Zootaxa 3682 (3): 441–453
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3682.3.5
http://zoobank.org/urn:lsid:zoobank.org:pub:69B9A846-616F-4774-B3F0-B796D2B90431

A revised taxonomy of crested newts in the *Triturus karelinii* group (Amphibia: Caudata: Salamandridae), with the description of a new species

B. WIELSTRA[1,5], S.N. LITVINCHUK[2], B. NAUMOV[3], N. TZANKOV[4] & J.W. ARNTZEN[1]

[1]*Naturalis Biodiversity Center, P. O. Box 9517, 2300 RA Leiden, The Netherlands*
[2]*Institute of Cytology, Russian Academy of Sciences, Tikhoretsky pr. 4, St. Petersburg, 194064, Russia*
[3]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Yurii Gagarin street, 1113 Sofia, Bulgaria*
[4]*National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tzar Osvoboditel boulevard, 1000 Sofia, Bulgaria*
[5]*Corresponding author. E-mail: ben.wielstra@naturalis.nl*

Abstract

We present a taxonomic revision of the crested newt *Triturus karelinii* sensu lato. Based on the presence of discrete nuclear DNA gene pools, deep genetic divergence of mitochondrial and nuclear DNA, and no indication of gene flow, we interpret this taxon as comprising two species: one covering the southern Caspian Sea shore, the Caucasus and the Crimea, i.e. the eastern part of the total range and another covering northern Asiatic Turkey and western Asiatic Turkey plus the south-eastern Balkan Peninsula, i.e. the central and western part of the total range. We acknowledge that the central/western species should likely be further subdivided into a central and a western taxon, but we prefer to await a more detailed genetic analysis of the putative contact zone, positioned in northwestern Asiatic Turkey. The name *T. karelinii* (Strauch, 1870) applies to the eastern species as the type locality is positioned along the coast of the Gulf of Gorgan, Iran. The name *T. arntzeni* has been applied to the central/western species with Vrtovać, Serbia as the type locality. We show that not *T. karelinii* sensu lato but *T. macedonicus* occurs at Vrtovać. Hence, the name *T. arntzeni* Litvinchuk, Borkin, Džukić and Kalezić, 1999 (in Litvinchuk *et al.*, 1999) is a junior synonym of *T. macedonicus* (Karaman, 1922) and should not be used for the central/western species. We propose the name *T. ivanbureschi* **sp. nov.** for the central/western species and provide a formal species description.

Key words: gene flow, phylogeny, *Triturus arntzeni*, *Triturus cristatus* superspecies, *Triturus ivanbureschi* **sp. nov.**, *Triturus karelinii*

Introduction

The genus *Triturus* (the marbled and crested newts) comprises five 'morphotypes', i.e. groups differing in relative body proportions (Arntzen, 2003). These five *Triturus* morphotypes are, arranged from a stocky to a slender built, composed of: 1) *T. marmoratus* (Latreille, 1800) and *T. pygmaeus* (Wolterstorff, 1905), 2) *T. karelinii* (Strauch, 1870), 3) *T. carnifex* (Laurenti, 1768) and *T. macedonicus* (Karaman, 1922), 4) *T. cristatus* (Laurenti, 1768), and 5) *T. dobrogicus* (Kiritzescu, 1903). The taxonomy of these five morphotypes is by now well established (Arntzen & Wielstra, 2010; Speybroeck *et al.*, 2010) with the exception of *T. karelinii* which, as previous studies suggest, comprises more than one species. For ease of communication we refer to the collective of species comprising *T. karelinii* as *T. karelinii* sensu lato (s. l.).

We here begin a revision of the taxonomy of *T. karelinii* s. l. We first review the phylogenetic studies that have been conducted on *T. karelinii* s. l. Second, we make the case that two species exist, distributed along the southern Caspian Sea shore, in the Caucasus and in the Crimea (referred to as the 'eastern species') and in northern Asiatic Turkey and western Asiatic Turkey plus the south-eastern Balkan Peninsula (referred to as the 'central/western species'). Third, based on an analysis of published and newly collected data, we demonstrate that the name *T. arntzeni* does not refer to the central/western species but — as the bulk of the data indicates — is a junior synonym

Accepted by M. Vences: 30 May 2013; published: 28 Jun. 2013

of *T. macedonicus*. Fourth, we provide a new name and a formal species description for the central/western *T. karelinii* s. l. species.

A review of phylogenetic studies with *Triturus karelinii* s. l.

The presence of deep mitochondrial DNA divergence within *T. karelinii* s. l. was first realized by Wallis and Arntzen (1989) and worked out in detail by Wielstra *et al.* (2010): *T. karelinii* s. l. comprises three distinct, geographically structured mitochondrial DNA lineages, with a level of genetic divergence on par with that of recognized *Triturus* species (Table 1). An eastern mitochondrial DNA lineage is distributed along the southern Caspian Sea shore, in the Caucasus and on the Crimea, a central mitochondrial DNA lineage is distributed in northern Asiatic Turkey, along the shore of the Black Sea, and a western mitochondrial DNA lineage is distributed in the south-eastern Balkan Peninsula and western Asiatic Turkey (Fig. 1).

TABLE 1. Genetic divergence (p-distances) among *Triturus* species and the three distinct clades comprising *T. karelinii* s. l. for the DNA barcoding fragments of the COI (above diagonal) and 16S (below diagonal) mitochondrial genes.

	T. marmoratus	*T. pygmaeus*	*T. cristatus*	*T. dobrogicus*	*T. carnifex*	*T. macedonicus*	*T. karelinii* west	*T. karelinii* central	*T. karelinii* east
T. marmoratus	-	0.052	0.135	0.154	0.159	0.162	0.157	0.151	0.162
T. pygmaeus	0.023	-	0.149	0.161	0.166	0.164	0.157	0.157	0.164
T. cristatus	0.052	0.048	-	0.081	0.078	0.078	0.086	0.074	0.083
T. dobrogicus	0.048	0.044	0.017	-	0.071	0.069	0.076	0.081	0.080
T. carnifex	0.052	0.044	0.027	0.029	-	0.029	0.071	0.080	0.066
T. macedonicus	0.040	0.036	0.023	0.025	0.011	-	0.074	0.081	0.064
T. karelinii west	0.052	0.044	0.015	0.027	0.027	0.023	-	0.052	0.063
*T. karelinii c*entral	0.054	0.054	0.027	0.029	0.042	0.038	0.027	-	0.069
T. karelinii east	0.063	0.057	0.029	0.031	0.040	0.036	0.025	0.036	-

Genetic data were taken from Wielstra & Arntzen (2011), MEGA 5.05 (Tamura *et al.*, 2011) was used to determine p-distances.

Based on a detailed phylogeographical survey of three nuclear DNA markers, the presence of three genetically distinct nuclear gene pools within *T. karelinii* s. l. was confirmed (Wielstra *et al.*, 2013a), that we refer to as an eastern, a central and a western *T. karelinii* s. l. nuclear gene pool. The distribution of these nuclear gene pools corresponds to the three mitochondrial DNA lineages, with the exception of northwestern Asiatic Turkey, where western *T. karelinii* s. l. mitochondrial DNA is observed in the central *T. karelinii* s. l. nuclear gene pool (Fig. 1). Wielstra *et al.* (2013a) suggested that partial geographical replacement of the western nuclear gene pool by the central *T. karelinii* s. l. nuclear gene pool coincided with asymmetric introgression of mitochondrial DNA. For a full biogeographical scenario of *T. karelinii* s. l., see Wielstra *et al.* (2013a); (Wielstra *et al.*, 2010).

The mitochondrial DNA phylogeny suggests that the central and western mitochondrial DNA lineages are each other's closest relatives and together have a sister-group relationship with the eastern mitochondrial DNA lineage (Wielstra & Arntzen, 2011; Wielstra *et al.*, 2010). A *T. karelinii* s. l. 'species tree' (i.e. a phylogeny based on multiple gene trees with their own coalescent models) based on the three nuclear DNA markers does not correspond to the mitochondrial DNA topology (see Wielstra *et al.*, 2013a for a detailed discussion on potential 'contamination' by interspecific gene flow). However, a preliminary *Triturus* species tree based on more nuclear DNA markers (Wielstra *et al.*, in prep.) shows no discrepancy with the mitochondrial DNA data and for now we consider the central and western *T. karelinii* s. l. nuclear gene pools to constitute a monophyletic assemblage.

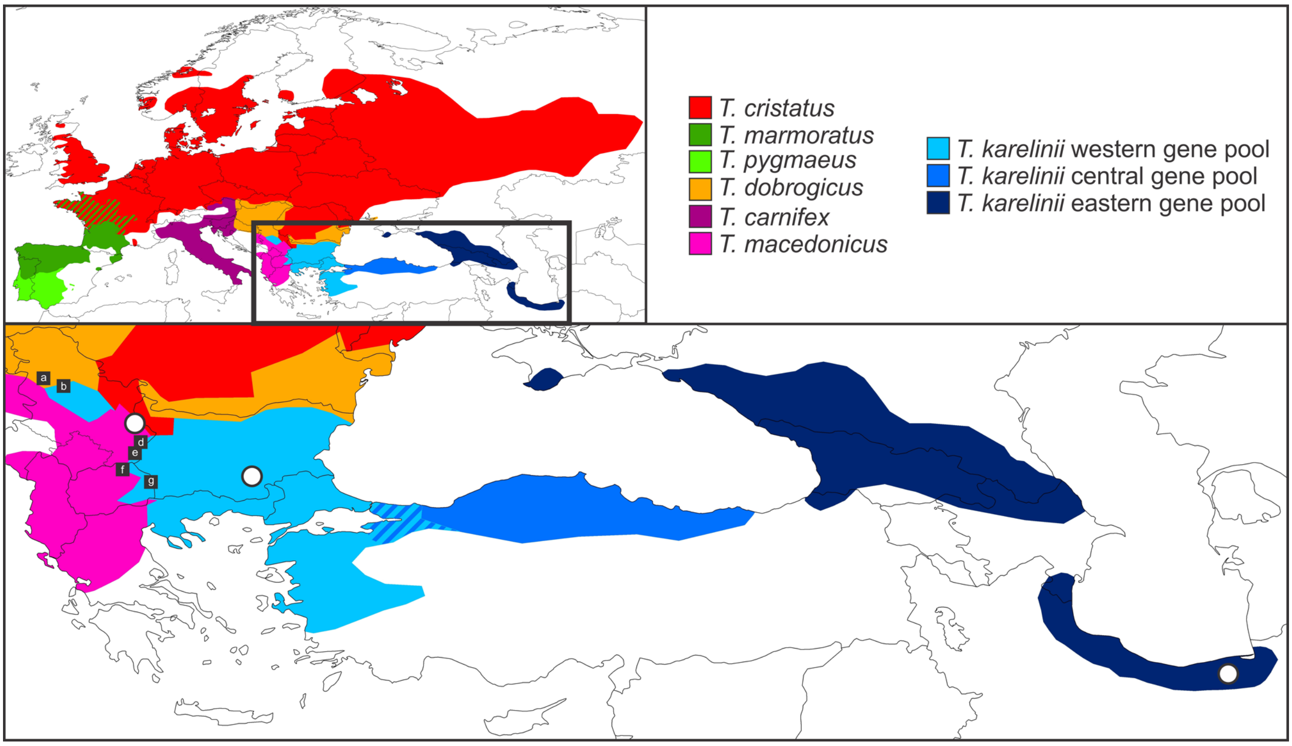

FIGURE 1. The distribution of *Triturus karelinii* s. l. The inset shows the distribution of *Triturus* (the red/green interdigitating shades refer to an area where *T. marmoratus* and *T. cristatus* are distributed sympatrically) and the cut-out shows the distribution of the three nuclear gene pools comprising *T. karelinii* s. l. in detail. The interdigitating shades of blue represent the area in which the central *T. karelinii* s. l. nuclear gene pool carries western *T. karelinii* s. l. mitochondrial DNA. The three white dots in the cut-out show type localities with, from left to right, Vrtovać, Serbia for *T. arntzeni*, Ostar Kamak, Bulgaria for *T. ivanbureschi* **sp. nov.** and the coast of the Gulf of Gorgan, Iran for *T. karelinii* sensu stricto. The black squares show six of the seven populations (a-b and d-g) representing locality 14 of *T. arntzeni* in Litvinchuk *et al.* (1999)); the left white dot corresponds to the seventh locality (c; see text for details).

The two components of the central and western *T. karelinii* s. l. assemblage occur in parapatry in northwestern Asiatic Turkey. Although nuclear DNA sequence data suggest genetic isolation (Wielstra *et al.*, 2013a), asymmetric introgression of mitochondrial DNA evidences recent genetic interaction (Wielstra *et al.*, 2013a). A study with 40 allozyme markers supports a scenario of recent genetic admixture, but the contact zone appears to be convoluted, or perhaps a mosaic (Arntzen & Wielstra, 2010). However, sampling was limited and the results not conclusive on their own. We anticipate that the central and western *T. karelinii* s. l. nuclear gene pools represent distinct species (see also Wielstra *et al.*, 2012), but we aim to study genetic interactions at the contact zone in more detail before formalizing our position.

The eastern *T. karelinii* s. l. nuclear gene pool appears to be disconnected from the central and western *T. karelinii* s. l. nuclear gene pools, with no records in the ca. 150 km in Turkey from Trabzon to Artvin and with no hint of genetic admixture (Wielstra *et al.*, 2013a; Wielstra *et al.*, 2010). We therefore consider the eastern *T. karelinii* s. l. nuclear gene pool and the combined central and western *T. karelinii* s. l. nuclear gene pools to represent two distinct species.

The name *T. arntzeni* is a junior synonym of *T. macedonicus*

Litvinchuk *et al.* (1999) described the subspecies *Triturus karelinii arntzeni* based on differences in genome size, protein variation and morphological characteristics. Subsequently, Espregueira Themudo *et al.* (2009) elevated this subspecies to species level, i.e. *Triturus arntzeni* Litvinchuk, Borkin, Džukić and Kalezić, 1999 (in Litvinchuk *et al.*, 1999). However, concern has been expressed that the type locality of *T. arntzeni*—Vrtovać, near Pirot in eastern Serbia (Fig. 1)—in fact represents the species *T. macedonicus* and not *T. karelinii* s. l. (Arntzen & Wielstra, 2010;

Stoyanov *et al.*, 2011). We review the species identity of crested newts from Vrtovać below. Because the bulk of evidence points towards *T. macedonicus* being the crested newt species occurring at Vrtovać, we identify the name *T. arntzeni* as a junior synonym of *T. macedonicus* and conclude that it does not represent a taxon newly distinguished in *T. karelinii* s. l.

Geography. The population in Litvinchuk *et al.* (1999) representing *T. arntzeni* comprises seven different localities (14a-g; see Fig. 1), all of which are situated at, or close to, various crested newt contact zones. Note that locality 14c concerns the actual type locality of *T. arntzeni*. For the latest overview of *Triturus* distribution, including a database of locality data, see Wielstra *et al.* (2013b). The species composition of the seven *T. arntzeni* populations we evaluate as follows:

- Locality 14a, Trbusać, Serbia is *T. dobrogicus* (Wielstra *et al.*, 2013b). Species identification is based upon the phenotype of material deposited at the Institute of Biological Research, “Siniša Stanković”, University of Belgrade (JWA, BW).
- Locality 14b, Trešnja, Serbia represents the western *T. karelinii* s. l. nuclear gene pool with *T. dobrogicus* mitochondrial DNA and some limited presence of, or genetic admixture with, *T. dobrogicus* (Arntzen *et al.*, submitted; Wallis & Arntzen, 1989).
- Locality 14c, Vrtovać, Serbia, is positioned inside the *T. macedonicus* range (Wielstra & Arntzen, 2012). Neighboring crested newt species are, in order of increasing distance, *T. cristatus*, *T. dobrogicus* and western *T. karelinii* s. l. nuclear gene pool (Fig. 1).
- Locality 14d, Vlasi, Serbia, has western *T. karelinii* s. l. nuclear gene pool and *T. macedonicus* in syntopy (Wielstra & Arntzen, 2012). Species identification is based on examination of animals observed in the field (JWA, BW).
- Locality 14e, Vlasina, Serbia has *T. macedonicus* (Wielstra & Arntzen, 2012). Species identification is based upon the phenotype of material deposited at the Institute of Biological Research, “Siniša Stanković”, University of Belgrade and of animals observed in the field (JWA, BW). The number of rib-bearing vertebrae (details below) reported in this population (n=7 with NRBV = 13, n=25 with NRBV = 14) is typical for *T. macedonicus* (Crnobrnja-Isailović *et al.*, 1997).
- Locality 14f, Stracin, Macedonia has the western *T. karelinii* s. l. nuclear gene pool and *T. macedonicus* in syntopy (Wielstra & Arntzen, 2012). Species identification is based upon the phenotype of material deposited at the Institute of Biological Research, “Siniša Stanković”, University of Belgrade (JWA, BW). Note that a population of *T. macedonicus* is located c. 4 kilometers to the west (Rugince, Macedonia in Wielstra & Arntzen, 2012).
- Locality 14g, Berovo, Macedonia is western *T. karelinii* s. l. nuclear gene pool. Species identification was confirmed based upon the phenotype of material deposited at the Institute of Biological Research, “Siniša Stanković”, University of Belgrade and of animals observed in the field (JWA, BW) and conforms to species identification by Kalezić and Hedgecock (1980). Another confirmed *T. karelinii* s. l. nuclear gene pool locality is located 9 kilometers to the northwest (Mitrašinci, Macedonia in Wielstra *et al.*, 2013a).

Throat and belly pattern. In the field crested newts are best identified by morphotype (see introduction) and by their coloration pattern (Arntzen, 2003; Arntzen & Wallis, 1999) as follows:

- *Triturus karelinii* s. l.: Stocky build. Heavily white-stippled sides. Ventral surface yellow-orange with many small to medium-sized, frequently angular black spots and continuous on the throat.
- *Triturus carnifex*: Medium stocky build. Little or no white stippling on sides. Throat color variable with white stipples. Ventral surface yellow with few large, roundish, ill-defined and muddy-gray to black spots.
- *Triturus macedonicus*: Medium stocky build. Sides densely white-stippled. Throat dark black or a muddied mix of black and yellow with many, medium sized white stipples. Ventral surface yellow to orange-yellow with a dense pattern of small, irregular spots.
- *Triturus cristatus*: Slender build. Heavily white-stippled sides. Throat a muddied mix of black and yellow with fine white stippling. Ventral surface yellow-orange with irregular black spots.
- *Triturus dobrogicus*: Very slender build. Heavily white-stippled sides. Black throat with large angular white spots (especially in males), ventral surface deep orange with many sharp, roundish black spots.

For a picture overview of throat and belly patterns of the different crested newt species, see Arntzen and Wallis (1999). Pictures of the throat and belly pattern of the holotype and paratypes of *T. arntzeni* (online Appendix 1), preserved material stored at the Institute of Biological Research, "Siniša Stanković", University of Belgrade, and live newts from the type locality as observed by several of us (JWA, BW) most resemble *T. macedonicus* and not *T. karelinii* s. l. In particular we point to the absence of the distinctive *T. karelinii* s. l. throat pattern, with small angular dark spots surrounded by yellow, similar to the pattern on the belly. These spots are also absent in the holotype of *T. arntzeni*. To ascribe the Vrtovać newts to *T. macedonicus* by coloration pattern alone is not straightforward: as noted by Freytag (1988), *T. macedonicus* overlaps considerably with *T. cristatus* in patterning and coloration.

Number of rib-bearing pre-sacral vertebrae. The *Triturus* morphotypes were initially distinguished with the help of the 'Wolterstorff index', a measure of overall shape, defined as 'forelimb length divided by inter-limb distance' (Wolterstorff, 1923). Arntzen and Wallis (1994) found the number of rib-bearing pre-sacral vertebrae (NRBV) in *Triturus* to represent a taxonomically discriminating character superior to the Wolterstorff-Index (the two are strongly negatively correlated across species). Accordingly, the *Triturus* morphotypes are characterized by a *modal* number of rib-bearing pre-sacral vertebrae (NRBV) (Arntzen & Wallis, 1999). Modal NRBV count increases from 12 in *T. marmoratus – T. pygmaeus*, via 13 in *T. karelinii* s. l., 14 in *T. carnifex – T. macedonicus* and 15 in *T. cristatus*, to 16/17 in *T. dobrogicus* (Arntzen, 2003; Arntzen & Wallis, 1999).

A disadvantage of NRBV (similarly applying to the Wolterstorff index) is that, although informative at the population level, it is not unambiguous at the individual level, due to intraspecific variation. Moreover, intermediate scores due to interspecific hybridization may point to a third species not involved (Arntzen & Wallis, 1994). We determined NRBV for 63 individuals from the *T. arntzeni* type locality (the holotype and 12 paratypes, deposited at the Zoological Institute, Russian Academy of Sciences, St. Petersburg; 46 specimens deposited at the Institute of Biological Research, "Siniša Stanković", University of Belgrade and four specimens deposited at Naturalis Biodiversity Center, Leiden, see online Appendix 2). Three specimens with different NRBV (13 versus 14) on either sides of the body were ignored. Eleven individuals (18.3%) have an NRBV count of 13, NRBV is 14 in n=44 (73.3%) and NRBV is 15 in n=5 (8.3%). The distribution of modal versus non-modal NRBV scores in the Vrtovać population is not significantly different from that of *T. carnifex / T. macedonicus* (G-test of independence, d. f. = 1, $P>0.05$; reference data in Arntzen, 2003). The NRBV count of 15 shown by the holotype of *T. arntzeni* is indicative since this value fits *T. macedonicus* better than it does *T. karelinii* s. l.

Genome size. The size of the genome may be a source of relevant taxonomic information (Green & Sessions, 2007; Kron *et al*., 2007). Litvinchuk *et al.* (1999) observed a difference in genome size between *T. arntzeni* and *T. karelinii* s. l., with non-overlapping confidence intervals. We here interpret the genome size of *T. arntzeni* from Vrtovać (n=13) not as 'different from other *T. karelinii'* (as in Litvinchuk *et al.*, 1999), but as 'not different from *T. macedonicus'* (Fig. 2; constituent data in online Appendix 3). It is important to note that these genome sizes also differ from those of *T. cristatus* and *T. dobrogicus*. In contrast to Litvinchuk *et al.* (1999) we do not invoke convergent evolution or introgressive hybridization to explain the data but conclude that newts from Vrtovać actually are *T. macedonicus*. The genome size of the holotype of *T. arntzeni* in particular falls within the range of *T. macedonicus* but outside of the range of *T. karelinii* s. l. The fourteenth specimen labeled as *T. arntzeni* in Litvinchuk *et al.* (1999) is from Tresnja (population 14b, see above) and has a genome size that is intermediate of *T. karelinii* s. l. and *T. dobrogicus*; it is excluded from Fig. 2.

Allozyme data. Litvinchuk *et al.* (1999) presented data for nine allozyme loci and analyzed these at the population level. We here re-analyze the data at the level of the individual and in a phylogenetic framework for 42 individuals with a complete dataset and 48 individuals with data missing at one locus (online Appendix 4). Unfortunately the dataset for the holotype of *T. arntzeni* is highly incomplete, but several paratypes can be included in the analysis. We first determine the number of distinctive gene pools that is best supported by the data probabilistically using BAPS v.5.3 (Corander *et al*., 2008). The recognized groups were clustered in a dendrogram on the basis of Rogers' genetic distance with BIOSYS-1 (Swofford & Selander, 1981).

All ten included *T. arntzeni* individuals and three *T. cristatus* are in one group. Other BAPS groups are composed as follows: all *T. karelinii* s. l. individuals with representatives of all three *T. karelinii* s. l. nuclear gene pools, the n=3 included *T. macedonicus* individuals, two *T. cristatus* groups and four *T. dobrogicus* groups and, finally, a mixed group with one *T. cristatus* and one *T. dobrogicus* (online Appendix 4). In the UPGMA dendrogram (Fig. 3) the BAPS group that contains all *T. arntzeni* clusters with *T. cristatus* and it does not cluster with either *T. karelinii* s. l. or *T. macedonicus*. A distance-Wagner tree yields the same result (data not shown). We further note that the two loci considered to distinguish *T. arntzeni* from *T. karelinii* s. l. by Litvinchuk *et al.* (1999) are actually represented by the same alleles in *T. cristatus* and *T. macedonicus*. However, the reference sample size is small, in particular for unquestionable *T. macedonicus* (n=3) and western *T. karelinii* s. l. nuclear gene pool (n=2).

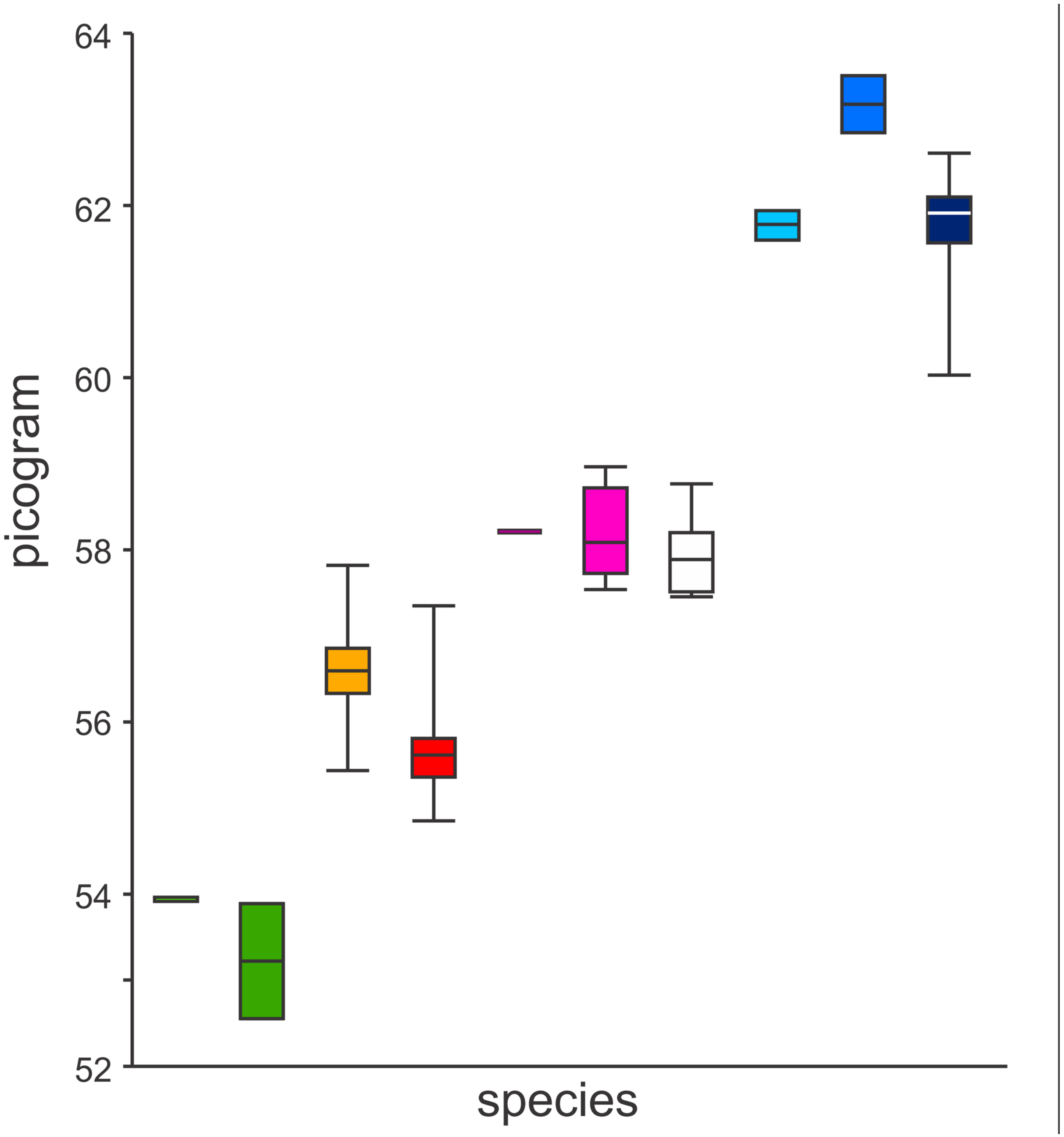

FIGURE 2. A comparison of the genome size (pg) of *Triturus arntzeni* with that of other groups of *Triturus* newts. From left to right, the groups are *T. pygmaeus* (n = 1), *T. marmoratus* (n = 2), *T. dobrogicus* (n = 115), *T. cristatus* (n = 183), *T. carnifex* (n = 3), *T. macedonicus* (n = 6), *T. arntzeni* (white; n = 13), and western (n = 2), central (n = 2) and eastern (n = 58) *T. karelinii* s. l. nuclear gene pool (colors correspond to those in Fig. 1). Shown are median, quartiles and range. Data are taken from (Litvinchuk *et al.*, 1999; Litvinchuk *et al.*, 2007) and provided in online Appendix 3. Note that the genome size of *T. arntzeni* is dissimilar to *T. karelinii* s. l. and is not different from that of *T. macedonicus* and *T. carnifex*.

Nuclear DNA sequence data. Wielstra *et al.* (2013a) provided a framework to cluster *Triturus* newts to species based on three nuclear DNA markers developed by Espregueira Themudo *et al.* (2009). Unfortunately, our attempts to obtain nuclear DNA sequence data from the type material (the holotype and 12 paratypes) of *T. arntzeni* were not successful, presumably because of degraded DNA. However, Wielstra *et al.* (2013a) presented sequence data for another five individuals from Vrtovać (voucher ID 2533-37 stored at Naturalis Biodiversity Center, Leiden). These individuals were all assigned to *T. macedonicus* (see Appendix S1 in Wielstra *et al.*, 2013a).

Mitochondrial DNA. As noted above we did not manage to obtain usable DNA extract and were not able to sequence mitochondrial DNA for the type material. However, we note that mitochondrial DNA could not be used to identify these newts anyway. This is because *T. macedonicus* in and around Serbia contain asymmetrically introgressed western *T. karelinii* s. l. mitochondrial DNA (the area for which this phenomenon has been documented is detailed in Wielstra & Arntzen, 2012). Similarly, the nearest Serbian *T. cristatus* populations contain asymmetrically introgressed western *T. karelinii* s. l. mitochondrial DNA (Wielstra & Arntzen, 2012; Wielstra *et al.*, 2013b). So, from the perspective of mitochondrial DNA, *T. macedonicus* and *T. cristatus* are indistinguishable in the Vrtovać region.

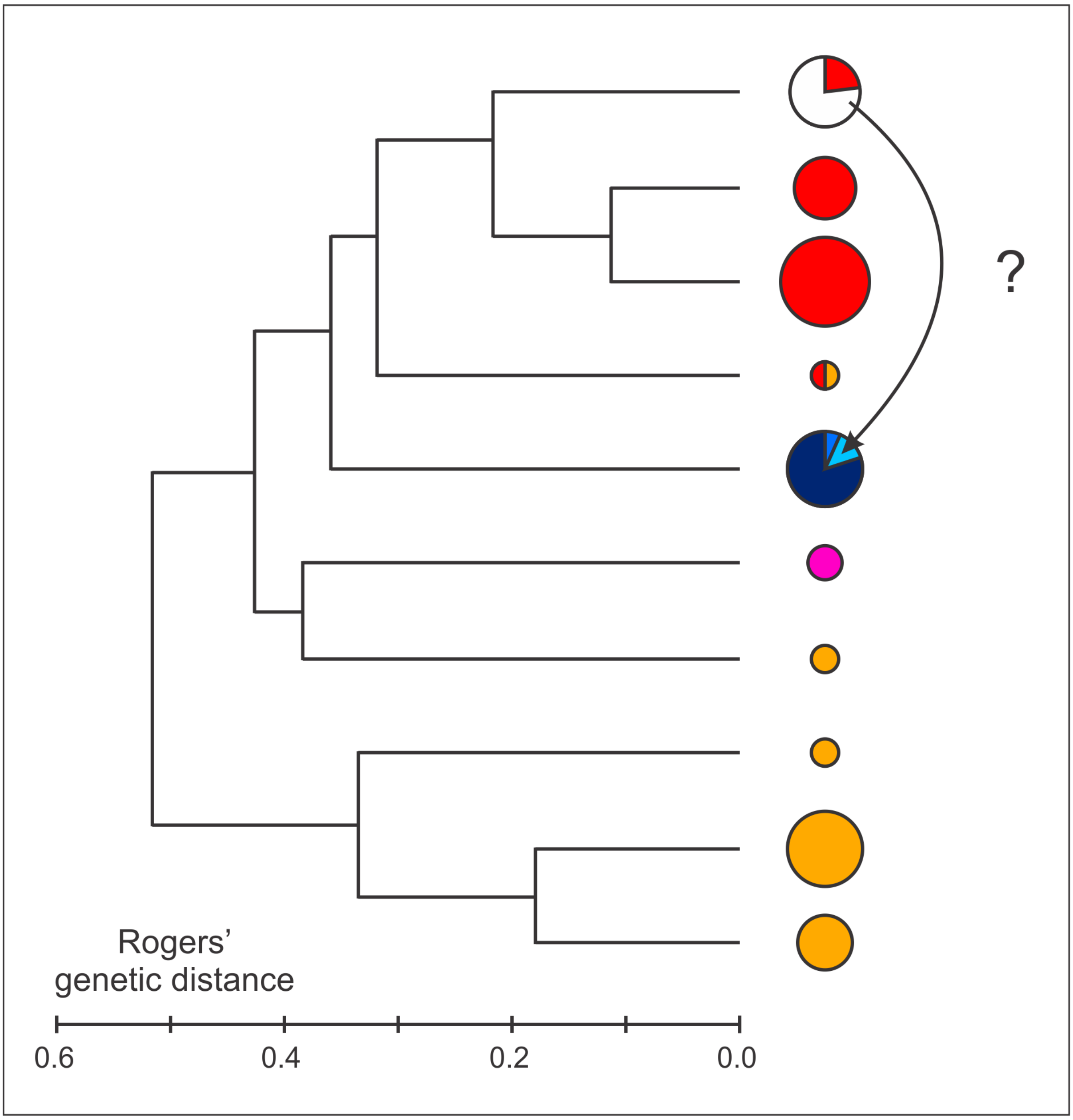

FIGURE 3. Genetic similarity among crested newts (*Triturus cristatus* superspecies) based on the allozyme data presented in Litvinchuk *et al.* (1999). Similarity among ten groups of crested newt, identified by Bayesian clustering, is expressed in an UPGMA dendrogram built with Rogers' genetic distance (for details see text and for data see online Appendix 4). Pie sizes reflect sample size. Color codes are as in Fig. 1 and individuals from the population of special interest (the type locality of *T. arntzeni*—Vrtovać) are shown in white. Note that this group does not cluster with *T. karelinii* s. l.

In conclusion, our analyses confirm that newts from Vrtovać, the type locality of *T. arntzeni*, including the holotype of *T. arntzeni*, are dissimilar to *T. karelinii* s. l. and resemble *T. macedonicus* (NRBV, genome size, nuclear DNA sequences) or *T. cristatus* (allozyme data). The throat and belly pattern suggest they are *T. macedonicus*, but we acknowledge that the distinction with *T. cristatus* is difficult. On balance, we consider the population from Vrtovać to represent *T. macedonicus* (with perhaps some influences of *T. cristatus*). Consequently, we consider the name *T. arntzeni* to be a junior synonym of *T. macedonicus* and we conclude that the name *T. arntzeni* should not be used for the central/western *T. karelinii* s. l. species.

Systematics of *T. karelinii* s. l.

The type locality of *T. karelinii* is "the northeast of the Iranian region along the southern shore of the Caspian Sea" (Strauch, 1870). Litvinchuk and Borkin (2009) restricted the type locality to the coast of the Gulf of Gorgan, Iran, based on the report of the expedition during which the type material was collected (Karelin, 1883). The type locality is within the range of the eastern *T. karelinii* s. l. species (Fig. 1). Therefore, the name *T. karelinii* sensu stricto should be applied to the eastern *T. karelinii* s. l. species. As a vernacular name we suggest Caucasian Crested Newt or Karelin's Crested Newt. A considerable number of localities (n = 235) are known from the former Soviet Union (reviewed in Litvinchuk & Borkin, 2009) but distribution data for Iran are sparse (n = 13 in Wielstra *et al.*, 2012). Its presence in the extreme north-east of Turkey is suspected but awaits confirmation (Wielstra *et al.*, 2013a).

Considering that no previously proposed name is applicable to the central/western *T. karelinii* s. l. species, we provide a new name, for which the authors are J. W. Arntzen and B. Wielstra:

Triturus ivanbureschi sp. nov. Arntzen & Wielstra

Type material. Holotype. RMNH.RENA.47200, an adult male from Ostar Kamak, Bulgaria (coordinates: 41.878°N, 25.853°E, elevation: 240 meter), collected 8 May 2012 by J.W. Arntzen, N. Tzankov and B. Wielstra (Fig. 4). **Paratypes.** Three males (RMNH.RENA.47201-47203) and 15 females (RMNH.RENA.47204-47218), with collection details identical to the holotype. For imagery, see online Appendix 5.

Diagnosis. The species is assigned to the genus *Triturus* because it possesses the characteristics of crested newts (the *T. cristatus* superspecies), in particular the combination of a denticulated crest, a dark brown dorsal coloration and orange ventral side covered with black spots. Based on mitochondrial DNA sequence data, crested newts from the type locality are firmly placed in the genus *Triturus*, within the western *T. karelinii* s. l. mitochondrial DNA lineage (Wielstra *et al.*, 2013b). As yet only molecular genetic data are available to distinguish *T. ivanbureschi* **sp. nov.** from its sister species *T. karelinii*. More details are provided in the section 'A review of phylogenetic studies with *Triturus karelinii* s. l.'. How to distinguish *T. ivanbureschi* **sp. nov.** + *T. karelinii* from the other crested newt species is discussed in the section 'The name *T. arntzeni* is a junior synonym of *T. macedonicus*'.

Description of holotype. A relatively robust crested newt, with a broad head (wider than neck) and well developed limbs. Four fingers and five toes, slightly fringed but interdigital webbing absent. Relative length of fingers 1 < 4 < 2 < 3. Relative length of toes 1 < 5 < 2 < 4 < 3. Skin granulated on dorsal and lateral sides and throat; tail and venter smooth. Gular fold inconspicuous. Swollen cloaca with papillae bordering cloacal slit. Denticulated crest, interrupted over the position of the legs and cloaca. Laterally compressed tail with evident dorsal fin and less conspicuous ventral fin. In life a brownish-blackish base color on the dorsolateral side, with scattered black flecks, most pronounced and marbled on the head. Bluish-white streak along the lateral side of the tail along the caudal vertebrae. A bright-orange ventral side and throat, dotted with small angular black spots; throat spots smaller, denser and more angular than belly spots. In the preserved state colors have slightly faded (Fig. 4). The number of rib-bearing pre-sacral vertebrae (NRBV) is 14, as determined from X-ray photographs (see online Appendix 6). The following measurements are in millimeters and have been determined with plastic Vernier calipers. Snout-vent length up to and including the hind-legs 54.0; Snout-vent length up to and including the cloaca 61.0; Total length 106.5; Inter-limb distance 28.0; Head length 14.8; Head width 11.3; Length of the right arm including the third finger 21.9; Length of the right third finger 8.5; Length of the right leg including the fourth toe 21.4; Length of the right fourth toe 8.5.

FIGURE 4. The holotype of *Triturus ivanbureschi* **sp. nov.** Pictures providing a ventral (a), lateral (b) and doral (c) view of the holotype of *T. ivanbureschi* **sp. nov.**, an adult male from Ostar Kamak, Bulgaria (museum number RMNH.RENA.47200).

Description of paratypes. The morphology of the paratypes resembles that of the holotype in general, but the pattern of black spots on the ventral side is highly variable among individuals (online Appendix 5). All paratypes have an NRBV count of 13, except RMNH.RENA.47205 (and probably RMNH.RENA.47202), which has an NRBV count of 14 (Table 2; online Appendix 6). Compared to males (such as the holotype), adult females have non-swollen cloacae, lack the denticulated crest and possess a less pronounced tail fin. In Table 2 the measurements taken for the holotype are provided for the paratypes.

Distribution. The European distribution encompasses the south-eastern Balkan Peninsula, covering most of Bulgaria, the eastern parts of Greece, Macedonia and Serbia, as well as European Turkey. An isolated distribution pocket is found in Serbia that is disconnected from the main range by other intervening *Triturus* species (*T. cristatus* in the north and *T. macedonicus* in the south; Arntzen & Wallis, 1999; Wielstra & Arntzen, 2012). The Asian distribution is restricted to Asiatic Turkey, along the coast of the Aegean Sea, the Marmara Sea and the Black Sea, reaching up to c. 300 kilometers inland (usually less) but not inner Anatolia. An outline of the distribution is provided in Fig. 1 (*T. ivanbureschi* **sp. nov.** encompasses the western and central gene pools of *T. karelinii* s.l., which meet in northwestern Asiatic Turkey). An incomplete listing of locality data is provided in Wielstra *et al.* (2012).

Etymology. We dedicate the newly recognized species of crested newt to the memory of Dr. Ivan Buresch (1885-1980), scientific director of the Institute of Zoology, Sofia (see pp. 234-235 in Adler, 2012). Dr. Buresch, together with his assistant Jordan Zonkov, laid the foundation for herpetology in Bulgaria and indeed the Balkan region with the publication of the four part monograph, '*Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel'* (Buresch & Zonkow, 1933, 1934, 1941, 1942). The work is published in the Bulgarian language with extensive summaries in German. The compound name *ivanbureschi* was chosen to avoid confusion with '*Triturus cristatus karelinii* forma *bureschi*' (Wolterstorff, 1925) (nomen illegitimum, Litvinchuk *et al.*, 1999; Mertens & Wermuth, 1960).

Proposed vernacular name. Balkan-Anatolian Crested Newt or Buresch's Crested Newt.

Discussion

We adopted a conservative approach in revising the taxonomy of *T. karelinii* s. l. For now we split the group into two species, namely *Triturus karelinii* and *Triturus ivanbureschi* **sp. nov.** The available data suggest the presence of a third taxon in northern Asiatic Turkey, distinct from a taxon in western Asiatic Turkey and the south-eastern Balkan Peninsula. Data are, however, somewhat ambiguous, in particular concerning the amount of gene flow (if any) between the putative taxa. We started surveying this area of interest in more detail. Our studies on *T. karelinii* s. l. underline the strength of molecular methods as a driving force in taxonomy. *Triturus ivanbureschi* **sp. nov.** and *T. karelinii* can as yet only be identified with molecular genetic data, with no documented diagnostic morphological features (Arntzen, 2003; Ivanović *et al.*, 2013; Ivanović and Arntzen, submitted; Litvinchuk & Borkin, 2009). We hope this paper will further stimulate what has been termed 'reverse taxonomy', i.e. the discovery of diagnostic morphological features for species initially identified based on genetic data only. Similarly, a detailed study on the natural history of *T. karelinii* s. l. and in particular the variation between the constituent species is much desired.

Online Appendices

The following additional data associated with this description have been deposited in the Dryad online data repository under DOI 10.5061/dryad.bg006:

Online Appendix 1—Pictures of the ventral side of the holotype and paratypes of *Triturus arntzeni*.
Online Appendix 2—Number of rib-bearing vertebrae (NRBV) for newts from Vrtovać, Serbia.
Online Appendix 3—Genome size data from Litvinchuk *et al.* (1999) for individual newts.
Online Appendix 4—Allozyme data from Litvinchuk *et al.* (1999) for individual newts and the BAPS group they were assigned to.
Online Appendix 5—Pictures of the ventral side of the holotype and paratypes of *Triturus ivanbureschi* **sp. nov.**
Online Appendix 6—X-ray photographs of the holotype and paratypes of *Triturus ivanbureschi* **sp. nov.**

TABLE 2. Number of rib-bearing pre-sacral vertebrae and measurements (in millimeters) for the holotype and paratypes of *Triturus ivanbureschi* **sp. nov.**

Museum number	Sex	Material	NRBV	SVL1	SVL2	Total	ILD	HL	HW	Arm	Finger3	Leg	Toe4
RMNH.RENA.47200	male	holotype	14	54.0	61.0	106.5	28.0	14.8	11.3	21.9	8.5	21.4	8.5
RMNH.RENA.47201	male	paratype	13	53.0	61.0	108.0	27.0	15.5	10.8	21.5	7.9	21.6	7.5
RMNH.RENA.47202	male	paratype	13/14	52.0	58.5	87.5	26.5	15.2	10.9	21.2	7.7	22.1	8.9
RMNH.RENA.47203	male	paratype	13	53.5	62.5	109.5	29.0	14.9	10.8	22.4	8.8	24.1	9.6
RMNH.RENA.47204	female	paratype	13	54.0	58.0	108.5	29.5	15.5	10.8	20.0	7.7	19.9	7.1
RMNH.RENA.47205	female	paratype	14	56.5	60.5	115.0	29.5	15.5	11.3	20.3	7.1	21.0	6.8
RMNH.RENA.47206	female	paratype	13	51.5	56.0	106.0	26.0	15.1	10.6	18.2	6.7	18.2	6.7
RMNH.RENA.47207	female	paratype	13	53.0	56.5	107.0	28.0	15.2	10.7	20.5	6.9	18.5	6.5
RMNH.RENA.47208	female	paratype	13	54.0	59.0	114.5	27.5	15.8	11.0	21.4	7.3	22.2	7.6
RMNH.RENA.47209	female	paratype	13	53.5	58.5	104.5	28.5	14.9	10.3	17.3	5.8	19.4	6.6
RMNH.RENA.47210	female	paratype	13	56.0	60.5	111.5	28.0	16.1	11.3	19.6	6.5	18.1	7.3
RMNH.RENA.47211	female	paratype	13	58.5	64.5	118.0	32.5	16.1	11.0	22.1	7.8	20.5	6.3
RMNH.RENA.47212	female	paratype	13	59.5	64.5	122.5	28.5	14.8	11.9	22.5	8.0	22.3	7.7
RMNH.RENA.47213	female	paratype	13	62.5	67.5	119.5	33.0	15.0	11.7	22.8	7.9	22.4	8.1
RMNH.RENA.47214	female	paratype	13	52.5	57.0	107.5	27.5	15.3	11.0	19.6	7.0	19.1	6.9
RMNH.RENA.47215	female	paratype	13	58.5	64.0	118.5	30.0	14.7	11.2	21.2	6.7	21.8	7.4
RMNH.RENA.47216	female	paratype	13	53.0	58.0	109.0	26.5	14.6	10.8	19.8	6.4	18.9	6.3
RMNH.RENA.47217	female	paratype	13	58.5	65.0	120.5	30.0	14.2	11.6	22.5	6.9	22.2	6.3
RMNH.RENA.47218	female	paratype	13	53.0	57.0	96.0	27.5	14.6	10.2	18.3	6.5	18.4	6.1

Abbreviations used are: NRBV = Number of rib-bearing pre-sacral vertebrae; SVL1 = Snout-vent length up to and including the hind-legs; SVL2 = Snout-vent length up to and including the cloaca; Total = Total length; ILD = Inter-limb distance; HL = Head length; HW = Head width; Arm = Length of the right arm including the third finger; Finger3 = Length of the right third finger; Leg = Length of the right leg including the fourth toe; Toe4 = Length of the right fourth toe. Note that for RMNH.RENA.47202 part of the tail is missing and for RMNH.RENA.47206 and RMNH.RENA.47213 the right leg was missing and measurements were taken from the other leg.

Acknowledgements

SNL was supported by grant RFBR 12–04-01277. M. Slijepcevic provided NRBV information and pictures for material from Vrtovać, deposited in the Institute of Biological Research, “Siniša Stanković”, University of Belgrade. Dirk van der Marel provided the X-ray photographs.

References

Adler, K. (2012) *Contributions to the history of herpetology. Vol. 3*. Society for the study of amphibians and reptiles, Vancouver, Canada, 564 pp.

Arntzen, J.W. (2003) *Triturus cristatus* Superspecies - Kammolch-Artenkreis (*Triturus cristatus* (Laurenti, 1768) - Nördlicher Kammolch, *Triturus carnifex* (Laurenti, 1768) - Italienischer Kammolch, *Triturus dobrogicus* (Kiritzescu, 1903) - Donau-Kammolch, *Triturus karelinii* (Strauch, 1870) - Südlicher Kammolch). *In:* Grossenbacher, K. & Thiesmeier, B. (Eds.), *Handbuch der Reptilien und Amphibien Europas. Schwanzlurche IIA*. Aula-Verlag, Wiebelsheim, pp. 421–514.

Arntzen, J.W. & Wallis, G.P. (1994) The ‘Wolterstorff Index’and its value to the taxonomy of the Crested Newt superspecies. *Abhandlungen und Berichte für Naturkunde, 17*, 57–66.

Arntzen, J.W. & Wallis, G.P. (1999) Geographic variation and taxonomy of crested newts (*Triturus cristatus* superspecies): Morphological and mitochondrial data. *Contributions to Zoology,* 68, 181–203.

Arntzen, J.W. & Wielstra, B. (2010) Where to draw the line? A nuclear genetic perspective on proposed range boundaries of the crested newts *Triturus karelinii* and *T. arntzeni*. *Amphibia-Reptilia,* 31, 311–322.
http://dx.doi.org/10.1163/156853810791769509

Buresch, I. & Zonkow, J. (1933) Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkan halbinsel. I. Teil: Schildkrötten (Testudinata) und Eidechsen (Sauria). *Bulletin des Institutions royales d'Histoire Naturelle, Sofia,* 6, 150–207.

Buresch, I. & Zonkow, J. (1934) Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkan halbinsel. II Feil: Schlangen (Serpentes). *Bulletin des Institutions royales d'Histoire Naturelle, Sofia*, 7, 106–188.

Buresch, I. & Zonkow, J. (1941) Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel III Teil: Schwanzlurche (Amphibia, Caudata). *Bulletin des Institutions royales d'Histoire Naturelle, Sofia,* 14, 171–237.

Buresch, I. & Zonkow, J. (1942) Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinsel IV Teil: Froschlurche (Amphibia, Salienta). *Bulletin des Institutions royales d'Histoire Naturelle, Sofia,* 15, 68–154.

Corander, J., Marttinen, P., Siren, J. & Tang, J. (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics,* 9, 539.
http://dx.doi.org/10.1186/1471-2105-9-539

Crnobrnja-Isailović, J., Džukić, G., Krstić, N. & Kalezić, M.L. (1997) Evolutionary and paleogeographical effects on the distribution of the *Triturus cristatus* superspecies in the central Balkans. *Amphibia-Reptilia*, 18, 321–332.
http://dx.doi.org/10.1163/156853897x00378

Espregueira Themudo, G., Wielstra, B. & Arntzen, J.W. (2009) Multiple nuclear and mitochondrial genes resolve the branching order of a rapid radiation of crested newts (*Triturus*, Salamandridae). *Molecular Phylogenetics and Evolution*, 52, 321–328.
http://dx.doi.org/10.1016/j.ympev.2009.03.024

Freytag, G.E. (1988) Erinnerungen an Willy Wolterstorff. Wie kompliziert ist die Rassengliederung des Kammolches (*Triturus cristatus* [LAURENTI, 1768])? (Amphibia, Caudata, Salamandridae). Ein blick in die Geschichte der Salamanderkunde. *Zoologische Abhandlungen für Tierkunde Dresden,* 44, 1–10.

Green, D.M. & Sessions, S.K. (2007) Chapter 6 - Karyology and cytogenetics. *In:* Heatwole, H. (Ed), *Amphibian Biology Volume 7*. Surrey Beatty & Sons, Chipping Norton, NSW, Australia, pp. 2757–2842.

Ivanović, A., Üzüm, N., Wielstra, B., Olgun, K., Litvinchuk, S.N., Kalezić, M.L., *et al.* (2013) Is mitochondrial DNA divergence of Near Eastern crested newts (*Triturus karelinii* group) reflected by differentiation of skull shape? *Zoologischer Anzeiger,* 252, 269–277.
http://dx.doi.org/10.1016/j.jcz.2012.08.005

Kalezić, M.L. & Hedgecock, D. (1980) Genetic variation and differentiation of three common European newts (*Triturus*) in Yugoslavia. *British Journal of Herpetology,* 6, 49–57.

Karaman, S.L. (1922) Beiträge zur Herpetologie von Mazedonien. *Glasnik Naucni Casopis Za Prirodne Nauke Zagreb, 34,* 591–612.

Karelin, G.S. (1883) Trip of G. S. Karelin on Caspian Sea. *In: Zapiski Imperatorskogo Russkogo Geograficheskogo Obschestva po Obschey Geografii (Otdeleniyam Geografii Matematicheskoy i Fizicheskoy),* Оникс, St. Petersburg, pp. 1–497.

Kiritzescu, C. (1903) Contribution à la faune des Batraciens de Roumanie. *Buletinul Societatii de Sciinte din Bucuresci, 12,* 243–265.

Kron, P., Suda, J. & Husband, B.C. (2007) Applications of flow cytometry to evolutionary and population biology. *Annual Review of Ecology Evolution and Systematics,* 38, 847–876.
http://dx.doi.org/10.1146/annurev.ecolsys.38.091206.095504

Latreille, P.A. (1800) *Histoire naturelle des salamandres de France, précédée d'un tableau méthodique des autres reptiles indigènes*. Imprimerie de Crapelet, Paris, 138 pp.

Laurenti, J.N. (1768) *Specimen Medicum, Exhibens Synopsin Reptilium Emendatam cum Experimentis circa Venena et Antidota Reptilium Austriacorum*. J. Thomae Trattnern, Vienna, 238 pp.

Litvinchuk, S.N. & Borkin, L.J. (2009) *Evolution, systematics and distribution of crested newts (*Triturus cristatus *complex) in Russia and adjacent countries*. Evropeisky Dom Press, St. Petersburg, 590 pp.

Litvinchuk, S.N., Borkin, L.J., Džukić, G., Kalezić, M.L., Khalturin, M.D. & Rosanov, J.M. (1999) Taxonomic status of *Triturus karelinii* on the Balkans, with some comments about other crested newt taxa. *Russian Journal of Herpetology,* 6, 153–163.

Litvinchuk, S.N., Rosanov, J.M. & Borkin, L.J. (2007) Correlation of geographic distribution and temperature of embryonic development with the nuclear DNA content in the Salamandridae (Urodela, Amphibia). *Genome*, 50, 333–342.
http://dx.doi.org/10.1139/g07-010

Mertens, R. & Wermuth, H. (1960) *Die Amphibien und Reptilien Europas*. Verlag Waldemar Kramer, Frankfurt am Main, 252 pp.

Speybroeck, J., Beukema, W. & Crochet, P.-A. (2010) A tentative species list of the European herpetofauna (Amphibia and Reptilia) - an update. *Zootaxa,* 2492, 1–27.

Stoyanov, A., Tzankov, N. & Naumov, B. (2011) *Die Amphiben und Reptilien Bulgariens*. Chimaira, Frankfurt am Main, 582 pp.

Strauch, A. (1870) Revision der Salamandriden-Gattungen nebst Beschreibung einiger neuen oder weniger bekannten Arten dieser Familie. *Mémoires de l'Académie impériale des sciences de St.-Pétersbourg Serie 7, 16,* 17–426.

Swofford, D.L. & Selander, R.B. (1981) BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity,* 72, 281–283.

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, 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121

Wallis, G.P. & Arntzen, J.W. (1989) Mitochondrial-DNA variation in the crested newt superspecies: Limited cytoplasmic gene flow among species. *Evolution,* 43, 88–104.
http://dx.doi.org/10.2307/2409166

Wielstra, B. & Arntzen, J.W. (2011) Unraveling the rapid radiation of crested newts (*Triturus cristatus* superspecies) using complete mitogenomic sequences. *BMC Evolutionary Biology,* 11, 162.
http://dx.doi.org/10.1186/1471-2148-11-162

Wielstra, B. & Arntzen, J.W. (2012) Postglacial species displacement in *Triturus* newts deduced from asymmetrically introgressed mitochondrial DNA and ecological niche models. *BMC Evolutionary Biology,* 12, 161.
http://dx.doi.org/10.1186/1471-2148-12-161

Wielstra, B., Baird, A.B. & Arntzen, J.W. (2013a) A multimarker phylogeography of crested newts (*Triturus cristatus* superspecies) reveals cryptic species. *Molecular Phylogenetics and Evolution*, 67, 167–175.
http://dx.doi.org/10.1016/j.ympev.2013.01.009

Wielstra, B., Beukema, W., Arntzen, J.W., Skidmore, A.K., Toxopeus, A.G. & Raes, N. (2012) Corresponding mitochondrial DNA and niche divergence for crested newt candidate species. *PLoS ONE, 7,* e46671.
http://dx.doi.org/10.1371/journal.pone.0046671

Wielstra, B., Crnobrnja-Isailović, J., Litvinchuk, S.N., Reijnen, B.T., Skidmore, A.K., Sotiropoulis, K., *et al.* (2013b) Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. *Frontiers in Zoology,* 10, 13.
http://dx.doi.org/10.1186/1742-9994-10-13

Wielstra, B., Espregueira Themudo, G., Güclü, Ö., Olgun, K., Poyarkov, N.A. & Arntzen, J.W. (2010) Cryptic crested newt diversity at the Eurasian transition: The mitochondrial DNA phylogeography of Near Eastern *Triturus* newts. *Molecular Phylogenetics and Evolution,* 56, 888–896.
http://dx.doi.org/10.1016/j.ympev.2010.04.030

Wolterstorff, W. (1905) Zwergformen der paläarktischen Urodelen. *Compte-rendu des Séances du Sixième Congrès International de Zoologie, Tenu à Berne du 14 au 16 août 1904. W. Kündig & fils, Genève,* 258–263.
http://dx.doi.org/10.5962/bhl.title.15748

Wolterstorff, W. (1923) Übersicht den Unterarten und Formen des *Triton cristatus* Laur. *Blätter für Aquarien-und Terrarienkunde,* 34, 120–126.

Wolterstorff, W. (1925) Katalog der Amphibien-Sammlung im Museum für Natur- und Heimatkunde. *Abhandlungen und Berichte aus dem Museum für Natur- und Heimatkunde zu Magdeburg,* 4, 155–234.