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Correspondence

Cracking cryptic species: external characters to distinguish two recently recognized banded newt species (*Ommatotriton ophryticus* and *O. nesterovi*)

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Cryptic species are species found to be genetically distinct, that previously went unrecognized due to their morphological similarity (Beheregaray & Caccone 2007, Bickford et al. 2007, Pfenninger & Schwenk 2007). With the exponential increase in genetic data, many cryptic species have been uncovered. However, their status as cryptic species would only be temporary if diagnostic morphological differences, corresponding to the genetically defined species boundary, were subsequently to be found. This way the genetic revolution could stimulate a renewed interest in morphology (Wielstra & Arntzen 2016).

Genetic studies in the family Salamandridae have regularly revealed cryptic species (e.g. Wielstra et al. 2013, Pabijan et al. 2017), with the most recent example provided by the banded newts (genus *Ommatotriton*) (Arntzen & Olgun 2000, Litvinchuk et al. 2005, Bülbül & Kutrup 2013, van Riemsdijk et al. 2017). A generally accepted split is *Ommatotriton vittatus* (Gray, 1835) versus *O. ophryticus* (Berthold, 1846). The latter has recently been further split into *O. ophryticus* sensu stricto (from here on *O. ophryticus*) and *O. nesterovi* (Litvinchuk, Zuiderwijk, Borkin & Rosanov, 2005). While morphological characters to distinguish *O. vittatus* from the other two species have been described (Table 3 in Borkin et al. 2003), how to separate *O. ophryticus* from *O. nesterovi* is currently unclear.

A superficial inspection of photographs taken in the field suggests two characters related to the 'band' in banded newts might be able to separate *O. ophryticus* from *O. nesterovi* (van Riemsdijk et al. 2018). In *O. nesterovi*, the lateral white band (**I**) appears to continue from the front limbs up to the eye and (**II**) appears to be disrupted by large specks on the tail (with a bluish hue in males), while this is not the case in *O. ophryticus* (Fig. 1). We conducted a survey of museum specimens of known origin to test the reliability of these characters to distinguish between *O. ophryticus* and *O. nesterovi*.

We surveyed adult specimens of *O. nesterovi* (n = 95 ♂♂; n = 160 ♀♀) and *O. ophryticus* (n = 181 ♂♂; n = 148 ♀♀) stored in the following museum collections: Zoological Museum of Adnan Menderes University (ZMADU), Aydın, Turkey; Karadeniz Technical University Zoology Research Laboratory (KZL), Trabzon, Turkey; Zoological Institute of the Russian Academy of Sciences, St. Petersburg (ZISP), Russia. Specimens could be unambiguously allocated to species based on their geographical origin, with *O. nesterovi* and *O. ophryticus*, as identified by genetic data, occurring west and east of the city of Samsun in Turkey (van Riemsdijk et al. 2017). For a detailed overview of material see the Supplementary material. For each specimen we determined whether the lateral white band continues from the front limbs up to the eye (Ia) or not (Ib), and whether the lateral white band is disrupted by large specks on the tail (IIa) or not (IIb). We also checked these characters in a less exhaustive survey of *O. vittatus* (n = 35 ♂♂; n = 73 ♀♀).

Table 1. Survey of two 'band characters' in the three banded newt species, genus *Ommatotriton*. For each species it is noted for how many individuals (I) the lateral white band continues from the front limbs up to the eye (Ia) or not (Ib), and (II) the lateral white band is disrupted by large specks on the tail (IIa) or not (IIb).

	All	Neck		Tail		♂♂	Neck		Tail		♀♀	Neck		Tail	
Species	n	Ia	Ib	IIa	IIb	n	Ia	Ib	IIa	IIb	n	Ia	Ib	IIa	IIb
O. nesterovi	255	247	8	250	5	95	87	8	94	1	160	160	0	156	4
O. ophryticus	329	72	257	32	297	181	49	132	17	164	148	23	125	15	133
O. vittatus	83	47	36	44	39	31	18	13	20	11	52	29	23	24	28

Results are summarized in Table 1 and Figure 2 and a detailed overview of the results is in the Supplementary material. We find that for almost all *O. nesterovi* the lateral white band continues from the front limbs up to the eye (Ia: 96.9% of total, 91.6% of ♂♂ and 100.0% of ♀♀) and the lateral white band is disrupted by large specks on the tail (IIa: 98.0% of total, 98.9% of ♂♂ and 97.5% of ♀♀). On the other hand, for the majority of *O. ophryticus* individuals the lateral white band does not continue from the front limbs up to the eye (Ib: 78.1% of total, 72.9% of ♂♂ and 84.5% of ♀♀) and the lateral white band is not disrupted by large specks on the tail (IIb: 90.3% of total, 90.6% of ♂♂ and 89.9% of ♀♀).

We see a higher frequency of occurrence of typical *O. nesterovi* character states in *O. ophryticus* than the other way around and most (but not all) of these morphological deviations occur close to the contact zone (Fig. 2). When such deviations are observed, the character state is often less extremely expressed as in the species for which it is typical. When looking at both characters in combination, there is 1 (0.4%) *O. nesterovi* individual (1 [1.1%] ♂) that show both character states typical for *O. ophryticus* and there are 8 (2.4%) *O. ophryticus* individuals (5 [2.8%] ♂♂ and 2 [1.4%] ♀♀) that show both character states typical for *O. nesterovi*.

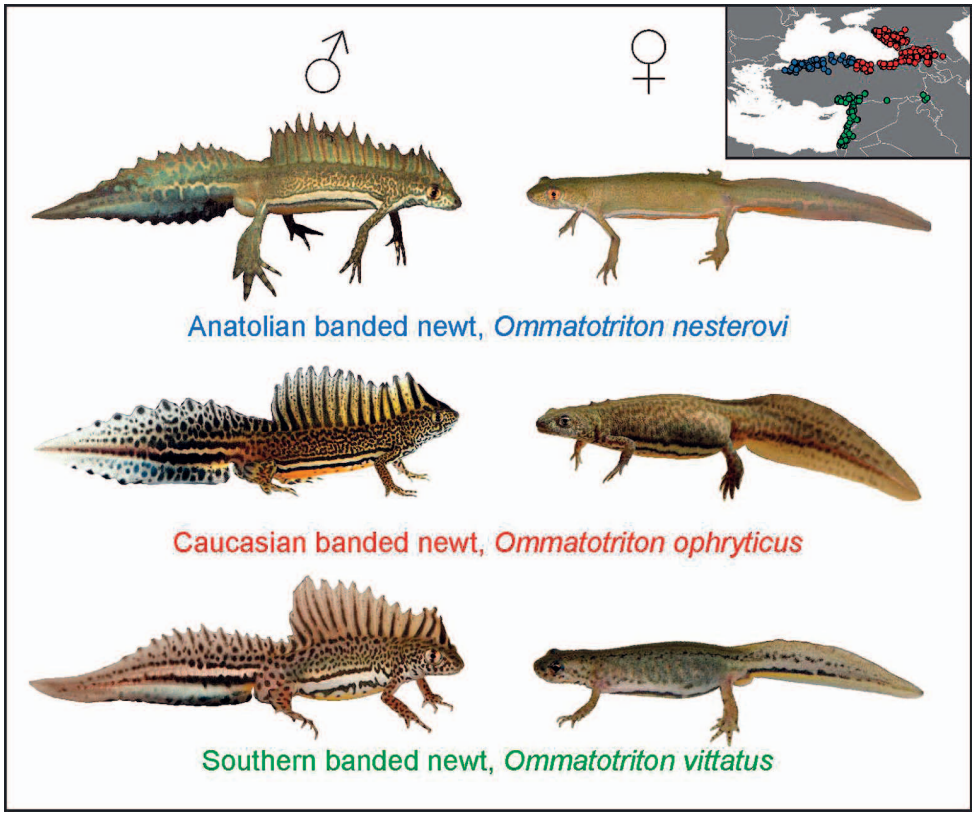

Figure 1. A male and female representative of each banded newt species, genus *Ommatotriton*, for which vernacular names are introduced here. The inset shows known localities for the three species, with colours corresponding to species names (based on van Riemsdijk et al. 2017).

Ommatotriton vittatus is easily distinguished from the other two species because the lateral white band is considerably broader (Fig. 1). The band only slightly more often than random extends from the front limbs towards the eye, as in *O. nesterovi* (although it is generally not so clearly demarcated), and is also only slightly more than random disrupted by specks on the tail, as in *O. nesterovi* (although smaller and never bluish).

We here studied the crypticness of the banded newts, a taxon that has had a turbulent taxonomical history, being formerly placed in a heavily polyphyletic *Triturus* as a single species, and later allocated to its own monotypic genus and split into first two and later three species (Arntzen & Olgun 2000, Litvinchuk et al. 2005, Steinfartz et al. 2007, Bülbül & Kutrup 2013, van Riemsdijk et al. 2017). We present two external characters related to the 'band', (I) whether the band continues from the front limbs up to the eye or not and (II) whether the band is disrupted by large specks on the tail (with a blue hue in males) or not, that distinguish *O. nesterovi* and *O. ophryticus* (Figs 1–2).

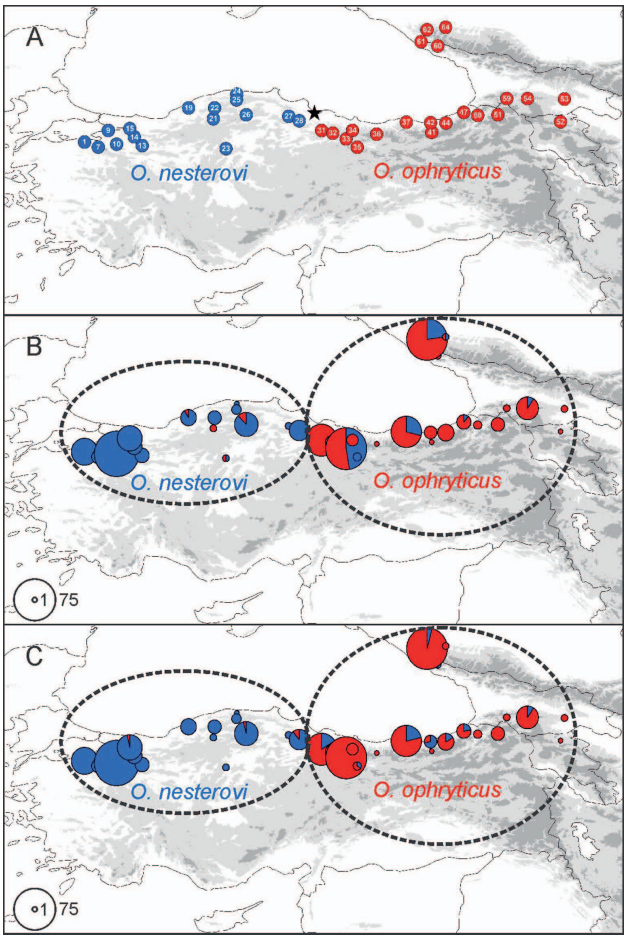

Figure 2. Geographical survey for two 'band' characters distinguishing two recently recognized banded newt species, genus *Ommatotriton*: Panel (A) shows the *O. nesterovi* (blue) and *O. ophryticus* (red) localities studied for morphology (with species allocation based on genetic data; van Riemsdijk et al. 2017); note that localities within 25 kilometres of each other have been merged; details are in the Appendix; the black star represents the city of Samsun. Panel (B) shows whether I the band continues from the front limbs up to the eye (Ia, typical for *O. nesterovi*, blue pie slice) or not (Ib, *O. ophtyricus*, red). Panel (C) shows whether II the band is disrupted by large specks on the tail (IIa, typical for *O. nesterovi*, blue pie slice) or not (IIb, *O. ophtyricus*, red). Pie diameter reflects sample size. Results partitioned by sex are in the Appendix.

We do notice the occurrence of character states typical of one of the two species present in the other at low frequency and more often see typical *O. nesterovi* character states in *O. ophryticus* than the other way around (Table 1). The deviation mostly occurs close to the contact zone (Fig. 2). As *O. ophryticus* and *O. nesterovi* are known to be able to hybridize under artificial conditions (van Riemsdijk et al. 2018), the mixed morphological signal we find might indicate that hybridization between the two species occurs in nature as well. Similarly, anthropogenic translocation might explain the localized occurrence of typical *O. nesterovi* characteristics in the Russian part of the range of *O. ophryticus* (Fig. 2). However, it should be noted that there is currently no evidence from genetic data for admixture between the two species in their natural range (van Riemsdijk et al. 2017) and the morphological 'deviation' we observe might well concern minor intraspecific variation.

Protecting biodiversity begins with recognizing it exists, so accurate species delineation plays an important role in conservation biology. With the massive increase in the availability of genetic data, many new species have been discovered that had not been previously recognized from morphology (Beheregaray & Caccone 2007, Bickford et al. 2007, Pfenninger & Schwenk 2007). Guided by such genetic insights, a fresh look can be taken from the point of morphology and 'cryptic species' may turn out to not be so cryptic after all.

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Supplementary material

Survey of two 'band' characters in the three banded newt species, genus *Ommatotriton*.