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A new Andean rocket frog (Amphibia: Anura: Leptodactylidae: *Leptodactylus*) from urban spaces at Mérida, Venezuela

Una nueva rana andina (Amphibia: Anura: Leptodactylidae: *Leptodactylus*) de espacios urbanos en Mérida, Venezuela

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ABSTRACT

A new species of *Leptodactylus* is described from the city of Mérida, in the Andean mountains of Venezuela. The new taxon, a member of the *Leptodactylus melanonotus* species group, is distinguished from all congeners by a combination of morphological and bioacoustic characters, including: moderate body size (adult males mean SVL 37.4 mm; female 44.5 mm); a single pair of irregular, often interrupted dorsolateral folds; slightly expanded (knob-shaped) toe tips; and a ventral color pattern with pale mottling fading posteriorly. The new species is further characterized by an advertisement call with an exceptionally high note repetition rate (~240 calls/min) and a high dominant frequency (2.4 kHz). We provide a detailed description of the holotype and variation within the type series, including data on coloration in life and preservative and description of the larval stage. The species is restricted to internal valleys within the Cordillera de Mérida, where it dwells in urban and peri-urban environments. We provide preliminary data on its ecology and conservation status.

Key words: Andes, Anura, conservation, *Leptodactylus*, natural history, taxonomy, Venezuela.

RESUMEN

Se describe una nueva especie de *Leptodactylus* de la ciudad de Mérida, en los Andes de Venezuela. El nuevo taxón, miembro del grupo de especies *Leptodactylus melanonotus*, se distingue de todos sus congéneres por una combinación de caracteres morfológicos y bioacústicos, que incluyen: tamaño corporal moderado (longitud hocico-cloaca, LHC, promedio en machos adultos de 37,4 mm; hembra de 44,5 mm); un único par de pliegues dorsolaterales irregulares y a menudo interrumpidos; puntas de los dedos de los pies ligeramente expandidas (en forma de pomo o botón); y un patrón de coloración ventral con un moteado pálido que se desvanece hacia la parte posterior. La nueva especie se caracteriza además por un canto de advertencia con una tasa de repetición de notas excepcionalmente alta (~240 cantos/min) y una frecuencia dominante elevada (2,4 kHz). Proporcionamos una descripción detallada del holotipo y de la variación dentro de la serie tipo, incluyendo datos sobre la coloración en vida y en preservativo, y descripción de la etapa larvaria. La especie está restringida a los valles internos de la Cordillera de Mérida, donde habita en entornos urbanos y periurbanos. Proveemos datos preliminares sobre su ecología y estado de conservación.

Palabras clave: Andes, Anura, conservación, historia natural, *Leptodactylus*, taxonomía, Venezuela.

INTRODUCTION

The biodiversity of the Venezuelan Andes is currently in a prolific stage of discovery and inventory. In the first decade of the 21st century alone, numerous new species were described from the Cordillera de Mérida, a mountain range approximately 400 km long and 80 km wide in northern South America. Most occurred in relatively undisturbed natural habitats. However, a distinct and yet undescribed taxon persists within the urban spaces of Mérida, one of the largest and highest cities in the Venezuelan mountains. The genus *Leptodactylus* is a diverse group of Neotropical frogs, currently organized into several major species groups. The *Leptodactylus melanonotus* group is characterized by having toes with lateral fringes and, at most, a single pair of dorsolateral folds. In Venezuela, this group includes several recognized taxa, e. g., *L. colombiensis*, *L. diedrus*, *L. leptodactyloides*, *L. magistris*, *L. petersi*, and *L. sabanensis*. Populations from the Venezuelan Andes were often referred to as a broad “Operational Taxonomic Unit” (OTU); however, researchers noted that this grouping might be too conservative and likely contained multiple species. Previous literature has occasionally mentioned the Andean populations under various names, including *L. podicipinus petersi*, *L. wagneri*, and the *nomen nudum* “*Leptodactylus meridensis*”. However, detailed morphological and bioacoustic analyses now confirm that the populations inhabiting the internal valleys of the Cordillera de Mérida represent a distinct evolutionary lineage. This new species is most similar to *L. colombiensis* but can be readily distinguished by: 1. its smaller size, distinct bioacoustic profile with a remarkably fast call repetition rate and 2. specific coloration patterns, i. e., greenish tinge in the groin. Herein we describe the new species, provide a comprehensive diagnosis against its congeners in the *L. melanonotus* group, and document its natural history and conservation requirements within the increasingly urbanized landscape of the Mérida terrace.

MATERIALS AND METHODS

Specimen collection and curation

All specimens examined in this study are deposited in the Collection of Amphibians and Reptiles of the Laboratorio de Biogeografía at the Universidad de Los Andes, Mérida, Venezuela (ULABG). A total of 10 specimens were collected for the species description; additional individuals were examined from existing holdings. Paratypes consist exclusively of topotypes, being specimens collected from the same locality as the holotype. Specimens considered to belong to the new species, but excluded from the

type series, are listed as additional specimens. The synonymy lists provided for the new taxon follow the concept of chresonymy (Smith & Smith 1972, La Marca 1994), i. e., the usage of names in previous literature. This is intended to clarify the taxonomic history of the populations from the Mérida terrace, which have been historically assigned to other taxa or mentioned as *nomen nudum*.

Morphometrics and character analysis

Measurements were taken with a digital caliper (Helios®, precision 0.01 mm). For the post metamorphic specimens, the following morphometric variables were recorded: snout-vent length (SVL); head length (HL) from the posterior corner of the mouth to the tip of the snout; head width (HW) at the angle of the jaws; eye-to-naris distance (EN) from the anterior corner of the eye to the center of the naris; internarial distance (IN); eye length (EYE) from the anterior to the posterior corner; horizontal diameter of the tympanum (T); hand length (HAND) from the proximal edge of the palmar tubercle to the tip of finger III; tibia length (TL) from the outer edge of the flexed knee to the heel; and foot length (FOOT) from the proximal edge of the outer metatarsal tubercle to the tip of toe IV. Adult status was confirmed via gonad inspection. Mature males were identified by the presence of open vocal slits in the floor of the mouth, a pair of black keratinized thumb spines, and enlarged testes. Mature females were identified by the presence of pigmented eggs and thickened, convoluted oviducts. Terminology and descriptive methods follow Heyer (1994) and La Marca *et al.* (2004).

Larval morphology

Tadpole developmental stages were determined following Gosner (1960), and morphological terminology follows Mijares-Urrutia (1998). Morphometric data for larvae included: body length (BL), tail length (TL), body height (BH), eye-to-nostril distance (END), eye-to-snout distance (ESD), eye diameter (ED), spiracle-to-snout distance (SSD), spiracle-to-dorsum distance (SDD), caudal musculature height (CMH), dorsal fin height (DFH), ventral fin height (VFH), anterior diastema width (ADW), posterior diastema width (PDW), oral disc width (ODW), body width (BW), inter-orbital distance (IOD), and inter-nostril distance (IND).

Bioacoustics

Advertisement calls were recorded in the field using a Sony TCS-310 tape recorder with an integrated microphone. Bioacoustic analyses were performed using Avisoft-Sonograph Pro (Windows), a PC-based spectrograph-generating program. Spectrograms display frequency

(kHz) on the vertical axis and time (s) on the horizontal axis. Relative amplitude (dB) is represented by a grayscale gradient, ranging from low (white) to high (black).

DESCRIPTION OF SPECIES

Leptodactylus trinacria sp. nov.

(Figs. 1-2)

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Common names

Mérida's Whistling Frog (La Marca 2016a, La Marca 2020), Sapito Silbador de Mérida (La Marca, 2015), Rana Silbadora de Mérida (La Marca 2016b).

Chresonomy

Leptodactylus podicipinus petersi. Rivero, 1961: 47 (*partim*).

Leptodactylus wagneri. Heyer, 1970: 45 (*partim*); La Marca, 1992: 63,107 (*partim*).

?*Leptodactylus fuscus*. Barrio-Amorós, 1998: 43 (*partim*).

Leptodactylus pallidirostris. Barrio-Amorós, 1998: 46 (*partim*).

Leptodactylus sp. 1. La Marca, 1999: 203; Barrio-Amorós, 2004: 98,170 (*partim*).

Leptodactylus sp. García, Albornoz & La Marca, 2007: 65; La Marca, 2015: 217; La Marca *et al.*, 2015a, b: 10; La Marca, 2016a: 3, b: 7; La Marca, 2017a, b: 5; La Marca & Castellanos, 2018a, b: 17; La Marca, 2020a: [2]; La Marca, 2021: 31.

"*Leptodactylus meridensis*" (*nomen nudum*) – Karlsdóttir, 2017a, b: 16; La Marca, 2022a, b: 7.

Holotype

ULABG 4873, adult male, with both vocal slits opened, a pair of keratinized black thumb spines on each hand and dark throat. Collected from the artificial lake at Museo de Ciencia y Tecnología of Mérida city (former "Central Azucarero Los Andes", CALA), Mérida state, Venezuela, 1320 m.asl, geographic coordinates WGS84: 8°33'44"N, 71°11'25"O (Fig. 1 A, B), collected by Enrique La Marca, Diego Cadenas, Luis Felipe Esqueda, Enzo La Marca and Francisco Nava, on 19 March 2002.

Paratypes (Topotypes)

ULABG 4871-4872, 4874-4879, adult males (vocal slits opened, keratinized black spines, dark throat), collected at the same locality of the holotype, but captured on 30 October 2001; ULABG 4880 (allotype, Fig. 1 C, D),

adult female (convoluted oviduct, large eggs up to 4.5 mm), same data as holotype.

Additional specimens

ULABG 1413, adult female, coming from Los Llanitos de Tabay, nr. Tabay, NE city of Mérida, estado Mérida, Venezuela. 1475 m.asl, 8°37'14"N, 71°6'14"W, collected on 28 marzo 1985 by Enrique La Marca.

ULABG 3061-3062, adult males from the Instituto de Geografía y Conservación de Recursos Naturales (IGCRN), Universidad de Los Andes (Mérida, Venezuela), 8°37'20"N, 71°8'20"W collected on 5 November 1991 by Hans Peter Reinthaler.

ULABG 3733, adult female, coming from Ejido, estado Mérida, Venezuela. 1100 m. asl., 8°33'30"N, 71°15'9"W, collected on 27 February 1994 by María José Praderio and Carlos Gottberg; ULABG 4286-4287, adult males, collected by Carlos Gottberg on 12 March 1996.

ULABG 4481, 4482, lots of a single tadpole each, coming from same locality as holotype and paratypes, collected on 16 September 2002 by María José Praderio.

Etymology

The specific epithet "trinacria" is a noun in apposition derived from the Ancient Greek *treis* (meaning three; Brown, 1956) and *ákrαι* (meaning the extreme point of a coast or territory; Rodríguez Adrados, 1980). Historically, the term served as the classical Greek designation for the island of Sicily, in reference to its triangular configuration. Herein, the name alludes to the triangular dorsal blotch located between the interorbital and scapular regions (Fig. 2), exhibited by several specimens of the new taxon, including the holotype. While this character is not diagnostic for the species and may occur in other *Leptodactylus* congeners, the name is formally dedicated to the author's father, Vincenzo La Marca Zirini, on the centenary of his birth in Sicily (22/I/1926). This nomenclature thus establishes a symbolic link between the author's ancestral Sicilian heritage and this new Andean frog.

Diagnosis

Previous work recognized that there was a Venezuelan Andes OTU" (Operational Taxonomic Unit) which purportedly contained specimens from the Andean states of Mérida, Táchira and Trujillo (Heyer 1994), as well as from other western and central Venezuelan states from mountains to lowlands. This Operational Taxonomic Unit was deemed to represent a single species ("although this may be too conservative a conclusion"; Heyer 1994:56). After examination of specimens from the interior part of the Andean Cordillera de Mérida, we consider that a single spe-

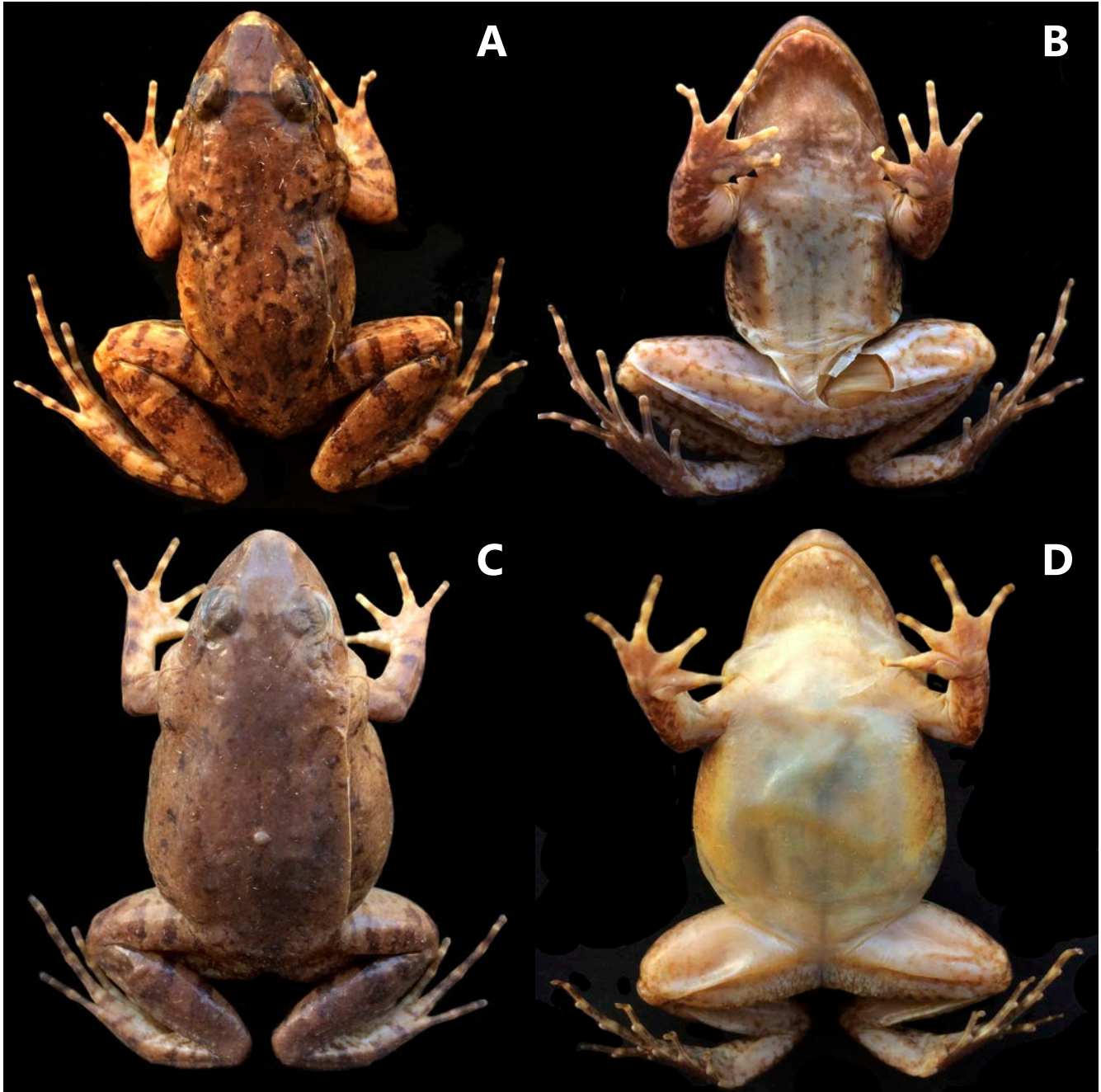


Figura 1. A, B: Dorsal and ventral views of preserved holotype (ULABG 4873) and C, D: allotype (ULABG 4880) of *Leptodactylus trinacria* sp. nov. Circular section on left thigh of holotype shows place where a sample tissue was taken for Bd testing. Photos: Enrique La Marca.

cies is involved, being well differentiated from the already described species. Nonetheless, future work analyzing audiospectrograms and DNA samples will be needed to fully resolve the taxonomic identity of other populations previously included in Heyer's (1994) Venezuela Andes OTU.

Leptodactylus trinacria sp. nov. is assigned to the *L. melanonotus* species group due to the presence of lateral

toe fringes and a pair of dorsolateral folds (sometimes interrupted). The new species is distinguished from its congeners by the following combination of characters: (1) moderate body size (mean SVL 37.4 mm in males, 44.5 mm in females); (2) a pair of irregular, frequently interrupted, or inconspicuous dorsolateral folds; (3) slightly expanded, knob-shaped toe tips; (4) a well-defined tym-



Figure 2. Dorsal view of a live *Leptodactylus trinacria* sp. nov. to show the triangular-shaped blotch between the interorbital and scapular regions. Photo: Enrique La Marca.

panum, with a diameter approximately 2/3 of the eye diameter; (5) ventral coloration with dense pale mottling on the throat and chest, fading posteriorly toward the inguinal region; (6) presence of a greenish tinge in the groin and on the hidden surfaces of the thighs in life; and (7) an advertisement call characterized by an exceptionally high note repetition rate (~ 240 notes/min) and a dominant frequency of 2.4 kHz.

Leptodactylus trinacria sp. nov. is a moderate-size species associated with internal valleys within the Andean Cordillera de Mérida, in western Venezuela. As all species in *L. melanonotus* species group, *L. trinacria* sp. nov. specimens have a single pair of dorsolateral folds. Dorsolateral folds tend to have a different degree of expression (Fig. 3), given by certain preserved conditions: poorly preserved specimens do not exhibit the folds that are distinctive in live specimens. Live specimens variably show the conditions of absent (Fig. 3B, E, F), indistinct or fragmented (Fig. 3A, C, D), and complete and moderate to long (Fig. 3G, H; 4); although the most frequent is indistinct or fragmented (Carvahlo *et al.* 2022).

Comparisons with other species

Specimens of the new species exhibits an upper lip stripe condition (following Heyer, 1994 and Carvahlo *et*

al. 2022) type “state B”, defined as extending from the posterior corner of the eye towards the base of the arm (Fig. 4); however, sometimes not easily discernible. This condition is also exhibited by *L. colombiensis* and *L. magistris*. The toe tips of the new species exhibit the “slightly expanded” condition “E” of Carvahlo *et al.* (2022), also defined as “knob-shaped swollen” (Fig. 5). The ventral color pattern follows condition “A” of Carvahlo *et al.* (2022), where the pale mottling is fading posteriorly (Fig. 6). The lower jaw may be covered with pale spots in both males and females (Fig. 6A, B). The ventral mottling concentrating towards the anterior central parts and fading progressively towards lower venter is also exhibited by *Leptodactylus colombiensis* and probably *L. magistris* (Carvahlo *et al.* 2022). The groin can be greenish in some specimens, easily differentiating the species from others in the *L. melanonotus* group that show a bright yellow coloration. The greenish tinge may be also present on concealed anterior and posterior parts of the thighs, and ventrolaterally at the posterior part of flank (Fig. 7).

Venezuelan species of *Leptodactylus* with toe fringes include members of the *L. melanonotus* and *L. latrans* groups of species. *Leptodactylus latrans* have at least four well-developed dorsolateral folds. All members of the *L. melanonotus* group have a single pair of dorsolateral folds,

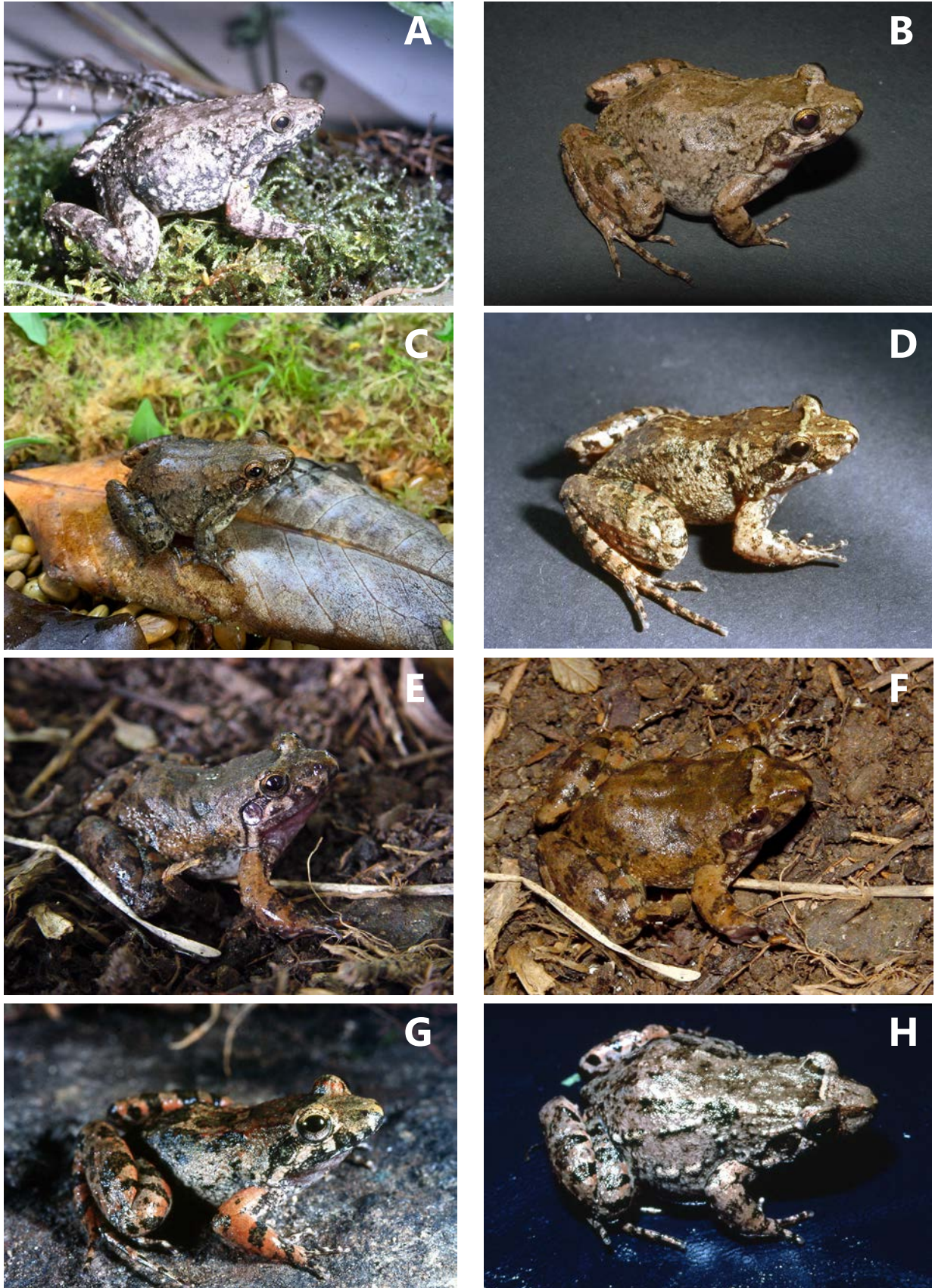


Figure 3. Live specimens, not collected, of *Leptodactylus trinacria* sp. nov. from the terrace of Mérida. Photos A-G: Enrique La Marca, Photo H: James R. Dixon.



Figure 4. Live specimen of *Leptodactylus trinacria* sp. nov. exhibiting a pale upper lip stripe extending from the posterior corner of the eye to base of arm. Photo by Christian Reymondin.

sometimes poorly developed. Other Venezuelan *Leptodactylus* with lateral toe fringes are *L. colombiensis* Heyer, 1994; *L. diedrus* Heyer, 1994; *L. leptodactyloides* (Anderson, 1945); *L. magistris* Mijares-Urrutia, 1997; *L. pascoensis* Heyer, 1994; *L. riveroi* Heyer & Pyburn, 1983; *L. sabaensis* Heyer, 1994; and *L. validus* Garman, 1888.

Leptodactylus trinacria sp. nov. most closely resembles *L. colombiensis*, which also has dorsolateral folds that are irregular and often interrupted. *Leptodactylus colombiensis* is a large-size species (SVL: females 38-62 mm, males to 36-56 mm) in which lip stripes, if present, extend only from the mid-eye level posteriorly. Most *L. colombiensis* individuals have some belly mottling (almost immaculate posterior belly in *L. trinacria* sp. nov.) and swollen toe tips (toe tips little or no expanded). Thigh length in *L. trinacria* sp. nov. is larger than that of *L. colombiensis*: males of *L. trinacria* sp. nov. with a mean value of 48.8% SVL (range 47%-51%), while *L. colombiensis* has a mean value of $43 \pm 3\%$ (range 37%-50%); the single female of *L. trinacria* sp. nov. has a shank length of 49% SVL, while that of females of *L. colombiensis* is $43 \pm 3\%$ SVL (range 37%-50%).

The advertisement call of new species has a higher dominant frequency (2.4 kHz vs. <2.0 kHz) and a faster calling rate (see below). The new species differs in bioacoustics from *Leptodactylus colombiensis* by having a higher dominant frequency (2.4 kHz vs. 1.47–1.98 kHz in *L. colombiensis*), lacks the low-frequency initial pulse character-

istic of *L. colombiensis* and exhibits a significantly higher dominant frequency (2.4 kHz vs. 1.47–1.98 kHz). The note repetition rate of the new species (~240 calls/min) is nearly seven times faster than that of *L. colombiensis* (~36 calls/min) and remains one of the highest recorded for any species within the *L. melanonotus* group (see Carvalho *et al.* 2022). This accelerated repetition rate remains an exceptional diagnostic feature within the *L. melanonotus* group.

Comparisons with other *Leptodactylus* species having toe fringes (except for *L. latrans* and its closest relatives): *L. bolivianus* Boulenger, 1898, is a larger species (SVL: females to 88 mm, males to 94 mm) and the dorsolateral folds are smooth, regular, and long. In the larger (SVL: females to 81 mm, males to 63 mm) *L. riveroi* Heyer & Pyburn, 1983, the entire upper lip and loreal region often have a broad and well-defined pale stripe. *Leptodactylus diedrus* lacks dorsolateral folds and the toe tips are usually expanded into small discs. The ventral and posterior thigh patterns do not merge but rather are in close contact. *Leptodactylus griseigularis* (Henle, 1981) is a slightly larger species (SVL: females 39-58 mm, males 35-51 mm) with indistinct pale lip stripes, and presence of large black thumb spines. *Leptodactylus leptodactyloides* specimens commonly have a thigh pattern with distinct pale stripes, and few specimens (10%) also have pale-spotted chins. Almost all specimens have melanophores on the belly resulting in a finely mottled pattern. *Leptodactylus natalensis* Lutz, 1930 occurs along coastal Brazil from the State of Rio Grande do Norte to the State of Rio de Janeiro; over half of the specimens of this species have toe tips larger than the narrow or just-swollen categories. The light posterior stripe is just distinct in some (9%) specimens. *Leptodactylus nesiotus* Heyer, 1994 from Trinidad is a smaller species (males 32-33 mm SVL) and has a broad pale stripe on the entire upper lip or at least to under the eye. *Leptodactylus magistris*, from a relatively isolated mountain range in Falcón State, Venezuela, shows keels on fingers II and III, a pale white line on lips with well-defined borders and a weak tarsal fold. *Leptodactylus pallidirostris* Lutz, 1930, from the Guianan shield region, is a smaller species (females 30-43 mm SVL, males 28-37 mm SVL). Belly bearing a fine mottling to dark blotches. Toe-tips expanded or with small discs. *Leptodactylus pascoensis*, from Amazonian flanks of the Andes in central Peru, is a larger species (SVL: females 52-67 mm, males 60-61 mm). Posterior pale lip stripes absent. Toe tips swollen or just swollen. Males with two large black thumb spines. *Leptodactylus petersi* (Steindachner, 1864), from greater Amazonia and the Guiana shield region, including Venezuela, has a belly pattern usually extensively mottled in an anastomotic pattern.

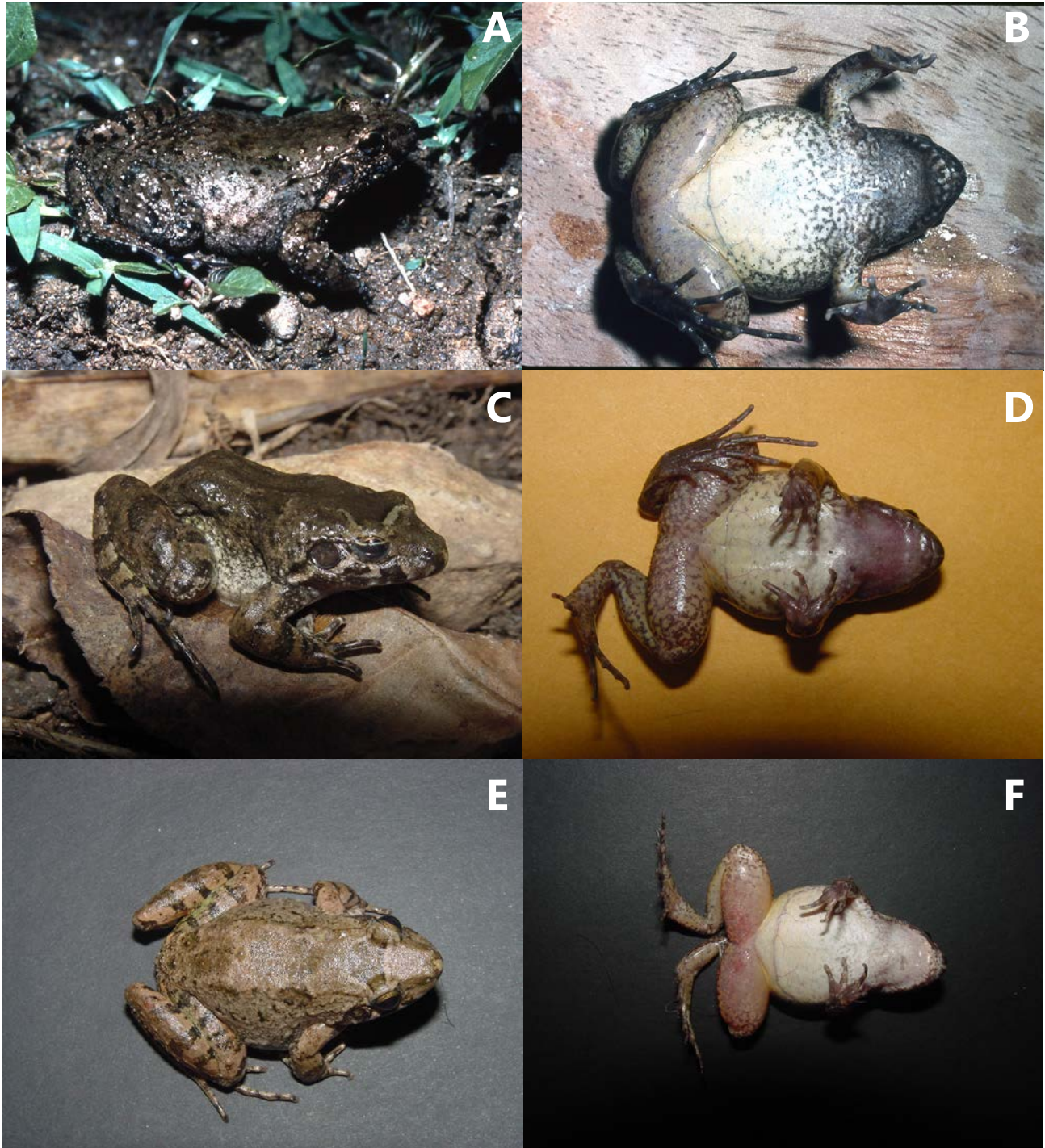


Figure 5. A-F. Specimens of *Leptodactylus trinacria* sp. nov. to show the slightly expanded or knob-shaped swollen toe tips. Photos: Enrique La Marca.

Toe tips usually just swollen (62%), some with swollen or just-expanded toe tips (34%). Posterior lip stripe usually (44%) indistinct to not discernible (43%). *Leptodactylus podicipinus* (Cope, 1862) occurs in Amazonia in Brasil, Bolivia, Paraguay and northeastern Argentina. It often has distinct pale belly spots. *Leptodactylus sabanensis*, from La

Gran Sabana, Venezuela has the most common pale lip stripe condition indiscernible, and, when discernible, they extend from the posterior corner of eye. *Leptodactylus validus* occurs in Guiana shield region, Trinidad, Tobago, and Lesser Antilles. In those individuals with discernible lip stripes, the stripes extend from the posterior corner of

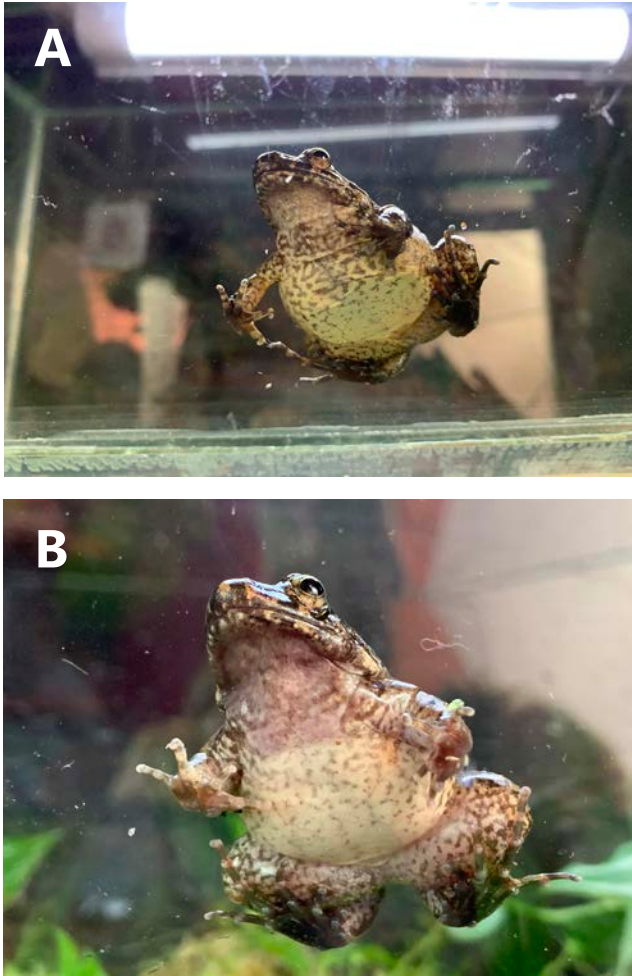


Figure 6. Ventral color pattern of (A) female and (B) male of *Leptodactylus trinacria* sp. nov. to show the pale ventral mottling that is fading posteriorly. Note that the lower jaw is covered with pale spots. Photos: Enrique La Marca.

the eye posteriorly. *Leptodactylus wagneri* (Peters, 1862), is a larger species (males 39-61 mm SVL, females 52-82 mm SVL) with long dorsolateral folds, and has moderately to extensively mottled bellies. In *L. trinacria* sp. nov. all discernible lip stripes extend from the posterior corner of the eye and the belly pattern is moderately mottled.

Description of holotype

Snout sub-ovoid in dorsal view; tip of snout rounded in dorsal and lateral views. Head 0.1 times wider than long. Inter-orbital space relatively smooth, without tubercles. Interorbital distance 0.6 times larger than upper eyelid width. *Canthus rostralis* straight, not well-defined. Loreal region slightly concave, descending smoothly to lips. Lips not flared. Nares elevated. Nares directed dorso-laterally and tilted about 45 degrees back and upward. Nares clos-

er to the tip of snout than to the anterior border of eye, about 62% of the eye-to-nares distance. Horizontal length of eye approximately 1.3 times the eye diameter and 1.1 times eye-to-nares distance. Horizontal length of eye 3 times larger than eye-to-tympanum distance. Internarial distance slightly shorter (0.9 times) than the eye-to-nares distance. Tympanum rounded, distinct, with a conspicuous tympanic annulus. Tympanum diameter 2.4 times larger than eye-to-tympanum distance. Supra-tympanic dermal fold distinct, covering the posterior part of eye to arm insertion. The post commissural gland is about twice as long as wide, single on left side and divided by a deep trench on the right side. Vocal sac single, internal, no external modification visible. Vocal slits well-developed, on posterior part of mouth, about 0.63 times shorter than the tongue length. Tongue $\frac{3}{4}$ larger than wide. Tongue sub-ovoid, about 30% adherent to floor of mouth, with posterior border round and slightly notched. Choanae sub-triangular, visible, not concealed by palatal shelf of maxillary arch; widely separated between them (about 6 times the width of a choana). Vomerine teeth in two long and almost straight series posterior to and almost entirely between choanae; odontophore processes elevated, slightly sub-triangular, approximately same width as length, separated between them about $\frac{1}{3}$ of its width. Maxilla and pre-maxilla toothed; small teeth.

Dorsum smooth, bearing pale pointed tubercles of various size; some tubercles faintly keratinized and more abundant towards the posterior region, particularly over the urostyle region. Dorsal folds absent. Skin of chest, venter and throat, smooth. Skin of venter, chest and throat, smooth. Chest spines absent. Ventral disc fold distinct. Arms not hypertrophied. Elbow and lateral posterior part of forearm with minute keratinized spicules. Ventral, external and lateral surfaces of forearm with small, pointed tubercles weakly keratinized. Ulnar ridge absent. Thenar tubercle conspicuous, oval, and approximately twice larger than wide. Palmar tubercle lobate, low-elevated and less prominent than thenar one. Supernumerary tubercles absent. Sub-articular tubercles elevated and rounded. Finger tips not expanded. Fingers without webbing. Keratinized spicules on the internal lateral surfaces of fingers II and III. Ridges and keels absent on lateral sides of fingers. First finger slightly larger than second. Finger lengths $II=IV < I < III$. First finger with two black nuptial conical spines; distal spine broader than proximal one, both relatively moderate in size and about 0.8 mm in length.

Cloacal opening well above the mid-line of thighs, covered by a short and crenulate dermal fold with shallow striae, giving a radiated appearance to the cloacal region. Dorsal skin on thighs and tarsi with numerous low-kerat-



Figure 7. Female specimen of *Leptodactylus trinacria* sp. nov. exhibiting a greenish tinge in groin and on concealed anterior and posterior parts of thighs, and ventrolaterally at the posterior part of flank. Same specimen as Figs. 5 A, B. Photo: Enrique La Marca.

tinized small and pointed tubercles. Thighs and shanks ventrally smooth; tarsi and metatarsi bearing dorsal and ventral small pointed tubercles scarcely or non-keratinized. A row of weakly keratinized small pointed tubercles from base of toe V to external border of the metatarsus and tarsus. A relatively large postcommissural gland, 2.6 mm in length, on posterior edge of lips. Ventrolateral gland bordering anterior 2/3 of belly. No other glands evident. Tarsal fold conspicuous, extending 7/9 distance of tarsus. Tarsal fold distinct, with numerous minute and weakly keratinized spicules on and along the medial and posterior portion of fold. Tarsal fold extending along tarsus, connecting with a membranous flap along the internal border of toe I. External metatarsal tubercle slightly oval (1.25 times larger than wide), elevated and truncate in lateral view. Internal metatarsal tubercle sub-spatulate (narrower at proximal portion), 2.5 times larger than wide in the middle, and about 2.5 times larger than the size of external metatarsal tubercle. Tarsi and soles of feet with several small, heterogeneous-size white tubercles, several tan-tipped. Subarticular tubercles large, round to oval, and round in lateral view. Toe-web formula: I1- ½ III1- ½

III ½ - ½ IV1- 1V. Toe tips slightly swollen. Toes bearing membranous keels and well-developed lateral folds. Tibio-tarsal articulation reaching to arm insertion when leg adpressed forward.

Measurements of holotype (in mm)

Snout to vent length 36.2, thigh length 16.5, shank length 17.8, head width 12.9, head length 12.0, tympanum diameter 2.7, eye diameter 3.4, eye to nostril distance 2.5, internarial distance 3.1, hand length 9.2, foot length 20.8.

Coloration of holotype in life

Throat dark gray; venter “dirty” white with a yellow tinge on the posterior end; groin with a yellowish-green wash. Dorsum dark olive-brown, with cream-colored areas delimiting a dark, cross-shaped pattern. Head with an inverted dark interorbital triangle bordered anteriorly by a narrow cream band. Anterior concealed surfaces of the thighs exhibit a faint reddish-orange tinge. Labial region with alternating cream and dark brown bands. A distinct cream-colored post-tympanic band extends from the low-

er posterior margin of the tympanum to the insertion of the arm (most prominent on the right side of the head).

Coloration of holotype in ethanol 70%

Dorsum marbled; large dark brown blotches on paler background, bordered by dark brown. Blotches more distinct posteriorly and at interorbital region. Irregular pale interorbital band, black-bordered posteriorly. A pair of narrow dark brown bands from the middle of upper eyelids converging diagonally to mid-posterior head, forming an interrupted triangle. Upper lip with wide alternating dark and pale brown bands, most posterior one separated from tympanum by a very narrow dark band. Tympanum pale brown; uppermost parts of tympanic annulus cream, surrounded by dark brown. Tip of snout cream, dorsally and laterally bordered by dark brown. Posterior articulation of mouth has postcommissural gland cream colored. Edge of chin dark with small irregular pale dots. Throat nearly uniformly brown; chest and belly cream, with irregular small dark brown blotches, especially on anterior part of belly. Ventral part of forearms are dark brown, except towards the anterior part near the arm insertion. Black (melanized) thumb spines on right hand, dark brown on left hand. Lower part of legs with irregular brown blotches on a pale-cream background, resembling anterior belly pattern. Palms brown. Inner metatarsal tubercle cream, bordered by dark brown. Under parts of fingers pale brown, with cream subarticular tubercles. Finger pads cream. Palms dark brown, with pale supernumerary tubercles. Toe pads cream to pale-brown. Metatarsal fold, toe fringes and toe lateral flaps tend to be cream to pale brown. Upper parts of extremities, except on forearms, banded; banding extends onto dorsal surfaces of fingers and toes. Supraclavical fold cream. Posterior surfaces of thighs with irregular pale and dark brown markings; no conspicuous pale or cream bands on thighs. Dark brown band-like pattern on posteroventral surfaces of thighs. Brown ventrolateral glands. Cloacal opening surrounded by dark brown. Testicles cream.

Variation within the type series

Dorsolateral folds never form a continuous uninterrupted line. Folds, in type specimens, are absent in 20%, are moderate in length in 30%, and long in 50%. Male specimen ULABG 4876 have several characters that depart from the description of the holotype as follow: eye diameter 2.5 larger than distance from eye to tympanum; tongue 50% free from floor of mouth; choanae separated among them by approximately three times the width of a choana; dorsum of arm and forearm with few spicules; arm with a single row of five to six not keratinized spicules;

palmar tubercle slightly heart-shaped; thick lateral keel on fingers II, III and IV, although less conspicuously on internal side of finger I; cloacal opening bordered by a striated series of thin and shallow lines forming a radiated pattern; tarsus and metatarsus smooth; foot web reaches the first tubercle on proximal side of first toe, and the first tubercle on distal side of toe III; outer border of toe V with a cutaneous keel that extends from distal end of toe to the base of the outer metatarsal tubercle. ULABG 4794 has odontophore processes that are about twice wider than long and a slightly conspicuous lateral ridge along the inner side of the first finger. In female specimen ULABG 4880 the choanae are separated between them by approximately 3 times the width of a choana. Thick lateral ridges present on all fingers. Thenar tubercle elongated. Thick fold from the base of the thenar tubercle, which connects with a thick ridge that runs along the outside of the 4th finger. Toe-web formula: I1- 1III1- ½ III1 - ½ IV1- 1V. It presents a rounded protuberance on the back, at the level of the anterior part of the sacrum (probably a parasite) embedded in the skin (Fig. 1C), without reaching the muscles.

Morphometric variation of complete type series, including holotype

Female 44.5 mm SVL, males 35.4-40.0 mm SVL ($x = 37.4 \pm 1.43$); female head length 14.9 mm, male head length 11.5-13.3 mm ($x = 12.3 \pm 0.6$); female head width 16.2 mm, male head width 12.0-14.1 mm ($x = 13.2 \pm 0.6$); female tympanum diameter 3.3 mm, male tympanum diameter 2.5-3.3 mm ($x = 2.87 \pm 0.25$); female thigh length 21.8 mm, male thigh length 16.9-19.8 mm ($x = 18.3 \pm 1.06$); female eye diameter 4.2 mm, male eye diameter 3.0-4.1 mm ($x = 3.5 \pm 0.3$); eye-to-nostril distance in females 4.0, eye-to-nostril distance in males 2.5-3.8 mm ($x = 3.3 \pm 0.4$); inter-nostril distance in female 4.0, inter-nostril distance in males 3.1-4.0 mm ($x = 3.4 \pm 0.3$); female hand length 11.0 mm, male hand length 8.3-10.3 mm ($x = 9.24 \pm 0.7$); female foot length 23.3 mm, male foot length 14.1-21.3 mm ($x = 20.1 \pm 2.27$). Measurements for every individual of the type series is provided in Table 1, and a summary of statistics for size and other selected measurements is given in Table 2.

Color variation

Paratypes, in life, had dorsum olive brown, with irregular dark brown blotches. A dark, incomplete inverted triangular blotch, with apex directed backwards, usually present and located between and behind eyes, always preceded by a cream inter-orbital narrow band. Lips barred with dark and pale brown bands. Pale stripe on the posterior lip region (cf. Heyer 1994: 30, Fig. 11) rather broad

Table 1. Selected measurements (in mm) of the type series of *Leptodactylus trinacria* sp. nov. Left column indicates museum catalog number at the Collection of Amphibians and Reptiles of the Biogeography Lab at University of Los Andes in Mérida, Venezuela. The holotype is indicated with an asterisk. All male specimens, except ULABG 4880 that is a female. See methodology for abbreviations; additionally, “Rep. cond.” in the last column stands for the reproductive condition, exhibited as indicated by the numbers 1 (both vocal slits opened), 2 (pair of keratinized black thumb spines), 3 (darkened throat), 4 (gray throat), 5 (convoluted oviducts), 6 (only right vocal slit opened), 7 (gray throat with darkened borders towards lips), 8 (*corpora adiposa*, “fat bodies”, present) and 9 (presence of mature eggs, 1.2 to 4.5 mm in diameter).

Museum Nr ULABG	SVL	TL	HW	HL	T	EYE	EN	IN	HAND	FOOT	Rep. cond.
4871	38.5	18.5	12.9	12.0	3.2	3.6	3.1	3.3	9.0	20.5	1,2,3,8
4872	37.8	19.3	13.3	13.0	2.9	3.0	2.5	3.1	9.7	20.6	1,2,3,8
4873 *	36.2	17.8	12.0	12.0	2.7	3.4	2.5	3.1	9.2	20.8	1,2,3
4874	35.8	16.9	12.8	12.0	2.8	3.5	3.3	3.1	8.4	14.1	2,3,6
4875	37.8	19.2	14.1	12.0	3.3	3.6	3.1	3.7	9.6	20.9	1,2,7
4876	35.4	16.9	13.0	12.0	2.5	3.5	3.8	4.0	8.9	20.9	1,2,3
4877	37.8	18.7	13.3	13.0	2.8	3.2	3.6	3.5	9.8	21.3	1,2,3
4878	37.5	17.5	13.3	13.0	2.9	4.1	3.3	3.5	8.3	20.8	1,2,3
4879	40.0	19.8	13.8	13.0	2.7	3.6	3.4	3.5	10.3	21.2	1,2,3,8
4880	44.5	21.8	16.2	15.0	3.3	4.2	4.0	4.0	11.0	23.3	5,9

Table 2. Summary of statistics for size and other selected measurements (in mm) of type series of *Leptodactylus trinacria* sp. nov. Abbreviations as indicated in the methodology section.

	Females (1)		Males (9)	
	Value +/- SD	Variation range	Value ± SD	Variation range
SVL	44.5	44.5	37.4 ± 1.43	35.4 – 40.0
TL	21.8	21.8	18.3 ± 1.06	16.9 – 19.8
HW	16.2	16.2	13.2 ± 0.6	12.0 – 14.1
HL	14.9	14.9	12.3 ± 0.6	11.5 – 13.3
T	3.3	3.3	2.87 ± 0.25	2.5 – 3.3
EYE	4.2	4.2	3.5 ± 0.3	3.0 – 4.1
EN	4.0	4.0	3.3 ± 0.4	2.5 – 3.8
IN	4.0	4.0	3.4 ± 0.3	3.1 – 4.0
HAND	11.0	11.0	9.24 ± 0.66	8.3 – 10.3
FOOT	23.3	23.3	20.1 ± 2.27	14.1 – 21.3

and extends from just past mid-eye in 100% of type specimens, although it is not clearly defined on left side of head of ULABG 4872. In some very dark specimens from the Mérida terrace, not collected, the pale posterior lip stripe was almost undistinguishable in 6 out of 10 examined specimens. White dots on the edge of the lower lips. Iris golden to bronze, with brown reticulation, most pronounced on the lower half. Olive-brown golden, with irregular dark brown markings. Very dark throat in males.

Pale dots on a dark chin usually present. Groin with a slight yellow tinge. Iris golden to bronze, with a brown reticule more pronounced on inferior half (*e. g.*, Fig. 3G). Posterior extremities dorsally brown with dark brown bands, especially noticeable on thighs; legs ventrally marmoreal. Posterior surface of thighs with a yellow-greenish tinge sometimes bearing a yellow-green line. Although males have usually a darker throat coloration, it is not a useful character to distinguish between males and females

of *Leptodactylus trinacria* sp. nov. Some adult males indeed have paler throats than adult females in some of the additional specimens examined. In 80% of the type series specimens there is a noticeable dark blotch, resembling an incomplete inverted triangle between and posterior to eyes, more noticeable when looking specimens submersed in liquid. In darker specimens, this blotch tends to merge with the background color. Posterior limbs barred and upper parts of thighs, shanks and tarsi barred with alternating pale- and dark-brown bars.

Tadpoles

Body elongated and oval in dorsal view, depressed (wider than deep); widest at the beginning of 1/3 of body length and in the last 1/3 length body. Snout broadly rounded in dorsal view. Eyes directed dorsolaterally. Nostrils located dorsolaterally and directed anterolaterally, slightly closer to tip-of-snout than to eye and about 32% distance from tip-of-snout to anterior border of eye. Nostril-openings are rounded. Edges of nostrils with a ring. Interorbital distance 22% of the internarial distance. Chondrocranial elements not visible through skin of head. Spiracle is below the middle line on left side of body, evident and forming a short tube almost completely attached to skin of body. Spiracle opening located at about 56% of body length from tip-of-snout. Cloacal tube short, mediomarginal and with median opening. Dorsal fin arises at body-tail junction. Dorsal fin equal in depth to the ventral fin at mid length of tail. Length of caudal musculature equal to total tail-length. Caudal musculature strong, with well-developed myomeres. The longitudinal tail axis straight. Tip-of-tail rounded. Posterior legs intra-parallel with respect to cloacal tube. Posterior legs without dermal folds and toes distinct (Fig. 8A-C).

Coloration in preservative. Dorsum is brown. Upper surface of flanks same as dorsum; Venter pale brown with numerous melanophores. Tail fins pale brown (numerous minute melanophores when viewed in high detail). Tail musculature cream, with skin bearing minute melanophores, same as fins. Legs brown; finger and toes with alternating cream and brown bands. Lateral line system arising on cheeks, continuing along the inferior border of eye and along dorsolateral part of body (with a medial inflexion towards internal part of back. There is a similar parallel line below the later. Both lines continue over the tail as a series of small white dots to middle part of tail (Fig. 8A-C).

Coloration in life. Pale brown with a greenish tinge, with numerous little dark spots. Dark markings tend to coalesce on top of dorsal fin and along the medial lateral part from the middle to posterior portion of tail; iris pale gold (Fig. 8D).

Oral apparatus. Oral disc with keratinized parts, relatively small, directed anteroventrally at tip-of-snout. Oral apparatus medium-sized in relation to body width. Oral disc trans-angular (*i. e.*, without lateral inflexions). A single row of small and blunt alternating papillae, separated by a wide rostral diastema on anterior margin of the oral apparatus. Posterior border of the oral apparatus with papillae in a mixed configuration. Beaks (“queratostoma” of Mijares-Urrutia 1998) partially keratinized. Upper beak broadly arched, bearing narrow lateral extensions; lower beak broadly V-shape. Acute serrations on both beaks; serrations on lower beak stronger than serrations on upper beak. Two upper and three lower rows of keratodonts (“denticles”, tooth row formula 2/3); upper rows equal in length to lower rows; upper rows complete; first lower with an inconspicuous medial gap; second and third lower rows entire. Slightly larger keratodonts on second upper row. Keratodonts on all rows well keratinized (Fig. 9).

Measurements of tadpoles (in mm)

The following data applies to tadpole ULABG 4881, in stage 40 of Gosner:

BL= 1.7; TL= 10.5; BH= 5.0; END= 1.6; ESD= 2.6; ED= 1.1; SSD= 6.9; SDD= 4.0; CMH= 2.2; DFH= 1.4; FH= 1.2; ODW= 0.6; BW= 6.9; IOD= 2.3; IND= 1.7.

Data for metamorphosing froglet ULABG 4882, in stage 44 of Gosner, as follows:

SVL= 9.1; TL= 2.5; IN=1.1; Tail bud= 2.7; END= 1.1; EYE= 0.7; IOD= 1.5.

Bioacoustics

Advertisement call of the new species (Fig. 10) is characterized by short, impulsive notes exhibiting steep upward frequency modulation. Spectrographic analysis reveals that the primary energy of the call is concentrated between approximately 1.8 kHz and 3.0 kHz. The dominant frequency is centered at 2.4 kHz, appearing as a sharp, near-vertical trace on the spectrogram: a visual result of its abrupt rise time and remarkably short duration (30–50 ms). The calling rate is exceptionally high, with approximately 4 calls per second (240 calls/min). The oscillogram (Fig. 10, bottom) displays pressure fluctuations with high-intensity peaks corresponding to each impulsive note. When cross-referenced with the values in previous work (Table 2, Carvalho *et al.* 2022), several significant details emerge. With a dominant frequency at 2,400 Hz, the new species places at the high end of the range for *L. melanonotus* (2,132–3,141 Hz) and *L. podicipinus* (2,218–3,258 Hz) and it is notably higher than the *L. wagneri* recorded in the table (1,572–1,898 Hz) and in *L. colombiensis* values stated as 1,470–1,980 Hz (de Sá *et*

A



B



C



D



Figure 8. Tadpole of *Leptodactylus trinacria* sp. nov. ULABG 4481. **A:** dorsal view, **B:** lateral view, **C:** ventral view, **D:** free-living larva, coloration in life. Photos: Enrique La Marca and Michelle Castellanos.

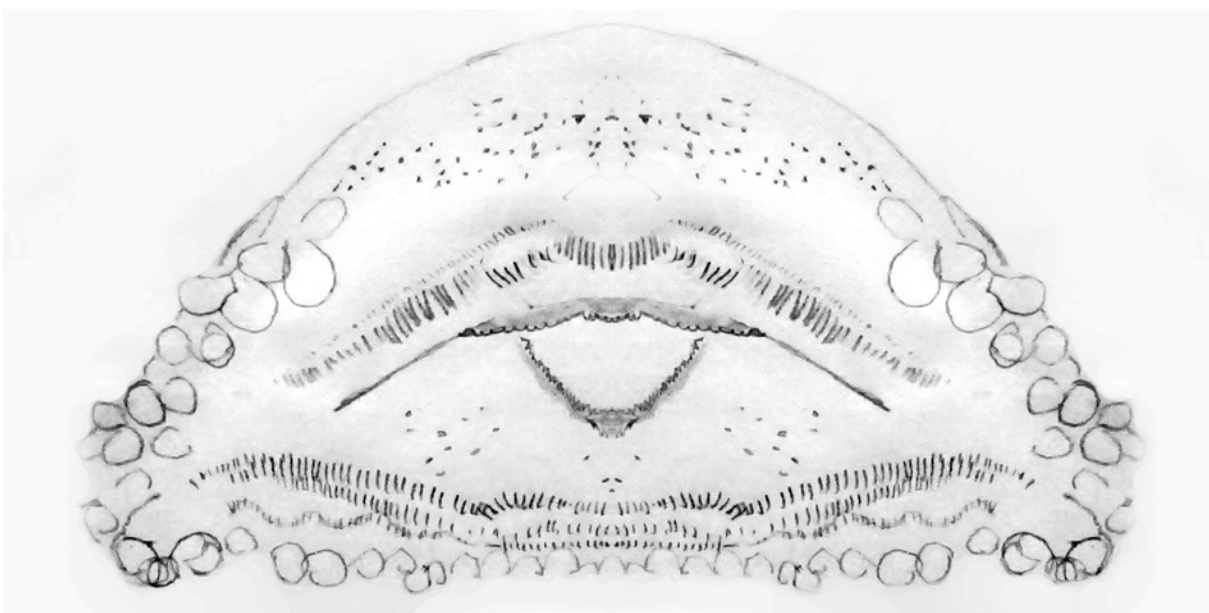


Figure 9. Mouth parts of tadpole ULABG 4481. From a pencil drawing by Enrique La Marca, with right part mirrored from left part due to slight deterioration of the later in the larval sample.

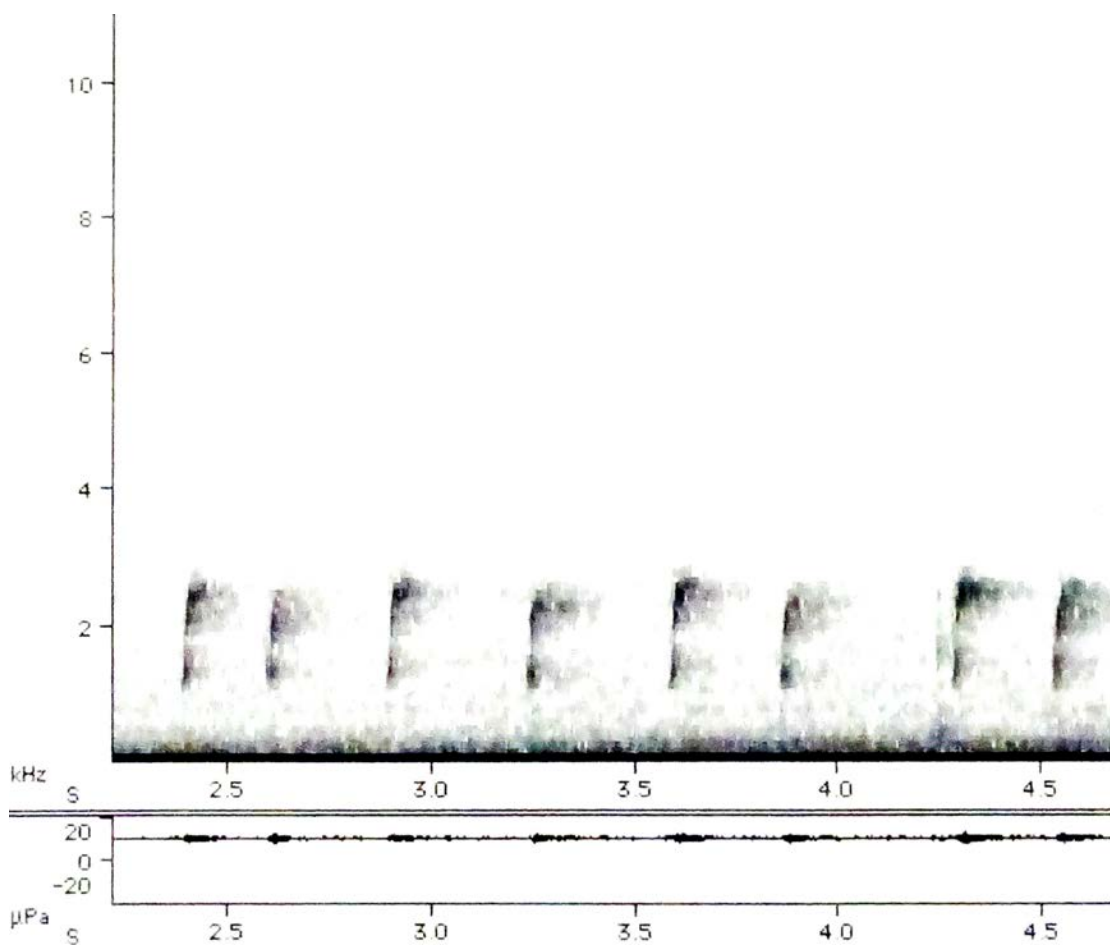


Figure 10. Audiospectrogram (above) and oscillogram (below) of an advertisement call of *Leptodactylus trinacria* sp. nov. from the type locality, recorded synchronously with collection of the type series. No time nor temperature registered.

al. 2014). Regarding the note repetition rate, the Mérida rate of ~ 240 calls/minute is exceptionally high. Only *L. brevipes* (150–330/min), *L. nesiotus* (264–288/min), and *L. natalensis* (163–296/min) reach such speeds; most other species in the group, including *L. wagneri* (50–55/min) and *L. colombiensis* (~ 36 /min), are significantly slower. As per the note duration, the 30–50 ms in duration for the Mérida frog is a perfect match for the “short-note” species such as *L. petersii* call type 1 (31–50 ms), *L. melanonotus* (28–48 ms), and *L. sabanensis* (29–45 ms).

Distribution

Populations of *Leptodactylus trinacria* sp. nov. have been found at several places in internal valleys of the Cordillera de Mérida close to the city of Mérida. Populations are known in inter-Andean valleys between the towns of Tabay and Ejido, along the mid valley of the Chama River, all within the metropolitan area of Mérida city. All these environments fall within the Life Zone of Premontane moist forest in the Holdridge’s system (Ewel *et al.* 1976), mainly dominated by seasonal semi deciduous forests, mostly intervened by human activities (Fig. 11). All surrounding environments are high elevation ecosystems dominated by cloud forests and paramo environments or

lower ones dominated by semiarid vegetation and tropical lowland humid forests.

There is a geographic distribution report for a *Leptodactylus* stated to come from “Amazonas: Río Pescado (= Sabana Grande), 100 m” (Heyer 1994: 114) that is obviously erroneous. The specimen, ULABG 1413, actually comes from the vicinities of Mérida city (see “Additional specimens” herein), and represents a *Leptodactylus trinacria* sp. nov.. It was listed within the “Venezuelan Andes OTU”, along with specimens from the states of Aragua, Barinas, Distrito Federal, Falcón, Guárico, Miranda, Táchira and Trujillo (Heyer 1994: 114). This grouping of what could actually be different evolutionary units may account for the resulting composite nature inferred for this taxonomic unit. For example, specimen UMMZ 216804, coming from Cerro Socopó (*sic*: Socopo), 1,260 m, most likely correspond to *Leptodactylus magistris*. Whereas, *L. trinacria* sp. nov. has not been collected in sympatry with any other member of the *L. melanonotus* species group. Within the *L. melanonotus* group, *L. colombiensis* is the geographically nearest species to *L. trinacria* sp. nov. *Leptodactylus colombiensis* is found in Colombia’s Caribbean drainages (Sá *et al.* (2014). Its presence in Táchira, Venezuela, previously noted by Barrio-Amorós & Chacón-

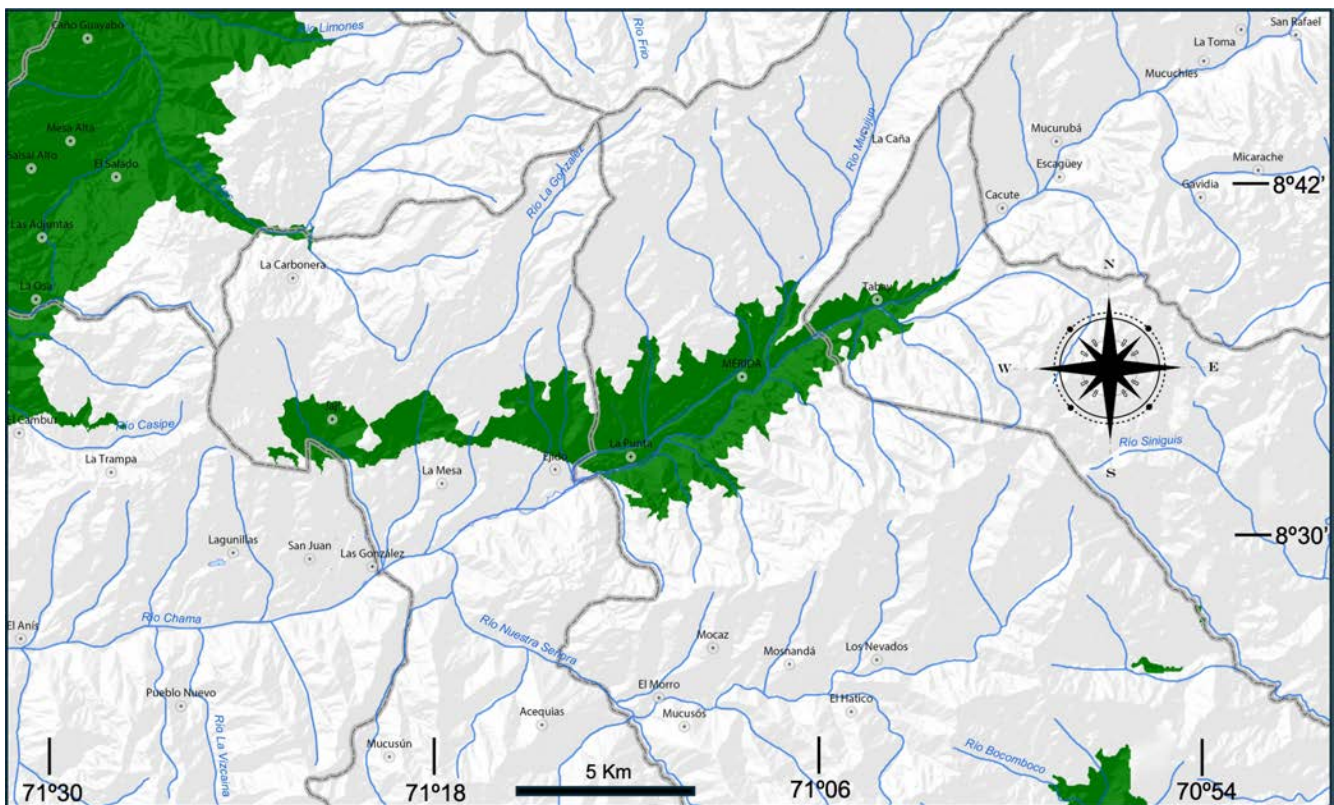


Figure 11. Distribution (center, in green) of the Premontane moist forest (Holdridge’s Life Zone) at Mérida city and surroundings, the region where all *Leptodactylus trinacria* sp. nov. specimens have been found.

Ortiz (2001), remains to be confirmed following de Sá *et al.* (2014).

Study area and natural history

Habitat and Ecology: The type locality (Fig. 12) is situated within the Premontane moist forest (Holdridge's Life Zone "bosque húmedo premontano" in Ewel *et al.* 1976). The region is characterized by a two-seasonal rainfall pattern: a primary dry season from late November to early March and a shorter, moderate dry period (locally termed "veranillo de San Juan") between June and August. High-precipitation peaks occur from early April to mid-May and again from mid-September to mid-November. Average annual temperatures range from 16.4°C to 17.8°C (García *et al.* 2007). Specimens of the type series were collected along the margins of an artificial lagoon in the city of Mérida. They were found among grasses growing on organic matter, *i. e.*, remnants of aquatic vegetation removed

during maintenance to prevent eutrophication. The site is currently bordered by urban residential areas. Some specimens were studied in swampy areas and within lentic pools of slow-moving rivulets in forest remnants at the Urbanización Campo Claro, on the Mérida terrace, 8°33'42"N, 71°12'46"W (Fig. 13). At the latter site, a freshwater crab (Pseudothelphusidae) was observed predated an unidentified tadpole, suggesting a potential predator for the species. At this locality, *Leptodactylus trinacria* **sp. nov.** was found to be sympatric with *Mannophryne collaris* (Boulenger, 1912).

Reproductive behavior

Specimens of *Leptodactylus trinacria* **sp. nov.** have been heard calling in the early morning (approx. 06:00 h), though calling may persist throughout the day during rainy periods; no nocturnal activity has been observed. Courtship behavior include inguinal amplexus, as observed in



Figure 12. Type locality of *Leptodactylus trinacria* **sp. nov.**, showing the artificial lake at Museo de Ciencia y Tecnología of Mérida city (within the former "Central Azucarero Los Andes", CALA), Mérida state, Venezuela, 1,320 m asl. Photo by Enrique La Marca on 18 February 2013.



Figure 13. Microhabitat of *Leptodactylus trinacria* sp. nov. at forest remnant in Urbanización Campo Claro, close to Río Albarregas, on the Mérida terrace. Photo: Enrique La Marca.

mating pairs maintained in captivity at the Rescue of Endangered Venezuelan Amphibian (REVA) ex situ conservation center in Mérida, Venezuela. The amplexus is inguinal and the male stimulates the female on the sides of the belly. During oviposition, the male on top of the female constructs a foam nest by beating the eggs and jelly with the hind limbs. These nests are constructed on the surface of lentic waters from where the tadpoles in early stages of development emerge (Fig. 14).

Larval biology and parental care

The larvae are exotrophic. Tadpoles exhibit larval schooling, moving together as a cohesive unit (Fig. 15), a trait shared with other members of the genus *Leptodactylus*. Furthermore, we observed larval attendance (parental care), where an adult female closely guarded batches of swimming tadpoles. This behavior has been documented both in the field and in captivity (La Marca 2017a, b).

Within the genus *Leptodactylus*, such parental care is otherwise only known in the closely related *L. latrans* group (de Sá *et al.* 2014).

Conservation status and legal framework

Leptodactylus trinacria sp. nov. represents the second member of the genus in Venezuela requiring immediate protection, alongside the non-Andean endemic *L. magistris*. With the data at hand, we are confident to state that *Leptodactylus trinacria* sp. nov. is an endangered species, specially within the limits of Mérida city. Overall, the populations of the new species have suffered a continuous decline in their original distribution area. The decline is mainly due to the reduced availability of suitable habitats because of human modification of their natural environment. This is an interesting case, joining to several others, in which a species is threatened with extinction even before being formally classified for science (La Marca

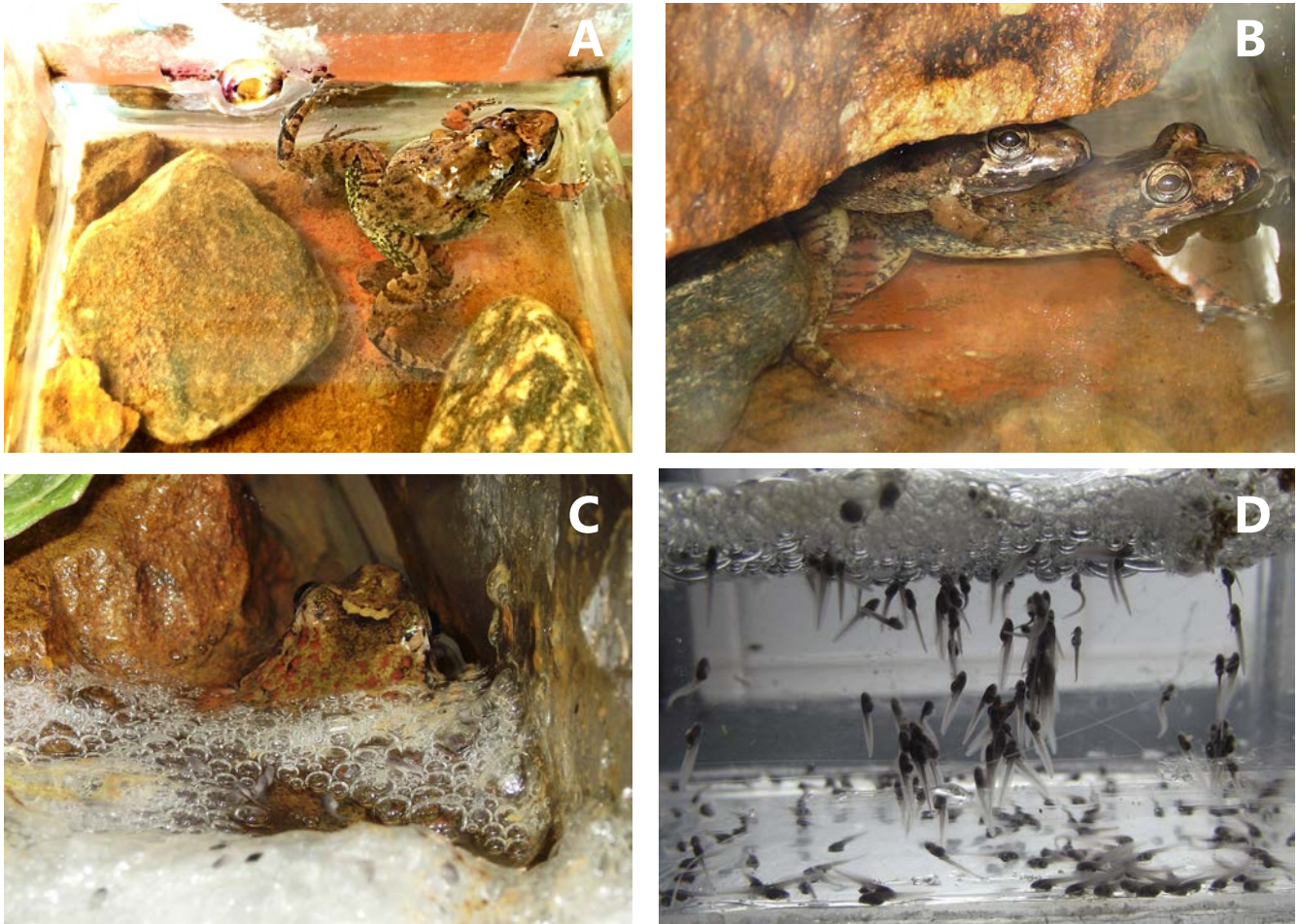


Figure 14. Reproductive behavior in *Leptodactylus trinacria* sp. nov. A: Amplexant pair (note inguinal amplexus). B: Male on top stimulates lateral sides of female. C: Female spawning after stimulation. D: Tadpoles emerging from foam nest. Photos: Enrique La Marca and Daniel Quihua. Archive of the Rescue of Endangered Venezuelan Amphibian (REVA) ex situ conservation center.

2017ab). No specific legislative measures have been enacted to protect the new species, nor is there official protection for its remaining habitats (La Marca 2016a, b). Before its formal description, the species was tentatively listed (La Marca 2015) in the Red Book of Venezuelan Fauna as Endangered (EN) B1ab(i,ii,iii). Based on more recent data, we propose an updated IUCN category of Endangered (EN) B1ab(iii,iv,v)+2ab(iii,iv,v).

Ex-situ initiatives and husbandry

In early 2014, an *ex-situ* conservation program was established at the Chorrros de Milla Zoological Park in Mérida (La Marca *et al.* 2015a, b). Founder individuals were sourced from forest remnants within the Mérida urban area. Following quarantine, adults were housed in large terraria designed to simulate their natural environment, featuring automated running water, native vegetation, leaf litter, and rock shelters. Environmental parameters –in-

cluding photoperiod, misting, and seasonal rainfall– were strictly regulated. Early reproductive efforts faced high larval mortality due to developmental diseases. However, after refining nutritional protocols and micro-ecological requirements, approximately two dozen froglets first successfully reached metamorphosis. Adults were maintained on a diverse diet of invertebrates, including *Drosophila melanogaster*, *Acheta domestica*, *Galleria mellonella*, *Tenebrio molitor*, *Sitophilus* spp., *Armadillidium vulgare*, and *Eisenia fetida*. Prey items were dusted with calcium and vitamin supplements, following established protocols already tested for *Mannophryne collaris* (La Marca 2016c, d). Larvae were raised using a specialized formula developed at the REVA Conservation Center (La Marca & Castellanos 2018a, b). Between 2014 and 2022, despite sociopolitical instability (La Marca & Castellanos 2017) and the COVID-19 pandemic (La Marca 2020b), the program successfully released several F1 generation of dozens



Figure 15. Larval schooling of *Leptodactylus trinacria* sp. nov. in the wild. Photo: Enrique La Marca.

of captive-born specimens into the Mérida Botanical Garden and a humid forest remnant in Campo Claro, near the Albarregas River (La Marca 2017a, b).

Habitat fragmentation and destruction

The primary threat to *Leptodactylus trinacria* sp. nov. is the near-total destruction of the seasonal premontane humid forests that once covered the Mérida terrace and other places at the middle Chama River basin. This degradation is largely driven by urban expansion and historical coffee plantations resulted in extensive deforestation. The species shares this precarious situation with the sympatric *Mannophryne collaris*; both serving as flagship taxa for the vanishing ecosystems of the Mérida terrace (La Marca, 2017a,b).

Emergent diseases

Analysis of museum specimens (ULABG 4871, 4873, 4875, 4878) tested negative for *Batrachochytrium dendrobatidis* (Bd). However, a *Leptodactylus* specimen (ULABG 4269) from nearby Chiguará tested positive (Lampo

et al. 2005, 2006). While the identity of the Chiguará specimen remains to be fully confirmed, the geographic proximity of this population suggests that the new taxon is likely exposed to the pathogen. It is currently unclear whether the relatively high ambient temperatures of its habitat mitigate fungal pathogenicity or if the populations possess a degree of resistance.

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Collecting permit (“Licencia de caza con fines científicos”) was issued by the Oficina Nacional de Diversidad Biológica, of the former Ministerio del Ambiente y de los Recursos Naturales Renovables, of the República Bolivariana de Venezuela, Oficio N° 41-0296 of 6 May 2001. Institutional permits for the captive breeding facilities for *Leptodactylus trinacria* sp. nov. were issued by the Ministerio del Poder Popular para el Ecosocialismo (formerly Ministerio del Ambiente y de los Recursos Naturales Renovables), under Providencia No. 0356 (17 October 2016); and the installation and operation of these facilities were officially registered under number DGDB-0002.

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