Axanthism in amphibians: A review and the first record in the wide-spread toad of the *Bufotes viridis* complex (Anura: Bufonidae)

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ABSTRACT. Axanthism in amphibians is a relatively rare color aberration reported less often than leucism or albinism. It is caused by lack of specific types of pigment cells in the skin, namely xanthophores, erythrophores, and iridophores. Here, we present the first case of occurrence of this aberration in a widely distributed toad of the green toad (*Bufotes viridis*) complex and provide an extensive review of axanthism occurrence in amphibians. So far it has been reported in more than 20 species from nine families of amphibians with the highest occurrence in the family Ranidae.

KEY WORDS: amphibia, *Bufo viridis*, color aberration, Slovakia.

INTRODUCTION

Coloration plays an important role in various aspects of animal life history. In many species of amphibians and reptiles cryptic or aposematic coloration enhances protection against visual predators, while at the same time it can provide valuable information to cognates about sex, fitness, maturity or availability for reproduction (Vitt & Caldwell, 2009). Therefore natural selection usually eliminates any aberrations that occur (Andrén & Nilson, 1981). However, there are several examples when specimens or even populations that are characterized by aberrant coloration, can survive and reproduce, e.g. melanic specimens of the common lizard *Zootoca vivipara* or an albinotic population of the snake *Elaphe climacophora* (Schulz, 1996; Gvoždík, 1999; Jambrich & Jandzik, 2012). Changes in coloration are also often related to life in the dark, e.g. in caves, as is known in the Mexican tetra *Astyanax mexicanus* (Mitchell et al., 1977; Wilkens, 1988), whose surface populations are normally pigmented, while the cave populations lack pigmentation. Another such example is the olm *Proteus anguinus*, a troglobiont urodele known in two forms: either completely unpigmented or with black pigmentation (Sket & Arntzen, 1994).

Color aberrations that have been described in vertebrates are albinism, amelanism, axanthism, erythrism, hypomelanism, leucism, melanism and piebaldism, though this list is not exhaustive and nomenclature is not consensual (Bechtel, 1995). In wild amphibians the literature reports albinism (white or yellowish body color and red eyes) and leucism (white or pinkish body color, dark eyes) as the most common forms of aberrant coloration (e.g. Werner, 1893; Smallcombe, 1949; Campanna, 1973; Dyrkacz, 1981; Bechtel, 1995; Mikulíček et al., 2001; Thomas et al., 2002; Pabijan et al., 2004; López & Ghirardi, 2011; Escoriza, 2012). They usually result from gene mutations affecting development and distribution of chromatophores and/or skin pigment production (Duellman & Trueb, 1994; Bechtel, 1995).

Axanthism characterized by blue, bluish or generally grey, dark body color, very dark patterns, and dark eyes, is one of the least known aberrations, though it is presumably as widespread as albinism (Bechtel, 1995). Skin of axanthic animals lacks xanthophores (Browder, 1968), erythrophores, and iridophores, which normally produce yellow and orange, red, and light-reflecting and scattering pigments, respectively. In some cases, the xanthophores might be unable to produce the pigment, despite the fact they are present (Berns & Narayan, 1970). The lack of iridophores typically results in duller and darker overall coloration. In contrast to the skin, the eyes of axanthic specimens may contain iridophores and melanophores (rarely also xanthophores; Bechtel, 1995; Frost-Mason & Mason, 1996). So far, axanthism has been reported either in a partial or a complete form in several species of amphibians (e.g. Liu, 1931; Dubois & Vachard, 1971; Dubois, 1979; Juszczyk, 1987; Bechtel, 1995; Vlček, 2003, 2008; Niccoli, 2013; for other references and detailed information see Table 1).

AXANTHISM IN THE GREEN TOAD *BUFOTES VIRIDIS*

The taxonomically complicated species complex of green toads [*Bufotes viridis* (Laurenti, 1768), complex; also known as *Bufo viridis* in traditional taxonomy] includes at least 14 morphologically similar species distributed across Europe, Asia and Northern Africa (Stöck et al., 2006, 2008; Frost, 2014). Coloration and pattern of these toads varies across their distribution range, but generally the background coloration is light to dark brown, covered with darker green patches creating a camouflage pattern. Tiny red spots might be present on various parts of the body. The belly is usually pale without a pattern and the iris is yellow or yellowish (Arnold & Ovenden, 2002). Interestingly, despite the variation in tint or intensity of both background color and pattern, color aberrations are relatively rare within this species complex. To the best of our knowledge, only albinism (Flindt, 1985; Andrä, 2011), erythrism (Lanza & Canestrelli, 2002) and retinal depigmentation (Engelmann & Obst, 1976) have been reported so far.

Here, we report an axanthic juvenile specimen (approx. SVL 30 mm) of *B. viridis*, encountered in Pezinok, SW Slovakia (48.31993° N 17.24085° E; June 28, 2009). The specimen was overall very dark with some bluish tint on the belly. Brighter parts were confined to the area around mouth, throat and eyes and some brighter spots were also observed on parotid glands, dorsum, and hind limbs. Skin patches that normally form a camouflage pattern were also darker, though the contrast between them and the dark background was hardly detectable. Some poison gland openings had white or yellow tips, and tiny yellowish spots were also present on flanks anterior to hind limbs (Fig. 1). Between 35 and 50 normally pigmented specimens of *B. viridis* were observed in the area at the same time. From the total number of about 150 observed specimens in the season of 2009, only the one reported here was axanthic (0.7 %).

AXANTHISM IN AMPHIBIANS

Axanthism remains a rarely observed and reported color aberration in amphibians when compared to other color aberrations (see Dubois, 1979; Bechtel, 1995). In partially axanthic specimens it is easy to overlook this aberration, and completely axanthic specimens can easily be confused with melanic specimens. These, similarly to the axanthic ones, are also dark and have a somewhat disrupted pattern. Distinction between melanism and axanthism is possible based on the slightly lighter coloration of axanthic specimens, in which also the pattern is typically discernible. This is also the case of the green toad specimen reported herein. The anatomical explanation has been offered from studies on axolotls, in which melanic specimens lack both xanthophores and iridophores in their skin, but unlike axanthic specimens, they also over-proliferate melanophores resulting in much darker coloration (FROST-MASON & MASON, 1996). According to our summarized literature data (see Table 1), three basic types of axanthism can be recognized in amphibians: (i) complete or partial blue body coloration (mainly in Ranidae, see Table 1 and references therein), (ii) complete or partial grayish or dark body coloration, (iii) normal body coloration with black eyes. These three types are not mutually exclusive and combinations can occur (e.g. blue coloration with black eyes).

CAUSES OF AXANTHISM

Although the genetic background of pigment cell formation in amphibians is relatively well known (NEVO, 1973; FROST-MASON & MASON, 1996; HOFFMAN & BLOUIN, 2000), proximate causes of axanthism remain unknown. Besides genetic mechanisms, environmental causes cannot be excluded, such as temperature fluctuations, food quality, parasitism or environmental pollution (cf. DUBOIS, 1979; VERSHININ, 2004; CABALLERO et al., 2012). Interestingly, many observations of axanthic specimens (mainly in the family Ranidae; see Table 1) were reported from 1960-1990s from urban areas of industrial countries with potentially high levels of environmental pollution (cf. DANDOVÁ et al., 1995). However, in some cases the darker coloration can offer an adaptive advantage, e.g. in thermoregulation, which is particularly important in ectothermic vertebrates (e.g. melanic *Vipera berus*; ANDRÉN & NILSON, 1981). Conversely, loss of cryptic or aposematic pattern and coloration might lead to an increased conspicuousness and decreased intensity of warning signals, respectively, and thus a higher predation risk (CHILDS, 1953; ANDRÉN & NILSON, 1981). It is possible that in axanthic specimens a genetic relationship exists between

Fig. 1 – Axanthic specimen of the green toad *Bufotes viridis* from Slovakia. A: dorsal view; B: lateral view; C–D: comparison with normally colored specimen of the same size and from the same locality.

TABLE 1

A summary of the axanthism occurrence in amphibians.

Species	N	Locality	Dorsal pattern	Background color	Eye color	Stage	Sex	Habitat	References
Ambystomatidae									
Ambystoma mexicanum	-	laboratory	dark	grey	black	larvae, adult	-	-	Frost et al., 1984
Salamandridae									
Lissotriton helveticus	1	Fontainebleau, France	visible	normal	black	adult	M	forest pond	Dubois et al., 1973
Taricha granulosa	1	Corvallis, Oregon, USA	any indication of the yellow-orange pigment	dorsal deep blackish-brown, ventral slightly lighter blackish-brown	black	adult	M	artificial pond	Livezey, 1960
Alytidae									
Alytes obstetricans	?	Arteixo, Spain	-	dark, light ventrally	black	juvenile	-	-	Galan et al., 1990
Bufonidae									
Anaxyrus woodhousii	1	North Carolina, USA	unspotted chest, warts on each of the largest dark spots	blue	-	-	-	backyard, urban area	Bechtel, 1995
Bufo bufo	3	Paris region, France	-	brown-grayish-pink abnormal pigmentation	black-brown	2 tadpoles, 1 adult	M (adult)	-	Dubois, 1969
Bufotes viridis	1	Pezinok, Slovakia	dark, partly visible	dark	black	juvenile	-	urban area	this study
Craugastoridae									
Craugastor phasma	1	Las Tablas, Costa Rica	not visible, fifteen to twenty black spots	gray white	black	adult	F	rocky stream bank	Lips & Savage, 1996
Dicroglossidae									
Euphlyctis cyanophlyctis	2	Yangdi Khola, Suikhet, Nepal	partly visible	grayish	black	adult	M	urban area	Dubois, 1976
Hylidae									
Acris crepitans	1	Harrison Lake, Charles City, USA	-	mottled pattern of tan/grey, green, and blue	-	juvenile	-	lake	Niccoli, 2013
Hyla arborea	1	Apetlon, Austria	-	dark	black	adult	-	-	Hinz, 1976
Hyla cinerea	1	Brazos County, Texas, USA	-	dark gray except green head and shoulder	black	adult	F	farm pond	Cain & Utesch, 1976
Hyla japonica	1	Konosu, Japan	-	blue	-	juvenile	F	-	Nishioka & Ueda, 1985a
Hyla japonica	1	Tottori, Japan	-	blue	-	juvenile	F	-	Nishioka & Ueda, 1985a
Hyla japonica	1	Hesaka, Japan	-	dark grayish-brown; dark grayish-olive	black	-	F	-	Nishioka & Ueda, 1985d
Hyla japonica	3	Yachiyo-cho, Japan	-	dark grayish-brown; dark grayish-olive	black	1 juvenile, 2 adult	2F, 1M	greenhouse	Nishioka & Ueda, 1985d

Ranidae									
Pelophylax esculentus	1	Poland	dark, partly visible	grayish	black	adult	-	-	Juszczyk, 1987
Pelophylax esculentus	1	Havířov, Czech Republic	normal, visible	normal	black	adult	M	natural wetlands	Vlček, 2003
Pelophylax esculentus	1	Oldenburg, Germany	dark, visible	normal	black	adult	F	fishpond	Fischer, 1999
Pelophylax lessonae	1	Žermanice, Czech Republic	partly visible	grayish	black	adult	M	flooded mine	Vlček, 2008
Pelophylax lessonae	1	Svatý Kříž, W Bohemia, Czech Republic	dark, partly visible	dark	black	adult	M	fishpond, urban area	Dandová et al., 1995
Pelophylax nigromaculatus	1	Wei-hsiu Yuan, China	-	blue	-	-	-	lotus pond	Liu, 1931
Pelophylax porosus	1	Maki-cho, Nishikambara-gun, Japan	yellowish dorso-lateral and dorso-median stripes	semitransparent, blackish	light yellowish-brown	adult	F	-	Nishioka & Ueda, 1985c
Pelophylax plancyi	7	Wei-hsiu Yuan, China	-	blue	-	subadults	-	lotus pond	Liu, 1931
Lithobates catesbeianus	1	Kentucky, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	69	MW Wisconsin, SE Minnesota, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	31	scattered throughout Maine, Vermont, Massachusetts, New York, New Jersey (USA), SE Canada	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	1	Virginia, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates clamitans	2	USA	-	blue and green	-	-	-	-	Berns & Narayan, 1970
Lithobates pipiens	1	Sapelo Island, Georgia, USA	partly visible	light sky-blue, green, dark brown	-	adult	F	-	Martof, 1964
Lithobates pipiens	3	MW Wisconsin, SE Minnesota, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates pipiens	6	scattered throughout Maine, Vermont, Massachusetts, New York, New Jersey (USA), SE Canada	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates pipiens	1	Georgia, USA	-	light blue, deep blue, blue-green	-	-	-	-	Berns & Uhler, 1966
Lithobates pipiens	1	Broadus, Montana, USA	visible, normal	Blue coloration over the back from the tips of the external nares to the anus	-	-	-	-	Black, 1967
Lithobates pipiens	-	laboratory	dark, visible	dark	black	tadpoles, adult	-	-	Richards et al., 1969
Lithobates sylvaticus	15	Innoko, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Lithobates sylvaticus	118	Kenai, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Lithobates sylvaticus	20	Tetlin, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Lithobates sylvaticus	1	Yukon Delta, USA	-	-	black	-	-	wild refuge	Reeves et al., 2008
Rhacophoridae									
Rhacophorus schlegelii	8	Okuyama, Ashiya, Hyogo, Japan	-	dark brown	black	adult	7F, 1M	rice field	Nishioka & Ueda, 1985b

the lack of certain types of chromatophores and harmful mutations, decreasing the chance of individual survival and/or genetic fixation of this aberration at the population level. All of this can underlie the relative rarity of this aberration in general and in adult specimens in particular (Dubois, 1979).

TAXONOMIC DISTRIBUTION OF AXANTHISM IN AMPHIBIANS AND FREQUENCY OF OCCURRENCE

Despite relatively broad taxonomic distribution of axanthism in amphibians (9 families, 23 species of the Americas, Asia and Europe, see Table 1), we can consider it a rare aberration. The largest number of examples was reported from the family Ranidae (23 cases from 9 species; cf. Berns & Uhler, 1966; Dubois & Vachard, 1971; Dubois, 1979) and it seems to be far more common in frogs than in any other group of amphibians (see Table 1). The data on intraspecific or intrapopulation frequencies are scarce and difficult to obtain. Berns and Uhler (1966) reported frequencies within different localities, finding two blue specimens out of 1000 normal frogs (0.2%) and 22 out of 7000 (0.3%) frogs for *Lithobates clamitans*. However, Dubois (1979) reported significantly higher frequencies – 101 (8.5%) of blue post-metamorphic specimens in 1186 *Pelophylax* sp. from France and four (3.2%) among 126 specimens from Iran, while the same author found only eight (0.2%) black-eyed frogs among 4651 *Pelophylax* sp. and one (0.1%) in 777 *Bufo bufo* (Dubois, 1969; Dubois, 1979).

Data on frequencies of other color aberrations occurring in amphibians (albinism, leucism, melanism etc.) are scattered or completely lacking. The literature record of albinism and leucism is extensive (see e.g. Werner, 1893; Smallcombe, 1949; Brame, 1962; Campanna, 1973; Dubois, 1979; Dyrkacz, 1981; Bechtel, 1995; Mikulíček et al., 2001; Mitchell, 2002; Pabijan et al., 2004; Spadola & Insacco, 2010; López & Ghirardi, 2011; Modesti et al., 2011; Toledo et al., 2011; Escoriza, 2012; Keely & Maldonado, 2013 and references therein), so it can be easily assumed that these two aberrations are more frequent than axanthism in natural populations. On the other hand, albinotic or leucistic specimens are more conspicuous and more interesting to record, while axanthic specimens could be misidentified as melanic or entirely overlooked, so this comparison should be treated with care. Contrary to albinism and leucism, the frequency of melanism and piebaldism seems to be lower than that of axanthism in amphibians (cf. Bechtel, 1995).

Axanthism represents an interesting color aberration of amphibians that deserves more attention. So far, it is only logical that most cases of its occurrence are known from countries where most of the herpetological research has been carried out, i.e. North America and Europe. With more studies appearing from the other parts of the world, we can expect that both taxonomic and geographical distribution of axanthism will expand.

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