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Malformations and body injuries in a hybrid zone of crested newts (Caudata: Salamandridae: *Triturus cristatus* superspecies)

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Abstract. Morphological abnormalities occur frequently in wild amphibian populations. We analysed malformation and injuries in the hybrid zone of three crested newt species, in the Czech Republic. In total, 274 individuals from 35 localities in South Moravia (Czech Republic) were examined during the period 2010-2014. Malformations were found in eight newts (2.9%) from seven localities. Injuries were recorded on 59 newts (21.5%). Proportions of tail crest injuries was significantly higher ($P < 0.1$) in males than in females and the probability of being injured was significantly higher ($P < 0.01$) for adult individuals. We discuss gene mutation, parasitism and predation as possible explanations for our observations.

Keywords. Amphibia, *Triturus cristatus*, morphology, abnormalities, polydactyly, limb damages, contact zone, Czech Republic

Pre- or post-natal malformations are regularly reported in different amphibian species (Canestrelli et al., 2006; Piha et al., 2006; Sas and Kovacs, 2006; Machado et al., 2010; Jarvis, 2011; Henle, et al. 2012; Gatti and Sannolo, 2014). In general, amphibian abnormalities include malformation and injuries, and according to Reeves et al. (2008) and Hassine et al. (2011), the most common malformations in amphibian are: i) eye abnormalities such as anophtalmia or microphtalmia, ii) skeletal injuries such as brachydactyly (short digits), ectrodactyly (missing digits) or ectromelia (partial limb formation), iii) skeletal malformations such as amelia (missing limb), polydactyly (extra digits), syndactyly (digits fused), brachygnathia (short jaw), iv) surficial abnormalities such as incomplete tail resorption or skin trauma (e.g., scars, edema). Along with colour abnormalities (e.g., leucism, albinism or axanthism) these malformations are recorded in the wild life of amphibians (Jablonski et al., 2014). Malformations could be a result of genetic mutation caused by environmental pollutants, parasites, diseases, prenatal stress, genetic predisposition or UV radiations (Blaustein and Johnson, 2003).

On the other hand, injuries in wild animals can originate from diseases, intraspecific aggression, and most of all by predation (Lima and Dill, 1990). Injuries are usually studied in animals that have the ability to autotomize a body part (Cooper et al., 2004; Maginnis, 2006) or to regenerate a missing part, which is after this process actually different from the original part (Maginnis, 2006). Frequency and types of injuries are probably connected with density and diversity of predators, with geographical differences in injury rates among population (Placyk and Burghardt, 2005) and sexes (sexual dimorphism) in the breeding season (Kopecký, 2013).

In this article, we present results of our study on morphological abnormalities found in crested newts from

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their hybrid zones in the Czech Republic. Studied localities are southern parts of the Czech Republic (Znojmo region), where the ranges of three crested newt species meet: *Triturus cristatus* (Laurenti, 1768), *T. dobrogicus* (Kiritzescu, 1903), and *T. carnifex* (Laurenti, 1768) (Wielstra et al., 2014). Their distribution is parapatric with mixed contact zone in South Moravia, the only known among these species in the Czech Republic (Mikulíček et al., 2012). Despite malformations of amphibians are relatively well known, the following paper provides a new point of view on their causes.

During the period 2010-2014 we recorded body abnormalities and injuries at *Triturus cristatus* superspecies in Znojmo region (South Moravia, Czech Republic). During the breeding season, 274 individuals (136 males, 127 females and 11 subadults) in the water phase from 35 localities, were examined. Newts were captured by funnel collapsible nylon baited traps (Bock et al., 2009; Madden and Jehle, 2013), sexed, measured and photographed from dorsal and lateral side of the body. Each locality was visited once, in case of sufficient number of caught newts (more than 10 individuals). Otherwise these localities were repeatedly visited. Individuals from all populations were recognized by belly pattern from photographs (according to Hagström, 1973; Jehle et al., 2011). Differences between males and females and between bigger and smaller individuals were performed on tail injuries by the software STATISTICA 12 (Hill and Lewicki, 2007), using a nonparametric Mann-Whitney U test.

Malformations were recorded at seven localities (Table 1, Fig. 1). Each malformation was recorded in different locality, except two cases (locality Podmolí 2). Artificial pond was the most frequent type of habitat with morphological abnormalities (four cases); two were situated to the wood ponds and other cases represented different types of ponds. The distance between the two nearest localities was 0.2 km (Podmolí 1 and Podmolí 2) separated by road. There is no evidence about identical belly pattern between newts in these localities. The longest distance between two localities was 18.8 km (Bojanovice and Chvalatice). Eight out of 274 individuals (2.9%) showed malformations of three different types: bidactyly, polydactyly and syndactyly (Table 1, Fig. 2). Bidactyly was recorded five times (1.9% of all individuals) while polydactyly two times (0.7%) and syndactyly one a time (0.4%). Overall, three male individuals (average SVL: 71.9 mm) and five females (86.8 mm) were recorded with malformations.

Fifty-nine out of 274 newts (21.5%) were injured. Male individuals were injured in 28 cases (47.5%) and females in 31 cases (52.5%). Injuries could be divided into six types. The first type consisted of damage to the front limb (including arms and fingers; Fig. 3E, G, H) and the second type consisted of a damage to the hind limb (Fig. 3F). Two types of injuries were recorded on tail: damage (ragged) on the tail crest and missing the tip of the tail (Fig. 3A-D). There was some interesting differences between males and females: while males were injured on the crest of tail, females were injured on the tip of the tail. The last two injuries types were recorded on the body (trunk) and the head. The most common injury was damage to the tail (32 cases, 71.2%, Fig. 4). The second most recorded was damage to the limb (15 cases, 25.4%). Furthermore, two atypical injuries were found: one female had fresh scars on the trunk and tail (Fig. 3I) and another female had a hole in the throat (Fig. 3J). The proportion of tail crest injuries was significantly higher for males (14 cases) compared to females ($P < 0.1$). The probability of injuries was significantly higher for bigger newts (average 76.9 mm) compared to smaller ones (average 72.1 mm; $P < 0.01$).

We observed three types of malformation in the *T. cristatus* superspecies (bidactyly, polydactyly and syndactyly). Several cases of morphological malformation among the genus *Triturus* are known from available literature: bidactyly in three individuals of *T. cristatus* from the United Kingdom (Jarvis, 2011), polydactyly in two *T. carnifex* from Italy (Gatti and Sannolo, 2014), polyme-

Table 1. Malformation recorded in *Triturus cristatus* superspecies during the study (SVL - snout-to-vent length; TL - tail length).

Figure	Sex	SVL	TL	Malformation	Locality	GPS	Altitude	Pond type	Date
1A	F	78.4	52.9	bidactyly	Čížov	48.88N, 15.88E	400 m	artificial	28 April 2010
1B	F	89.9	69.8	polydactyly	Onšov	48.90N, 15.84E	460 m	natural forest	22 April 2011
1C	F	94	80.9	bidactyly	Podmolí 2	48.84N, 15.93E	406 m	artificial	25 May 2011
1D	M	75.4	56.1	bidactyly	Podmolí 2	48.84N, 15.93E	406 m	artificial	25 May 2011
1E	F	90	60.8	bidactyly	Podmolí 1	48.84N, 15.93E	412 m	fish pond	27 April 2012
1F	F	82	54	bidactyly	Lukov	48.86N, 15.89E	444 m	forest wetland	29 April 2013
1G	M	66.4	51.6	polydactyly	Bojanovice	48.94N, 16.00E	350 m	artificial	10 May 2013
1H	M	73.9	52.8	syndactyly	Chvalatice	48.93N, 15.74E	446 m	artificial	2 April 2014

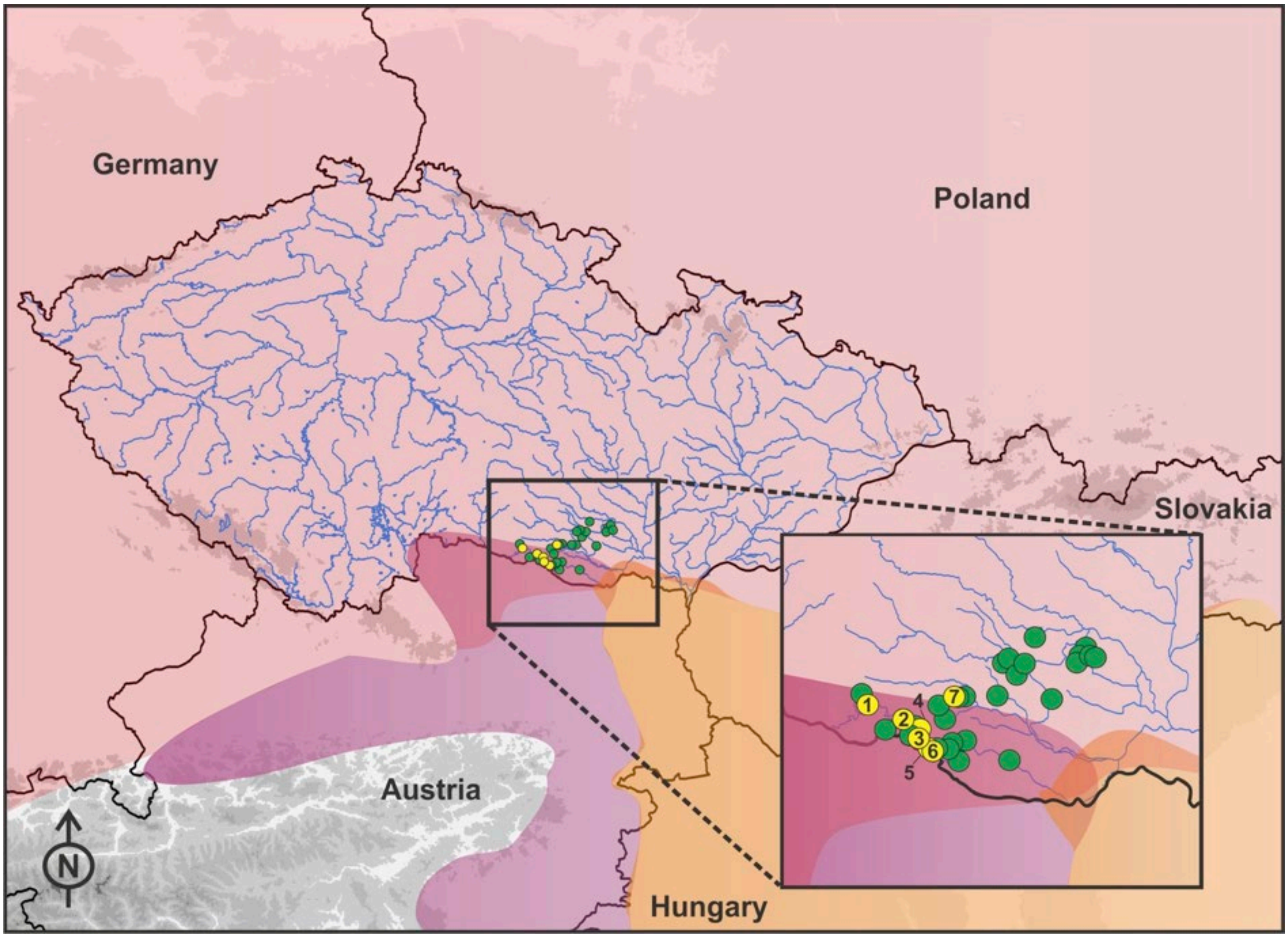

Fig. 1. Distribution map of *Triturus cristatus* superspecies (*T. cristatus* - pink, *T. carnifex* - purple, *T. dobrogicus* - orange; The colours in the overlap indicate putative contact or hybrid zones; sensu Wielstra et al., 2014) in study area and locations of localities with records of malformations (1 - Chvalatice, 2 - Onšov, 3 - Lukov, 4 - Čížov, 5 - Podmolí 2, 6 - Podmolí 1, 7 - Bojanovice; see Table 1.).

ly in *T. marmoratus* (Recuero-Gil and Campos Asenjo, 2002) and malformation of digits between hybrids of *T. cristatus* and *T. marmoratus* in western France (Arntzen and Wallis, 1991). Malformation rates in amphibians do not exceed 5% in healthy populations (Blaustein and Johnson, 2003). In comparison with other authors studying crested newts (Jarvis, 2011; Gatti and Sannolo, 2014; Mester et al., 2015), the malformation rate found in this study is relatively high. On the other hand, malformation rate shows much higher fluctuations among other species of Caudata: e.g., about 3.9% at *Calotriton arnoldi* (Martínez-Silvestre et al., 2014) or about 40-90% at *Cryptobranchus alleganiensis* (Hiler et al., 2005).

Amphibians seem particularly prone to malformation (e.g., polydactyly). The extreme sensibility to environmental changes and habitat loss are well known in amphibians, including crested newts (Beebee and Griffiths, 2005). However, we have no data to suppose that pollution (some localities are situated into national park, where we can assume low levels of pollution) caused observed abnormalities. Five main factors could possibly cause malformations: i) hyper-regeneration after predator attempts or accidents (Eaton et al., 2004; Ballengée and Sessions, 2009), ii) exposition to high UV-B radiation (Blaustein et al., 1997; Pahkala et al., 2003), iii) chemical pollution from industry and agriculture (Kiesecker, 2002; Piha et al., 2006), iv) degradation of the environment (Houlahan et al., 2000) and v) parasite infection (e.g., *Ribeiroia* trematodes; Kiesecker, 2002; Johnson and Chase, 2004). In addition, body malformations could also be caused by hybridization. Different crested newt species cross in narrow hybrid zones (Arntzen et al., 2014). Arntzen and Wallis (1991) discussed the relatively high proportion of malformation within *Triturus* hybrid zone caused by collapsed genetic homeostasis. A much lower proportion of malformation is reported away from crest-

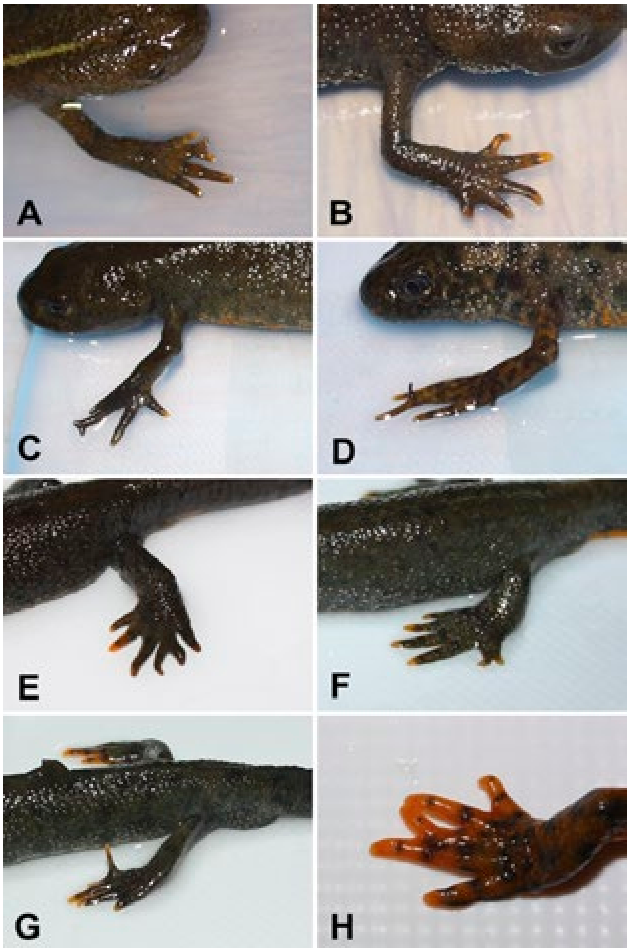

Fig. 2. Malformation recorded during the study of crested newt (for details see Table 1).

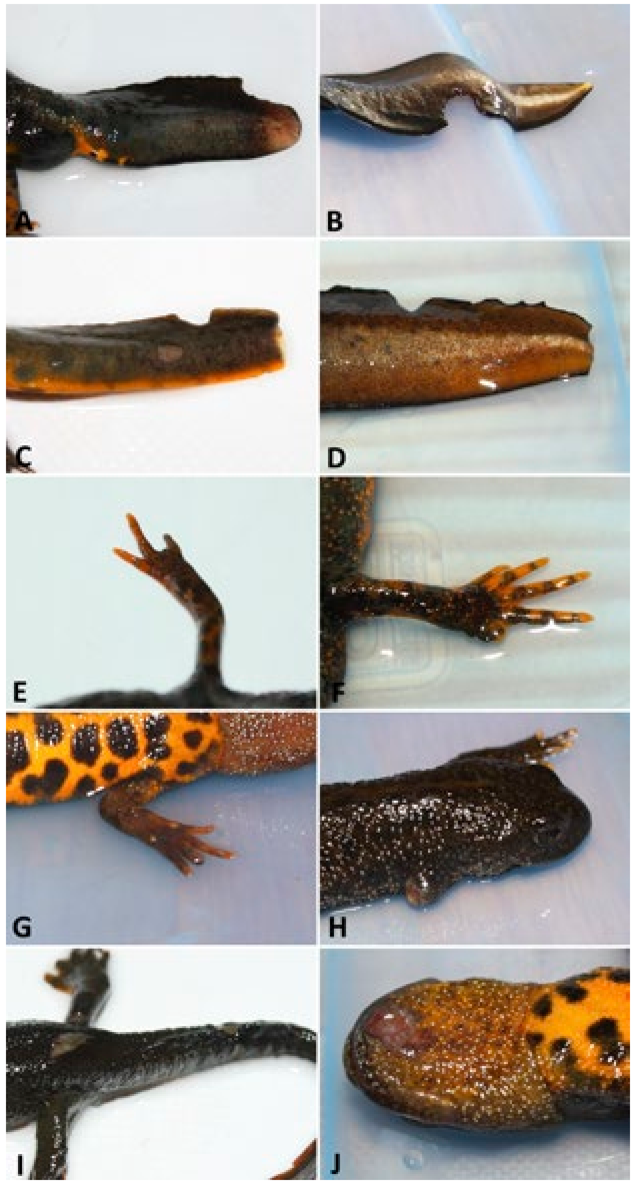

Fig. 3. Examples of injuries recorded during the study of crested newts (A-D tail damage, E-H missing toes or limb, I-J scars and tissues damage).

ed newt hybrid zones (Jarvis, 2011; Gatti and Sannolo, 2014, Mester et al., 2015). Therefore, hybridization could be the likely explanation for our results.

Crested newts are active swimmers, therefore, the injuries of tail or limbs could negatively affect their fitness. Under natural conditions, injured individuals are easily liable to become a prey for predators (Kopecký, 2013). The relatively high percentage of injured individuals in our study (compare with Székely and Nemes, 2003; Kopecký, 2013) could be probably caused by predators, such as water birds, small mammals or invertebrates (e.g., dragonfly nymph, Bowerman et al., 2010). Moreover, some injuries could emerge from stocking fish for fishing, such as *Carassius gibelio* (Bloch, 1782), *Pseudorasbora parva* (Temminck and Schlegel, 1846) and *Ameiurus nebulosus* (Lesueur, 1819) (Lusk et al., 2010).

Taking into account the syntopic occurrence of the three crested newt species, competition within species is also possible. Considerable sexual dimorphism during breeding phase is well known, in which males are more brightly coloured than females (e.g., Griffiths, 1996; Jehle et al., 2011). Moreover, males are territorial and fights between them are known (Griffiths, 1996). Thus, we can expect that males are probably more frequently injured than females (cf. Kopecký, 2013). Our results concerning tail injuries partly support this hypothesis. As a simple explanation, we consider male tails to be more visually attractive for predators and they have different shape

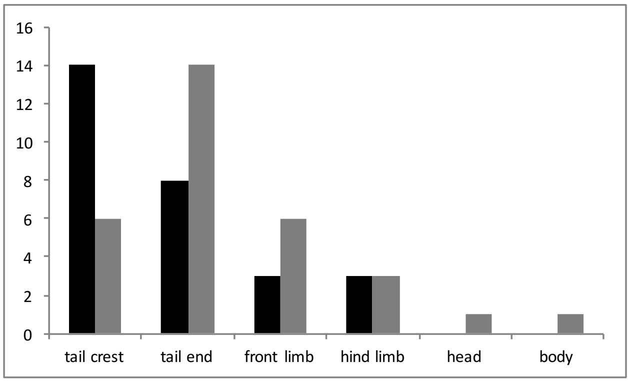

Fig. 4. Frequency of body part injuries in the crested newts (black bar - male, grey bar - female).

and dimension than female tails. Kopecký (2013) did not find any intersexual difference, although injuries at tail tip were more frequent (but not significantly) in females. Kopecký (2013) offers an explanation for mechanical damage to tail during underground movement in narrow spaces at terrestrial phase/hibernation. It is undisputed, that all types of injuries (loss of limbs, fingers or tail) can significantly impair walking, running, swimming, gliding, diving and could be a risk for disease infection (Cooper et al., 2004; Maginnis, 2006; Marvin, 2010). Our results suggest that larger (and presumably older) individuals are injured more often probably because body size at crested newts increases significantly with age (Rehák, 1983; Halliday and Verrell, 1988). Moreover, older animals will also accumulate injuries on their body. Nevertheless, these accumulations do not need to be necessarily lethal for amphibians as was found by Mott and Steffen (2013) who reported the association between body size and non-lethal injuries in amphibians.

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