

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/346036897>

Recent findings of *Isthmohyla pictipes* (Anura: Hylidae) in Costa Rica: variation and implications for conservation

Article in *Zootaxa* · November 2020

DOI: 10.11646/zootaxa.4881.3.4

CITATION

1

READS

809

9 authors, including:



Wagner Chaves-Acuña

Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"

11 PUBLICATIONS 12 CITATIONS

[SEE PROFILE](#)



Jeremy Klank

University of Costa Rica

4 PUBLICATIONS 4 CITATIONS

[SEE PROFILE](#)



Erick Arias

University of Costa Rica

19 PUBLICATIONS 91 CITATIONS

[SEE PROFILE](#)



Federico Bolaños

University of Costa Rica

90 PUBLICATIONS 4,074 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Evolution and Diversity in Phyllomedusinae Günther, 1858 (Amphibia, Anura): an evolutionary approach of genotype and phenotype [View project](#)



JAPU Q'EROS [View project](#)



Recent findings of *Isthmohyla pictipes* (Anura: Hylidae) in Costa Rica: variation and implications for conservation

WAGNER CHAVES-ACUÑA^{1,9}, GERARDO CHAVES², JEREMY KLANK^{2,3}, ERICK ARIAS^{2,4}, FEDERICO BOLAÑOS^{2,3}, ALEX SHEPACK⁵, TWAN LEENDERS⁶, JOHN COSSEL⁷ & JULIÁN FAIVOVICH^{1,8}

¹División Herpetología, Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”– Consejo Nacional de Investigaciones Científicas y Técnicas, Ángel Gallardo 470, C1405DJR, Buenos Aires, Argentina.

²Museo de Zoología, Centro de Investigaciones en Biodiversidad y Ecología Tropical, Universidad de Costa Rica, 11501-2060, Montes de Oca, San José, Costa Rica.

✉ cachi13@gmail.com; <https://orcid.org/0000-0002-4301-6569>

³Escuela de Biología, Universidad de Costa Rica, 11501-2060, Montes de Oca, San José, Costa Rica.

✉ jklank97@gmail.com; <https://orcid.org/0000-0003-4451-4436>

✉ federico.bolanos@ucr.ac.cr; <https://orcid.org/0000-0002-7935-6418>

⁴Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, AP 70-153 Ciudad Universitaria, Ciudad de México, México.

✉ eapiedra@gmail.com; <https://orcid.org/0000-0002-4449-1070>

⁵Department of Biological Sciences, Florida International University, 11200 SW 8th St, Miami, Florida 33199, USA.

✉ alex.shepack@gmail.com; <https://orcid.org/0000-0003-0623-0969>

⁶Roger Tory Peterson Institute of Natural History, 311 Curtis Street, Jamestown, New York 14701, USA.

✉ tleenders@rtpi.org; <https://orcid.org/0000-0003-4166-5001>

⁷Biology Department, Northwest Nazarene University, 623 South University Boulevard, Nampa, Idaho 83686, USA.

✉ jocossel@nnu.edu; <https://orcid.org/0000-0002-1707-5063>

⁸Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

✉ jfaivovich@gmail.com; <https://orcid.org/0000-0001-7157-8131>

⁹Corresponding author: ✉ wchaves512@gmail.com; <https://orcid.org/0000-0002-6669-5701>

Abstract

We report recent findings of *Isthmohyla pictipes* (Cope, 1875) in the Cordillera de Talamanca, Costa Rica, roughly two decades after it was last registered. We provide notes on microhabitat use, color variation, external morphology of adults and larvae, and geographic variation, and discuss some taxonomic characters employed to differentiate *I. pictipes* from *I. tica* (Starrett, 1966) and *I. xanthosticta* (Duellman, 1968). We also report fluorescence on the ventral surfaces of *I. pictipes*. Our findings are expected to shed light on the taxonomy of this species and should be useful in further population assessments and conservation plans.

Key words: Cerro de la Muerte, fluorescence, Hylinae, Hylini, threatened species

Introduction

In the late 1980s, a great number of anuran species declined across the Central American Isthmus (Lips *et al.* 2006; Crawford *et al.* 2010), a region with an extraordinary diversity of amphibians (Campbell 1999; Savage & Bolaños 2009; Sasa *et al.* 2010; Salazar-Zúñiga *et al.* 2019). *Isthmohyla* Faivovich *et al.*, 2005 is a hylid genus that currently comprises 14 species endemic to rainforests of Central America (Faivovich *et al.* 2018), and from which 12 species are considered threatened to some degree (IUCN 2020). With the exception of *I. insolita*, which inhabits riparian forests in Honduras (McCranie *et al.* 1993), all other species of *Isthmohyla* are distributed in Costa Rica and Panama (Duellman 2001; Faivovich *et al.* 2018). These hylids were once conspicuous members of major mountain ranges in Costa Rica and western Panama (Savage 2002), but now only a few species are found in stable numbers (García-Rodríguez *et al.* 2012; IUCN 2020).

Isthmohyla pictipes (Cope, 1875) is a moderate-sized species (SVL females 40.9–45.1 mm; SVL males 31.8–39.0 mm) that inhabits streams 1,500–2,800 masl (Duellman 2001; Savage 2002; Bolaños *et al.* 2008). Its distribution ranges from the northern slope of the Cordillera Central in Costa Rica through the highlands of the Cordillera de Talamanca, to the southernmost limit of its distribution in La Amistad International Park, between Costa Rica and Panama (Bolaños *et al.* 2008). Presumably, this species may also occur in western Panama (Auth 1994) but the presence of *I. pictipes* in that country still requires confirmation (R. Ibañez, pers. comm.). This species was hypothesized to breed year-round (Duellman 1970) although calling males were found to be more abundant during the dry season and at the beginning of the wet season (Starrett 1966; Duellman 1970; Savage 2002).

The databases of the Museum of Zoology of the Universidad de Costa Rica (UCR) and VertNet (2020) show that an increasing number of expeditions between 1957 and 1979, mainly conducted at the slopes of the Cordillera Central, account for approximately 90% ($n=202$) of the historical collection activity assigned to *I. pictipes* in public collections (see Appendix 1). Bolaños *et al.* (2008) reported that this species has not been documented throughout its range across the Cordillera Central, but “it continues to be seen in the Cerro de la Muerte and several other localities within its range”. However, this statement was solely based on fieldwork performed by two of us (FB and GC) in the mid-1990s at Cuericí Biological Station (hereafter Cuericí), located in the Cerro de la Muerte area, on the Pacific Slope of the Cordillera de Talamanca (see Appendix 2). *Isthmohyla pictipes* was last collected in 1994 at Cuericí 2,700 masl (UCR 11823–24).

Early studies implicated the chytrid fungus *Batrachochytrium dendrobatidis* as the likely cause of well-documented amphibian declines of stream-breeding species at protected sites where *I. pictipes* was known to occur (Lips 1998; Lips *et al.* 2003). Tadpoles of this species were collected from Las Tablas at eastern Cordillera de Talamanca in 1990 by K. Lips (VertNet 2020; LACM 180438, its identity corroborated by us); however, she did not mention *I. pictipes* in her assessment of amphibian declines from this locality (Lips 1998). Recent surveys in other highland areas that surround sites of historical and potential occurrence for *I. pictipes* have not yielded any reports either (Abarca 2012; Hertz *et al.* 2012; Acosta-Chaves *et al.* 2015). Yet, most of the species’ range has not been properly surveyed by herpetologists (Bolaños 2009; García-Rodríguez *et al.* 2012; IUCN 2020).

Here we report recent findings of adults and larvae of *I. pictipes* in the Cordillera de Talamanca, Costa Rica, roughly two decades after it was last registered. We provide field observations and natural history notes for this relatively little known species, and comment on its variation of coloration in life, external morphology, and report the occurrence of fluorescence. We compare our findings with previous diagnoses and with those of other closely related species (*I. tica* [Starrett, 1966] and *I. xanthosticta* [Duellman, 1968]).

Material and methods

Data were collected at two sites in the Cerro de la Muerte area, Cordillera de Talamanca, southeastern Costa Rica: 1) during June 2103, February 2015, and January and March 2016 we conducted field observations near San Gerardo de Dota (9°34'16.45"N, 83°47'54.84"W, ~2500 masl), consisting of ~70 person-hours (ph) of search effort; and 2) during October 2019 and January–February 2020 we surveyed a second major population at the headwaters of Río Buena Vista in a montane old growth oak forest at Cuericí, a 303.5 ha private reserve (9°33'18.01"N, 83°40'03.23"W, 2475 masl), totaling ~22 ph of search effort. This reserve is located 75 km NW from the type locality of *I. pictipes* at Cerro Kamuk, Cordillera de Talamanca, 1,520–2,135 masl (Arias & Chaves 2014; see Fig. 1).

We conducted diurnal searches for tadpoles and nocturnal surveys between 7:00–10:30 p.m. along small, narrow streams (<1 m wide) with numerous cascades and waterfalls. Frogs were captured by hand and kept overnight to record color variation during the day. Once photographed, all uncollected individuals were returned unharmed to their location of capture. In the laboratory, we determined fluorescence emission by visual inspection of the individuals using a handheld LED UV flashlight with a peak intensity of 365 nm.

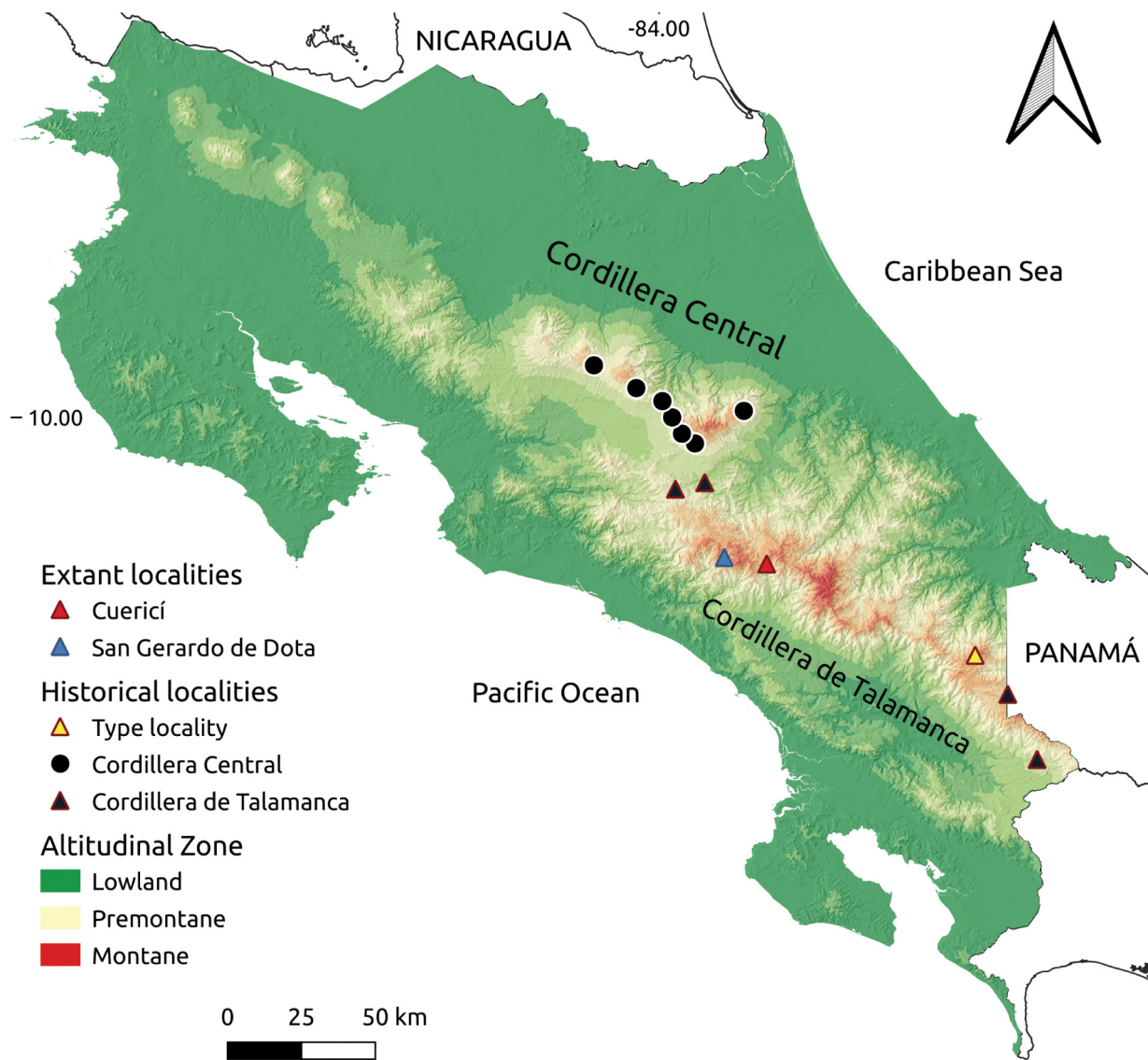


FIGURE 1. Distribution map of *Isthmohyla pictipes* in Costa Rica showing new records from Cuericí and San Gerardo de Dota, the type locality, and museum records from historical localities in the Cordillera Central and the Cordillera de Talamanca.

Collected larvae were preserved in 5% formaldehyde. Developmental stages and body measurements were determined according to Gosner (1960) and Altig & McDiarmid (1999), respectively. Fourteen measurements were taken: total length, body length, tail length, maximum body width, maximum tail height, tail muscle height, tail muscle width, dorsal fin height, ventral fin height, eye diameter, interorbital distance, eye-snout distance, nostril-eye distance, and oral disc width. Tadpoles were measured to a 0.1 g accuracy using ImageJ (Abramoff & Magalhaes 2004). Adult specimens were fixed in 10% formaldehyde and stored in 70% ethanol. Measurements (rounded to the nearest 0.1 mm) of SVL (snout-vent length), eye diameter, and tympanum diameter were taken using ImageJ for collected specimens. A caliper was used in living individuals to measure SVL. Weight was measured with a digital scale to the nearest 0.1 g. Sex was determined by the presence of dark colored nuptial pads in males. Specimens were deposited at UCR (adults: UCR 23363–23366, larvae: UCR 23382). We performed the comparisons of adult specimens based on observations of museum material from UCR and on literature information for color traits in life.

We obtained meteorological records from the Centro de Investigaciones Geofísicas of the UCR located in Cuericí, 215 m from the stream where we conducted fieldwork. The environmental temperature was 11.7–13.2°C (mean = 12.3°C) during October 2019 with a total precipitation of 444.2 mm and a mean relative humidity of 98.4%, and 11.5–14.9°C (mean = 12.9°C) during January 2020 with a total precipitation of 16.6 mm and a mean relative humidity of 85.7%. Air temperature during March 2020 (~13°C) was obtained using a Kestrel 3000 weather meter.

Results

On 16 June 2013, we received a report (M. Mooring, pers. comm.) of an unknown frog that had been found near a high gradient stream in the area of San Gerardo de Dota. The frog was seen on trailside vegetation, ~1 m above the forest floor about 10 m from the stream's edge at ~11:00 a.m., and although the frog was active, it may have been disturbed from its resting site (T. Mooring, pers. comm.). While a photograph of the frog suggested that it was *I. pictipes* (Fig. 2A), we did not find any additional frogs during a subsequent daytime visit to the exact location where the frog had been observed. We visited the site again on 17 February 2015 and conducted a diurnal search for tadpoles in ~100 m stretch of the stream, but did not encounter tadpoles of any species. Upon returning to the stream that evening, we encountered two individuals of *I. pictipes* at about 7:00 p.m. (Fig. 2B–E). One female was sitting on the upper surface of vegetation alongside the stream about 1 m above the surface of the water (Fig. 2B, D–E). Approximately 100 m upstream, we found a male on a boulder, near a streamside pool at the base of a waterfall (Fig. 2C, F–G); although we did not notice nuptial pads in this individual, we suspect that it was a male based on the approximate tympanic membrane/eye diameter ratio (reported to be 0.3–0.5 by Duellman 1970, but see below). Syntopic frog species (*Craugastor melanostictus* and *C. podiciferus*) were encountered at this stream.

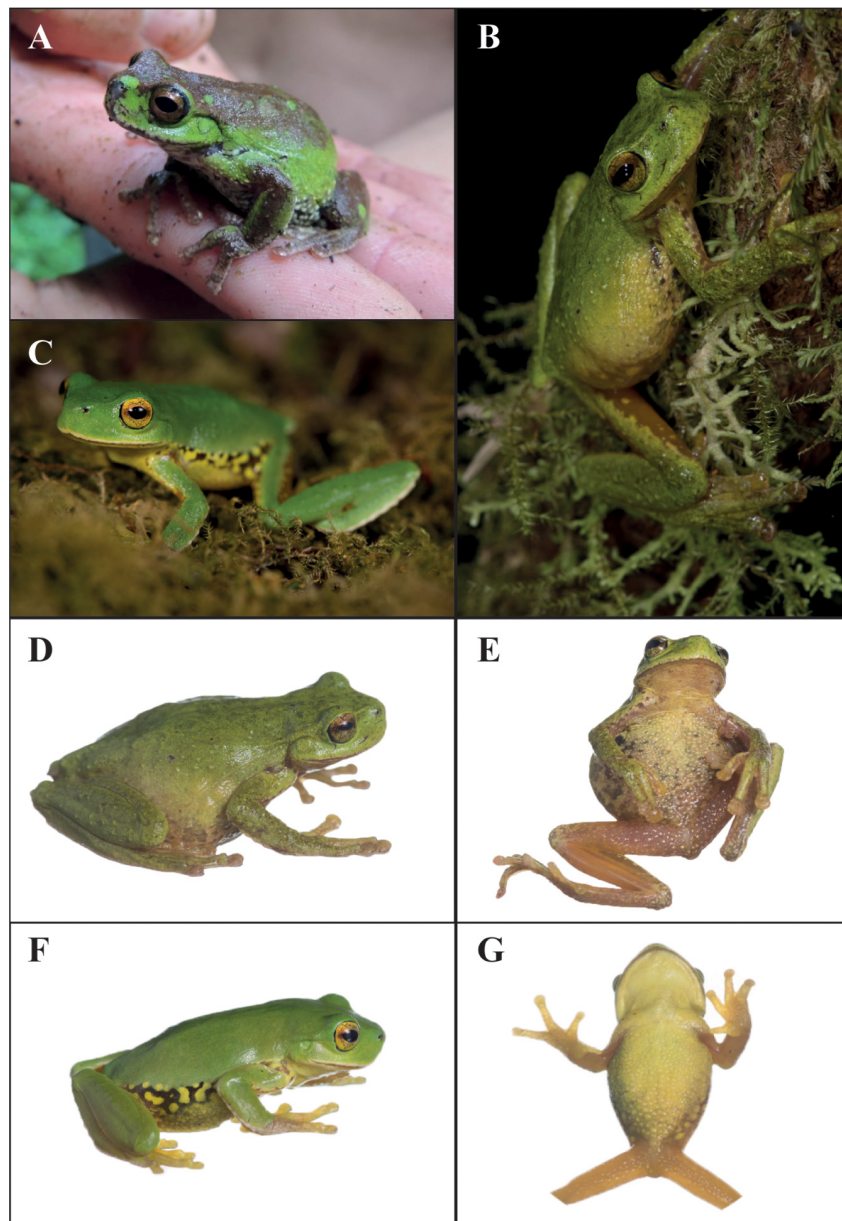


FIGURE 2. *Isthmohyla pictipes* from San Gerardo de Dota (not collected). (A): Adult female with diurnal coloration. (B, D–E): Adult female with a green uniform dorsum. (C, F–G): Adult male with white stripes on the outer edges of the forearms and on the upper lip.

On 14 October 2019 we found tadpoles of *I. pictipes* in shallow, small pools (<20 cm depth), clustered in groups of up to five individuals, hidden within the leaf litter at the bottom of a slow-moving stream in Cuericí. We collected five larvae in developmental stage 25 (Fig. 3; see Appendix 3 for body measurements). On 21 January 2020, ~50 m upstream from where the pools with tadpoles were located, we collected three females (Fig. 4A–D) and one male (Fig. 4E, F), within an area of ca. 2 m² on large rocks at a cascade in the headwaters of this stream. When handled, the frogs exuded sticky, transparent skin secretions from the entire body, which were not irritating to the human skin.

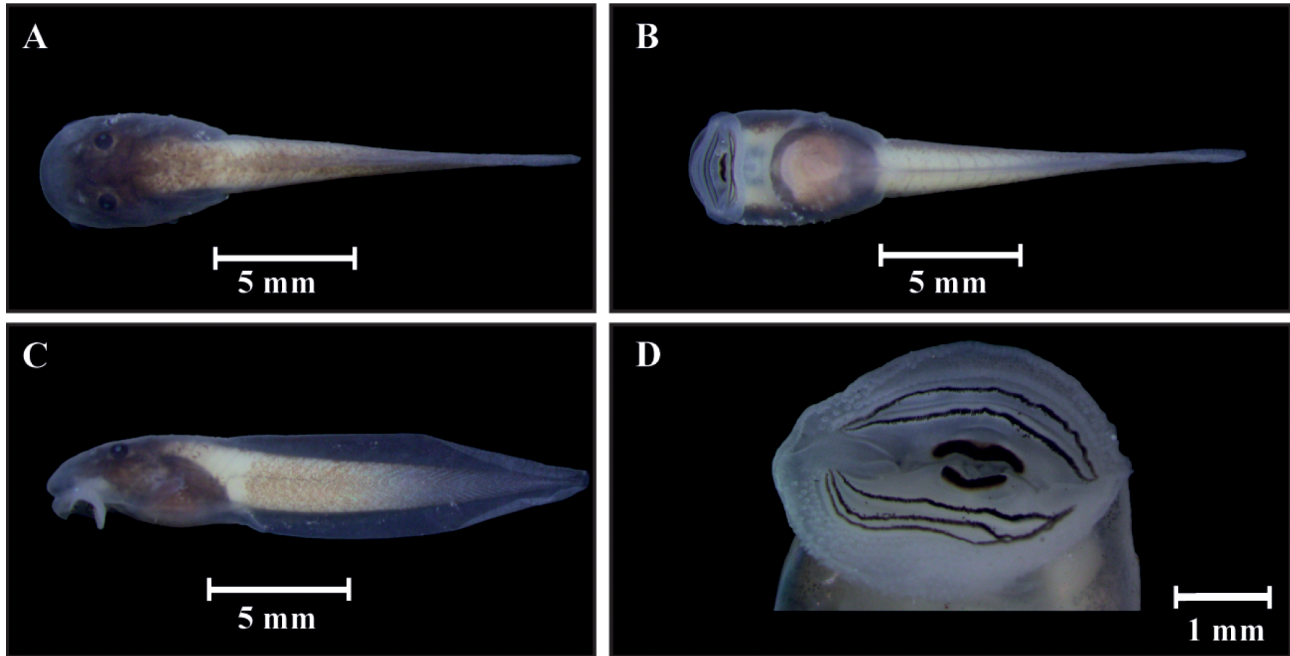


FIGURE 3. Tadpole of *Isthmohyla pictipes* (UCR 23382) at stage 25 (Gosner 1960). (A): Dorsal view. (B): Lateral view. (C): Ventral view. (D): Oral disc.

In subsequent surveys during 10–11 March 2020 we recorded three males (SVL 27.8–40 mm, weight 1.4–4.7 g; Fig. 4G–I) and five females (SVL 32.2–48.9 mm, weight 3.3–11.4 g; Fig. 4J–L) along the headwaters of a nearby low gradient stream. With the exception of one female that was found below a man-made water retention structure, all frogs were found spread out over about 160 m of stream course. All individuals were clustered around small cascades, and found either perched on leaves (e.g., *Anthurium* sp.), vines, sticks, roots above the cascade, or on rocks in the splash zone. All were located from ground level to 1.30 m above the surface of the water, but most were found less than 60 cm above the water level. Except for exhibiting lighter colors during the day, frogs did not exhibit great color variation between day and night (Fig. 4G–H, J–K). Throughout the sampling periods, we did not register any calling males, gravid females, juveniles, or egg clutches.

Discussion

To date, *I. pictipes* is currently listed as Endangered on the IUCN Red List of Threatened Species because its extent of occurrence is less than 5,000 km², its distribution is severely fragmented, the number of mature individuals is considered to be decreasing, and there is continuing decline in the extent and quality of its habitat outside of protected areas (Bolaños *et al.* 2008; IUCN 2020). However, in a review of the assessment made on 2019 the status of *I. pictipes* was changed to Critically Endangered because it is suspected that the surviving population is very small, possibly fewer than 250 mature individuals, with no more than 50 mature individuals occurring in each subpopulation (IUCN SSC Amphibian Specialist Group, in press).

Larvae of *I. pictipes* were described by Starrett (1966) and Duellman (1970), and our specimens agree in all points with their descriptions (Fig. 3). The presence of an enlarged oral disc with an M-shaped upper jaw sheath, single submarginal papillae on the posterior labium, and multiple submarginal papillae on the anterior labium are

character states shared by *I. pictipes* with other closely related stream-breeders like *I. rivularis* (Taylor, 1952) and *I. tica* (Duellman 2001; Faivovich *et al.* 2018). The former is known to occur sympatrically with *I. pictipes* (Duellman 2001), while *I. tica* has been registered as common at sites like Las Tablas and the Orosi-Tapantí region at the eastern and western limits of the Cordillera de Talamanca, respectively (VertNet 2020). Neither of these species have been recorded in Cuericí, but there are records of *I. rivularis* at Cerro de la Muerte, only 8 km SW from our findings (Museum of Comparative Zoology, MCZ A-29062–63).

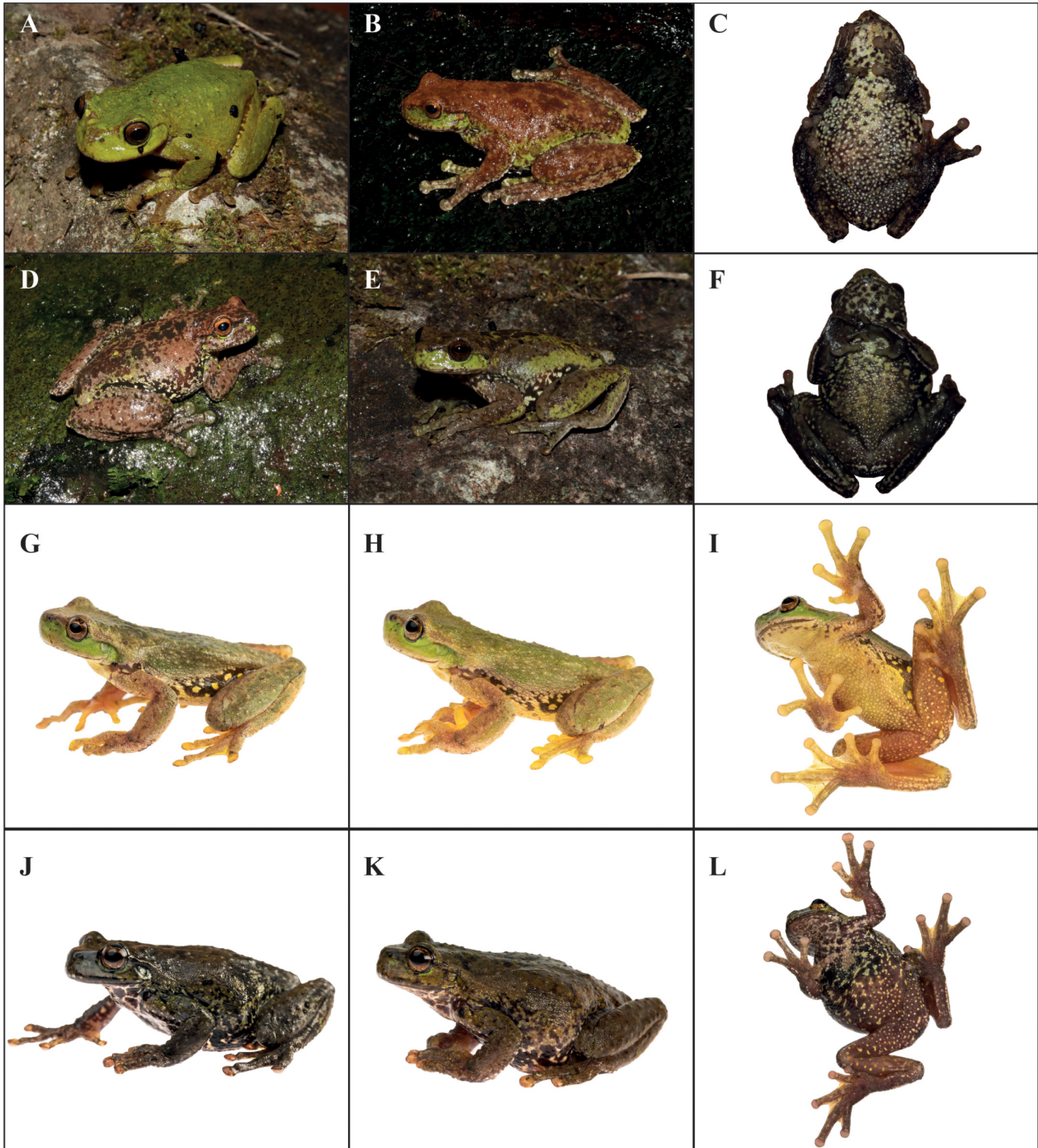


FIGURE 4. *Isthmohyla pictipes* from Cuericí. Note the highly variable coloration among all individuals. (A): UCR23366, adult female with a green uniform dorsum, SVL 49.9 mm. Note the bronze colored canthal stripe. (B–C): UCR23364, dorsum and venter of adult female, SVL 52.2 mm. Note the white crenulated dermal flap on the heel. (D): UCR23365, adult female, SVL 54.1 mm. (E–F): UCR23363, dorsum and venter of adult male, SVL 38.8 mm. Color variation during day and night with corresponding ventral photos of an uncollected adult male (G–I) and female (J–L). (G, J): nighttime. (H, K): daytime. (I, L): ventral views.

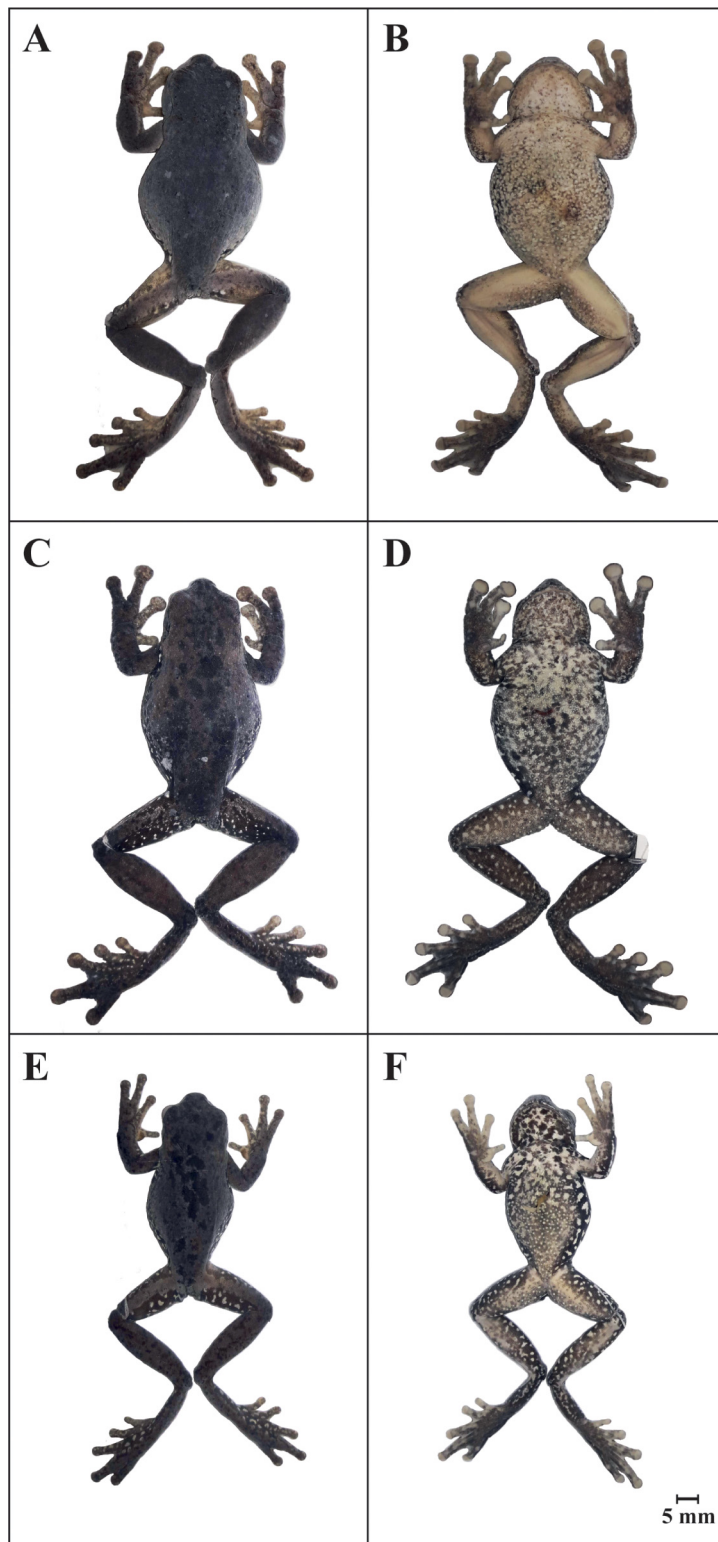


FIGURE 5. Dorsal and ventral views of *Isthmohyla pictipes* adults collected in Cuericí, Costa Rica. (A–B): Female (UCR 23366). (C–D): Female (UCR 23364). (E–F): Male (UCR 23363).

Tadpoles of *I. pictipes* are distinguished from those of *I. rivularis* (characters in parentheses) by the absence of submarginal papillae in the lateral folds (lateral folds with papillae), the presence of a single row of marginal papillae (multiple rows of large papillae bordering the lips, and one or two rows of small papillae also bordering the lower lip), rounded snout in dorsal view (truncate), and because the depth of the tail musculature is greater than that of the dorsal and ventral fins at mid-length of the tail (as deep as either fin). Tadpoles of *I. pictipes* differ from those of *I.*

tica (characters in parentheses) in that the body is half as wide as deep (much wider than deep, and widest behind the eyes), the nostrils are situated about one fourth the distance from the eyes to the tip of the snout (nostrils situated about midway between the eyes and the tip of the snout), the cloacal tube is median (dextral), the tail is about two times the length of the body (about three times the length of the body), and by the presence of two rows of small papillae on the anterior lip (two small rows and one large row of submarginal papillae on the upper lip).

The collected adult specimens agree with previous characterizations of the species (Starrett 1966; Duellman 1970), as follows: (1) snout is truncate and rounded in lateral and dorsal view, respectively; snout is moderately short; (2) the tympanum is located posteroventrally to the eye and separated from it by a distance equal to about half the diameter of the tympanum; (3) slightly protuberant nostrils situated approximately two thirds the distance from the eyes to the tip of the snout; (4) canthus rostralis rounded, but distinct; (5) color in life, in males the dorsum is green mottled with brown or black, and in females the dorsum is uniform green; the belly is yellow with a variable suffusion of brown or gray reticulations; the flanks, and the anterior and posterior surfaces of thighs in both sexes are brown with creamy yellow spots; the ventral surfaces of the hands and feet are partly or entirely brown; (6) webbing formula on hand I 2–3⁺ II 2–3⁺ III 3–2⁺ IV; (7) webbing formula on foot I 1⁺–2⁺ II 1⁺–2⁺ III 1⁺–2 IV 2^{1/2}–1^{1/2} V; (8) the cloacal opening is directed posteroventrally at the midlevel of the thighs; (9) dermal fringe extending from the inner metatarsal tubercle to the base of the disc of first toe; dermal fringe present on the lateral edge of the fifth toe.

Aside from differences in osteology (e.g., broad skull without a quadratojugal, a small frontoparietal fontanelle, and a reduced sphenethmoid that does not extend anteriorly between the nasals) and vocalizations (e.g., low frequency trill-like call), not considered here, *I. pictipes* was also distinguished by exhibiting color variation between sexes (Duellman 1970). Although described as sexually dichromatic (Duellman 1970), our findings show that the color differences between sexes are rather subtle. Aside of the typical green morph (Fig. 2B, D–E), we report a second female morph that combines green or dark brown with green mottling on the dorsum (Fig. 4B–D). We also noted differences in the coloration of the belly, chest, flanks, and thighs, and in the shape of the snout in ventral profile between these two forms (Fig. 5A–D).

In uniform green females, the belly is yellow suffused with dark spots, and the chest is pale yellow (Fig. 2E); the color of the flanks is lighter than the color of the dorsum (Fig. 2B, D); the anterior and posterior surfaces of the thighs are tan with pale yellow spots, and the snout is rounded in ventral view (Fig. 5A, B). We noted a bronze canthal stripe in a green female (Fig. 4A), also present in the closely related *I. debilis* (Taylor, 1952) and *I. xanthosticta* (Duellman 2001). In the mottled forms, the belly and chest are creamy white suffused with a dark reticulation (Fig. 4C); the flanks and thighs have numerous bright yellow spots (Fig. 5C, D), similar in overall ventral appearance to males from this locality (Fig. 5E, F). The colors of the flanks vary from green or reticulated with white, to being colored similar to the dorsum (Fig. 4B, D, K); the snout is angular in ventral profile (Fig. 5D).

The distribution and size of the yellow spots in the flanks and the posterior surface of the thighs is variable in *I. pictipes*. In some individuals, the discrete light-colored spots are big and widely dispersed from the groin to the axilla, even extending onto the hind limbs and feet. In other frogs these large spots only cover the area between the groin and the axilla, but light speckling might be present on the lower surface of the thigh. Occasionally, only small yellow markings are present on the posterior surface of the thighs, with no light markings on other parts of the hind limbs and flanks. The flanks may be partly or completely covered by a dark brown color.

Duellman (1968, 1970) reported that the presence of large, well-defined yellow spots on dark colored flanks and thighs, together with a bronze colored canthal stripe, vestigial webbing in the hands, and the presence of white stripes on the outer edges of the lips, forearm, tarsus, and above the cloacal opening are character states that distinguish *I. xanthosticta*, a species known only from its female holotype (Duellman 1968, 2001), from *I. pictipes*. However, the male that we detected in the San Gerardo de Dota region closely resembles the description provided for *I. xanthosticta*. With the exception of the bronze canthal stripe, it matches all of the aforementioned character states, in addition to having a uniform green dorsum, an unmarked venter, and yellow ventral surfaces of the limbs (Fig. 2F, G). Most of these character states (e.g., yellow spots of variable sizes on the flanks and thighs, a bronze canthal stripe, and white stripes on the lips, limbs, and above the cloacal opening) were also present in specimens from Cuericí (Fig. 4) and in other referred material collected at this locality (see Appendix 4) and at the southern slope of Volcán Barva, Cordillera Central (see Appendix 5). Other reports have noticed a series of white tubercles on the ventrolateral edge of the forearms (Starrett 1966) and a white line on the upper lip in some individuals of *I. pictipes* (Duellman 1970). Similar markings have been reported in individuals of other congeners like *I. debilis*, *I.*

rivularis, *I. tica* and *I. xanthosticta* (Duellman 1970). *Isthmohyla pictipes* is clearly a more variable species than was previously thought and additional research is needed to better assess this intraspecific variation and overlap with other putative, related species. Given the known variability in morphometry and color pattern within a relatively small sample of *I. pictipes*, a re-evaluation of the taxonomic status of *I. xanthosticta* may be warranted.

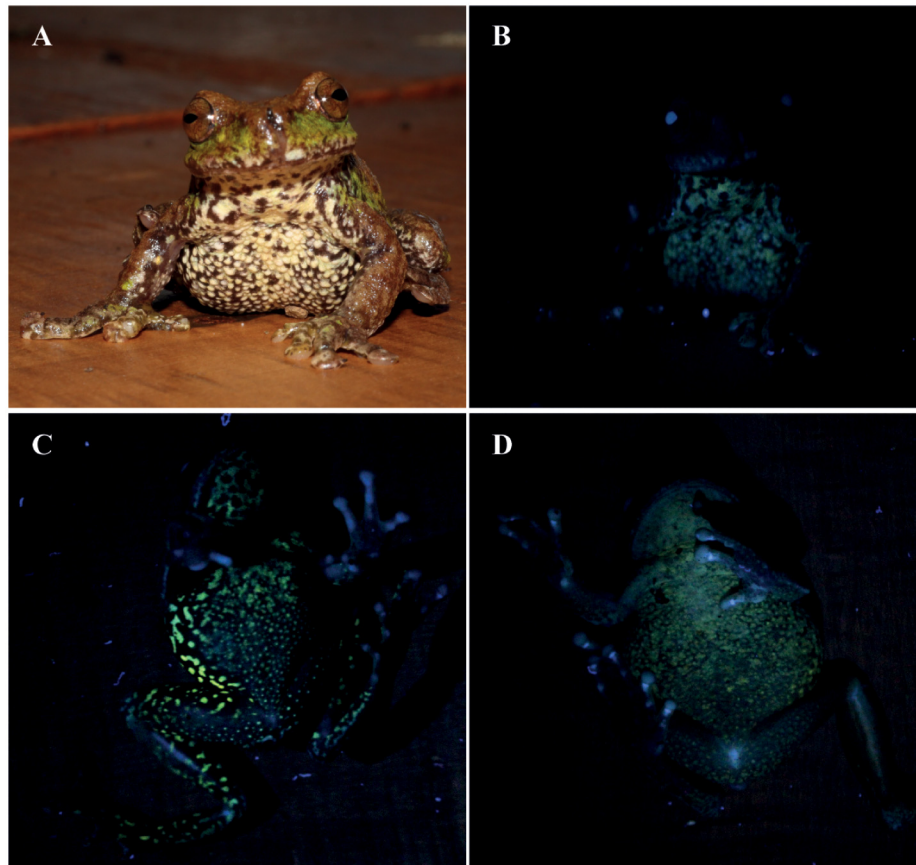


FIGURE 6. Fluorescence emission in *Isthmohyla pictipes* under ultraviolet-blue light exposure (365 nm) in the laboratory. (A–B): Female (UCR23364) shown under visible light and UV-Blue flashlight. (C): Female (UCR23365) showing bright yellow fluorescence of the spots that extend from below the forelimb to the base of the toes. (D): Fluorescent bones and tendons seen by transparency in sparsely pigmented areas of limbs and pelvic region of female (UCR23366).

Previous studies reported little variation in external morphology and size proportions between populations of *I. pictipes* (Duellman 1966; Starrett 1966; Duellman 1970). However, only a few specimens from the Cordillera de Talamanca have been included in those analyses (Starrett 1966; Savage & Heyer 1969; Duellman 2001). Our findings complement those of Starrett (1966), who included a male specimen (SVL 39 mm) collected at Cerro de la Muerte (Los Angeles County Museum, LACM 150197, formerly CRE 274), ~5.5 km W from Cuericí. In *I. pictipes*, the disc of the first inner finger is reduced in comparison to the discs of the other fingers (see Appendix 4). Also, the dorsum of *I. pictipes* from Cerro de la Muerte is tuberculate, a character state only mentioned by Savage & Heyer (1969), but not on subsequent characterizations of the species (Duellman 1970, 2001; Savage 2002). Savage & Heyer (1969) examined material from the Cordillera Central and the Cordillera de Talamanca and referred to the skin of the dorsum as variable from smooth to slightly tuberculate. We noted the presence of a small dermal flap on the heel (see Fig. 4B), sometimes crenulated and white in color (see Appendix 6).

The fact that no females from the Cordillera de Talamanca have been described before may explain the size differences reported here in comparison to smaller females from Río Poasito, south slope of Volcán Poás at the northwestern most distribution range of *I. pictipes* (Duellman 1966, 1970). Indeed, sample bias could account for the differences in SVL, but also for the variation in the tympanum/eye diameter ratio reported here (females = 0.3–0.4; male = 0.5). Duellman (1970) reported that this ratio is slightly larger in females than males; however, these proportions are 0.3–0.5 in both sexes. Similarly, Duellman (1970) indicated that the tympanum is “much smaller” in *I. pictipes* than in *I. tica*, but the tympanum size in the former may overlap with that of *I. tica* (reported

to be 0.5–0.6 in Duellman 1970). Thus, the tympanum/eye diameter ratio may not be a valid taxonomic character to distinguish between *I. pictipes* and *I. tica*. The presence of transverse bars on the legs and forearms, and a rounded snout in lateral profile in *I. tica* are other differences between these two hylids (Duellman 2001), aside from those already mentioned above. More thorough studies, including a reassessment of interspecific variation and molecular approaches are required to elucidate the taxonomy of *I. pictipes* and related congeners.

In the laboratory, we detected fluorescence emission in the ventral surfaces of both sexes of *I. pictipes* (Fig. 6). This phenomenon was recently reported in frogs by Taboada *et al.* (2017a; 2017b), describing it in lymph and dermal glands of *Boana punctata* and isolating the fluorescent compounds. Since this report, fluorescence has been reported in all extant orders of amphibians (Deschepper *et al.* 2018; Gray 2019; Lamb & Davis 2020), although evidently due to different fluorescent compounds. In *I. pictipes*, there is yellow fluorescence emitted by chromatophores in the gular and abdominal regions (Fig. 6A, B), and in the conspicuous bright spots that extend along the flanks and hind limbs (Fig. 6C); furthermore, the bones and possibly tendons fluoresce in the areas where skin has sparse pigmentation in ventral surfaces of hands, feet, limbs, and pelvic region (Fig. 6D).

Together with our findings, the recent records of *I. angustilineata* (Taylor, 1952) (Nishida 2006) and *I. rivularis* (Olsen and Cossel 2014; Jiménez *et al.* 2019), and the anecdotal accounts of *I. tica* (pers. obs) could indicate a trend towards reappearance in stream-breeding congeners that have remained undetected for several years or even decades in Costa Rica. It is noteworthy that many of these frogs have undergone severe population declines due to chytridiomycosis (Lips 1998; Lips *et al.* 2003; Stuart *et al.* 2008), but are now found again at several sites where they had disappeared. The finding of numerous adults and larvae of *I. pictipes* is a significant result for the conservation of *I. pictipes*. However, our findings were made in a relatively small geographic area (ca. 15 km²) and this species is still missing throughout much of its range in spite of searches. Given that *I. pictipes* is mostly threatened by habitat alteration (agriculture, trout farms), habitat fragmentation, and habitat loss (Bolaños *et al.* 2008), we consider Cuericí as an important biological reserve for the protection of this endangered species. We recorded most frogs at the headwaters of streams, and therefore this type of microhabitat should be considered for the conservation of *I. pictipes*. Basic aspects of the reproductive biology of this species have not been described yet, including amplexus and oviposition site. Long term monitoring would be instrumental to better understand the natural history, reproductive biology, and population health of *I. pictipes*.

Acknowledgements

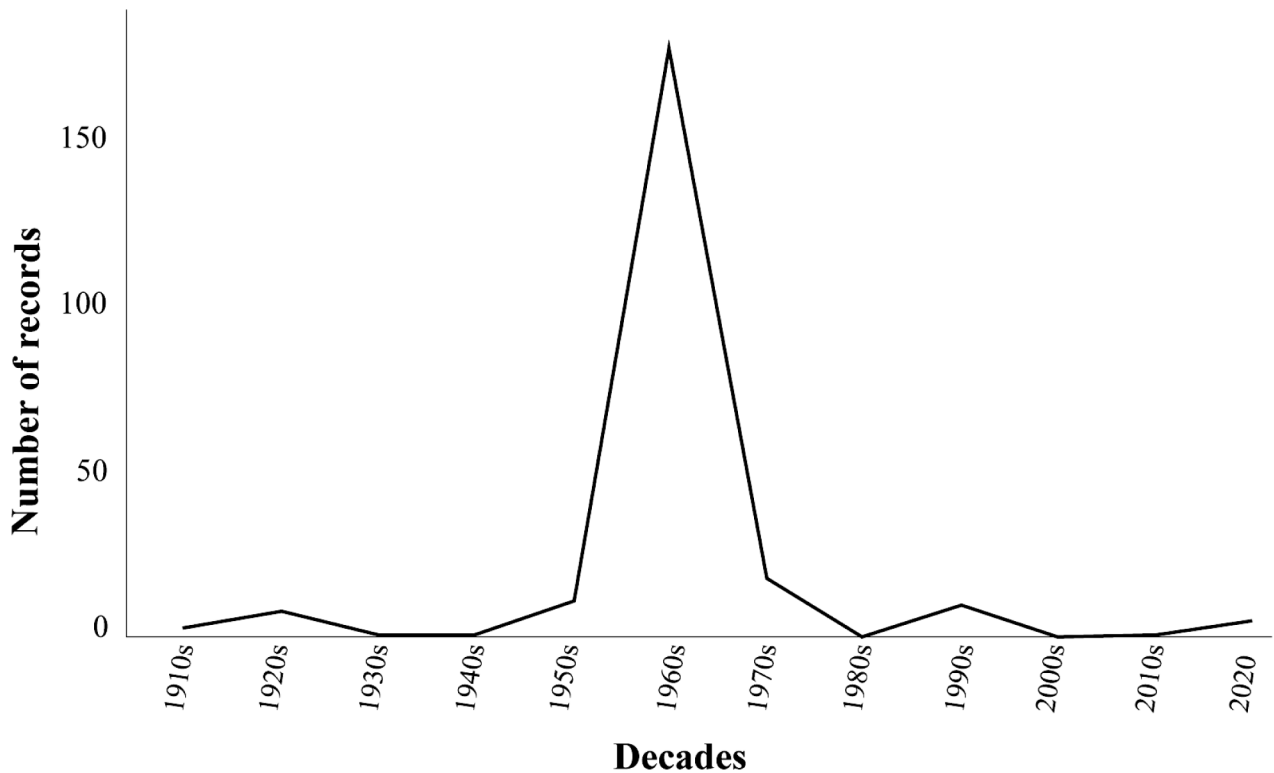
We would like to thank Carlos Solano, Ana Solano, “Beto” Mena, and Mayela Córdoba for their help and logistic support to conduct this research at Cuericí Biological Station. We thank Emilia Moreno, Roberto Ibañez, and Karen Lips for their helpful comments on previous versions of this work. Mike and Tim Mooring shared their findings of *I. pictipes* at San Gerardo de Dota; Neftali Camacho and Greg Pauly shared photographs on LACM/CRE records of *I. pictipes*. The Centro de Investigaciones Geofísicas of the UCR kindly provided environmental data. All specimens were collected under permit no. ACLAP-111-2019 in accordance with the regulations and protocols established by the Sistema Nacional de Areas de Conservación in Costa Rica. JF thanks ANPCyT (PICT 820-2015) and FAPESP (procs. 2013/50741-7, 2018/15425-0). FB and GC support on 1994 was from National Science Foundation (NSF) Grant DEB-9200081 to Jay M. Savage.

References

- Abarca, J. (2012) Cambios en la estructura de la comunidad de anuros (Amphibia: Anura) en el Cerro Chompipe, Costa Rica. *Cuadernos de Investigación UNED*, 20, 9–15.
- Abramoff, M. & Magalhaes, P. (2004) Image processing with imageJ. *Biophotonics International*, 11, 36–42.
- Acosta-Chaves, V., Chaves, G., Abarca, J., García-Rodríguez, A. & Bolaños, F. (2015) A checklist of the amphibians and reptiles of Río Macho Biological Station, Provincia de Cartago, Costa Rica. *Checklist*, 11, 1784. <https://doi.org/10.15560/11.6.1784>
- Altig, R. & McDiarmid, R.W. (1999) Body plan. Development and morphology. In: McDiarmid, R. & Altig, R. (Eds.), *Tadpoles. The Biology of Anuran Larvae*. The University of Chicago Press, Chicago, Illinois, pp. 24–51.
- Arias, E. & Chaves, G. (2014) 140 years after William M. Gabb’s climb to Cerro Pico Blanco. *Mesoamerican Herpetology*, 1, 176–180.

- Auth, D.L. (1994) Checklist and bibliography of the amphibians and reptiles of Panama. *Smithsonian Herpetological Information Service*, 98, 1–59.
<https://doi.org/10.5479/si.23317515.98.1>
- Bolaños, F. (2009) Situación de los anfibios de Costa Rica. *Biocenosis*, 22, 95–108.
- Bolaños, F., Chaves, G. & Savage, J. (2008) *Isthmohyla pictipes*, The IUCN Red List of Threatened Species.
<https://doi.org/10.2305/IUCN.UK.2008.RLTS.T55603A11335341.en>
- Campbell, J.A. (1999) Distribution patterns of amphibians in Middle America. In: Duellman, W.E. (Ed.), *Patterns of distribution of amphibians: A global perspective*. The Johns Hopkins University Press, Baltimore, Maryland, pp. 111–210.
- Cope, E.D. (1875 [“1876”]) On the Batrachia and Reptilia of Costa Rica. *Journal of the Academy of Natural Sciences of Philadelphia*, Series 2, 8, 93–154.
- Crawford, A., Lips, K. & Bermingham, E. (2010) Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *Proceedings of the National Academy of Sciences of the USA*, 107, 13777–13782.
<https://doi.org/10.1073/pnas.0914115107>
- Deschepper, P., Jonckheere, B. & Matthys, J. (2018) A light in the dark: the discovery of another fluorescent frog in the Costa Rican rainforests. *Wilderness and Environmental Medicine*, 29, 421–422.
<https://doi.org/10.1016/j.wem.2018.03.004>
- Duellman, W.E. (1966) Taxonomic notes on some Mexican and Central American hylid frogs. *University of Kansas Publications, Museum of Natural History*, 17, 263–279.
<https://doi.org/10.5962/bhl.part.7132>
- Duellman, W.E. (1968) Descriptions of new hylid frogs from Mexico and Central America. *University of Kansas Publications, Museum of Natural History*, 17, 559–578.
<https://doi.org/10.5962/bhl.part.7138>
- Duellman, W.E. (1970) The Hylid Frogs of Middle America. Monograph of the Museum of Natural History, University of Kansas, 1, 1–753.
- Duellman, W.E. (2001) *Hylid Frogs of Middle America. Vols. 1 & 2*. Society for the Study of Amphibians and Reptiles, Ithaca, New York, 1158 pp.
- Faivovich, J., Haddad, C.F.B., Garcia, P.C.A., Frost, D.R., Campbell, J.A. & Wheeler, W.C. (2005) Systematic review of the frog family Hylidae, with special reference to Hyliinae: phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History*, 294, 1–240.
[https://doi.org/10.1206/0003-0090\(2005\)294\[0001:SR0TFF\]2.0.CO;2](https://doi.org/10.1206/0003-0090(2005)294[0001:SR0TFF]2.0.CO;2)
- Faivovich, J., Pereyra, M.O., Luna, M.C., Hertz, A., Blotto, B.L., Velázquez, C.R., Almazán, J.R., McCranie, J.R., Sánchez, D.A., Baeta, D., Araujo-Vieira, K., Köhler, G., Kubicki, B., Campbell, J.A., Frost, D.R., Wheeler, W.C. & Haddad, C.F.B. (2018) On the monophyly and relationships of several genera of Hyliini (Anura: Hylidae: Hyliinae), with comments on recent taxonomy changes in hylids. *South American Journal of Herpetology*, 13, 1–32.
<https://doi.org/10.2994/SAJH-D-17-00115.1>
- García-Rodríguez, A., Chaves, G., Benavides-Varela, C. & Puschendorf, R. (2012) Where are the survivors? Tracking relictual populations of endangered frogs in Costa Rica. *Diversity and Distributions*, 2012, 204–212.
<https://doi.org/10.1111/j.1472-4642.2011.00862.x>
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Gray, R.J. (2019) Biofluorescent lateral patterning on the Mossy Bushfrog (*Philautus macroscelis*): the first report of biofluorescence in a rhacophorid frog. *Herpetology Notes*, 12, 363–364.
- Hertz, A., Lotzkat, S., Carriza, A., Ponce, M., Kohler, G. & Streit, B. (2012) Field notes on findings of threatened amphibian species in the central mountain range of western Panama. *Amphibian & Reptile Conservation*, 6, 9–30.
- IUCN (2020) The IUCN Red List of Threatened Species. Version 2019-3. Available from: <https://www.iucnredlist.org> (access on 19 October 2020)
- IUCN SSC Amphibian Specialist Group (2020) *Isthmohyla pictipes*, The IUCN Red List of Threatened Species. [in press]
- Jiménez, R.R., Ballesteros, E., Astorga, J.D., Rodríguez, E. & Alvarado, G. (2019) *Isthmohyla rivularis* (American Cinchona Plantation Treefrog). *Herpetological Review*, 50, 322.
- Lamb, J. & Davis, M.P. (2020) Salamanders and other amphibians are aglow with biofluorescence. *Scientific Reports*, 10, 2821.
<https://doi.org/10.1038/s41598-020-59528-9>
- Lips, K. (1998) Decline of a tropical montane amphibian fauna. *Conservation Biology*, 12, 106–117.
- Lips, K., Reeve, J.D. & Witters, L.R. (2003) Ecological traits predicting amphibian population declines in Central America. *Conservation Biology*, 17, 1078–1088.
<https://doi.org/10.1046/j.1523-1739.2003.01623.x>
- Lips, K., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P. & Collins, J.P. (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proceedings of the National Academy of Sciences*, 103, 3165–3170.
<https://doi.org/10.1073/pnas.0506889103>

- McCranie, J.R., Wilson, L.D. & Williams, K.L. (1993) New species of tree frog of the genus *Hyla* (Anura: Hylidae) from northern Honduras. *Copeia*, 1993, 1057–1062.
<https://doi.org/10.2307/1447084>
- Nishida, K. (2006) Encounter with *Hyla angustilineata* Taylor, 1952 (Anura: Hylidae) in cloud forest of Costa Rica. *Brenesia*, 66, 78–81.
- Olsen, A.C. & Cossel, J.O. (2014) Observations of a remnant population of the critically endangered frog *Isthmohyla rivularis* on the Monteverde Cloud Forest Preserve, Costa Rica. *Herpetological Review*, 45, 205–208.
- Salazar-Zúñiga, J.A., Chaves-Acuña, W., Chaves, G., Acuña, A., Abarca-Odio, J.I., Lobón-Rovira, J., Gómez-Méndez, E., Gutiérrez-Vannucchi, A.C. & Bolaños, F. (2019) The most frog-diverse place in Middle America, with notes on the conservation status of eight threatened species of amphibians. *Amphibian & Reptile Conservation*, 13, 304–322.
- Sasa, M., Chaves, G. & Porras, W. (2010) The Costa Rican herpetofauna: Conservation status and future perspectives. In: Wilson, L.D., Townsend, J.H. & Johnson, J.D. (Eds.), *Conservation of Mesoamerican amphibians and reptiles*. Eagle Mountain Publications, Eagle Mountain City, Utah, pp. 510–603.
- Savage, J.M. (2002) *The Amphibians and Reptiles of Costa Rica: A Herpetofauna between Two Continents, between Two Seas*. The University of Chicago Press, Chicago, xx + 934 pp.
- Savage, J.M. & Bolaños, F. (2009) A checklist of the amphibians and reptiles of Costa Rica: additions and nomenclatural revisions. *Zootaxa*, 2005, 1–23.
<https://doi.org/10.11646/zootaxa.2005.1.1>
- Savage, J.M. & Heyer, W.R. (1969) The tree-frogs (family Hylidae) of Costa Rica: diagnosis and distribution. *Revista de Biología Tropical*, 16, 1–127.
- Starrett, P.H. (1966) Rediscovery of *Hyla pictipes* Cope, with description of a new montane stream *Hyla* from Costa Rica. *Bulletin of the Southern California Academy of Sciences*, 65, 17–28.
- Stuart, S.N., Hoffmann, M., Chanson, J.S., Cox, N.A., Berridge, R.J., Ramani, P. & Young, B.E. (2008) *Threatened amphibians of the world*. Lynx Edicions, Barcelona and IUCN, Gland, 758 pp.
- Taboada, C., Brunetti, A.E., Pedron, F.N., Neto, F.C., Estrin, D.A., Bari, S.E., Chemes, L.B., Lopes, N.P., Lagorio, M.G. & Faivovich, J. (2017a) Naturally occurring fluorescence in frogs. *Proceedings of the National Academy of Sciences*, 114, 3672–3677.
<https://doi.org/10.1073/pnas.1701053114>
- Taboada, C., Brunetti, A.E., Alexandre, C., Lagorio, M.G. & Faivovich, J. (2017b) Fluorescent frogs: a herpetological perspective. *South American Journal of Herpetology*, 12, 1–13.
<https://doi.org/10.2994/SAJH-D-17-00029.1>
- Taylor, E.H. (1952) A review of the frogs and toads of Costa Rica. *University of Kansas Science Bulletin*, 35, 577–942.
<https://doi.org/10.5962/bhl.part.4328>
- VertNet (2020) Available from: <http://vertnet.org/about/classicnetworks.html> (access 27 July 2020)



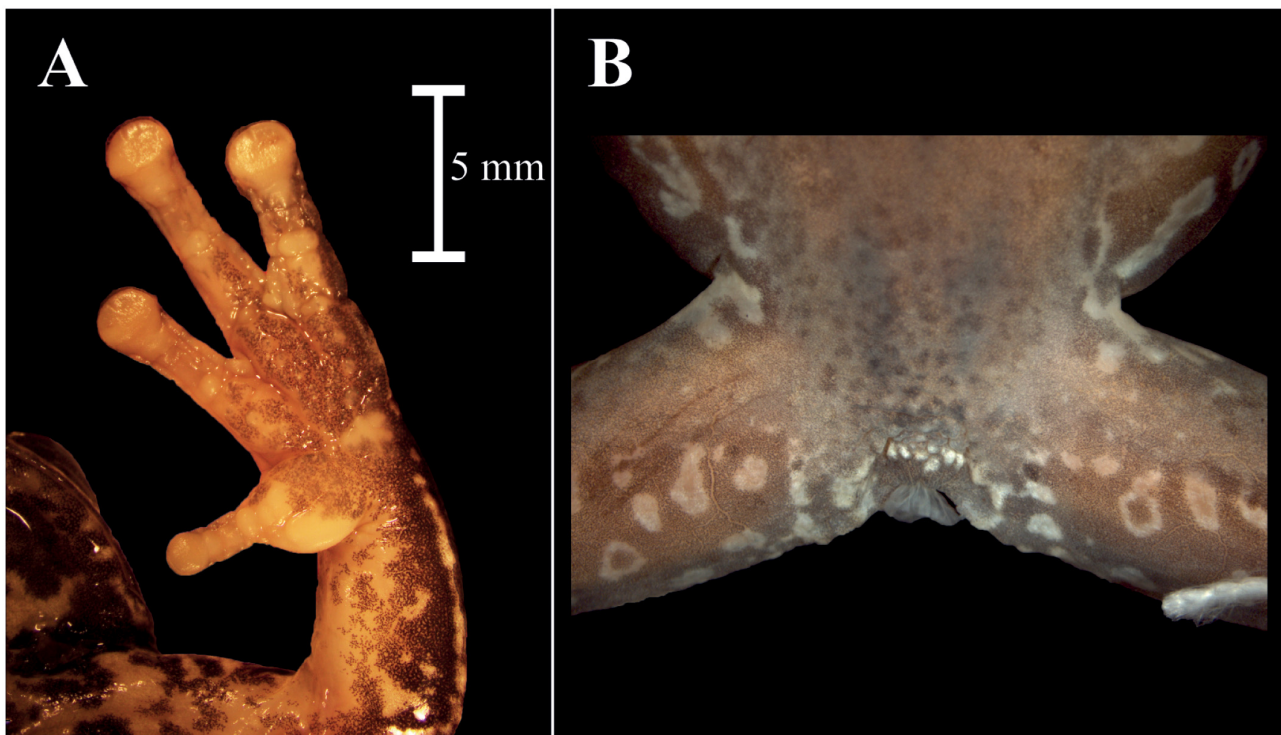
APPENDIX 1. Collection activity records of *Isthmohyla pictipes* since 1910 based on the database of the Zoology Museum of the Universidad de Costa Rica, the California Academy of Sciences, Kansas University, Los Angeles County Museum, Museum of Comparative Zoology, University of Michigan Museum of Zoology, and the National Museum of Natural History obtained from VertNet (2020).

APPENDIX 2. Number of males and females of *Isthmohyla pictipes* recorded during 1994 and 1995 at Cuericí Biological Station, Cerro de la Muerte, Cordillera de Talamanca, Costa Rica.

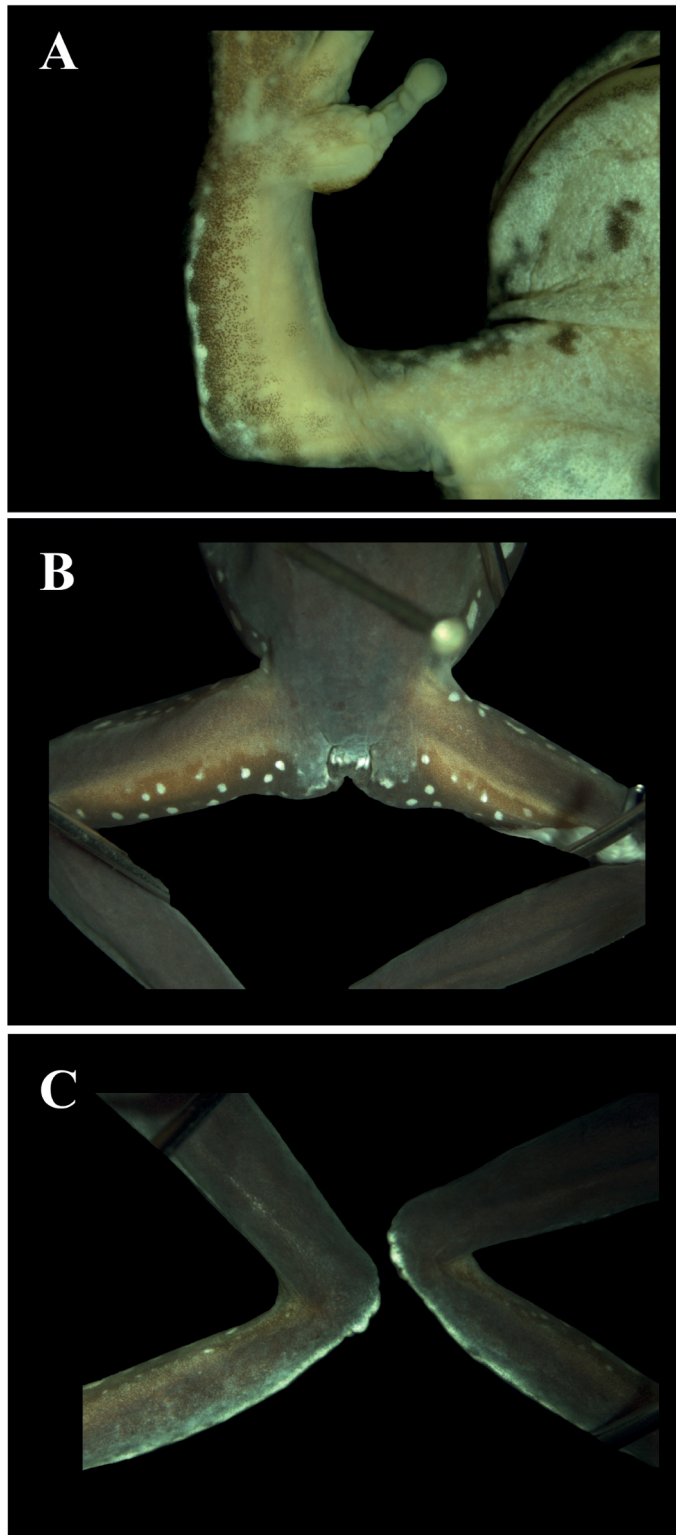
Date	Females	Males
19/03/94	2	4
10/04/94	-	3
08/05/94	1	2
12/06/94	-	5
12/07/94	1	2
07/08/94	-	2
09/10/94	-	2
07/05/95	1	-

APPENDIX 3. Measurements (mm) for five tadpoles of *Isthmohyla pictipes* (UCR 23382) in developmental stage 25 (Gosner 1960) collected from Cuericí. Values are presented as average \pm standard deviation (minimum–maximum).

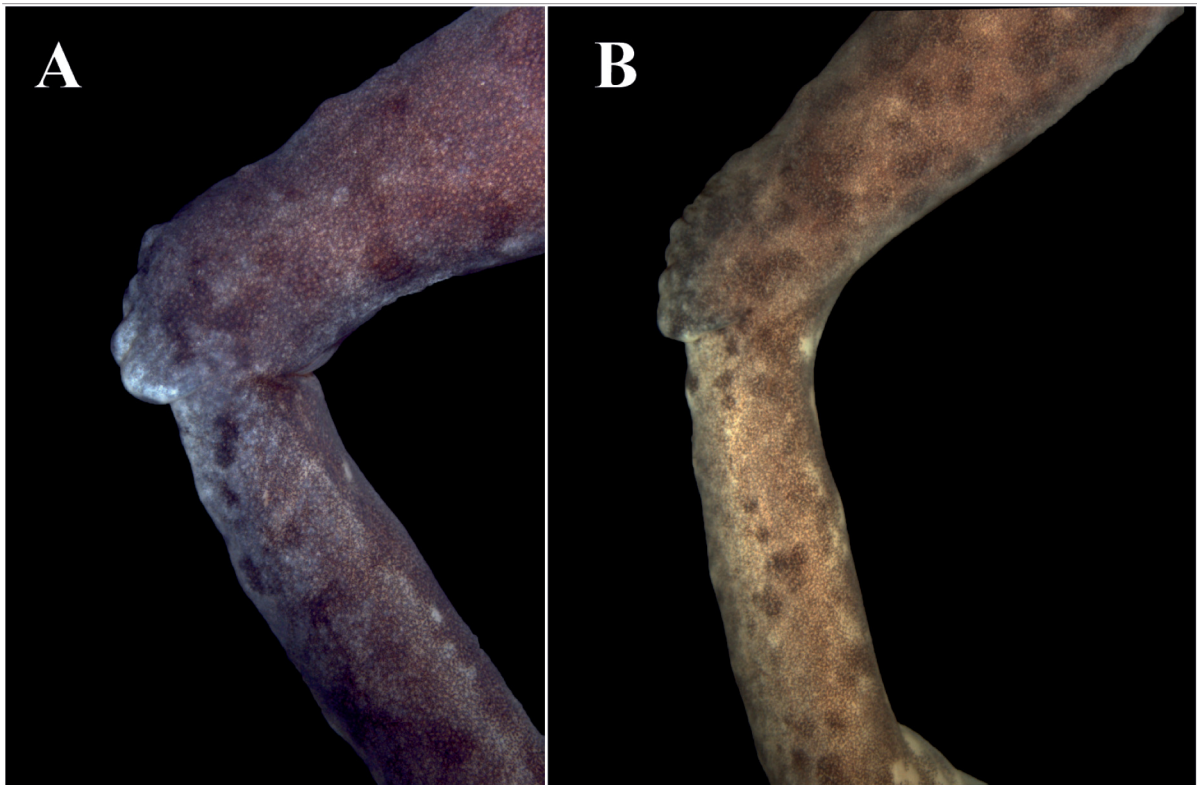
Character	Measurements (mm)
Total length	21.1 \pm 1.9 (19–23.4)
Body length	7.1 \pm 0.6 (6.3–7.7)
Tail length	14 \pm 1.4 (12.2–15.7)
Maximum body width	3.8 \pm 0.2 (3.5–4.1)
Maximum tail height	3.5 \pm 0.2 (3.2–3.8)
Tail muscle height	2.3 \pm 0.3 (2–2.7)
Tail muscle width	2.3 \pm 0.3 (2–2.7)
Dorsal fin height	1.1 \pm 0.3 (0.7–1.6)
Ventral fin height	1 \pm 0.3 (0.6–1.3)
Eye diameter	0.8 \pm 0.1 (0.7–0.9)
Interorbital distance	2.3 \pm 0.1 (2.2–2.4)
Eye-snout distance	2.5 \pm 0.2 (2.2–2.8)
Nostril-eye distance	0.5 \pm 0.04 (0.4–0.5)
Oral disc width	3.6 \pm 0.2 (3.2–3.9)



APPENDIX 4. Color variation in *Isthmohyla pictipes* from Cuericí. Presence of white stripes (A): on the forearm (UCR 23363) and (B): above the cloacal opening (UCR 11753).



APPENDIX 5. Adult male of *Isthmohyla pictipes* (UCR 1624) collected from Río Las Vueltas showing white stripes on (A): the forearm, (B): above the cloacal opening and (C): on the tarsus, extending onto the heel and feet; note the presence of calcar tubercles.



APPENDIX 6. Calcar tubercles on protuberant dermal flaps of the heel of *Isthmohyla pictipes* collected from Cu-
erici. (A): Colored white (UCR 11823) and (B); colored like the limbs (UCR 11753).