1412$) showed well differences between most subspecies, in the exception of *T. v. vulgaris* and *T. v. lantzi*.

Triturus montandoni is endemic to the Carpathian Mountains. Two small and geographically isolated populations from the main species range are situated in western Ukraine. These are the Maloe Opol'e Eminence in Lvov Oblast' and Gutyi Mountains in the Transcarpathians (Litvinchuk et al., 2003). We failed to find any significant differences between the both isolated populations and newts from the main range of the species.

According to our data, the banded newt is suggested to consist of two species: *T. ophryticus* and *T. vittatus* (Litvinchuk et al., 2005). In the northern banded newt (*T. ophryticus*), significant differences between populations from northwestern Turkey and from northeastern Turkey and the Caucasus were expressed in genome size (Table 1), allozyme data ($D_{\text{Nei}'72} = 0.383$; $n = 30$), and trunk vertebrae count (the modal number 12 vs. 13; $n = 219$). Therefore, we allocate the western group of *T. ophryticus* to a separate subspecies (Litvinchuk et al., 2005).

We examined the genome size variation in *T. alpestris* ($n = 73$) as well. We failed to reveal any significant differ-

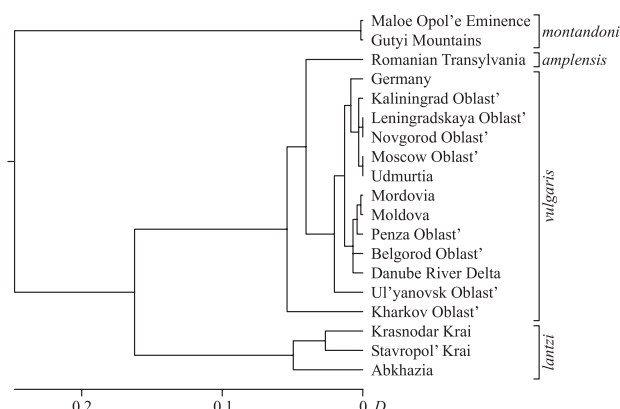


Fig. 3. UPGMA dendrogram for the *Triturus vulgaris* group based upon Nei's (1972) genetic distance.

ences between *T. a. alpestris*, *T. alpestris*, *T. a. montenegrinus*, and *T. a. serdarus* from Montenegro (Table 1). Moreover, the amount of nuclear DNA in four geographically isolated populations of *T. a. alpestris* from the Ukrainian Carpathians, Maloe Opol'e Eminence, Romanian Bihor Mountains, and Montenegro was quite similar.

Thus, we consider that genome size together with other cytogenetic, molecular and morphological characters could be used for analysis of geographical differentiation and speciation in urodelans.

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