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Natural history and conservation of the Nurse Frog of the Serranía del Perijá *Allobates ignotus* (Dendrobatoidea: Aromobatidae) in northeastern Colombia

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Abstract. We describe new findings on the geographic distribution, habitat uses, relative abundance, tadpoles, and advertisement call of *Allobates ignotus*. Data of habitat uses and relative abundance were collected during five annual fieldtrips of seven days each one, from April 2010 to June 2014, in a mining zone of Canime Creek Basin, La Jagua de Ibirico, Cesar Department, northeast Colombia. Tadpoles and advertisement call were obtained in La Veguita Creek Basin, Manaure Balcón del Cesar, Cesar. We report six new localities with presence of *A. ignotus*, all between 7-96 km in straight line to the type locality, from 194-1236 m a.s.l. This species is recorded for the first time in the Tropical Dry Forest Life Zone and in La Guajira Department. Four substrate categories used by *A. ignotus* were detected (leaf-litter, rocks, naked floor, and lower branches), and differential use was highlighted. The larval morphology of *A. ignotus* was described, based on 11 specimens between 25-29 stages. Like other *Allobates* tadpoles, the gap of the second teeth row is "wide". The advertisement call of *A. ignotus* is composed by a series of high frequency ascending pulsed notes. Possibly, the high frequency of the call is due to the acoustic disturbance generated by the streamflow noise of the creeks. In 2014, a decrease of relative abundance of *A. ignotus* in Canime Creek was detected. This, coupled to restricted distribution and loss of habitat quality are sufficient criteria to suggest the category of vulnerable for *A. ignotus*.

Keywords. Advertisement call, Colombian Andes, Colombian Caribbean, geographic distribution, habitat uses, relative abundance, tadpoles, tropical dry forest.

INTRODUCTION

The Serranía del Perijá is a mountain system of the northern South America, whose highest points make up the northern boundary between Colombia and Venezuela. Because its geographic position, the Serranía del Perijá functioned in the past as a biological corridor between Andes and Caribe, showing biogeographical affinities with the Sierra Nevada de Santa Marta (= Santa Marta Mountains) and the Cordillera Oriental (= Colom-

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bian Eastern Andes) (Hernández-Camacho et al., 1992). Currently, the Serranía del Perijá is considered of great importance from an amphibian biodiversity approach by the high endemicity, deserving to constitute a distinct ecogeographic unit (Romero-Martínez and Lynch, 2012).

In Colombia, 14 species of the genus *Allobates* Zimmerman and Zimmerman, 1988 (Aromobatidae) are recognized, broadly distributed in the wet and dry forests of the western and eastern lowlands of the country (Acosta-Galvis, 2017). However, there are gaps in the knowledge of the Colombian *Allobates* about of their taxonomy and natural history (Anganoy-Criollo, 2012). For the species of this country, there are some notes on the abundance, habitat use, conservation and feeding ecology of *Allobates juanii* (Cáceres-Andrade and Urbina-Cardona, 2009; Astwood-Romero et al., 2016), and a bit more information for *Allobates femoralis* and *Allobates talamancae* (*v. gr.* Junca and Eterovick, 2007; Ringler et al., 2009; Hopkins and Lahanas, 2011; Montanarin et al., 2011; Keafer et al., 2012; Lechelt et al., 2014), even if these data do not come from Colombian populations. For all other Colombian *Allobates*, the information on their geographic distribution, habitat use, and calls, is mostly provided only by the original (re-)descriptions.

Allobates ignotus Anganoy-Criollo, 2012 (Fig. 1) is an aromobatid frog, only known from its original description, endangered, and endemic to the Serranía del Perijá (IUCN SSC Amphibian Specialist Group, 2017). In

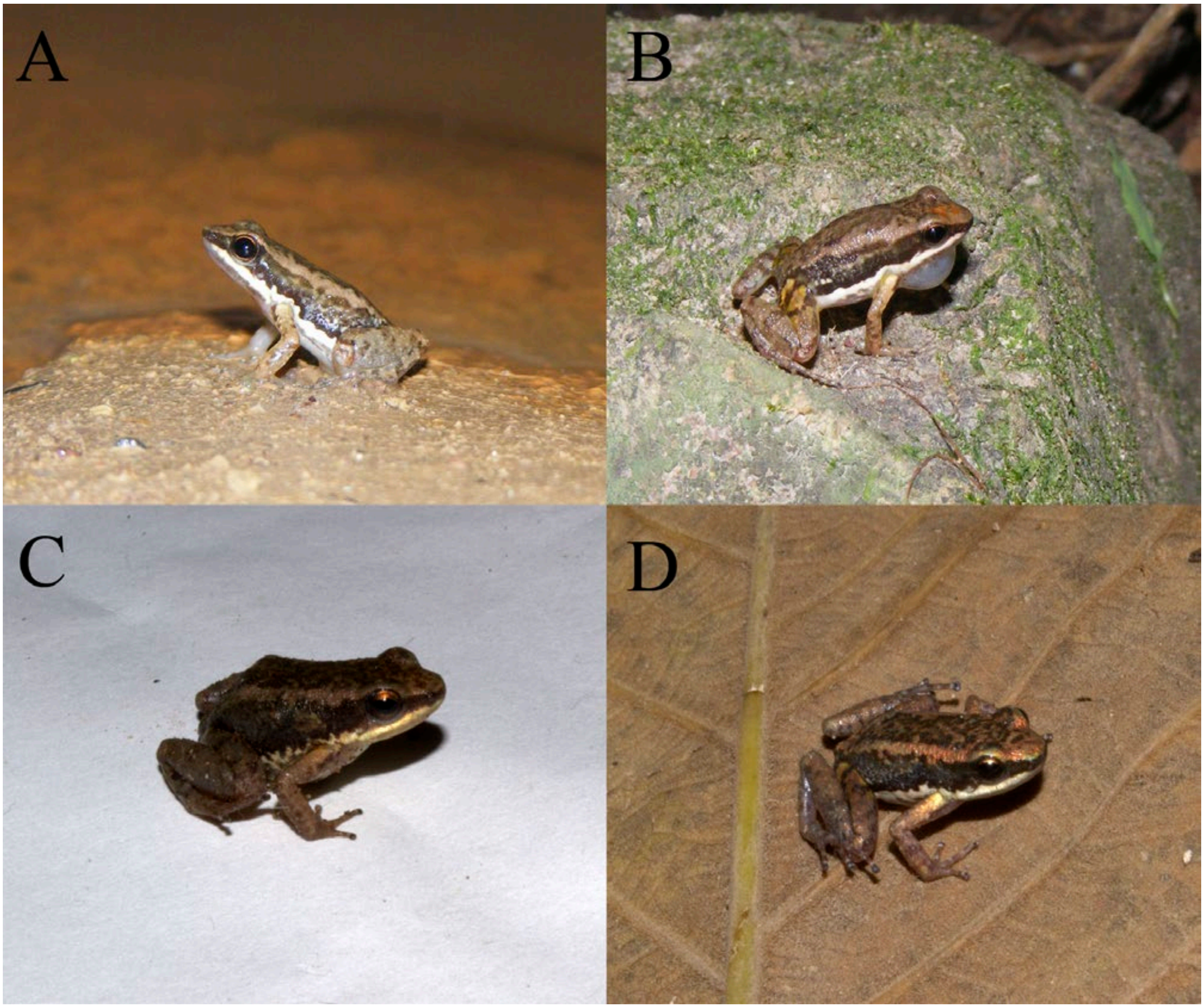

Fig. 1. Specimens of *Allobates ignotus* from Canime Creek (A), La Frontera's way (B), La Veguita Creek (C), Cesar department, Colombia; and Quebrada Nicaragua (D), La Guajira department, Colombia. Photographs by H.D. Granda-Rodríguez (A), A. del Portillo-Mozo (B), and J.D. Jiménez-Bolaño (C, D).

the distribution of this species, only three localities are known (Anganoy-Criollo, 2012; Acosta-Galvis, 2017) with very few available information. As a consequence, being many aspects of the biology, ecology, and natural history of *A. ignotus* unknown, it is hard to determine the conservation status, threats, and measures to species protection. In this paper, we report new data on several aspects of the natural history (geographic distribution, free-swimming tadpole, advertisement call, habitat use, and abundance) and conservation status of *A. ignotus*.

MATERIALS AND METHODS

Study area

All records of *Allobates ignotus* were collected on the foothills and midlands on the western flank of the Serranía del Perijá. These localities have a bimodal rainfall regime, with high to moderate water deficit in dry periods (Rangel-Ch. and Carvajal-Cogollo, 2012). The first rainy peaks is from April to May and second from September to October. The two periods of low rains are from December to March and in July (Rangel-Ch. and Carvajal-Cogollo, 2009). The foothills of the Serranía correspond to the "Zonobioma Tropical Alternohígrico" (follow to Hernández-Camacho and Sánchez 1992) or to the Tropical Dry Forest Life Zone (*sensu* Holdridge, 1967). The midlands of the Serrania is a biome known as "Orobioma de la Selva Subandina" (Hernández-Camacho and Sánchez, 1992) or as Moist Forest, Wet Forest and Rain Forest life zones (Holdridge, 1967). The latter has a low water deficit in dry periods (Rangel-Ch. and Carvajal-Cogollo, 2009).

Habitat use and abundance

We obtained the relative abundance, habitat and microhabitat uses data in three sections of the Canime Creek (Sector 1: 09°33'16.10"N, 73°16'00.90"W, 250 m a.s.l.; Sector 2: 09°36'10.20"N, 73°16'53.70"W, 200 m a.s.l.; Sector 3: 09°34'22.42"N, 73°15'52.24"W, 194 m a.s.l.), a quaternary tributary of the Cesar River, in the municipality of La Jagua de Ibirico, Cesar Department, northeast Colombia. Surveys were conducted in a mining area, where most of the land corresponds to coal exploitation zones and deposits of waste material. Grasslands, wooded savannas, and cattle pastures occupy another larger percentage of the land use. The tropical dry forest cover is established in a smaller proportion, only in the margins of streams, in mosaics of gallery forest, bushes, stubble, and deciduous forest.

We performed five field trips to the Canime Creek, one in dry season (February 2011) and four in rainy season (April 2010, August 2012, April 2013, and June 2014), for a total of seven days for each trip. For frogs sighting, we used the Visual Encounter Survey (VES) (Crump and Scott, 1994). Daily, two observers conducted random walks between 09:00-12:00 h and 15:00-16:00 h, for a total sampling effort of 109 h × observer. We recorded the substrate just under the specimen at the time of sighting. We collected some voucher specimens, sacrificed on immersion to chlorobutanol solution (10%). All collected vouchers were deposited in the Amphibian Collection of the Centro de Colecciones Biológicas de la Universidad del Magdalena, at Santa Marta, Colombia (CBUMAG:ANF). To confirm the species level determination, the collected voucher was directly compared to the type series of *A. ignotus* deposited in the Amphibian Collection of the Instituto de Ciencias Naturales at Universidad Nacional de Colombia (ICN 55434, holotype; 55427-433, 55435-439, paratypes)

Relative abundance was calculated as the number of individuals in each sample relative to capture effort, expressed in individuals/hours × observer (RA = Ind/h × obs) (Lips, 1999). We performed a homogeneity Chi square test (χ^2) in order to reject the null hypothesis that there is no differential use of the microhabitat by *A. ignotus*. The expected values were calculated equitably from each substrate category detected in field, assuming that all substrates had the same probability to be occupied.

Free-swimming tadpoles

From December 2015, we collected tadpoles in La Veguita Creek (10°23'12.57"N, 73°03'19.03"W, 633 m a.s.l.), La Veguita Village, Municipality of Manaure Balcón del Cesar, Cesar department, Colombia. This creek is surrounded for a secondary tropical dry forest relict, on a slope of approximately 20°, with a more or less staggered configuration of small waterfalls and pools, and abundant boulders. La Veguita Creek is the repository of waste waters of the municipality, showing grayish color. We found the tadpoles on the marginal puddles of the stream. These puddles had a low depth (< 20 cm) and recorded water temperature was 28 °C. The puddle bottom was very sediment, but column was transparent. Tadpoles were sacrificed in an immersion in the formaldehyde solution (10%). We assigned the tadpoles to *Allobates ignotus* because the metamorphic individuals observed in the same site have adult features (dorsolateral, oblique, and ventrolateral stripes, and color pattern) of *A. ignotus*. In addition, the only one dendrobatoid frog detected in the study area was this species.

Description was based on a set of 11 specimens (CBUMAG:ANF 936), collected by J.D. Jiménez-Bolaño, J.A. Rincón, and A.C. Montes-Correa. No other species of *Allobates* (or any other dendrobatoid frog) were found in La Veguita Creek. Here, we provided a description of the free-swimming tadpoles at stages 25-29 (according to Gosner, 1960). No other developmental phases were available (i.e., neither back-riding tadpoles nor metamorphic tadpoles). Measurements (in mm) and proportions are provided for all tadpoles. Larval terminology were determined according to Altig and McDiarmid (1999). To describe the attached type of the vent tube to the ventral fin, we follow the Figure 3.5 of Altig and McDiarmid (1999). Description and measurement of the number of marginal papillae on each lip followed Anganoy-Criollo (2013) and disposition of the row of marginal papillae followed Sánchez (2013). Values of the gap of the second tooth row of other species of *Allobates* were obtained from the measurements or illustrations of the oral disc of free-swimming tadpoles provided in each description. Measurements were taken with a manual caliper (0.01 mm) and with an ocular micrometer of a Carl Zeiss Stemi 2000 stereomicroscope (0.001 mm). For morphological characters the following abbreviations were used: (AL gap) anterior lip gap, (A-2 gap) medial gap in second anterior tooth row, (BH) body height, (BL) body length, (BW) body width, (END) eye-nostril distance, (IND) internostril distance, (IOD) interorbital distance, (LJW) lower jaw sheath width, (ODW) oral disc width, (P-AL) papillae on the right side of anterior lip, (P-PL) papillae on posterior lip in a space of 1 mm, (SH) spiracle height at its base, (SL) spiracle length, (SND) snout-nostril distance, (SOH) spiracle opening height, (SSD) snout-spiracle distance, (TAL) tail length, (TMW) tail muscle width at its base, (TMH) tail muscle height at its base, (TL) total length, (UJW) upper jaw sheath width, (VT) vent tube length.

Advertisement call

From December 2015, we collected three recordings of advertisement calls of three males in La Veguita Creek, village of La Veguita, municipality of Manaure Balcón del Cesar, department of Cesar, Colombia. Digital recordings were made at a sampling rate of 44 kHz and 16 bit resolution with a SONY LCD-PX 240 Voice Recorder with built-in microphone. We collected 281 sec of total recording. One of the males was preserved as a voucher specimen. Calls were recorded at 16:20 pm, with a temperature of 25.4 °C under shade. Calls were analyzed in PRAAT 6.0.13 for Windows (Boersma and Weenink, 2007). Each sound file of each individual contained four to six calls, which were analyzed individually. The acoustic parameters measured were: call duration (sec), number of notes per call, note duration (sec), internote interval (sec), and rate of notes per sec (notes/sec); as well as the spectral properties, such as dominant frequency and frequency range, with respective mean and standard deviation (SD) (Littlejohn, 2001; Köhler et al., 2017). Graphics was produced with the R package Seewave (Sueur et al., 2008).

RESULTS

Geographic distribution

Based on museum specimens and field observations, we recorded six new localities with confirmed presence of *Allobates ignotus* (Fig. 2); five localities in Cesar Department (three sectors in the Canime Creek, 194-250 m a.s.l., La Jagua de Ibirico; La Frontera's way, 1236 m a.s.l., Agustin Codazzi; La Veguita Creek, 570 m a.s.l., Manaure Balcón del Cesar) and one locality in La Guajira Department (Nicaragua Creek , 905 m a.s.l., La Jagua del Pilar). The new localities are between 7-96 km in straight line from the type locality, between 194-1236 m a.s.l. The life zone of these localities is tropical dry forest and the transition with the low montane wet forest. The new records widen the occurrence of the species to 605 km^2, and represent the first record of *A. ignotus* in tropical dry forest and La Guajira Department.

Habitat uses

In the three sections of the Canime Creek, La Jagua de Ibirico lowlands, we recorded 187 individuals of *Allobates ignotus*, all restricted to low flow and shallow creeks, with abundant gallery forest of closed canopy. *Allobates ignotus* were not recorded in the deciduous forest, bushes, stubble, or pastures adjacent to the riparian forest.

In the gallery forest interior, we determined four categories of microhabitat (substrate) occupied by *Allobates ignotus:* leaf-litter, rocky substrate (boulders-likes), fallen trunks, and lower branches (<50 cm to floor). *Allobates ignotus* uses differentially the available substrata (χ^2 = 51.65, df = 3, P < 0.001, Table 1): 46.64% were recorded active on the leaf-litter; a slightly lower percentage

Table 1. Frequency and homogeneity Chi square test (χ^2) of microhabitat uses by *Allobates ignotus* in Canime Creek Basin, Cesar department, Colombia.

Substrata	N	χ^2	df	P
Leaf-litter	76	18.301		
Rocks	65	7.124		
Fallen trunks	30	6.001		
Lower branches	16	20.226		
Total	187	51.652	3	<0.001

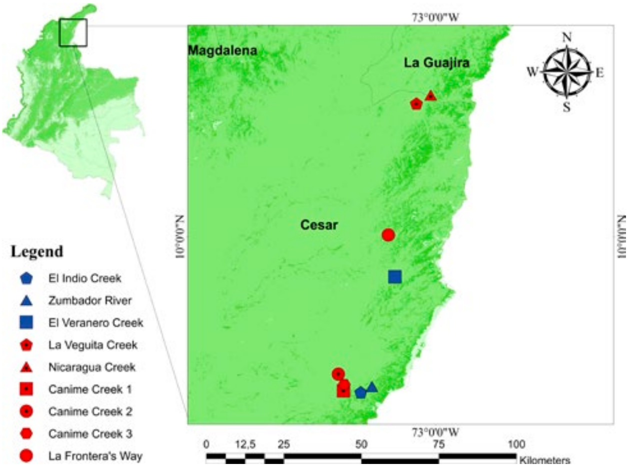

Fig. 2. Geographic distribution of *Allobates ignotus* in Colombia. Blue (light grey in the printed version) symbols for known localities and red (dark grey in the printed version) symbols for new records. Development by Lorena Benitez-Cubillos.

(34.76%) occurred on rocky substrates; fallen trunks and lower branches were used to a lesser extent (16.04% and 8.56%, respectively).

Free-swimming tadpoles

All measurements and proportions of free living tadpoles are given in Table 2. Tadpoles have an ovoid body in dorsal view, wider at mid-body; depressed in lateral view (Fig. 3A-C), barely higher at level of intestines (BH/BW = 0.67-0.80; BW/BL = 0.57-0.71), shorter than half of the total length (BL/TL = 0.27-0.39). Snout rounded in dorsal and lateral view (Fig. 3A, C). The nostrils are rounded or slightly ovoid, positioned dorsally, and opening directed dorsolaterally; located about midway between the eye and the tip of snout; nostril 25-36% of eye diameter; inner margin with a thick fleshy ring and very low fleshy projection dorsally, sometimes no visible. The internarial distance is equivalent to 53-75% of interorbital distance. Interorbital distance 43-55% of body width. Eyes dorsal, directed dorsolaterally. Eye diameter larger than nostril-eye distance (except a tadpole in stage 27, with eye about equal to the nostril-eye distance) and 39-51% of interorbital distance. Spiracle sinistral, slightly longer than high (0.9-1.2 times) with cylindrical shape (i.e., the spiracle height is the same through of spiracle length). Spiracle directed posterodorsally is located below longitudinal midline and on vertical midline of body. Spiracular opening ending 63-77% of body length (from tip of snout), and is barely separated from body. Vent tube short, 8-15% of body length, attached to right side of ventral fin in B-type; opening of vent tube with posterior direction and smooth edge.

Inner intestines were visible through translucent skin; the longitudinal axis of intestines is sinistral to body; guts in 5-10 coils in ventral view (Fig. 3B). Tail length of 61-73% of total length. At body-tail junction, tail muscle height from 45-66% of body height. Myotomes higher than wide at body-tail junction, except in a tad-

Table 2. Measurements (in mm) of the free-swimming tadpoles of *Allobates ignotus*. The range (mean ± standard deviation) for linear measurements are reported; only the range for proportions. For abbreviations, see "Tadpoles" section in Materials and Methods.

Measurement	Stage 25 (n = 2)	Stage 26 (n = 4)	Stage 27 (n = 3)	Stage 28-29 (n = 2)
TL	8.7-16 (12.4 ± 5.2)	9.6-14.9 (12 ± 2.2)	13.9-18.2 (16.2 ± 2.2)	17.8-21 (19.4 ± 2.3)
BL	3.2-4.3 (3.8 ± 0.8)	3.6-4.9 (4.3 ± 0.5)	5-6.7 (5.9 ± 0.9)	7-7.6 (7.3 ± 0.4)
BW	2-2.9 (2.5 ± 0.6)	2.4-3.3 (2.9 ± 0.4)	3.4-4.2 (3.9 ± 0.4)	4.5-4.6 (4.6 ± 0.1)
BH	1.6-2.2 (1.9 ± 0.4)	1.6-2.6 (2.2 ± 0.4)	2.4-3 (2.8 ± 0.3)	3.2-3.3 (3.3 ± 0.1)
TAL	5.5-11.7 (8.6 ± 4.4)	6-10 (7.7 ± 1.7)	8.9-11.5 (10.3 ± 1.3)	10.8-13.4 (12.1 ± 1.8)
Eye	0.56	0.6-0.7 (0.6 ± 0.1)	0.7-0.8 (0.7 ± 0.1)	0.8-0.9 (0.8 ± 0.1)
Nostril	0.2	0.2	0.2	0.2
IOD	1.1-1.4 (1.3 ± 0.2)	1.3-1.6 (1.4 ± 0.1)	1.7-1.9 (1.8 ± 0.1)	2
IND	0.7-0.9 (0.8 ± 0.1)	0.8-1 (0.9 ± 0.1)	0.9-1.3 (1.2 ± 0.2)	1.4-1.5 (1.4 ± 0.1)
SND	0.2-0.4 (0.3 ± 0.1)	0.4-0.7 (0.5 ± 0.2)	0.4-0.6 (0.5 ± 0.1)	0.9-1 (0.9 ± 0.04)
END	0.4-0.5 (0.4 ± 0.03)	0.4-0.5 (0.4 ± 0.1)	0.5-0.7 (0.6 ± 0.1)	0.7-0.8 (0.7 ± 0.1)
SL	0.6-1 (0.8 ± 0.3)	0.7-1.2 (0.9 ± 0.2)	0.8-1 (0.9 ± 0.1)	1.2-1.3 (1.3 ± 0.1)
SH	0.5-1 (0.8 ± 0.3)	0.6-1.1 (0.9 ± 0.2)	0.8-1.1 (1 ± 0.1)	1.4-1.5 (1.4 ± 0.1)
SOH	0.1-0.3 (0.2 ± 0.1)	0.1-0.2 (0.2 ± 0.04)	0.2-0.3 (0.3 ± 0.02)	0.4
SSD	2-3.3 (2.7 ± 0.9)	2.7-3.4 (3.1 ± 0.3)	3.8-4.8 (4.3 ± 0.5)	5.3-5.5 (5.4 ± 0.1)
VT	0.4	0.4-0.5 (0.4 ± 0.03)	0.6-0.9 (0.8 ± 0.1)	1.0
TMW	0.8-0.9 (0.9 ± 0.1)	0.8-1.2 (1 ± 0.2)	1.1-1.7 (1.4 ± 0.3)	1.8
TMH	1-1.3 (1.2 ± 0.2)	1-1.3 (1.1 ± 0.2)	1.5-1.8 (1.7 ± 0.2)	2-2.1 (2.1 ± 0.1)
ODW	1.4-2.2 (1.8 ± 0.2)	1-1.6 (1.4 ± 0.3)	1.5-2 (1.8 ± 0.3)	2.1-2.4 (2.3 ± 0.2)
AL gap	0.7-1 (0.8 ± 0.2)	0.8-1 (1 ± 0.1)	1.1-1.2 (1.2 ± 0.1)	1.4-1.6 (1.5 ± 0.2)
UJW	0.5-0.6 (0.5 ± 0.1)	0.5-0.8 (0.6 ± 0.1)	0.7-0.9 (0.8 ± 0.1)	0.9-1.1 (1.0 ± 0.1)
LJW	0.3	0.3-0.5 (0.4 ± 0.1)	0.6-0.7 (0.6 ± 0.1)	0.7-0.8 (0.7 ± 0.1)
A-2 gap	0.3	0.3	0.3-0.4 (0.3 ± 0.03)	0.4
P-AL	5	5-7	7-9	10-15
P-PL	23-25	13-19	14-17	16-18

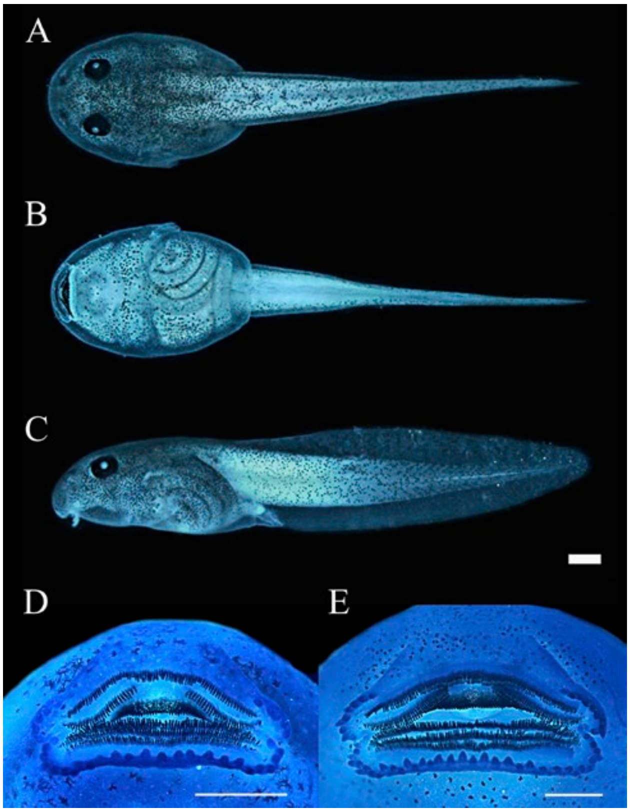

Fig. 3. Tadpole in stage 27 of *Allobates ignotus*, in dorsal (A), ventral (B), and lateral (C) view. Oral disc of a tadpoles in stage 26 (D) and in stage 29 (E). See the upper jaw sheath shape (D) and the lower tooth rows (E). Scale bar equal to 1.0 mm (A, B, C) and 0.5 mm (D, E). Photographs by M. Anganoy-Criollo.

pole in stage 26 that have myotomes as high as wide. The myotomes are straitened gradually towards the tip of the tail. At mid-tail, dorsal fin barely higher than ventral fin and myotomes higher than dorsal fin. Dorsal fin originating at body-tail junction; dorsal fin lower on first anterior third of the tail, posteriorly of same height through tail length, sometimes higher at mid-tail. Ventral fin of same height through all of tail length. Tip of tail rounded. Maximum tail height less, equal to, or higher than body height, 88-116% of body height.

Lateral line system symmetrical on both sides of body with supraorbital, anterior pit, infraorbital, oral, temporal infraorbital, supratemporal, dorsal, middle and ventral trunk lateral lines. Lateral lines were visible from stage 27 (except for one tadpole in stage 26 with supraorbital lateral line). In one tadpoles in stage 27 only the supraorbital, infraorbital lateral, dorsal and middle trunk line are present, and in other tadpoles (stages 27-29) the lines already mentioned are observable. The stitches in all lateral lines are equidistant between each of them and predominantly rounded (see additional changes below). Pale white spot(s) (cumuli of neuromasts), and pale line between eye-nostril are absent.

Oral disc positioned and ventrally directed, laterally emarginated, and surrounded by marginal papillae, except on anterior lip; sub-marginal papillae absent (Fig. 3D, E). Oral disc width 42-52% of body width. Anterior lip gap 60-88% of oral disc width. Marginal papillae arranged in rows varying by lip and stage from 1-2 rows (see below). Papillae abundant on anterior and posterior lip, 5-7 papillae (stages 25-27), 15 (stage 28), and 10 (stage 29) on right side of anterior lip, and 23-25 papillae on entire posterior lip (stage 25, due that posterior lips is less than 1 mm wide) and from 13-19 papillae/mm on posterior lip (stages 26-29). Papillae low and narrow on anterior and posterior lip, barely elongated, papilla width (at base) third quarter or two third than papilla height. Tips of papillae rounded to sub-acuminate. Labial tooth row formulae (LTRF) 2(2)/3; second upper row (A-2) with moderate gap, from 17-28% of oral disc width. Length of first upper row (A-1) equal to second upper row (A-2). First lower row (P-1) equal to second and third lower row (P-2, P-3) (n = 4); P-1 equal to P-2 and P-3 slightly shorter than P-1 and P-2 (n = 4); or P-1 equal than P-3 and these slightly shorter than P-2 (n = 3). Upper rows sub-equal (commonly) or slightly shorter (rarely) than first and second lower rows. Jaw sheath keratinized and black or dark brown. Upper jaw sheath (UJS) low in height, with long and thin lateral processes; UJS height 11-18% of UJS width. UJS width 37-50% of oral disc width. Anterior edge of UJS well defined (commonly) or indistinct (rarely), forming a convex shape; posterior free edge of UJS barely sinuous, with low serrations; mid-UJS with very low notch (Fig. 3E), 20-22% of UJS height. Lateral processes without serrations. Tips of serrations rounded on middle of UJS and acuminate to sub-acuminate towards ends. Shelf on concealed UJS absent. Lower jaw sheath (LJS) thin, V-shaped with proximal ends slightly open. Only distal half of LJS pigmented black, LJS height (black pigmented area) 50-100% of UJS height. Serrations on LJS low and with rounded tips.

In preservative, the skin of all body of the free-swimming tadpoles is translucent; body dorsally and laterally with abundant dark brown (stages 25-27) or less brown dots (stages 28-29), distributed uniformly on body, without darkening the body, forming a dark brown region around of nostril. Ventrally with dark brown dots (stages 25-27) or brown dots (stages 28-29), distributed uniformly from posterior oral disc to middle intestines, without darkening the venter. Under magnification, the dark brown dots of venter are asterisk-like (stages 25-27) or rounded (stages 28-29) dots (Fig. 3B). Intestines yellowish brown, visible in lateral

Table 3. Variation of the rows of marginal papillae of the oral disc of tadpoles of *Allobates ignotus* through of the available stages of development. Whether there was variation, this is reported as range. Conventions: (A) papillae on anterior lip, (B) papillae on postero-lateral side of posterior lip, and (C) papillae on middle of posterior lip; (1) one row, (2) one biseriated row, (3) two rows of papillae, (*n*) number of tadpoles. Stages refer to Gosner (1960).

Stages (n)	A	B	C
25 (2)	1	1	1-2
26 (4)	1	1	1
27 (3)	1	2	1-2
28-29 (2)	1	3	1-3

and ventral views and partially in dorsal view. Myotomes white and fins white translucent with brown dots covering the dorsal fin, myotomes and dorsal portion of the ventral fins, distributes uniformly (stages 25-27) and forming some weak reticulate pattern (stages 28-29). Nostril white; spiracle and tube vent translucent and oral disc white with a few brown dots on anterior lip.

The variation in free-swimming tadpoles (stages 25-29) (Table 3) is as it follows: in dorsal view, one tadpole in s25 has the body slightly more rounded than the rest of tadpoles. The marginal papillae row number varies between stages 25 and 29. In the anterior lip, the marginal papillae are disposed in one row from stages 25 to 29. In the posterolateral side of posterior lip, the marginal papillae are arranged in one row in stage 25-26, one biseriated row in stage 27 and two row in stage 28-29. On the posterior region of posterior lip, the marginal papillae are in one row in stages 25-27 and 29, in one biseriated row in one tadpole in stage 25 and 27, and in two rows in stage 28.

The lateral line system also varies between these tadpoles. The supraorbital lateral, dorsal and middle trunk lines are incomplete. The supraorbital lateral line of one tadpole in stage 26 and other in stage 27 extends from oral disc to nostril; whereas, the dorsal and middle trunk lines of the stage 27 ranges from body to mid-tail. The stitches are rounded in supraorbital, anterior pit, infraorbital, temporal infraorbital, supratemporal lateral and dorsal trunk lines, but are slightly elongate and parallel to the line in the oral lateral and middle trunk lines, although in middle trunk line the stitches are ovoid in the stage 29. The stitches are oval in ventral trunk line.

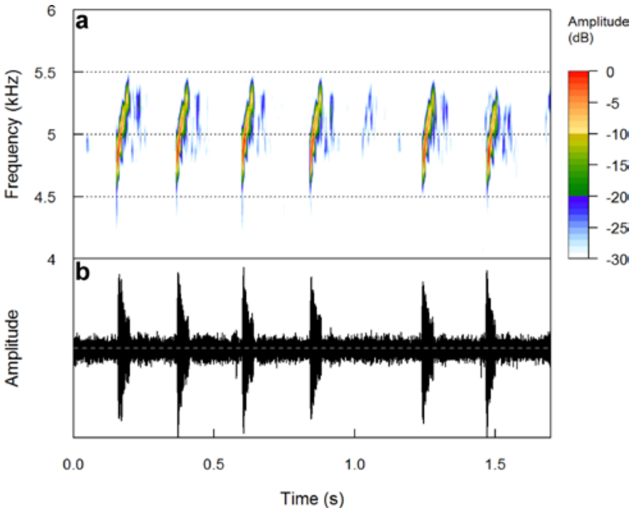

Fig. 4. Spectrogram (a) and oscillogram (b) of the advertisement call of *Allobates ignotus* of La Veguita Creek, La Veguita Village, Manuaure Balcón del Cesar, Cesar department, Colombia. Air temperature 25.4 °C (CBUMAG:ANF:00933). Total duration = 1.70 sec, 512 bands resolution.

Advertisement call

The advertisement call of *Allobates ignotus* is composed by a series of pulsed notes (x = 32; SD = 27.44; range = 6-116 notes; n = 13 calls), with a mean duration of 8.168 sec (SD = 6.36; range = 1.35-27.53 sec; n = 13 calls). The mean duration of the note was 0.046 sec (SD = 0.009; range = 0.026-0.085 sec; n = 456 notes), emitted at a rate of 3.94 notes per sec (SD = 0.61; range= 2.32-4.46), in an internote interval of 0.211 sec (SD = 0.110; range = 0.043-1.790 sec; n = 443) (Fig. 4b). The call frequency was between 4600-14000 Hz, and the dominant frequency was 4942.82 Hz (SD = 204.3; range = 5312-6662Hz; n = 13). Pulse has three distinct harmonics, 7490-7993 Hz, 9784-10065 Hz, and 12834-13132 Hz, respectively (Fig. 4a). Our observations in La Veguita Creek indicate that *A. ignotus* may be a philopatric species regarding to vocalization site, since an uncollected male vocalized for four hours approximately on the same rock. When this frog perceived disturbances in the vegetation caused by our capturing attempts, it escaped quickly, but returned in a few minutes to continue vocalizing in the same place. This situation was repeated about six times.

Relative abundance

The surveys performed from 2010 to 2014 showed an abrupt decrease in relative abundance of *Allobates ignotus* in Canime Creek Basin in the last year (Fig. 5). In April 2010, we recorded 0.46 ind/h × obs (n = 25, rare) of this frog; while in February 2011 observed 0.55 ind/h × obs (n = 30, common); in August 2012, we obtained 0.92 ind/h × obs (n = 50, abundant); and April 2013 we calcu-

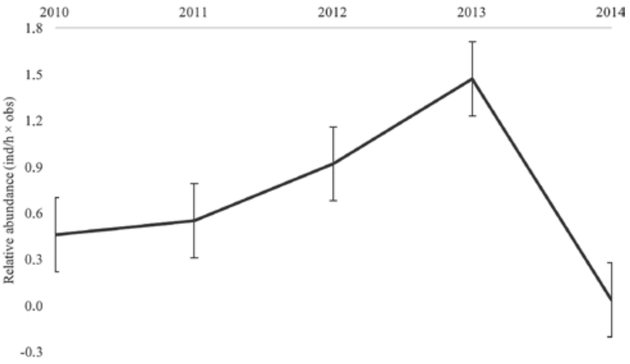

Fig. 5. Temporal variation of relative abundance of *Allobates ignotus* in Canime Creek Basin, Cesar department, Colombia.

lated 1.47 ind/h × obs (n = 80, very abundant). Nevertheless, in June 2014, we found 0.04 ind/h × obs (n = 2, very rare). Although we did not find tadpoles or amplectant couples in any of the fieldtrips to Canime Creek Basin, we observed and listened males vocalizing in all field expeditions in this basin, except to June 2014.

DISCUSSION

Geographic distribution and habitat use

Allobates ignotus was previously known in only three localities in the low wet forest of the western foothills of the Serranía del Perijá, with an extent of occurrence of 364 km^2 and 180 m altitudinal range (400 to 580 m a.s.l., Anganoy-Criollo, 2012; IUCN SSC Amphibian Specialist Group, 2017). With the records reported in the present study, we extended the distribution range of this frog *by 241* km^2, with a total extent of occurrence at 605 km^2 and a altitudinal range from 194 m to 1236 m. This records are the first for the Tropical Dry Forest Life Zone and for the department of La Guajira. The lowest localities where *A. ignotus* was found fall within the altitudinal range (0-249 m a.s.l.) with the greatest amphibian diversity in Colombia (284 species according to Acosta-Galvis, 2012).

Amphibians from dry environments require physiological, ecological, and behavioral strategies for dehydration tolerance (Urbina-Cardona et al., 2014). Habitat selection is an essential aspect for amphibians of dry ecosystems by the occupation of humid microhabitats in dehydrating environments (Bentley, 1966). Little is known about habitat use and mechanism to avoid the drying of dendrobatoids frog from tropical dry forests. Nevertheless, some species such as *Dendrobates truncatus* (Dendrobatidae) have relatively well described ecological and behavioral strategies that allow them to withstand drought conditions in Caribbean tropical dry forest. Strategies like displacement to humid refuges or permanently staying in them allow taking advantage of the temperature and humidity provided by some microhabitats available (Cuentas et al., 2002). We do not know the strategies used by *A. ignotus* for dehydration tolerance, but this frog is active all year round (we found vocalizing males in dry and rainy season). It is possible that the occupation of rocky substrate and leaf litter by *A. ignotus* corresponds to some mechanism for temperature and humidity capture (Feber, 1982; Navas, 1996). However, this hypothesis needs additional studies that evaluate the physical and structural variables of the habitat that influence the distribution and density of the frogs (Giaretta et al., 1999; van Sluys et al., 2007).

Free-swimming tadpoles

Prior to Grant et al. (2006), most species of *Allobates* were grouped in the *Colostethus* sensu lato. Coloma (1995) described the larval external morphology of the species that were allocated in *Colostethus* sensu lato. Afterwards, Anganoy-Criollo (2013) provided larval features that are shared between the species previously included in *Colostethus* sensu lato and now split in *Allobates, Aromobates, Mannophryne, Rheobates* (Aromobatidae), *Colostethus* and *Hyloxalus* (Dendrobatidae). The general morphology of the tadpoles of *A. ignotus* resembles well the one described in Coloma (1995) and in Anganoy-Criollo (2013). However, both these works report that A-2 gap is short or narrow, though explicit measurements of A-2 gap are not provided in these descriptions. On the opposite, the A-2 gap of *Allobates ignotus* appears nor short or narrow. Anganoy-Criollo (2013) tells about a narrow or short A-2 gap of *Hyloxalus subpunctatus* (Dendrobatidae) being 4-15% of OD width, while the A-2 gap of *A. ignotus* is 17-28% of OD width, therefore markedly wider. Previous descriptions of tadpoles of other *Allobates* also showed "wide" A-2 gap; for example, in *A. brunneus* (30% of ODW; Lima et al., 2009), *A. caeruleodactylus* (35% of ODW; Caldwell et al., 2002), *A. femoralis* (21-35% of ODW; Lescure, 1976; Silverstone, 1976), *A granti* (52% of ODW; Kok et al., 2006), *A. grillisimilis* (30% of ODW; Simões et al., 2013), *A. hodli* (23% of ODW; Simões et al., 2010), *A. kingsburgyi* (38% of ODW; Castillo-Trenn, 2004), *A. magnussoni* (22% of ODW; Lima et al., 2014), *A. niputidea* (although not explicitly reported in the text of the description, but evident from the drawing of the tadpole; Grant et al., 2007), *A. paleovarzensis* (35% of ODW; Lima et al., 2010), *A. subfolionidificans* (23% of ODW; Lima et al., 2007), *A. sumtuosus* (25% of ODW; Simões and Lima, 2012), *A.*

talamancae (35 or 31% of ODW; Breder, 1946; Savage, 2002), and *A. tapajos* (25% of ODW; Lima et al., 2015).

In Dendrobatoidea, this wide A-2 gap was also found in some species of dendrobatid genus *Andinobates* (Silverstone, 1975; Myers and Daly, 1980; Ruiz-Carranza and Ramírez-Pinilla, 1992), *Ameerega* (Haddad and Martins, 1994; Twomey and Brown, 2008; Poelman et al., 2010), some *Phyllobates* (Siverstone, 1976; Donnelly et al., 1990; Savage, 2002), and *Ranitomeya* (Brown et al., 2008; von May et al., 2008; Twomey and Brown, 2009; Perez-Peña et al., 2010). The observed variability of the A-2 gap among the above genera suggests it is a potentially meaningful trait from both a taxonomic and phylogenetic perspective; therefore, an adequate characterization of A-2 gap width variability in the superfamily Dendrobatoidea would be worthwhile.

The systematic value of the larval morphology has been used in the phylogenetic relationships history of poison frogs (e.g., Savage, 1968; Silverstone, 1975, 1976; Myers, 1987; Grant et al., 2006; Sánchez, 2013) showing that there is correspondence between larval features and dendrobatoids taxonomy (e.g., reduction in labial tooth row, oral disc without emargination, umbelliform oral disc, notch on UJS). At this moment, despite that the "wide" A-2 gap is present in several poison frog genera, the "wide" A-2 gap of the tadpoles of *A. ignotus* is shared with other *Allobates* species, which supports the assignation of *A. ignotus* within the genus *Allobates*, where Anganoy-Criollo (2012) provisionally located this species based on adult characters. Molecular and phenotypical characters known for the genus *Allobates* (Grant et al., 2006, 2017) suggest that the "wide" A-2 gap may be a derived and shared character for this genus; however a phylogenetic analysis is needed to test this hypothesis.

Advertisement call

Comparisons of advertisement calls within others species of the genus *Allobates* are limited by the reduced number of calls described. Calls of most of the Colombian *Allobates* are unknown. The advertisement call of *A. ignotus* is composed by a sequence of pulsed notes (6-116 notes/call) with ascending modulation frequency, like to other aromobatid frogs as *A. talamancae* (5-15 notes/call, Lechelt et al., 2014), *A. humilis* (7-16 notes/call, La Marca et al., 2002), *A. myersi* (5-10 notes/call, Simões and Lima, 2011), *A. paleovarsensis* (3-21 notes/call, Lima et al., 2010), and *Aromobates saltuensis* (2 notes/call, Barrio-Amorós and Santos, 2012), from which it differs by the greater number of notes per call. Although in the genus *Allobates* the advertisements calls are highly variable, some species converge their calls in the long trains of notes, such as *A. crombiei* (25-59 notes/call, Lima et al., 2012), *A. goianus* (2-41 notes/call, Carvalho et al., 2016), and *A magnussoni* (not specified in the call description, Lima et al., 2014).

Some *Allobates* can produce their advertisement calls at a dominant frequency similar to *A. ignotus* (4942 Hz), such as *Allobates algorei* (5065 Hz, Barrio-Amorós and Santos, 2009), *A. femoralis* (2853 Hz), *A. goianus* (4946-5230.79 Hz, Bastos et al., 2011; Carvalho et al., 2016), *A. humilis* (4200 Hz, La Marca et al., 2002), *A. myersi* (3400 Hz, Simões and Lima, 2011), *A. sumptuosus* (6500 Hz, Kok and Ernst, 2007), *A.* aff. *brunneus* (5450 Hz, Lötters et al., 2003); however, these species occupy distant geographic areas and are easily morphologically distinguishable from *A. ignotus*. On the other hand, only two aromobatid frogs geographically close to *A. ignotus* have advertisement calls described, *A. talamancae* and *Rheobates palmatus*. The advertisement call of *A. talamancae* differs from that of *A. ignotus* by the slightly higher dominant frequency (5036 Hz, Lechelt et al., 2014). Despite the advertisement call of *R. palmatus* consists of a long train of notes, this differs from the advertisement call of *A. ignotus* by the lower dominant frequency (2083-2700 Hz, Lüddecke, 1999; Bernal et al., 2006). For other dendrobatoid with trans-Andean distribution or geographically close to *A. ignotus*, such as *A. niputidea* (Grant et al., 2007), *A. wayuu* (Acosta-Galvis et al., 1999), *Aromobates* aff. *saltuensis* (Anganoy-Criollo, 2012, see specimens examined), *Aromobates totuko* (Rojas-Runjaic et al., 2011), *Colostethus inguinalis* (Grant, 2004), and "*Colostethus*" *ruthveni* complex (Granda-Rodríguez et al., 2014); there are no descriptions of their advertisement calls.

Relative abundance and conservation status

Habitat degradation and fragmentation are the principal factors in the amphibian decline and extinction, since they generate drastic changes on the amphibian community structure (Mazerolle, 2003). Some dendrobatoid frogs are very sensitive and have declined their populations due to forest fragmentation and degradation, e.g., *A. juanii* in the eastern foothills of the Cordillera Oriental of Colombia (Cáceres-Andrade and Urbina-Cardona, 2009) and *Anomaloglossus stepheni* (Aromobatidae) in the central Amazon (Funk and Mills, 2003). On the other hand, dendrobatoid frogs like *Epipedobates boulengeri* (Dendrobatidae) can tolerate structural changes on their habitat, although environment variables like microhabitat temperature are strongly associated with the relative abundance of this frog, conditioning their establishment (Urbina-Cardona and Londoño-Murcia, 2003).

In the three localities reported herein, *Allobates ignotus* showed fluctuations in their relative abundance during the study period, and we found the lowest abundance in the last year. These areas are immersed in a zone of hydrocarbons exploitation activity. Fragmentation and degradation of riparian forest of Canime Creek by mining activity, plus the stream flow reduction by severe droughts (recent and predicted for future, see Nakaegawa and Vergara, 2010), can be both short and long term threats, due to the decrease of habitat availability and quality. In addition, the influence of pollution with chemical and solid mining wastes on populations of these frogs should be assessed, since these factors are known to affect the development and survival of amphibians (Rowe et al., 1996). All these potential threats can act synergistically and cause population declines in *A. ignotus.*

Although *Allobates ignotus* was previously categorized as endangered species [EN B1ab(iii), IUCN SSC Amphibian Specialist Group. 2017], this decision was based on the limited data provided by Anganoy-Criollo (2012). Therefore, we re-evaluate the conservation status of the species with the data provided herein. Following the IUCN criteria (IUCN, 2012), we suggest categorizing *Allobates ignotus* as Vulnerable [VU B2ab (iii)]; given to the decrease of the relative abundance in the three localities of the Canime Creek Basin, the extent of occurrence lesser than 2000 km^2 (605 km^2), and loss of habitat quality by degradation of creeks and forests where this species lives.

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APPENDIX

Examined specimens

Allobates ignotus. - Colombia: *Cesar*: La Jagua de Ibirico: Zumbador River (580 m a.s.l.), Nueva Granada Village, ICN 55434 (holotype), ICN 55435-436 (paratypes); El Indio Creek (520 m a.s.l.), La Victoria de San Isidro Village, ICN 55427-433; Canime Creek 1 (250 m a.s.l.), CBUMAG:ANF:00254-256; Canime Creek 2 (200 m a.s.l.), CBUMAG:ANF:0069; Canime Creek 3 (194 m a.s.l.), CBUMAG:ANF:00270-272. Becerril: El Veranero Creek (400 m a.s.l.), El 11 Village, ICN 55437-439 (paratypes). Agustín Codazzi: La Frontera's way (1236 m a.s.l.), La Frontera Village, CBUMAG:ANF:00937. Manaure Balcón del Cesar: La Veguita Creek (633 m a.s.l.), La Veguita Village, CBUMAG:ANF:00933, 00935-936. *La Guajira*: La Jauga del Pilar, Nicaragua Creek, El Plan Village, CBUMAG:ANF: 00934.