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Population observations and microhabitat use of *Isthmohyla tica* (Anura: Hylidae) in San Ramón, Costa Rica

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Abstract

Population observations and microhabitat use of *Isthmohyla tica* (Anura: Hylidae) in San Ramón, Costa Rica. *Isthmohyla tica,* the Tica Frog, is a critically endangered species endemic to Costa Rica and supposedly Panama that has only been seen in a few sites in recent decades. To aid in its conservation, we assessed the population status and microhabitat preferences of this frog in a section of the Danta stream at Rancho Alegre farm, San Ramón, Costa Rica. We conducted 18 nocturnal surveys and collected data on perch types, vegetation associations, stream characteristics, and environmental variables for each sighting of the Tica Frog. To identify potential microhabitat preferences, we compared these features between sites with or without frogs as well as between sites with juvenile or adult individuals. We recorded 13 individuals, comprising three juveniles and 10 adults. Nine adults were male and one female, with the disparity likely due to the conspicuous vocalizations of males. Juvenile and adult frogs occupied areas with similar stream widths (t = 0.91; p = 0.38). However, juveniles were found closer to the water (t = 5.14, p = 0.01) and adjacent to shallower areas of the stream compared to adults (t = 2.50, p = 0.03). Frogs predominantly perched on *Piper* sp. (30.7%) and on the upper surface of leaves (69.2%). The height at which the Tica Frog was recorded (0.1 to 2.2 m) was similar for both age groups (t = 0.86, p = 0.44). We did not find significant differences in canopy cover (average = 74.6%), diameter at breast height (≤ 3.14 cm), vegetation density (5.0 plants/m²), or plant richness (1–4 species) between juveniles and adults. Similarly, we did not observe differences in environmental variables between these groups. Furthermore, vegetation characteristics and environmental variables did not differ between sites with the

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Tica Frog and nearby sites without them. Our findings suggest that the measured variables do not significantly influence the microhabitat selection of the Tica Frog. This novel information on the population status and microhabitat use of *I. tica* is needed for the development of management strategies for the conservation of this critically endangered species.

Keywords: Amphibians, Conservation, Critically endangered, Environmental variables, Microhabitats, Population, Vegetation characteristics.

Resumen

Observaciones poblacionales y uso del microhábitat de *Isthmohyla tica* (Anura: Hylidae) en San Ramón, Costa Rica. *Isthmohyla tica,* la rana Tica, es una especie en peligro crítico de extinción, endémica de Costa Rica y supuestamente de Panamá que solo ha sido avistada en unos pocos sitios en las últimas décadas. Para contribuir a su conservación, evaluamos el estado de la población y las preferencias de microhábitat de esta rana en un tramo de la quebrada Danta en Rancho Alegre, San Ramón, Costa Rica. Realizamos 18 censos nocturnos y recolectamos datos sobre tipos de perchas, asociaciones vegetales, características de la quebrada y variables ambientales para cada avistamiento de esta rana. Comparamos estas características entre individuos juveniles y adultos, así como entre sitios con y sin la presencia de la rana, con el fin de identificar posibles preferencias de microhábitat. Registramos 13 individuos, tres juveniles y 10 adultos, nueve machos y una hembra. Esta disparidad posiblemente se deba a que los machos son más fáciles de detectar debido a sus vocalizaciones. Tanto juveniles como adultos ocuparon áreas con similares anchos de la quebrada (t = 0.91; p = 0.38). Sin embargo, los juveniles se encontraron más cerca del agua (t = 5.14, p = 0.01) y en zonas laterales más superficiales de la quebrada (t = 2.50, p = 0.03). Las ranas se posaron predominantemente en *Piper* sp. (30.7%) y en la superficie superior de las hojas (69.2%). La altura a la que se registró la rana Tica (0.1 a 2.2 m) fue similar para ambos grupos (t = 0.86, p = 0.44). No encontramos diferencias significativas en la cobertura de dosel (promedio = 74.60%), diámetro a la altura del pecho (≤ 3.14 cm), densidad de vegetación (5.0 plantas/m²) o riqueza de especies entre juveniles y adultos. Del mismo modo, no observamos diferencias en las variables ambientales entre estos grupos. Además, no encontramos diferencias en las características de la vegetación o en las variables ambientales entre los sitios con y sin ranas. Nuestros hallazgos sugieren que las variables medidas no influyen significativamente en la selección de microhábitat de la rana Tica. Esta información es crucial para desarrollar estrategias de manejo y conservación para esta especie críticamente amenazada.

Palabras clave: Anfibios, Características de la vegetación, Conservación, Microhábitats, Peligro crítico, Población, Variables ambientales.

Resumo

Observações populacionais e uso de micro-hábitats de *Isthmohyla tica* (Anura: Hylidae) em San Ramón, Costa Rica. *Isthmohyla tica* é uma espécie criticamente ameaçada de extinção, endêmica da Costa Rica e, supostamente, do Panamá, que só foi vista em algumas localidades nas últimas décadas. Para contribuir para sua conservação, avaliamos o estado da população e as preferências de micro-hábitat dessa perereca em um trecho do riacho Danta, fazenda Rancho Alegre, em San Ramón, Costa Rica. Realizamos 18 observações noturnas e coletamos dados sobre tipos de poleiros, associações com a vegetação, características do riacho e variáveis ambientais para cada observação. Para identificar potenciais preferências de micro-hábitat, comparamos essas características entre localidades com ou sem as pererecas, bem como entre locais com indivíduos juvenis ou adultos. Registramos 13 indivíduos, incluindo três juvenis e 10 adultos. Nove adultos eram machos e um era fêmea, com a disparidade provavelmente devida às vocalizações conspícuas dos machos. As rãs juvenis e adultas ocupavam áreas com larguras do curso d'água semelhantes (t = 0,91; p = 0,38).

No entanto, os juvenis foram encontrados mais perto da água (t = 5,14, p = 0,01) e adjacentes a áreas mais rasas do riacho em comparação com os adultos (t = 2,50, p = 0,03). As pererecas empoleiraram-se predominantemente em *Piper* sp. (30,7%) e na superfície superior das folhas (69,2%). A altura em que os animais foram registrados (0,1 a 2,2 m) foi semelhante para ambas as faixas etárias (t = 0,86, p = 0,44). Não encontramos diferenças significativas na cobertura do dossel (média = 74,6%), diâmetro à altura do peito (≤ 3,14 cm), densidade da vegetação (5,0 plantas/m²) ou riqueza de plantas (1–4 espécies) entre juvenis e adultos. Da mesma forma, não observamos diferenças nas variáveis ambientais entre esses grupos. Além disso, as características da vegetação e as variáveis ambientais não diferiram entre os locais com a espécie e as localidades próximas sem a espécie. Nossas descobertas sugerem que as variáveis medidas não influenciaram significativamente a seleção de micro-hábitats por essa espécie. Essas novas informações sobre o estado populacional e o uso de micro-hábitats de *I. tica* são necessárias para o desenvolvimento de estratégias de manejo para a conservação dessa espécie criticamente ameaçada.

Palavras-chave: Anfíbios, Características da vegetação, Conservação, Criticamente ameaçada, Micro-hábitats, População, Variáveis ambientais.

Introduction

Amphibians are the most endangered class of vertebrates globally, with approximately 41% of described species classified as threatened and an increasing number facing a high risk of extinction (Carné and Vieites 2024). In Costa Rica, declines in numerous amphibian species have been observed. The country is home to 221 amphibian species, of which about 100 are listed under some threat category and four are considered extinct (Zumbado-Ulate *et al.* 2019, Rodríguez *et al.* 2020, BiodataCR 2024, Sasa *et al.* 2025).

Frogs of the genus *Isthmohyla* are second among groups of anurans with the highest number of critically endangered species in Costa Rica; some are observed only rarely and are listed among priority species for conservation (Bolaños 2009, Chaves-Acuña *et al.* 2020). *Isthmohyla tica* (Starrett, 1966) is one of these priority species.

Populations of *I. tica* have declined dramatically since the late 1980s, to the point that the species was reported absent from many sites with historic populations. By 2007, it was feared to be extinct (Bolaños 2009). Declines of *I. tica* populations have been attributed to chytridiomycosis, a disease caused by the fungus *Batrachochytrium dendrobatidis* Longcore, Pessier, and D. K. Nichols, 1999 (Gratwicke *et al.* 2016). No evidence of this pathogen was found in 26 *I. tica* (collected between 1961 and 2011) specimens at the Museum of Zoology at the University of California-Berkeley and the University of Costa Rica (De León *et al.* 2018). Therefore, the population decline of this tree frog remains enigmatic and has occurred despite the availability of protected areas with preserved forests (Cheng *et al.* 2011, Rodríguez *et al.* 2020).

Isthmohyla tica is endemic to Costa Rica and supposedly Panama, meaning its distribution is limited, which heightens its vulnerability to extinction. In Costa Rica, it was historically found in the mid-elevations of the Tilarán, Central, and Talamanca mountain ranges, at altitudes ranging from 720 to 1750 m above sea level (Sasa *et al.* 2010). Since 2007, it has only been reported in the Bosque Eterno de los Niños in Monteverde along with a single individual that was sighted at Tapantí National Park (Abarca 2016, IUCN SSC Amphibian Specialist Group and NatureServe 2020, Hidalgo-Mora *et al.* 2022). According to the information we compiled, stable populations have only been reported in the Chutas sector of the Bosque Eterno de los Niños (Hidalgo-Mora *et al*. 2022).

In Panama, the species was "rediscovered" in 2010, with sightings of three individuals at two sites within the Parque Internacional La Amistad (Hertz *et al.* 2012, IUCN SSC Amphibian

Specialist Group and NatureServe 2020). It is thought that the populations in Monteverde, Costa Rica and western Panama are likely not the same species (IUCN SSC Amphibian Specialist Group and NatureServe 2020). Similarly, another population described in the Los Santos region of Costa Rica likely represents a different, undescribed species (G. Chaves pers. comm.).

In the current scenario, the species has been reported at only two localities in Costa Rica, and the records are sporadic with a small number of individuals observed during each visit (Rodríguez *et al.* 2020). Due to this situation, the IUCN SSC Amphibian Specialist Group and NatureServe (2020) have classified the *I. tica* as a critically endangered species with declining populations for over two decades. It is estimated that there are no more than five subpopulations throughout its range, and it is likely that each subpopulation has fewer than 50 mature individuals (IUCN SSC Amphibian Specialist Group and NatureServe 2020). If the Los Santos population in Costa Rica and the Panama populations are indeed different species, then the species' status is even more concerning.

The first recent sighting of *Isthmohyla tica* in Costa Rica occurred in Monteverde in 2010, after 15 years without any reports (García-Rodríguez *et al.* 2012, Hertz *et al.* 2012). In 2020, we also identified *I. tica* at the Rancho Alegre farm in San Ramón de Alajuela, Costa Rica. We aimed to study this population in order to generate baseline information on biological and ecological characteristics, which in turn could contribute to future management plans and conservation strategies for this species. To this end, our objective was to assess the status and microhabitat use of the population in terms of the vegetation, perch types, and environmental variables in a section of the Danta stream at Rancho Alegre farm. Additionally, we sought to compare microhabitat variables between sites with *I. tica* and nearby sites that did not have individuals of this species in this same section of the stream.

Materials and Methods

Study Species

Isthmohyla tica has a short, truncated snout in profile. The dorsum and upper body surfaces display visible greenish-brown tubercles, and the hind limbs are marked with dark transverse bands (Savage 2002). Adult males range from 27 to 34 mm in snout–vent length, while females range from 33 to 42 mm. The toes are relatively short and broad, and digits III and IV bear large, wide discs that are equal to, or slightly larger than, the diameter of the tympanum (Savage 2002).

Savage (2002) suggested that *I. tica* lays its eggs beneath rocks in streams, and that the tadpoles adhere to the substrate using a large oral funnel. Nevertheless, many fundamental aspects of its reproductive and developmental biology, such as type of amplexus, oviposition behavior, parental care, hatching time, microhabitat preferences, feeding, and duration of metamorphosis, remain unknown (Savage 2002, IUCN SSC Amphibian Specialist Group and NatureServe 2020).

Study Area

The study area is located in the western part of San Ramón Canton, Alajuela Province, within the Peñas Blancas District, specifically along the Danta Stream (10°20'13" N, 84°46'21" W; 1,492 m a.s.l.; Figure 1). The site lies within the Lower Montane Rain Forest life zone (Calvo 1989). The climate is humid, with average annual precipitation exceeding 5,000 mm, and March being the only dry month. Temperatures vary between 9 and 24°C (Calvo 1989). Common vegetation includes ferns, epiphytes, and a variety of trees from the Lauraceae, Meliaceae, Melastomataceae, Rubiaceae, and Sapotaceae families (Calvo 1989). The Danta Stream is 2,303 m long with lotic waters; however, for this study, we focused on a 1,200 m section. A waterfall blocked access upstream to the

northeast and served as a natural reference point marking the start of our sampling transect.

Data Collection

We conducted 18 sampling sessions, each consisting of two nights in a row per month from December 2020 to August 2021. Visits to the study site between September and November were not conducted because they are risky due to severe storms and flash floods. We established defined band transects to estimate presence or absence, relative abundance, microhabitat association, and activity patterns of anuran species (Heyer *et al.* 2014). This method involves limited searches for individuals per unit of time along permanent transects (Heyer *et al.* 2014). We divided the selected section of the Danta stream into three 400 m long sectors, designated as A, B, and C (Figure 1). During each sampling night, we surveyed all three sectors, but we randomized the order in which the sectors were surveyed to avoid biases in the behavior and distribution of the species according to its activity peaks.

We marked 24 defined band transects, with eight transects per sector. Each transect measured 2 m perpendicularly from the stream's edge on both sides by 50 m along the stream. We systematically surveyed the transects, alternating between odd-numbered transects (1, 3, 5, 7… 23) on the first night and even-numbered transects (2, 4, 6, 8… 24) on the following night. To cover both sides of the stream, we always surveyed both banks simultaneously for 30 min. We used a stopwatch to control the time, pausing it each time we found a frog to record the respective data. Each survey included both visual searches to locate individual *I. tica* and auditory searches to find males.

We conducted surveys from 17:30 h to approximately midnight (between 23:30 and 24:00 h). For each individual, we recorded the geographical coordinates, time, date, activity (calling, amplexus, or resting), stage (juvenile or adult), and sex (determined by vocalizations and the presence of nuptial pads in males). In cases where the sex of an individual was uncertain, we captured the frog to accurately determine its characteristics and subsequently released it at the same location where it was found. Capture was conducted using gloves to minimize the risk of disease transmission, although we acknowledge that certain types of gloves may negatively affect amphibians (Gutleb *et al.* 2001, Cashins *et al.* 2008).

For each record of *Isthmohyla tica,* we noted the following biophysical habitat characteristics: type of perch (leaf, branch, vine, stem, sand, soil, leaf litter, pebble, or stone), position on vegetative substrate (upper or lower leaf surface), plant species, height above ground, and distance of the perch from the stream edge. Additionally, we recorded data on the width and depth of the water at the center of the stream.

To characterize the vegetation, we marked 2 × 5 m plots for the tree stratum (diameter at breast height; DBH > 10 cm) and 1 × 1 m plots for the herbaceous stratum (DBH <10 cm). These plots were established in locations where *I. tica* was present and were also paired randomly with plots in locations where the frogs were absent (Tocher *et al.* 1997). We characterized the vegetation according to the following variables: (a) species richness, defined as the number of plant species; (b) vegetation density of the tree and herbaceous strata, measured as abundance per unit area; (c) DBH of each individual plant; and (d) canopy cover, estimated using a densiometer (Tocher *et al.* 1997).

Regarding climatic variables, we used a Be8910 Ontranki® multifunction anemometer to measure air temperature (°C), relative humidity (%), and wind speed (m/s). We collected these data at a height of 1.50 m above the ground. We obtained precipitation values (mm) from the meteorological station in Monteverde, 3.41 km from the Danta stream. Additionally, we gathered data on moonlight percentage using the website of the Astronomical Applications Department of the United States Naval Observatory (https://aa.usno.navy.mil/data/index). Meteorological variables

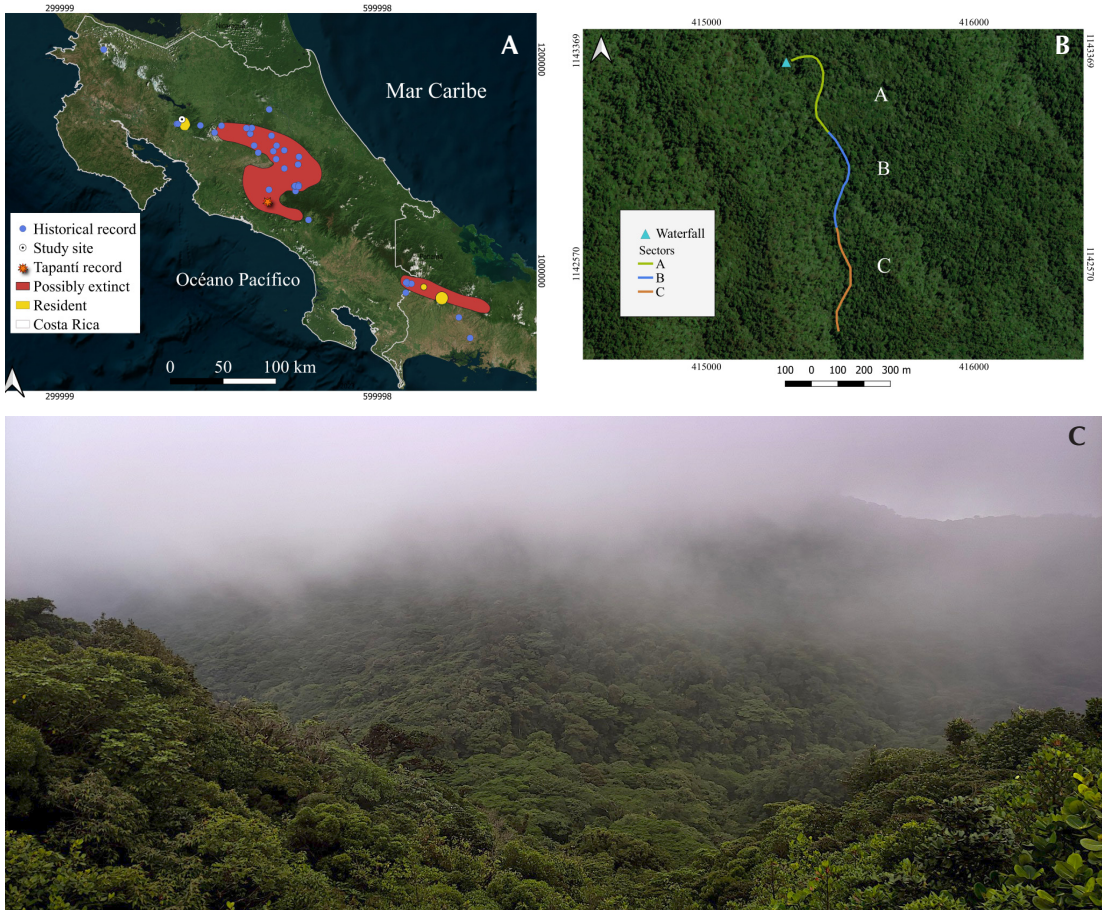

Figure 1. (**A**) Historical records (red; IUCN SSC Amphibian Specialist Group and NatureServe 2020), and current known areas of *Isthmohyla tica* in Costa Rica and Panama. (**B**) Sampling sectors along the Danta stream at the study site. (**C**) General view of the study site at Rancho Alegre, San Ramón, Alajuela, Costa Rica.

were recorded in locations where *I. tica* was present, as well as during sampling sessions in which no individuals were found.

Data Analysis

To analyze the microhabitat characteristics of Tica Frog, *Isthmohyla tica,* we calculated descriptive statistics of each variable, including the mean, standard deviation, and range, as well as created contingency tables for count data. We tested whether any vegetation or environmental variables predicted the presence or absence of *I. tica* using generalized linear models (GLM) with a binomial error distribution. We attempted to include random variables to account for different transects and sampling nights, but with low sample size and the fact that we typically only found a single frog on a given night or transect (with a single exception of a metamorph and a male both located on 25 March 2021), mixed models led to non-convergence due to singularity. Additionally, inclusion of interactions between different vegetation and environmental variables

in models led to model non-convergence, likely due to low sample sizes and high co-linearity among fixed model predictors (|r| = 0.37 to 0.71), so here we report the results of GLMs with individual predictors. Separately, we compared sites with adult and juvenile individuals with respect to vegetation, environmental, perch, and stream characteristics using either a *t*-test or Mann-Whitney U test as appropriate after testing each variable for normality with a Shapiro-Wilk test. All statistical analyses were performed using R 4.1.2 (R Core Team 2021).

Results

We obtained 13 records of *Isthmohyla tica,* of which three were juvenile individuals and 10 were adults. Among the adults there were nine males and one female. The most commonly used substrate for perching was the upper surface of leaves, accouting for 69.23% of observations (Figure 2). Candelillos (*Piper* spp.) were the most frequently used substrate plants (30.76%), while *Cyathea* sp. and *Heliconia tortuosa* Griggs were used less often (15.38% each). Other plant species were recorded only once each (Figure 3).

Perch height ranged from 0.1 to 2.2 m above the ground, and the distance from the stream ranged from 0.1 to 2.1 m (Table 1). Stream depth ranged from 3 to 60 cm (Table 1). Forest canopy cover averaged 74.6% (Table 2). Sites with *I. tica* had an average plant density of 5.0 plants/ m^2 (Table 2), and a low average DBH (≤ 3.14 cm), characteristic of an herbaceous stratum (Table 2).

The average temperature at the study site was 22.42 (± 1.81°C SD), with values ranging from 19.6°C to 24.8°C (Table 3). Both precipitation and relative humidity were high (Table 3). Sixty-two percent of *I. tica* observations were associated with the presence of rain or drizzle at the time of encounter, while 38% were made on nights without precipitation (Table 3). Additionally, 38% of encounters included males vocalizing in the presence of rain or drizzle.

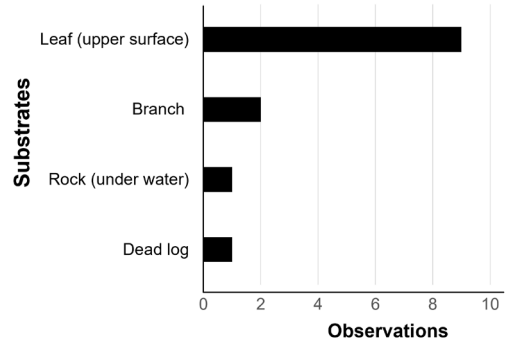

Figure 2. Types of substrates upon which *Isthmohyla tica* was found adjacent to the Danta stream, San Ramón, Costa Rica (*N* = 13).

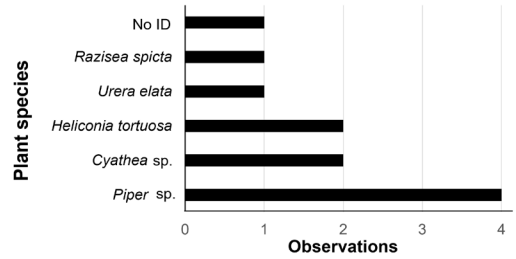

Figure 3. Plant species upon which *Isthmohyla tica* was found perching adjacent to the Danta stream, San Ramón, Costa Rica (*N* = 11).

Males vocalized at wind speeds of up to 8.2 km/h (Table 3). Forty-six percent of sightings occurred during the waxing crescent moon, followed by 31% during the full moon, and 15% during the new moon. There was a single sighting during the waning crescent moon. Males vocalized during the full moon, new moon, and waxing crescent moon.

Vegetation association variables did not predict the presence or absence of *I. tica* in transects (vegetation density: Z = -0.64, p = 0.56; plant richness: Z = -0.92, p = 0.35; canopy cover: Z = 0.69, p = 0.48; plant DBH: Z = -0.63, p = 0.52). Similarly, environmental variables did not differ between sites with and without frogs (temperature: Z = 0.50, p = 0.61; humid-

ity: Z = -0.41, p = 0.67; moonlight illumination: Z = 0.52, p = 0.60; precipitation: Z = -1.30, p = 0.54), with the exception of a trend suggesting that as wind speed increases, the likelihood of the presence of *I. tica* decreases (Z = 1.85, p = 0.06).

Juveniles were found closer to the water (t = 5.14, p = 0.01) and in shallower areas of the stream (t = 2.50, p = 0.03) than adults, but stream width did not differ between sites with juveniles or adults (t = 0.91; p = 0.38). Perch height was similar between juveniles and adults (t = 0.86, p = 0.44). We did not find differences between sites with juveniles or adults in either vegetation parameter (canopy cover: t = -1.01, p = 0.36; plant DBH: t = 0.30, p = 0.77; vegetation density: t = -1.12, p = 0.34; plant richness: t = -0.06, p = 0.95), or environmental parameters (temperature: t = 0.28, p = 0.78; humidity: t = -0.30, p = 0.77; precipitation: U = 13, p = 0.79; moonlight illumination: t = 0.51, p = 0.63; wind speed: t = 1.26, p = 0.26).

Table 1. Characteristics of the perches and the Danta stream where *Isthmohyla tica* was found in San Ramón, Costa Rica (N = 13).

Variable	Average ± SD	Range
Height above ground (m)	0.89 ± 0.70	0.1–2.2
Distance from the stream (m)	0.95 ± 0.75	0.1–2.1
Stream depth (cm)	21.54 ± 15.97	3–60
Stream width (m)	2.33 ± 0.93	0.78–4.17

Table 2. Characteristics of the plants in plots at the sites where *Isthmohyla tica* was found in the Danta stream, San Ramón, Costa Rica (N = 13).

Variable	Average ± SD	Range
Canopy cover (%)	74.60 ± 10.76	57–92
DBH (cm)	1.82 ± 0.74	0.90–3.14
Plant richness (number of species)	2.61 ± 1.28	1–4
Vegetation density (plants/m^2)	5.00 ± 2.88	1–10

Table 3. Characterization of the environmental variables at the sites where *Isthmohyla tica* was found in the Danta stream, San Ramón, Costa Rica (N = 13).

Variable	Average ± SD	Range
Humidity (%)	82.21 ± 6.29	71.8–91.5
Temperature (°C)	22.42 ± 1.74	19.6–24.8
Wind speed (km/h)	5.47 ± 2.63	1–10.1
Precipitation (mm)	4.97 ± 5.33	0–15.2
Moon illumination (%)	54.28 ± 35.58	3.3–99.7

Discussion

This study provides fundamental ecological data on *Isthmohyla tica,* an endemic Costa Rican frog species listed as Critically Endangered by the IUCN (IUCN SSC Amphibian Specialist Group and NatureServe 2020). Although individual frogs have been observed on vegetation along stream edges during both the rainy and dry seasons (Duellman 1970), we present some of the first data on the microhabitat characteristics and population dynamics of *I. tica,* based on 13 individuals encountered at Quebrada Danta, San Ramón, Costa Rica.

Of the 10 adult *I. tica* individuals we encountered, nine were males, and only one was a female. This disparity is likely due to the males' distinctive vocalizations, which increase their detectability compared to females (Emerson and Boyd 1999). We recorded an average of 0.72 frogs per night, a relatively low count, but consistent with findings from other locations (Abarca 2016, Hidalgo-Mora *et al.* 2022). For instance, only a single individual was observed in Tapantí National Park in 2012 (Abarca 2016). Similarly, very few sightings and individuals per visit have been reported in the Bosque Eterno de los Niños in Monteverde (Rodríguez *et al.* 2020).

Apparently, *Isthmohyla tica* primarily used leaves as perches, consistent with its arboreal behavior (Savage 2002). Frogs were predominantly found on leaves of *Piper* spp. This preference may be associated with its ability to camouflage and blend into its surroundings, particularly given the green tones of its skin (Figure 4). A choice-behavior study would be necessary to draw conclusions about substrate preferences.

The proximity of *I. tica* to the stream in terms of height and horizontal distance may be necessary for accessing food, such as arthropods near the water, and could serve as a means of escape from predators (Restrepo 2018). Additionally, proximity to appropriate water bodies is crucial during the breeding season for the development of tadpoles (Savage 2002), and may explain why we found juveniles closer to the edge of the stream than adults. Juveniles recently metamorphosized from tadpoles, which is a transition that requires an adaptation process from aquatic to terrestrial environments and may require them to stay closer to the water than adults that are already acclimated to the terrestrial environment. However, it could simply be that the metamorphs were leaving the water when we found them. The height at which we found frogs could represent an estimation bias, because we sampled at ground level, which makes it difficult to find frogs above 2 m. Thus, frogs at greater heights may not have been detected. However, 46.15% of the detections occurred while following vocalizations, and in none of those cases did we find frogs at heights exceeding 2.20 m, unlike frogs in other clades such as centrolenids that frequently vocalize at heights above 2 m (Duarte-Marín *et al.* 2023).

Canopy cover at sites used by *Isthmohyla tica* was high (74.6%), which likely helps reduce desiccation by limiting solar exposure, maintaining moisture, and providing camouflage within the surrounding vegetation (McDiarmid 1978, Stückler *et al.* 2023, Spranger *et al.* 2024). Plant DBH is not as well studied in relation to amphibian microhabitats, but may indicate the types of perches available to *I. tica,* such as stems or trunks. Some studies have found that the presence of trees with large diameters and woody debris affect the presence of certain frog species (Acosta-Chaves *et al.* 2015, Pabijan *et al.* 2023). The average DBH at sites with *I. tica* was low ($\leq$ 3.14 cm), characteristic of an herbaceous layer. This may be because within the 2 m sampled on each side of the river, the area is prone to increased flow. Certain sectors of this microhabitat regenerate annually due to the increased stream flow during the rainy season, which washes away some vegetation. For example, on a visit after the study in September 2021, we found that part of the microhabitat where we had previously sighted *I. tica* had been washed away by the strong current after a storm (Figure 4).

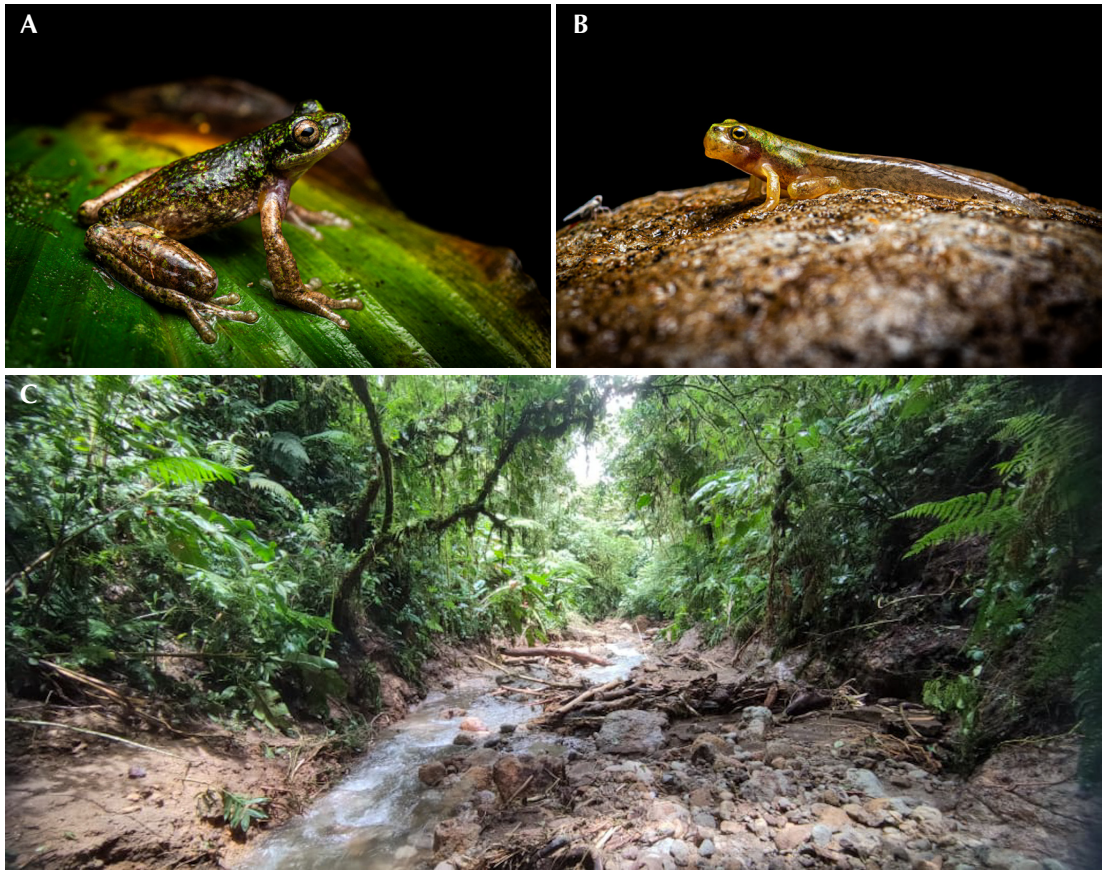

Figure 4. (**A**) Adult *Isthmohyla tica,* (**B**) metamorphosing tadpole of *I. tica,* (**C**) the Danta stream after a storm in September 2021. San Ramón, Alajuela, Costa Rica.

We observed a tendency for *I. tica* to be more abundant and for males to vocalize more when wind speeds were lower, although this pattern was not statistically supported. Wind speed can influence relative humidity, calling activity, and desiccation risk in amphibians (Burggren and Moalli 1984, Prasad *et al.* 2022). Other studies suggest that tolerance to desiccation varies among species, with some hylids being relatively resilient (Ralin and Rogers 1972), while abundance and calling activity in related species may be more strongly linked to rainfall and temperature (Arguedas *et al.* 2022).

Lunar phases can affect the activity and detection of anuran species (Acosta-Chaves *et al.* 2015, Pérez and Medina 2018). A study in Brazil found a positive correlation between amphibian vocalization and darker lunar phases (Lima *et al.* 2021). Two tree frogs, *Agalychnis spurrelli* Boullenger, 1913 and *A. saltator* Taylor, 1955, are explosive breeders that may be influenced by lunar phases (Ortega-Andrade *et al.* 2011, Arguedas *et al.* 2022, 2023). Our limited observations suggest that lunar phases may also influence the behavior of *I. tica,* although further studies are needed to understand this aspect of its ecology.

In the case of *I. tica,* reproduction is concentrated in the dry season when precipitation is lower (Duellman 1970, Savage 2002, Leenders

2016). Accordingly, we found juveniles in March, April, and May, the least rainy months. The study site has an average annual precipitation greater than 5000 mm, and the stream maintains its flow year-round (Calvo 1989), providing this frog with suitable ecological conditions for reproduction during the dry season. Tadpoles of *I. tica* have oral disc adaptations that allow them to cling to rocks and avoid being swept away by the current (Savage 2002). When water flow increases and currents are stronger, tadpoles would need to expend more energy to cling to rocks or could be carried away by the current. Tadpoles may be more likely to survive to metamorphosis during the dry season when the current flow is slower. The reproductive activity of amphibians is strongly influenced by exogenous factors such as rainfall and air temperature (Bertoluci and Rodrigues 2002), and is commonly associated with the rainy season in the Neotropics (e.g., Wells 2007). Reproductive activity of Neotropical species can indeed increase or decrease during either the rainy or dry seasons (Bertoluci and Rodrigues 2002).

We found a low relative abundance of juveniles compared to adults across the months of sampling. Given that the breeding period of this species is in the dry season from February to May (Duellman 1970, Savage 2002, Leenders 2016) and most of the sampling took place outside of this period, this phenology and relative abundance of life phases is to be expected.

Although our analyses are limited by a small sample size ($N = 13$), the absence of significant differences in microhabitat variables between sites with and without *I. tica* suggests that the species may rely on streams in general, rather than on specific microhabitat features (Duellman 1970). By contrast, some amphibians show strong habitat specialization, such as *Craugastor rhyacobatrachus* (Campbell and Savage 2000), which depends on streams and inhabits clear, cascading mountain streams in humid montane forests, often associated with rocky sections of torrential watercourses (Savage 2002), and *Atelopus varius* (Lichtenstein and Martens, 1856), which prefers rocky substrates and log accumulations in streams and shows higher encounter rates in forested habitats than in pastures (Leenders 2016, Gómez-Hoyos *et al.* 2020). Future studies should assess the degree of microhabitat flexibility in *I. tica* and examine additional factors not measured here, including food availability, predator pressure, and disease dynamics. Such work is urgently needed to clarify the ecological requirements of this critically threatened species and to inform conservation strategies for its persistence and recovery.

Conclusion

This study represents the first documentation of the microhabitat use of the critically endangered frog *Isthmohyla tica* in Costa Rica. Our findings reveal a remnant population with low relative abundance in the riparian forest of Rancho Alegre farm, San Ramón. The presence of both juvenile and adult individuals indicates active reproduction in the study area, offering encouraging evidence for the species' persistence. These values are important as a baseline; however, long-term monitoring over several years will be essential to determine whether the population trends are positive or negative and to assess how this information can best guide conservation actions. We therefore recommend the implementation of a sustained monitoring program in the Danta stream and other known populations to ensure the long-term survival and potential recovery of this species.

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