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New reports of amelanism, axanthism, erythrism, and melanism in amphibians from northern Greece

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Colouration is a critical aspect of animal biology, influencing a wide range of ecological and evolutionary processes, including both inter- and intraspecific communication (Galeano and Harms, 2016; Araujo et al., 2020). Aberrant colour patterns, while rare in natural populations, can have profound consequences for individual fitness. Amphibians are a good example of this. Antipredator colouration in this group can be either cryptic, offering prey camouflage (Vitt and Caldwell, 2013; Rojas, 2017), or aposematic, alerting potential predators to avoid the individual due to potential toxicity (Poulton, 1890; Noonan and Comeault, 2008; Pedroso-Santos et al., 2022). At the same time, colouration can provide valuable information to conspecifics regarding sex (Sztatecsny et al., 2012), fitness (Vitt and Caldwell, 2013), maturity (Vitt and Caldwell, 2013), mate quality (Gomez et al., 2009), or reproductive availability (Vitt and Caldwell 2013), and generally plays a significant role in communication (Galeano and Harms, 2016; Araujo et al., 2020). The colour phenotype is also involved in displays of dominance during male-to-male competition (Duellman and Trueb, 1994; Yang et al., 2020) and aids in thermoregulation (Clusella-Trullas et al., 2007). In many amphibian species it can also be highly plastic and influenced by factors like stress, environmental background, and temperature (Nilsson Sköld et al., 2013).

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Colouration in amphibians is the end product of the interaction of light with three distinct types of specialized, pigment-containing cells located in the dermis layer called chromatophores (Duellman and Trueb, 1994; Bechtel, 1995). Chromatophore units are organized into a layered structure, with xanthophores at the surface, containing pigments such as pteridines and carotenoids that absorb blue-violet light and appear yellow (Bagnara and Hadley, 1968, 1973). Beneath them lie iridophores, which reflect light in the blue-to-green spectrum, and melanophores at the base, which contain dark melanin pigments that absorb red and orange wavelengths (Bagnara and Hadley, 1968, 1973). This arrangement allows many frogs to acquire their stereotypical green colouration, as it facilitates the reflection of intermediate wavelengths such as green through the skin (Taylor and Bagnara, 1972; Bagnara and Hadley, 1973).

Aberrant colourations have been observed in several amphibian species (Kolenda et al., 2017; Marushchak et al. 2021; Allain et al., 2023; Iković et al., 2025) in various parts of the world. Here, we describe six such cases of rare colour aberrations in amphibians from northern Greece, namely amelanism and erythrism in Fire Salamanders, *Salamandra salamandra* (Linnaeus, 1758); erythrism in a Greek Stream Frog, *Rana graeca* Boulenger, 1891; axanthism in a Marsh Frog, *Pelophylax ridibundus* (Pallas, 1771) and a Common Treefrog, *Hyla arborea* (Linnaeus, 1758); and melanism in a Balkan Spadefoot Toad, *Pelobates balcanicus* Karaman, 1928.

An amelanistic Fire Salamander. Melanin deficiency may occur because of mutations in tyrosinase genes (Miura et al., 2018; Deng et al., 2023) and can potentially lead to phenotypes such as albinism, leucism, piebaldism, amelanism, and hypomelanism (Henle et al., 2017). Amelanism is defined as the complete absence of melanin or melanophores (Henle et al., 2017), a condition distinct from albinism which is characterized not only by the absence of brown or black melanin-based pigmentation, but also by the lack of other pigments, including pheomelanins, pteridines, and carotenoids (Schartl et al., 2016).

An amelanistic phenotype in *Salamandra salamandra* is characterised by the complete lack of black pigmentation in the skin and eyes, while the yellow markings retain their colouration. Such melanin-deficient records of *S. salamandra* have been documented across various parts of the species' range in the European continent. Their distribution appears to be widespread in Central and Western Europe, with a higher frequency in regions such as Germany, France, and the Iberian Peninsula (Balogová et al., 2022). Except for Croatia, observations in the Balkan region are completely lacking (Balogová et al., 2022).

The observation was made on 23 November 2024 during fieldwork conducted in the Drama region, near the border with Serres Prefecture (Fig. 1). An amelanistic juvenile *S. salamandra* (Fig. 2A) was observed on the margin of a small ephemeral pond. In the wider area, eight additional juveniles exhibiting the typical phenotype were recorded. Exact locality coordinates for this and all other records presented here are withheld to discourage poaching but can be made available upon legitimate scientific request. The observation was documented *in situ* photographically, and habitat characteristics were recorded. To our knowledge, this constitutes the first report of an amelanistic Fire Salamander from Greece and the southern Balkans.

An erythristic Fire Salamander. Erythrism is typically characterized by an abnormal increase in red pigmentation on an animal's skin, due to an excessive deposition of erythrophores (Jablonski et al., 2022). The eyes of erythristic animals may or may not be affected. In cases where the eyes are affected, red pupil colouration results from the absence of melanosomes, which makes the underlying blood vessels visible. The same process is responsible for the red pupil colouration in albinistic animals (Allain et al., 2023).

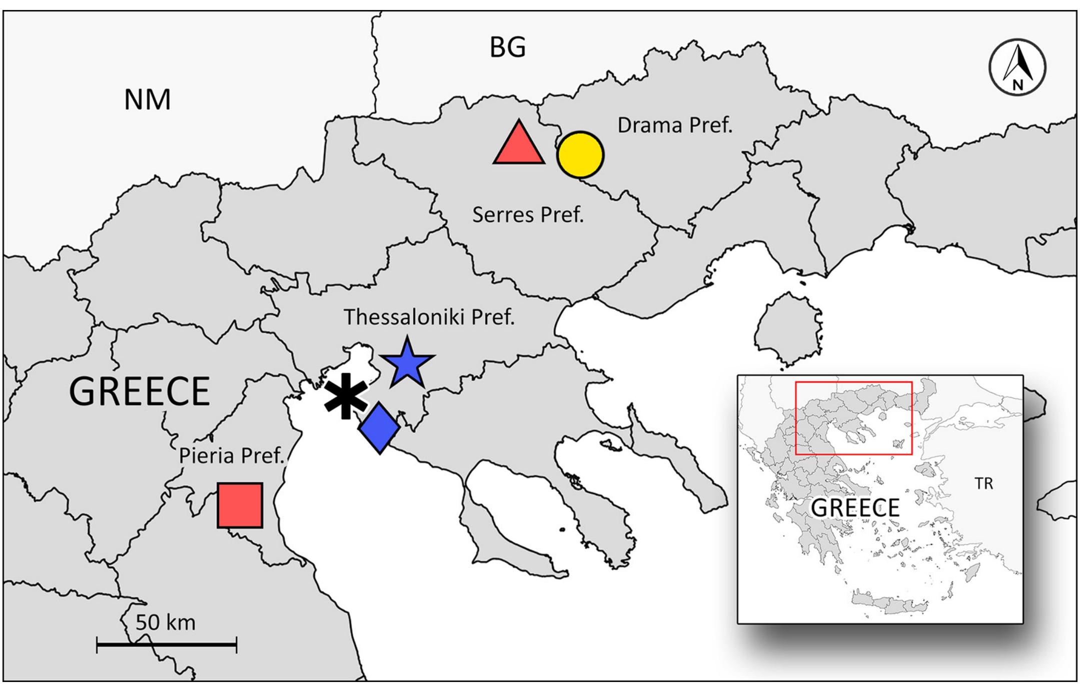

Figure 1. Localities for six observations of aberrant amphibian colour phenotypes in northern Greece. These include an amelanistic *Salamandra salamandra* from Drama Prefecture (yellow circle), an erythristic *S. salamandra* from Serres Prefecture (red triangle), an erythristic *Rana graeca* from Mt. Olympus, Pieria Prefecture (red square), an axanthic *Pelophylax ridibundus* from Thessaloniki Prefecture (blue star), an axanthic *Hyla arborea* from Thessaloniki Prefecture (blue diamond), and a melanistic *Pelobates balcanicus* from Thessaloniki Prefecture (black asterisk). The inset shows a map of Greece with the red frame marking the area of the larger map.

Figure 2. Aberrantly coloured amphibians from northern Greece. (A) An amelanistic juvenile *Salamandra salamandra* from Drama Prefecture. (B) An erythristic adult *S. salamandra* from Serres Prefecture (C) An erythristic *Rana graeca* from Mt. Olympus, Pieria Prefecture. (D) An axanthic *Pelophylax ridibundus* from Thessaloniki Prefecture. (E) An axanthic *Hyla arborea* from Thessaloniki Prefecture. (F) A melanistic *Pelobates balcanicus* from Thessaloniki Prefecture. Photos by Ilias Strachinis (A–C, F), Thomas Daftsios (D), and Spyridon Matthaiakis (E).

However, the evaluation of a red/reddish colouration can be subjective, as it depends on the observer's ability to perceive redness (Hofer et al., 2005; Bosten, 2022), the stage of ecdysis the animal may be in (Jablonski et al., 2022), and, finally, erythrism itself can vary in different shades of red, like reddish-brown (a mixture of red with blue or black pigments) or orange (a mixture of red with yellow pigments).

Erythrism may occur as a result of gene mutations controlling pteridine and carotenoid synthesis (Majerus and Mundy, 2003; Peterschmitt et al., 2009) but it can also be a consequence of diet (Hudon and Mulvihill, 2018), iron deposition in the environment (Mcgraw et al., 2005; Kolenda et al., 2017), or unidentified factors (Bukaciński and Bukacińska, 1997) in various species. Two different forms of individuals exhibiting abnormal red pigmentation have been identified: (1) cases in which xanthophores are substituted by erythrophores while the other pigment cell types remain unaffected, and (2) cases in which integumentary pigmentation is largely absent, with erythrophores being the only pigment cells present (Henle et al., 2017).

Erythristic phenotypes have been documented across several amphibian families, with occurrences reported in various species. In the Order Urodela, erythristic individuals have been recorded in species from the family Plethodontidae, such as *Plethodon cinereus* (Moore and Ouellet, 2014), and within the Salamandridae, erythristic phenotypes have been documented in *S. salamandra* (Rivera et al., 2001).

During fieldwork conducted in Serres Prefecture over a 12-month survey period between 2024 and 2025, the authors observed six erythristic adult *S. salamandra* (Fig. 2B) near small, shallow streams. These streams are located in very humid environments characterised by dense vegetation, including abundant deciduous trees such as oaks (genus *Quercus*) and other hydrophilic flora. Numerous salamander larvae exhibiting a range of yellow to pink colouration were also found in these streams. Several individuals were collected and translocated into the laboratory until metamorphosis. After keeping the larvae under laboratory conditions, where metamorphosis was observed sooner than their counterparts in the streams of origin, the pink-coloured larvae developed into orange-coloured juveniles, while yellow larvae retained their yellow pigmentation. A noticeable variation in colouration intensity was present among individuals of both groups.

An erythristic Greek Stream Frog. In the Order Anura, erythrism has been observed in various species, including in the ranids *Rana temporaria* (West and Allain, 2020) and *R. dybowskii* (Matushkina, 2023), the hylid *Pseudacris crucifer* (McAlpine and Gilhen, 2018), and the pelobatid *Pelobates fuscus* (Kolenda et al., 2017). Here, we report the first record of an erythristic *R. graeca* from Pieria Prefecture, Greece.

The erythristic female *R. graeca* (Fig. 2C) was encountered opportunistically on 18 September 2017 during the filming of a national television documentary on Greek wildlife presented by one of the authors (IS) on Mount Olympus (Fig. 1). Several additional *R. graeca* were observed in the surrounding area, but these all exhibited a colouration consistent with the typical phenotypic range of the species.

An axanthic Marsh Frog. Even though "blue" frogs have been recorded since 1885 (Haller, 1885), axanthism is one of the least understood colour aberrations in anurans (Jablonski et al., 2014). Axanthism results from the absence or dysfunction of xanthophores (Browder, 1968; Berns and Narayan, 1970; Bechtel, 1995), which may stem from mutations in genes responsible for xanthophore formation. Erythrophores and iridophores can also be affected in axanthic individuals (Jablonski et al., 2014), while their eyes exhibit dark colouration due to the presence of melanophores and iridophores (Frost et al., 1984; Frost-Mason and Mason, 1996). The precise causes of axanthism remain unidentified (Jablonski et al., 2014). Besides genetic mutations (Nakajima et al., 2021) and hormonal activity (Isoldi et al., 2010), ecological factors such as temperature fluctuations, food quality, parasitism, or environmental contamination, cannot be excluded (Vershinin, 2004; Caballero et al., 2012; Marushchak et al., 2021). Jablonski et al. (2014) categorised the three primary types of axanthism according to body colouration, which can occur either independently or in combination: completely or partially blue, completely or partially greyish or darker, or exhibiting normal body colouration with black eyes.

Axanthic phenotypes have been described in more than 20 amphibian species across nine families, with the highest number of records within the Ranidae (Jablonski et al., 2014). They have also been observed in common species of the Salamandridae, including *Mesotriton alpestris* (Fiorenza, 2012) and *S. salamandra* (Lucchini and Pizzigalli, 2020); in the bufonids *Bufotes viridis* (Jablonski et al., 2014; Schluckebier et al., 2022) and *Bufo bufo* (Allain et al., 2023); in the hylid *Dendropsophus minutus* (Araújo et al., 2020); and in the phyllomedusid *Phyllomedusa vaillantii* (Pedroso-Santos et al., 2022). In the Ranidae, axanthism has been reported in several species, including *Lithobates clamitans* and *L. catesbeianus* (Lindemann et al., 2020), *L. sphenocephalus* (Le Sage et al., 2018), and *Rana temporaria* (Julian, 2023). In the genus *Pelophylax*, axanthism has been observed in *P. ridibundus* (Allain et al., 2023) and *P. perezi* (Martinez Silvestre et al., 2016). We here report the first documented case of axanthism in *P. ridibundus* from Greece.

On 11 April 2024, during fieldwork aimed at recording reptiles and amphibians in the area of Thessaloniki (Fig. 1), an axanthic adult *P. ridibundus* (Fig. 2D) was observed in a seasonal pond with emergent vegetation adjacent to a dirt road. Approximately 15 additional individuals exhibiting the typical colouration were recorded in the same pond. The axanthic individual was captured and maintained under laboratory observation to assess whether the blue colouration persisted over time. The colouration remained unchanged after two months, suggesting that the phenomenon was unlikely to be hormonally induced. No additional individuals exhibiting blue or bluish colouration were detected at this site, either during the present survey or in previous years.

An axanthic Common Treefrog. In addition to the earlier field observations, an adult *Hyla arborea* exhibiting unusual blue colouration (Fig. 2E) was also recorded in Thessaloniki Prefecture (Fig. 1). The frog was observed on 17 May 2025 in a small pond near a wetland south of the city of Thessaloniki during a targeted amphibian night survey. This observation represents the first documented case of blue colouration in *H. arborea* in Greece. Similar cases of blue colouration in *Hyla* species have previously been reported from Japan, Germany, Bosnia and Herzegovina, and Slovakia (Zimić and Ćurić, 2024). The blue colouration of those individuals may have resulted from a physiological response to temperature fluctuations at the beginning and/or end of the activity season, potentially associated with elevated stress levels (Zimić and Ćurić, 2024).

A melanistic Balkan Spadefoot Toad. Melanism is defined as a condition in which the body, or parts of it, are completely black, and may occur either as partial or complete melanism (Henle et al., 2017). It refers to individuals exhibiting darker pigmentation compared to the typical phenotype and may appear as a polymorphism within species or as consistent variation among closely related taxa (True, 2003). Melanistic individuals are characterized by an overproduction of eumelanin, usually due to an increased number of melanophores, often accompanied by a reduction in xanthophores and iridophores as a result of genetic defects affecting pigment cell differentiation (Bagnara et al., 1978; Majerus and Mundy, 2003). Melanism has been associated primarily with mutations in a small number of nuclear genes, including the melanocortin-1 receptor (Mc1r) and the agouti signalling protein (ASIP) (Malacinski, 1978; Majerus and Mundy, 2003; Kingsley et al., 2009; Caro and Mallarino, 2020). Population density has also been reported as a factor affecting the occurrence of melanistic individuals (Hagen et al., 2003).

Melanistic phenotypes have been documented in several amphibian taxa. In anurans, dark or melanistic pigment variants have been described in *Rana temporaria* (Alho et al., 2010), *Lithobates pipiens*, *L. clamitans*, *L. sylvaticus*, *Ptychadena mascareniensis* (Richards and Nace, 1983), *Strongylopus fasciatus* (van Dijk, 1997), *L. chiricahuensis* (Fernandez and Bagnara, 1993), and *L. berlandieri* (Sanders and Smith, 1971). In urodeles, melanistic phenotypes or dark pigmentation have been documented in *Ambystoma mexicanum* (Malacinski, 1978), *Salamandra salamandra*, *Euptroctus asper*, and *Triturus marmoratus* (Rivera et al., 2001; Henle et al., 2017).

During an amphibian monitoring program conducted in the Aggelochori area, Thessaloniki, Greece (Fig. 1), within the framework of the European ACCOLAGOONS (Actions for the Conservation of Coastal Habitats and Significant Avifauna Species) Programme, a melanistic, dark-coloured male Balkan Spadefoot Toad, *Pelobates balcanicus* (Fig. 2F) was observed at night near a breeding pond, on 18 March 2016. Although numerous conspecifics were recorded throughout the wider Natura 2000 site (GR1220005), no other individuals exhibited a dark colour phenotype. To our knowledge, this represents the first documented record of such a morphotype in *P. balcanicus*.

Conclusions

Documented records of aberrant colour phenotypes in amphibians from Greece and the broader Balkan region remain scarce (Strachinis and Tsarouhas, 2021; Iković et al., 2025; Zimić and Ćurić, 2024). This apparent rarity may be due to several non-mutually exclusive factors. First, such phenotypes may indeed be inherently rare within natural populations (Henle et al., 2017). Second, individuals exhibiting aberrant colouration may experience reduced survival due to increased predation pressure (Childs, 1953), or colour aberrations may be associated with other deleterious mutations (Browder, 1972; Sanabria et al., 2010). Additionally, these colour variants may be less likely to be selected during reproduction, in species where positive colour assortative mating occurs, thereby favouring typical phenotypes (Maan and Cummings, 2008; Yang et al., 2016; Gade et al., 2016). Finally, the limited number of records may also reflect the fact that systematic field surveys and targeted documentation of amphibian diversity in Greece have intensified only over the past decade through national biodiversity monitoring programs implemented under EU obligations, as well as Natura 2000 Management Body monitorings.

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