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Short Note

The position of the *Triturus carnifex-T. dobrogicus* newt hybrid zone in central Croatia compared to that of *Bombina* fire-bellied toads

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Abstract. Hybrid zones are research arenas where shared range boundaries allow testing the strength of reproductive isolation between related species. However, the ecological component in hybrid zone properties is frequently ignored. For lowland and upland species in the genera *Triturus* (large-bodied newts) and *Bombina* (fire-bellied toads) in central Croatia it is here shown that hybrid zones are displaced from the elevation gradient and instead align with lowland forest cover. Among species pairs, the resembling ecological profiles do not work out similarly, signifying a higher propensity for long distance and riverine dispersal in *B. bombina* than in *T. dobrogicus*. This line of research – one step up from the comparison of related species – constitutes a promising avenue towards the better understanding of pattern and process in the formation, development and maintenance of species hybrid zones and their ecological attributes.

Keywords: crested newts, ecology of hybrid zones, in situ hybrid zone comparison, parapatry, riverine dispersal corridors, selection against hybrids.

The broadly similar distributions of large-bodied newts (genus *Triturus*) and fire-bellied toads (genus *Bombina*) across central Europe, with hybrid zones that run approximately in parallel, provide a valuable opportunity for comparative hybrid zone research that is one step up from the mere comparison of species in hybrid zones. Whereas the *Bombina* hybrid zone has been studied extensively (see Zacho et al., 2025 for a review), comparable data for central European *Triturus* remain scarce. To address this gap, I document the position of the *Triturus carnifex-T. dobrogicus* hybrid zone in central Croatia, within an area for which detailed distributional and ecological data are available for *Bombina bombina* and *B. variegata* (Szymura, 1988; Nürnberger et al., 1995; Bugter et al., 1997; MacCallum et al., 1998; Atkinson, 2001). Similarities and differences between the two species pairs may provide insight into the role of landscape features on hybrid zone positioning and on species propensities for dispersal and colonization.

Nineteen populations of crested newts were sampled in 1996 along a transect in central Croatia (fig. 1), situated within the wider study area previously investigated for fire-bellied toads. Sampling included the Jastrebarsko lowlands, where *B. bombina* occurs as a pen-enclave, almost completely surrounded by *B. variegata* (Atkinson, 2001; Arntzen, 2025). Adult newts were captured by dip-netting and

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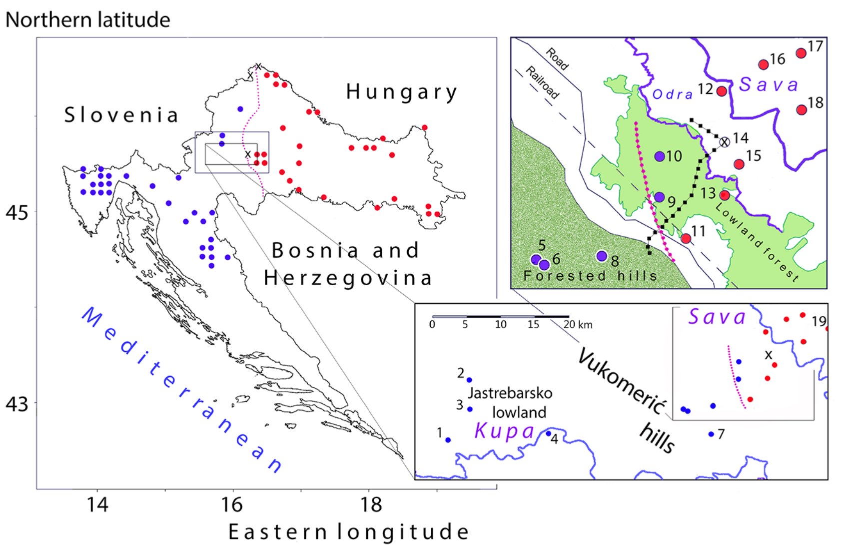

Figure 1. Documented occurrences of *Triturus carnifex* (blue symbols) and *T. dobrogicus* (red symbols) in Croatia (Croatian Biologer Community, 2019; data available at https://biologer.hr/groups/15/species/171%20and%20172). Data presentation follows the UTM10 grid cell system. Three cells with both species are shown by a cross. The interrupted purple line shows the centre of the species hybrid zone as estimated in the literature (van Eden et al., 2026). First inset: study localities in the Jastrebarsko lowland area (populations 1-4) and the transect populations 7 and 19, relative to the Vukomerić hills and the rivers Kupa and Sava. The area covered corresponds to the yellow box of fig. 2. Second inset: populations at (14, crossed) and adjacent to the hybrid zone centre plotted over a forest map with the blotched line approximating the centre of the hybrid zone. Note that this hybrid zone position is at odds with the projection from the literature. The base map is redrawn from Bugter et al. (1997).

identified using colour pattern characteristics (Arntzen and Wallis, 1999; Fahrbach and Gerlach, 2018) and morphometrics. Morphological variation was quantified with the Wolterstorff index (Arntzen and Wallis, 1994), a measure of body shape based on forelimb length relative to trunk length. The index has low values in the slender-bodied *T. dobrogicus*, higher values in the more robust *T. carnifex* and intermediate values for species hybrids (Arntzen, 2003: Table 30).

Genetic data were obtained from freshly deposited eggs by enzyme electrophoresis at two diagnostic loci, *Gpi* with two common and two rare alleles and *Pgm* with two common alleles. Experimental procedures followed Rafiński and Arntzen (1987), and observed allele frequencies are presented in table A1 in the Appendix. Genetic variation was summarized by Principal Coordinate Analysis (PCoA) implemented in Adegenet (Jombart, 2008), to obtain individual scores on the first axis. Distributional data for *Bombina* were gathered from the literature (Nürnberger et al., 1995; MacCallum et al., 1998; Atkinson, 2001).

High Wolterstorff index values were recorded in eight individuals from populations 5 and 9, identifying them as *T. carnifex*, whereas low values in 15 individuals from populations 12, 13 and 15 identified *T. dobrogicus*. One female from population 11 showed an intermediate value (table A2 in the Appendix). Genetic data were obtained from all 19 populations ($5 \leqslant N \leqslant 18$; $\Sigma N = 260$). The first PCoA axis explained 89.8% of the total variation. Individual scores were strongly bimodal, with ten populations showing negative values (mean -1.60, standard deviation 0.34, $N = 133$) and eight populations showing positive values (1.84 ± 0.60, $N = 114$), corresponding to the morphologically identified *T. carnifex* and *T. dobrogicus*, respectively. Population 14 was intermediate (0.24 ± 0.82, $N = 13$), indicating

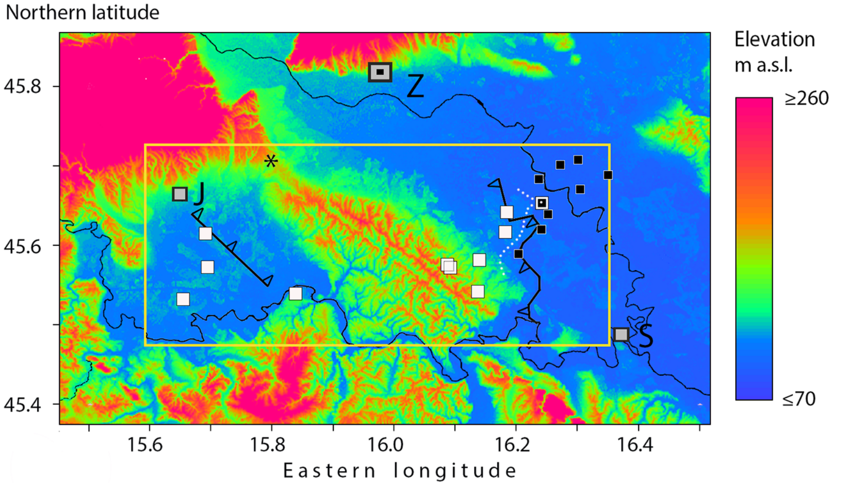

Figure 2. Distribution of *Triturus carnifex* (large open square symbols) and *T. dobrogicus* (small solid square symbols) over the study transect in-between Zagreb (Z) and Sisak (S) and the Jastrebarsko (J) area plotted over an elevation map (see colour legend). The hybrid population 14 is shown by symbols combined. The white stippled line shows the approximate position of the *T. carnifex*-*T. dobrogicus* contact zone. The solid black lines show the central location of the *Bombina bombina*-*B. variegata* hybrid zone with triangles indicating the *B. variegata* side. For the extent of the *B. bombina* pen-enclave in the Jastrebarsko area see Arntzen (2025: Supporting Information 3). The asterisk indicates a saddle over the Vukomerić hills that is a potential corridor for lowland species to disperse into to the Jastrebarsko area, alternative to the valley of the Kupa river (for discussion, see text). The base map is from Arntzen (2025).

admixture between the species (table A1 and fig. A1 in the Appendix). The two species are spatially separated with *T. carnifex* in the Jastrebarsko area and at the hilly side of the transect whereas *T. dobrogicus* is found adjacent to the river Sava (fig. 1). The contact zone between *T. carnifex* and *T. dobrogicus* in central Croatia is situated between the Vukomerić hills and the river Sava, in agreement with previous studies. Its general position corresponds with the hybrid zone centre inferred from geographically dispersed genetic samples (van Eden et al., 2026). However, some lowland forest populations are not *T. dobrogicus*, as predicted, but *T. carnifex* (9 and 10) or genetically admixed (14) (fig. 1). Although the study transect was intended to be linear, the inevitable spatial scatter in sampling localities revealed an angular species contact in which the *T. carnifex* range exhibits a prong towards the Sava. This extension of the *T. carnifex* range relative to the Vukomerić hills coincides with an extensive lowland forest. Distributional data for fire-bellied toads show that the *B. variegata* range bulges out similarly, at the expense of *B. bombina*, also following forest cover more closely than the elevation gradient (Bugter et al., 1997; MacCallum et al., 1998; Arntzen, 2025) (fig. 2). However, neither *T. carnifex* nor *B. variegata* occupy the lowland forest deeper towards Sisak (see fig. 2) and it would be interesting to model the cross-forest position of hybrid zones for species pairs in concert.

In spite of the positional congruence observed at the transect area, the comparison of *Triturus* and *Bombina* also shows a remarkable difference, because the Jastrebarsko area in the Kupa river drainage is occupied by the lowland species *B. bombina* where it is not accompanied by its ecological counterpart *T. dobrogicus* but by *T. carnifex*. The local distribution of *B. bombina* strongly suggest that colonization of the Jastrebarsko area was along the Kupa river and not, for example, over the saddle of the Vukomerić hills (fig. 2). Neither of these potential dispersal corridors seem to have been used by *T. dobrogicus*. I see two explanations for the observed discrepancy, as follows.

While the lowland habitat characteristics of *B. bombina* and *T. dobrogicus* are broadly similar (Vörös et al., 2016), this may not apply to their propensity for long-distance and riverine dispersal. Already von Méhély (1905) noted the presence of *B. bombina* inside *B. variegata* territory, at an elevation distance of ca. 400 m from the *Bombina* hybrid zone (cf. Arntzen et al., 2025). As noted, *B. bombina* made it into the Jastrebarsko lowlands along a riverine corridor whereas *T. dobrogicus* did not. Similarly, *B. bombina* colonized Transylvania, central Romania, along rivers (Arntzen, 2025) and *T. dobrogicus* did not (cf. Cogălniceanu et al., 2013), to which it may be noted that it concerns interactions with the different species *T. cristatus*.

The other explanation is that the ecological signatures of the *Triturus* and *Bombina* hybrid zones are not always coinciding as they are in central Croatia. The two-species distribution modelling by Vörös et al. (2016) indicates that *B. bombina* reaches higher up elevated areas than *T. dobrogicus*. The difference between the species pairs amounts to ca. 20 km with more of a patchy aspect in *Triturus* than in *Bombina* (Vörös et al., 2016). These spatial-ecological characteristics may render the Jastrebarsko area more difficult to reach for *T. dobrogicus* than for *B. bombina*.

A recurrent question in hybrid zone research is if paired species (such as *T. carnifex-T. dobrogicus* and *B. variegata-B. bombina*) are in competition. This is a meaningful question because competition limits where species can (co)exist and determines whether the hybrid zone is stable or moving. Removal experiments could be conclusive to determine the degree of niche overlap among contributing species, but are in vertebrates not realistically achievable. However, if it concerns species engaging in a *moving* hybrid zone such as in *Bombina* (Arntzen, 1978, 2025) competition is likely because the area vacated by the regressing species (emulating removal) is filled by the advancing species, indicating niche overlap and suggestive of competition. Alternatively, competition has been invoked through the occupancy of niches typical of the counterpart species in allopatry, but this argument loses significance with the distance involved because eco-geographical variation could be at play. The *T. carnifex-T. dobrogicus* hybrid zone is said to be stable (van Eden et al., 2026) and would therewith be uninformative on niche overlap dynamics and/or competition. However, as argued above, the absence of *T. dobrogicus* from the nearby Jastrebarsko lowland is best explained by restricted accessibility rather than environmental unsuitability, consistent with the hypothesis of interspecific competition.

The association of abutting range borders with an elevation gradient can come as no surprise when lowland species (*T. dobrogicus*, *B. bombina*) are compared with upland species (*T. carnifex*, *B. variegata*) but the more stringent association of *T. carnifex* at the species interface with lowland forestation than with elevation, as in *Bombina variegata*, is a novel finding, to which it must be noted that the *Triturus* component of the replicated hybrid zone system is in need of more data. It would also be worthwhile to study wider areas (if not central Europe entirely), to see if and how the landscape affects the newt and toad hybrid zones in concert. Important differences between the species pairs are though to be expected. Whilst hybrid zone widths are not widely different among genera (3.1 km in *Triturus* and 5.1 km in *Bombina*), estimates on the effective selection against hybrids differ by an order of magnitude (0.018 versus 0.170), in line with a four-fold difference in lifetime dispersal estimates (0.21 km in *Triturus* versus 0.89 km in *Bombina*) (Szymura and Barton, 1986; van Eden at al., 2026; see Barton and Gale, 1993). These intrinsic differences among counterpart species pairs engaging in hybrid zones may also play a role in the reconstruction of their post-glacial recolonization histories, from whenever (and wherever) range borders turned from allopatric to parapatric.

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Appendix

Table A1. Information on the position of 19 crested newt populations studied in central Croatia along with sample sizes, allele frequencies observed over two enzyme loci, the genetic information condensed along the first axis of a Principal Coordinate Analysis (PCoA1) and the allocated species status.

Population No.	Longitude (E)	Latitude (N)	Sample size	*Gpi*				*Pgm*		PCoA1	Inferred *Triturus* species
				a	*b*	*c*	*d*	*a*	*b*		
1	15.651	45.534	15	0.97	0.00	0.00	0.03	1.00	0.00	−1.726	*carnifex*
2	15.692	45.618	15	1.00	0.00	0.00	0.00	1.00	0.00	−1.758	*carnifex*
3	15.693	45.576	5	1.00	0.00	0.00	0.00	1.00	0.00	−1.758	*carnifex*
4	15.838	45.543	14	0.96	0.00	0.04	0.00	1.00	0.00	−1.723	*carnifex*
5	16.090	45.575	11	1.00	0.00	0.00	0.00	1.00	0.00	−1.566	*carnifex*
6	16.093	45.575	15	0.80	0.00	0.00	0.20	1.00	0.00	−1.424	*carnifex*
7	16.138	45.543	15	1.00	0.00	0.00	0.00	0.83	0.17	−1.758	*carnifex*
8	16.141	45.583	15	0.87	0.03	0.10	0.00	0.97	0.03	−1.528	*carnifex*
9	16.185	45.619	15	0.83	0.13	0.00	0.03	0.93	0.07	−1.326	*carnifex*
10	16.185	45.642	13	0.92	0.08	0.00	0.00	1.00	0.00	−1.605	*carnifex*
11	16.205	45.591	18	0.06	0.94	0.00	0.00	0.08	0.92	1.964	*dobrogicus*
12	16.237	45.685	12	0.00	1.00	0.00	0.00	0.21	0.79	1.824	*dobrogicus*
13	16.240	45.622	9	0.28	0.72	0.00	0.00	0.22	0.78	1.242	*dobrogicus*
14	16.242	45.654	13	0.58	0.42	0.00	0.00	0.53	0.46	0.243	Intermediate
15	16.251	45.641	15	0.30	0.67	0.00	0.03	0.00	1.00	1.609	*dobrogicus*
16	16.273	45.703	15	0.10	0.90	0.00	0.00	0.00	1.00	2.042	*dobrogicus*
17	16.301	45.709	15	0.07	0.93	0.00	0.00	0.39	0.61	1.298	*dobrogicus*
18	16.305	45.671	15	0.00	1.00	0.00	0.00	0.00	1.00	2.241	*dobrogicus*
19	16.356	45.690	15	0.00	1.00	0.00	0.00	0.00	1.00	2.241	*dobrogicus*

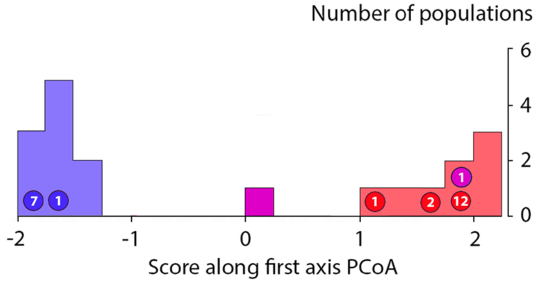

Figure A1. Distribution of scores along the first axis of a Principal Coordinate Analysis (PCoA) on genetic data. Populations 1-10 are shown in blue and are composed of *Triturus carnifex*; populations 11-13 and 15-19 are shown in red and are composed of *T. dobrogicus*. The genetically intermediate population 14 is shown in purple. Populations studied for the Wolterstorff index are indicated by round symbols with colours corresponding to phenotype and with numbers in white showing sample sizes.

Table A2. Unprocessed morphometric data on 24 adult crested newts (13 males and 11 females) in six populations across the *Triturus carnifex-T. dobrogicus* hybrid zone in central Croatia.

Population number	Sex	Forelimb length (mm)*	Interlimb distance (mm)	Wolterstorff index
5	Female	24.2	44	55.0
5	Female	22.6	38	59.5
5	Female	25.4	41	62.0
5	Male	20.7	33	62.7
5	Male	23.4	37	63.2
5	Male	23.5	34	69.1
5	Male	27.8	33	84.2
9	Male	23.7	37	64.1
11	Female	21.7	43	50.5
12	Female	21.0	57	36.8
12	Female	15.0	38	39.5
12	Female	22.0	55	40.0
12	Female	19.0	42	45.2
12	Female	22.0	48	45.8
12	Male	19.0	51	37.3
12	Male	19.0	41	46.3
12	Male	20.0	43	46.5
12	Male	20.5	41	50.0
12	Male	23.0	43	53.5
12	Male	26.0	47	55.3
12	Male	24.0	41	58.5
13	Female	18.4	51	36.1
15	Female	20.5	42	48.8
15	Male	22.5	44	51.1

*Forelimb distance measured with callipers, interlimb distance measured on plasticised millimetre paper.