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## Description of a new species of crested newt, previously subsumed in *Triturus ivanbureschi* (Amphibia: Caudata: Salamandridae)

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### Abstract

Multilocus molecular data play a pivotal role in diagnosing cryptic species (i.e. genetically distinct but morphologically similar species). A multilocus phylogeographic survey has provided compelling evidence that *Triturus ivanbureschi* *sensu lato* comprises two distinct gene pools with restricted gene flow. We conclude that this taxon had better be treated as two distinct (albeit morphologically cryptic) species. The name *T. ivanbureschi* should be restricted to the western species, which is distributed in western Asiatic Turkey plus the south-eastern Balkan Peninsula. No name is as yet available for the eastern species, which is distributed in northern Asiatic Turkey. We propose the name *T. anatolicus* sp. nov. for the eastern species and provide a formal species description.

**Key words:** gene flow, introgression, phylogeny, *Triturus anatolicus* sp. nov., *Triturus karelinii*

### Introduction

**Diagnosing cryptic species.** Speciation unaccompanied by morphological change has led to an underestimation of biodiversity (Bickford *et al.*, 2007; Pfenninger & Schwenk, 2007). With the advent of molecular data, many ‘cryptic species’—genetically distinct species that were previously unrecognized due to their morphological similarity—have been revealed (Beheregaray & Caccone, 2007; Bickford *et al.*, 2007). DNA barcoding is an important tool in the search for cryptic diversity, but deals with mtDNA exclusively (Hebert *et al.*, 2003). Although a phylogeographical survey with mtDNA provides an efficient manner to identify *potential* cryptic species, the biological significance of geographical populations with differentiated mtDNA should be explored before making taxonomical decisions (Balloux, 2010; DeSalle, 2006; Moritz & Cicero, 2004).

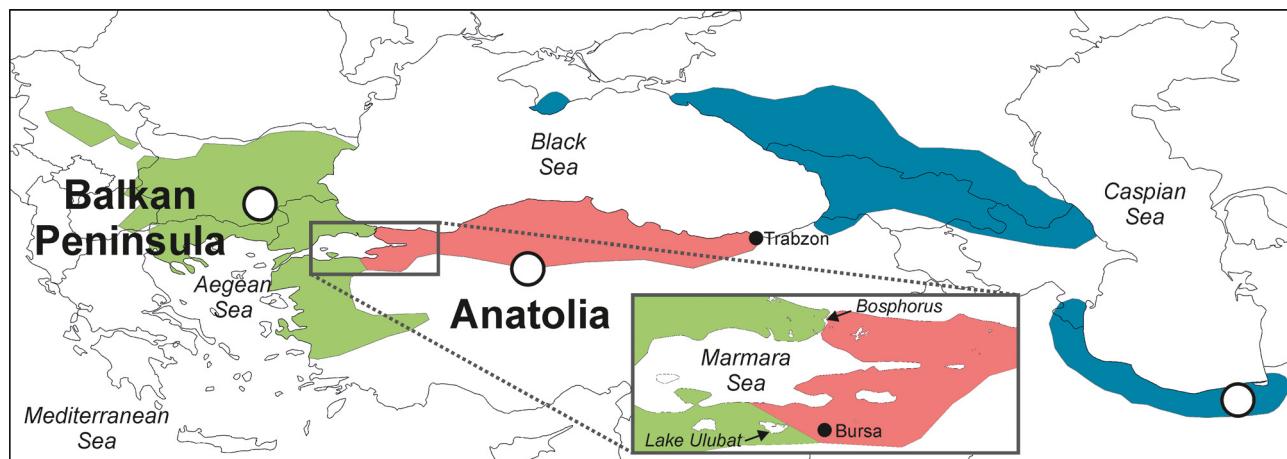
Indeed, mtDNA divergence may fail to reflect the presence of different species: a deep divergence that arose during historic allopatry can be accompanied by unlimited gene flow upon secondary contact (Pereira & Wake, 2009) and in species with low dispersal and/or small population sizes, phylogeographic breaks in mtDNA can originate in the face of unimpeded nuclear gene flow (Irwin, 2002). Even if mtDNA does reflect the presence of distinct cryptic species, its spatial distribution will regularly deviate from the actual species boundary (Toews & Brelsford, 2012). This is because mtDNA is relatively susceptible to introgression (Petit & Excoffier, 2009), and positive selection (Chan & Levin, 2005) or hybrid zone movement (Currat *et al.*, 2008) can subsequently cause a geographical asymmetry in mtDNA introgression to arise.

For candidate species identified based on differentiated mtDNA, the best evidence for evolutionary independence would be a congruent genetic divergence of many unlinked markers, distributed across the nuclear genome (Avise, 2000; Edwards, 2009). With the advent of next-generation sequencing, consulting a considerable number of nuclear markers for a large number of individuals of non-model species has come within reach (Ekblom & Galindo, 2011; Twyford & Ennos, 2012). We developed a next-generation sequencing protocol for marbled and crested newts, genus *Triturus* (Wielstra *et al.*, 2014a) that can be applied to address taxonomical questions. A

recent study confirmed that one currently recognized crested newt species actually consists of two cryptic species (Wielstra *et al.*, submitted). We present a taxonomic revision here.

**The crested newt *Triturus ivanbureschi* comprises two species.** *Triturus ivanbureschi* Arntzen and Wielstra 2013 (in Wielstra *et al.*, 2013b) comprises two candidate species and we refer to the collective as *T. ivanbureschi sensu lato*. The two candidate species comprising *T. ivanbureschi sensu lato* were initially identified based on their deep genetic divergence of mtDNA, on par with recognized crested newt species (Wallis & Arntzen, 1989; Wielstra *et al.*, 2010). There are no known morphological differences to distinguish the two (Arntzen, 2003; Ivanović *et al.*, 2013). A phylogeographical survey employing three nuclear introns provided support for the hypothesis of the two candidate species representing distinct species (Wielstra *et al.*, 2013a). Ambiguous separation based on allozyme data (Arntzen & Wielstra, 2010) and the observation of recent mtDNA introgression (Wielstra *et al.*, 2013a) supported a scenario of recent genetic interaction and suggested that the alternative hypothesis of ongoing gene flow could not be excluded (Wielstra *et al.*, 2013b).

A study employing 52 nuclear markers and samples from across the putative hybrid zone, confirms that gene flow between the two candidate species that make up *T. ivanbureschi sensu lato* is restricted (Wielstra *et al.*, submitted). Here we consider the two candidate species to represent distinct, albeit morphologically cryptic species. The first species covers the western part of the total range of *T. ivanbureschi sensu lato*, namely western Asiatic Turkey and the south-eastern Balkan Peninsula, and the second covers the eastern part, namely northern Asiatic Turkey. The two species meet at a hybrid zone in northwestern Asiatic Turkey (Wielstra *et al.*, submitted). An overview of the distribution of the two species is provided in Fig. 1 and a summary of the genetic data as analyzed with Structure 2.3.3 (Pritchard *et al.*, 2000) is provided in Fig. 2. The name *T. ivanbureschi sensu stricto* applies to the western species, considering that the type locality is in Bulgaria (Wielstra *et al.*, 2013b). As no name is as yet available for the eastern species (Wielstra *et al.*, 2013b) we provide one here, together with a formal species description.



**FIGURE 1.** The two cryptic species comprising *Triturus ivanbureschi sensu lato* are distributed in Asiatic Turkey and the south-eastern Balkan Peninsula. A rough distribution outline for *T. ivanbureschi sensu stricto* is shown in green and for *T. anatolicus sp. nov.* in red. The two taxa meet at a hybrid zone in northwestern Asiatic Turkey. A rough distribution outline for *T. karelinii sensu stricto* – with which *Triturus ivanbureschi sensu lato* until recently was considered to be conspecific – is in blue. Records of *Triturus* newts are absent from most of NE Turkey, suggesting that the ranges of *T. anatolicus sp. nov.* and *T. karelinii sensu stricto* do not meet. White dots represent type localities for, from left to right, *T. ivanbureschi sensu stricto*, *T. anatolicus sp. nov.* and *T. karelinii sensu stricto*.

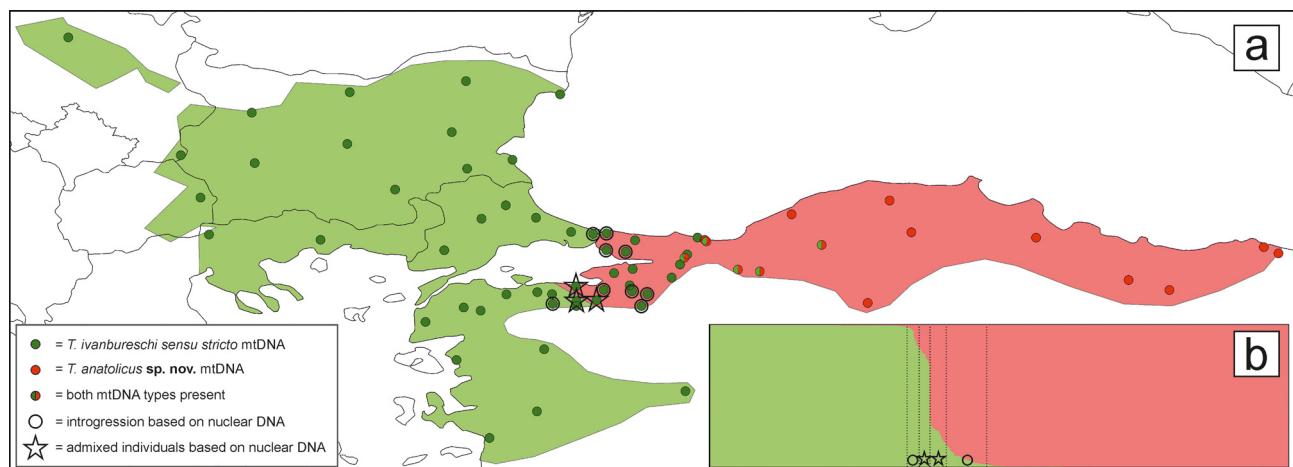
## Results

### Description of a new crested newt species

#### *Triturus anatolicus* sp. nov.

**Type material. Holotype.** RMNH.RENA.48232, an adult male (Fig. 3) from Gölköy, near Kalecik, Turkey

(coordinates: 40.077°N, 33.341°E, elevation: 1230 meter a.s.l.; Fig. 4), collected 6 April 2014 and donated to Naturalis Biodiversity Center by Kurtuluş Olgun from the Department of Biology at Adnan Menderes University, Aydın, Turkey. **Paratypes.** Six males (RMNH.RENA.48233-48238), six females (RMNH.RENA.48239-48245), and a juvenile (RMNH.RENA.48246), with collection details identical to the holotype. For imagery, see Fig. 3 and online Appendix 1 and 2.



**FIGURE 2.** Genetic data for *Triturus ivanbureschi* *sensu lato* presented in Wielstra *et al.* (submitted) illustrate the sharp geographical overturn between the genotypes representing its two constituent species. Panel (a) shows the 67 sampled populations as dots with the colored background representing a rough distribution outline of the two species based on nuclear DNA. Populations are colored according to the mtDNA type present; western populations of *Triturus anatolicus* sp. nov. possess mtDNA of *Triturus ivanbureschi* *sensu stricto*. Those populations harboring individuals showing introgression and genetically admixed individuals based on nuclear DNA (as defined below) are highlighted. Panel (b) shows a Structure plot representing  $Q$  scores with which the 428 sampled individuals were allocated to two different species ( $k = 2$  was the optimal number of gene pools inferred in the Structure analysis). The individuals are ordered based on the  $Q$  score with which they are allocated to *T. ivanbureschi* *sensu stricto*, from high to low values. Individuals allocated to their respective species with  $Q \geq 0.987$  are considered genetically pure. Individuals showing introgression are defined as being allocated to a species with  $0.987 > Q > 0.85$  and genetically admixed individuals as being allocated to either species with  $Q \leq 0.85$ . Those individuals that are not genetically pure are highlighted in panel (b). Note that nuclear introgression is geographically asymmetric.

**Diagnosis.** The new 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 (in males in breeding costume), a dark brown dorsal coloration and an orange ventral side covered with black spots. Based on mtDNA sequence data, crested newts from the type locality are firmly placed in the genus *Triturus*, within the distinct ‘central *T. karelinii* *sensu lato*’ mtDNA lineage (Wielstra *et al.*, 2010). Although identification based on mtDNA is possible across the majority of the range of the new species, mtDNA derived from *T. ivanbureschi* has introgressed at the western part of the range (Wielstra *et al.*, submitted; Fig. 2). The pattern of asymmetric introgression is mirrored by certain individual nuclear DNA markers and we consider this to represent a genomic footprint of hybrid zone movement (Wielstra *et al.*, submitted; Fig. 2). As yet only multilocus nuclear DNA sequence data distinguish *T. anatolicus* sp. nov. unambiguously from *T. ivanbureschi* – and from *T. karelinii*, with which both *T. anatolicus* sp. nov. and *T. ivanbureschi* were until recently considered conspecific (Wielstra *et al.*, submitted). No morphological features are currently known to distinguish the three species comprising *T. karelinii* *sensu lato*. How to separate *T. anatolicus* sp. nov., *T. ivanbureschi* and *T. karelinii* from the other crested newt species is discussed in Wielstra *et al.* (2013b).

**Description of holotype.** A relatively robust crested newt, with a broad head (wider than neck) and well developed limbs. Four fingers and five toes. Toes fringed but interdigital webbing absent. Relative length of fingers left  $1 < 4 < 2 < 3$ , right  $4 < 1 < 3 < 2$  (3 and 4 possibly regenerated). Relative length of toes on both sides  $1 < 5 < 2 < 4 < 3$ . Skin granulated on dorsal and lateral sides, tail base and throat; tail and venter smooth. Gular fold inconspicuous. Swollen cloaca with papillae bordering cloacal slit. Denticulated crest, indented 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 deep-orange ventral side and throat, dotted with small angular black spots; throat spots smaller and denser than belly spots. In the preserved state colors have slightly faded (see online Appendix 1).

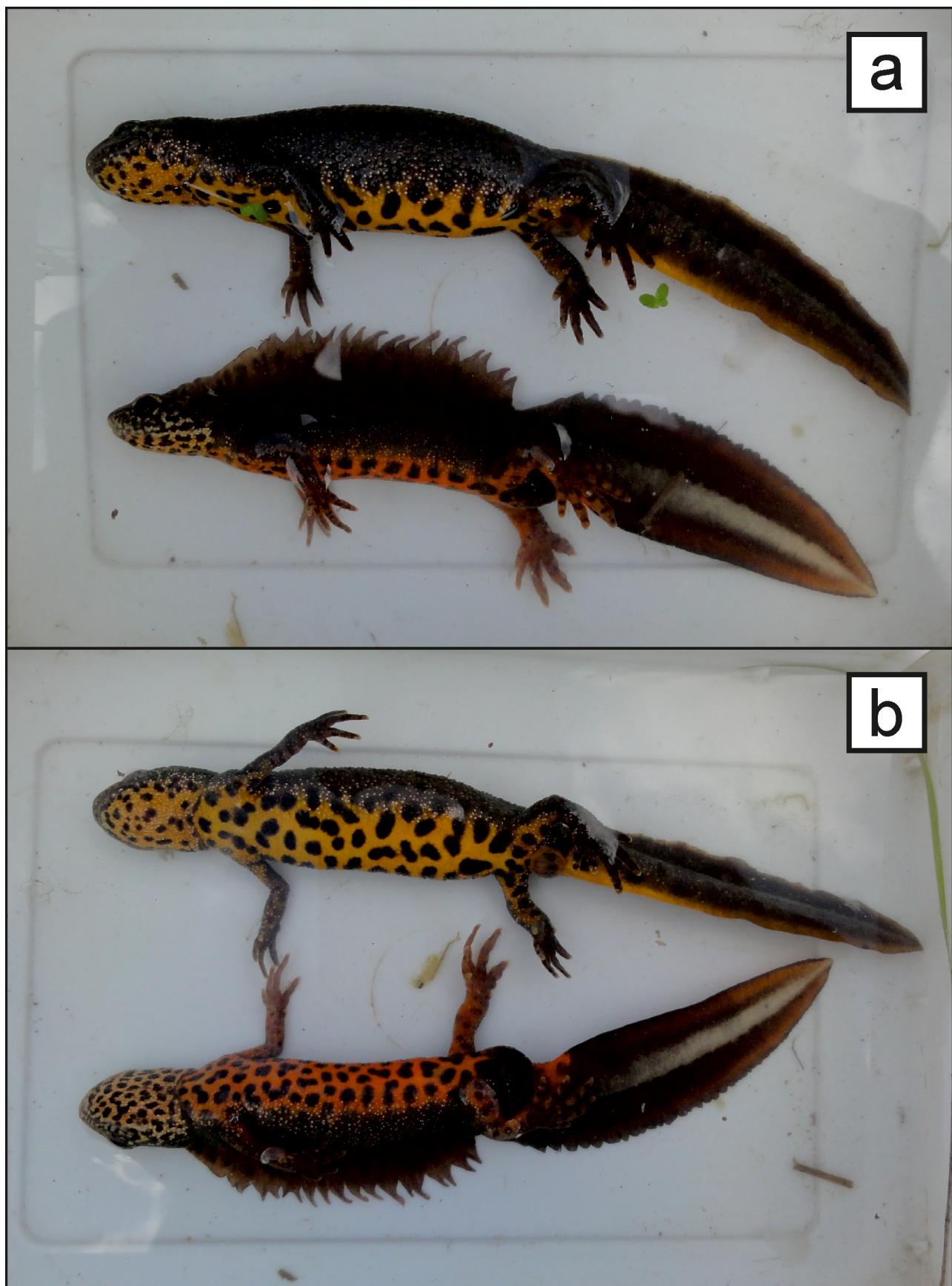
**TABLE 1.** Number of rib-bearing pre-sacral vertebrae and measurements (in millimeters) for the holotype and paratypes of *Triturus anatolicus* sp. nov.

Museum number	Sex	Material	NRBV	SVL1	SVL2	TL	ILD
RMNH.RENA.48232	male	holotype	13	53	61	108	27
RMNH.RENA.48233	male	paratype	13	63	71	126	34
RMNH.RENA.48234	male	paratype	12	53	60	110	29
RMNH.RENA.48235	male	paratype	13	53	61	109	26
RMNH.RENA.48236	male	paratype	13	50	58	100	27
RMNH.RENA.48237	male	paratype	13	56	64	115	30
RMNH.RENA.48238	male	paratype	13	53	60	110	29
RMNH.RENA.48239	female	paratype	13	57	62	118	31
RMNH.RENA.48240	female	paratype	13	62	68	133	32
RMNH.RENA.48241	female	paratype	13	58	63	122	33
RMNH.RENA.48242	female	paratype	13	58	62	123	30
RMNH.RENA.48243	female	paratype	13	62	67	117 **	33
RMNH.RENA.48244	female	paratype	13	65	70	131	36
RMNH.RENA.48245	female	paratype	13	56	60	111	31
RMNH.RENA.48246	juvenile	paratype	13	51	55	102	29

continued.

Museum number	Sex	Material	HL	HW	Arm	Finger3	Leg	Toe4
RMNH.RENA.48232	male	holotype	16.2	10.6	18.7	5.6 *	22.1	9.5
RMNH.RENA.48233	male	paratype	18.4	12.0	26.9	10.4	26.6	11.6
RMNH.RENA.48234	male	paratype	16.5	11.5	22.7	8.6	25.9	10.4
RMNH.RENA.48235	male	paratype	16.3	11.2	22.0	7.9	22.9	8.8
RMNH.RENA.48236	male	paratype	15.5	10.7	20.2	7.7	21.3	8.6
RMNH.RENA.48237	male	paratype	16.7	11.9	19.1	5.5	23.7	9.3
RMNH.RENA.48238	male	paratype	16.6	11.5	20.8	7.8	22.8	9.4
RMNH.RENA.48239	female	paratype	16.8	11.2	21.5	6.8	21.9	8.1
RMNH.RENA.48240	female	paratype	18.5	13.0	23.6	8.9	24.1	9.5
RMNH.RENA.48241	female	paratype	17.2	11.5	20.8	7.9	22.2	7.8
RMNH.RENA.48242	female	paratype	17.1	11.4	20.9	7.8	21.1	8.1
RMNH.RENA.48243	female	paratype	18.6	13.3	23.4	9.0	22.9	7.1
RMNH.RENA.48244	female	paratype	18.7	13.5	24.4	9.5	23.0	8.6
RMNH.RENA.48245	female	paratype	16.1	11.0	20.7	7.2	22.6	8.0
RMNH.RENA.48246	juvenile	paratype	15.2	10.3	17.6	6.6	18.6	6.7

Abbreviations used are: NRBV = Number of rib-bearing pre-sacral vertebrae; SVL1 = Snout-vent length up to and including the insertion of the hind-legs; SVL2 = Snout-vent length up to and including the cloaca; TL = 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. \* Finger c3/4 regenerated; \*\* Deformed tail tip.



**FIGURE 3.** The holotype and one of the paratypes of *Triturus anatolicus* sp. nov. in life. Lateral (a) and ventral (b) view of a male (below, holotype, RMNH.RENA.48232) and a female (above, paratype, RMNH.RENA.48239).



**FIGURE 4.** The type locality of *Triturus anatolicus* sp. nov. at Gölköy, Turkey.

The number of rib-bearing pre-sacral vertebrae (NRBV) is 13, as determined from X-ray photographs (see online Appendix 2). The following measurements are in millimeters and have been determined with plastic Vernier calipers. Snout-vent length up to and including the hind-legs 53; Snout-vent length up to and including the cloaca 61; Total length 108; Inter-limb distance 27; Head length 16.2; Head width 10.6; Length of the right arm including the third finger 18.7; Length of the right third finger 5.6; Length of the right leg including the fourth toe 22.1; Length of the right fourth toe 9.5.

**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 1). Compared to males (such as the holotype), adult females have non-swollen cloacae, lack the denticulated crest and possess a less pronounced tail fin. All paratypes have an NRBV count of 13 except one, which has an NRBV count of 12 (Table 1; online Appendix 2). In Table 1 the same measurements as taken for the holotype are provided for the paratypes.

**Distribution.** The distribution encompasses Asiatic Turkey south of the Black Sea, reaching up to c. 200 kilometers inland (usually less), but not into inner Anatolia. To the west the new species reaches the Bosphorus at the northern side of the Marmara Sea. On the southern side of the Marmara Sea it meets *T. ivanbureschi* sensu stricto, east of Lake Ulubat and west of the city of Bursa. The two species form a hybrid zone here (Wielstra *et al.*, submitted). To the east the new species reaches the town of Yomra, just east of the city of Trabzon. The nearest known *Triturus* localities further east are from the extreme NE of Turkey, over 150 km away, and probably concern *T. karelinii* sensu stricto (Wielstra *et al.*, 2013a). An outline of the distribution of the new species is provided in Fig. 1. A database of distribution records is provided in Wielstra *et al.* (2014b).

**Etymology.** The specific epithet reflects the distribution of the new *Triturus* species. *Triturus anatolicus* sp. nov. is endemic to Anatolia. It is the only *Triturus* species to which this applies. It should be noted that the range of *T. ivanbureschi* sensu stricto covers western Anatolia (Wielstra *et al.*, 2013a; Wielstra *et al.*, submitted) and the range of *T. karelinii* sensu stricto probably protrudes into northeastern Anatolia (Wielstra *et al.*, 2010).

**Proposed vernacular name.** We propose to use the vernacular name Anatolian Crested Newt for *T. anatolicus* sp. nov. This name highlights its status as an Anatolian endemic. We suggest to use the vernacular name Balkan Crested Newt for *T. ivanbureschi* sensu stricto (rather than Balkan-Anatolian Crested Newt previously applied to *T. ivanbureschi* sensu lato). Although *T. ivanbureschi* sensu stricto also partially occurs in Anatolia, the main part of its range is in the Balkan Peninsula.

## Conclusion

We have taken a cautious approach in revising the taxonomy of *T. karelinii* sensu lato. We first split the group into *T. karelinii* sensu stricto and *T. ivanbureschi* sensu lato and preferred to await a detailed analysis of the putative hybrid zone between the two candidate species within *T. ivanbureschi* sensu lato before considering whether further taxonomic change was warranted (Wielstra et al., 2013b). By applying a next-generation sequencing protocol for *Triturus* (Wielstra et al., 2014a) to a detailed sampling in and around the putative hybrid zone (Wielstra et al., submitted) we could confirm the existence of an as yet undescribed cryptic species in *T. ivanbureschi* sensu lato. This gave us the confidence to describe *T. anatolicus* sp. nov. in the present paper. Our studies on *Triturus* illustrate how ‘shallow genomics’ (Zilversmit et al., 2002), in which a comprehensive but tractable portion of the total genome is employed to approximate evolutionary history, can be a driving force in taxonomy. As yet no diagnostic morphological characters are known to distinguish the three species comprising *T. karelinii* sensu lato. This raises the question: are the three crested newt species truly cryptic, or have they not been studied closely enough? Considering that previous morphological studies have mainly focused on the species meeting in the Balkan Peninsula rather than *T. karelinii* sensu lato (Arntzen, 2003) we suspect the latter. A future morphological study, using the genetic results as a guidance, should prove illuminating.

## Online appendices

The following supplementary materials are available on the Zootaxa server:

**Online Appendix 1**—Pictures of the ventral side of the holotype and paratypes of *Triturus anatolicus* sp. nov.

**Online Appendix 2**—X-ray photographs of the holotype and paratypes of *Triturus anatolicus* sp. nov.

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