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RECORDS ON MALFORMATIONS IN *BUFO BUFO, BUFOTES VIRIDIS,* AND *BOMBINA BOMBINA* FROM THE CZECH REPUBLIC

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Abstract. Developmental anomalies and malformations in amphibians serve as a marker of extreme or unstable environmental conditions and can affect the reproduction and viability of amphibians. In the Czech Republic, anomalies were reported for amphibians from the Elbe and the Danube rivers basins, but not from the Oder River. Here, we report anomalies in three anuran species, i.e., in *Bufo bufo* (n = 250), *Bufotes viridis* (n = 13) and *Bombina bombina* (n = 24), from the Oder River Basin (Czech Republic). We found eight types of anomalies in 16 out of the 287 individuals analyzed (six types of limb malformations and two anomalies of coloration). The most frequent malformation recorded in all the three species was brachydactyly. Flavism was found in *Bufo bufo* and *Bufotes viridis*, while all the remaining malformations were recorded only in *Bufo bufo*. The monitoring of the number and types of malformations in different populations is crucial in the light of emerging disease threats to European amphibians.

INTRODUCTION

Research findings on developmental anomalies and malformations in amphibians are of great concern to researchers (e. g. Lunde and Johnson 2012; Jablonski et al. 2014; Henle et al. 2017; Pinelli et al. 2019; Svinin et al. 2022). Anomalies in amphibians serve as crucial indicators of environmental health, with instances revealing local pollution sources such as industrial factories. Amphibians are susceptible to environmental changes since their development takes place outside the maternal organism, eggs lack typical amniotic internal and external protective envelopes, as a result of which developing organisms are much more exposed to various biotic, abiotic, and anthropogenic factors from the environment than amniotes (e.g. Carey and Bryant 1995; deWijer et al. 2003; Slaby et al. 2019). Mapping and monitoring anomalies in amphibian populations is important from two perspectives. Firstly, a large number of detected malformed individuals can be a marker of unstable or disbalanced environmental conditions (e.g. Taylor et al. 2005; Lunde and Johnson 2012; Henle et al. 2017; Pinelli et al. 2019; Katrushenko 2020). Secondly, severe anomalies affect the reproduction and viability of amphibians, e.g., limb malformations may disrupt amplexus and cause locomotion disabilities, making individuals more vulnerable to predation (Johnson and Sutherland 2003; Wilson et al. 2005). The identification of anomalies and the understanding of their origins are pivotal for effective environmental monitoring and conservation efforts.

Proper identification of anomalies is a challenging task requiring their differentiation from injuries using rigorous methodologies like histological examination and morphometric analyses. The information on amphibian anomalies registered in the Czech Republic, a territory that encompasses three international river basins (the Elbe, the Oder, and the Danube), is rather limited. Mačát et al. (2015) studied malformations and body injuries in the Znojmo region (Danube Basin) where three species of crested newts, namely *Triturus cristatus* (Laurenti, 1768), *Triturus dobrogicus* (Kiritzescu, 1903) and *Triturus carnifex* (Laurenti, 1768), meet in hybrid zones. Besides multiple tail injuries, there were three types of malformations recorded: bidactyly, polydactyly, and syndactyly (Mačát et al. 2015). Zavadil et al. (1997) described cases of myiasis (the infestation of a live animal's body by fly larvae) in *Bufo bufo* (Laurenti, 1768) from the Cheb district (the

Elbe Basin). Two albino tadpoles of the same species were registered by Wenig (1913). Albinism was also shown in *Pelophylax lessonae* (Camerano, 1882) from the region (Dandová et al. 1995; Kotlík and Zavadil 1997). Koleska and Holer (2018) described a juvenile *Pelophylax esculentus* (Linnaeus, 1758) with an absent distal part of the hindlimb.

The aim of the study is to report anomalies in three anuran species, i.e., *Bufo bufo* (Linnaeus, 1758), *Bufotes viridis* (Laurenti, 1768), and *Bombina bombina* (Linnaeus, 1761), from the Oder River Basin.

MATERIALS AND METHODS

A total of 250 *B. bufo* individuals were examined in Šilheřovice (49.8910N, 18.2810E) on 30 March 2022 within the survey aimed to monitor migrating common toads and their road mortality risks during the breeding season (permit ID 0245/2022/ŽPZ/O, contracting authority – the Moravian-Silesian Region of the Czech Republic). We caught almost every toad sitting on the road and examined its external morphology to find any anomalies in limbs, eyes, or coloration. Individuals with anomalies were photographed, and all toads were released close to the site where they were caught.

Thirteen *Bufotes viridis* individuals were collected in Starý Bohumín (49.9289N, 18.3496E) on 12 May 2022. Twenty-four *Bombina bombina* individuals were caught in Jistebník, the Velký Roh pond (49.7370N, 18.1167E) during two monitoring rounds on 16 May 2022 and 4 June 2022. All collections were performed during the monitoring of the Sites of European Importance within the Natura 2000 Network (permit ID 05172/SOPK/22, contracting authority – the Agency for Nature Conservation and Landscape Protection of the Czech Republic). Toads were further examined for the presence of anomalies in limbs, eyes, or coloration. All the anomalies were photographed and the examined toads were released close to the spot where they were caught.

We described types of anomalies according to Henle et al. (2017a). The alternative classifications mostly differ in definitions of terms hemimely and ectromely, and word endings, e. g. hemimelia *vs*. hemimely (Meteyer 2000; Nekrasova 2008; Katrushenko 2020).

For *B. viridis* and *B. bombina,* the frequencies of anomalies were not calculated due to the small number of the individuals collected. The frequency of anomalies in the sample of *B. bufo* (A_f) individuals was calculated using the following formula: $A_f = (N_a/N)*100\%$, where N_a is the number of individuals with anomalies in a sample and N is the total number of individuals in a sample. The partial frequency of anomalies (A_{pf}) was estimated as $A_{pf} = (N_{at}/N_a)*100\%$, where N_{at} is the number of individuals with a certain type of anomaly (Marushchak and Muravynets 2018). Differences in frequencies of anomalies between samples were estimated with Fisher's exact test.

RESULTS

We found eight types of anomalies in 16 individuals of *B. bufo*, *B. viridis*, and *B. bombina* (Table 1). Twelve (4.8%) of the examined 250 *B. bufo* individuals (237 males and 13 females) had anomalies of limbs and coloration (Figure 1 (A–F), Table 1). All toads with anomalies were males, except for one female with a locomotion disability who could not move her hindlimbs properly, probably due to a mechanical injury. Otherwise, the structure of the hindlimbs of that individual was normal.

Two males (Figure 1D) had a totally disturbed structure of the foot. We described this anomaly as ectrodactyly, meaning a combination of oligodactyly and brachydactyly.

Two *B. bufo* individuals in our sample had a distal part of the hindlimb absent, and one individual lacked the distal part of the forelimb (Figure 1 B, C). Here, we define such an anomaly as ectromely, according to

Table 1. Summarized data on the anomalies found in three species of Anura. Asterisks denote anomalies found in the same individual. N_a – number of individuals with anomalies, A_{pf} – partial frequencies of anomalies (calculated for *B. bufo* only).

Anomaly N_a (A_{pf}, %)	*Bufo bufo* (n = 250)	*Bufotes viridis* (n = 13)	*Bombina bombina* (n = 24)
Limbs			
Brachydactyly	3 (23)	1	2
Ectromely	3* (23)	–	–
Ectrodactyly	2 (15)	–	–
Oligodactyly	1* (8)	–	–
Motor disability	2 (15)	–	–
Coloration			
Flavism (yellow/orange patches)	1 (8)	1	–
Dark brown patches	1 (8)	–	–

Meteyer (2000). However, some authors describe it as hemimelia (Katrushenko 2020). Such discordance in terminology complicates the comparison of different studies' results, especially when photos of the registered anomalies are not provided. In total, 1.2% of toads in our sample had ectromely.

Among the examined 13 *B. viridis* males, two had anomalies in the form of brachydactyly of the right forearm and abnormal coloration represented by the yellow patch on the left side of the body (Figure 2, Table 1).

Two *B. bombina* (24 individuals examined in total; sex was not identified) had anomalies represented by

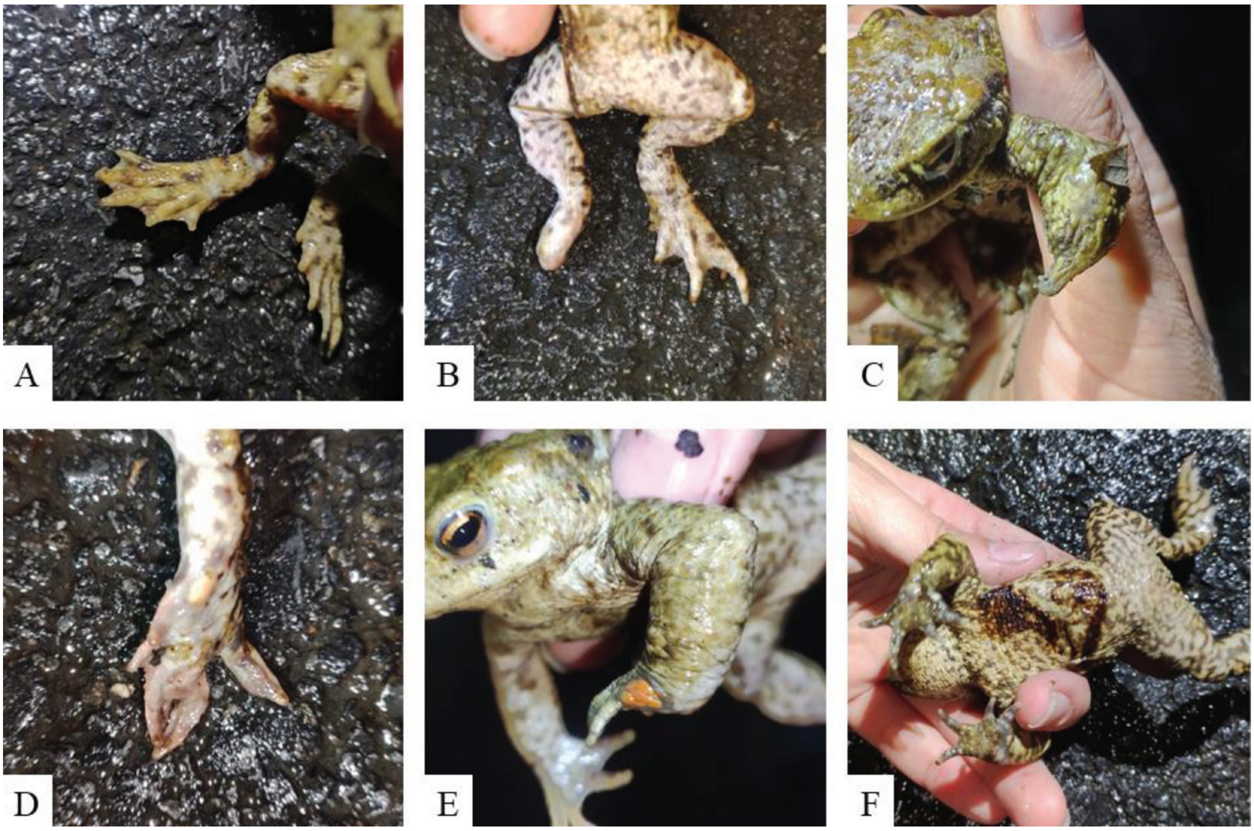

Figure 1. Anomalies found in *B. bufo*: A – Brachydactyly, B – Ectromely of hindlimb and oligodactyly, C – Ectromely of the forelimb, D – Ectrodactyly, E, F – Coloration (orange and brown patches, respectively). (Photographed by Eleonora Pustovalova).

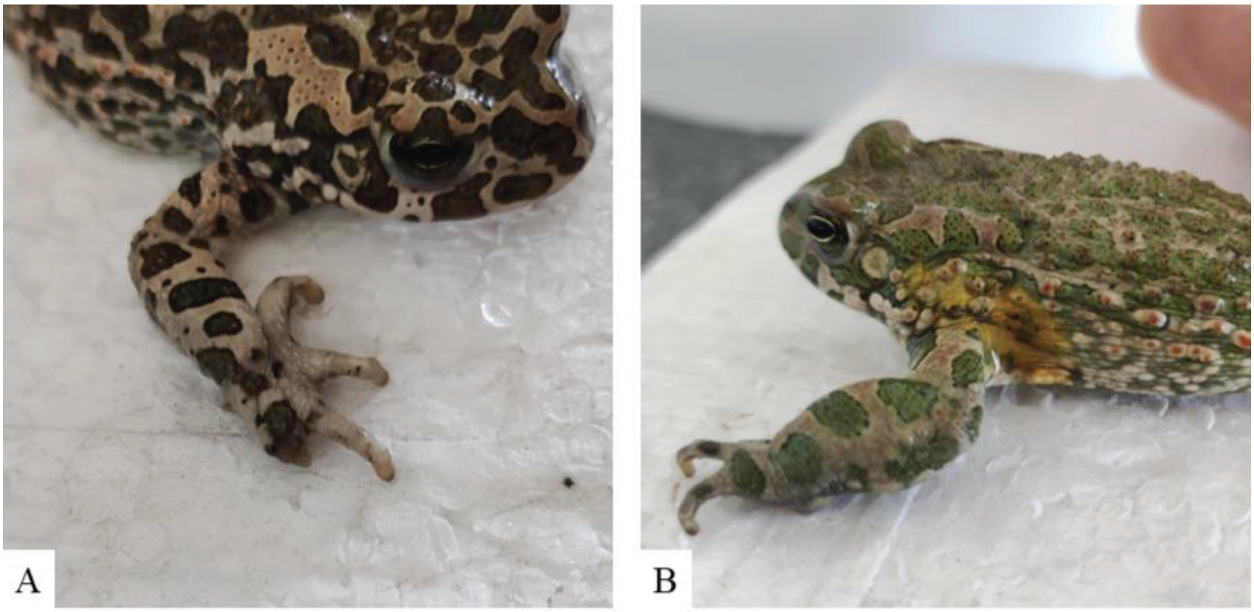

Figure 2. Anomalies found in *B. viridis*. A – brachydactyly on the right forearm; B – yellow patch (flavism). (Photographed by Anna Fedorova).

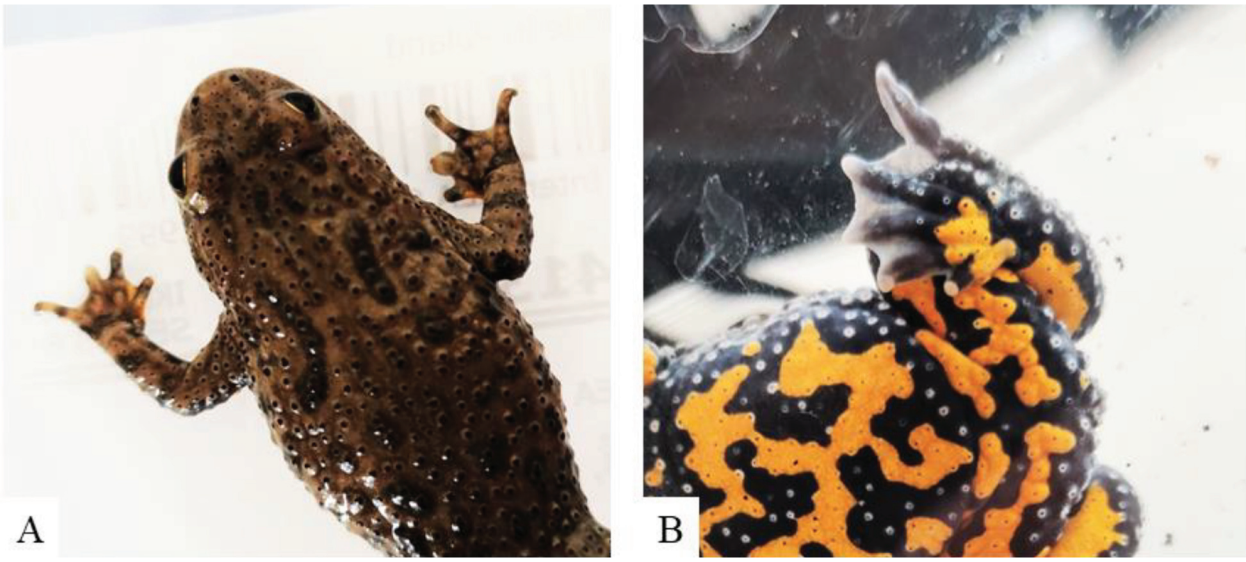

Figure 3. Brachydactyly in two individuals of *B. bombina*: A – on both forelimbs; B – on the left hindlimb. (Photographed by Anna Fedorova).

brachydactyly only (Figure 3, Table 1). One of the individuals had short digits on both forearms, however, the right forearm had traits of an old mechanical injury (part of the bone sticks out surrounded by a large amount of presumably connective tissue) rather than a developmental anomaly (Figure 3A).

DISCUSSION

Malformed limbs are usually the most common malformations in amphibians, which can be caused by numerous factors, ranging from mutations and chemical pollution to parasites and predators (Henle et al. 2017a and citations therein). In our study, brachydactyly appeared to be the most common anomaly found in all the three anuran species studied. This type of anomaly was previously reported for *B. bufo* (e.g. Katrushenko 2020; Deaton 2022), *B. viridis* (e.g. Henle et al. 2017a; Katrushenko 2020), and *B. bombina* (e.g. Katrushenko 2020) outside the territory of the Czech Republic. Brachydactyly, characterized by shortened toes due to the reduced number of phalanges, is one of the mildest anomalies that should not lead to any severe consequences, decreasing the viability of individuals. Therefore, finding a notable number of adult toads with brachydactyly is not surprising. The partial frequency of brachydactyly (Table 1) was ~2.4 times lower in our sample than in the sample of *B. bufo* individuals from Kyiv (Ukraine) reported by Marushchak and Muravinets (2018).

In terms of severity, ectrodactyly and oligodactyly are similar to brachydactyly. These anomalies were reported previously for *B. bufo* from other localities outside the Czech Republic (e.g. Rostand 1948; Marushchak & Muravinets 2018; Katrushenko 2020). The only individual with a totally disturbed foot structure (described as ectrodactyly) in our sample would require X-ray examination for precise identification of the anomaly type.

The total absence of a distal part of the limb (ectromely) is a more severe anomaly. It can affect the viability of an individual, especially during the breeding season when toads migrate long distances and are at risk of road kills or predation. The incidence of ectromely was shown to be more frequent among juveniles than among adults of *Pelophylax* frogs (Fedorova et al. 2023). One of the individuals registered by us had ectromely of the right hindlimb accompanied by oligodactyly of the left hindlimb, which could make it difficult for the individual to move (Figure 1B). Ectromely of forelimbs (Figure 1C) can also influence the reproduction since such individuals have a lower ability to perform a proper amplexus. The proportion of *B. bufo* toads with ectromely in our sample did not differ from the frequency of ectromely among the *B. bufo* adults reported by Wisniewski (1979) (p = 0.2182). Significantly more toads lacking distal parts of limbs were found in samples of juveniles: ~10 % in a sample from Veith and Viertel (1993) compared to 1.2% in our sample ($p < 0.0001$). Therefore, the frequency of ectromely is much lower among adults than among juveniles, which means that this anomaly influences the viability of toads.

In general, the frequency of limb anomalies in our sample did not differ from the frequencies reported in other studies on *B. bufo*. For example, Gittins (1983) conducted a similar survey on a road around the lake during the breeding season and reported 2.5 % limb anomalies, which does not differ from our results (p = 0.1338). Katrushenko (2020) collected a sample of 554 *B. bufo* individuals (Kharkiv region, Ukraine) and identified 3.2% of individuals with limb anomalies, which also does not differ from our results ($p = 0.5652$).

Two individuals of *B. bufo* and one *B. viridis* had color patches on different parts of their bodies (Figure 1E, F, 2B): the homogeneous orange patch on the forelimb of one *B. bufo* male, heterogeneous dark brown ventral patches on another *B. bufo* male, and the yellowish coloration on the left side of a *B. viridis* male. The rest of the body coloration was normal and species-specific. The abnormal coloration varying in shades of yellow is called flavism and seems rare in amphibians. It has been previously registered in some species of Anura (Kolenda 2017), including abnormal ochre patches in a juvenile *B. viridis* (Henle et al. 2017a; Szkudlarek et al. 2022), orange patches in *B. bufo* (Marushchak et al. 2021) and full-body flavistic (almost albinistic) female *B. bufo* (Thomas et al. 2002). Usually, *B. bufo* and *B. viridis* have uniform light brown or heterogeneous greenish cryptic coloration, respectively. The presence of bright-colored patches is likely to decrease an individual's viability since it vividly stands out from the overall cryptic coloration and makes toads more visible for predators.

Brown spots on the belly of *B. bufo* from two German populations were previously reported by Mattes (2013), who assumed that they were caused by a fungal infection. Further, Kliemt (2017) observed similar dark spots on juvenile *B. bufo* and confirmed its fungal origin. Brown patches on the belly of the individual from our sample looked very similar to those described by Mattes (2013) and Kliemt (2017). In our case, fungal infection is also a possible cause, but only a detailed investigation in this area may confirm our assumption.

The frequency of malformations in amphibians often serves as an indicator of population health. In the light of emerging disease threats to European amphibian populations such as ranaviruses (Stöhr et al. 2015; Campbell et al. 2020) or *Batrachochytrium* fungi (Spitzen-van der Sluijs et al. 2016; Kärvemo et al. 2018), detection of amphibian anomalies and malformations is vital, as some can serve as infection gateways into amphibian bodies. Monitoring the number and types of malformations in a population is the first step in identifying its cause and setting up appropriate measures.

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