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Authors: Mikulíček, Peter, Horák, Aleš, Zavadil, Vít, Kautman, Ján, and Piálek, Jaroslav

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Hybridization between three crested newt species (*Triturus cristatus* superspecies) in the Czech Republic and Slovakia: comparison of nuclear markers and mitochondrial DNA

Peter MIKULÍČEK[1,2], Aleš HORÁK[3,4], Vít ZAVADIL[5], Ján KAUTMAN[6] and Jaroslav PIÁLEK[2]

[1] *Comenius University, Faculty of Natural Sciences, Department of Zoology, Mlynská dolina B-1, 842 15 Bratislava, Slovak Republic; e-mail: pmikulicek@fns.uniba.sk*
[2] *Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, 603 65 Brno, Czech Republic; e-mail: jpialek@brno.cas.cz*
[3] *Biology Centre of Academy of Sciences of the Czech Republic, v.v.i., Institute of Parasitology, Branišovská 31, 370 05 České Budějovice, Czech Republic; e-mail: ogar@paru.cas.cz*
[4] *University of South Bohemia, Faculty of Sciences, Department of Molecular Biology, Branišovská 31, 370 05 České Budějovice, Czech Republic*
[5] *ENKI, o.p.s., Dukelská 145, 379 01 Třeboň, Czech Republic; e-mail: arnoviza@seznam.cz*
[6] *Slovak National Museum – Museum of Natural History, Department of Zoology, Vajanského nábrežie 2, 814 36 Bratislava, Slovak Republic; e-mail: zoolog@snm.sk*

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Abstract. Crested newts (*Triturus cristatus* superspecies) are a group of closely related species with parapatric distributions that are likely to interbreed where their ranges meet. Coexistence of three species of the complex (*Triturus cristatus*, *T. dobrogicus* and *T. carnifex*) has been recently confirmed in central Europe. In this study we aim to elucidate the distribution of crested newts in contact zones in the Czech Republic and Slovakia, and determine the extent of hybridization and introgression using nuclear (microsatellites and Randomly Amplified Polymorphic DNA, RAPD) and mitochondrial DNA (mtDNA) markers. Nuclear markers reveal hybrid zones between *T. cristatus* and *T. dobrogicus* at the foothills of the Carpathians in southern Slovakia, and between *T. cristatus* and *T. carnifex* in the southern parts of the Czech Republic. Analysis of mitochondrial cytochrome *b* sequences reveals *T. cristatus* and *T. dobrogicus*-specific haplotypes in contact zones in southern Slovakia. Surprisingly, most *T. carnifex* and individuals with mixed ancestry between *T. carnifex* and *T. cristatus* possess haplotypes specific for *T. dobrogicus*, most likely as a result of historical mtDNA introgression. Only one *T. carnifex*-specific haplotype carried by a single specimen is found in the Czech Republic. Our study shows that genetic structure of central European populations of crested newts is complex and influenced by historical and contemporary hybridization.

Key words: hybrid zone, introgression, mtDNA, microsatellites, RAPD, Salamandridae

Introduction

Natural hybridization is considered to be an important evolutionary phenomenon, which can drive reinforcement of mating preferences and give rise to fully isolated species, or alternatively, can result in the origin of new evolutionary lineages (for current review see the Marie Curie SPECIATION Network 2012). Even though the evolutionary mechanisms involved in the process of hybridization are universal, the outcomes of particular hybridization episodes can differ. Hybridization thus can lead to the origin of natural hybrid taxa with sexual or

asexual reproduction, introgression of genetic traits without the establishment of a hybrid zone, or more commonly, to the formation of transition hybrid zones (Arnold 1997, Allendorf et al. 2001). Most studies focusing on natural hybridization rely on the analysis of two species (subspecies, chromosomal races or genetically divergent populations; e.g. Lukáčová et al. 1994, Babik et al. 2003, Yanchukov et al. 2006, Macholán et al. 2007, Bailey et al. 2012, Horn et al. 2012). However, comparatively few studies have addressed patterns of hybridization between two taxa, which can exchange genetic material indirectly *via* a third species (Grant et al. 2005, Alves et al. 2008, McDonald et al. 2008, Keck & Near 2009, Nevado et al. 2011). Comparison of three or more hybridizing species with different levels of genetic divergence and ecological isolation can shed light on the evolution of reproductive isolating barriers maintaining species boundaries and on the role of natural/sexual selection and genetic drift in speciation (Coyne & Orr 2004).

Of the six currently recognized crested newt species of the *Triturus cristatus* superspecies group (Steinfartz et al. 2007, Espregueira Themudo et al. 2009, Wielstra et al. 2010, Wielstra & Arntzen 2011), three of them live in central Europe: *Triturus cristatus* (Laurenti, 1768), *Triturus dobrogicus* (Kiritzescu, 1903) and *Triturus carnifex* (Laurenti, 1768). Their present distribution is parapatric (Fig. 1) with contact zones occurring in central Europe and the Balkans (Wallis & Arntzen 1989, Crnobrnja-Isailović et al. 1997, Arntzen & Wallis 1999, Mikulíček et al. 2004, Maletzky et al. 2008, Arntzen & Wielstra 2010). The most northerly and widely distributed *T. cristatus* is found from the British Isles to the Ural Mountains, *T. dobrogicus* is restricted to the lowlands of the Middle and Lower Danube River basins, and *T. carnifex* is distributed from the Apennine Peninsula and the northern Adriatic part of the Balkans to central Europe (Arntzen & Borkin 1997, Arntzen 2003). Crested newts diverged rapidly during the late Miocene in the Balkan Peninsula. Divergence time between sister species *T. cristatus* and *T. dobrogicus* was estimated at ca. 8.8 Ma, the split between *T. carnifex* and *T. cristatus/T. dobrogicus* was estimated at ca. 9.3 Ma (Wielstra & Arntzen 2011). Crested newt species differ phenotypically and ecologically. *Triturus dobrogicus*

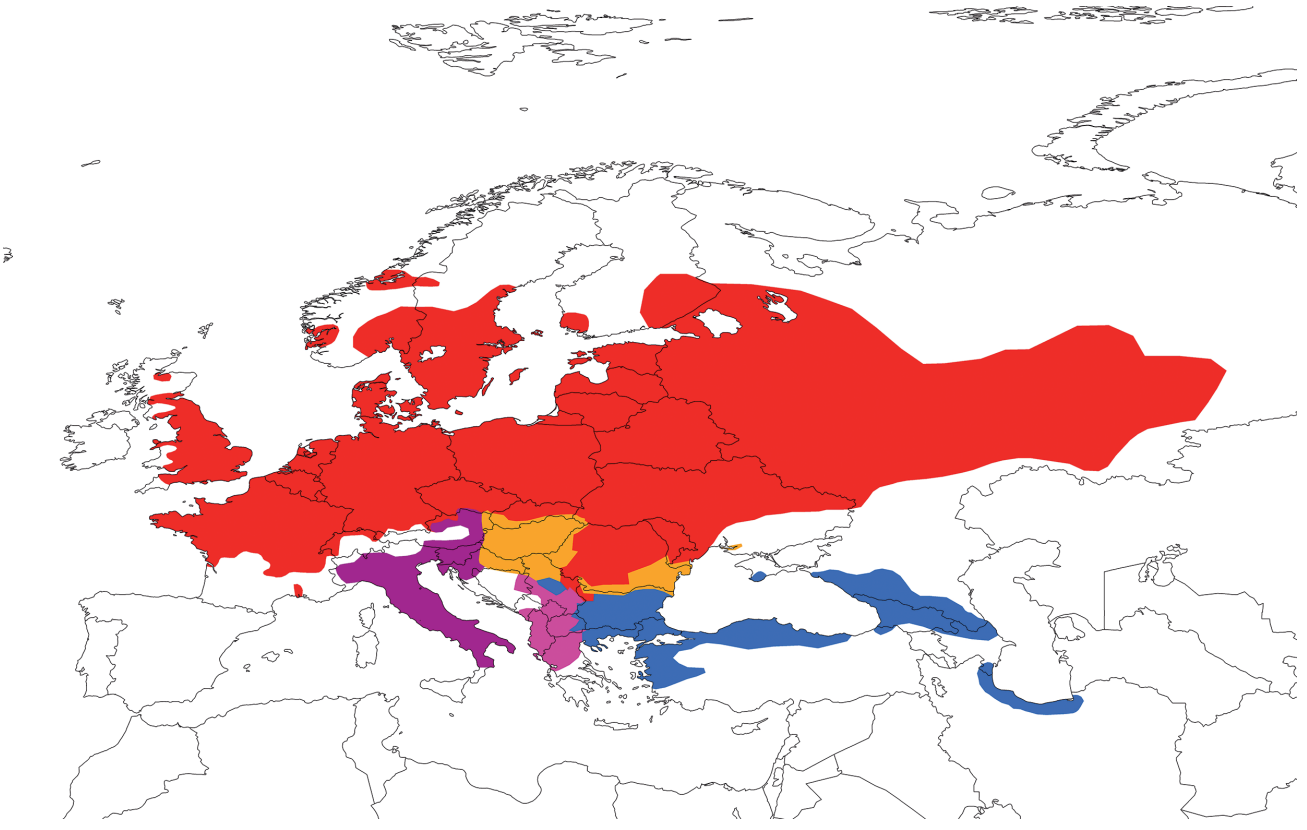

Fig. 1. *The distribution of the* Triturus cristatus *superspecies. Three crested newt species,* T. cristatus *(red),* T. dobrogicus *(orange) and* T. carnifex *(purple), come into contact in central Europe.* Triturus macedonicus *(pink) and* T. karelinii group *(blue) are distributed in the Balkans, Turkey and the Caucasian-Caspian region. The map is based on Wielstra & Arntzen (2011) and was kindly provided by Ben Wielstra.*

has relatively slender body and short legs, *T. carnifex* is relatively robust and has long legs, and *T. cristatus* reveals intermediate phenotype with medium built body and medium sized legs. It can be expected that the relative length of the trunk, tail and limbs in the crested newts is important for locomotion in terrestrial and aquatic environment and is associated with habitat selection. For instance, the robust bodies with longer limbs can be advantageous for more efficient terrestrial movement, while a longer tail can increase swimming speed (Arntzen & Wallis 1999, Gvoždík & Van Damme 2006, Vukov et al. 2011). These eco-morphological predictions fit with the ecology of the crested newt taxa. *Triturus dobrogicus* is the most aquatic species, annually spending almost six months in water (Jehle et al. 1997). An aquatic phase of *T. cristatus* and *T. carnifex* lasts five and four months, respectively (Arntzen & Wallis 1999).

Table 1. *Sampling sites, their abbreviations, the country, coordinates and number of crested newts analyzed for nuclear markers (n_{nuc}) and mtDNA (n_{mtDNA}). n/a – not available.*

Sampling site	Acronym	Country	Latitute	Longitude	n_{nuc}	n_{mtDNA}
Tuscany – Massa Marittima	TUS	Italy	43° 01′ N	10° 53′ E	2	1
Fülöpháza	FUL	Hungary	46° 53′ N	19° 24′ E	15	3
Matena	MAT	Slovenia	45° 58′ N	14° 31′ E	21	5
Uppsala	UPP	Sweden	59° 49′ N	17° 40′ E	3	3
Rapšach – Bosna	BOS	Czech Republic	48° 53′ N	14° 56′ E	5	5
Čertoryje	CER	Czech Republic	49° 27′ N	17° 23′ E	4	n/a
Chlumec	CHL	Czech Republic	49° 03′ N	15° 28′ E	1	n/a
Citonice	CIT	Czech Republic	48° 53′ N	15° 56′ E	7	3
Hostim	HOS	Czech Republic	49° 01′ N	15° 54′ E	1	1
Horní Slatina	HS	Czech Republic	49° 05′ N	15° 33′ E	5	5
Horní Újezd	HU	Czech Republic	49° 09′ N	15° 50′ E	3	n/a
Jevišovice	JEV	Czech Republic	48° 59′ N	15° 59′ E	3	n/a
Kurovice	KUR	Czech Republic	49° 17′ N	17° 30′ E	11	3
Lanžhot	LAN	Czech Republic	48° 43′ N	16° 58′ E	5	5
Mašovice	MAS	Czech Republic	48° 52′ N	15° 59′ E	14	3
Moravský Krumlov	MKR	Czech Republic	49° 02′ N	16° 19′ E	1	n/a
Nové Mlýny	NM	Czech Republic	48° 51′ N	16° 44′ E	1	1
Nová Říše	NR	Czech Republic	49° 09′ N	15° 35′ E	10	n/a
Pastviny	PAS	Czech Republic	50° 04′ N	16° 33′ E	10	3
Podmolí	POD	Czech Republic	48° 51′ N	15° 55′ E	8	3
Řečice	REC	Czech Republic	49° 08′ N	15° 21′ E	3	4
Sedlec	SED	Czech Republic	49° 10′ N	16° 07′ E	1	n/a
Tasovice	TAS	Czech Republic	48° 49′ N	16° 09′ E	12	3
Třebětice	TRB	Czech Republic	49° 03′ N	15° 30′ E	7	4
Třebohostice	TRE	Czech Republic	50° 02′ N	14° 44′ E	11	3
Únanov	UNA	Czech Republic	48° 53′ N	16° 07′ E	11	1
Zblovice	ZBL	Czech Republic	48° 57′ N	15° 42′ E	10	3
Žerůtky	ZER	Czech Republic	48° 55′ N	15° 58′ E	19	3
Beša	BES	Slovakia	48° 32′ N	21° 57′ E	1	n/a
Bratislava – Čunovo	CUN	Slovakia	48° 01′ N	17° 12′ E	2	n/a
Devínske jazero	DJ	Slovakia	48° 17′ N	16° 57′ E	19	4
Domica	DOM	Slovakia	48° 28′ N	20° 28′ E	7	2
Jovsa	JOV	Slovakia	48° 48′ N	22° 04′ E	10	3
Krasňany (Žilina)	KRA	Slovakia	49° 12′ N	18° 53′ E	2	n/a
Leles	LEL	Slovakia	48° 28′ N	22° 01′ E	1	1
Prešov	PRE	Slovakia	48° 59′ N	21° 15′ E	12	n/a
Revúca	REV	Slovakia	48° 41′ N	20° 07′ E	7	n/a
Bratislava – Rusovce	RUS	Slovakia	48° 03′ N	17° 09′ E	20	3
Silica	SIL	Slovakia	48° 34′ N	20° 32′ E	6	1
Svätá Mária	SVM	Slovakia	48° 27′ N	21° 49′ E	15	1
Komjatice – Torozlín	TOR	Slovakia	48° 09′ N	18° 12′ E	1	n/a
Teplý vrch	TV	Slovakia	48° 28′ N	20° 08′ E	11	2
Veľký Blh	VB	Slovakia	48° 26′ N	20° 06′ E	19	3
Veškovce	VES	Slovakia	48° 33′ N	22° 06′ E	17	3

Triturus dobrogicus is restricted to lowlands where gene flow among populations is high because of floods and continuous habitats along rivers (Vörös & Arntzen 2010). *Triturus cristatus* and *T. carnifex* inhabit mainly plains and hilly areas, though the latter species can occupy also mountainous regions in the Alps (Arntzen 2003). Eco-morphological divergence can result in restrictions to interspecific hybridization between the crested newts in parapatric regions.

The goal of the present study was to elucidate distribution of *T. cristatus*, *T. dobrogicus* and *T. carnifex* in contact zones in the Czech Republic and Slovakia and explore the occurrence and geographical extent of interspecific hybridization. To this aim we used three types of molecular markers, differing in their mode of inheritance: nuclear highly polymorphic microsatellites, nuclear species-specific Randomly Amplified Polymorphic DNA (RAPD) and matrilineal mtDNA.

Material and Methods

Sampling

Newts (n = 354) were caught in 44 sampling sites during the breeding season in the years 1997-2003 (Table 1). A tail tip or a finger was removed and stored in 96 % ethanol. To establish allele frequencies of microsatellite loci in the parental species, reference populations located away of the contact zones were sampled in Slovenia and Italy (*T. carnifex*), southern Slovakia and Hungary (*T. dobrogicus*), and the northern part of the Czech Republic, northern Slovakia and Sweden (*T. cristatus*). For analysis of mtDNA, we also used reference sequences of cytochrome *b* of *T. cristatus*, *T. dobrogicus*, *T. carnifex*, *T. macedonicus*, *T. arntzeni*, *T. karelinii* and *T. marmoratus* originating from the study of Steinfartz et al. (2007).

DNA markers and laboratory techniques

Total genomic DNA was extracted following standard procedures including proteinase K treatment and phenol-chloroform extraction.

An approximately 380 bp long fragment of mitochondrial cytochrome *b* (*cytb*) was amplified using TRI*cytb*-f (5′-CCTCACAGGCCTATTCCTAGC-3′) and TRI*cytb*-r (5′-TAGAAGAGATACCTGTTGGGT-3′) primers under the PCR conditions described in Maletzky et al. (2008). Amplicons of expected length were gel-purified and both strands were sequenced using ABI Prism technology. Sequence reads were edited and assembled using Editseq and SeqMan software (part of DNAStar suite). The nucleotide contigs were translated into amino acids, checked for presence of open reading frame disrupting indels, aligned using ClustalW and back-translated using BioEdit 7.09 (Hall 1999). Sequences were deposited in GenBank under the accession numbers EU030807-EU030937.

Eighteen diagnostic RAPD markers (Table 2) and seven microsatellite loci (*Tcri13*, *Tcri29*, *Tcri32*, *Tcri35*, *Tcri36*, *Tcri43*, and *Tcri46*; Krupa et al. 2002) were used as nuclear markers. Diagnostic RAPDs and PCR conditions were chosen according to Mikulíček & Piálek (2003). Analysis of microsatellites was performed according to Mikulíček et al. (2007).

Table 2. *RAPD markers specific for the crested newt species. The symbol "+" indicates that the marker was present in a species.*

Primer	Marker (bp)	*T. cristatus*	*T. dobrogicus*	*T. carnifex*
OPA-07	700	+		
	720		+	
OPA-08	700		+	
	790		+	
OPA-15	850			+
OPA-16	700		+	
OPA-17	820	+		
	900	+		
	950		+	
OPA-18	700	+		
	710		+	
OPA-19	950			+
	1000	+		
OPA-20	600		+	+
	880	+		
OPD-12	300	+		
	700		+	+
	900	+		

Data analysis

Phylogenetic analyses based on cytochrome b *sequence variation*

A Bayesian phylogenetic tree (BI) was constructed in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) under the GTR + I + G model of evolution with the Markov chain set to 2×10^6 generations, every 100th tree was sampled and the first 5×10^5 generations were omitted from phylogeny reconstruction. Maximum parsimony (MP) non-parametric bootstrap support was calculated using PAUP* 4.0b10 (Swofford 2003). Maximum likelihood (ML) bootstrapping was performed under the HKY + I + G (second model according to the Akaike Information Criterion) as implemented in Modeltest (Posada & Crandall 1998) using Phyml 2.4.4 (Guindon & Gascuel 2003). Bootstraps were computed from 1000 replicates.

Hybrid index based on RAPD markers

Species-specific RAPD markers were used for computation of arithmetic hybrid indices (HI) between species pairs *T. cristatus* × *T. dobrogicus* and *T. cristatus* × *T. carnifex*. Both species pairs were analyzed separately because no introgression of RAPD markers was found between *T. dobrogicus* and *T. carnifex* (see results). "Pure" *T. cristatus* individuals were given a score of 1, "pure" *T. dobrogicus* or *T. carnifex* individuals, respectively, were given a score of 0. The lack of any *T. dobrogicus*/*T. carnifex* marker or the presence of any *T. cristatus* marker increased the index value of a particular individual up to a maximum of 1. To compare association between RAPD and microsatellite markers in estimation of admixture, the correlation between HI scores (based on RAPDs) and the admixture proportion of each individual (*q* according to Pritchard et al. 2000; based on the analysis of microsatellites with $K = 3$ implemented in Structure 2.3.3) was calculated using Spearman rank order correlation.

Genetic diversity at microsatellite loci and estimation of admixture

Number of alleles, allele frequencies, and observed (H_O) and expected (H_E) heterozygosity were calculated using GenAlEx 6.1 (Peakall & Smouse 2006). Hardy-Weinberg (HW) equilibrium and linkage disequilibria (LD) were computed using GENEPOP 3.3 (Raymond & Rousset 1995). Deviations from HW equilibrium were measured by coefficient of inbreeding – F_{IS} (Weir & Cockerham 1984). Negative F_{IS} values indicated heterozygote excess, positive values indicated heterozygote deficiency. Estimation of exact P-values of F_{IS} was performed using Markov chain algorithm based on 10^4 iterations. The significance of LD between pairs of loci was computed using the Fisher's exact test through 10^4 iterations.

Two model-based methods were used to estimate the proportion of admixture from multilocus genotype data applying a Bayesian approach implemented in the programs Structure 2.3.3 (Pritchard et al. 2000) and NewHybrids 1.1 beta (Anderson & Thompson 2002). Structure analysis was performed only with microsatellite data. The ancestry of newts was investigated assuming that each hybrid genotype belongs to more than one of the three inferred clusters ($K = 3$), corresponding to *T. cristatus*, *T. dobrogicus* and *T. carnifex* (c.f. Randi & Lucchini 2002, Pierpaoli et al. 2003, Gay et al. 2007). In a first procedure, all individuals were assigned to the inferred clusters without any *prior* population information. In a second procedure, *prior* population information for individuals from reference populations was used. Individuals from populations in or close to presumable contact zones were assigned to the clusters without using any *prior* information. All Structure analyses were based on runs of 10^6 iterations, following a burn-in period of 10^4 iterations.

NewHybrids 1.1 beta (Anderson & Thompson 2002) was used to compute the posterior probability that an individual in a sample belongs to one of four defined hybrid classes (F1, F2, and backcross hybrids with one or another parental species) or two parental species. The analysis was performed with microsatellite and RAPD data simultaneously. Data for *T. cristatus* × *T. dobrogicus* and *T. cristatus* × *T. carnifex* populations were analyzed separately. Individuals from reference populations were *a priori* assigned as pure parental genotypes and were not considered to be a part of the admixture. Each dataset was analyzed five times and probability scores were based on runs of 10^6 iterations, following a burn-in period of 10^4 iterations. Only individuals assigned to a particular genotype class with a probability $P \geq 0.900$ were considered in the NewHybrids analyses.

Results

Analysis of mtDNA haplotypes

We characterized 379 bp of *cytb* gene for 105 individuals belonging to the *T. cristatus* superspecies and one *T. marmoratus* specimen. With the exclusion of the outgroup (i.e. *T. marmoratus*, *T. karelinii* and *T. arntzeni*) used for rooting the phylogenetic trees, we identified 42 haplotypes, defined by 63 polymorphic sites (49 transitions, 13 transversions, no indels). The reference sequences of *T. dobrogicus* (Bulgaria, Hungary, Romania), *T. carnifex* (Slovenia, Italy) and *T. cristatus* (Romania, UK) originating from this study and work of Steinfartz et al. (2007) were used to identify the mitochondrial *cytb* haplotypes of central European samples. The Bayesian-based phylogeny separated the central European specimens into three clades, corresponding to *T. cristatus*, *T. dobrogicus* and *T. carnifex* (Fig. 2). The first split was between *T. carnifex*/*T. macedonicus* and other two species. *Triturus cristatus* and *T. dobrogicus* represented sister clades. The bootstrap support for basal groups was rather low in any of the tree reconstructing methods employed (ML, MP, BI), however, they all gave consistent topology, differing only by the mutual position of *T. karelinii* and *T. arntzeni* (irrelevant for the purpose of this study).

Sequences of reference populations all grouped with species-specific haplotype clades (Table 3). However,

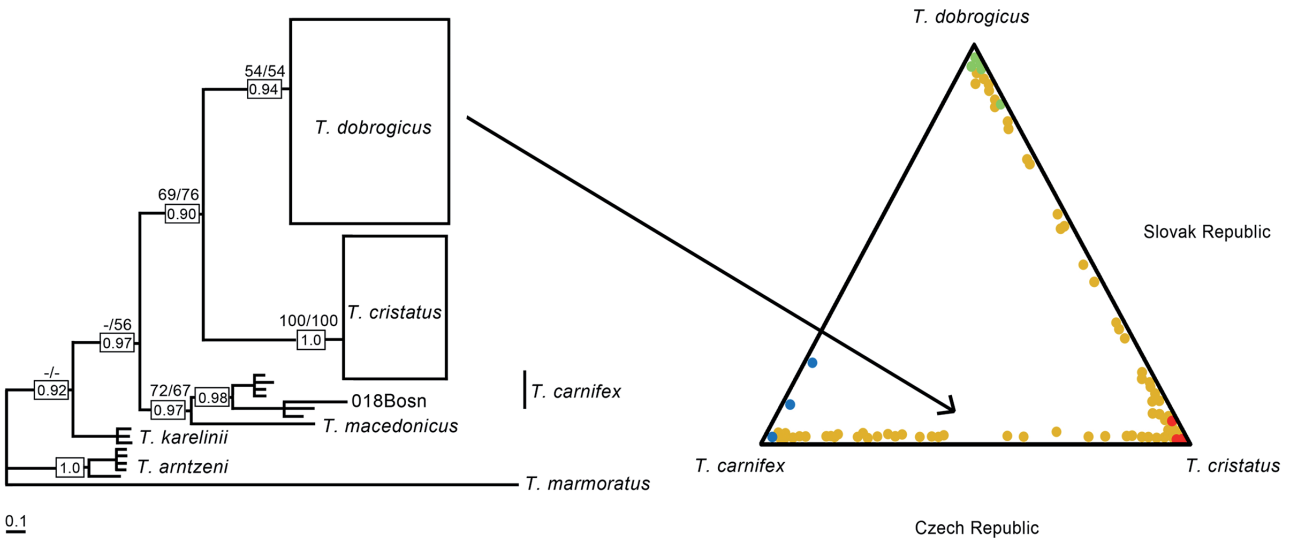

Fig. 2. *Bayesian phylogenetic tree of crested newts based on cytochrome* b *sequences and the triangle plot of an admixture parameter* q *(Structure) based on microsatellites (see Material and Methods for details). Each individual is represented by a colour point. Parental species* T. cristatus *(red),* T. dobrogicus *(green) and* T. carnifex *(blue) were assigned to a particular cluster using* prior *population information. No* prior *information was used for individuals from potential contact zones (orange points). An arrow indicates that* T. carnifex *from the southern part of the Czech Republic and* T. carnifex × T. cristatus *hybrids possessed predominantly* T. dobrogicus*-specific mtDNA.*

the position of haplotypes from near the possible contact zones was less straightforward (Table 3, Fig. 3 and 4). Samples from southwestern Moravia with "*carnifex*"-like morphology (Piálek et al. 2000) had predominantly "*dobrogicus*" *cytb* sequence. Further to the west, three localities with mixture of "*dobrogicus*" and "*cristatus*" haplotypes (HS, REC, TRB), and one with "*carnifex*" and "*dobrogicus*" (BOS), were

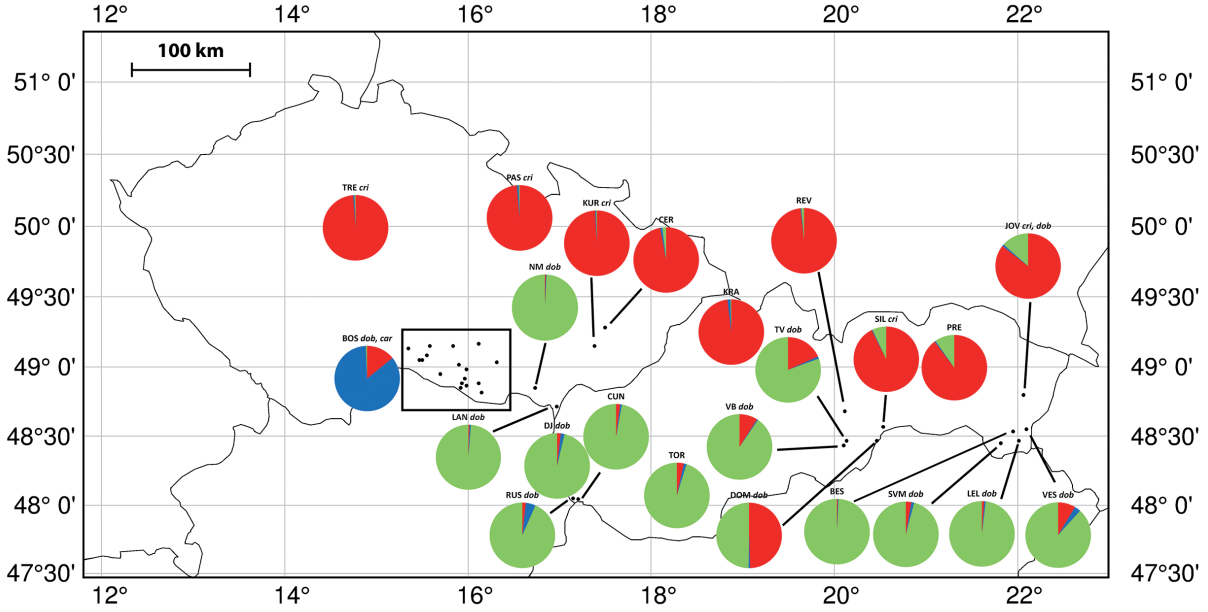

Fig. 3. *The proportion of admixture (*q*) between* T. cristatus *(red),* T. dobrogicus *(green) and* T. carnifex *(blue) in the Czech and Slovak populations estimated on the basis of microsatellite data. Species-specific mtDNA haplotypes (*cri, dob, car*) are indicated above the pie charts. A detailed genetic structure of the populations from the southern part of the Czech Republic (an open frame) is given in the Fig. 4.*

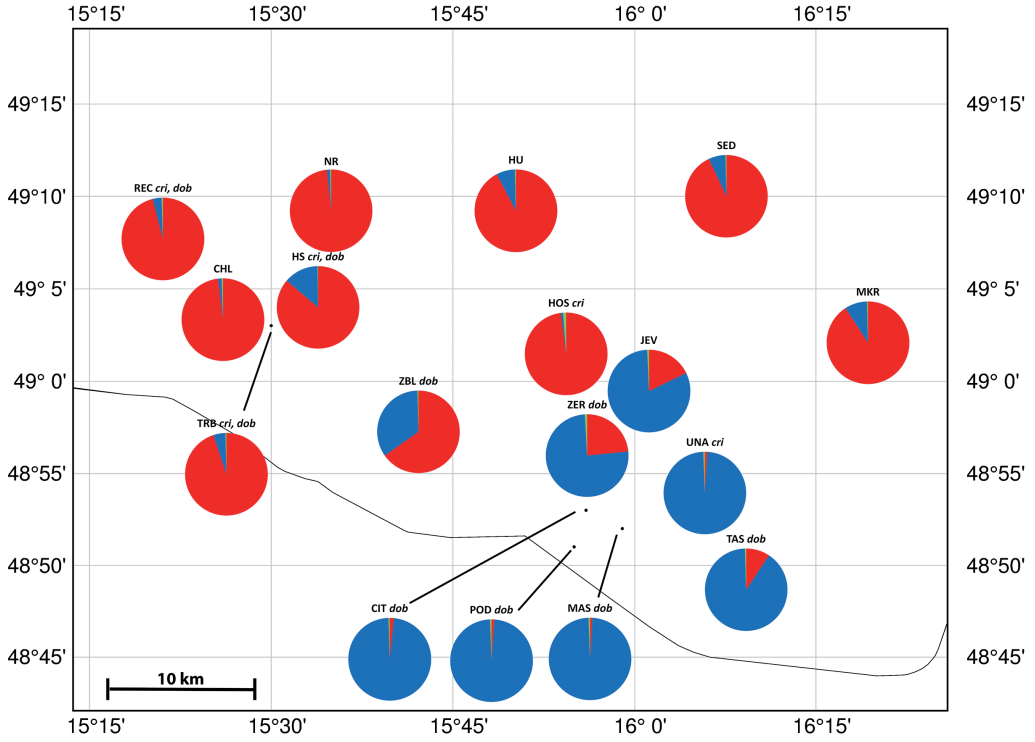

Fig. 4. *The proportion of admixture (*q*) between* T. cristatus *(red),* T. dobrogicus *(green) and* T. carnifex *(blue) in the southern part of the Czech Republic estimated on the basis of microsatellite data. Species-specific mtDNA haplotypes (*cri, dob, car*) are indicated above the pie charts. Individuals 018BOSN and 588ZBL were not included in estimation of the admixture (see Results).*

detected. Only one *T. carnifex*-specific haplotype carried by a single specimen from BOS (018BOSN) was found in the Czech Republic. In Slovakia the only locality with a mixed haplotype profile ("*cristatus*" and "*dobrogicus*") was JOV; other populations from Slovakian contact zones contained either "*cristatus*" or "*dobrogicus*" haplotypes.

Hybrid index based on RAPDs

Newts from reference populations possessed only species-specific RAPD markers compared to individuals from contact zones that possessed markers of *T. cristatus* and *T. dobrogicus* (Slovakia) or *T. cristatus* and *T. carnifex* (southern part of the Czech Republic; Table 3). Individuals possessing both *T. dobrogicus* and *T. carnifex* markers were not detected.

Genetic diversity at microsatellite loci

In the crested newt populations all loci were highly polymorphic, showing 15-66 different alleles per locus (total number of alleles 248, average per locus 35.4, SD 17.2; Table 4). Locus *Tcri*32 was not amplified in *T. dobrogicus* and *T. carnifex*. *Triturus dobrogicus* had the highest number of alleles, followed by *T. cristatus* and *T. carnifex*.

All loci but two (*Tcri*13 and *Tcri*36) showed significant heterozygote deficit (positive F_{IS} values) likely caused by the presence of null alleles at least in some reference samples (Appendix 1). Significant deficit of heterozygotes was observed predominately in *T. dobrogicus* populations (Table 3). Average values of heterozygosity were slightly lower in *T. cristatus* (H_O = 0.571, H_E = 0.562) than *T. carnifex* (H_O = 0.611, H_E = 0.656) and *T. dobrogicus* (H_O = 0.707, H_E = 0.824). Pairwise linkage disequilibria (LD), estimated over all reference samples within a species, were sufficiently low to assume the studied microsatellite loci are physically unlinked or freely recombining. Linkage disequilibria within populations cannot be estimated because of limited sample sizes. Within populations situated in contact zones, significant deficit of heterozygotes estimated over loci (Table 3, Appendix 1) was observed in JOV, PRE and SIL (*T. cristatus* × *T. dobrogicus* contact zone), and BOS, POD and ZBL (*T. cristatus* × *T. carnifex* contact zone).

Estimation of admixture

Newts from reference populations were assigned to the correct Structure clusters (corresponding to the parental species) with a probability *q* 0.883-0.992,

Table 3. *Summary of population genetic structure of crested newts based on microsatellites, species-specific RAPDs and mtDNA.* H_O *– observed heterozygosity;* H_E *– expected heterozygosity;* F_{IS} *– coefficient of inbreeding and test for heterozygote deficiency;* q-cri, q-car, q-dob *– probability of each individual to belong to one of the three inferred clusters corresponding to the parental species* T. cristatus, T. carnifex *and* T. dobrogicus *(mean* q *values per population);* HI^{RAPD} *– hybrid index based on RAPDs averaged per population (1.000 – "pure"* T. cristatus*, 0.000*^{D} *– "pure"* T. dobrogicus*, 0.000*^{C} *– "pure"* T. carnifex*, values in italics indicate* T. cristatus × T. dobrogicus *admixture, roman values between 0 and 1 indicate* T. cristatus × T. carnifex *admixture); Genotype class (NewHybrids:* car, cri, dob *– parental* T. carnifex, T. cristatus, T. dobrogicus, car-*bx*cri *–* T. carnifex *backcrossed with a hybrid* T. carnifex × T. cristatus, cri-*bx*car, cri-*bx*dob *–* T. cristatus *backcrossed with a hybrid* T. cristatus × T. carnifex *and* T. cristatus × T. dobrogicus, dob-*bx*cri *–* T. dobrogicus *backcrossed with a hybrid* T. dobrogicus × T. cristatus*; mtDNA – species-specific haplotypes.*

Site	H_O	H_E	F_{IS}	*q-cri*	*q-car*	*q-dob*	HI^{RAPD}	Genotype class	mtDNA
MAT*	0.611	0.656	0.069^{NS}	0.005	0.988	0.007	0.000^{C}	*car*	*car*
TUS*	-	-	-	0.006	0.502	0.492	0.000^{C}	*car*	*car*
MAS	0.495	0.551	0.069^{NS}	0.009	0.985	0.006	0.166	*car, car*-bx*cri*	*dob*
CIT	0.551	0.626	0.121^{NS}	0.016	0.979	0.005	0.257	*car*-bx*cri*, F2	*dob*
UNA	0.586	0.495	$–0.194^{NS}$	0.010	0.984	0.006	0.319	*car*-bx*cri*	*cri*
POD†	0.338	0.580	0.392***	0.011	0.983	0.006	0.300	*car*-bx*cri*, F2	*dob*
TAS	0.423	0.464	0.059^{NS}	0.093	0.902	0.005	0.291	*car*-bx*cri*, F2	*dob*
ZER	0.655	0.713	0.066^{NS}	0.234	0.757	0.009	0.401	*car*-bx*cri*, F2	*dob*
BOS	0.634	0.760	0.187**	0.141	0.853	0.006	0.578	F2	*car, dob*
JEV	-	-	-	0.178	0.815	0.007	0.700	F2	n/a
ZBL†	0.604	0.664	0.095*	0.652	0.344	0.004	0.654	*cri, car*-bx*cri*	*dob*
HS	0.571	0.613	0.075^{NS}	0.862	0.136	0.002	0.820	*cri*-bx*car*	*cri, dob*
REC	-	-	-	0.960	0.035	0.005	0.850	*cri*	*cri, dob*
TRB	0.601	0.627	0.036^{NS}	0.949	0.047	0.004	0.874	*cri*	*cri, dob*
NR	0.608	0.550	$–0.116^{NS}$	0.984	0.012	0.004	0.950	*cri*	n/a
HU	-	-	-	0.923	0.073	0.004	1.000	*cri*	n/a
KUR	0.555	0.510	$–0.089^{NS}$	0.991	0.005	0.004	1.000	*cri*	*cri*
PAS*	0.671	0.601	$–0.125^{NS}$	0.986	0.010	0.004	1.000	*cri*	*cri*
CHL	-	-	-	0.981	0.014	0.005	1.000	*cri*	n/a
REV*	0.551	0.640	0.023^{NS}	0.983	0.004	0.013	1.000	*cri*	n/a
TRE*	0.509	0.496	0.150^{NS}	0.988	0.007	0.005	1.000	*cri*	*cri*
UPP*	-	-	-	0.968	0.005	0.027	1.000	*cri*	*cri*
KRA*	-	-	-	0.981	0.015	0.004	1.000	*cri*	n/a
HOS	-	-	-	0.979	0.009	0.012	1.000	*cri*	*cri*
SED	-	-	-	0.930	0.066	0.004	1.000	*cri*	n/a
MKR	-	-	-	0.908	0.088	0.004	1.000	*cri*	n/a
CER	-	-	-	0.970	0.011	0.019	1.000	*cri*	n/a
PRE	0.726	0.825	0.125*	0.897	0.006	0.097	*0.948*	*cri, cri*-bx*dob*	n/a
JOV	0.776	0.835	0.085*	0.855	0.010	0.135	*0.852*	*cri, cri*-bx*dob*	*cri, dob*
SIL	0.510	0.698	0.247**	0.926	0.004	0.070	*0.793*	*cri, cri*-bx*dob*	*cri*
BES*	-	-	-	0.004	0.004	0.992	0.000^{D}	*dob*	n/a
CUN*	-	-	-	0.020	0.009	0.971	0.000^{D}	*dob*	n/a
SVM*	0.919	0.920	0.275^{NS}	0.027	0.014	0.959	0.000^{D}	*dob*	*dob*
LAN	0.567	0.589	0.060^{NS}	0.006	0.007	0.987	0.000^{D}	*dob*	*dob*
LEL*	-	-	-	0.011	0.008	0.981	0.000^{D}	*dob*	*dob*
TOR*	-	-	-	0.034	0.014	0.952	0.000^{D}	*dob*	n/a
DJ*	0.581	0.813	0.235**	0.018	0.019	0.963	0.000^{D}	*dob*	*dob*
RUS*	0.572	0.825	0.178***	0.017	0.049	0.934	0.000^{D}	*dob*	*dob*
FUL*	0.761	0.901	0.042***	0.029	0.018	0.953	0.000^{D}	*dob*	*dob*
VES*	0.843	0.898	0.003*	0.086	0.031	0.883	0.000^{D}	*dob*	*dob*
NM	-	-	-	0.004	0.004	0.992	*0.190*	*dob*	*dob*
TV	0.713	0.817	0.064^{NS}	0.183	0.011	0.806	*0.108*	*dob*-bx*cri*	*dob*
VB	0.664	0.771	0.069^{NS}	0.090	0.006	0.904	*0.129*	*dob, dob*-bx*cri*	*dob*
DOM	0.653	0.728	0.111^{NS}	0.495	0.008	0.497	*0.198*	*dob, dob*-bx*cri*	*dob*

** Reference populations, † individuals 018BOSN and 588ZBL were not included in estimation of the admixture (*q*, see Results).*

Table 4. *Microsatellite allele variation in reference crested newt populations. Number of private alleles is given in parentheses.*

Locus	Size range (bp)	Number of alleles			
		total	*T. cristatus*	*T. dobrogicus*	*T. carnifex*
*Tcri*13	80–128	24	9 (4)	13 (9)	9 (3)
*Tcri*29	224–348	26	11 (0)	19 (8)	3 (3)
*Tcri*32	408–464	15	12 (12)	not amplified	not amplified
*Tcri*35	184–332	37	7 (0)	36 (19)	11 (1)
*Tcri*36	176–472	66	11 (7)	43 (39)	7 (7)
*Tcri*43	216–508	49	10 (0)	24 (26)	13 (7)
*Tcri*46	220–348	31	8 (0)	25 (15)	6 (4)
Sum		248	68 (23)	160 (116)	49 (25)
Mean		35.4	9.7	26.7	8.2
SD		17.2	1.8	11.0	3.6

with the exception of two *T. carnifex* from TUS, which were split between the "*carnifex*" and "*dobrogicus*" cluster (Table 3, Fig. 2 and 3). Specimens originating from the contact zones were either assigned to the parental species or showed mixed ancestry between *T. cristatus* × *T. dobrogicus* and *T. cristatus* × *T. carnifex* (Fig. 2, 3 and 4). Surprisingly, two newts from the Czech populations BOS (018BOSN) and ZBL (588ZBL) were assigned to the "*dobrogicus*" cluster with 0.986 and 0.984 probability, respectively, although both localities are situated outside the distribution range of *T. dobrogicus*. Neither BOS nor ZBL possessed any RAPD markers specific for *T. dobrogicus*. Moreover, when the Structure analysis was performed only with reference *T. carnifex* and *T. carnifex*-like populations, these two individuals were assigned to one cluster together with TUS samples with probabilities 0.988 (018BOSN) and 0.970, respectively (588ZBL; Appendix 2).

Besides parental genotypes, NewHybrids identified backcross and F2 hybrids (Table 3) in a contact zone of *T. carnifex* × *T. cristatus* (MAS, CIT, UNA, POD, TAS, BOS, ZER, JEV, ZBL, HS) and backcross hybrids in contact zones of *T. cristatus* × *T. dobrogicus* (CER, PRE, JOV, SIL, TV, VB, DOM). No F1 hybrids were detected (P = 0.000-0.039). 14.5 % of individuals were assigned to a particular genotype class with a probability lower than 0.900 and therefore they were not considered in the NewHybrids analyses.

Comparison of RAPD-based HI scores and the Structure-based admixture parameter *q* brought concordant results. The correlation between both admixture estimates was highly significant in the species pair *T. cristatus* × *T. dobrogicus* [Spearman rank order correlation, $r = 0.767$, $t(N-2) = 9.577$, $P < 0.001$] as well as *T. cristatus* × *T. carnifex* [$r = 0.836$, $t(N-2) = 15.808$, $P < 0.001$].

Discussion

The analysis of species-specific RAPD markers, microsatellites and mtDNA revealed hybridization between three crested newt species in the Czech Republic and Slovakia. The admixed populations between *T. cristatus* and *T. dobrogicus* were detected on the basis of nuclear as well as mitochondrial markers at the foothills of the Carpathians, corroborating previous evidence for hybridization based on morphological variation (Lác 1957, 1963, Fuhn & Freytag 1961, Shcherbak & Shcherbak 1980, Kautman & Zavadil 2001) and allozymes (Horák 2000, Morozov-Leonov et al. 2003). Populations from the southern part of the Czech Republic were either assigned to *T. carnifex* or showed admixture between *T. cristatus* and *T. carnifex* in nuclear markers, but possessed predominately *T. dobrogicus* mtDNA. Mitochondrial haplotypes carried by these newts were also found in *T. dobrogicus* from southeastern Moravia (an eastern historical part of the Czech Republic) and southwestern Slovakia. Hybridization between *T. cristatus* and *T. carnifex* was also reported in northern Austria, but newts from this hybrid zone possessed "*cristatus*" or "*carnifex*" mtDNA (Maletzky et al. 2008).

The width of studied hybrid zones between the crested newt species is difficult to estimate directly because of the limited number of analyzed populations in the transects. The populations are scattered and isolated due to present-day habitat fragmentation and represent only fragments of the former hybrid zones (c.f. Gollmann 1996). The shortest straight geographic distance between *T. cristatus*-like (SIL) and *T. dobrogicus*-like (DOM) populations was ca. 11 km, the same estimation between *T. cristatus* (HOS) and *T. carnifex* (CIT) populations was ca. 15 km. These results corroborate findings of Wallis &

Arntzen (1989) based on mtDNA variation that hybrid zones between the crested newt species are relatively narrow regions with limited gene flow.

Comparison of mtDNA and nuclear markers in the Czech populations shows that "*dobrogicus*" mtDNA is currently distributed in the areas where *T. dobrogicus* itself likely never occurred. Present distribution of *T. dobrogicus* and *T. carnifex* (possessing "*dobrogicus*" mtDNA) in southern Moravia reveals ca 40 km gap of abused agriculture land (Zavadil et al. 1994, Piálek et al. 2000, Reiter & Hanák 2000). Hybridization between *T. carnifex* and *T. dobrogicus* followed by unidirectional mtDNA introgression could occur in the regions of central Europe, where both species came into contact in the past. *Triturus carnifex* possessing "*dobrogicus*" mtDNA could then spread to the areas of the present-day hybridization with *T. cristatus*. The spread of *T. dobrogicus* further to the west could be limited due to preferred association of this species to basins of the large rivers and lowlands (Arntzen & Borkin 1997, Arntzen 2003). *Triturus carnifex* individuals thus serve as vehicles transferring "*dobrogicus*" mtDNA into the *T. cristatus* or admixed *T. cristatus* × *T. carnifex* populations. Discordant mobility of nuclear and mitochondrial markers through contact zones is described from numerous studies (e.g. Garcia-Paris et al. 2003, Leache & Cole 2007) and has been recently, though to a lesser extent, reported also in *T. cristatus* complex from Austria (Maletzky et al. 2008). One can only speculate about evolutionary processes behind the spread of "*dobrogicus*" mtDNA into *T. carnifex*. In general, mtDNA can relatively easily cross species boundaries and introgress from one species into another as a result of interspecific hybridization (Ballard & Whitlock 2004, Mallet 2005, Ďureje et al. 2012). However, the extent and direction of mtDNA introgression varies between hybridizing species and depends on demographic processes (Currat et al. 2008), cytonuclear and cytonuclear × environment interactions (Gompert et al. 2008, Arnqvist et al. 2010), assortative mating (Lamb & Avise 1986, Helbig et al. 2005), sex-biased dispersal (Petit & Excoffier 2009), sex-biased survival of hybrids (Arntzen et al. 2009) or positive selection (Bachtrog et al. 2006).

The only Czech *T. carnifex* haplotype possessed by one individual from the locality BOS (018BOSN) was found to be more related to those from Apennines rather than the Balkans. This specimen was also assigned to the same cluster with Tuscany samples in the Structure analyses based on microsatellites. However, the assumption about "Apennine" origin of this specimen is complicated by the Balkan origin of *T. carnifex* populations from northern Austria and the adjacent part of Germany (mtDNA data, Maletzky et al. 2008), and eastern Austria (allozyme data, Arntzen 2001). This suggests that these particular parts of Austria and Germany had to be colonized independently, with no genetic traces of presumably original "Apennine" sequence type. Alternatively, the Czech Republic could be colonized *via* some alternative way. Even allochthonous origin, in *T. carnifex* reported also from Bavaria (Franzen et al. 2002), Switzerland (Arntzen & Thorpe 1999) and England (Brede et al. 2000) cannot be fully discarded, although there are no data to support this.

Two types of postzygotic selection can act in hybrid zones. Endogenous selection determines the fitness of hybrids through interactions between genes originated from distinct species (genomic incompatibilities), irrespective of habitat across the hybrid zone. On the contrary, exogenous selection is mediated by environmental variation such that fitness of hybrids depends on habitat type (Barton & Hewitt 1985, Arnold 1997, Jiggins & Mallet 2000). It could be assumed that both types of selection may act against the crested newt hybrids, although the evidence is indirect. For instance, artificial male hybrids between *T. cristatus* × *T. carnifex* are fertile and able to produce F2 and backcross generations, but they reveal disturbed meiosis and production of dysfunctional gametes, what can be interpreted as a result of genomic incompatibilities (Callan & Spurway 1951, Macgregor et al. 1990). In the case of exogenous selection, parental genotypes reveal adaptation to alternative ecological conditions. Within studied crested newts, marked ecological and eco-morphological differences exist mainly between *T. dobrogicus* and the other species (Arntzen & Wallis 1999, Arntzen 2003). While *T. cristatus* and *T. carnifex* occur mainly in hilly areas and in mountain valleys in central Europe, *T. dobrogicus* is restricted to lowlands. Most of Slovak *T. cristatus* populations are situated at an altitude of 250-550 m a.s.l. On the other hand, nearly all *T. dobrogicus* populations occur at an altitude below 250 m a.s.l. (Kautman & Zavadil 2001). An experimental study of Vinšálková & Gvoždík (2007) also revealed that larvae and juveniles of *T. dobrogicus* preferred higher temperatures than the same life stages of *T. carnifex*, which corresponds to their altitudinal distribution. It can be assumed that elevation together with relief and temperature are the most important ecological factors limiting hybridization between *T. dobrogicus* and other crested newts. Such marked ecological differences

are not known between *T. carnifex* and *T. cristatus*. However, Arntzen & Thorpe (1999) pointed out habitat preferences between these species in an area where *T. carnifex* was introduced. While *T. cristatus* preferred pools containing an abundance of aquatic vegetation, *T. carnifex* thrived in disturbed quarries with little or no vegetation. Whether such ecological conditions limit the distribution and hybridization in natural contact zones remains unknown.

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Appendix 1. *F_{IS} and P-values of the Hardy-Weinberg exact test computed per locus and population. Only populations with N ≥ 5 were analyzed.*

Population	*Tcri*13		*Tcri*29		*Tcri*32		*Tcri*35		*Tcri*36		*Tcri*43		*Tcri*46	
	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P	F_{IS}	P
RUS	0.020	0.585	0.309	**0.032***	-	-	0.644	**0.000*****	0.199	0.091	0.039	0.399	0.556	**0.000*****
BOS	0.111	0.105	–0.067	0.863	-	-	0.091	0.514	0.149	0.192	0.250	0.172	0.474	**0.008****
CIT	0.020	0.664	0.094	0.399	-	-	0.250	0.287	–0.043	1.000	–0.448	1.000	1.000	**0.016***
CER	–0.200	1.000	–0.200	1.000	–0.200	1.000	0.200	0.365	–0.500	1.000	–0.200	1.000	–0.200	1.000
DJ	–0.120	0.811	0.225	**0.012***	-	-	0.737	**0.000*****	–0.004	0.693	0.057	0.082	0.616	**0.000*****
DOM	0.016	0.662	0.077	0.458	-	-	0.351	**0.022***	0.178	0.169	0.143	0.334	–0.105	1.000
FUL	0.288	0.107	0.032	0.285	-	-	0.131	0.098	–0.111	1.000	0.097	**0.015***	0.522	**0.000*****
HS	–0.067	1.000	–0.143	0.873	0.714	**0.048***	–0.333	1.000	0.351	0.091	-	-	–0.212	1.000
JOV	0.187	**0.016***	–0.286	1.000	0.091	0.199	0.143	0.278	–0.143	1.000	0.187	0.121	0.338	0.079
KUR	–0.163	1.000	–0.250	1.000	0.474	0.158	–0.370	0.970	–0.216	0.228	0.124	**0.038***	–0.346	1.000
LAN	–0.091	0.873	–0.280	1.000	-	-	0.273	0.333	–0.200	1.000	–0.391	1.000	0.733	**0.048***
MAS	–0.143	1.000	0.012	0.117	-	-	–0.220	0.893	0.030	0.565	–0.073	0.808	0.841	**0.001*****
MAT	–0.020	0.675	0.169	0.278	-	-	0.048	0.193	–0.111	0.891	0.020	0.558	0.394	**0.036***
NR	-	-	0.184	0.100	–0.200	0.498	0.060	0.507	–0.333	0.911	–0.286	1.000	–0.200	1.000
PAS	0.244	0.202	–0.312	1.000	–0.029	0.557	–0.426	0.970	–0.156	0.909	–0.200	1.000	–0.134	0.895
POD	0.082	0.611	0.155	0.311	-	-	0.833	**0.000*****	0.157	0.057	0.467	0.143	1.000	0.200
PRE	0.132	0.180	0.150	0.079	0.371	0.115	0.316	**0.009****	0.128	0.240	–0.091	1.000	–0.084	0.866
REV	0.357	0.152	0.178	0.308	0.593	**0.022***	0.094	0.399	–0.154	0.851	–0.034	0.651	–0.200	1.000
SIL	0.070	0.550	0.024	0.654	1.000	0.200	0.167	0.131	0.245	0.182	0.484	0.079	0.143	0.536
SVM	0.000	0.651	–0.109	1.000	-	-	0.028	0.621	0.003	0.219	0.119	0.057	–0.045	1.000
TV	–0.196	1.000	0.243	**0.018***	1.000	0.200	–0.047	0.413	–0.059	1.000	0.314	0.053	–0.023	0.669
TAS	0.730	**0.008****	–0.224	1.000	-	-	0.043	0.667	–0.200	0.883	–0.128	0.873	0.475	**0.019***
TRB	–0.154	0.851	–0.364	1.000	0.070	0.550	0.714	**0.021***	–0.105	1.000	0.040	0.645	0.273	0.619
TRE	–0.065	1.000	–0.274	0.992	0.399	**0.038***	0.132	0.369	monomorphic		–0.212	0.943	–0.158	0.874
UNA	–0.250	0.951	–0.111	0.507	-	-	–0.565	0.995	–0.050	0.287	–0.212	1.000	–0.023	0.653
VB	0.027	0.551	0.021	0.503	1.000	0.200	0.036	0.160	0.016	0.378	0.060	0.414	0.115	0.152
VES	0.243	0.190	0.189	**0.018***	-	-	–0.056	1.000	0.043	0.395	–0.042	1.000	0.057	0.118
ZBL	0.130	0.047	–0.231	1.000	0.263	0.047	0.200	0.266	0.159	0.262	–0.068	0.137	0.301	0.215
ZER	0.087	0.172	–0.024	0.493	0.416	**0.040***	0.338	**0.006****	–0.122	0.889	–0.062	0.815	0.013	0.537

Appendix 2. *Population differentiation between* T. carnifex *and* T. carnifex*-like populations (Structure 2.3.3). Direct posterior probabilities for* K *(Ln likelihood) as well as* ad hoc *statistic* ΔK *(Evanno et al. 2005) were estimated assuming uniform* prior *values on* K *of one to ten. Admixture and non-correlated allele model was applied. All individuals were assigned to inferred clusters without using any* prior *population information. The analyses were based on runs of* 10^6 *iterations, following a burn-in period of* 10^4 *iterations. A series of five independent runs for each* K *was made with the same parameters to test the accuracy of results.*
The highest Ln likelihood value was obtained with K *= 4,* ΔK *statistics found appropriate clustering with* K *= 2. Individuals 018BOSN and 588ZBL were assigned to the cluster 2 (run with* K *= 4), showing high similarity with newts from Tuscany (TUS171 and TUS172).*

	K = 2		*K* = 4			
	cluster 1	*cluster 2*	*cluster 1*	*cluster 2*	*cluster 3*	*cluster 4*
MAT858	0.995	0.005	0.987	0.005	0.005	0.004
MAT859	0.995	0.005	0.987	0.004	0.005	0.004
MAT860	0.994	0.006	0.981	0.007	0.008	0.004
MAT861	0.997	0.003	0.988	0.005	0.003	0.003
MAT861A	0.996	0.004	0.988	0.004	0.004	0.003
MAT862	0.995	0.005	0.988	0.004	0.005	0.003
MAT863	0.997	0.003	0.990	0.004	0.003	0.003
MAT864	0.995	0.005	0.988	0.004	0.005	0.004
MAT865	0.994	0.006	0.984	0.005	0.007	0.004
MAT865A	0.995	0.005	0.988	0.004	0.005	0.003
MAT866	0.997	0.003	0.989	0.004	0.003	0.003
MAT867	0.995	0.005	0.989	0.003	0.004	0.004
MAT868	0.995	0.005	0.987	0.003	0.006	0.004
MAT869	0.997	0.003	0.989	0.004	0.003	0.003
MAT870	0.995	0.005	0.986	0.005	0.005	0.004
MAT871	0.994	0.006	0.986	0.004	0.006	0.004
MAT872	0.994	0.006	0.987	0.003	0.005	0.004
MAT873	0.994	0.006	0.987	0.004	0.006	0.004
MAT874	0.993	0.007	0.985	0.004	0.004	0.007
MAT877	0.992	0.008	0.981	0.005	0.005	0.009
MAT880	0.997	0.003	0.989	0.004	0.003	0.003
TUS171	0.991	0.009	0.074	**0.911**	0.010	0.005
TUS172	0.991	0.009	0.012	**0.974**	0.010	0.005
018BOSN	0.995	0.005	0.005	**0.988**	0.004	0.004
019BOSN	0.004	0.996	0.004	0.005	0.787	0.204
020BOSN	0.018	0.982	0.013	0.004	0.938	0.045
021BOSN	0.005	0.995	0.005	0.005	0.429	0.561
022BOSN	0.005	0.995	0.003	0.007	0.111	0.878
023BOSN	0.005	0.995	0.005	0.007	0.597	0.391
CIT563	0.005	0.995	0.004	0.004	0.984	0.009
CIT564	0.183	0.817	0.057	0.004	0.934	0.004
CIT566	0.006	0.994	0.006	0.008	0.175	0.811
CIT567	0.006	0.994	0.006	0.004	0.970	0.020
CIT568	0.006	0.994	0.005	0.006	0.968	0.022
CIT569	0.005	0.995	0.004	0.004	0.980	0.013
CIT570	0.005	0.995	0.004	0.004	0.983	0.010
HS743	0.009	0.991	0.009	0.015	0.014	0.961
HS744	0.004	0.996	0.004	0.004	0.012	0.981
HS745	0.008	0.992	0.005	0.006	0.005	0.985
HS750	0.004	0.996	0.004	0.004	0.008	0.985
HS751	0.004	0.996	0.004	0.003	0.010	0.983
JEV812	0.011	0.989	0.011	0.010	0.125	0.854
JEV813	0.008	0.992	0.015	0.013	0.232	0.739
JEV814	0.006	0.994	0.008	0.005	0.121	0.866
MAS169	0.067	0.933	0.112	0.010	0.791	0.087
MAS170	0.009	0.991	0.006	0.005	0.978	0.011
MAS550	0.014	0.986	0.009	0.005	0.978	0.008
MAS552	0.017	0.983	0.012	0.004	0.975	0.009
MAS553	0.721	0.279	0.494	0.007	0.487	0.013
MAS554	0.175	0.825	0.038	0.008	0.946	0.008
MAS555	0.024	0.976	0.011	0.009	0.968	0.012
MAS556	0.204	0.796	0.058	0.018	0.915	0.010

MAS557	0.010	0.990	0.007	0.004	0.976	0.013
MAS558	0.009	0.991	0.005	0.004	0.986	0.005
MAS559	0.006	0.994	0.005	0.005	0.969	0.021
MAS560	0.011	0.989	0.006	0.006	0.982	0.006
MAS561	0.007	0.993	0.005	0.005	0.971	0.018
MAS562	0.010	0.990	0.008	0.005	0.971	0.016
POD804	0.025	0.975	0.014	0.009	0.964	0.012
POD805	0.072	0.928	0.045	0.006	0.911	0.037
POD806	0.012	0.988	0.008	0.004	0.979	0.009
POD807	0.006	0.994	0.005	0.006	0.792	0.197
POD808	0.019	0.981	0.017	0.004	0.899	0.080
POD809	0.004	0.996	0.004	0.004	0.969	0.023
POD810	0.007	0.993	0.006	0.007	0.908	0.079
TAS168	0.010	0.990	0.006	0.015	0.951	0.027
TAS611	0.004	0.996	0.004	0.005	0.226	0.765
TAS612	0.005	0.995	0.004	0.007	0.945	0.044
TAS613	0.004	0.996	0.004	0.004	0.267	0.725
TAS614	0.003	0.997	0.003	0.003	0.970	0.024
TAS615	0.004	0.996	0.004	0.004	0.851	0.142
TAS616	0.005	0.995	0.004	0.005	0.972	0.019
TAS617	0.006	0.994	0.005	0.005	0.951	0.039
TAS618	0.008	0.992	0.006	0.003	0.974	0.017
TAS619	0.003	0.997	0.003	0.003	0.894	0.099
TAS620	0.007	0.993	0.007	0.008	0.508	0.476
TAS621	0.005	0.995	0.006	0.004	0.177	0.813
UN691	0.004	0.996	0.003	0.003	0.988	0.006
UN692	0.004	0.996	0.003	0.003	0.988	0.006
UN693	0.004	0.996	0.004	0.003	0.971	0.022
UN694	0.003	0.997	0.003	0.004	0.969	0.024
UN695	0.003	0.997	0.003	0.004	0.827	0.166
UN696	0.005	0.995	0.004	0.004	0.987	0.005
UN697	0.391	0.609	0.215	0.030	0.727	0.028
UN715	0.003	0.997	0.003	0.004	0.981	0.012
UN716	0.003	0.997	0.003	0.004	0.960	0.033
UN717	0.004	0.996	0.003	0.003	0.984	0.009
UN746	0.006	0.994	0.004	0.003	0.982	0.011
584ZBL	0.013	0.987	0.010	0.007	0.050	0.933
585ZBL	0.005	0.995	0.004	0.003	0.005	0.988
586ZBL	0.005	0.995	0.005	0.004	0.010	0.980
587ZBL	0.116	0.884	0.033	0.039	0.010	0.918
588ZBL	0.754	0.246	0.004	**0.970**	0.017	0.009
589ZBL	0.006	0.994	0.005	0.004	0.008	0.984
590ZBL	0.006	0.994	0.005	0.004	0.008	0.984
591ZBL	0.005	0.995	0.004	0.003	0.016	0.976
592ZBL	0.004	0.996	0.003	0.003	0.005	0.989
593ZBL	0.006	0.994	0.004	0.004	0.006	0.986
ZER572	0.006	0.994	0.006	0.005	0.954	0.036
ZER573	0.003	0.997	0.003	0.005	0.208	0.783
ZER574	0.004	0.996	0.003	0.004	0.083	0.910
ZER575	0.004	0.996	0.006	0.003	0.111	0.880
ZER576	0.004	0.996	0.003	0.007	0.008	0.982
ZER577	0.005	0.995	0.006	0.004	0.320	0.670
ZER578	0.004	0.996	0.005	0.004	0.119	0.871
ZER579	0.006	0.994	0.007	0.005	0.054	0.933
ZER580	0.004	0.996	0.005	0.006	0.125	0.864
ZER581	0.006	0.994	0.006	0.006	0.122	0.866
ZER583	0.005	0.995	0.004	0.006	0.176	0.814
ZER647	0.003	0.997	0.003	0.004	0.168	0.824
ZER648	0.003	0.997	0.003	0.004	0.625	0.368
ZER650	0.003	0.997	0.003	0.003	0.097	0.897
ZER653	0.004	0.996	0.004	0.004	0.176	0.816
ZER663	0.004	0.996	0.003	0.004	0.108	0.884
ZER664	0.007	0.993	0.005	0.009	0.010	0.976
ZER666	0.004	0.996	0.004	0.004	0.086	0.905
ZER667	0.004	0.996	0.005	0.004	0.178	0.814