

Fluorescent Frogs: A Herpetological Perspective

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Abstract. In this paper we present information on fluorescent frogs that will be of use to interested researchers. We describe practical details and provide new data on the phenomenon in *Boana punctata*, while reporting for the first time its occurrence in the closely related *B. atlantica*. Both species leave persistent fluorescent marks on the substrate in field and lab conditions, the biological role of which, if any, remains unknown. We discuss briefly the heuristic criteria employed to identify other anuran species most likely to be fluorescent—at least in a similar way to that described in *B. atlantica* and *B. punctata*—and provide a list of 281 species in seven families (Arthroleptidae, Centrolenidae, Hemiphractidae, Hylidae, Hyperoliidae, Mantellidae, and Rhacophoridae) that should be tested for the occurrence of fluorescence. Finally, we discuss some general aspects related to anuran coloration, fluorescence, and criteria to assess its biological role, if any, commenting on the role of pteridins and hypothetical situations where these could lead to fluorescence in a different way than how we see it in *B. atlantica* and *B. punctata*.

Key-Words. Anura; Coloration; Fluorescence; Hylidae; *Boana atlantica*; *Boana punctata*.

INTRODUCTION

Three terms are commonly misused when describing different optical phenomena in nature. These are fluorescence, phosphorescence and bioluminescence. Despite the fact that they are often conflated, each of them denotes a particular physical phenomenon. Fluorescence and phosphorescence refer to the emission of light after direct excitation by light absorption of the emitting molecule. In fact, fluorescence is caused by fluorophores, which are pigments that absorb and re-emit part of the absorbed light as radiation of lower energy. While non-fluorescent pigments typically convert all the absorbed light into other types of energy (e.g., vibrational energy), fluorophores only convert part of it, emitting the remainder almost immediately as photons. In the latter case, if a certain amount of energy reaches a surface, part of the light will be reflected and another part will emerge as fluorescence of a different color (longer wavelengths) than that of the excitation beam (Johnsen, 2012; Lacowicz, 1999). Phosphorescence is a similar phenomenon except that the lifetime of the excited state reached by light absorption is greater and light emission lasts longer. Finally, bioluminescence refers to chemiluminescence that occurs in living organisms (Valeur and Berberan-Santos, 2011;

Johnsen, 2012). In chemiluminescence, the light emitting molecules are excited by the energy provided by a chemical reaction and not by the absorption of photons, and de-excitation to the ground state results in light emission.

A practical and simple distinction among the three phenomena is as follows. If a specimen only glows while being illuminated and stops glowing when the light is switched off, it is fluorescent. If a specimen continues glowing for some time after initial exposure to light, it is phosphorescent. Finally, if an animal emits light without any source of illumination, it is bioluminescent. Hence, without going deeper into the physical complexities of these phenomena, the easiest way to compare fluorescence with phosphorescence is by means of their lifetimes. Fluorescence is a fast phenomenon, and fluorescent substances stop glowing in less than nanoseconds when the excitation light is turned off. Phosphorescent substances remain glowing for longer periods, which could be as long as a few milliseconds or even minutes and hours, and, hence, imply a lifetime about a million times longer than that in fluorescence. There are many examples of phosphorescence, most of which do not come from biology (e.g., glow in the dark stickers; Johnsen, 2012).

Recently, Taboada et al. (2017) described the first case of fluorescence in amphibians. These authors

reported UV-A/blue light induced fluorescence in *Boana punctata*¹ (Schneider, 1799), studied its anatomical basis and traced the phenomenon to a new class of molecules they called hylins that are stored in lymph and dermal glands. In addition to being visually stunning when induced by UV-A/blue light, the authors showed that the emission generated under natural conditions accounts for 18–30% of the total emerging light from the individuals, the rest being the reflected component. The only other report we are aware of regarding a putative photochemical phenomenon in amphibians is the curious reference by Boie (1827) to Rollander's then-unpublished notes on his trip to Surinam in 1755 and 1756, where he mentions a case of apparent bioluminescence in calling males of *Trachycephalus typhonius* (Linnaeus, 1768) (Hylidae Rafinesque, 1815). This phenomenon has never been reported again in *T. typhonius*, despite the species' widespread distribution, and the glowing observed by Rollander has been more plausibly interpreted as a persistence of active remains of luciferin-luciferase complex after eating fireflies (Lavilla et al., 2010).

Although Taboada et al. (2017) employed *Boana punctata* as their model species to study fluorescence, they noted that fluorescence might also occur in other anuran species distributed in seven families (Arthroleptidae Mivart, 1869, Centrolenidae Taylor, 1951, Hemiphractidae Peters, 1862, Hylidae, Hyperoliidae Laurent, 1943, Mantellidae Laurent, 1946, Rhacophoridae Hoffmann, 1932 (1858)). While that statement might be informative enough for a general readership, it is certainly not so for herpetologists or other scientists interested in comparative research, and we intend to amend that here. In this paper we discuss briefly the heuristic criteria employed to identify the anuran species predicted to be fluorescent, present a list of those species, and report fluorescence in *B. atlantica* (Caramaschi and Velosa, 1996). We also present new data on fluorescence in *B. punctata* and discuss some general aspects related to anuran coloration and fluorescence.

MATERIALS AND METHODS

Prediction of possibly fluorescent species

Besides the occurrence of the fluorescent compounds, the most relevant character state for fluorescent emission to be evident—at least in the way we detected it in *Boana punctata*—is the occurrence of translucent skin (Taboada et al., 2017). Even though, theoretically, there is

no need to have this trait to obtain a fluorescent surface, the scarcity of pigments that are highly absorbent in the visible range of the spectrum allows fluorescence emission to emerge from the skin without being fully resorbed.

Overall, the taxonomic distribution of skin translucency in anurans is very poorly known, but it is more evident when associated with another two characters: (1) the occurrence of white peritonea and urinary bladder and (2) high concentration of biliverdin (Barrio, 1965a; Jones, 1967). To a certain extent, the three character states occur independently of each other as well, although much less frequently than when they are associated. Unfortunately, the occurrence of these three characters is seldom explicitly included in taxonomic descriptions; however in most cases they are easily identifiable from good quality photographs. On the basis of these, and the information available from literature, we compiled a list of species more likely to show fluorescence.

Specimen collection

Adult specimens of *Boana punctata* were observed in the field at night and collected on the outskirts of the city of Santa Fe, Santa Fe, Argentina. Adult specimens of *B. atlantica* were observed in the field at night in Lagoa Encantada, Ilhéus, Bahia, Brazil (14°37.450'S, 039°05.396'W).

Fluorescence recording

Fluorescence emission in living specimens of *Boana punctata* is detected by the naked human eye when illuminated with a UV-Blue flashlight. There are several options for this kind of flashlight available in the market, usually with peak intensities ranging from the ultraviolet (around 365 nm) and reaching the blue wavelengths of the visible spectrum (around 470 nm). Commonly, it is easy to find LEDs with full width at half maximum (FWHM) of about 20 nm. We have tried different sources of illumination and found that 400 nm (violet) flashlights allow for excellent fluorescence excitation and good contrast in *B. punctata*. Ideally, the access to a spectrofluorometer to characterize the excitation-emission matrices of the fluorescent specimen is the most precise way to select a proper illuminator for fluorescence detection in living animals (Taboada et al., 2017). However, for exploratory research in the field, a set of different LEDs should be tested to encompass a broad region of the UV-visible spectrum (e.g., 365 nm, 400 nm, 450 nm, 530 nm), especially taking into account the possibility of diverse molecular origins of fluorescence in other species. When illuminated, frogs fluoresce, but the visible content of the light source is also reflected by the frog as well as by the surrounding. To overcome this, the perception of fluorescence can

¹ As *Hypsiboas*. Dubois (2017) reinterpreted Gray's (1825) highly inconsistent and confusing text as proposing *Boana* Gray, 1825 as a subgenus and not as a synonym of *Hyla* Laurenti, 1768, as interpreted by Faivovich et al. (2005). This led to the availability of *Boana* Gray, 1825 and priority over *Hypsiboas* Wagler, 1830.

be enhanced by using long pass filters that block the illumination but not the emission wavelengths. There are several commercial options available at low prices in the market. We have tried yellow glasses, which block the blue and ultraviolet light with good results. Of course, our color perception of fluorescence emission will depend largely on the cut-off wavelength of the long pass filter. Ideally, a filter that blocks most part of the illumination radiation should be chosen, and this will depend on the excitation source employed.

Confocal images and gland secretions of *Boana punctata*

Small strips of skin (9–16 mm²) were excised from the dorsum and lateral region, immediately embedded in TissueTek OCT Compound (Sakura Finetek), and frozen at -20°C. 10 µm sections were mounted with glycerol, covered with quartz coverslips, and subjected to confocal imaging (CI). CIs were obtained in a light laser TCS SP8 confocal microscope (Leica, Germany) using 405 nm (laser intensity: 0.81%) and 488 nm (laser intensity: 2.85%) laser lines for excitation. Emission detection was regulated by an acousto-optical beam splitter, tuned at 430–490 nm and 500–560 nm, respectively. Gain and offset of PMT were set at 619 V and -11 and 681 V and 0, respectively.

Samples of skin glandular secretions were taken from dorsal and lateral skin. The skin of the frog was moistened with distilled water, and an electrical stimulus (1–2 V, pulse length 2–4 ms) was applied during 8–10 s using two platinum electrodes that were rubbed gently along the dorsum of the animal. The secretion was washed with ultrapure water (20–30 mL), immediately frozen in liquid N₂, and lyophilized.

RESULTS

Predicted fluorescence in other anurans

Based on our criteria, our literature survey resulted in a tentative list of 281 species from seven anuran families that should be tested for the occurrence of fluorescence (Table 1). The number of potentially fluorescent species per family ranges from two (Artholeptidae) to as many as 59 (Hylidae) and 137 (Centrolenidae). There are eight cases in species of *Boana* Gray, 1825, *Gastrotheca* Fitzinger, 1843, *Hyperolius* Rapp, 1842 where there are different color phases, one of which corresponds to the translucent skin, high biliverdin concentration and, at least in some cases, white peritonea. Furthermore, we identified four cases in some species of *Hyloscirtus* Peters, 1882 and of the *Boana albopunctata* group where these characteristics occur in juveniles but are lost in adults, which have heavily

pigmented skin, and, depending the species, may or may not have green tissues or white peritonea.

Fluorescence in *Boana atlantica*

Adult specimens of *Boana atlantica* in the field and in the laboratory fluoresced when illuminated with a UV-Blue flashlight (Fig. 1A–D). In this species, traces of the fluorescence from the frogs in the field and in the laboratory were found on the substrate on which the animals were standing (Fig. 1B, C). The mental gland of males showed a slightly more yellowish fluorescence compared to the rest of the body (Fig. 1D).

Fluorescence in *Boana punctata*

Adult and juveniles specimens of *Boana punctata* are well known (e.g., Barrio, 1965b; Duellman, 1978; Lescure and Marty, 2000; Prado et al., 2005) to be active during dusk and night and inactive during the day when they remain perched from floating vegetation in the center of large water bodies exposed to strong solar irradiance. The coloration of individuals is markedly different at night, when they present a yellow-green coloration with numerous red dots distributed evenly over the dorsal skin (Fig. 2A), compared to daytime, when the red dots are almost invisible (Fig. 2B).

When illuminated with a UV-Blue flashlight, frogs fluoresced (Fig. 2C–D). This phenomenon was observed in specimens in the field and captivity. As noted above, maximum contrast was obtained when using an emission cut-off filter to block the wavelengths present in the excitation source, which removes the light reflected either by the frogs and the surroundings and only allows the fluoresced photons to pass. Although this rendered excellent results, fluorescence from *Boana punctata* was also clearly visible by the naked eye without any filter (Fig. 2D).

Confocal images using 405 nm laser lines for excitation showed that fluorescence is present in serous glands, as observed previously in dorsal skin samples (Taboada et al., 2017: fig. 2b), and in sexually dimorphic skin glands (SDSGs), as observed in specialized mucous glands in skin samples from the flanks (Fig. 2E). Also, using 488 nm laser line for excitation, we observed faint green fluorescence corresponding to xanthopore pigment cells (Fig. 2E), below the epidermis.

The lyophilized secretions from dorsal skin glands, which have a yellowish color under white light, fluoresce under UV light (Fig. 2F, G). Latex gloves used to handle the frog, exhibited similar fluorescence to that observed from the frogs (Fig. 2H); we do not have replicate quantitative data, but the glove remained fluorescent for at least 12 weeks after handling.

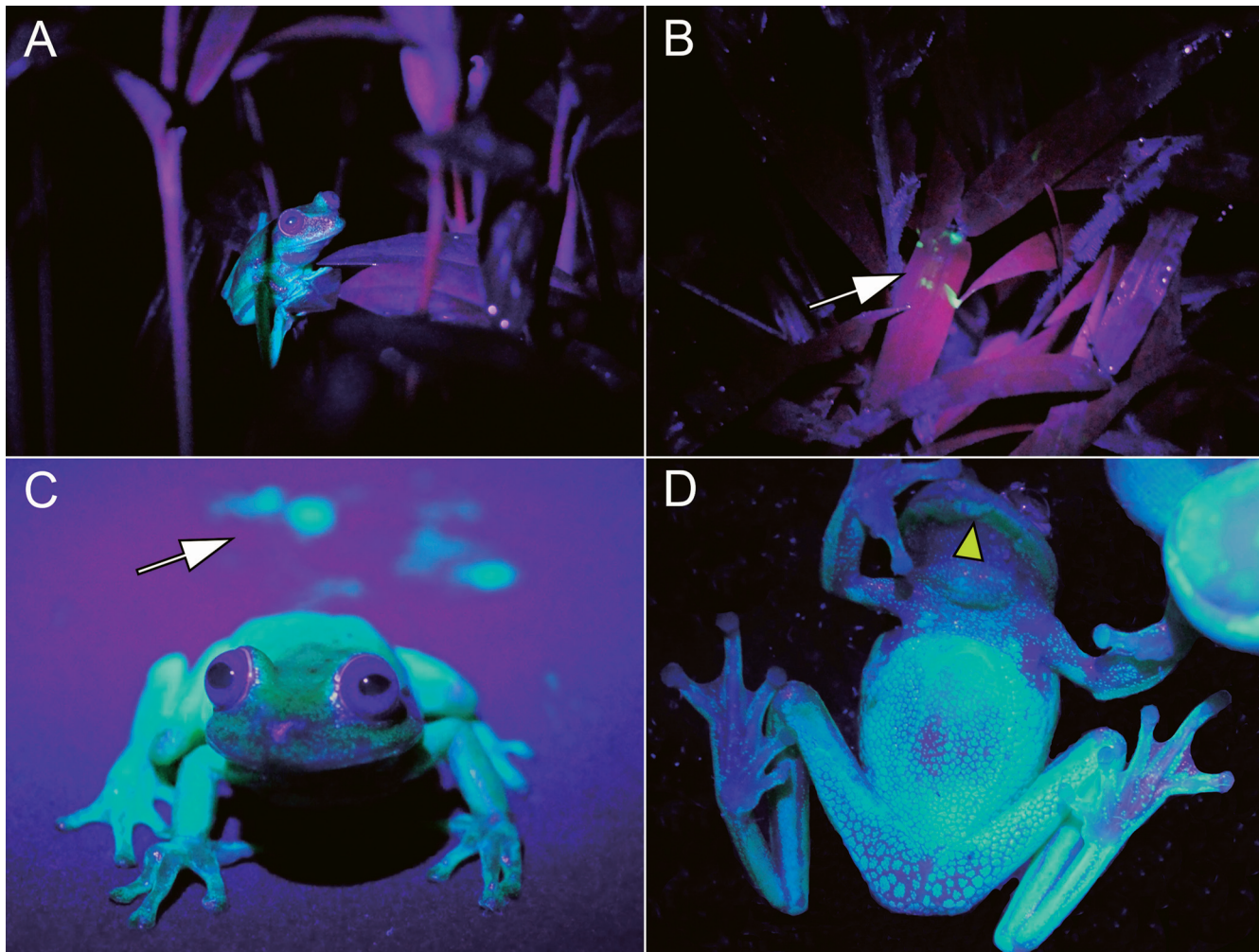


Figure 1. Fluorescence in *Boana atlantica* under UV-blue light (400 nm). **(A)** Adult male perched on vegetation in the field. **(B)** Fluorescent traces (arrow) found in the vegetation where specimens were standing. **(C)** Adult male and traces of fluorescence on the substrate (arrow) in the laboratory. **(D)** Ventral view of a male specimen in the laboratory. Note the slightly more yellowish fluorescence of the mental gland (arrowhead) compared to acinar glands from other parts of the body.

DISCUSSION

Predicted fluorescence in *Boana atlantica* and other frogs

Our finding of fluorescence in *B. atlantica* is a corroboration of the prediction, based on the shared occurrence of translucent skin, white peritonea and urinary bladder, and high concentration of biliverdin, as well as the close phylogenetic relationship with *B. punctata* (Faivovich et al., 2005, 2013), that *B. atlantica* is a fluorescent frog (Taboada et al., 2017: supp. info. 20), making this the second report of fluorescence in amphibians. Although *B. atlantica* has not been included in published phylogenetic analyses, it is morphologically and acoustically similar with *B. punctata* and difficult to differentiate (Caramaschi and Velosa, 1996; Napoli and Cruz, 2005).

Based on the same criteria, we (see also Taboada et al. 2017: supp. info. 20) hypothesize that *Boana*

cinerascens might be fluorescent as well. Given the ease with which fluorescence can be detected in both the field and laboratory, we are hopeful that many more of the 281 species with the characters that we associate with macroscopic fluorescence emission will be tested both in the Neotropics and around the world.

Nevertheless, due to the difficulty in finding explicit reference to the occurrence of high concentration of biliverdin (evidenced by green tissues, like bones, muscle, lymph, etc.), translucent skin, and white peritonea and/or urinary bladder in the literature, our list should be seen as strictly tentative. It provides a heuristic tool that reduces the number of potentially fluorescent species from the more than 6,700 known anurans (Frost, 2017) to the 281 species that are most likely to show fluorescence as we find it in *Boana punctata*. All included species have the morphology commonly associated with treefrogs in their different families, but they are just a subset of all treefrogs, and actually, also a subset of all treefrogs known to

have a high concentration of biliverdin, as evidenced by the green bones or other tissues. Until they are examined, we have no way of knowing how many of the species in the list will be actually fluorescent. Taboada et al. (2017) tested *Aplastodiscus leucopygius* and *A. perviridis* using,

respectively, LC-DAD (liquid chromatography with diode-array detector) and visual inspection with a UV-Blue light (400 nm) and did not detect fluorescence. Nevertheless, considering the high levels of homoplasy shown by the character states that we consider as possible predictors

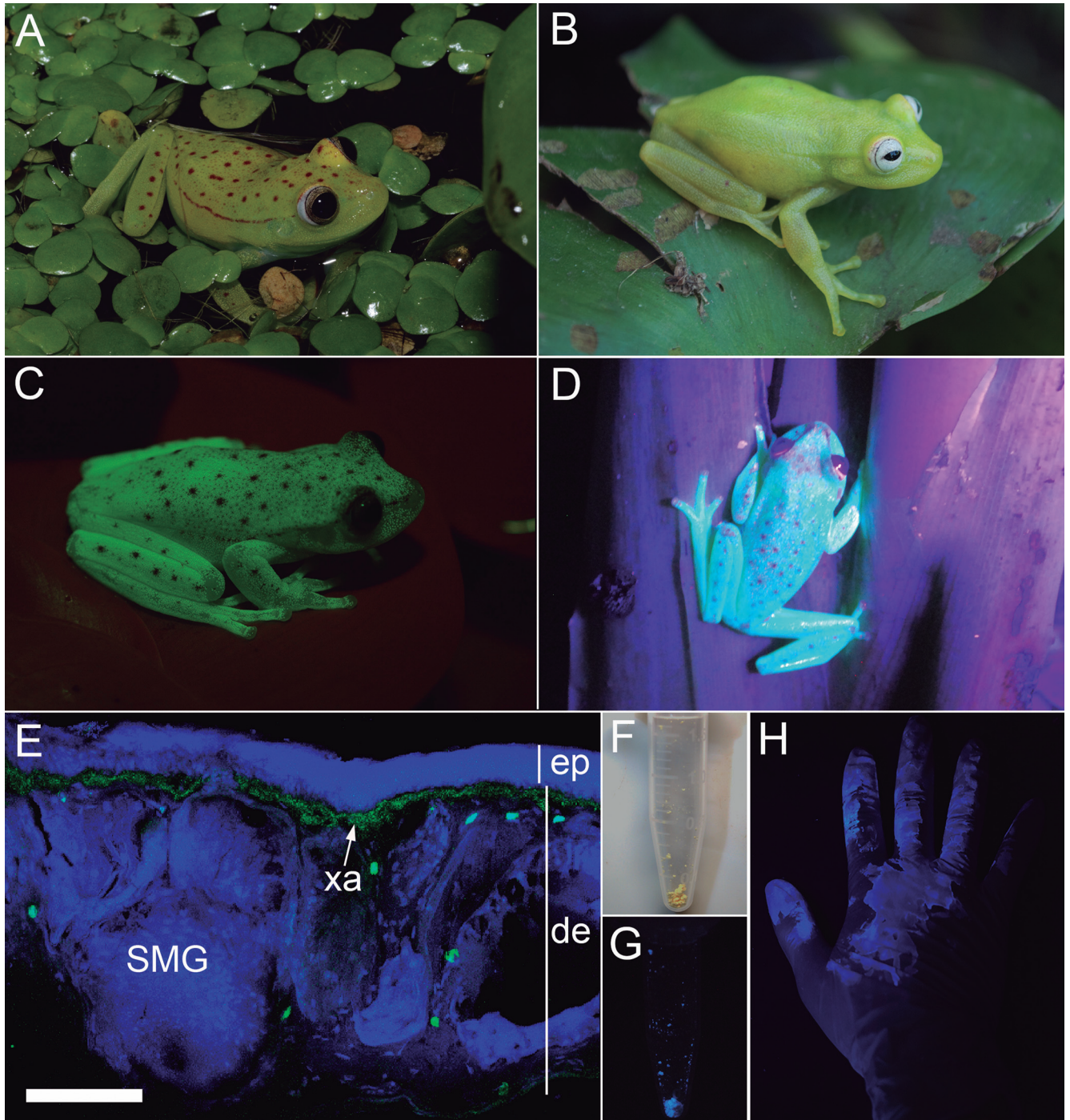


Figure 2. Fluorescence and glandular source of fluorescence in *Boana punctata*. **(A)** Adult male under white light during the night. **(B)** Adult male under natural light during daytime. **(C)** Adult male under UV-blue light (400 nm), with photograph using a long-pass emission filter (516 nm); **(C)**. **(D)** Same as **(C)** but without emission filtering. **(E)** Confocal image of a transverse section of fresh skin samples from the flanks of *B. punctata* using 405 nm laser line (blue) and 488 nm laser line (green). Blue fluorescence emission is observed from epidermis (ep), dermis (d), and specialized mucous glands (smg), whereas green fluorescence emission is observed well below the epidermis in the xanthophore layer (xa). **(F-G)** Lyophilized glandular secretion from dorsal skin under white light **(F)**, and UV-blue light (400 nm; **G**). **(H)** Gloves observed under UV-blue light (400 nm) after manipulating a male specimen of *B. punctata*.

Table 1. List of species predicted to be fluorescent based on the co-occurrence of a high concentration of biliverdin, translucent skin, and white peritonea. * Species included on the basis of these characters being evident in photographs from the cited papers. † Species in which only juveniles have these characters. ‡ Species in which translucent skin is only evident ventrally. § Species in which only one of the known color phases includes translucent skin. ¶ Species in which we did not detect fluorescence (see text).

TAXON	SOURCE	TAXON	SOURCE
Arthroleptidae		Espadarana	
Leptopelis		<i>E. andina</i> (Rivero, 1968)	Guayasamin et al., 2009
<i>L. grandiceps</i> Ahl, 1929	Schiøtz, 1999*; Gvoždik et al., 2014*	<i>E. audax</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009
<i>L. uluguriensis</i> Barbour and Loveridge, 1928	Schiøtz, 1999*; Gvoždik et al., 2014*	<i>E. callistomma</i> (Guayasamin and Trueb, 2007)	Guayasamin et al., 2009
Centrolenidae		<i>E. durrellorum</i> (Cisneros-Heredia, 2007)	Guayasamin et al., 2009
Centrolene		<i>E. prosoblepon</i> (Boettger, 1892)	Guayasamin et al., 2009
<i>C. altitudinale</i> (Rivero, 1968)	Guayasamin et al., 2009	Nymphargus	
<i>C. antioquiense</i> (Noble, 1920)	Guayasamin et al., 2009	<i>N. anomalus</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009
<i>C. bacatum</i> Wild, 1994	Guayasamin et al., 2009	<i>N. armatus</i> (Lynch and Ruiz-Carranza, 1996)	Guayasamin et al., 2009
<i>C. ballux</i> (Duellman and Burrowes, 1989)	Guayasamin et al., 2009	<i>N. bejaranoi</i> (Cannatella, 1980)	Guayasamin et al., 2009
<i>C. buckleyi</i> (Boulenger, 1882)	Guayasamin et al., 2009	<i>N. buenaventura</i> (Cisneros-Heredia and Yáñez-Muñoz, 2007)	Guayasamin et al., 2009
<i>C. charapita</i> Twomey et al., 2014	Guayasamin et al., 2009	<i>N. cariticommatum</i> (Wild, 1994)	Guayasamin et al., 2009
<i>C. condor</i> Cisneros-Heredia and Morales-Mite, 2008	Guayasamin et al., 2009	<i>N. chami</i> (Ruiz-Carranza and Lynch, 1995)	Guayasamin et al., 2009
<i>C. daidaleum</i> (Ruiz-Carranza and Lynch, 1991)	Guayasamin et al., 2009	<i>N. chancas</i> (Duellman and Schulte, 1993)	Guayasamin et al., 2009
<i>C. gemmatum</i> (Flores, 1985)	Guayasamin et al., 2009	<i>N. cochranae</i> (Goin, 1961)	Guayasamin et al., 2009
<i>C. heloderma</i> (Duellman, 1981)	Guayasamin et al., 2009	<i>N. cristinae</i> (Ruiz-Carranza and Lynch, 1995)	Guayasamin et al., 2009
<i>C. hesperium</i> (Cadle and McDiarmid, 1990)	Guayasamin et al., 2009	<i>N. garciae</i> (Ruiz-Carranza and Lynch, 1995)	Guayasamin et al., 2009
<i>C. huilense</i> Ruiz-Carranza and Lynch, 1995	Guayasamin et al., 2009	<i>N. grandisonae</i> (Cochran and Goin, 1970)	Guayasamin et al., 2009
<i>C. hybrida</i> Ruiz-Carranza and Lynch, 1991	Guayasamin et al., 2009	<i>N. griffithsi</i> (Goin, 1961)	Guayasamin et al., 2009
<i>C. lemniscatum</i> Duellman and Schulte, 1993	Guayasamin et al., 2009	<i>N. ignotus</i> (Lynch, 1990)	Guayasamin et al., 2009
<i>C. lynchi</i> (Duellman, 1980)	Guayasamin et al., 2009	<i>N. lasgralarias</i> Hutter and Guayasamin, 2012	Guayasamin et al., 2009
<i>C. muelleri</i> Duellman and Schulte, 1993	Guayasamin et al., 2009	<i>N. laurae</i> Cisneros-Heredia and McDiarmid, 2007	Guayasamin et al., 2009
<i>C. notostictum</i> Ruiz-Carranza and Lynch, 1991	Guayasamin et al., 2009	<i>N. luminosus</i> (Ruiz-Carranza and Lynch, 1995)	Guayasamin et al., 2009
<i>C. paezorum</i> Ruiz-Carranza et al., 1986	Guayasamin et al., 2009	<i>N. luteopunctatus</i> (Ruiz-Carranza and Lynch, 1996)	Guayasamin et al., 2009
<i>C. peristictum</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009	<i>N. mariae</i> (Duellman and Toft, 1979)	Guayasamin et al., 2009
<i>C. pipilatum</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009	<i>N. megacheirus</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009
<i>C. sabini</i> Cattenazi et al., 2012	Guayasamin et al., 2009	<i>N. mixomaculatus</i> Guayasamin et al., 2006	Guayasamin et al., 2009
<i>C. sanchezi</i> Ruiz-Carranza and Lynch, 1991	Guayasamin et al., 2009	<i>N. nephelophila</i> (Ruiz-Carranza and Lynch, 1991)	Guayasamin et al., 2009
<i>C. savagei</i> (Ruiz-Carranza and Lynch, 1991)	Guayasamin et al., 2009	<i>N. ocellatus</i> (Boulenger, 1918)	Guayasamin et al., 2009
<i>C. scirtetes</i> (Duellman and Burrowes, 1989)	Guayasamin et al., 2009	<i>N. oreonympha</i> (Ruiz-Carranza and Lynch, 1991)	Guayasamin et al., 2009
<i>C. solitaria</i> (Ruiz-Carranza and Lynch, 1991)	Guayasamin et al., 2009	<i>N. phenax</i> (Cannatella and Duellman, 1982)	Guayasamin et al., 2009
<i>C. venezuelense</i> (Rivero, 1968)	Guayasamin et al., 2009	<i>N. pluvialis</i> (Cannatella and Duellman, 1982)	Guayasamin et al., 2009
Chimerella		<i>N. posadae</i> (Ruiz-Carranza and Lynch, 1995)	Guayasamin et al., 2009
<i>C. corleone</i> Twomey et al., 2014	Guayasamin et al., 2009	<i>N. prasinus</i> (Duellman, 1981)	Guayasamin et al., 2009
<i>C. mariaelena</i> (Cisneros-Heredia and McDiarmid, 2006)	Guayasamin et al., 2009	<i>N. rosada</i> (Ruiz-Carranza and Lynch, 1997)	Guayasamin et al., 2009
Cochranella		<i>N. ruizi</i> (Lynch, 1993)	Guayasamin et al., 2009
<i>C. erminea</i> Torres-Gastello et al., 2007	Guayasamin et al., 2009	<i>N. siren</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009
<i>C. euknemos</i> (Savage and Starret, 1967)	Guayasamin et al., 2009	<i>N. spilotes</i> Ruiz-Carranza and Lynch, 1997	Guayasamin et al., 2009
<i>C. granulosa</i> (Taylor, 1949)	Guayasamin et al., 2009	<i>N. sucre</i> Guayasamin, 2013	Guayasamin et al., 2009
<i>C. guayasamini</i> Twomey et al., 2014	Guayasamin et al., 2009	<i>N. truebae</i> (Duellman, 1976)	Guayasamin et al., 2009
<i>C. litoralis</i> (Ruiz-Carranza and Lynch, 1996)	Guayasamin et al., 2009	<i>N. vicenteruedai</i> Velásquez-Álvarez et al., 2007	Guayasamin et al., 2009
<i>C. mache</i> Guayasamin and Bonaccorso, 2004	Guayasamin et al., 2009	<i>N. wileyi</i> Guayasamin et al., 2006	Guayasamin et al., 2009
<i>C. nola</i> Harvey, 1996	Guayasamin et al., 2009	Teratohyla	
<i>C. phryxa</i> Aguado-Vedia and Harvey, 2006	Guayasamin et al., 2009	<i>T. adenocheira</i> (Harvey and Noonan, 2005)	Guayasamin et al., 2009
<i>C. resplendens</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009		

Table 1. Continued.

TAXON	SOURCE	TAXON	SOURCE
<i>T. amelie</i> (Cisneros-Heredia and Meza-Ramos, 2007)	Guayasamin et al., 2009	<i>H. tricolor</i> Castroviejo-Fisher et al., 2011	Rada pers. comm.
<i>T. midas</i> (Lynch and Duellman, 1973)	Guayasamin et al., 2009	<i>H. valerioi</i> (Dunn, 1931)	Rada pers. comm.
<i>T. pulverata</i> (Peters, 1873)	Guayasamin et al., 2009	<i>H. vireovittatum</i> (Starrett and Savage, 1973)	Rada pers. comm.
<i>T. spinosa</i> (Taylor, 1949)	Guayasamin et al., 2009	" <i>Centrolene</i> " <i>azulae</i> (Flores and McDiarmid, 1989)	Guayasamin et al., 2009
Vitreorana		" <i>Centrolene</i> " <i>guanacaram</i> Ruiz-Carranza and Lynch, 1995	Guayasamin et al., 2009
<i>V. antisthenesi</i> (Goin, 1963)	Guayasamin et al., 2009	" <i>Centrolene</i> " <i>quindianum</i> Ruiz-Carranza and Lynch, 1995	Guayasamin et al., 2009
<i>V. baliomma</i> Pontes et al., 2014	Guayasamin et al., 2009	" <i>Centrolene</i> " <i>robledoi</i> Ruiz-Carranza and Lynch, 1995	Guayasamin et al., 2009
<i>V. castroviejoi</i> (Ayarzagüena and Señaris, 1997)	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>balionota</i> (Duellman, 1981)	Guayasamin et al., 2009
<i>V. eurygnatha</i> (Lutz, 1925)	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>duidaeana</i> (Ayarzagüena, 1992)	Guayasamin et al., 2009
<i>V. franciscana</i> Santana et al., 2015	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>euhystrix</i> (Cadle and McDiarmid, 1990)	Guayasamin et al., 2009
<i>V. gorzulae</i> (Ayarzagüena, 1992)	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>geijskesi</i> (Goin, 1966)	Guayasamin et al., 2009
<i>V. helenae</i> (Ayarzagüena, 1992)	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>megista</i> (Rivero, 1985)	Guayasamin et al., 2009
<i>V. parvula</i> (Boulenger, 1895)	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>ramirezi</i> Ruiz-Carranza and Lynch, 1991	Guayasamin et al., 2009
<i>V. ritae</i> (Lutz, 1952)	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>riveroi</i> (Ayarzagüena, 1992)	Guayasamin et al., 2009
<i>V. uranoscopa</i> (Müller, 1924)	Guayasamin et al., 2009	" <i>Cochranella</i> " <i>xanthocheridia</i> Ruiz-Carranza and Lynch, 1995	Guayasamin et al., 2009
Ikakogi		Hemiphractidae	
<i>Ikakogi tayrona</i> (Ruiz-Carranza and Lynch, 1991)	Guayasamin et al., 2009	Gastrotheca	
Celsiella		<i>G. albolineata</i> (Lutz and Lutz, 1939)	Machado, pers. comm.
<i>C. revocata</i> (Rivero, 1985)	Guayasamin et al., 2009	<i>G. helenae</i> ⁺ Dunn, 1944	Duellman, 2015
<i>C. vozmedianoi</i> (Ayarzagüena and Señaris, 1997)	Guayasamin et al., 2009	<i>G. longipes</i> (Boulenger, 1882)	Duellman, 2015*
Hyalinobatrachium		<i>G. fulvorufa</i> (Andersson, 1911)	Duellman, 2015; Trevine, pers. comm.
<i>H. anachoretus</i> Twomey et al., 2014	Rada pers. comm.	Hylidae Pelodyadinae	
<i>H. aureoguttatum</i> (Barrera-Rodriguez and Ruiz-Carranza, 1989)	Rada pers. comm.	Litoria	
<i>H. bergeri</i> (Cannatella, 1980)	Rada pers. comm.	<i>L. lutea</i> (Boulenger, 1887)	Tyler, 1968; Menzies, 2006
<i>H. cappellei</i> Van Lidth de Jeude, 1904	Rada pers. comm.	<i>L. thesaurensis</i> (Peters, 1877)	Tyler, 1968; Menzies, 2006
<i>H. carlesvilai</i> Castroviejo-Fisher et al., 2009	Rada pers. comm.	Hylidae Hylinae	
<i>H. chirripoi</i> (Taylor, 1958)	Rada pers. comm.	Aplastodiscus	
<i>H. colymbiphylum</i> (Taylor, 1949)	Rada pers. comm.	<i>A. albofrenatus</i> group	
<i>H. dianae</i> Kubicki et al., 2015	Rada pers. comm.	<i>A. albofrenatus</i> (Lutz, 1924)	Lutz, 1949
<i>H. durantii</i> (Rivero, 1985)	Rada pers. comm.	<i>A. arildae</i> (Cruz and Peixoto, 1987)	Berneck et al., 2016
<i>H. esmeralda</i> Ruiz-Carranza and Lynch, 1998	Rada pers. comm.	<i>A. ehrhardti</i> (Müller, 1924)	Berneck et al., 2016
<i>H. fleischmanni</i> (Boettger, 1893)	Rada pers. comm.	<i>A. eugenioi</i> (Carvalho-e-Silva and Carvalho-e-Silva, 2005)	Berneck et al., 2016
<i>H. fragile</i> (Rivero, 1985)	Rada pers. comm.	<i>A. weygoldti</i> (Cruz and Peixoto, 1987)	Berneck et al., 2016
<i>H. guairarepanense</i> Señaris, 2001	Rada pers. comm.	<i>A. albosignatus</i> group	
<i>H. iaspidiense</i> (Ayarzagüena, 1992)	Rada pers. comm.	<i>A. albosignatus</i> (Lutz and Lutz, 1938)	Lutz, 1949
<i>H. ibama</i> Ruiz-Carranza and Lynch, 1998	Rada pers. comm.	<i>A. cavicola</i> (Cruz and Peixoto, 1987)	Berneck et al., 2016
<i>H. kawense</i> Castroviejo-Fisher et al., 2011	Rada pers. comm.	<i>A. flumineus</i> (Cruz and Peixoto, 1985)	Berneck et al., 2016
<i>H. mesai</i> Barrio-Amorós and Brewer-Carias, 2008	Rada pers. comm.	<i>A. ibirapitanga</i> (Cruz et al., 2003)	Berneck et al., 2016
<i>H. mondolfii</i> Señaris and Ayarzagüena, 2001	Rada pers. comm.	<i>A. leucopygius</i> ⁻ (Cruz and Peixoto, 1985)	Berneck et al., 2016
<i>H. munozorum</i> (Lynch and Duellman, 1973)	Rada pers. comm.	<i>A. musicus</i> (Lutz, 1949)	Lutz, 1949
<i>H. orientale</i> (Rivero, 1968)	Rada pers. comm.	<i>A. perviridis</i> group	
<i>H. orcostale</i> (Rivero, 1968)	Rada pers. comm.	<i>A. lutzorum</i> Berneck et al., 2017	Berneck et al., 2017*
<i>H. pallidum</i> (Rivero, 1985)	Rada pers. comm.	<i>A. perviridis</i> ⁻ Lutz, 1950	Berneck et al., 2016
<i>H. pellucidum</i> (Lynch and Duellman, 1973)	Rada pers. comm.	<i>A. sibilatus</i> group	
<i>H. ruedai</i> Ruiz-Carranza and Lynch, 1998	Rada pers. comm.		
<i>H. talamancae</i> (Taylor, 1952)	Rada pers. comm.		
<i>H. tatayoi</i> Castroviejo-Fisher et al., 2007	Rada pers. comm.		
<i>H. taylori</i> (Goin, 1968)	Rada pers. comm.		

Table 1. Continued.

TAXON	SOURCE	TAXON	SOURCE
<i>A. sibilatus</i> (Cruz et al., 2003)	Berneck et al., 2016	<i>S. orophilus</i> (Lutz and Lutz, 1938)	Lutz and Lutz, 1938
Boana		<i>S. palustris</i> Bokermann, 1966	Haddad et al., 2013*
<i>B. albopunctata</i> group		<i>S. pauloalvini</i> Bokermann, 1973	Haddad et al., 2013*
<i>B. albopunctata</i> ¹ (Spix, 1824)	Maffei et al., 2011	<i>S. planicola</i> (Lutz and Lutz, 1938)	Lutz and Lutz, 1938
<i>B. heilprini</i> (Noble, 1923)	Jones, 1967	<i>S. prasinus</i> Bokermann, 1973	Haddad et al., 2013*
<i>B. lanciformis</i> ¹ (Cope, 1871)	Ron, pers. comm.	<i>S. surdus</i> (Cochran, 1953)	Haddad et al., 2013*
<i>B. raniceps</i> ¹ (Cope, 1862)	Faivovich pers. obs.	Hyperoliidae	
<i>B. benitezi</i> group		Alexeteron	
<i>B. nymphe</i> Faivovich et al., 2006	Faivovich et al., 2006	<i>A. jynx</i> Amiet, 2000	Amiet, 2012*
<i>B. ornatissima</i> (Noble, 1923)	Hoogmoed, 1979	<i>A. obstetricans</i> (Ahl, 1931)	Amiet, 2012*
<i>B. sibleszi</i> (Rivero, 1972)	Hoogmoed, 1979	Hyperolius	
<i>B. pellucens</i> group		<i>H. acutirostris</i> ^v Buchholz and Peters, 1875	Amiet, 2012*
<i>B. pellucens</i> (Werner, 1901)	Duellman, 1971	<i>H. argus</i> [*] Peters, 1874	Harper et al., 2010*
<i>B. rubracyla</i> (Cochran and Goin, 1970)	Rivera-Correa, pers. comm.	<i>H. burguessi</i> Loader et al., 2015	Loader et al., 2015*
<i>B. rufitela</i> (Fouquette, 1961)	Duellman, 1970	<i>H. camerunensis</i> [*] Amiet, 2004	Amiet, 2012*
<i>B. pulchella</i> group		<i>H. chlorosteus</i> E.G. Boulenger, 1915	E.G. Boulenger, 1915
<i>B. guentheri</i> [*] (Boulenger, 1886)	Kwet et al., 2010*	<i>H. davenporti</i> Loader et al., 2015	Loader et al., 2015*
<i>B. marginata</i> (Boulenger, 1887)	Garcia et al., 2001	<i>H. endjami</i> ^v Amiet, 1980	Amiet, 2012*
<i>B. poaju</i> (Garcia et al., 2008)	Garcia et al., 2008	<i>H. frontalis</i> Laurent, 1950	Schiøtz, 1999*
Hyloscirtus		<i>H. guttulatus</i> [*] Günther, 1858	Amiet, 2012*
<i>H. armatus</i> group		<i>H. koehleri</i> Mertens, 1940	Amiet, 2012*
<i>H. charazani</i> ¹ (Vellard, 1970)	Lötters et al., 2005	<i>H. leucotaenius</i> Laurent, 1950	Greenbaum and Kusamba, 2016*
<i>H. bogotensis</i> group		<i>H. mosaicus</i> ^v Perret, 1959	Amiet, 2012*
<i>H. alytolylax</i> (Duellman, 1972)	Duellman, 1972; Guayasamin et al., 2015*	<i>H. nienokouensis</i> Rödel, 1998	Schiøtz, 1999*
<i>H. callipeza</i> (Duellman, 1989)	Duellman, 1989*	<i>H. ocellatus</i> [*] Günther, 1858	Amiet, 2012*
<i>H. colymba</i> (Dunn, 1931)	Duellman, 1970	<i>H. pardalis</i> [*] Laurent, 1948	Amiet, 2012*
<i>H. denticulatus</i> (Duellman, 1972)	Duellman, 1972	<i>H. parkeri</i> Loveridge, 1933	Du Preez and Carruthers, 2009*
<i>H. estevezi</i> (Rivero, 1968)	Rivero, 1968	<i>H. pseudargus</i> Schiøtz and Westergaard, 1999	Harper et al., 2010*
<i>H. jahni</i> (Rivero, 1961)	La Marca, 1985*	<i>H. pusillus</i> (Cope, 1862)	Du Preez and Carruthers, 2009*
<i>H. lascinius</i> (Rivero, 1970)	Rojas-Runjaic et al., 2016*	<i>H. riggenbachi</i> ⁺ (Nieden, 1910)	Amiet, 2012*
<i>H. mashpi</i> Guayasamin et al., 2015	Guayasamin et al., 2015*	<i>H. tanneri</i> Schiøtz, 1982	Loader et al., 2015*
<i>H. palmeri</i> (Boulenger, 1908)	Duellman, 1970	<i>H. wermuthi</i> Laurent, 1961	Schiøtz, 1999*
<i>H. phyllognathus</i> (Melin, 1941)	Duellman, 1972	<i>H. nasutus</i> group	
<i>H. piceigularis</i> (Ruiz-Carranza and Lynch, 1982)	Rivera-Correa, pers. comm.	<i>H. adpersus</i> Peters, 1877	Channing et al., 2013*
<i>H. platydactylus</i> (Boulenger, 1905)	La Marca, 1985*	<i>H. benguellensis</i> [*] (Bocage, 1893)	Channing et al., 2013*; Du Preez and Carruthers, 2009*
<i>H. simmonsii</i> (Duellman, 1989)	Duellman, 1989*	<i>H. dartavellei</i> Laurent, 1943	Channing et al., 2013*
<i>H. torrenticola</i> (Duellman and Altig, 1978)	Duellman and Altig, 1978*	<i>H. friedmanni</i> Mercurio and Rödel, 2013	Channing et al., 2013*
Osteopilus		<i>H. howelli</i> Du Preez and Channing, 2013	Channing et al., 2013*
<i>O. wilderi</i> (Dunn, 1925)	Jones, 1967	<i>H. igbettensis</i> Schiøtz, 1963	Channing et al., 2013*
Sphaenorhynchus		<i>H. inyangae</i> Channing, 2013	Channing et al., 2013*
<i>S. botocudo</i> Caramaschi et al., 2009	Caramaschi et al., 2009*	<i>H. microps</i> Günther, 1864	Channing et al., 2013*
<i>S. bromelicola</i> Bokermann, 1966	Bokermann, 1966; Araujo-Vieira pers. comm.	<i>H. nasicus</i> Laurent, 1943	Channing et al., 2013*
<i>S. canga</i> Araujo-Vieira et al., 2015	Araujo-Vieira et al., 2015	<i>H. nasutus</i> Günther, 1865	Channing et al., 2013*
<i>S. caramaschii</i> Toledo et al., 2007	Toledo et al., 2007*	<i>H. poweri</i> Loveridge, 1938	Channing et al., 2013*
<i>S. carneus</i> (Cope, 1868)	Goin, 1957	<i>H. rwandae</i> Dehling et al., 2013	Channing et al., 2013*
<i>S. lacteus</i> (Daudin, 1800)	Jones, 1967	<i>H. viridis</i> Schiøtz, 1975	Channing et al., 2013*
<i>S. mirim</i> Caramaschi et al., 2009	Caramaschi et al., 2009*	Mantellidae	
		Boophis	
		<i>B. luteus</i> group	
		<i>B. luteus</i> (Boulenger, 1882)	Glaw and Vences, 2007*

Table 1. Continued.

TAXON	SOURCE
<i>B. anjanaharibeensis</i> Andreone, 1996	Glaw and Vences, 2007*
<i>B. elenae</i> Andreone, 1993	Glaw and Vences, 2007*
<i>B. englaenderi</i> Glaw and Vences, 1994	Glaw and Vences, 2007*
<i>B. andreonei</i> Glaw and Vences, 1994	Glaw and Vences, 2007*
<i>B. andohahela</i> Andreone et al., 1995	Glaw and Vences, 2007*
<i>B. septentrionalis</i> Glaw and Vences, 1994	Glaw and Vences, 2007*
<i>B. jaegeri</i> Glaw and Vences, 1992	Glaw and Vences, 2007*
<i>B. sandra</i> Glaw et al., 2010	Glaw et al., 2010*
<i>B. albipunctatus</i> group	
<i>B. ankaratra</i> Andreone, 1993	Glaw and Vences, 2007*
<i>B. boppa</i> Hutter et al., 2015	Hutter et al., 2015*
<i>B. schuboeae</i> Glaw and Vences, 2002	Glaw and Vences, 2007*
<i>B. albipunctatus</i> Glaw and Thiesmeier, 1993	Glaw and Vences, 2007*
<i>B. sibilans</i> Glaw and Thiesmeier, 1993	Glaw and Vences, 2007*
<i>B. miadana</i> Glaw et al., 2010	Glaw et al., 2010*
<i>B. haingana</i> Glaw et al., 2010	Glaw et al., 2010*
<i>B. luciae</i> Glaw et al., 2010	Glaw et al., 2010*
<i>B. tampoka</i> Köhler et al., 2008	Glaw et al., 2010*
<i>B. rappidodes</i> group	
<i>B. ankarafensis</i> Penny et al., 2014	Penny et al., 2014*
<i>B. rappidodes</i> (Ahl, 1928)	Glaw and Vences, 2007*
<i>B. bottae</i> Vences and Glaw, 2002	Glaw and Vences, 2007*
<i>B. tasymena</i> Vences and Glaw, 2002	Glaw and Vences, 2007*
<i>B. erythodactylus</i> (Guibé, 1953)	Glaw and Vences, 2007*
<i>B. viridis</i> Blommers-Schlösser, 1979	Glaw and Vences, 2007*
<i>B. mandraka</i> group	
<i>B. mandraka</i> Blommers-Schlösser, 1979	Glaw and Vences, 2007*
<i>B. liami</i> Vallan et al., 2003	Glaw and Vences, 2007*
<i>B. sambirano</i> Vences and Glaw, 2005	Glaw and Vences, 2007*
<i>B. solomaso</i> Vallan et al., 2003	Glaw and Vences, 2007*
<i>B. ulftunni</i> group	
<i>B. baetkei</i> Köhler et al., 2008	Köhler et al., 2008*
<i>B. liliana</i> Köhler et al., 2008	Köhler et al., 2008*
<i>B. ulftunni</i> Wollenberg et al., 2008	Wollenberg et al., 2008*
Guibemantis	
<i>G. liber</i> * (Peracca, 1893)	Glaw and Vences, 2007*
<i>G. pulcher</i> (Boulenger, 1882)	Glaw and Vences, 2007*
<i>G. tasifotsy</i> Lehtinen et al., 2012	Lehtinen et al., 2012*
Mantidactylus	
<i>M. argenteus</i> (Methuen, 1920)	Glaw and Vences, 2007*
Rhacophoridae	
Chiromantis	
<i>C. samkosensis</i> Grismer et al., 2007	Grismer et al., 2007
Gracixalus	
<i>G. gracilipes</i> (Bourret, 1937)	Bain and Nguyen, 2004
<i>G. supercornutus</i> (Orlov et al., 2004)	Rowley et al., 2011*
<i>G. quangi</i> Rowley et al., 2011	Rowley et al., 2011*
Rhacophorus	
<i>R. prominanus</i> Smith, 1924	Harvey et al., 2002
<i>R. achantharrhena</i> Harvey et al., 2002	Harvey et al., 2002
<i>R. dulitensis</i> Boulenger, 1892	Inger and Stuebing, 1996

of the occurrence of fluorescence, it would be surprising if fluorescence in anurans is restricted exclusively to the species most closely related to *B. atlantica* and *B. punctata*.

Glandular secretions and persistence of fluorescence

Our observations indicate that individuals of *Boana atlantica* and *B. punctata* can leave persistent fluorescent secretions on substrate. The presence of numerous exocrine skin glands in *B. punctata* (Brunetti et al., 2012) is suggestive of a mostly glandular source for this fluorescence. In *B. punctata*, the compound that is responsible for fluorescence is named Hyloin-G1 (in contrast to Hyloin-L1 and Hyloin-L2 that occur in muscle and lymph) identified in the glandular secretions from dorsal skin (Taboada et al., 2017). Given its phylogenetic proximity to *B. punctata*, we assume fluorescence in *B. atlantica* is caused by the same compounds, although we caution that the molecular basis of fluorescence in *B. atlantica* has yet to be examined.

In order to identify the anatomical source of fluorescence in *Boana punctata*, Taboada et al. (2017) used electrical stimuli to obtain glandular secretions from dorsal skin. This methodology stimulates secretion of serous glands but not mucous glands (Brunetti et al., 2016), indicating that Hyloin-G1 is stored in serous skin glands. Because serous glands are evenly distributed at high densities in all body regions (Brunetti et al., 2012), we can attribute each fluorescent spot in the skin of living specimens of *B. punctata* (Fig. 2C) and—we infer—*B. atlantica* (Fig. 1D) to the fluorescence of Hyloin-G1 stored in these glands.

In our CIs from lateral glands of *Boana punctata*, we observed strong fluorescence in sexually dimorphic skin glands (SDSGs; specifically, specialized mucous glands; see Brunetti et al., 2012) similar to those recorded in the serous glands of this species (Taboada et al., 2017). At this point, it is not possible to determine whether fluorescence from SDSGs is also due to Hyloin-G1. The more yellowish fluorescence emitted from the mental gland of *B. atlantica* might be possibly explained by (1) the different gland types and density that occur in mental and lateral glands of males compared to other body regions or (2) the blue emission of hyloins filtered by the different composition of other blue absorbing pigments in chromatophores. Additional research is required to test these alternative hypotheses.

Field observations of *Boana punctata* by Brunetti et al. (2014) have shown that males of this species exhibit territorial behavior evidenced by site fidelity, acoustic interactions, and aggression against male intruders, usually involving physical contact. Those authors described a behavior in which a male emits territorial calls at different

distances around the center of its territory. In *B. atlantica*, males have been observed calling on perches and in water and with females found near the males (Camurugi and Juncá, 2013). These observations are compatible with the territoriality of males and mating strategy recorded in *B. punctata*. Whether fluorescent marks on the substrate left by *B. atlantica* and *B. punctata* play any role as signals for potential mates and/or rival males—or play any biological role at all—remains to be elucidated.

Other sources of fluorescence in amphibians

Translucency may not be a *sine qua non* for fluorescence in amphibians, but it is an important trait if fluorescent compounds are distributed in deep regions of the skin, like glands and lymph beneath the pigmentary cells. A thick layer of pigmentary cells immediately below the epidermis in species with opaque skin would prevent excitation light from reaching the deeper layers and resorb whatever small amount of fluorescence were emitted. This is probably one of the reasons why pteridins in the connective tissue (fluorescent compounds broadly distributed in amphibians) do not contribute to macroscopic fluorescence in frogs, at least in the experimental setups assayed by Taboada et al. (2017). Taking into account the structural organization of the dermal chromatophore unit in the skin of frogs (Bagnara et al., 1968) with outer xanthophores (closer to epidermis), middle iridophores, and inner melanophores, there could still be other hypothetical conditions where fluorescence (if present) would be detected in species with opaque skins. One of them implies xanthophores, considering their outer—and hence less attenuated—localization within the skin and their high pteridine content. Since the latter compounds are brightly fluorescent upon purification, fluorescence from xanthophores could occur in frogs skin.

However, despite the chemical composition of xanthophores, transverse sections in bullfrog skin (Hama and Obika, 1959) showed that fluorescence is not discernible in any chromatophores. The same was reported by Taboada et al. (2017) for *B. punctata*, *B. raniceps* (Cope, 1862), and *Scinax nasicus* (Cope, 1862) for excitation at 405 nm. This implies that within pigmentary cells there must be at least a quenching mechanism that accounts for the fluorescence emission deactivation and, hence, the lack of macroscopic fluorescence. When cross sections of skin of *B. punctata* were analyzed by confocal imaging using the cyan 488 nm laser line, xanthophores displayed a faint green fluorescence. The chemical origin of the latter is unknown, although it could be ascribed to certain pteridines. The fact that fluorescence can be detected from chromatophores when a different combination of excitation source and emission filter is used is not unexpected. In fact, by using appropriate combinations of powerful

excitation lights and filters it should be expected to see many surfaces and animals fluorescing to a certain extent simply as a byproduct of their chemical composition. Whether or not this fluorescence can be detected macroscopically and be of any biological relevance under natural conditions will depend on the nature and photochemistry of the fluorophores, as well as several characteristics of the skin (e.g., presence of quenchers, abundance of other absorbing pigments).

The biological relevance of fluorescence will depend on: (1) the relative contribution of the fluoresced photons to the total emerging light from the animals (i.e., fluorescence + reflectance) under natural environmental irradiance conditions (Taboada et al., 2017) and (2) the visual sensitivity of the animals for which seeing this fluorescence would be of some biological relevance. Taking these conditions together we expect fluorescence to be biologically relevant when—among other variables—excitation wavelengths correspond to a region of the spectrum where animals have low visual sensitivity (like the violet edge of the spectrum) and emission is not only intense but also matches the peak of visual sensitivity, as occurs in the hyloin-based fluorescence of *Boana punctata* (Taboada et al., 2017) and possibly, *B. atlantica*. However, given that neither condition seems to be fulfilled with the faint fluorescence observed from xanthophores, this kind of fluorescence does not seem to be relevant from a biological point of view and instead may be more likely interpreted as a byproduct of the chemical nature of certain pigments presents in the cells. Naturally, further research will be necessary to analyze quantitatively this fluorescence emission in several amphibian species and properly seize its contribution to animal coloration.

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