ISTHMIAN CENTRAL AMERICAN FROGS WITH DIFFERENT BIOFLUORESCENCE PATTERNS

RANAS DEL ISTMO CENTROAMERICANO CON DIFERENTES PATRONES DE BIOFLUORESCENCIA

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Received: 2023-08-02. **Accepted**: 2023-10-25. **Published**: 2023-11-28.
Editor: Adrian García-Rodríguez, Costa Rica.

Resumen.– La biofluorescencia se refiere al brillo fluorescente en colores azules, verdes y rojos que emiten los organismos cuando son expuestos a la luz azul o ultra-violeta. Este fenómeno ha sido documentado en diversos grupos animales, siendo el hallazgo más reciente en anfibios. La función de la biofluorescencia en anfibios aún es desconocida, aunque se hipotetiza que puede estar asociada a la selección sexual, comunicación y agudeza visual. En este trabajo se describen los patrones biofluorescentes de seis especies de anuros en Centroamérica ístmica. Asímismo, se mencionan cinco especies que no reaccionaron con fluorecencia ante el estúmulo de luz azul. Debido a que en algunos trabajos existen discordancias con respecto a la presencia o ausencia de biofluorescencia entre especies, recomendamos el uso de luz azul de 440–460 nm y un filtro amarillo #12, los cuales se han probado exitosamente para la detección de biofluorescencia en anfibios.

Palabras clave.– Fluorescencia, anfibios, luz azul, Costa Rica, Panamá.

Abstract.– Biofluorescence is the fluorescent glow in blue, green, and red colors that organisms emit when exposed to blue or ultra-violet light. This phenomenon has been documented in different animal groups, the most recent finding being in amphibians. The function of biofluorescence in amphibians is still unknown, although it is hypothesized that it may be associated with sexual selection, communication, and visual acuity. In this work, we described the biofluorescence patterns for six species of anurans occurring in Isthmian Central America. Likewise, five species that did not react with fluorescence to the stimulus of blue light are also mentioned. Because in some works there are inconsistencies regarding the presence or absence of biofluorescence between species, we recommend the use of blue light of 440–460 nm and a yellow #12 filter, which have been successfully tested for the detection of biofluorescence in amphibians.

Keywords.– fluorescence, amphibians, blue light, Costa Rica, Panama.

Biofluorescence is a bright displayed in organisms exposed to blue or ultra-violet light, thus is observed in fluorescent colors (blues, greens, and reds). This phenomenon is physically explained by the higher energy wavelengths of light that are absorbed and reemitted at lower energy wavelengths (Taboada et al., 2017, 2017b). Hypothesized functions for biofluorescence include sexual selection (Arnold et al., 2002), visual communication (Marshall & Johnsen, 2017), camouflage (Claes & Mallefet, 2008), prey attraction (Haddock et al., 2005), and mimicry (Kohler et al., 2019). The biofluorescence has been mainly documented in arthropods (Gaffin et al., 2012), birds (Arnold et al., 2002), cnidarians (Salih et al., 2000), fishes (Sparks et al., 2014), mammals (Kohler et al., 2019), reptiles (Prötzel et al., 2018), and recently in amphibians (Taboada et al., 2017a, 2017b; Lamb & Davis, 2020).

In amphibians, biofluorescence has been reported for the three orders: anurans, salamanders and caecilians. Nevertheless, the evidence remains restricted to 16 families, and 46 species, which represent 0.5% (AmphibiaWeb, 2023) of the total amphibian diversity (Taboada et al., 2017b; Deschepper et al., 2018; Gray, 2019; Chavez-Acuña et al., 2020; Lamb & Davis, 2020; Whitcher, 2020; Marjan et al., 2022; Cox & Fitzpatrick, 2023; Kong et al., 2023). However, some species do not respond to ultra-violet or blue light (Thompson et al., 2019; Marjan et al., 2022). In most cases, biofluorescence is just reported as visual

Tabla 1. Patrones de biofluorescencia en especies de anuros de Panamá (P) y Costa Rica (CR) por exposición a luz azul. Especie, familia, color y breve descripción de la fluorescencia. Los términos de patrones se basan en el catálogo de colores de Köhler (2012).

Table 1. Biofluorescence patterns in anuran species of Panama (P) and Costa Rica (CR) by exposure to blue excitation light. Species, family, color, and a short fluorescence description. Patterns terms are based on Köhler (2012) color catalogue.

Species	Family	Color	Pattern	Country
Craugastor fitzingeri	Craugastoridae	Green	Dorsal green marks surrounding dark coloration patterns /Lateral suffusion / Uniform in lower ventral.	CR
Diasporus diastema	Eleutherodactylidae	Green	Dorsal reticulum / Green bones and jaw with ventral stipples	CR
Boana rufitela	Hylidae	Green	Dorsal uniform green	CR
Smilisca manisorum	Hylidae	Red / Green	Red dorsal flecks / Ventral suffusion in green	CR
Engystomops pustulosus	Leptodactylidae	Green / Orange	Dorsal suffusion / Upper ventral in green and tubercules in orange.	P
Pristimantis ridens	Strabomantidae	Green	Dorsal suffusion / green bones and jaw in ventral view	CR

records and/or quantifying the intensity with a spectrometer. However, the physiological causes of fluorescence production have been documented only for *Boana punctata*, where was discovered that the pigmentary skin cells emerge from lymph and glandular structures (Taboada et al., 2017a). Its function in amphibians is still unknown, and some hypotheses include sexual selection, visual communication (Lamb & Davis, 2020; Cox & Fitzpatrick, 2023), and improved visual acuity (Taboada et al., 2017a). According to this lack of knowledge regarding biofluorescence in amphibians, further efforts to report and investigate the presence and function of biofluorescence are necessary.

In this study, we briefly explore two sites in 2023; one in Panama (Gamboa; 9.120584 N, 79.693988 W, 57 m a.s.l.) on April 7th, and the second in Costa Rica (Heredia Province; 10.429947 N, 84.006142 W, 59 m a.s.l.) on May 2 to 7th. Both sites correspond to secondary forests and the surveys were conducted on designed trails in these areas. We did visual searching during the night, and every anuran found was exposed to blue excitation light (Nightsea Xite Flashlight, 440–460 nm) and observed with yellow barrier filter glasses (Lamb & Davis, 2020). When anurans showed brightness, they were photographed dorsally and ventrally using a digital Lumix DC-FZ80 Panasonic camera with a Tiffen #12 Yellow Filter. Color fluorescence pattern of each individual photograph was analyzed according to Köhler (2012).

We observed eleven anuran species from seven families: Bufonidae, Craugastoridae, Dendrobatidae, Eleutherodactylidae, Hylidae, Leptodactylidae, and Strabomantidae. We found different patterns of biofluorescence in six species (Table 1). The species that did not show biofluorescence were *Rhaebo haematiticus* (Bufonidae), *Rhinella horribilis* (Bufonidae), *Craugastor bransfordii* (Craugastoridae), and *Oophaga pumilio* (Dendrobatidae) from Costa Rica, and *Rhinella alata* from Panama.

The greenest brightness was shown in *Engystomops pustulosus* (Leptodactylidae) (Fig. 1B-C), in which the individuals showed fluorescence in the dorsal area. Particularly, this species was emitting breeding calls in a small puddle (1.5 m x 1.5 m approx.), and the visualization was difficult despite the calls. But, when we used the blue light, the individuals were easily seen (7 approx.) showing a clear fluorescent pattern that stood out from the ground. Similarly, breeding calls were heard when we found individuals of *Diasporus diastema* (Eleutherodactylidae). This species showed a dorsally reticular pattern of biofluorescence that contrasts with the pale coloration that characterizes this species (Fig. 1D-F). In contrast, *Craugastor fitzingeri* (Craugastoridae) showed light biofluorescence laterally (Fig. 1H), but it was concentrated on the lower ventral surface (Fig. 1I). *Boana rufitela* (Hylidae) showed a dorsal bright green glow (Fig. 2B), which is very similar to the biofluorescence reported for its congener

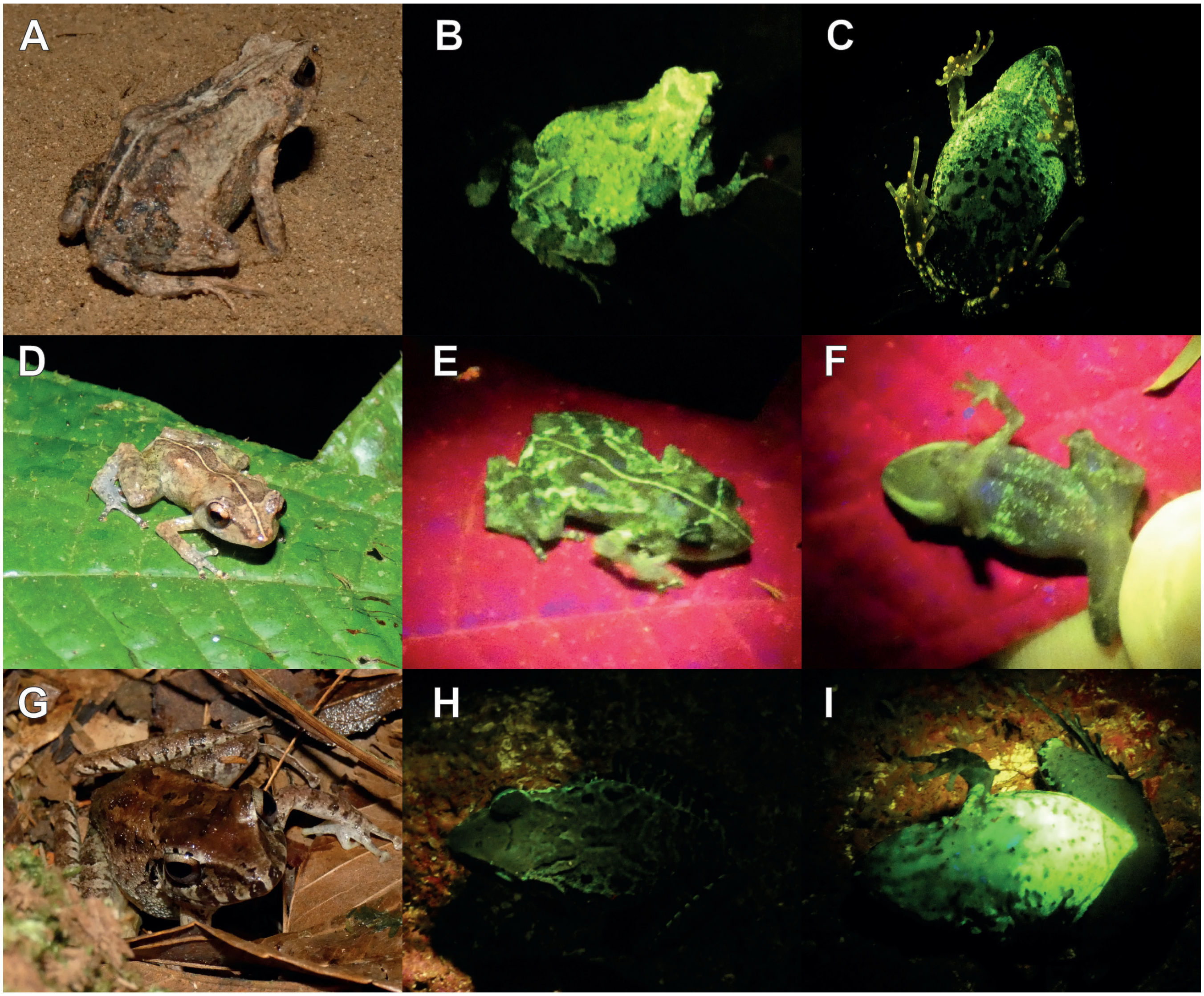

Figura 1. Fotografías de anuros centroamericanos bajo luz blanca (primera columna) y luz azul (segunda y tercera columna). El orden es según la intensidad de brillo observada (alto a bajo). *Engystomops pustulosus* (A, B, C), *Diasporus diastema* (D, E, F) y *Craugastor fitzingeri* (G, H, I).

Figure 1. Photographs of Central American anurans under white light (first column) and blue light (second and third column). The order is according to the brightness intensity observed (high to low). *Engystomops pustulosus* (A, B, C), *Diasporus diastema* (D, E, F) and *Craugastor fitzingeri* (G, H, I).

Boana punctata (Nowogrodzki, 2017; Taboada et al., 2017a). The ventral view of *B. rufitela* was not examined. *Smilisca manisorum* (Hylidae) presented a weak dorsal fluorescence with some red spots (Fig. 2D), but green spread ventrally (Fig. 2E). Finally, *Pristimantis ridens* (Strabomantidae) showed a soft dorsal green suffusion that seems more notable in the flanks (Fig. 2G), but the bones were green bright in the ventral view (Fig. 2H).

Our results add new records of biofluorescence in anuran species of Isthmian Central America. Especially, species belonging to the families Eleutherodactylidae, Craugastoridae, and Strabomantide which had not been surveyed previously. We observed different patterns and intensities in fluorescence, which *Engystomops pustulosus* and *Diasporus diastema* showed the most intense brightness (Fig. 1A-F). We also found two species from the family Hylidae that displayed very different fluorescence patterns. This suggests that fluorescence variation should be evaluated in lower taxonomic hierarchies and in different families.

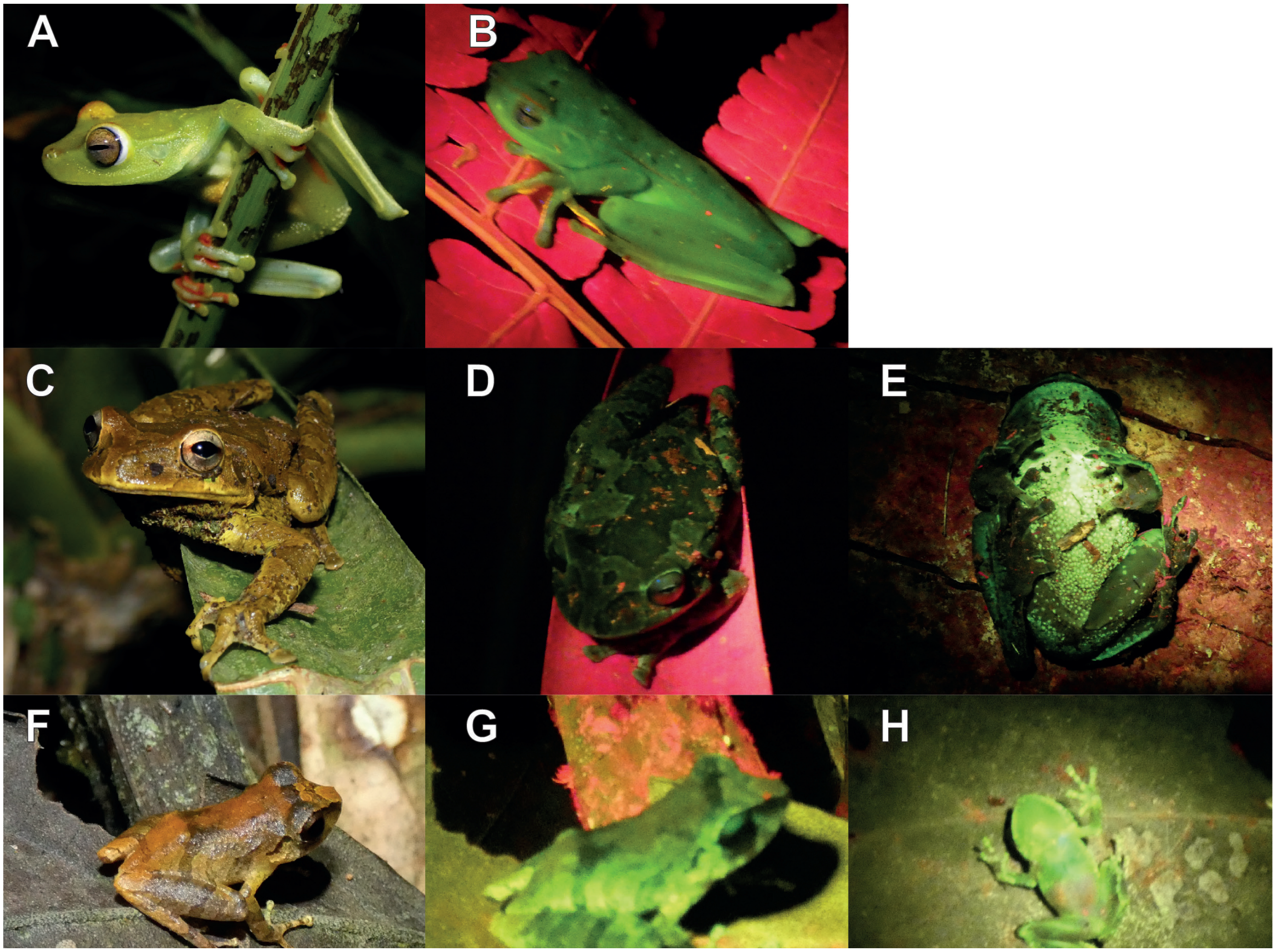

Figura 2. Fotografías de anuros centroamericanos bajo luz blanca (primera columna) y luz azul (segunda y tercera columna). El orden es según la intensidad de brillo observada (alto a bajo). *Boana rufitela* (A, B), *Smilisca manisorum* (C, D, E) y *Pristimantis ridens* (F, G, H).

Figure 2. Photographs of Central American anurans under white light (first column) and blue light (second and third column). The order is according to the brightness intensity observed (high to low). *Boana rufitela* (A, B), *Smilisca manisorum* (C, D, E), and *Pristimantis ridens* (F, G, H).

According to published studies, some other species from the families surveyed here have been previously reported. For example, fluorescence was reported in *Leptodactylus fragilis* (Leptodactylidae) (Whitcher, 2020), suggesting that fluorescence could be observed in more members of this family. The Hylidae family is the most surveyed for fluorescence presence (Taboada et al., 2017b; Deschepper et al., 2018; Chavez-Acuña et al., 2020; Whitcher, 2020). *Boana rufitela* was predicted to be fluorescent (Taboada et al., 2017b), and Deschepper (2018) reported the fluorescence in this species until a wavelength of 365 nm. In contrast, Thompson et al. (2019) did not observe biofluorescence in *B. rufitela*. This could be explained by the absence of a long pass filter. The observation of fluorescence emission is highly recommended through a yellow long-pass filter (glasses or a camera filter). The filter is used to eliminate the influence of reflected light with a wavelength longer than 500 nm in human vision (Taboada et al., 2017a, 2017b). Due to Amphibians' eyes possessing types of retinal rods that enable nocturnal scotopic vision, humans need to use a long pass filter that blocks most of the illumination and allows us to observe the color perception as amphibians do (Taboada et al., 2017b).

Hylid species *Boana atlantica* and *B. punctata* also were reported to leave fluorescent traces in sites where they stood or in the gloves after manipulation (Taboada et al., 2017b). We also observed these traces on gloves when *Smilisca manisorum* was handled, this may be explained by glandular secretions with fluorescence components such as Hyloin-G1 as has been reported by Taboada et al. (2017a, 2017b). We consider that secretion marks are an important element that could be addressed when fluorescence is observed in amphibians, due to trying to find the origin (gland and/or lymph) and the possible function of this fluorescence.

According to our experience finding the species *Engystomops pustulosus*, we suggest the use of blue light (440–460 nm) could be a good detection method in a short range (2–3 m) for this species. Although in some cases the short range is not optimal (Alvarez et al., 2022), normal visual detection is difficult due to the camouflage color pattern and the tiny size of some amphibians. Instead, blue light and the use of filter glasses enhance the detection of fluorescent individuals in the field.

Acknowledgments.– We follow the ethical standards of Costa Rica and Panama for amphibian handling. We expose amphibians using blue light without any injury, *in situ*, and only manipulated the specimens for ventral photographs. We thank Resilience Institute Bridging Biological Training and Research (RIBBiRT) for the facilities to visit the Biological Station La Selva in Costa Rica, and all the training workshop participants for helping in the field explorations. Erick Arias for helping in the species identification.

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