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Two new species of *Elachistocleis* Parker, 1927 (Anura: Microhylidae: Gastrophryninae) from Colombia

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Abstract

The genus *Elachistocleis* consists of small to medium size species of Neotropical microhylids. It is the second largest genus of New World microhylids and an important component of the anuran diversity in the Neotropical region. Herein, based on morphological, acoustical, and molecular evidence, we describe two new species and their advertisement calls. The new species have reticulated venter and inhabit the Orinoco basin of Colombia.

Key words: *Elachistocleis*, new species, Colombia, cryptic species, Gastrophryninae

Introduction

The genus *Elachistocleis*, Parker 1927 currently consists of 20 described species (Frost 2020) representing the second most diverse genus of Neotropical microhylids; their combined distribution extends from Panama to south-eastern Brazil and Uruguay and occurring also in the island of Trinidad (de Sá *et al.* 2012). Ventral color patterns are commonly used to describe new species and were used to cluster *Elachistocleis* into two species groups: one with immaculate ventral surfaces, a narrow femoral stripe, and no inguinal spots, e.g. *E. bicolor* group, and the other one with ventral surfaces mottled, vermiculated, or blotched, a broad femoral stripe, and inguinal spots; in addition, this phenotype can also have markings on posterior surface of knees and/or feet, e.g. *E. cesarii* group (Caramaschi & Jim 1983; Lavilla *et al.* 2003).

As currently defined, *Elachistocleis* is monophyletic and a relatively young clade (~23 my) of Neotropical anurans (de Sá *et al.* 2012). *Elachistocleis* is the sister group to the North American radiation consisting of the genera *Hypopachus* and *Gastrophryne* (de Sá *et al.* 2012, Streicher *et al.* 2012). *Elachistocleis* species are mostly fossorial which could explain overall conserved external morphology across species due to environmental constraints. The traits exhibited by *Elachistocleis* (e.g., fossoriality, morphologically conserved, with high genetic distinctiveness of populations) fit the general concept of a "cryptic species complex", i.e., two or more genetic independent lineages that are morphologically similar and placed under the same species name (Struck *et al.* 2018).

Caramaschi (2010) careful assessment of the taxon *Elachistocleis ovalis* (Schneider, 1799) concluded the assigned name to the species to be a *nomem dubium* (i.e., doubtful name) applied to a *species inquirenda* (i.e., species of doubtful identity). Consequently, although he assigned names to five populations, this precise action left other populations across the genus geographical range as *Elachistocleis* sp. Herein, we use molecular, morphological, and acoustic information to describe and name two new species from current *Elachistocleis* sp. populations with reticulated belly, a broad femoral stripe, and inguinal spots in Colombia.

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Material and methods.

Specimens were euthanized using Benzocaine 2% (Chen & Combs 1999), fixed in 10% formalin, and preserved in 70% ethanol. Specimens, tissues and comparative material used herein are deposited in the biological collections of the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia (IAvH-Am). Other specimens examined are listed in Appendix 1. We follow previous criteria for the definition of descriptions and diagnostic characters (Duellman 2001; Sánchez-Nivicela *et al.* 2020). Measurements were taken with a digital caliper under a stereomicroscope to the nearest 0.1 mm: SVL (snout-vent length), HL (head length, from snout to angle of the jaw), HW (head width, between the angle of jaws), ED (eye diameter, between anterior and posterior corner of the eye), IOD (inter-orbital distance, distance between anterior corner of the eyes), IND (inter-nostril distance), END (eye-nostril distance, from the anterior corner of the eye to the posterior margin of nostril), THL (thigh length, from the center of the cloaca opening to the outer edge of the flexed knee), TBL (tibia length, from the outer edge of the flexed knee to the heel), FL (foot length, from tibio-tarsal articulation to tip of fourth toe), HDL3 (hand length, from the base of the thenar tubercle to the tip of the third finger), HDL4 (hand length from the base of the thenar tubercle to the tip of the fourth finger), FAL (forearm length), FD3 (diameter of third finger disk), FT4 (diameter of fourth toe disk). Fingers and toes are numbered and abbreviated as follows: Fingers I–IV = FI–IV, Toes I–V = TI–V.

Bioacoustical analyses. Calls were recorded using a Marantz PMD670 digital recorder with a unidirectional Sennheiser MKH 60 P48, positioned about to 1 m from the calling males. We used a digital thermohygrometer EXtECH Instrument Model 445702 to record air temperature and humidity for each recorded individual. Acoustic analysis was conducted using Raven Pro 1.5 (Charif *et al.* 2010) for Windows to measure temporal and spectral traits of the advertisement calls using FFt (Fast Fourier transformation) = 256 and overlap = 50 software with a sampling frequency of 44.1 KHz and 16–bit resolution, and the package Seewave (Sueur *et al.* 2008) in R (R Core Team 2014) to create graphs. The criteria for the definition and descriptions of acoustic parameters included temporal and spectral variables following previous work (De La Riva *et al.* 1996; Kwet & Di Bernardo 1998; Lavilla *et al.* 2003; Toledo *et al.* 2010; Nunes De Almeida & Toledo 2012; Da Fonseca *et al.* 2012; Pereyra *et al.* 2013; Köhler *et al.* 2017; Marinho *et al.* 2018; Emmrich *et al.* 2020). The measured temporal variables included the following: calls (main acoustic unit in a frog vocalization), notes (smaller subunits of calls separated from one another), number of notes per call (number of distinct acoustical signals recognizable in a call), note rate (number of notes per minute), note duration (duration individual note in seconds), pulses (number of sound waves contained in each note), and inter-note interval (from the beginning of one note to the beginning of the next note). The spectral structure was evaluated with the dominant frequency (frequency of highest energy determined by the Fourier calculation), and the number of harmonics (number of harmonic frequencies present in the call). All advertisement calls are available and deposited in figshare (for identification and use by other researchers, call collection numbers are provided along the call descriptions).

Molecular analyses. Total genomic DNA was extracted from ethanol-preserved liver or muscle tissues using Qiagen DNeasy kit (Valencia, California, USA). We used four molecular markers (mtDNA: 12S, 16S; and nDNA: brain-derived neurotrophic factor [BDNF] and Seven in absentia (SINA), amplified using previously published primer sets and PCR profiles (de Sá *et al.* 2012; de Sá *et al.* 2019). Sequences were deposited in Genbank with the following accession numbers: MZ018667–MZ018675, MZ092897– MZ092916, MZ101348– MZ101349, MZ101634– MZ101635, MZ130956– MZ130963 MZ133760– MZ133775. We performed a multiple loci alignment using an iterative procedure to compute a series of alignment/tree pairs in SATé-II (Liu *et al.* 2012), using default settings. Uncorrected pairwise genetic distances were calculated for a segment of 527 bp of the 3' part of the 16S gene in MEGA7 (Kumar *et al.* 2016).

Phylogenetic analysis. We included molecular data from *Elachistocleis* species with geographic distribution overlapping or adjacent to the new species such as *E. pearsei*, *E. panamensis*, and *Elachistocleis*. sp. (two populations formerly known as *E. ovalis*). We concatenated the genes into a supermatrix and ran a Maximum Likelihood phylogenetic estimation using RAxML v8 (Stamatakis 2014) in the Cyberinfrastructure for Phylogenetic Research (CIPRES; Miller *et al.* 2015). We used the GTR + CAT model with 100 bootstrap replicates. Bootstrap values higher than 80 were considered as good support. Trees were visualized and edited using FigTree. Figure 11 shows relationships of the two new species..

Results

The integration of molecular phylogenetic, species specific analysis of acoustic vocalization, and morphological analyses recovered two new species of occurring in the "Llanos Orientales" of Colombia, an ecosystem of plains, 200–600 mts above sea level, consisting of a savanna like vegetation that also extends into Venezuela and Brazil. The new species exhibit overall standard body size and traits previously associated with the genus *Elachistocleis* and in the phylogenetic analysis the species clusters with other species in the genus.

Elachistocleis sikuani sp. nov.

(Figures 1, 3)

Holotype. IAvH-Am–11748 (field number ARA 5924. Figs 1, 3) an adult female collected on 3 December 2010 by Andrés R. Acosta-Galvis and Luis Daniel Prada.

Type locality (Fig. 2). Colombia, Casanare Department, Maní Municipality, vereda El Viso, Caracaro Farm, a locality that is part of the "Llanos Orientales". 04°5'19.8"N,072°21'56.6"W, 191 m a.s.l.

Paratypes (6 specimens) (Fig. 3, Table 1). IAvH-Am–13074, adult male, IAvH-Am–13071–13073 and IAvH-Am–13075, adult females, collected on 13 May 2016 by Andrés R. Acosta-Galvis, Rafael O. de Sá, Travis Cuddy, and Luis Daniel Prada in Colombia, Casanare Department, Maní Municipality, Guaranito Farm, a locality that is part of the "Llanos Orientales", 04°53'42.8"N,0 72°22'0.5"W, 187 m a.s.l., IAvH-Am–11791 adult male collected on 20 April 2011 by Andrés R. Acosta-Galvis in Colombia, Casanare Department, Orocué Municipality, Vereda Cumaco, site Cachicamo, Mare Stream 4°49'34.79"N, 71°46'14.35"W, 152 m a.s.l.

Referred specimens (3 specimens). IAvH-Am–11747 an juvenile, collected on 26 November 2010 by Andrés R. Acosta-Galvis and Luis Daniel Prada., same locality as the holotype, IAvH-Am–11364 adult female, IAvH-Am–11365–11366 juveniles, collected on 19 November 2013 in Colombia, Casanare Department, Maní Municipality, Vereda Santa María, Corocito Farm, a locality that is part of the "Llanos Orientales". 04°38'11.2"N,0 72°05'31"W, 153 m a.s.l.

Etymology. The specific epithet *sikuani* refers to the native south American ethnic groups of Sikuani, Guahibo, or Jivi (Jiwi) who inhabit the Llanos del Orinoco, between the Guaviare, Meta, and Arauca River basins, area that includes the distribution range of the new species.

Diagnosis (Figs. 1, 3). A medium size species of *Elachistocleis* (males SVL= 27.3–28.8 mm, χ = 28.0 1.1 mm, females SVL = 28.7–33.2 mm, χ = 31.6 1.8 mm), diagnosed by the following combination of characters: (1) an ovoid body form, (2) triangular head, slightly wider than long, (3) a barely developed and complete occipital fold, (4) snout rounded in lateral and dorsal views, (5) *canthus rostralis* slightly concave and loreal region is convex, (6) nostrils laterally oriented, (7) post-commissural glands well defined, (8) tympanic annulus and membrane absent, (9) dentigerous process of vomer absent, (10) upper jaw projects beyond the lower jaw, (11) tongue large and ovoid, occupying the entire buccal floor, (12) choanae ovoid, large, and widely separated, (13) arms slender without tubercles on forearm, (14) hands without interdigital membranes and supernumerary tubercles, (15) finger lengths are I<II<IV<III, (16) subarticular tubercles ovoid, as wide as fingers, (17) thenar tubercle prominent and palmar tubercle bilobate, (18) foot lacks interdigital membranes and supernumerary tubercles, toes lack discs, (19) inner plantar tubercle equal in size to subarticular tubercles, (20) relative toe lengths I<II<V<III<IV, (21) THL is slightly longer than TBL, (22) dorsal surface of body with sparse dermal spines, (23) cloacal area lacks glands and tubercles, but bears poorly distinct dermal spines, (24) in preservative dorsal coloration dark gray to dark brown with scattered cream spots, (25) ventral surfaces of fore and hind limbs mottled, (26) mid-dorsal line absent, (27) light colored, broad, and irregular femoral stripe on posterior surface of thighs, (28) axillae with a light color spot, (29) groin light spotted, (30) all toes fringed and slightly webbed in females and male, (31) dermal spines absent on ventral surfaces, except on chin of adult breeding males.

Description of Holotype (Fig. 1). Body small (SVL = 31.6 mm), slender, and slightly ovoid, head triangular in shape, broader than long (Table 1), snout short, snout tip rounded, nostrils located closer to the tip of snout than to the eye, slightly protuberant, directed laterally, inter-nostril distance smaller than eye–nostril distance and smaller than eye diameter (Table 1), *canthus rostralis* slightly defined, loreal region slightly convex, lips flared, eyes small, slightly protruding, inter-orbital slightly concave, occipital fold complete, tympanum indistinct, upper jaw

FIGURE 1. *Elachistocleis sikuani* **sp. nov.**, preserved holotype, adult female, IAvH-Am–11748 (SVL = 31.6 mm). A Dorsal view B Ventral view C Lateral surfaces. D. Ventral view of hand E. Ventral view of foot. Scale bar = 10 mm. Photographs by Andrés Acosta-Galvis.

FIGURE 2. Type locality of *Elachistocleis sikuani* **sp. nov.** at Caracaro Farm, Man Municipality, Casanare Department. Photos: Andrés Acosta-Galvis.

TABLE 1. Measurements (in mm) of the Holotype and Paratypes of *Elachistocleis sikuani*. See abbreviations in Methods.

	IAvH-Am–11748 Female (holotype)	IAvH-Am–13071 Female	IAvH-Am–13073 Female	IAvH-Am–13072 Female	IAvH-Am–13075 Female	IAvH-Am–11364 Female	IAvH-Am–11791 Male	IAvH-Am–13074 Male
SVL	31.6	33.0	33.2	31.8	28.7	33.0	28.8	27.2
HL	6.5	5.1	6.6	6.1	6.0	6.6	6.7	6.2
HW	7.5	6.3	8.4	7.3	6.8	7.2	6.5	5.8
ED	2.2	2.0	2.1	2.3	1.9	1.4	2.2	2.1
IOD	3.2	2.7	3.7	3.2	3.5	3.1	2.7	2.4
IND	2.1	2.1	2.0	2.1	2.0	2.2	2.0	2.1
END	1.9	2.2	2.3	2.5	2.0	2.6	1.9	1.9
THL	10.7	10.3	11.6	11.9	11.7	11.7	10.5	10.1
TBL	10.3	10.4	10.9	10.9	10.4	12.1	9.0	9.3
FL	11.9	11.0	12.5	12.5	11.4	12.1	10.0	10.5
HDL3	6.1	6.4	6.4	6.7	6.1	7.4	5.8	5.5
HDL4	4.3	4.5	5.1	4.4	4.2	5.3	4.0	4.0
FAL	4.9	4.1	5.4	4.8	4.3	5.3	4.2	4.1
FD3	0.5	0.4	0.6	0.7	0.5	0.6	0.4	0.4
FT4	0.6	0.7	0.6	0.6	0.5	0.5	0.4	0.5

projecting beyond the lower one, tongue big, ovoid, and covers the entire buccal floor, premaxillae, maxillae, and vomerine teeth absent, choanae small, subovoid, widely separated, positioned anterolaterally to eye. Arms slender and lacking tubercles on forearm. Hands not webbed, fingers slightly fringed with not expanded and rounded tips, fingers lacking dermal spines, finger lengths I<II<IV<III, subarticular tubercles well developed and rounded, proximal subarticular tubercles larger than others, supernumerary tubercles absent, thenar tubercle well developed and subovoid, palmar tubercle bifid. Legs short, moderately robust, knee and heel lacking tubercles, tibial and tarsal ridges absent. Foot not webbed, toes slightly fringed, toe tip rounded lacking disks, subarticular tubercles well developed and ovoid, supernumerary tubercles absent, an oval inner, but no outer, metatarsal tubercle. Toe lengths I<II<V<III<IV, toes lacking dermal spines, tibia length slightly shorter than thigh length, combined thigh, and tibia lengths approximately 43.3% of snout-vent length, foot length approximately 37.9% of snout-vent length. Skin smooth, dorsal surfaces of body lacking dermal spines. Throat smooth, without dermal spines on chin and snout. Cloaca with para-cloacal tubercles or glands.

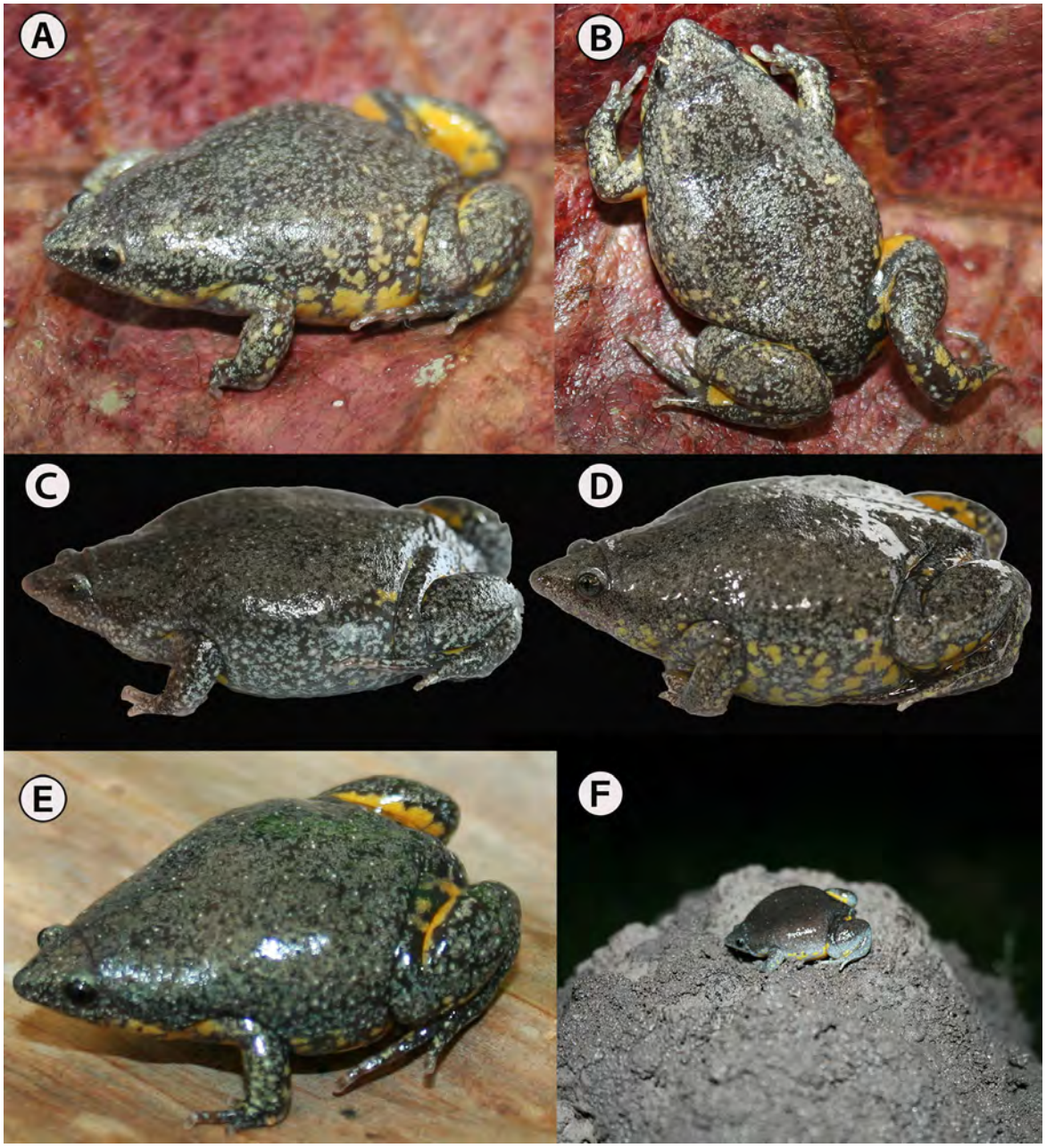

FIGURE 3. *Elachistocleis sikuani* **sp. nov.**, live specimens. A Lateral view of Holotype, adult female, IAvH-Am–11748 (SVL= 31.6 mm) B Dorsal view of Holotype, adult female, IAvH-Am–11748 C Paratype, adult female, IAvH-Am–13071 (SVL = 33.0 mm), D Paratype, adult female, IAvH-Am–13073 (SVL = 33.2 mm). E-F Lateral view, subadult female, IAvH-Am–11747 (SVL= 27.8 mm) Photographs by Andrés Acosta-Galvis.

FIGURE 4. Live specimens (lateral view) of *Elachistocleis* currently known from Colombia. A *Elachistocleis panamensis*, Pelaya Municipality, Cesar Department, IAvH-Am–10555 (adult male, SVL = 25.4 mm). B *Elachistocleis pearsei*, Tolu Viejo Municipality, Sucre Department, IAvH-Am–8392 (adult male, SVL = 33.6 mm). C *Elachistocleis sikuani*, Orocu Municipality, Casanare Department, IAvH-Am–11791 (adult male, SVL = 28.8 mm). D *Elachistocleis tinigua*, Macarena Municipality, Meta Department, IAvH-Am–15179 (juvenile, SVL = 15.9 mm). Photographs by Andrés Acosta-Galvis.

Color of holotype in preservative (Fig. 1). Dorsum dark brown with a few small and diffuse cream spots and blotches, dorsal surface of limbs dark brown with cream blotches and small spots, particularly on proximal forelimb, palm of hands pale cream and marbled brown on subarticular region, foot dark brown with pale cream blotches, belly surface pale cream with dark brown reticulated pattern, throat dark brown with small irregular cream spots. Ventral surface of anterior thighs pale cream, medial region of thighs and ventral surfaces of tibia pale cream with dark brown reticles. Body and limbs lack distinct lines.

Color of holotype in life (Fig. 3 A–B). Dorsal body and limbs surfaces pale gray with black reticulations, cephalic flank black with small, pale gray, and irregular spots, yellow pale blotches in the anterior region of the arm, flanks dark brown with dense pale yellow blotches, surface of venter pale cream with pale brown reticulations and dense pale yellow blotches, throat pale brown with pale cream irregular spots, axillary region, groin, and anterior thigh pale yellow, ventral surfaces of thighs pale cream with pale brown reticulations and pale yellow blotches, iris pale gray.

Measurements of Holotype (in mm). SLV 31.6, HDL3 6.1, HDL4 4.3, HL 6.5, HW 7.5, ED 2.2, IOD 3.2, IND 2.1, END 1.9, THL 10.7, TBL 10.3, FL 11.9, FAL 4.9, FD3 0.5, TD4 0.6.

Variation of type series (Figs. 3, 6; Table 1). In this section, coloration refers to live specimens and is based on field notes and digital photographs, unless otherwise noted. Flanks coloration varies from pale yellow with distinct pale-yellow blotches (e.g., IAvH-Am–11748, Fig. 3A) to moderate (e.g., IAvH-Am–13073, Fig. 3D), and diffuse (e.g., IAvH-Am–13071–2, Fig. 3C; IAvH-Am–11747, Fig. 3E-F; IAvH-Am–11791, Fig. 4C). Femoral light stripe varies from a longitudinal band (e.g., IAvH-Am–13073–4) to irregular and broad (e.g., IAvH-Am–13072). The post-commissural with light-colored spots (i.e., pale yellow) that can vary from densely pigmented (e.g., IAvH-Am–11747–8, IAvH-Am–11791) to moderate (e.g., IAvH-Am–13071–3). The variation in the skin texture is noteworthy in both sexes, noticeable dermal spines on chin of adult males (e.g., IAvH-Am–11791 IAvH-Am–13074) that are

absent in females (e.g., IAvH-Am–11364, IAvH-Am–11747–8, and IAvH-Am–13071–3). HL approximately equal to HW in adult females, HL 78.0–92.2 % of HW whereas in adult males HL is longer HL 102.8–107.1 % of HW.

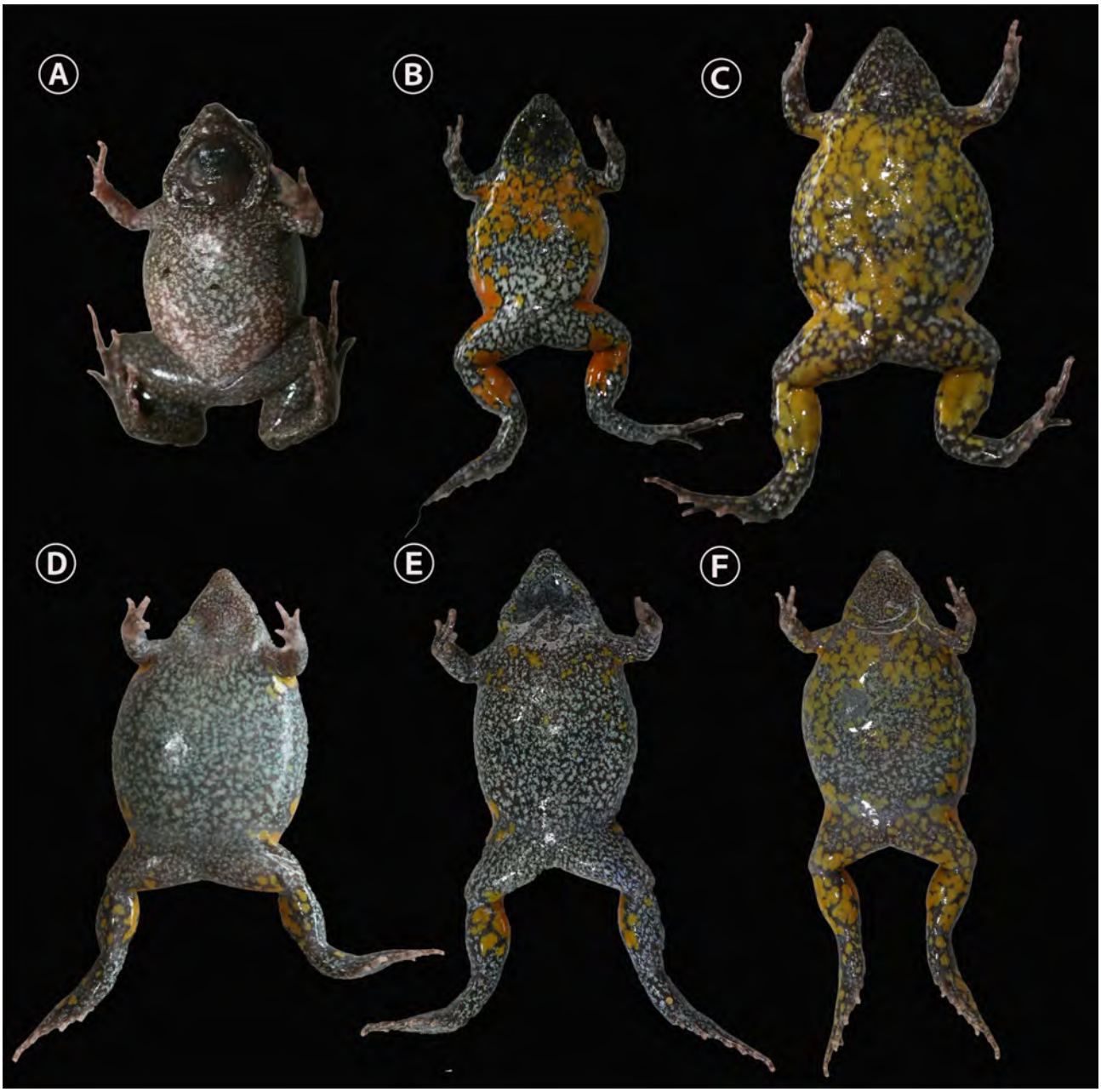

FIGURE 5. Live specimens (Ventral view) some *Elachistocleis* currently known from Colombia. A *Elachistocleis panamensis*, Cimitarra Municipality, Santander Department, IAvH-Am–13051 (adult male, SVL = 21.4 mm). B *Elachistocleis pearsei*, Cimitarra Municipality, Santander Department, IAvH-Am–13052 (adult male, SVL = 31.4 mm). C *Elachistocleis pearsei*, Cimitarra Municipality, Santander Department, IAvH-Am–13053 (adult female, SVL = 43.5 mm). D Paratype of *Elachistocleis sikuani*, Man Municipality, Casanare Department, IAvH-Am–13071 (adult female, SVL = 33.0 mm). E Paratype of *Elachistocleis sikuani*, Man Municipality, Casanare Department, IAvH-Am–13074 (adult male, SVL = 27.2 mm). E Paratype of *Elachistocleis sikuani*, Man Municipality, Casanare Department, IAvH-Am–13073 (adult female, SVL = 33.2 mm). Photographs by Andrés Acosta-Galvis.

Advertisement calls analysis (Fig. 7). Males of *Elachistocleis sikuani* called at night on ephemeral ponds formed after heavy rains in the typical open savannas environment, that in the area, have numerous termite mounds. The analyzed call belongs to voucher specimen: IAvH-Am–13074 (https://doi.org/10.6084/m9.figshare.16943425) recorded on May 14, 2006 at 19:12 hrs., air temperature of 28.7°C, and 71% relative humidity and unvouchered

male (https://doi.org/10.6084/m9.figshare.16944232), recorded from Guaranito farm, at 187 m a.s.l., in the Municipality of Maní (4°53'42.8"N, 72°22'0.5W), Casanare Department, Colombia on May 13, 2016 at 19:00 hrs., air temperature of 25.8°C, 68% relative humidity. The call of *Elachistocleis sikuani* **sp. nov.** (n = 21 calls) consists of a sustained trill made by a long, strong, and continuous note (one pulsed note, Guild C *sensu* Emmrich *et al.* 2020) lasting between 1158 and 3106 milliseconds (χ = 2429 ms 0.49) and interval between notes between 13365 and 21215 ms (χ = 18129 ms 2.72), dominant frequency between 1042 and 1842 Hz (χ = 1521 Hz 342.7). Each note consists of a series of sustained and long multi-pulses (260–410 pulses per note) with a rate of 236 to 269 pulses per seconds (χ = 261.2 pulses/s.), the duration of each pulse is 3.0 msec. The calls have an ascendant modulation frequency where the first segment consist of 33 to 49 initial pulses (χ = 38.1) with lower frequencies between 2695 and 3231.9 Hz (χ = 3056.3 Hz) and the maximum frequency, at the end of the call, between 4863 and 5028 Hz (χ = 4941.2 Hz).

Tadpole. Unknown

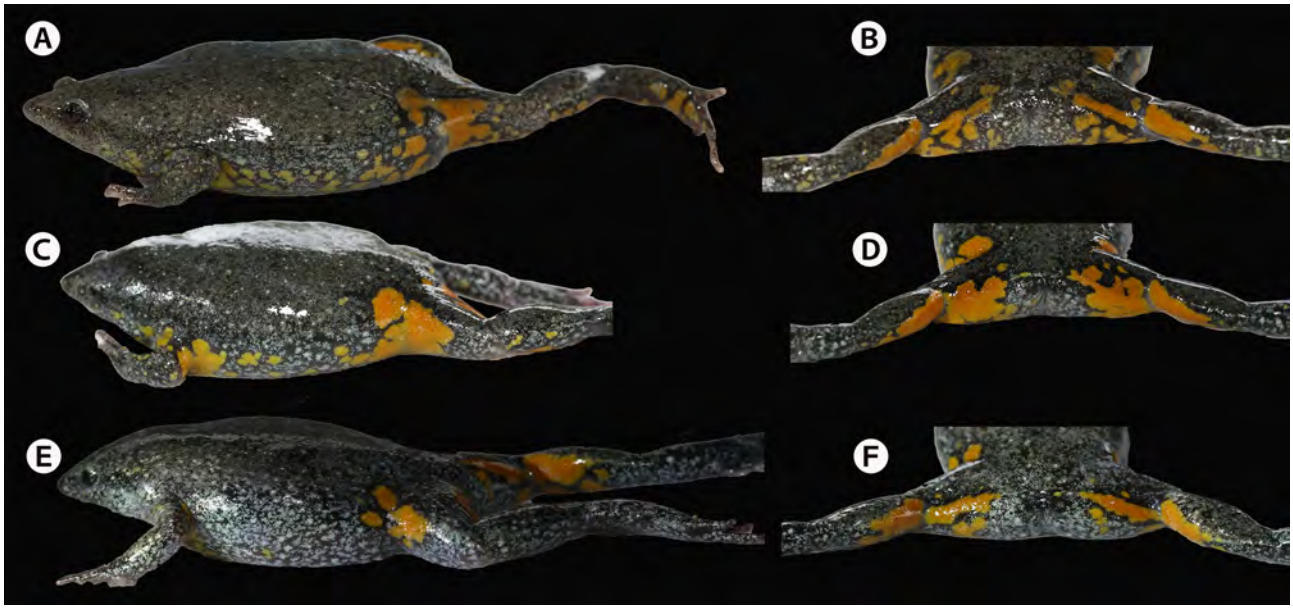

FIGURE 6. Color pattern variation of flanks and posterior thigh in life of type series of *Elachistocleis sikuani* **sp. nov.** A, B. Paratype adult female IAvH-Am–13073, C, D: Paratype adult female IAvH-Am–13072, E, F. Paratype adult male IAvH-Am–13074.

Distribution. *Elachistocleis sikuani* **sp. nov.** is known from the savanna like environments called "Llanos Orientales" in the Casanare Department; all localities are associated with the sub-basin of the Meta River, at elevation between 138 to 325 m a.s.l. The localities are within the Llanos ecoregion (Dinerstein *et al.* 1995) in the Orinoco region (Fig. 7).

Natural history. The specimens were found active during the rainy season (March to May and August to December). A species typical of plains habitats, being reported in grasslands and flooded forested areas in aeolian savannas, the annual rainfall in localities varies between 1400 to 5600 mm with bimodal seasonality. *Elachistocleis sikuani* **sp. nov.** is active entirely in the rainy season when seasonal ponds are established in flooded grasslands, being remarkably active after very heavy rains or during moderate rains. The species was active between 21.6–27.6 C° and 71–91% relative humidity during the dry season between December and March. The species remains submerged in the sandy substrate or under termite mounds in open savannas, but it can also be found buried in the substrate mixed with gallery forests. Males vocalize partially submerged and hidden at the base of flooded grasslands or in seasonal ponds. The amplexus is axillary and performed submerged in shallow pools.

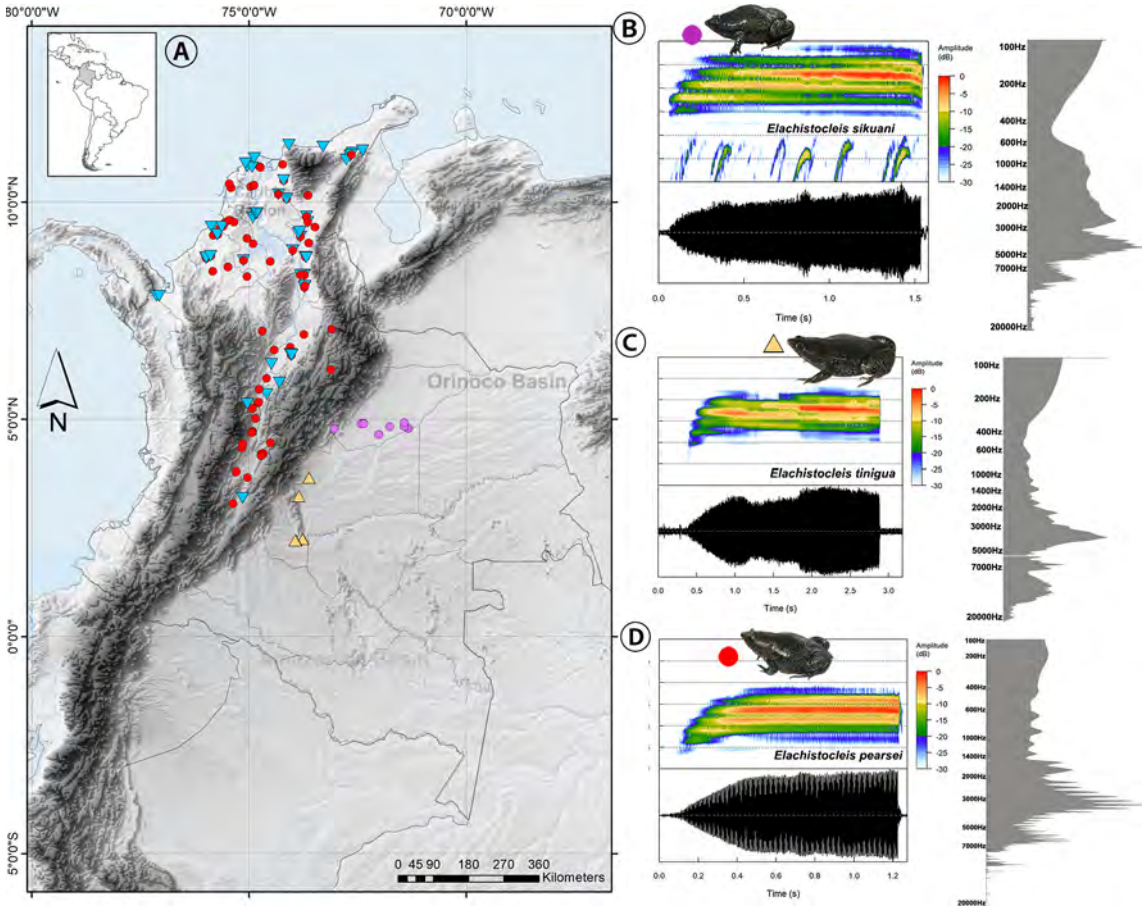

FIGURE 7. A. Distribution of species in the genus *Elachistocleis* from Colombia, *Elachistocleis sikuani* sp. nov (violet dots), *Elachistocleis tinigua* sp. nov (yellow triangle), *Elachistocleis pearsei* (red dots), and *Elachistocleis panamensis* (blue triangle) for localities see appendix I. B. Vocalization of *Elachistocleis sikuani* **sp. nov.**, Adult male specimen (specimen IAvH-Am–13074, track IAvH-CSA–34356), SVL: 27.3 mm, featured as a picture on the map) from Maní Municipality, Casanare Department, Colombia, Spectrogram (above),oscillogram (below), and Power spectrum (right) of one note recorded from the male. C. Vocalization of *Elachistocleis tinigua* **sp. nov.**, Adult male specimen (specimen IAvH-Am–15252, track IAvH-CSA–34357, SVL: 34.1 mm, featured as a picture on the map) from La Macarena Municipality, Meta Department, Colombia, Spectrogram(above), oscillogram (below), and Power spectrum (right) of one note recorded from the male. D. Vocalization of *Elachistocleis pearsei*. Adult male featured as a picture on the map) from Cimitarra Municipality, Santander Department, Colombia, Spectrogram (above), oscillogram (below), and Power spectrum (right) of one note recorded from the male.

Elachistocleis tinigua sp. nov.

Holotype. IAvH-Am–15251 (field number ARA 8311, Fig. 8) an adult female collected on 3 July 2018 by Andrés R. Acosta-Galvis and Duberley Rayo.

Type locality (Fig. 9). Colombia, Meta Department, La Macarena Municipality, vereda Los Alpes, left margin of Losada River, near Los Alpes School, 02°13’7.1” N, 073°55’16.3” W, 234 m a.s.l.

Paratypes (4 specimens) (Fig. 10, Table 2). IAvH-Am–15200 adult female collected on 1 July 2018., IAvH-Am–15252 collected on 3 July 2018, IAvH-Am–15275 collected on 4 July 2018, adult males, by Andrés R. Acosta-Galvis and Duberley Rayo, same locality as the holotype.

Referred specimens (29 specimens). IAvH-Am–14816, IAvH-Am–14829, juveniles collected on 1 July 2018 in Colombia, Meta Department, La Macarena Municipality, El Silencio Lake. 2°14’55.21”N, 73°45’27.40”W, 223 m a.s.l., IAvH-Am–15172–15199 juveniles, same locality and date as holotype.

Referred specimens. MPUJ- 6118–21 (field numbers DXA 044, 082–4, respectively),

Adult males, from Colombia, Meta Department, San Martin Municipality, vereda Montebello, Tocancipá Farm,

3°39’21.7”N, 73°36’49.40”W, 424 m a.s.l, collected on 4 May 2007, by Xamara Albarán and Andrés R. Acosta-Galvis.

Etymology. The specific epithet tinigua honors the now extinct native ethnic group of the Tiniguas who lived in the Manacacias, Yarí, Caguán, and Guayabero river basins within the distribution range of the species. The last two inhabitants of this ethnic group survived until 1994.

Diagnosis (Fig. 4, 8, 10). A medium size species of *Elachistocleis* (adult males SVL = 30.4–34.1 mm, χ = 32.2 2.6 mm, females SVL = 35.1–38.8 mm, χ = 36.9 2.6 mm), diagnosed by the following combination of characters: (1) an ovoid body form, (2) triangular head, slightly longer than wide, (3) a barely developed and complete occipital fold, (4) snout rounded in lateral and dorsal views, (5) *canthus rostralis* slightly concave and loreal region is convex, (6) nostrils laterally oriented, (7) post-commissural glands distinct in adult males, barely noticeable in adult females, (8) tympanic annulus and membrane absent, (9) dentigerous process of vomer absent, (10) upper jaw projects beyond the lower jaw, (11) tongue large, ovoid, and occupying the entire buccal cavity, (12) choanae ovoid, large, and widely separated, (13) arms slender without tubercles on forearm, (14) hands lack interdigital membranes and supernumerary tubercles, (15) finger lengths are I<II<IV<III, (16) subarticular tubercles, ovoid and as wide as fingers, (17) thenar tubercle prominent and palmar tubercle bilobate, (18) foot lacks interdigital membranes and supernumerary tubercles, toes lack discs, (19) inner plantar tubercle equal in size to subarticular tubercles, (20) relative toe lengths I<II<V<III<IV, (21) THL is slightly longer than TBL, (22) dorsal surface of body with sparse dermal spines, (23) cloacal region lacks glands, but bears tubercles, (24) in preservative, dorsal coloration dark gray to dark brown with scattered cream spots, (25) ventral surfaces of fore and hind limbs mottled, (26) mid-dorsal line absent, (27) longitudinal line on posterior surface of thighs (= femoral stripe) broad, light, and irregular, (28) axillae and groin lightly spotted, (29) all toes fringed and slightly webbed in females and males, (30) dermal spines absent on ventral surfaces, except on chin of adult breeding males.

Description of Holotype. (Fig. 8). Body size medium (SVL = 38.8 mm), body slender, slightly ovoid, head triangular in shape, longer than broad (Table 2), snout short, snout tip rounded (Fig. 8), nostrils located closer to the tip of snout than to the eye, protuberant, directed laterally (Fig. 8), inter-nostril distance smaller than eye–nostril distance and wider than eye diameter (Table 2), *canthus rostralis* slightly defined, loreal region slightly convex, lips flared, eyes small, slightly protruding, inter-orbital slightly concave, occipital fold complete, tympanum indistinct, upper jaw projecting beyond lower one, tongue is big, ovoid, and covers three quarters of the buccal cavity, premaxillae, maxillae, and vomerine teeth absent, choanae small, almost ovoid, widely separated, positioned anterolaterally to eye.

TABLE 2. Measurements (in mm) of the Holotype and Paratypes of *Elachistocleis tinigua*. See abbreviations in Methods.

	IAvH-Am–15251 Female (holotype)	IAvH-Am–15200 Female	IAvH-Am–15252 Male	IAvH-Am–15275 Male
SVL	38.8	35.1	34.1	30.4
HL	8.2	9.2	12.7	10.9
HW	8.0	7.9	6.7	7.1
ED	1.6	1.5	2.4	2.5
IOD	4.6	4.5	4.0	3.7
IND	2.5	2.7	2.4	2.2
END	2.5	2.8	2.4	2.3
THL	17.3	14.3	12.0	11.0
TBL	16.3	14.2	11.8	11.1
FL	14.7	14.3	11.5	11.6
HDL3	5.8	6.1	9.1	9.4
HDL4	8.0	8.2	7.3	7.0
FAL	6.3	6.3	5.5	5.6
FD3	0.7	0.5	0.5	0.5
FT4	0.7	0.7	0.6	0.6

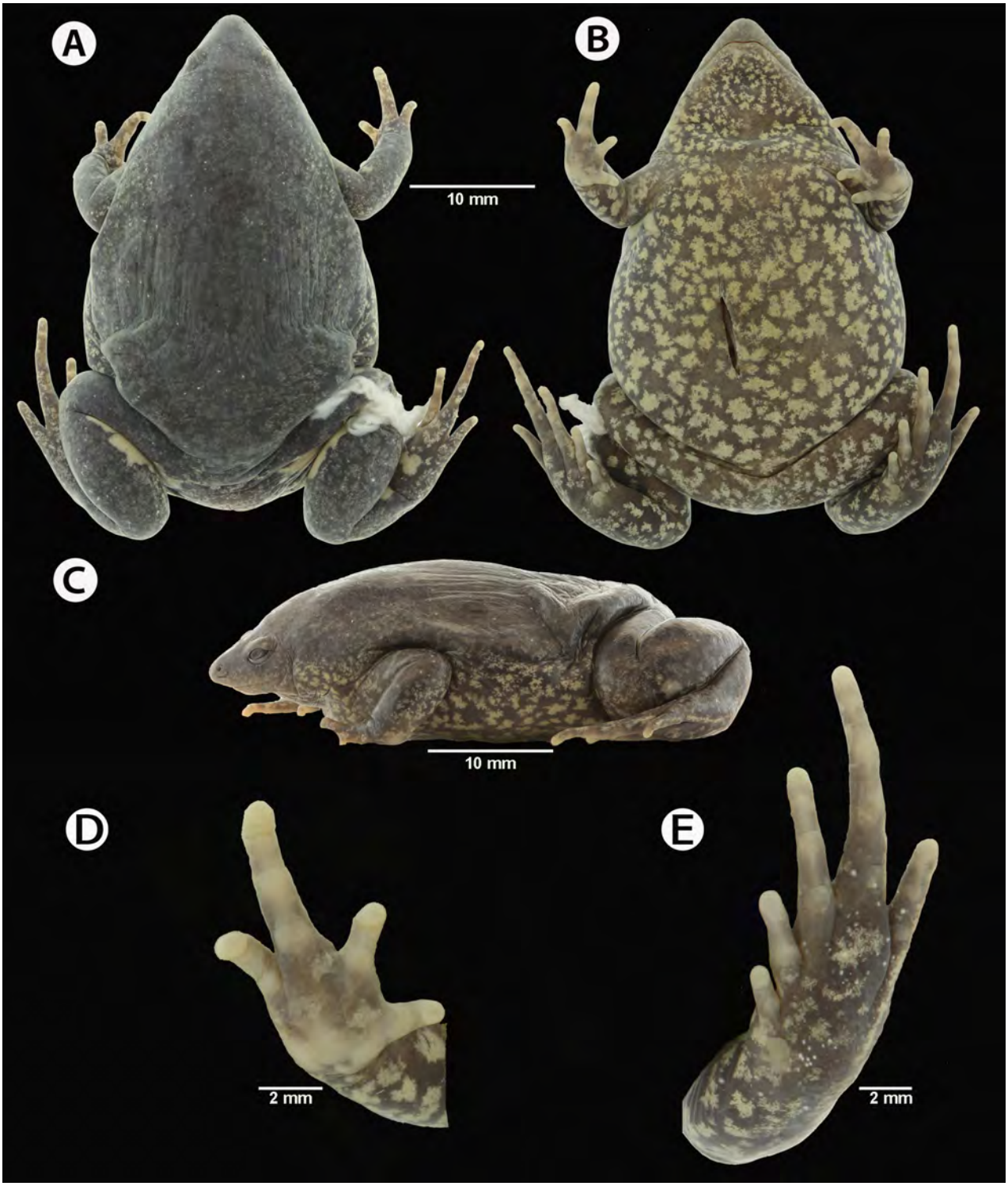

FIGURE 8. *Elachistocleis tinigua* **sp. nov.**, preserved holotype, adult female, IAvH-Am–15251 (SVL = 38.8 mm). A Dorsal view B Ventral view C Lateral surfaces. D. Ventral view of hand E. Ventral view of foot. Scale bar = 10 mm. Photographs by Andrés Acosta.

Arms are slender, lacking tubercles on forearms. Hands not webbed, fingers tips rounded, not expanded, and not fringed, fingers lacking dermal spines, finger lengths I<II<IV<III, subarticular tubercles well developed and rounded, proximal subarticular tubercles larger than others, supernumerary tubercles absent, thenar tubercle well-developed and subovoid, palmar tubercle longitudinally divided. Legs short, moderately robust, knee and heel lacking tubercles, tibial and tarsal ridges absent. Foot not webbed, toes not fringed, toe tip rounded lacking disks, subarticular tubercles well-developed and rounded, supernumerary tubercles absent, inner metatarsal tubercle oval,

no outer metatarsal tubercle. Toe lengths I<II<V<III<IV, toes lack dermal spines, tibia length slightly shorter than thigh length, combined thigh and tibia lengths approximately 43.3% of snout-vent length, foot length approximately 59.5% of snout-vent length. Skin smooth, dorsal surfaces of body lacking dermal spines. Throat smooth, lacks dermal spines on chin and snout. Cloaca with para-cloacal tubercles or glands.

Color of holotype in preservative (Fig. 8). Dorsum gray with diffuse black reticulations, a few small white spots, dorsal surface of limbs similar to dorsum, dorsal surfaces of tibia with irregular cream spots on the inner margin, palm of hands pale cream and marbled brown below subarticular region, foot dark brown, with blotches pale cream, and a few small white spots, ventral surfaces of anterior thighs, tibia, throat, and belly, dark brown with irregular cream spots, and absence of distinct lines on the body and limbs.

Color of holotype in life (Fig. 10 A–B). Dorsum surfaces and limbs pale dark brown with light gray reticulations and spots, cephalic region and flanks with coloration similar to dorsal surfaces, ventral surfaces dark brown with irregular yellowish cream spots, axillary region, groin, and anterior thigh orange, iris dark brown.

Measurements of Holotype (in mm). SLV 38.8, HDL3 5.89, HDL4 8.0, HL 8.2, HW 8.0, ED 1.6, IOD 4.6, IND 2.5, END 2.5, THL 17.3, TBL 16.3, FL 14.7, FAL 6.3, FD3 0.7, TD4 0.7.

Variation (Fig. 10, Table 2). Herein, coloration refers to live specimens and is based on field notes and digital photographs, unless otherwise noted. Dorsal coloration varies from dark brown with light gray reticulations (e.g., IAvH-Am–15200, IAvH-Am–15251, Fig. 10) to black with light gray reticulations (e.g., IAvH-Am–15172, IAvH-Am–15175–6, IAvH-Am–15178, IAvH-Am–15181, IAvH-Am–15252, Fig. 10) or uniformly dark brown (e.g., IAvH-Am–15275, Fig. 10). Axillary spots can be present or absent (e.g., IAvH-Am–15172, IAvH-Am–15275, Fig. 10); if present they are orange and visible in lateral view (e.g., IAvH-Am–15251–2, IAvH-Am–15173, IAvH-Am–15178, Fig. 8). Dermal spines on chin are absent (e.g., IAvH-Am–15200, IAvH-Am–15251–2) or present and prominent (e.g., in adult male IAvH-Am–15275). The ratio of TBL added to THL with respect to SVL is 54.9–59.5 % in adult females, in adult males is 46.7–47.7%.

Call analysis (Fig.7). Males of *Elachistocleis tinigua* **sp. nov.** called at night on ephemeral ponds and from within natural ground depressions filled with water (< 10 cm deep), these are the result of rainwater that circulates freely on the surface of land in open grasslands.

The analyzed call belongs to voucher specimens: IAvH-Am–15252 (https://doi.org/ 10.6084/m9.figshare.16944193) and IAvH–Am–15275 (https://doi.org/ 10.6084/m9.figshare.16944190), were recorded respectively from Vereda Los Alpes near Losada River, at 255 m a.s.l., in the Municipality of Macarena (2°13’12.4”N, 73°55’27.1W), Meta Department, Colombia, on July 3 and 4, 2018 between 20:18–20:49 hrs., air temperature between 25.6–27.7 °C and relative humidity between 61–72%; and MPUJ– 6118–21 (https://doi.org/ 10.6084/m9.figshare.16944205) recorded from Vereda Montebello, Tocancipá farm, at 350 m a.s.l., in the Municipality of San Martin (3°39’21.7”N, 73°36’49.5W), Meta Department, Colombia on 4 May 2007 (Table 4).

The call of *Elachistocleis tinigua* **sp. nov.** (n = 50 calls) consists of a sustained trill (one pulsed note, Guild C *sensu* Emmrich *et al.* 2020) made by a long, strong, and continuous note lasting between 1350 and 3753 ms (χ = 2457 ms 0.58) and interval between notes between 1400 and 18684 ms (χ = 6553 ms 3.71), the dominant frequency is between 2954 and 4655 Hz (χ = 3780 Hz 445). Each note consists of a series of sustained and long multi-pulses (348–926 pulses per note) with a rate of 206 to 236 pulses per seconds (χ = 223.7 pulses/s.), the duration of each pulse is 3.0 ms. The calls have an ascendant modulation frequency where the first segment consist of 12 to 48 initial pulses (χ= 27.2) with lower frequencies between 1759.6 and 2678.5 Hz (χ= 2256.6 Hz) and the maximum frequency, at the end of the call, between 4347 and 4702 Hz (χ= 4514.9 Hz).

Distribution (Fig.7). *Elachistocleis tinigua* **sp. nov.** is currently known from three localities in the transitional areas between the Orinoco and the Amazon basins in Meta Department, all the localities are located within sub-basins of the Guayabero and Guaviare rivers, at an elevation between 182 to 424 m a.s.l.

Tadpole. Unknown.

Natural history. *Elachistocleis tinigua* **sp. nov.** was collected in open areas (grasslands) associated to tropical forests. It is a nocturnal species, the specimens were found active during the period of greatest rainfall (i.e., July). The annual rainfall in the localities where the species are found varies between 2000 to 4800 mm with monomodal seasonality. *Elachistocleis tinigua* **sp. nov.** breeds in small pools in areas associated with runoff at about 5% angle. Males vocalize submerged and hidden at the base of seasonal ponds. At the time of collection of the type series, an individual of a false fer-de-lance snake, i.e., *Leptodeira annulata* (IAvH-R–8837), was observed feeding on a female at 18:33 h (Fig. 9 B).

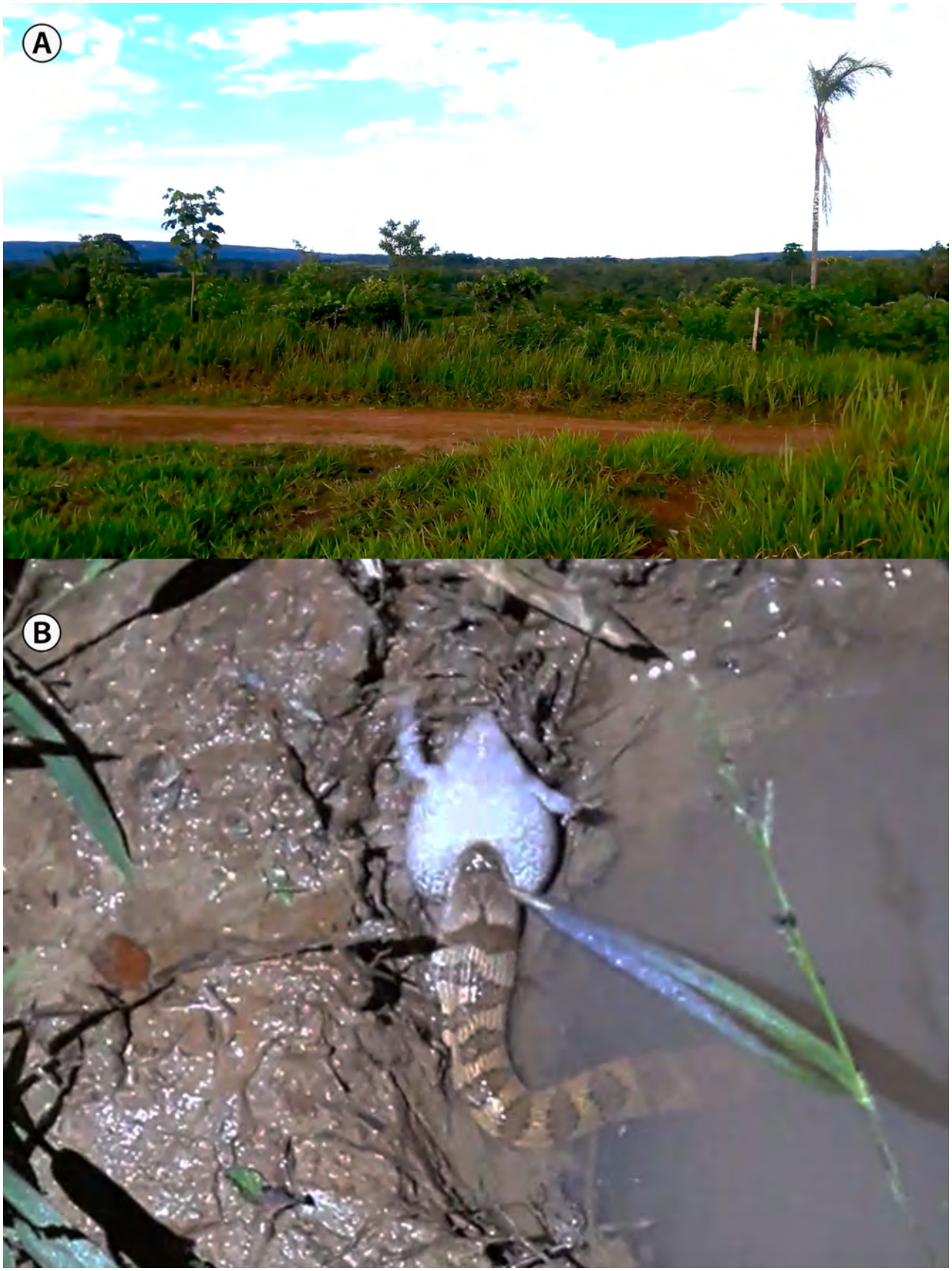

FIGURE 9. A. Type locality of *Elachistocleis tinigua* **sp. nov.** at Vereda Los Alpes, La Macarena Municipality, Meta Department. B. Predation on specimen by the of the false fer-de-lance snake *Leptodeira annulata* in type locality, Photos: Andrés Acosta-Galvis.

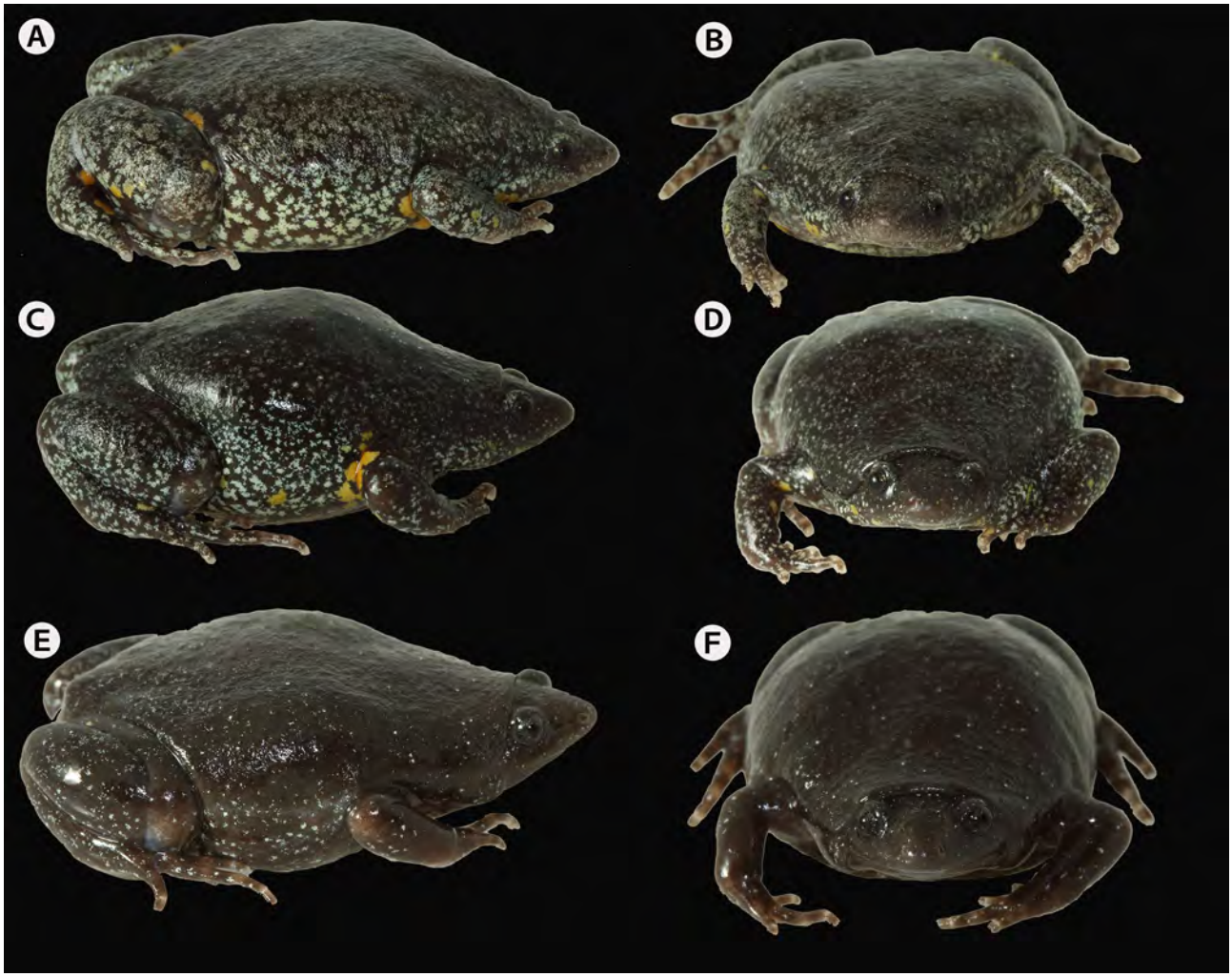

FIGURE 10. Lateral and frontal view of *Elachistocleis tinigua* **sp. nov.**, live specimens. A and B. Holotype, adult female, IAvH-Am–15251 (SVL= 38.8 mm), B and C. Paratype adult male, IAvH-Am–15252 (SVL= 34.1 mm), D and E Paratype, adult male, IAvH-Am–15275 (SVL = 30.1 mm). Photographs by Andrés Acosta.

Discussion

Elachistocleis currently consists of 20 described species (Frost, 2021) making it the second most speciose genus, after *Chiasmocleis*, of the Neotropical microhylid subfamily Gastrophryninae. Cochran and Goin (1970) applied the name *Elachistocleis ovalis* to specimens from Departments in the eastern region of Colombia: Casanare (USNM 152032–3), Meta (La Macarena, CJG 2340; Villavicencio, CNHM 81816, USNM 152204–8, MLS 132, 132[a]), Granada, on Rio Ariari, (USNM 151967–76), and Vichada (from Guaviare river, USNM 152316) along with a disjunct population from the Caribbean region (Córdoba Department, USNM 152158, considered here as *E. pearsei*). Ruiz *et al.* (1996) and Acosta-Galvis (2000) follow this proposal and point out the extent of the distribution of *E. ovalis* in the Caribbean region and the Magdalena Valley (considered here as *E. pearsei*). However, recent contributions have made it possible to establish a clear geographical segregation, albeit with low morphological support, in which *E. pearsei* has a restricted distribution in the lowlands in the Caribbean region, the Magdalena Valley of Colombia, and the North of the Pacific region (Ruthven 1914; 1922; Stebbins & Hendrickson 1959; Renjifo & Lundberg 1999; Cuentas *et al.* 2002; Bernal *et al.* 2005; Acosta-Galvis *et al.* 2006; Galvan *et al.* 2009; Reinoso *et al.* 2009; Romero & Lynch, 2010; Medina-Rangel *et al.* 2011; Llano *et al.* 2011; Romero & Lynch 2012; Acosta-Galvis 2012; Paternina *et al.* 2013; Rodríguez *et al.* 2014; Blanco *et al.* 2015; Angarita *et al.* 2015; Moreno-Arias *et al.* 2020).

Elachistocleis populations across the Neotropical region that were formerly assigned to *E. ovalis* were left without a name by Caramaschi's (2010) nomenclatural action. In Colombia, some of these populations are geographically limited to the lowlands in the Orinoco and North of the Amazonian basins (Lynch 2006; Acosta-Galvis & Alfaro, 2011; Angarita *et al.* 2013; Angarita 2014; Pedroza *et al.* 2014; Acosta-Galvis 2018; Acosta-Galvis *et al.* 2018; Blanco-Torres *et al.* 2019; Medina Rangel *et al.* 2019). Some of those populations have already been described as new species, e.g., *E. nigrogularis* (Jowers *et al.* 2021). Herein, we described two populations (i.e., formerly refer to *E. ovalis*) as new species from Colombia.

Currently, there are two known species of *Elachistocleis* in Colombia, *E. pearsei* (Ruthven, 1914) and *E. panamensis* (Dunn, Trapido, and Evans 1948). The two new species are distinct from these known species, and from each other morphologically and in their advertisement calls. In Colombia, *Elachistocleis panamensis* is sympatric with *E. pearsei* in areas of the Magdalena Valley, Caribbean, and north of Pacific region of Colombia; p-uncorrected distances for the 16S ribosomal marker are given in Table 3. The genetic divergence for the 16S marker reported between the new species is 0.8–1.2%; however, low levels of genetic divergence are common among animals inhabiting non-heterogenous environments as is the case of fossoriality; indeed, previous work have demonstrated that fossorial species have significantly lower genetic diversity than species living aboveground (Gorman and Kim1976; Gorman and Taylor 1977; Kim *et al.* 1978; Schmidt *et al.* 2021; Nevo *et al.* 1990). Previously, we reported that Otophryninae split from Gastrophryninae between 65–60 mya whereas the genus *Elachistoclies* originated about 22 mya (de Sá *et al.*, 2012), our data on the genetic divergence of *Synapturanus* is slightly above 2%.

TABLE 3. Summary of p-uncorrected genetic distances in percent for a 527 bp segment at the 3' end of the mitochondrial 16S rRNA gene.

	E. tinigua	*E. sikuani*	*E. panamensis*	*E. pearsei*
E. tinigua	0.0–1.1	1.5–2.1	6.1–6.9	1.1–2.1
E. sikuani		0.0–0.4	6.7–7.3	1.5–1.9
E. panamensis			0.0–0.6	5.7–6.7

We secured tissue samples of *E. sikuani* and *E. tinigua* and our phylogenetic analysis show the species are closely related to each other and sister to the clade formed by *E. pearsei*, *E. panamensis*, and a currently unnamed *Elachistocleis* species (Fig. 11)

The new species are compared to other species of *Elachistocleis* occurring in northern South America; character states of the compared species are in parentheses. *Elachistocleis sikuani* is distinguished from *E. panamensis* (Dunn, Trapido, & Evans, 1948) by the absence of a paravertebral pattern (symmetric pattern constituted by dark spots, resembling a "pine tree", Fig. 4), dorsum dark gray with scattered cream spots (grayish brown, Fig. 4), mid-dorsal stripe absent (most commonly present, sometimes absent), uniform dorsal and lateral coloration (biseriate, usually the ventrolateral pattern is darker with diffuse white spots), axillae and groin light spotted (not spotted), and medium size species with SVL 27.3–33.2 mm (small species, SVL 18.4–27.6 mm). *Elachistocleis sikuani* differs from *Elachistocleis pearsei* (Ruthven, 1914) by having a belly with irregular pale orange spots scattered towards the ventrolateral margins (densely pigmented belly, with irregular light orange spots), a medium species with SVL 27.3–33.2 mm (SVL 31.7–47 mm), and post-commissural glands well defined (barely noticeable). In life, *Elachistocleis sikuani* differs from *E. surinamensis* by having pale yellow blotches on the inner thighs (red blotches) and lacks a light vertebral stripe (evident).

Elachistocleis tinigua is distinguished from *E. panamensis* (Dunn, Trapido, & Evans, 1948) by the absence of a paravertebral pattern (symmetric pattern constituted by dark spots, resembling a "pine tree"), mid-dorsal stripe absent (most commonly present, sometimes absent), dorsal and lateral coloration pattern uniform (pattern biseriate, usually the ventrolateral pattern is darker with diffuse white spots), medium size species with a SVL 28.7–33.2 mm (small species, SVL 18.4–27.6 mm). The new species differs from *Elachistocleis pearsei* (Ruthven 1914) by usually having irregular and diffuse light orange spots on belly (densely pigmented belly, with irregular light orange spots), and well-defined post-commissural glands (barely noticeable). *Elachistocleis tinigua* differs from *E. surinamensis* by having in life light yellow blotches in the internal region of the legs (red blotches) and the absence of light vertebral stripe (evident).

In our phylogenetic analysis, the two new species described herein, *Elachistocleis tinigua* and *E. sikuani*, turned out to be phylogenetically sister species. These two allopatric lineages are separated by only a low genetic distance

of 1.5–2.1% in the 16S gene (Table 3). However, there are several morphological and bioacoustic differences that support their status as separate species.

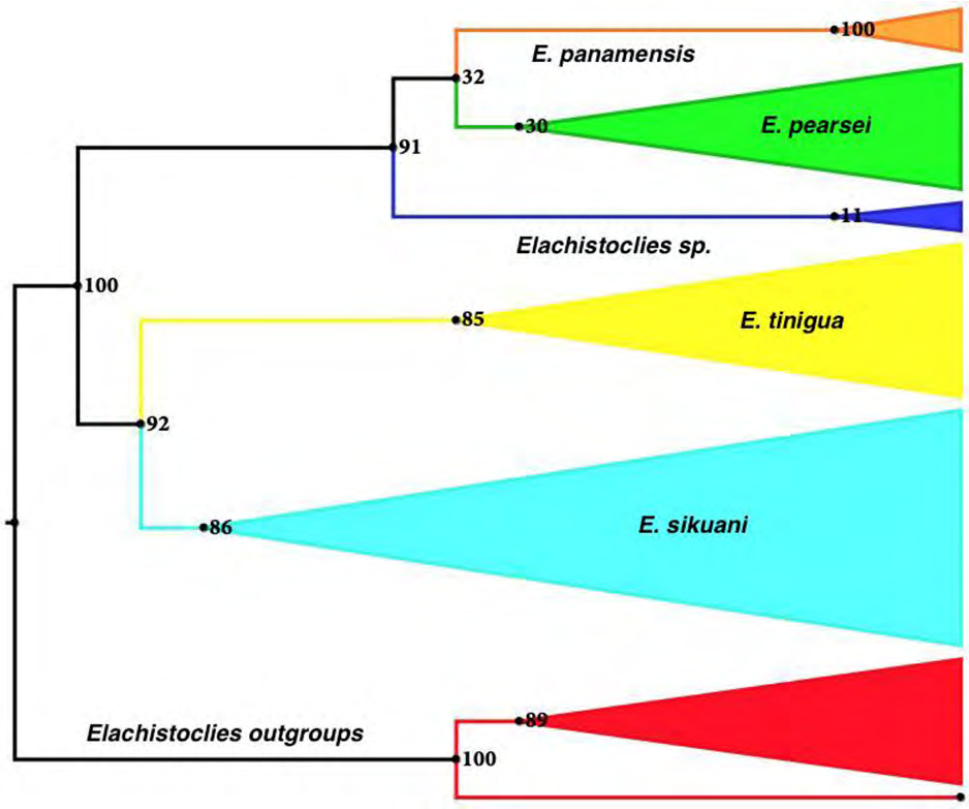

FIGURE 11. Phylogenetic relationships of *Elachistocleis tinigua* **sp. nov.** and *E.sikuani* **sp. nov.** The two new species cluster within *Elachistocleis* as the sister group to *E. pearsei*, *E. panamensis*, *Elachistocleis* sp. Bootstrap values supporting the branches of the new species are high (>80). A large-scale phylogenetic analysis of the genus will confirm or clarify the placement of the new species within *Elachistocleis*.

Elachistocleis sikuani can be separated from *E. tinigua* by the smaller size of adult specimens: SVL 28.7–33.2 mm (SVL 35.1–38.8 mm in adult females) and SVL 27.2–28.8 mm in adult males (SVL 30.4–34.1 mm in adult males), dorsum dark gray with scattered cream spots (grayish brown with scattered white spots), a post-commissural with light spots (usually absent), belly usually with a high density of irregular pale orange spots (diffuse light orange spots), the ratio TBL added to THL with respect to SVL is 36.7–48.2 % in adult females (54.9–59.5% in adult females). Also, the two new species consistently differ from each other in various advertisement call parameters. the call duration is shorter in *Elachistocleis sikuani* and has a higher dominant frequency (average values of two individuals 4179–4308 Hz, vs. 3742–3911 Hz in six individuals of *E. tinigua*). This, however, is likely explained by the smaller body size of *E. sikuani* (27.2-28.8 mm vs 30.4-34.1 mm of SVL in adult males of *E tinigua*), as in general, the dominant frequency in anuran calls is inversely related to body size (Preininger *et al.* 2007; Gingras *et al.* 2012; de Mello *et al.* 2021; Vences, pers. com.). Another difference, however, is seen in the number of pulses per note which is lower in *E. sikuani* (Table 4); this difference appears to be stable across individuals, despite some overlap in the values measured (262-410, vs. 333-786), and it is also reflected in shorter call duration in *E. sikuani* (despite greater overlap among the two species in this variable). These differences were verified for individuals from two populations of *E. tinigua* (La Macarena Municipality and San Martin Municipality), and without important differences in recording temperature, suggesting they represent consistent differences between these two lineages.

Also, the morphological differences in body size, limb length and coloration were verified in multiple individuals in populations of *E. sikuani* and populations of *E. tinigua* and were found to be consistent.

As a conclusion, the concordance of differences in genetics, coloration, morphology and especially, advertisement calls, justifies considering *E. sikuani* and *E. tinigua* as separate species.

TABLE 4. Bioacustical parameters of *E. sikuani* sp nov. and *E. tinigua* sp nov.

Measurements	*E. sikuani* IAvH-Am–13074	*E. sikuani* unvouchered male	*E. tinigua* IAvH–Am–15275	*E. tinigua* IAvH-Am–15252	*E. tinigua* MPUJ 6118	*E. tinigua* MPUJ 6119	*E. tinigua* MPUJ 6120	*E. tinigua* MPUJ 6121
Recording localities	Guaranito farm, 187 m a.s.l., Municipality Man (453'42.8"N, 7222'0.5W), Casanare Department. .		Vereda Los Alpes, 255 m a.s.l., Municipality Macarena (213'12.4"N, 7355'27.1W), Meta Department.		Vereda Montebello, Tocancip farm, 350 m a.s.l., Municipality San Martin (339'21.7"N, 7336'49.5W), Meta Department.			
Temperature (C)	28.7	28.8	27.7		27.3	27.3	23	22.9
HR (%)	71	72	72	62	72	72	89	88
Acoustical collection code	IAvH-CSA –34356	_–	IAvH-CSA 34358	IAvH-CSA 34357	MPUJ 6118	MPUJ 6119	MPUJ 6120	MPUJ 6121
n notes measured / n call measured	17	4	22	10	4	8	6	8
Call duration (s) *	1.2941+ 0.17 (1.066–1.542)	2.0392+ 0.68 (1.28–2.684)	2.23+ 0.54 (1.066–1.542)	2.36 + 0.52 (1.481–3.186)	2.19 + 0.59 (1.682–3.048)	3.038 + 0.61 (2.125–3.753)	3.007 + 0.36 (2.576–3.405)	2.484 + 0.49 (1.783–3.223)
Inter-call interval duration (s) *	18.64+ 2.9 (13.39–22.68)	11.15+ 4.2 (11.15–14.11)	4.64+ 2.04 (1.44–8.00)	4.5+ 2.06 (1.41–6.88)	11.8+ 6.03 (7.22–18.68)	10.52+ 4.22 (4.77–14.88)	10.50+ 4.22 (5.40–16.64)	6.872+ 1.14 (5.28–8.73)
Call dominant frequency (Hz) *	4179.9 + 558 (3491.7–4897.1)	4308.7 + 500 (3897.9–4898.7)	3742.18 +414.5 (3168.6–4265.8)	3758.3 +433.3 (3183.3–4370.1)	3660.2 +446.4 (3263.1–4081.4)	3823.6 +480.5 (3096.4–4655.6)	3819.5 +582.6 (2954.4–4436.3)	3911.3 +515.5 (3397–4437.4)
Pulses per note	347.8 + 53.47 (262–410)	296.3 + 38.14 (260–352)	590.5 + 138.7 (333–786)	599.7 + 53.13 (460–622)	477.7 + 124.9 (348–614)	635.6 + 74.59 (538–752)	693.5 + 140 (567–926)	530.8 + 116.3 (364–713)

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APPENDIX 1. Locality Records in Colombia.

Museum acronyms are: CZUT (Colección Zoológica de la Universidad del Tolima) IAvH-Am (Amphibian collection of the Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Boyac, Colombia), ICN:(Institute of Natural Sciences, Colección de Anfibios National University, Bogot-Colombia) MPUJ: (Lorenzo Uribe Museum of Natural History, Colección de Anfibios Pontificia Universidad Javeriana, Bogot-Colombia), UMAG (Magdalena University, Santa Marta, Colombia), UMMZ (University of Michigan, Museum of Zoology, Michigan,USA), USNM (National Museum of Natural History, Division of Amphibians and Reptiles, Washington USA).

Elachistocleis pearsei. COLOMBIA: Antioquia Department, Antioquia Department, Remedios Municipality, ICN13918, Ciénaga de Chiqueros, 130 m a.s.l. Atlántico Department, Campo de La Cruz, Algodonal, ICN 45490–9, ICN46122–3, ICN46129, Sabanagrande Municipality, Ciénaga del Convento, ICN46169, to Magdalena River, ICN49023. Bolívar Department, Ach Municipality, Corregimiento Puerto Isabel, IAvH-Am–082, Cartagena Municipality, IAvH-Am–9130, Turbaco Municipality, Matute, Guillermo Pineres Botanical Garden., 100 m a.s.l., ICN 14043, Zambrano Municipality, Monterrey Forestal, site Andaluz, 70 m a.s.l., 0937’48”N, 7454’44”W, IAvH-Am–6876. Boyac Department, Puerto Boyac Municipality, Vereda Balastrera, Velásquez stream, 280 m a.s.l., IAvH-Am–7753. Caldas Department, La Dorada Municipality, Vereda El Gigante, La Española Farm, near to the Purnio river, 248 m a.s.l., 52310.9”N,744643.6”W, IAvH-Am–13027-13030. Cesar Department, Aguachica Municipality, Ciénaga Musanda, 37 m a.s.l.(Paternina *et al.* 2013), Ciénaga Doña María, 37 m a.s.l.(Paternina *et al.* 2013), Chimichagua Municipality, La Felicidad farm, 64 m a.s.l.(Moreno-Arias *et al.* 2009), Ciénaga de Zapatosa (Paternina *et al.* 2013), Caño Largo- Tordecilla, 918’58”N,7349’50”W,103 m a.s.l.,IAvH-Am–11278,Chiriguan Municipality, Corregimiento de La Loma, km 16 road between Loma-La Jagua, Borrego Farm, Carboniferous Project La Loma, IAvH-Am–4933, IAvH-Am–4936-8,El Copey Municipality, La Joya Farm,104’18.14”N,7407’70(Blanco *et al.* 2015), El Paso Municipality, Ciénaga La Pachita (Paternina *et al.* 2013), Ciénaga Mata de Palma, Gamarra Municipality, Ciénaga de Baquero (Paternina *et al.* 2013),Ciénaga de Juncal (Paternina *et al.* 2013), La Gloria Municipality, Ciénaga de Morales (Paternina *et al.* 2013), Pelaya Municipality, Ciénaga de Costilla(Paternina *et al.* 2013). Córdoba Department, Caño Betanci, Junquilla, USNM 152158(Cochran and Goin, 1970), Ayapel Municipality, Cucharita farm, ICN48282, Montería Municipality, El Diluvio Farm, 64 m a.s.l., ICN48230-48268, ICN48272-48281, Ciénaga Martinica, ICN48269-ICN48270, site Los Araujos, ICN48311-5, ICN48919-20, Pueblo Nuevo Municipality, Toronto Farm, 30 m a.s.l., ICN48294-48314, Santa Cruz de Lorica Municipality, Cubinca Farm and Fish Station of Corporacion de los valles del Sin y San Jorge, 20 m a.s.l., ICN48283-ICN48293, ICN48992-3, Ceiba Tejado, Ciénaga Pantano Bonito, ICN 48862. Caldas Department, La Dorada Municipality, Vereda San Roque, Natural Private Reserve Rio Manso, 245 m a. s.l., MPUJ 3098, MPUJ 3397, MPUJ 3421-24, Vereda El Gigante, La Española Farm, Right bank of the Purnio river, 490 m a.s.l., MPUJ 9041-3. Cundinamarca Department, Viot Municipality, Natural Private Reserve Camino Verde, 620 m a.s.l., 0426’52”N,74 30’ 31”W, IAvH-Am–14610. Huila Department, Aipe Municipality, San Francisco,550 m a.s.l., MPUJ 1740, Neiva Municipality, Vereda Tamarindo, Hocol Prívate Reserve, Research and Education Center, La tribuna farm,570 m a.s.l., MPUJ 3612, MPUJ 4287. La Guajira Department, La Albania Municipality, Cerrejon,115’26.68 N, 7238’38.84”W(Blanco *et al.* 2015). Magdalena Department, Ciénaga Municipality, Candelaria along the Frío River, IAvH-Am–6330. Fundación Municipality, UMMZ 45566-45571, UMMZ 45573,UMMZ 45575-45581, USNM 51215, Sabanas de San Angel, “La Gloria project is part of “Reforestadora de la Costa (REFOCOSTA S.A.S.),1010’29.2”N, 7419’38.052”W, UMAG 710, UMAG 720 (Angarita *et al.* 2015). Santander Department, Barrancabermeja Municipality, Corregimiento El Centro, Vereda Campo 14, Family Pimentel´s farm, 111 m a.s.l., IAvH-Am–16578, Cimitarra Municipality, Farm near road Ruta del sol-Cimitarra, 166 m a.s.l., 62836.4”N,740153.6”W, IAvH-Am–13050, in pond near to Cimitarra 17 kms to West,166 m a.s.l., 62751.7”N,740149” W, IAvH-Am–13052-3,Floridablanca Municipality, IAvH-Am–4750 , Puerto Parra Municipality, Puerto Parra. Sucre Department, Colos Municipality, Primates Station, El Sereno stream, 386 m a.s.l., IAvH-Am–10482. Coveñas Municipality, around the town, ICN 1203, Galeras Municipality, Farmer of Pedro Hernández, ICN49094-6, San Benito Abad, site La Caimanera, 0927,1’N,7454’26,7”W, 25 m a.s.l., IAvH-Am–8219, San Marcos Municipality, Vereda La Florida, Crocodylia farm, 34 m a.s.l., ICN 49114-ICN 49126, 0839’40” N,7508’05”W, IAvH-Am–6665, Tol Viejo Municipality, La Estanzuela farm 4km West of Tolu, Zoocriadero La Oculta, IAvH-Am–5471, Vereda Macajan, Mundo Nuevo Farm, 43 m a.s.l., 0934’38,6”N,7526’58,6”W, IAvH-Am–8391–3. Vereda Macajan, Los Navas Forest, site El Cañito, 934’39”N,7527’40”W, 40 m a.s.l, IAvH-Am–8438. Tolima Department, Carmén de Apicala Municipality, 318 m a.s.l, ICN56411-8, near 3 Km of Melgar Municipality, road to El Carmen de Apicala, banks of the Sumapaz river, 530 m a.s.l, ICN1231, Armero-Guayabal Municipality, El Cardonal farm,400 m a.s.l, MPUJ 1446-7, El Carmén de Apicala Municipality, road to Carmen de Apicala from the central highway, ICN52778, Ibagu Municipality, El Totumo, 1015 m a.s.l., CZUT 350 (Bernal *et al.* 2005), IAvH-Am–10028, Mariquita Municipality Mariquita

town, MLS 134, CNHM81817, (Cochran & Goin,1970), Ortega Municipality, Balsillas, El Antojo Farm, 455 m a.s.l., MPUJ 1739, Venadillo Municipality, ICN1206-7, Miravalle Farm, ICN1233-6, 4 Km. south of Venadillo, 430 m a.s.l, ICN52123.

Elachistocleis panamensis. COLOMBIA: Atlántico Department, Juan de Acosta Municipality, 50 m a.s.l., ICN49060, Baranoa Municipality, 112 m a.s.l., ICN49061. Tubar Municipality, 100 m a.s.l., ICN49062-6. Puerto Colombia Municipality, 100 m a.s.l., MPUJ7196. Bolívar Department, Zambrano Municipality, IAvH-Am–8639-41, IAvH-Am–9145-7, Hacienda Monterrey, 20 m a.s.l., 333'56"N,7635'3"W, ICN50104-7, IAvH-Am–7629, IAvH-Am–7631, IAvH-Am–9137. Boyac Department, Puerto Boyac, Police Inspection Puerto Romero, 550'15.07"N, 7420'26.84"W, 251 m a.s.l., ICN44699.Cesar Department, Aguachica Municipality, Cienaga Musanda, 81'48.00"N, 7343'48.00"W, 37 m. a.s.l. (Paternina *et al.* 2013), Ciénaga Doña María, 8 3'52.26"N, 7344'19.01"W, 37 m a.s.l. (Paternina *et al.* 2013), Chimichagua Municipality, Ciénaga de Zapatosa, 911'59.99"N, 7349'12.00"W, 29 m a.s.l. (Paternina *et al.* 2013), Municipality El Copey, Finca La Joya, 104'18.14"N,74◦07'70"W (Blanco *et al.* 2015), Finca Las Varas, 916'20"N,7351'35"W, 121 m a.s.l., IAvH-Am–11279, Caño Largo- Tordecilla,918'59"N,7349'5 2"W, 113 m a.s.l., IAvH-Am–11280, El Paso Municipality, Ciénaga La Pachita, 939'35.99"N, 7340'48.00"W, 41 m a.s.l. (Paternina *et al.* 2013), Ciénaga Mata de Palma, 931'48.00"N, 7339'0.00"W, 34 m a.s.l. (Paternina *et al.* 2013), Gamarra Municipality, Ciénaga de Baquero, 819'4.10"N, 7343'42.41"W, 36 m a.s.l. (Paternina *et al.* 2013), Ciénaga de Juncal, 818'27.29"N, 7343'53.14"W, 34 m a.s.l. (Paternina *et al.* 2013), Municipality La Gloria, Ciénaga de Morales, 819'12.00"N, 7349'12.00"W, 40 m a.s.l. (Paternina *et al.* 2013), Pelaya Municipality, Ciénaga de Costilla, 843'47.95"N, 7342'0.01"W, 40 m a.s.l. (Paternina *et al.* 2013), site El Lucero, Brisa de Mar farm, 843'12.00", 7340'48.00"W, 190 m a.s.l., IAvH-Am–10555, Municipality San Martin, Ciénaga del Congo, 852'48.00"N, 7359'60.00"W, 25 m a.s.l. (Paternina *et al.* 2013). Caldas Department, Saman Municipality, Tasajos Camp, La Miel II, 523'22.40"N, 7455'52.63"W, 530 m a.s.l., ICN34933. Choc Department, Riosucio Municipality, Sautat, 750'1.13"N, 77 3'57.83"W, FMNH 74908. Córdoba Department, Cispat Municipality, ICN43419-21, Montería Municipality, El Diluvio Farm, 843'12.00"N, 7558'48.00"W, 64m a.s.l., ICN48687-ICN48709, Los Araujos, ICN48921. Cundinamarca Department, Puerto Salgar Municipality, Magdalena river, Montecristo farm, 250 m a.s.l. MLS 278. Huila Department, km 10 road between Villa Vieja and Baraya Municipalities, 311'24.00"N, 75 8'60.00"W, 465 m a.s.l., ICN11707-09, ICN11712-14. La Guajira Department, Dibulla Municipality, Mingueo, RPB526-7, Montes de Oca, 1115'24.08"N, 7221'41.22"W, 106 m a.s.l. (Galvis *et al.* 2011), Arroyo Cerrejon, 11 3'6.67"N, 7240'11.16"W, Arroyo Palomino, 1058'15.98"N, 7245'35.31", 143 m a.s.l., Río Rancheria, sector La Batea, 11 7'49.38"N, 7237'3.65"W, 97 m a.s.l., Río Rancheria, sector Los Pozos, 11 5'21.13"N, 7238'56.67"W, 105 m a.s.l. (Blanco *et al.* 2013), La Albania Municipality, Cerrejon, 11◦5'26.68 N, 72◦38'38.84"W (Blanco *et al.* 2015). Sucre Department, San Marcos Municipality, La Florida,34 m a.s.l., ICN49343. Magdalena Department, Fundación Municipality, UMMZ 45576, UMMZ 48266, Sabanas de San Ángel, "La Gloria project is part of "Reforestadora de la Costa (REFOCOSTA S.A.S.),1010'29.2"N, 7419'38.052"W, UMAG 719, Tayrona National Natural Park, Neguanje site, IAvH-Am–7398-9. Santander, Department, Cimitarra Municipality, Farm near road Ruta del sol-Cimitarra,166 m a.s.l.,62836.4"N,74015 3.6"W, IAvH-Am–13051, near Puerto Araujo Ruta del Sol, 2 kms eastern of Zambito, 135 m a.s.l., 61555.2"N,742854.9" W, IAvH-Am–13057, 2 kms northwestern of La Luz,166 m a.s.l.,62751.7"N,740144.0"W, IAvH-Am–13079, 3 kms to eastern of the la Sonora,135 m a.s.l.,62940.0"N,740257.0"W, IAvH-Am–13088–9, Deviation from Ruta del Sol to Cimitarra,135 m a.s.l., 740257.0", IAvH-Am–13801, Vereda El Tigre, La Dorada farm ,IAvH-Am–15642-3. Sucre Department, Tol Viejo Municipality, Zoocriadero La Oculta, IAvH-Am–5470.