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Axanthic green toads, *Bufotes viridis* (Anura: Bufonidae), from Cologne, Germany

Rieke Schluckebier[1], Maria Sachs[1], and Miguel Vences[2,*]

The Green Toad, *Bufotes viridis* (Laurenti, 1768), stands out among Central European amphibians by its characteristic colour pattern, with a beige-grey dorsal ground colour with sometimes a reddish shade, and numerous green markings, irregularly distributed across the dorsal and lateral sides, including fore- and hindlimbs (Stöck et al., 2008). In males, these markings are light green, mostly sharply delimited but, especially on the dorsal side, they can be slightly blurry (Fig. 1). In females, the markings are darker green and more distinctly separated from the beige ground colour (Fig. 1A vs. 1B), and the bigger warts at the lateral side of the head are often reddish. The ventral side usually is of light greyish colour, often uniform or speckled with a few green spots; the iris is typically greenish (Stöck et al., 2008).

Here we report on several green toad individuals strongly differing from the species' usual appearance, observed during nocturnal surveys in the Westhovener Aue, Cologne, Germany, in 2018 and 2021 (geographical coordinates: 50.9037ºN, 7.0092ºW). On 8 May 2018 we captured 20 green toads in total and four of them (20%) showed an abnormally dark colour pattern. All four dark-coloured toads were adult males. Specimens were released at the site. On 10 May 2021 we surveyed the same site and captured three dark-coloured individuals, again all adult males, among an observed total of approximately 25 to 30 green toads. These toads were much darker than the other toads found at the same site, almost without obvious green colour. The characteristic dorsal and lateral markings were barely visible but could be recognised when carefully inspecting especially the area of the head, including the parotoid glands, where sometimes a greenish shade remained. The ventral side was much lighter, somewhat translucent, with a distinct pinkish colour especially on the gular region but also on posterior abdomen and ventral side of shanks. Variable parts of the ventral side were pigmented blackish (Fig. 1C). The eyes were completely black. Other than the deviant colour, these toads did not seem to differ obviously in body size or by other physical abnormalities from the other individuals.

The surveys took place during the peak of the species' mating season, and all individuals were found at the shore or in shallow water, in the main water body of the habitat. On both occasions, many males were emitting their characteristic trills, and some were in amplexus with females. We did not observe the dark-coloured males calling or mating but cannot exclude they were sexually active as all of them seemed vital and were mixed among the normal-coloured individuals. Since we did not photograph in detail the specimens found in 2018, we could not assess whether the 2021 observations included recaptures.

A large number of anomalies have been recorded from amphibians, many of which concern colour pattern (Henle et al., 2017). Abnormal black eyes, such as those in the recorded green toads, have been observed in at least 29 species of amphibians, almost all of which were anurans (Henle et al., 2017). While the direct genetic causes underlying black-eyedness in amphibians have not yet been determined, several factors have been associated with this anomaly, for instance pollution, temperature, absence of natural predators, or inbreeding (Henle et al., 2017). Additionally, eye colour variation of individuals within a population can correlate with different dorsal body colouration (Glaw and Vences, 1997). The number of black-eyed individuals is usually very low within a population (Henle et al., 2017).

Along with Jablonski et al. (2014) who observed a very similar case, we classify the dark-coloured green toads observed at the Westhovener Aue as axanthic.

[1] University of Cologne, Institute of Zoology, Zülpicher Str. 47b, 50674 Köln, Germany.
[2] Braunschweig University of Technology, Zoological Institute, Mendelssohnstr. 4, 38106 Braunschweig, Germany.
[*] Corresponding author. E-mail: m.vences@tu-braunschweig.de

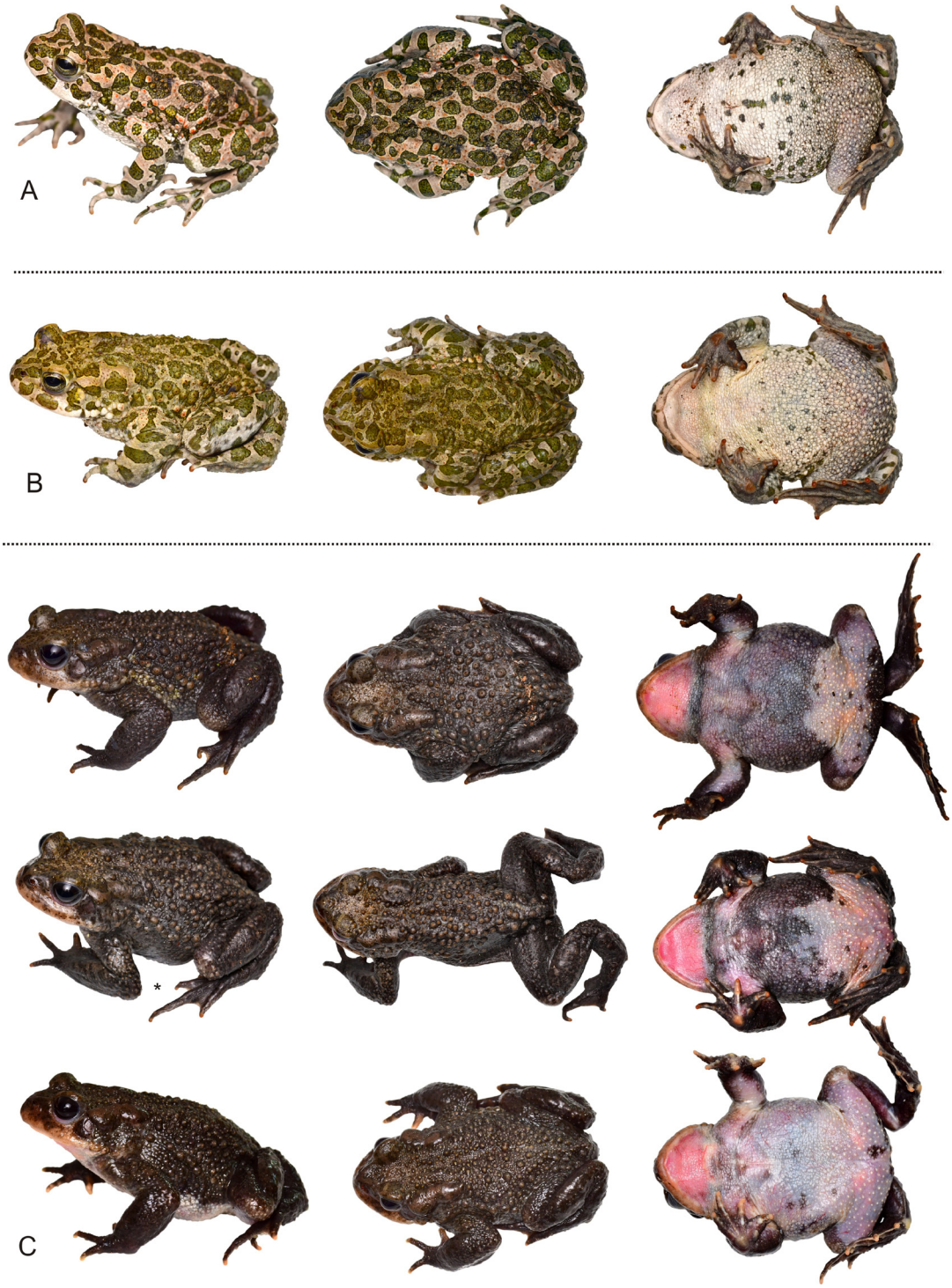

Figure 1. Comparison of normal colouration and abnormal colouration in green toads (*Bufotes viridis*) from the Westhovener Aue, Cologne, Germany, photographed in May 2021 by M. Vences. In the first two rows (A) a female and (B) a male green toad (top to bottom) are shown. Rows three to five show (C) three of the axanthic male individuals. A small asterisk marks one lateral picture that was mirrored for graphical consistency with the orientation of the other pictures.

Axanthism describes a genetic mutation that causes a lack or disfunction of xanthophores, which produce yellow pigments (pteridines, carotinoids) in the animal's skin (Browder, 1968; Bechtel, 1995). Erythrophores (responsible for red/orange pigmentation) and iridophores (reflecting short wavelengths) can also be affected within axanthic animals (Frost et al., 1986; Jablonski et al., 2014). In contrast to melanistic specimens, axanthics do not over-proliferate melanophores (Frost-Mason and Mason, 1996). According to Browder (1968) the lack of xanthophores and iridophores causes melanophores to show through stronger, resulting in a darker body colouration. Therefore, axanthic specimens show a slightly lighter body colouration in which often the pattern is still visible, compared to melanistic individuals (Jablonski et al., 2014). Additionally, a reduced number of iridophores can cause parts of the ventral body side to appear pink (Browder, 1968).

Jablonski et al. (2014) define three types of axanthism which can be differentiated by body colouration (completely or partially blue, completely or partially greyish or darker, or a normal body colouration with black eyes) and can occur in combination. For the green toads of the Westhovener Aue it is most accurate to classify them at least as partially axanthic, showing a combination of two types of axanthism (black eyes and partially darker body colouration; Jablonski et al., 2014).

While melanism or partial melanism (i.e., abnormally dark, completely, or partially black colour) has been described from various anuran species (Henle et al., 2017), axanthism is one of the least mentioned anomalies in colouration of amphibians, although it presumably occurs as often as better-known colour anomalies, such as albinism or leucism (i.e. partial or partial reduction of chromophores) (Bechtel, 1995; Lunghi, 2017). A juvenile green toad from Slovakia, phenotypically similar to the specimens of the Westhovener Aue has been reported as axanthic by Jablonski et al. (2014). Kolenda et al. (2017) observed another juvenile green toad with a darker spot on its dorsal body side, as well as an adult common toad (*Bufo bufo*) with darker skin colouration on the dorsal body side, both of which they considered being partially axanthic. Whether the very dark-colored juvenile of *Bufotes balearicus* reported by Bruni et al. (2020) qualifies as axanthic or rather melanistic is somewhat uncertain; this specimen appeared dorsally almost uniformly blackish without recognisable pattern, had black eyes, but an apparently normally coloured, perhaps somewhat translucent ventral side. The specimen was found by Bruni et al. (2020) together with other juveniles having more localised black spots and markings on the dorsum which were very similar to those reported in juvenile green toads and natterjack toads (*Epidalea calamita*) from the Cologne area in Germany by Vences and Glaw (1988) and Glaw and Vences (1989). However, it is doubtful if this "black spot" anomaly in otherwise normal-coloured juveniles is related to the axanthism or melanism affecting the full dorsal surface in other individuals. A further interesting observation of Bruni et al. (2020) concerns a green toad with only black eyes but otherwise normally coloured body. This latter example confirms that colour anomalies can affect the eyes and body separately. Given that both the pigment cells of iris and body skin are derived from the neural crest, this indicates that either the neural crest cells of the two tissues undergo separate cell regulation processes, or the mutations underlying colour anomalies may affect their migration in different ways (Amat et al., 2013).

Abnormally dark pigmentation can be influenced by several different external factors (disease, fungal infection, chemicals, high doses of UV-B irradiation) and often has genetic causes (Henle et al., 2017). However, in the Westhovener Aue, there is no evidence for pollution or disease, and all specimens were apparently in good health. Yet, we cannot exclude that environmental pollution in this urban area might have affected some toads at a certain point in time; as suggested by Papezikova et al. (2020) for snakes and Marushchak et al. (2021) for amphibians), the correlation of environmental factors such as chemical pollution and human footprint with frequency of colour anomalies merits additional study. As another possible explanation, low genetic variability in a natural population presumably can result in axanthic phenotypes (Oliveira et al., 2020). Since the population in the Westhovener Aue is genetically isolated from other populations within the same area this could be an explanation for the occurrence of the phenotypically different individuals, although its allelic variation was not found to be particularly low (Vences et al., 2019). In the absence of evidence from pollution or inbreeding in our case, we hypothesise that most likely the cause of the colour anomaly was a spontaneous mutation in a gene controlling pigment production, for instance production of carotinoids or pteridines. It would be tempting to assume a sex-linked mutation given that all encountered specimens were males; however, since females are typically much more rarely observed at the

breeding waters, we here refrain from this speculation. Similarly, the data at hand do not allow to assess whether this mutation is vertically propagated in the population, i.e., whether axanthic individuals are reproducing. Given the known longevity of *Bufotes viridis* of up to 15 years (Sinsch et al., 2007), is well possible that all encountered individuals were full siblings.

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References

Amat, F., Wollenberg, K.C., Vences, M. (2013): Correlates of eye colour and pattern in mantellid frogs. Salamandra **49**: 7–17.

Bechtel, H.B. (1995): Reptile and amphibian variants: colors, patterns, and scales. Krieger Publishing Company, Malabar, Florida.

Browder L.W. (1968): Pigmentation in *Rana pipiens*: I. Inheritance of the speckle mutation. Journal of Heredity **59**: 163–167.

Bruni, G., Di Mitri, A., Grecchi, L., Di Nicola, M.R. (2020): "Translucent" colour aberrations in *Bufotes balearicus* (Anura: Bufonidae) and *Hyla perrini* (Anura: Hylidae) from Italy. Herpetology Notes **13**: 57–60.

Frost, S.K., Epp, L.G., Robinson. S.J. (1986): The pigmentary system of developing axolotls. IV. An analysis of the axanthic phenotype. Journal of Embryology and Experimental Morphology **95**: 117–130.

Frost-Mason, S.K., Mason, K.A. (1996): What insights into vertebrate pigmentation has the axolotl model system provided? International Journal of Developmental Biology **40**: 685–693.

Glaw, F., Vences, M. (1989): Zur Verbreitung von Wechselkröte (*Bufo viridis* Laurenti, 1768) und Kreuzkröte (*Bufo calamita* Laurenti, 1768) im nördlichen Rheinland. Jahrbuch für Feldherpetologie **3**: 61–75.

Glaw, F., Vences, M. (1997): A review of anuran eye colouration: definitions, taxonomic implications and possible functions. – pp. 125–138 in Böhme, W., Bischoff, W., Ziegler, T. (eds.): Herpetologia Bonnensis (Proceedings of the eighth ordinary general meeting of the Societas Herpetologica Europaea). Bonn (SEH), 416 pp.

Henle, K., Dubois, A., Vershinin, V. (2017): A review of anomalies in natural populations of amphibians and their potential causes. Mertensiella **25**: 57–164.

Jablonski, D., Alena, A., Vlček, P., Jandzik, D. (2014): Axanthism in amphibians: A review and the first record in the widespread toad of the *Bufotes viridis* complex (Anura: Bufonidae). Belgian Journal of Zoology **144**: 93–101.

Kolenda, K., Najbar, B., Najbar, A., Kaczmarek, P., Kaczmarski, M., Skawiński, T. (2017): Rare colour aberrations and anomalies of amphibians and reptiles recorded in Poland. Herpetology Notes **10**: 103–109.

Lunghi, E., Ficetola, G.F., Barzaghi, B., Vitillo, C., Mulargia, M., Manenti, R. (2017): Melanism in European plethodontid salamanders (Amphibia, Plethodontidae, *Hydromantes*). Spixiana **40**: 157–160.

Oliveira, C.R. de, Mascarenhas, W., Cardozo Ribeiro, S., Borges Nojosa, D.M. (2021): Axanthism in *Pithecopus gonzagai* Andrade et al., 2020 (Anura: Phyllomedusidae). Cuadernos de Herpetología **35**: 157–159.

Marushchak, O.Y., Nekrasova, O.D., Tytar, V.M., Smirnov, N.A., Korshunov, O.V., Pupins, M., et al. (2021): A GIS approach to the study of colour anomalies in amphibians of Ukraine reveals the deleterious effect of human impacts. Herpetology Notes **14**: 1239–1251.

Papezikova, S., Oselsky, M., Papezik, P., Jablonski, D. (2020): Albinism in *Natrix tessellata* (Serpentes: Natricidae). Phyllomedusa **19**: 165–176.

Sinsch, U., Leskovar, C., Drobig, A., König, A., Grosse, W.-R. (2007): Life-history traits in green toad (*Bufo viridis*) populations: indicators of habitat quality. Canadian Journal of Zoology **85**: 665–673.

Stöck, M., Roth, P., Podloucky, R., Grossenbacher, K. (2008): Wechselkröten unter Berücksichtigung von *Bufo viridis viridis* Laurenti, 1768; *Bufo variabilis* (Pallas, 1769); *Bufo boulengeri* Lataste, 1879; *Bufo balearicus* Böttger, 1880 und *Bufo siculus* Stöck, Sicilia, Belfiore, Lo Brutto, Lo Valvo und Arculeo, 2008. In Handbuch der Reptilien und Amphibien Europas. Band 5 (Froschlurche II), 413–498.

Vences, M., Glaw, F. (1988): Kreuzkröten mit schwarzen Flecken im Raum Köln. Jahrbuch für Feldherpetologie **2**: 127–128.

Vences, M., Perl, R.G.B., Giesen, K., Schluckebier, R., Simon, K., Schmidt, E., et al. (2019): Development of new microsatellite markers for the Green Toad, *Bufotes viridis*, to assess population structure at its northwestern range boundary in Germany. Salamandra **55**: 191–198.

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