

A field-based survey of fluorescence in tropical tree frogs using an LED UV-B flashlight

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Biological fluorescence is a widespread phenomenon that occurs in a diversity of organisms, ranging from microorganisms to vertebrates (Lagorio et al., 2015). Among vertebrates, fluorescence has been described in fishes (Garcia and Perera, 2002; Michiels, et al., 2008; Sparks et al., 2014; Gruber et al., 2015; Gruber et al., 2016), sea turtles (Gruber and Sparks, 2015), lizards (Prötzel et al., 2018; Sloggett, 2018), birds (Pearn et al., 2001; Stradi et al., 2001; Arnold et al., 2002; Weidensaul et al., 2011), opossums (Meisner, 1983; Pine et al., 1985), squirrels (Goutte et al., 2019), and recently, in the bones of two anuran species in the genus *Brachycephalus* (Kohler et al., 2019), and in the South American tree frogs *Boana punctata* (Schneider, 1799) (Taboada et al., 2017a) and *Boana atlantica* (Caramaschi and Velosa, 1996) (Taboada et al., 2017b). Although present in a diversity of vertebrates, the ecological function of fluorescence is not well understood but some studies provide evidence of a role in visual communication (e.g., mate choice, antipredator defence; Lagorio et al., 2015; Kohler et al., 2019). Among anurans, the chemical basis of fluorescence has only been studied in *B. punctata* and is due to a class of compounds called hyloins, which

are present in both lymph and skin glands (Taboada et al., 2017a). However, the taxonomic distribution of fluorescence among other anurans (ca. 7,000 species), and in particular its presence in other members of *Boana* (>90 species), remains largely unknown.

We surveyed 22 tree frog species from Costa Rica and Colombia in the families Phyllomedusidae, Centrolenidae, and Hylidae (including 5 members of *Boana*) for the presence of fluorescence (Table 1). Although our sampling is taxonomically diverse, the frogs included in the present study were collected opportunistically as part of larger studies in Sarapiquí and the Osa Peninsula, Costa Rica, and during a Field Museum rapid inventory in Bajo Caguán-Caquetá, Colombia. Adult frogs were captured by hand, and the presence of fluorescence was determined by visual inspection of individuals using a handheld LED UV-flashlight with a peak intensity of 385 nm. One additional *B. rufitela* was examined using a handheld LED UV-flashlight with a peak intensity of 365 nm.

Biological fluorescence at a peak intensity of 385 nm was detected only in *B. punctata* from Colombia, and was not detected in the 21 other species examined, including other members *Boana* (Table 1; Figure 1).

The recent discovery by Taboada et al. (2017a,b) of fluorescence in *B. punctata* and *B. atlantica* suggests that other tree frogs may also possess this ability; however, in our survey, we only detected evidence of fluorescence in *B. punctata* (Table 1; Figure 1). Taboada et al. (2017b) suggest that fluorescence is most likely to occur in anurans with a white peritonea and urinary bladder, a high concentration of biliverdin, and translucent skin, and provide a comprehensive list candidate species to survey for fluorescence, which include members of the families Arthroleptidae, Centrolenidae, Hemiphractidae, Hylidae, Hyperoliidae, Mantellidae, and Rhacophoridae (see Table 1 of Taboada et al., 2017b). Although we examined several species from Centrolenidae and Phyllomedusidae

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Table 1. Anuran species examined for fluorescence.

Species	Family	Fluorescence	Location
<i>Agalychnis callidryas</i> (Cope, 1862)	Phyllomedusidae	No	Costa Rica
<i>Phyllomedusa tarsius</i> (Cope, 1868)	Phyllomedusidae	No	Colombia
<i>Phyllomedusa vaillantii</i> (Boulenger, 1882)	Phyllomedusidae	No	Colombia
<i>Cochranella granulosa</i> (Taylor, 1949)	Centrolenidae	No	Costa Rica
<i>Espadarana prosoblepon</i> (Boettger, 1892)	Centrolenidae	No	Costa Rica
<i>Hyalinobatrachium capellei</i> (Van Lidth de Jeude, 1904)	Centrolenidae	No	Colombia
<i>Hyalinobatrachium fleischmanni</i> (Boettger, 1893)	Centrolenidae	No	Costa Rica
<i>Hyalinobatrachium valerioi</i> (Dunn, 1931)	Centrolenidae	No	Costa Rica
<i>Sachatamia albomaculata</i> (Taylor, 1949)	Centrolenidae	No	Costa Rica
<i>Sachatamia ilex</i> (Savage, 1967)	Centrolenidae	No	Costa Rica
<i>Teratohyla midas</i> (Lynch and Duellman, 1973)	Centrolenidae	No	Colombia
<i>Teratohyla pulverata</i> (Peters, 1873)	Centrolenidae	No	Costa Rica
<i>Teratohyla spinosa</i> (Taylor, 1949)	Centrolenidae	No	Costa Rica
<i>Boana cinerascens</i> (Spix, 1824)	Hylidae	No*	Colombia
<i>Boana lanciformis</i> (Cope, 1871)	Hylidae	No	Costa Rica
<i>Boana punctata</i> (Schneider, 1799)	Hylidae	Yes	Colombia
<i>Boana rosenbergi</i> (Boulenger, 1898)	Hylidae	No	Costa Rica
<i>Boana rufitela</i> (Fouquette, 1961)	Hylidae	No [^]	Costa Rica
<i>Dendropsophus ebraccatus</i> (Cope, 1874)	Hylidae	No	Costa Rica
<i>Dendropsophus rhodopeplus</i> (Günther, 1858)	Hylidae	No	Colombia
<i>Dendropsophus sarayacuensis</i> (Shreve, 1935)	Hylidae	No	Colombia
<i>Scinax garbei</i> (Miranda-Ribeiro, 1926)	Hylidae	No	Colombia
<i>Trachycephalus typhonius</i> (Linnaeus, 1758)	Hylidae	No	Colombia

* Individual was not tested under ideal lighting conditions, should be re-tested.

[^] Note that fluorescence was detected in *Boana rufitela* by Deschepper *et al.* (2018).

(not included as a candidate family), we did not detect evidence of fluorescence (Table 1). Among members of Hylidae, Taboada *et al.* (2017a) report the absence of fluorescence in *Aplastodiscus leucopygius* (Cruz and Peixoto, 1984), *A. perviridis* (Lutz, 1950), *Boana prasina* (Burmeister, 1856), and *Boana raniceps* (Cope, 1862). It should be noted that Taboada *et al.* (2017a) reported faint fluorescence in histological skin sections of *Scinax nasicus* (Cope, 1862), however this was not observed in living animals and therefore would not be detected using the methods employed in the present study. In the present study, we find no evidence of fluorescence in the five species of *Boana* (aside from *B. punctata*) or *Scinax garbei* (Table 1). Included in our sample was *Boana cinerascens* (Spix, 1824), which according to Taboada *et al.* (2017a,b) is one of the most

closely related species to *B. punctata*, making it a good candidate to examine for fluorescence, yet we found no evidence of fluorescence. Unfortunately, we were not able to examine the individual *B. cinerascens* under optimal lighting conditions. While we were able to examine all other species at night, we were only able to examine this species at dawn, in darkness provided by a tent. Given that *B. cinerascens* has been identified as a prime candidate for fluorescence, we urge for further testing of the fluorescent properties of this species. Also included in our sample were *Boana lanciformis* (Cope, 1871) and *Boana rufitela* (Fouquette, 1961), both of which are listed as candidate species for fluorescence by Taboada *et al.* (2017b), yet we found no evidence of fluorescence. However, only juvenile *B. lanciformis* possess a white peritonea and urinary bladder, a high



Figure 1. Fluorescence of venter of two *Boana punctata* individuals under UV light.

concentration of biliverdin, and translucent skin (Taboada et al., 2017b), and therefore should be the target of future investigations.

Deschepper et al. (2018) recently reported fluorescence in *B. rufitela* in a field survey with a UV light similar to the one used in our study (Deschepper et al. [2018]: 365 nm UV light). We examined *B. rufitela* in the present study using both a 365nm and 385nm UV light, but did not find evidence of fluorescence (Table 1). The difference in results between our two studies is uncertain, and will likely need corroboration with additional specimens and spectroscopic methods, but it is possible that fluorescence varies within frog species. To date, there is no published evidence of intraspecific variation of fluorescence in frogs, but variation in fluorescence has been documented in other taxa. For example, sexual dichromy has been documented in the number of fluorescent tubercles present in chameleons (Prötzel et al., 2018), intraspecific variation in fluorescence has been described in cave-dwelling anchialine crustaceans (Glenn et al., 2013), and intraindividual variation exists in scorpions, which can vary in fluorescence intensity throughout their molt cycle (Stachel et al., 1999). In the

present study, when collecting and handling *B. punctata* for photographs, a patchy distribution of brighter spots of fluorescence on the epidermis of the frogs was noted, which appeared to be associated with regions of the frog that exuded skin secretion upon handling. Individuals held for long periods emitted enhanced fluorescence across the skin. Taboada et al. (2017b) reported fluorescent secretions in *B. punctata* and *B. atlantica* and suggested a glandular source for these secretions. Our field observations require additional investigation, but certainly suggest the possibility that fluorescence can vary within frog species, perhaps dependent on the release of skin secretions that reflect UV light.

Although the present study is not comprehensive and there are many taxonomic gaps in our sampling, our results suggest that fluorescence is not a widespread phenomenon among anurans, and in particular the tree frogs we examined. However, our results are based on the use of a 385 nm LED UV-flashlight, and as suggested by Taboada et al. (2017b), future field-based surveys should utilize a variety of flashlights to capture a broader region of the UV spectrum when examining anurans for fluorescence. Future studies

should also consider confirming our findings using the spectroscopic methods described by Taboada *et al.* (2017a). The ecological function of fluorescence in the nocturnal tree frog *B. punctata* will require further investigation; however, Taboada *et al.* (2017a) provide empirical evidence that fluorescence can enhance individual brightness, especially under low light conditions, and suggest a potential role for fluorescence in visual communication.

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