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Fluorescence in amphibians and reptiles: new cases and insights

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Abstract. Fluorescence in amphibians and reptiles has emerged as a prominent study subject in recent years, with research focused on understanding its function and diversity. As the knowledge of fluorescence in vertebrates is still understudied, we surveyed amphibian and reptile species in montane and lowland Atlantic Forest sites to evaluate presence or absence of fluorescence. By randomly sampling species, we found evidence of fluorescence in amphibians of the genera *Scinax*, *Brachycephalus* and *Hylodes*, and reptiles of the genera *Bothrops*, *Enyalius* and *Hemidactylus*. Our findings increase the list of known species that may benefit from fluorescent patterns. Fluorescence was either ocular, dermal, or subdermal related to the skeleton or ossified dermal structures. Whether these species are able to see and interpret the different the patterns generated by fluorescent structures is yet to be discovered.

Keywords. Biofluorescence; Coloration; Herpetofauna; Natural History; Ontogeny; Sexual dimorphism; Terrestrial vertebrates; Visual communication.

Running title: Fluorescence in amphibians and reptiles

Biofluorescence was first described in the early 16th century through the study of medicinal herbs by a Spanish researcher (Lagorio et al., 2015). However, the first *in situ* observation was only reported in the 20th century for green-blue algae (Tswett, 1911). Cockayne (1924) published the first studies about biofluorescent animals. Since then, fluorescence has been the subject of investigation by numerous researchers. Among vertebrates, biofluorescence was primarily reported for marine species (Wucherer and Michiels, 2012; Sparks et al., 2014), while research on terrestrial tetrapods has only gained attention in recent years (Prötzel et al., 2021). For amphibians and reptiles, three main types of fluorescence are known. In reptiles, bone fluorescence stands out (Prötzel et al., 2018; Sloggett, 2018; Jeng, 2019; Top et al., 2020; Pinto et al., 2021; Maria et al., 2022), along with dermal fluorescence (Paul and Mendyk, 2021; Prötzel et al., 2021). In amphibians, in addition to dermal (Taboada et al., 2017a, b; Deschepper et al., 2018; Chaves-Acuña et al., 2020; Whitcher, 2020) and bone fluorescence (Goutte et al., 2019; Rebouças et al., 2019; Nunes et al., 2021), ocular fluorescence was reported recently (Deschepper et al., 2018; Alvarez et al., 2022).

Although the evolution of biofluorescence is still barely understood (Macel et al., 2020), some hypotheses have been proposed to explain its function. These include prey attraction (Haddock and Dunn, 2015; Paul and Mendyk, 2021), predator avoidance (Rebouças et al., 2019), camouflage (Sparks et al., 2014), visual communication (Goutte et al., 2019; Gray, 2019; Alvarez et al., 2022), visual recognition, mate choice, and sexual attraction (Hausmann et al., 2003; Prötzel et al., 2018).

In this study, we describe and illustrate fluorescence in four amphibian and four reptile species, and provide a list of amphibians that apparently did not display fluorescence when observed under UV light.

We used an UltraFire WF-5016 flashlight with a wavelength of 365 nm to test UV light fluorescence of several amphibians and reptiles. To maximize the chances of finding fluorescence, we caught and exposed the amphibians to light on all body sides, including the ocular region. When detecting the presence of UV fluorescence, we photographed the animal using a Nikon D7100 digital camera with a 100mm Sigma macro lens, with an aperture of f/5, ISO sensitivity of 3200, and a shutter speed of 1/200. After the tests, we recorded the presence or absence of fluorescence in a field spreadsheet and released the animals into the same location where we captured them. We categorized the fluorescence as dermal when detected on the surface of the animal's skin or in soft tissues, as bone fluorescence when reflected in areas such as the skull or vertebral column, and as ocular fluorescence when the fluorescence was displayed in the animal's eyes.

Individuals of *Brachycephalus nodoterga* were found in March 2022 in the Núcleo Santa Virgínia of the Parque Estadual da Serra do Mar, Natividade da Serra, São Paulo, Brazil. The specific location was known as "trilha do campinho" (23.866667ºS, 45.568611ºW, 855 m a.s.l.). Natividade da Serra is in mosaic-like Atlantic Forest vegetation, consisting of primary and secondary forests in different stages, bordered by eucalyptus plantations and pastures. The rainy season in this area occurs from October to March, while the drier season spans April to September. We searched opportunistically for other amphibians and reptiles from May to June 2023, during routine monitoring in the area of the NGO Projeto Dacnis (23.462947°S, 45.132943°W; 15-500 m a.s.l.). Projeto Dacnis encompasses a private reserve spanning 136 ha within the Atlantic Forest in Ubatuba, São Paulo, Brazil. The area is a swampy forest in low-lying areas and patches of primary and secondary dry forest on steep terrain. The climate is humid with rainfall incidence throughout the year. Finally, we also tested one individual of *Bokermannohyla alvarengai* in August 2023 in Monumento Natural Estadual Várzea do Lajeado e Serra do Raio, Serro, Minas Gerais, Brazil. The location is close to Caminho dos Escravos, in the district of São Gonçalo do Rio das Pedras (18.43019ºS, 43.464654ºW, 1165 m a.s.l.). The Serro region is predominantly covered by high-altitude savannah vegetation, with rocky and sandy fields and humid floodplains. There is also Atlantic Forest, with secondary forests, and areas deforested for agricultural use. The climate is characterized by two well-defined seasons, cold and dry winter, between April and September, and hot and humid summer, between October and March.

In total we tested 122 individuals of 25 amphibian and four reptile species (Table 1). Among the tested amphibians, five species displayed fluorescence: *Brachycephalus nodoterga* had dermal bones fluorescence on the dorsum (Fig. 1A–B); *Scinax argyreornatus* displayed dermal fluorescence on the dorsum, inguinal region, jaw, and upper part of the head (Fig. 1C–F); *Bokermannohyla alvarengai* presented dermal fluorescence on the entire dorsum, but in the blue spectrum (Fig. S1); *Hylodes phyllodes* and *H. asper* showed fluorescence only on their eyes (Fig. S2). Among reptiles, all four tested species displayed fluorescence. The lizard *Enyalius perditus* had fluorescence on the skull, with more evident reflections in males, both on the back and the lateral side of the head (Fig. 2A–F). A juvenile *Hemidactylus mabouia* gecko displayed fluorescence in both the skull and the vertebral column (Fig. 2G–I). The adult, photographed from a distance, showed fluorescence only on the upper part of the head and jaw. *Bothrops jararaca* and *B. jararacussu* displayed fluorescence only on the tail tip of juveniles (Fig. S3). From the three *B. jararaca* individuals (total length 28, 43, and 62 cm), the largest individual showed fluorescence only at a small portion of the tail tip.

Bone fluorescence in *Brachycephalus nodoterga* showed a distinct pattern from *B. ephippium*, *B. pitanga* and *B. rotenbergae* (Goutte et al., 2019; Nunes et al. 2021). This difference is due to the amount and distribution of dorsal ossified plates in these species (Goutte et al., 2019; Nunes et al. 2021). UV light fluorescence in a species of the genus *Scinax* and *Bokermannohyla* is here reported for the first time, despite fluorescence being recorded in other genera and species of hylid treefrogs (Taboada et al., 2017 a, b; Deschepper et al., 2018; Chaves-Acuña et al., 2020; Whitcher, 2020). Fluorescence in frogs could be related to intraspecific communication as a visual signal that complements acoustic signalling (Goutte et al., 2019; Gray, 2019) and can contribute to achromatic vision and the detection of other individuals in low-light environments (Lamb and Davis, 2020). Fluorescence in frogs of the genus *Hylodes* is also reported for the first time here. Furthermore, ocular fluorescence is reported only for four other anuran species in the genera *Boana* (Hylidae) and *Rana* (Ranidae) (Deschepper et al., 2018; Alvarez et al., 2022). Deschepper et al. (2018) suggest that fluorescent eyes are related to intraspecific recognition, whereas for Alvarez et al. (2022) this fluorescence type may be related to interspecific communication among sympatric species, thus avoiding predatory conflicts or disputes for food and territory.

Bone fluorescence for a species of the genus *Enyalius* is a novel information, similar to that reported by Prötzel et al. (2018) in chameleons of the genus *Caluma*, where males display more cranial fluorescence than females. In lizards, bone fluorescence has also been reported for the gekkonid genera *Chondrodactylus* (Sloggett, 2018), *Cyrtodactylus* (Jeng, 2019; Top et al., 2020), *Kolekanos* (Pinto et al., 2021), and *Hemidactylus* (Maria et al., 2022). Maria et al. (2022) reported fluorescence on the head and mandible of *Hemidactylus platyurus*, as well as a more pronounced fluorescence in juvenile individuals, similarly to what we observed for *H. mabouia*. Bone fluorescence in lizards was suggested to play a role in interspecific visual communication, serving as a secondary visual communication system that does not compromise their camouflage, as well as for attracting sexual partners (Prötzel et al., 2018; Top et al., 2020).

Our record of tail tip fluorescence in two pit viper species of the genus *Bothrops* is a novel information, although Paul and Mendyk (2021) already reported tail tip fluorescence in eight pit viper genera known or suspected to display tail luring to attract prey. Juveniles of both *B. jararaca* and *B. jararacussu* feed predominantly on frogs and use caudal luring to attract and catch this prey type (Sazima, 1991, 1992; Hartmann et al., 2003; Sazima, 2006). As frogs have UV light sensitivity (Thomas et al., 2022), this sense may be used to detect prey, and the fluorescence of the two snakes' tail tips could play an important role: attracting frogs at night (Sazima, 1991; Sazima and Haddad, 1992).

The noticeable decrease in tail tip fluorescence of the largest *B. jararaca* individual (not adult yet, see Sazima, 1992) is likely related to ontogenetic diet changes. Adult individuals ambush or actively hunt rodents and small mammals, and no longer display caudal luring (Sazima, 1991; Hartmann et al., 2003). Fluorescence decrease on the tail tip of large individuals was already reported in other vipers, also related to ontogenetic diet changes (Paul and Mendyk, 2021).

We failed to detect UV fluorescence in an additional 20 species tested. However, we do not exclude the possibility of fluorescence in those species. We illuminated them with a wavelength of 365 nm, and suggest experiments with longer wavelengths, as some species may only display fluorescence when exposed to lights of 400–415 nm (Whitcher, 2020).

As we showed, fluorescence in anurans and reptiles may be widespread (Deschepper et al., 2018), especially when considering that studies on fluorescence in terrestrial tetrapods have only begun to increase in recent years (Prötzel et al., 2021). Therefore, testing other species in different localities may reveal fluorescence of numerous other species, as well as provide insights into the ecological and evolutionary relevance of such coloration patterns.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 14922

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TABLES

Table 1. Amphibians and reptiles tested under UV light (wavelength 365 nm) and fluorescence type when present (bold).

Species	**Fluorescence**	**Type**	**Nº ind. tested**
Anura			
Brachycephalidae			
Brachycephalus nodoterga	**Yes**	**Bone**	**15**
Ischnocnema sp. (aff. *guentheri*)	No		6
Bufonidae			
Dendrophryniscus haddadi	No		4
Rhinella ornata	No		3
Craugastoridae			
Haddadus binotatus	No		3
Cycloramphidae			
Cycloramphus boraceiensis	No		9
Phyllomedusidae			
Phasmahyla sp. (aff. *cruzi*)	No		5
Pithecopus rohdei	No		3
Hylidae			

Boana albomarginata	No		3
Boana faber	No		2
Boana semilineata	No		2
Bokermannohyla alvarengai	**Yes**	**Dermal**	**1**
Bokermannohyla hylax	No		2
Itapotihyla langsdorffii	No		4
Scinax argyreornatus	**Yes**	**Dermal**	**9**
Scinax hayii	No		3
Scinax littoralis	No		5
Scinax perpusillus	No		3
Hylodidae			
Hylodes asper	**Yes**	**Ocular**	**6**
Hylodes phyllodes	**Yes**	**Ocular**	**5**
Hemiphractidae			
Fritziana mitus	No		2
Gastrotheca albolineata	No		1
Leptodactylidae			
Adenomera marmorata	No		5
Leptodactylus latrans	No		3
Physalaemus atlanticus	No		3
Squamata			
Leiosauridae			
Enyalius perditus	**Yes**	**Bone**	**6**
Gekkonidae			
Hemidactylus mabouia	**Yes**	**Bone**	**4**

Viperidae			
Bothrops jararaca	**Yes**	**Dermal**	**3**
Bothrops jararacussu	**Yes**	**Dermal**	**2**

CAPTIONS TO FIGURES

Figure 1. Amphibian species with fluorescence: *Brachycephalus nodoterga* photographed with flash (A) and UV light (B); *Scinax argyreornatus* photographed with flash (C); another *Scinax argyreornatus* with a dorsal stripe, photographed with flash (E); and the same individuals under UV light (D and F).

Figure 2. Lizard species with fluorescence: Male *Enyalius perditus* photographed with flash (A) and under UV light (B-C); Female *Enyalius perditus* photographed with flash (D) and under UV light (E-F); *Hemidactylus mabouia* photographed with flash (G) and, under UV light, an adult (H) and a juvenile (I).

Figure 1

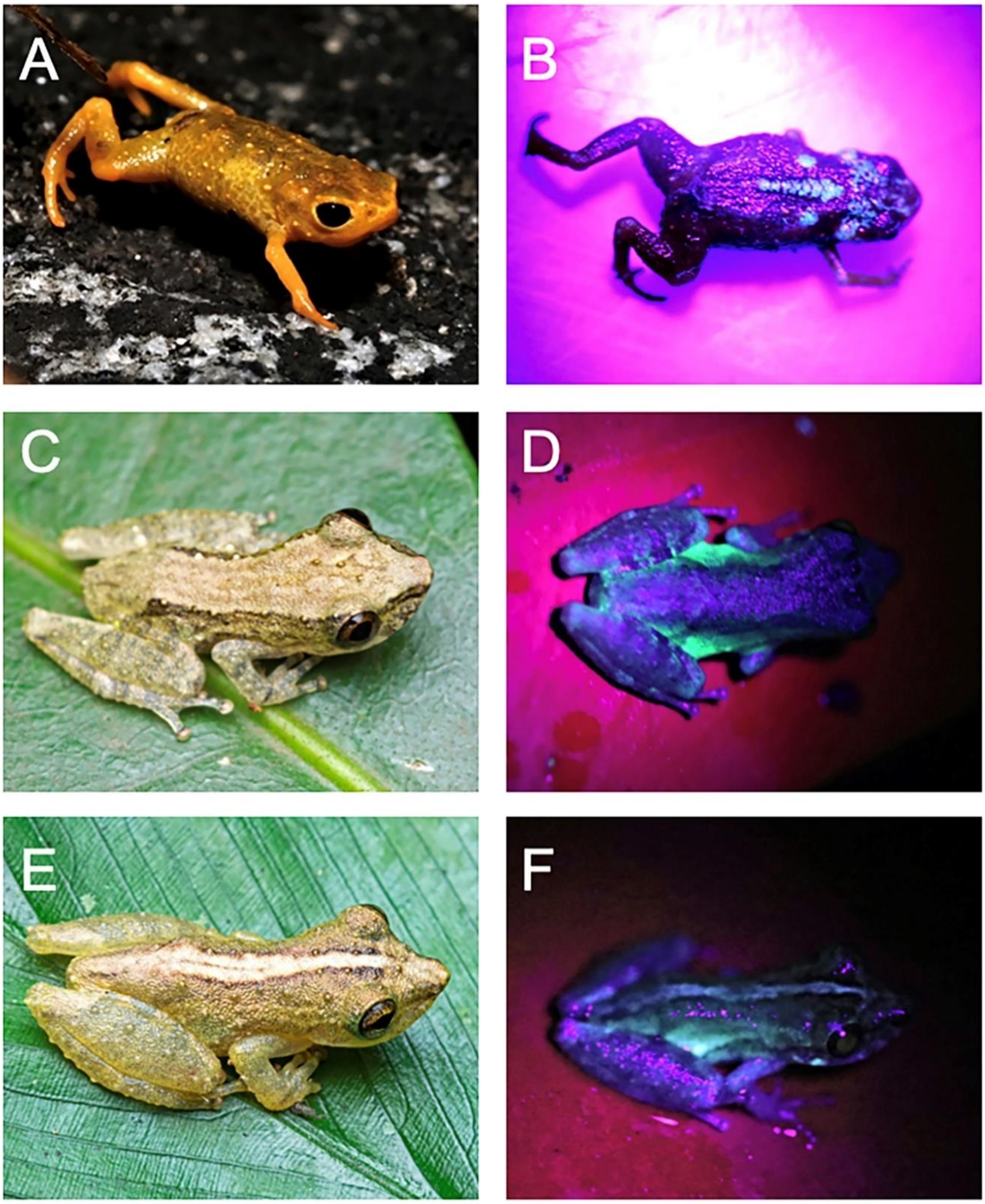

Figure 2