

## High frequency of hindlimb malformation in froglets *Pelophylax* sp. in Ukraine

ANNA FEDOROVA\*, ELEONORA PUSTOVALOVA & MYKOLA DROHVALENKO

Department of Zoology and Animal Ecology, V.N. Karazin Kharkiv National University, Kharkiv, 61022, Ukraine

\*Corresponding author e-mail: [anna.fedorova@karazin.ua](mailto:anna.fedorova@karazin.ua)

Hindlimb anomalies are the most widespread among all developmental anomalies of tailless amphibians (Meteyer et al., 2000; Ballengee & Sessions, 2009). Severe limb malformation may reduce the viability and reproduction success of an individual. Herein we report noticeable hindlimb malformations in froglets of European water frogs *Pelophylax* Fitzinger, 1843, from one particular locality.

In September 2018, we collected a sample of 21 water frog froglets in the old artificial pond, the Lower Dobrytskyi pond (NP "Homilshanskilisy", Kharkiv region, Ukraine, 49° 37'40" N, 36° 16'58" E). It was previously shown that the Lower Dobrytskyi pond was inhabited by a population system of hybrid edible frogs *Pelophylax esculentus* Linnaeus, 1758, (both diploid and triploid forms) and one of its parental species, the marsh frog *Pelophylax ridibundus*, Pallas, 1771 (Meleshko et al., 2014; Kryvoltsevych et al., 2022). Adult hybrids are usually easily distinguishable from *P. ridibundus* by a complex of morphological traits that include colouration of hips and vocal sacs (for males) and shape of the inner metatarsal tubercle (e.g. Kierzkowski et al., 2013). However, these differences are not reliable for the identification of

subadults, consequently we refer to all the froglets only as *Pelophylax*. We assessed the froglets sex and ploidy, by examination of gonadal morphology and chromosomal slides prepared from intestine and/or bone marrow (Macgregor & Varley, 1983). For this purpose, the froglets were euthanised with ethylacetate vapours and dissected; specimens were further stored in 96 % ethanol. Data from the investigation of ploidy is described in detail in Fedorova et al. (2018). Water frogs are not listed as Endangered species either in IUCN Red List or Red Data Book of Ukraine. All manipulations were undertaken according to the Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research (ASIH, 2004).

In most literature sources, the terminology concerning hindlimb malformations lacks consistency. Here we follow Nekrasova (2008) by referring to the total absence of any part of the limb as ectromelia and shortened limb as hemimelia. The disturbed body plan of the limb is described as taumelia.

Among the 21 froglets studied, 13 were females, and eight were males. All froglets were diploid. Seven individuals (33 %, five females and two males) had severe malformations of



Figure 1. *Pelophylax* froglets with serious hindlimb malformations- A.–E. Ectromelia, F. Hemimelia, and G. Taumelia (arrow) and hemimelia

hindlimbs. Five individuals had ectromelia (Fig. 1A–E), one had hemimelia (Fig. 1F), and one had hemimelia accompanied by taumelia (Fig. 1G). The presence of malformations was not associated with the sex of froglets ( $\chi^2 = 0.2074$ ,  $p = 0.6488$ ).

Our previous research on 190 adult water frogs from the same locality showed only one frog with a missing hindlimb and one with taumelia (Kryvoltsevych et al., 2022). Based on that research and our data on the froglets presented in the current study we applied Fisher's exact test to estimate the difference between the frequency of hindlimb malformations among froglets and among adults. The test results showed a significant difference (two-tailed,  $p < 0.0001$ ), indicating that such types of malformations may affect the viability of frogs.

Revealing the primary reason of each malformation would be a complex task because malformations could result from either natural or anthropogenic factors, including parasitic infections, exposure to UV radiation and chemical pollutants, physical traumas, and genetic defects (Johnson et al., 1999; Ouellet, 2000; Henle et al., 2017). Furthermore, Ballengee & Sessions (2009) showed that the most likely reason for missing limbs and missing limb segments is predation by dragonfly nymphs who can injury or even amputate the limbs of tadpoles. The extent of subsequent limb regeneration, and as a result the extent of limb deformity, depends on the tadpole's stage of development: younger tadpoles have a greater chance of full limb regeneration. The local occurrence of this phenomenon may indicate a link to genetic traits of the quite complex local population system. Such frequent limb malformation has not been reported from other *Pelophylax* populations in this national park, although they have been monitored for almost a decade.

Currently, we are unable to state why there was such a high frequency of missing hindlimbs in these froglets. Additional investigations with larger samples (including both larvae and metamorphosed individuals) are required.

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