

NOTES ON GEOGRAPHIC DISTRIBUTION, ADVERTISEMENT CALL AND HABITAT OF THE STARRETT'S TREE FROG *ISTHMOHYLA TICA* (HYLIDAE) IN THE ZONA DE LOS SANTOS, COSTA RICA

NOTAS DE DISTRIBUCIÓN GEOGRÁFICA, LLAMADO DE ANUNCIO Y HÁBITAT DE LA RANA ARBÓREA DE STARRETT *ISTHMOHYLA TICA* (HYLIDAE) EN LA ZONA DE LOS SANTOS, COSTA RICA

ESTEBAN HIDALGO-MORA¹, JONATHAN NAVARRO-PICADO² & JUAN G. ABARCA^{3*}

¹Laboratorio de Ecología Funcional y Ecosistemas Tropicales, Escuela de Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica.

²Escuela de Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica.

³Laboratorio de Recursos Naturales y Vida Silvestre, Escuela de Ciencias Biológicas, Universidad Nacional, Heredia, Costa Rica.

*Correspondence: barcazajuan@gmail.com

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Resumen.— Las ranas centroamericanas del género *Isthmohyla* han sufrido un declive dramático en las tierras altas. Muchas especies de este género se consideran en peligro crítico, incluida *Isthmohyla tica*, una especie endémica de Costa Rica y Panamá. Encontramos una población de *I. tica* dentro de ecosistemas agrícolas cafetaleros en Tarrazú, San José, Costa Rica y describimos nuevos tipos de cantos en esta especie. El descubrimiento de esta población representa los registros más recientes en la Zona de Los Santos desde el último informe en 1963; hasta ahora, solo se conocían poblaciones estables en el Bosque Eterno de los Niños, Monteverde. La presencia de esta especie en peligro de extinción, junto con otros anfibios amenazados, refleja la importancia de comprender mejor su presencia en estos agroecosistemas y asegurar la conservación de parches boscosos dentro de los cafetales.

Palabras clave.— Agroecosistemas, anfibios, canto, conservación, *Isthmohyla*, redescubrimiento.

Abstract.— Central American hylids frogs of the genus *Isthmohyla* have suffered dramatic decline in highlands. Many species of this genus are considered critically endangered, including *Isthmohyla tica*, an endemic species of Costa Rica and Panama. We found a population of *I. tica* inside agricultural coffee ecosystems in Tarrazú, San José, Costa Rica and we describe new advertisement calls of this species. The discovery of this population represents the most recent records in the Zona de Los Santos since the last record in 1963; until now, only stable populations were known in the Children's Eternal Rainforest, Monteverde. The presence of this endangered species, among with other threatened amphibians, reflects the importance of better understanding their presence in these agro-ecosystems and ensuring the conservation of forest patches inside coffee plantations.

Keywords.— Advertisement call, agro-ecosystems, amphibians, conservation, *Isthmohyla*, rediscovery.

In the family Hylidae, the genus *Isthmohyla* comprises 14 species endemic to rainforests of Central America (Chaves-Acuña et al., 2020). *Isthmohyla tica* was described as *Hyla tica* by Andrew and Priscilla Starred and Thomas Uzzell in 1966 in a stream near Turrialba Volcano, Cartago, Costa Rica (Duellman, 1970). In general, *I. tica* prefers cloud forest habitats and is common to find them on vegetation at the edges of the streams. Also, males can be found calling from rocks, bushes and trees near the stream (Savage, 2002). The species have been found at elevations from 836 to 1920 meters in Central and Talamanca Cordilleras in

Costa Rica and Western Panamá (Duellman, 1970). Last time the species was officially reported in the Zona de Los Santos region it was found at Tarrazú river 1 km South of San Cristobal Sur in 1963 (Duellman, 1970; GBIF, 2021).

Isthmohyla tica the same as the whole group of hylids present in highlands has been affected by many environmental changes and anthropogenic pressures that cause dramatic decline (Lips et al., 2003). At the beginning of the 80's, herpetologists started to recognize an amphibian decline attributed to habitat

destruction and pesticide pollution among the principal causes, but between 1987 and 1988 some species showed a decline even in protected areas around the world (Savage, 2002; Whitfield et al., 2007). Costa Rica was not the exception of this pattern of decline (Savage, 2002). This situation is reflected in the unstable populations of most of the highlands hylid frogs (García-Rodríguez et al., 2012) and the fact that the Starrett's Treefrog has critically endangered (CR) status according to the IUCN red list (IUCN, 2020).

In recent years, some populations of these threatened species, specifically in the genus *Isthmohyla*, have been reported in some locations (Nishida, 2006; Olsen & Cossel, 2014; Jiménez et al., 2019; Chaves-Acuña et al., 2020). Reports of any threatened species are very important to ensure their conservation. We report recent observations of habitat, advertisement call variation and distribution of *I. tica* in the Zona de Los Santos, Costa Rica.

Records

During a monitoring of amphibians and reptiles diversity made on 8 September 2021 at the agricultural ecosystems of Tarrazú, San José, Costa Rica (SINAC-ACC-PI-re-018-2021), we found a gravid adult female of *I. tica* at 19:25 h. The individual was detected on a plant of the cucurbit family at 50 cm above the ground next to a stream in a coffee plantation in San Lorenzo district (9.62° N, 84.04° W; elev. 1,380 m a.s.l.), specifically in a town called Santa Marta. The frog was still and perched on the leaf. We took several photographs where some characteristics such as color of the thighs, size of the tympanum, dorsal coloration and presence of eggs were made evident (Fig. 1A). Climatic conditions during our sampling were rainy (large drops but not intensely). During the afternoon of that same day and prior to the sampling, it had rained heavily.

We had another sighting a day later at another site located 3 km northeast of the place of the first observation. The place is known as La Libia (9.63° N, 84.02° W; elev. 1,501 m a.s.l.) belonging to the district of San Lorenzo which in turn belongs to the canton of Tarrazú as mentioned above. At 18:15 h on 9 September 2021, we were monitoring another little creek adjacent to a coffee plantation (SINAC-ACC-PI-re-060-2021). We found another gravid adult female of *I. tica* on a naturalized exotic grass called *Coix lacryma-jobi*, popularly known in Costa Rica as “lágrimas de San Pedro”. The plant was located right next to the streamlet. The frog was one meter above the ground. Evidence through photography was taken in order to register some characteristics such as color of the thighs, web foot, tympanum size, dorsal coloration and presence of eggs. The presence of eggs in this

individual was not so noticeable (Fig. 1B). Weather conditions while searching for frogs and hours before were very similar to those present the previous day.

Additionally, we obtained three more records in La Libia on September 18 of the same year. On this occasion, we sampled from 18:00 h to 20:00 h. We spotted three males by their calls; once we managed to direct our attention in the direction from which the call was coming, we looked harder in the plants and in the soil. The first individual was found calling on the vegetation, about 15 cm above the stream (Fig. 1C). The second was on vegetation about 50 cm above the ground. The third one was seen calling on some semi-dry branches that lay in the middle of the creek. The branches were just above a fast-flowing current and the individual was one meter above the ground. The first individual found was captured and taken to the place where we stayed in order to take pictures and some measurements.

All individuals were identified as *Isthmohyla tica* based on dorsal pattern coloration of mottled green and brown dorsally with distinct brown transverse bands on the limbs; rounded snout profile, moderate sized tympanum (height one-half diameter of eye); posterior and inferior surfaces of thighs orange brown; and venter dull white without black spots (Duellman, 1970; Savage, 2002; Leenders, 2016; Cossel & Kubicki, 2019). Also, the identity of the species was confirmed by Mark Wainwright. Contrary to what is reported in the literature, we found a lot of variation in skin texture, the first impressions of males did not seem to have tuberculated skin, however a change in skin texture towards a more tuberculated pattern was also observed, in accordance with what was indicated in the literature (Duellman, 1970; Savage, 2002; Leenders, 2016; Cossel & Kubicki, 2019) (Fig. 1D). We think that like other species, amphibians may have slight variations in skin texture and may not always be a diagnostic aspect for identification in the field (Guayasamin et al., 2015).

Before observing the males, we reproduce calls of *Isthmohyla tica* and *I. rivularis* included in the Cossel & Kubicki (2019) guide. Surprisingly, the males of *I. tica* present in La Libia responded only to the song of *I. rivularis*. We also proceeded to record the song of *I. tica* once the males gave their response.

Call analysis

Advertisement calls of three uncollected males of the *Isthmohyla tica* from La Libia were recorded in the sampling site during September, 2021. Calls were recorded using a digital camera Canon PowerShot SX740 HS. The digital recorders were positioned 30 cm to 50 cm from each male. Recordings were converted to WAV format for the analysis and are housed in

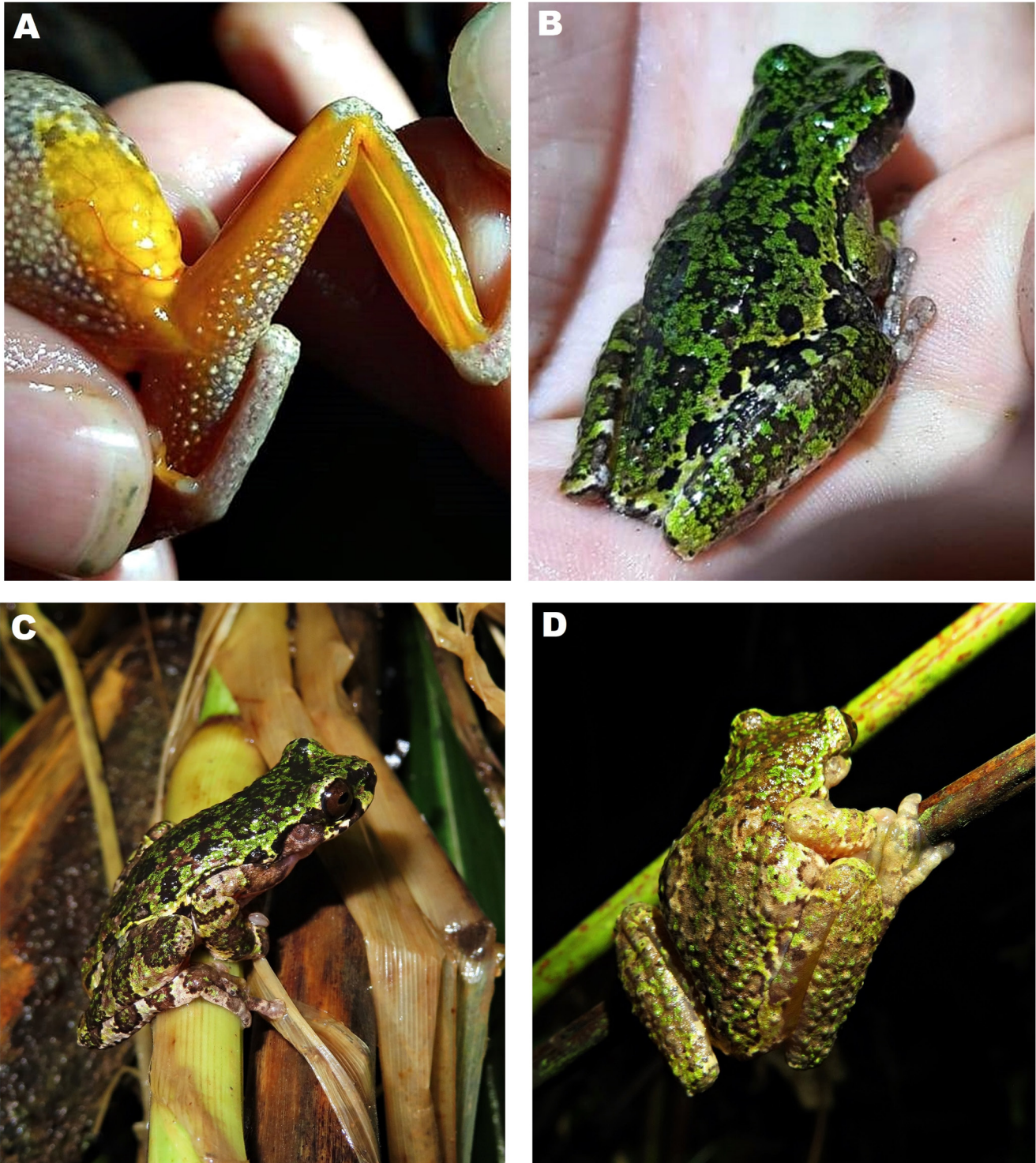


Figura 1. A) Hembra adulta de *Isthmohyla tica* con presencia de huevos de Santa Marta. B) Hembra adulta de *I. tica* de La Libia. C) Macho adulto de *I. tica* sobre la vegetación en el riachuelo de La Libia. La textura de la piel era ligeramente tuberculada. D) Macho adulto de *I. tica* sobre la vegetación junto al arroyo de La Libia. Nótese lo tuberculado en la parte posterior de este espécimen.

Figure 1. A) Adult female of *Isthmohyla tica* with presence of eggs from Santa Marta. B) Adult female of *I. tica* from La Libia. C) Adult male of *I. tica* on the vegetation above the small stream from La Libia. The skin texture was slightly tuberculata. D) Adult male of *I. tica* on the vegetation next to the stream from La Libia. Note the tuberculata on the back in this specimen.

the bioacoustic repository of FonoZoo (FZ-SOUND-CODE: 12988-12989). Advertisement call parameters were measured in RAVEN 1.4 (Bioacoustics Research Program, 2015). Raven parameters were set as follows: Hann window type, window size of 1024 samples, 3 dB filter bandwidth of 61.9 Hz, time grid 90% overlap, time grid size of 102 samples, 43.1 Hz frequency grid spacing. To avoid the overlap with background noise of the river and for the graphic representation of the advertisement call we delete frequency below fundamental frequency using the Filter out active selection tool in RAVEN 1.4. The following temporal and spectral parameters were inferred: number of notes, fundamental and dominant frequency (measured through the function Peak Frequency), call duration, note duration, note period, inter-note interval and pulses per note. Terminology of call measurements follows Kok & Kalamandeen (2008).

The first call recorded from *Isthmohyla tica* from La Libia (Call 1) is composed by a series of “chips” notes similar to a cricket (Fig. 2, Table 1). A call group consists of 9 to 224 notes ($n = 8$) of variable duration: 2 to 51 seconds (s) in the records, and is repeated at intervals of about 10 to 60 s ($n = 8$). Each note of amplitude-unmodulated contains a single pulse, has duration of 0.033 to 0.060 s ($n = 40$, average = 0.045 s), and with a note period of 0.218 to 0.378 s ($n = 40$, average = 0.239) and inter-note intervals of 0.176 to 0.331 s. ($n = 40$, average = 0.194); average note repetition rate is 4.1 notes/s. This call has a dominant frequency of 4.6-4.8 kHz ($n = 8$, average = 4.7). No harmonics were detected. A video of this recording call can be seen at: <https://youtu.be/HuGCqKEyNtA>. Calls were heard throughout the night from 17:00 to 03:00 h.

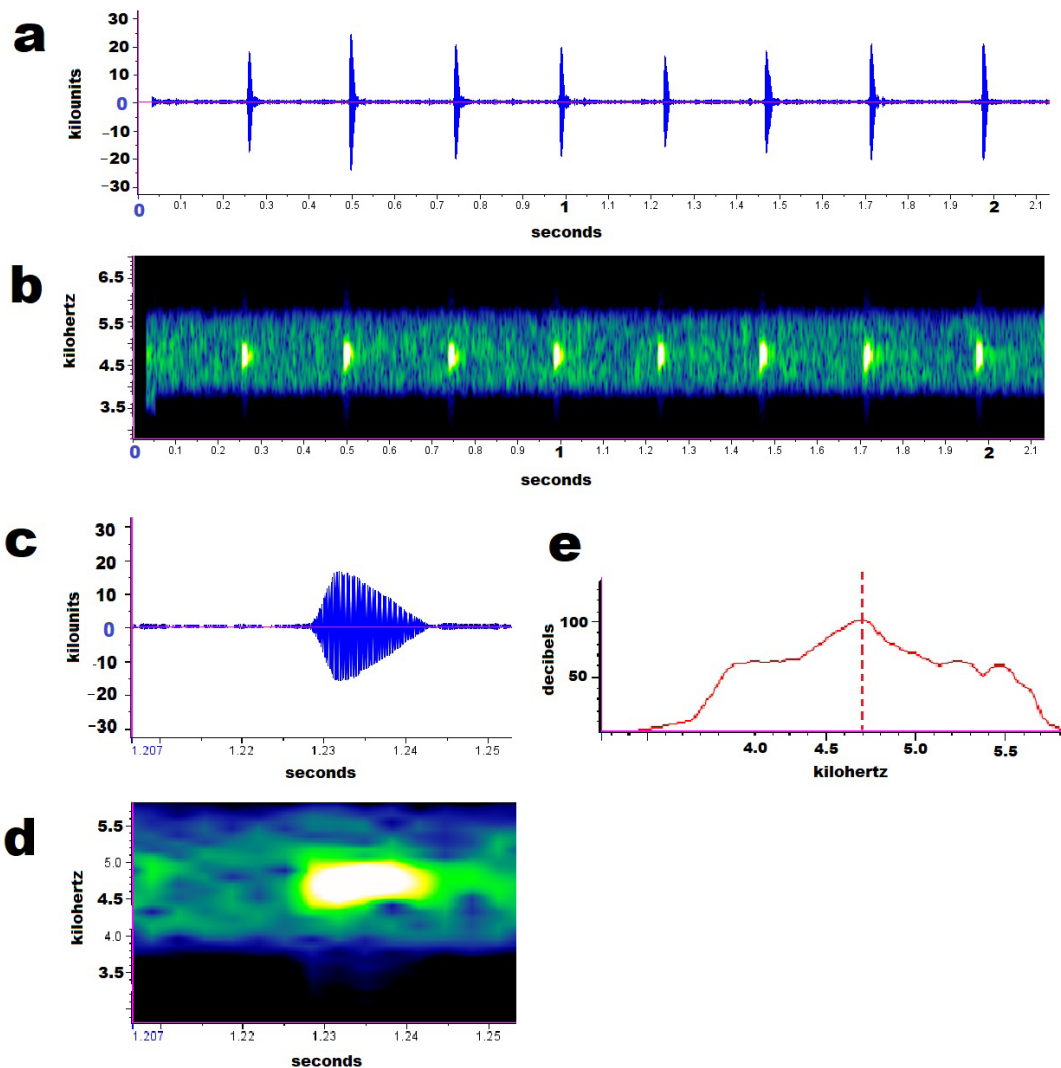


Figura 2. *Isthmohyla tica* Canto 1. De La Libia: (a-b) oscilograma y espectrograma con 8 notas (c-d) oscilograma y espectrograma de una sola nota (e) espectro de potencia de 8 notas. La línea roja punteada indica la frecuencia dominante.

Figure 2. *Isthmohyla tica* Call 1. From La Libia: (a-b) oscillogram and spectrogram with 8 notes (c-d) oscillogram and spectrogram of a single note (e) power spectrum of 8 notes. Dotted red line indicates the dominant frequency.

Tabla 1. Características de los cantos de *I. tica* e *I. rivularis*, con los promedios en paréntesis. *Obtenido de Duellman, 1970.

Table 1. Characteristics of calls of *I. tica* and *I. rivularis*, with means in parentheses. *From Duellman, 1970.

| Specie | n | Call type | Notes per call group | Duration call group (s) | Pulses per call | Dominant frequency (kHz) | Emphasized harmonics (kHz) |
|-------------------------------|---|-----------|----------------------|-------------------------|-----------------|--------------------------|----------------------------|
| <i>Isthmohyla tica</i> | 8 | 1 | 9.0 - 224 (82.00) | 2.2 - 51 (19.19) | 1 | 4.688 - 4.867 (4764) | no |
| <i>Isthmohyla tica</i> | 2 | 2 | 20-23 (21.5) | 0.013 - 0.020 | 1 | 1.839 - 1.862 (1850) | 3.723 - 3.740 (3731) |
| <i>Isthmohyla tica</i> * | 5 | 3 | 3 - 5 | 0.38 - 0.60 | 2 - 4 | 1.980 - 2.300 | 4.420 - 4.840 |
| <i>Isthmohyla rivularis</i> * | 7 | - | 12-137 | 5 - 66 | 3 | 2.160 - 2.880 | no |

A second call (Call 2) taken from La Libia is a distress call composed by a one note “chip”, emitted at irregular intervals. Each note of amplitud-unmodulated contains a single pulse, and has duration of 0.016 to 0.037 s (n = 9, average = 0.022). The dominant frequency is 1.839 – 1.862 kHz (n = 2, average: 1850 kHz) with one harmonic in 3.723 – 3.740 KHz (n = 2, average = 3.731 kHz) (Fig. 3). This call was observed when the individual was held by the researcher and when it was temporarily collected for taking pictures.

There is a third call for *I. tica* (Call 3), that was historically reported as a series of three to five crickets like “chirps”, with

intervals of 1.5 s. whit 2 to 4 pulses, with two emphasized frequencies at about 2.2 and 4.7 kHz (Duellman, 1970; Savage, 2002). We never heard this call or similar in the La Libia population. Calls from Monteverde reported by Cossel & Kubicki (2019) and provided by Mark Wainwright correspond to what was previously reported by Duellman (1970) and Savage (2002) for *I. tica* (Table 1).

The *I. tica* Call 1 heard in La Libia is more similar to the call reported for *I. rivularis*. Therefore, we also compared the call of *I. rivularis* published on Amphibia-web (2021), with *I. tica* Call 1; in table 1 and figure 4 the most evident differences are observed.

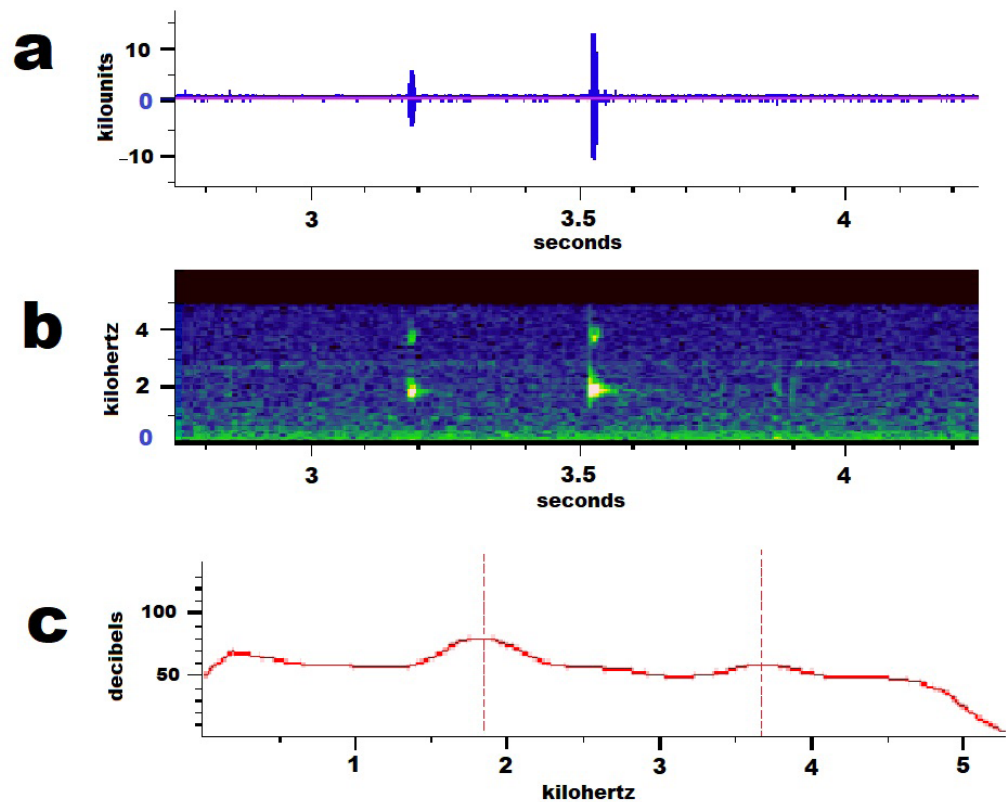


Figura 3. *Isthmohyla tica* Canto 2. De La Libia: (a) oscilograma (b) espectrograma y (c) espectro de potencia de 2 notas no pulsadas. La línea roja punteada indica la frecuencia dominante y el armónico.

Figure 3. *Isthmohyla tica* Call 2. From La Libia: (a) oscillogram (b) spectrogram and (c) power spectrum of 2 unpulsed notes. Dotted red line indicates the dominant frequency and harmonic.



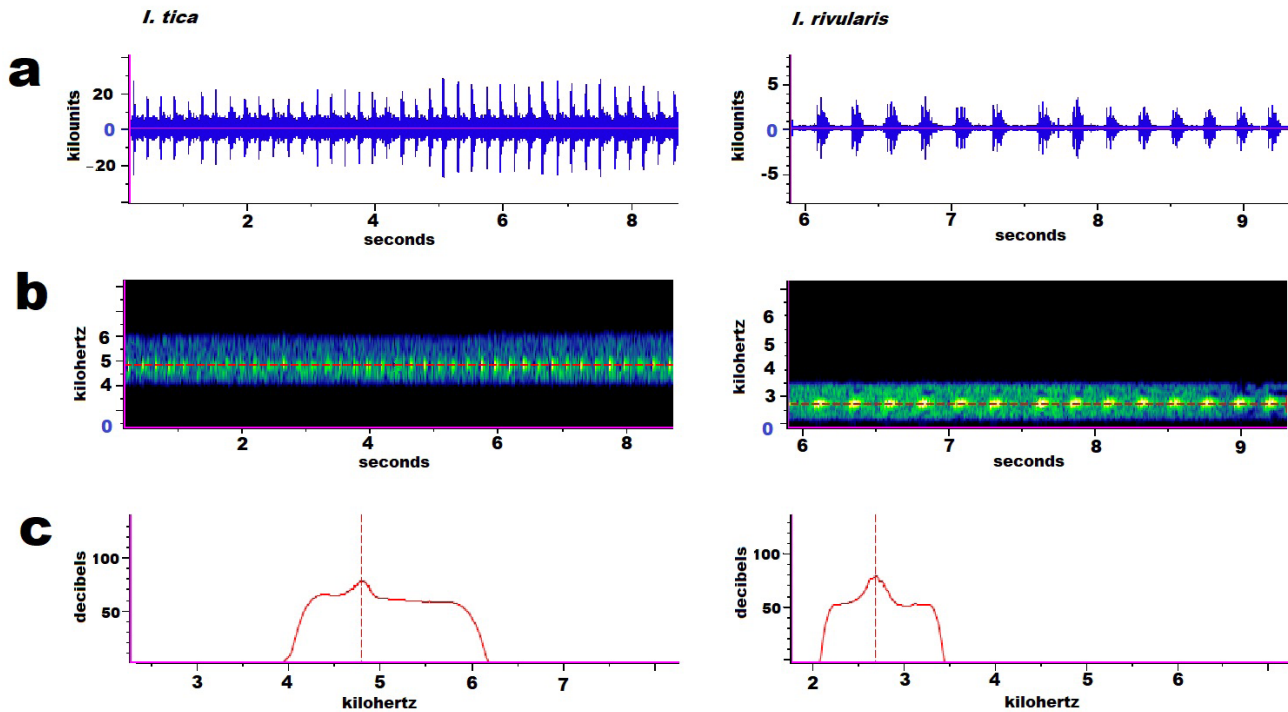


Figura 4. Comparación del Canto 1 de *Isthmohyla tica* de La Libia (panel izquierdo) e *I. rivularis* (panel derecho). Oscilograma, espectrograma y espectro de potencia de varias notas de llamada. La línea roja punteada indica la frecuencia dominante. Nótese la frecuencia dominante más baja y las notas pulsadas en *I. rivularis*.

Figure 4. Comparison of *Isthmohyla tica* Call 1 from La Libia (left panels) and *I. rivularis* (right panels). Oscillogram, spectrogram and power spectrum of several call notes. Dotted red line indicates the dominant frequency. Note lower dominant frequency and pulsed notes in *I. rivularis*.

Although both calls are a series of continuous “chirps” and sustained for several seconds, even minutes, the call of *I. rivularis* has a lower dominant frequency (1.98 to 2.30 kHz, Duellman, 1970) and each note of its call has three clear pulses with duration from 0.080 to 0.144 s. Although the note period is similar in both calls, both the length of the note and the interval between notes are different in the two calls. The lower dominant frequency and the presence of three pulses in the note differentiate the song of *I. rivularis* from the *I. tica* Call 1 described in this report.

Taking into account that in other species there may be considerable variation in call types, including variations in *I. rivularis* (Mark Wainwright *com. pers.*), it was expected that *I. tica* also had variations in call repertoire. It is not clear why they exhibit these variations, or when they use one or another type of calls; more research is needed to clarify this aspect. In terms of conservation, it is important to recognize such variations in calls in the field, since threatened populations may pass undetected, which would otherwise serve to justify the application of conservation strategies in these areas.

Distribution and presence in coffee plantations

Isthmohyla tica has been observed in the Premontane Wet Forest and Lower Montane forest, and marginally to Rainforest from 836 to 1920 m a.s.l. (Duellman, 1970; Savage, 2002). The species has been reported in different locations in the Tilarán, Central and Talamanca mountain ranges in Costa Rica, and in Chiriquí western of Panama (Savage, 2002). This species was widely distributed in the mountainous regions of the country, and was even considered relatively common in known sites (Savage, 2002). But at some point it was no longer observed and it is believed that it suffered a dramatic population decline, being the year 1996 the last observation of this species in the country (García-Rodríguez et al., 2012). It was even considered as possibly extinct in 2007 (Leenders, 2016). In 2010, several reports occurred in Panama (Hertz et al., 2012) and Monteverde (Leenders, 2016). In 2010 and 2012 *I. tica* was recorded in Children's Eternal RainForest (IUCN, 2020). Another single individual was spotted in Tapanti National Park in 2012 (Abarca, 2016). Until now, only stable populations were known in the sector known as Chutas of the Children's Eternal Rainforest (Luis Solano *com. pers.*)

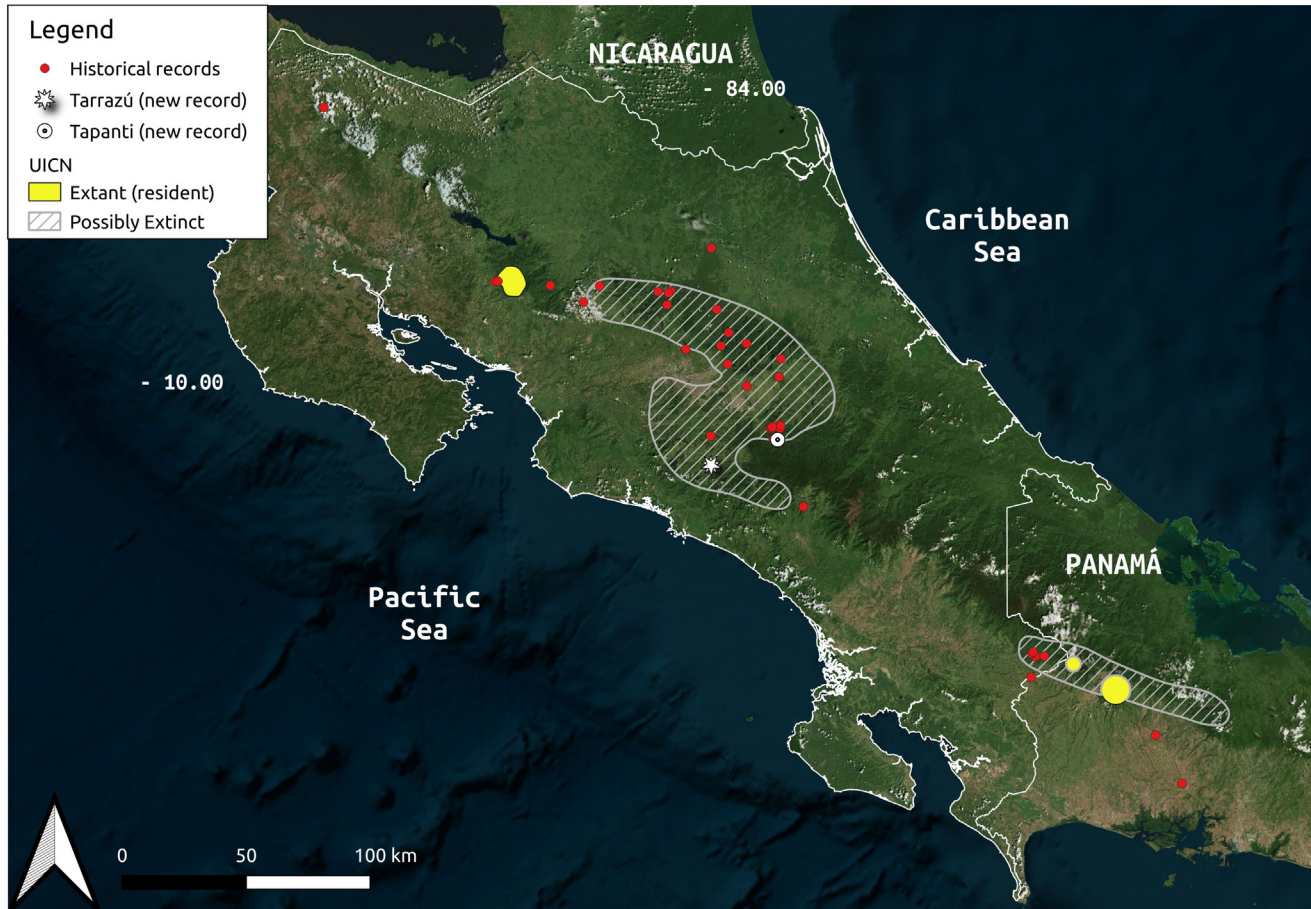


Figura 5. Mapa de distribución de *Isthmohyla tica* según registros de IUCN (2020) y Gbif (2021), incluidos los registros de Tapanti y la Zona de los Santos.

Figure 5. Distribution map of *Isthmohyla tica* according to IUCN (2020) and Gbif records (2021), including the records of Tapanti and the Zona de los Santos.

In the region known as the Zona de Los Santos, the last reports of the species were in 1963 (GBIF, 2021). At that time the area suffered a considerable change in ecosystems, because from 1950, through technological packages, the cultivation of coffee expanded in the Zona de Los Santos, causing that between 1960 and 2000 the cantonal production of coffee increased up to ten times (Picado et al., 2009). The populations that we find in La Libia and Santa Marta are within the areas that the IUCN (2020) considered as possibly extinct (Fig. 5). Currently, the predominant landscape in this area are coffee plantations, which is typical of the Zona de Los Santos, however in many plantations there is a mixture of remnants of very small forests, sometimes a few meters wide on each side of the rivers. Individuals of *I. tica* observed in La Libia and Santa Marta were in a small vegetation of secondary growths and pastures, always very close to the coffee plants (Fig. 6). The presence of this endangered species is added to that of other equally endangered species, which have been observed in coffee plantations, such

as *Duellmanohyla legleri* (Abarca et al., 2021) and *Agalychnis annae* (Hidalgo-Mora et al., 2021) in these areas of Los Santos. The presence of these species reflects the importance of better understanding their distribution and presence in the coffee plantation agroecosystems, and ensuring the conservation of those forest patches on the banks of the rivers.

Usually, *I. tica* has been reported in primary or little altered forests (Leenders, 2016; Cossel & Kubicki, 2019); however, we were able to verify its presence in relatively altered perturbed environments, in places where agricultural treatments are carried out, such as the use of fungicides, in addition to the nearby presence of human settlements. While we recognize the importance of conserving forested areas within protected areas, there are environments that have not been considered as important sites for the conservation of threatened species. In general, these environments have been undervalued due to their anthropogenic use. However, the tolerance of many species of



Figura 6. Paisaje del agroecosistema de los cafetales en La Libia (izquierda) y Santa Marta (derecha) donde se avistó *I. tica*.

Figure 6. Landscape of the agro-ecosystem of the coffee plantations in La Libia (left) and Santa Marta (right) where *I. tica* was spotted.

amphibians to anthropic alteration is known (Hidalgo-Mora et al., 2021).

While some agricultural practices can affect amphibians, others can be tolerated, so it is necessary to recognize which of these practices can be less harmful to threatened species. In addition, the presence of threatened species in agro-ecosystems can represent not only an important reason for research, but it can also generate some motivation on the part of the owners and people of the community to protect them. Many times the presence of threatened species can be used in favor of producers to give added value to their products, this is how the presence of threatened frogs in coffee plantation ecosystems could help improve the perception of these animals by coffee producers, and at the same time improve the income of the producer by offering an environmentally friendly product while protecting endangered species.

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