

## Two cases of unclear hindlimb malformation in Bombina variegata

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Limb malformations in anurans are recently frequently reported worldwide (e. g. Ouellet 2000, Sessions 2003, Mônico *et al.* 2016, Sousa & Costa-Campos 2016). Although malformations are unlikely to cause major population declines, this problem might represent an emerging threat (Hoppe 2000). Herein we report noticeable limb malformations at *Bombina variegata* (Linnaeus, 1758), a small anuran species found throughout most of central and southern Europe from France through Balkans to southern Greece (Sillero *et al.* 2014). It occurs mostly on disturbed sunny habitats of temporary character such as flooded wheelruts, cattle troughs or drainage ditches (Speybroeck *et al.* 2016).



**Figure 1.** Observed individuals of *Bombina variegata* with a hind limb malformations from Shëndelli Mts., Albania: (A) ventral view, (B) dorsal view; and Lacul Poiana Mărului, Romania: (C) ventral view, (D) dorsal view.

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On 28<sup>th</sup> June 2014 in a small pond of the foothills of Shëndelli Mts., southern Albania (40.379°N, 20.030°E, 776 m elev.), we observed an adult female of *B. variegata* with a malformation of the right hind limb (Fig. 1A, B). Ectromelia of femur and tibiofibula with a presumably functional joint was present. Tarsal bones and digits were completely missing. Posterior part of the limb was shaped into a point with a tip part of soft tissue. Despite this malformation, observed individual was in a good nutritional condition, fully mobile, without any other visible injuries.

Second observation was made on  $16^{\text{th}}$  June 2016 near a road leading to Lacul Poiana Mărului (45.428°N, 22.466°E, 523 m elev.), Romania. We found an adult female of *B. variegata* with a limb malformation very similar to the one described above (Fig. 1C, D). Ectromelia of calcanium and talus on the left hind limb was present. Digits were completely missing. Again, posterior part of the limb was shaped into a point with a tip part of soft tissue. Although malformed, this adult female seemed, again, to be capable of regular feeding, according to its nutritional condition. No additional injuries were present. Many more individuals (>50) of *B. variegata* were observed on the locality but none were malformed such as the one described.

Missing or malformed limbs as one of the most common anuran deformities (Ballengée & Sessions 2009) are considered to be a result of various possible teratogenous causes. Ouellet et al. (1997) and Lannoo (2008) discuss environmental pollution by pesticides and other chemicals used in agriculture as one of the factors causing malformed body parts during amphibian ontogenesis. Blaustein et al. (1997) proved UV-B radiation to be causing deformities of anuran embryos. Johnson et al. (1999) and Kiesecker (2002) blame parasitic infestation by trematodes for incorrect limb development; and there are other suggested causes discussed such as microbes or other diseases (Sessions & Ruth 1990). It was proven that some anuran species develop body malformations caused by nitrogenous fertilizers during larval stage and these deformities affect their movement after metamorphosis (e. g. Marco et al. 1999). However, missing part of a hind limb could be also caused by previous attempt of predation (Ballengée & Sessions 2009). The Yellowbellied toad has several potential predators such as mammals, water birds, reptiles (Bajger 1980) or even invertebrates such as Aeshna cyanea (Vorndran et al. 2002) predating on tadpoles. Notwithstanding, in our records is remarkable a shape of the posterior parts of the malformed limbs; both are formed into a point. Considering this shape and similar previous findings of Meteyer et al. (2000), we may presume that the limbs were exposed to the teratogenous factor before metamorphosis. However, missing parts of limbs are obviously difficult to interpret due to various possible causes. Therefore we cannot be certain which factor (or factors) caused malformations we observed. Nevertheless, we lean to the environmental pollution by chemicals used in agronomy as a presumed cause of the origin.

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## References

- Bajger, J. (1980) Diversity of defensive responses in populations of fire toads (*Bombina bombina* and *Bombina variegata*). *Herpetologica*, 133–137.
- Ballengée, B. & Sessions, S. K. (2009) Explanation for missing limbs in deformed amphibians. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, 312 (7), 770–779.
- Blaustein, A. R., Kiesecker, J. M., Chivers, D. P. & Anthony, R. G. (1997) Ambient UV-B radiation causes deformities in amphibian embryos. *Proceedings of the National Academy of Sciences*, 94 (25), 13735–13737.
- Hoppe, D. M. (2000) History of Minnesota frog abnormalities: do recent findings represent a new phenomenon? *Journal of the Iowa Academy of Science*, 107, 86–89.
- Johnson, P. T., Lunde, K. B., Ritchie, E. G. & Launer, A. E. (1999) The effect of trematode infection on amphibian limb development and survivorship. *Science*, 284 (5415), 802–804.
- Kiesecker, J. M. (2002) Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? *Proceedings of the National Academy of Sciences*, 99 (15), 9900–9904.

- Lannoo, M. (2008) The collapse of aquatic ecosystems: malformed frogs. University of California Press, Berkeley, 288 pp.
- Marco, A., Quilchano, C. & Blaustein, A. R. (1999) Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. *Environmental Toxicology and Chemistry*, 18 (12), 2836–2839.
- Meteyer, C. U., Loeffler, I. K., Fallon, J. F., Converse, K. A., Green, E., Helgen, J. C., Kersten, S., Levey, R., Eaton-Poole, L. & Burkhart, J. G. (2000) Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. *Teratology*, 62 (3), 151–171.
- Mônico, A. T., Silva-Soares, T. & Clemente-Carvalho, R. B. G. (2016) *Pipa carvalhoi* (Carvalho's Surinam Toad; Sapo d'água). Hindlimb malformation. *Herpetological Review*, 47 (1), 115.
- Ouellet, M., Bonin, J., Rodrigue, J., DesGranges, J. L., & Lair, S. (1997) Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. *Journal of wildlife diseases*, 33 (1), 95-104.
- Ouellet, M. (2000) Amphibian deformities: current state of knowledge. 617–661. In: Ecotoxicology of Amphibians and Reptiles. D. W. Sparling, G. Linder, and C. A. Bishop (eds.). Society of Environmental Toxicology and Chemistry, Pensacola, Florida.
- Sessions, S. K. & Ruth, S. B. (1990) Explanation for naturally occurring supernumerary limbs in amphibians. *Journal of Experimental Zoology*, 254 (1), 38–47.
- Sessions, S. K. (2003) What is causing deformed amphibians? 168–186. In: Amphibian Conservation. R. D. Semlitsch (ed.). Smithsonian Institution, Washington, D.C. 324 pp.
- Sillero, N., Campos, J., Bonardi, A., Corti, C., Creemers, R., Crochet, P.-A., Crnobrnja-Isailović, J., Denoël, M., Ficetola, G. F., Gonçalves, J., Kuzmin, S., Lymberakis, P., de Pous, P., Rodríguez, A., Sindaco, R., Speybroeck, J., Toxopeus, B., Vieites, D. R. & Vences, M. (2014) Updated distribution and biogeography of amphibians and reptiles of Europe. *Amphibia-Reptilia*, 35 (1), 1–31.
- Sousa, C. J. & Costa-Campos, C. E. (2016) *Leptodactylus podicipinus* (Pointedbelly Frog). Malformations. *Herpetological Review*, 47 (1), 112-113.
- Speybroeck, J., Beukema, W., Van Der Voort, J., Velikov, I. & Bok, B. (2016) Field Guide to the Amphibians and Reptiles of Britain and Europe. Bloomsbury Publishing. 432 pp.
- Vorndran, I. C., Reichwaldt, E. & Nürnberger, B. (2002) Does differential susceptibility to predation in tadpoles stabilize the *Bombina* hybrid zone? *Ecology*, 83 (6), 1648-1659.