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The Tadpoles of Two *Atelopus* Species (Anura: Bufonidae) from the Sierra Nevada de Santa Marta, Colombia, with Notes on their Ecology and Comments on the Morphology of *Atelopus* Larvae

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Abstract. We describe for the first time the tadpole of two endemic harlequin frogs of the Sierra Nevada de Santa Marta, north of Colombia: *Atelopus nahumae* and *A. laetissimus*. In addition, we provide further morphological data for a third species, *A. carrikeri*. We also discuss some external morphological features for the tadpoles of these species and compare them with data currently available in the literature for the genus and with other larvae deposited in the amphibian collection of the Instituto de Ciencias Naturales of the National University of Colombia. The examined characters comprise eight morphometric variables and many traits of external morphology related with the oral apparatus, abdominal disc, fins, and dorsal color pattern. The tadpoles of *A. nahumae* and *A. laetissimus* are gastromyzophorous and morphologically similar, sharing a great number of features with their congeners. The tadpoles of *A. nahumae, A. laetissimus,* and *A. carrikeri* contrast morphometrically; total length and tail width are the morphometric traits that are useful to differentiate among them. They are also differentiated by the size of the abdominal sucker and spiracle. In *A. carrikeri,* the abdominal sucker is large relative to that of *A. nahumae* and *A. laetissimus;* the spiracle of *A. laetissimus* and *A. carrikeri* is not visible in ventral and dorsal view, whereas it is large and conspicuous in *A. nahumae.* The tadpoles of the species from Sierra Nevada de Santa Marta differ from most of their congeners found in the Cordillera Oriental and Central of Colombia by lacking a dark band on the fins and caudal musculature. We recorded abiotic factors of the microhabitat where tadpoles of *A. nahumae* were observed (temperature, dissolved oxygen, and water depth). Our results indicate that the probability of finding *A. nahumae* tadpoles depends on the depth of the stream.

Keywords. *Atelopus carrikeri; A. laetissimus; A. nahumae;* Gastromyzophorous; Larval features; Microhabitats; Ontogeny.

Resumen. Describimos por primera vez el renacuajo de dos especies de sapitos Harlequines de la Sierra Nevada de Santa Marta, Colombia: *Atelopus nahumae* y *A. laetissimus*. Adicionalmente, proveemos información morfológica adicional del renacuajo de una tercera especie, *A. carrikeri*. Discutimos algunas características de la morfología externa de las larvas de estas especies y las comparamos con la evidencia reportada en la literatura para otros renacuajos del género y con larvas depositadas en la colección de anfibios del Instituto de Ciencias Naturales de la Universidad Nacional de Colombia. Los caracteres examinados incluyen ocho variables morfométricas y un amplio número de caracteres de la anatomía externa relacionados con el aparato oral, el disco abdominal, las aletas y el patrón de color dorsal. Los renacuajos de *A. nahumae* y *A. laetissimus* son considerados de tipo gastromizóforo y similares morfológicamente, compartiendo un gran número de caracteres con sus congéneres. Las larvas de *A. nahumae, A. laetissimus* y *A. carrikeri* contrastan morfológicamente, siendo la longitud total y el ancho de la cola las variables morfométricas que permiten diferenciar las especies. A su vez, estos se diferencian por el tamaño de la ventosa abdominal. En *A. carrikeri* esta ventosa es más grande al ser comparada con las de *A. nahumae* y *A. laetissimus*. Además, el espiráculo en vista dorsal y lateral es largo y conspicuo en *A. nahumae,* mientras que en *A. laetissimus* y *A. carrikeri* no es visible. Los renacuajos encontrados en la Sierra Nevada de Santa Marta difieren de la mayoría de sus congéneres encontrados en la Cordillera Oriental y Central de Colombia, debido a que los primeros no presentan una banda oscura sobre las aletas y la musculatura caudal. Por último, registramos algunos factores abióticos del microhabitat donde se encuentran los renacuajos de *A. nahumae* (temperatura, oxígeno disuelto y profundidad del agua). Nuestros resultados indican que la probabilidad de encontrar renacuajos de *A. nahumae* depende de la profundidad del agua en el arroyo.

INTRODUCTION

Atelopus Duméril and Bibron, 1841 (commonly known as harlequin frogs) is one of the most threatened amphibian genera on the planet due to several non-exclusive factors, including infection by the pathogen fungi *Batracochytrium dendrobatidis,* climate change, and habitat destruction (La Marca et al., 2005; Stuart et al., 2008;

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Lötters, 2009). Species of this genus inhabit a wide variety of ecosystems ranging from Tropical rain forests at sea level to paramos at approximately 4800 m above sea level (asl) and are distributed from Costa Rica in Central America to Bolivia in South America (Gawor et al., 2012; Frost, 2018). During the breeding season, it is common to observe solitary or amplexed individuals alongside streams; at the time of oviposition, the females attach a string of eggs to submerged rocks and vegetation. Tadpoles are usually found on sandy substrates or attached to rocks in streams (Starrett, 1967; Lötters, 1996; Karraker et al., 2006; Crump 2009). To date, 94 species of *Atelopus* are recognized, of which 46 are found in Colombia (Frost, 2018). However, although Colombia is inhabited by the greatest number of species of *Atelopus,* the tadpoles of only five Colombian species have been described or illustrated, including *A. subornatus* Werner, 1899 (Lynch, 1986; Enciso-Calle et al., 2017), *A. mittermeieri* Acosta-Galvis et al., 2006 (tadpole described and illustrated in the species description), *A. carrikeri* Ruthven, 1916 (Rueda-Solano et al., 2015), *A. spumarius* Cope, 1871 (Duellman and Lynch, 1969), and *A. ardila* Coloma et al., 2010 (Gómez Castillo, 1982, 1993).

Here, we describe the external morphology of tadpoles of *Atelopus nahumae* Ruiz-Carranza et al., 1994 and *A. laetissimus* Ruiz-Carranza et al., 1994. Both species inhabit forests between 1,500–2,800 m asl and are endemic to the Sierra Nevada of Santa Marta (SNSM), an isolated mountain range located in northern Colombia. We compared external morphological features of the tadpoles of *A. nahumae* and *A. laetissimus* with those of *A. carrikeri,* another species endemic to the SNSM but distributed at higher elevations (2,900–4,500 m asl; Rueda-Solano et al., 2015) and with larval descriptions available in the literature. In addition, the distribution of some characters/states related to dorsolateral color pattern and some oral disc structures are reported and discussed for the tadpoles described herein and for some undescribed *Atelopus* tadpoles deposited in the Instituto de Ciencias Naturales of the National University of Colombia in Bogotá. We explore diagnostic information useful to delimit *A. nahumae* and *A. laetissimus* species, which will serve as a basis for future comparative studies that seek to elucidate the diversity of morphological characters within *Atelopus.* Finally, we recorded microhabitat preferences of these two species.

MATERIALS AND METHODS

Collection sites

We collected tadpoles from four localities distributed across an elevational gradient in the SNSM (Fig. 1). Tadpoles of *Atelopus laetissimus* were collected from two localities: the Estación Experimental San Lorenzo, San Lorenzo stream, northwestern slope of the SNSM, 2,100 m asl (11°06′54.96″N, 74°03′03.46″W; WGS84), 11 April 2015, and in the Serranía de Cebolletas San Pedro de la Sierra, Pascual stream, western slope of the SNSM, 2,200 m asl (10°54′03.70″N, 73°55′04.50″W), 31 March 2016. Tadpoles of *A. nahumae* were collected on 12 April 2015, at the headwaters of the Gaira river, Serranía de San Lorenzo, northwestern slope of the SNSM, 1,560 m asl (11°10′02.0″N, 74°10′41.5″W). Tadpoles of *A. carrikeri* were collected in the Serranía de Cebolletas on the western slope of the SNSM, 3,500 m asl (10°54′03″N, 73°55′05″W).

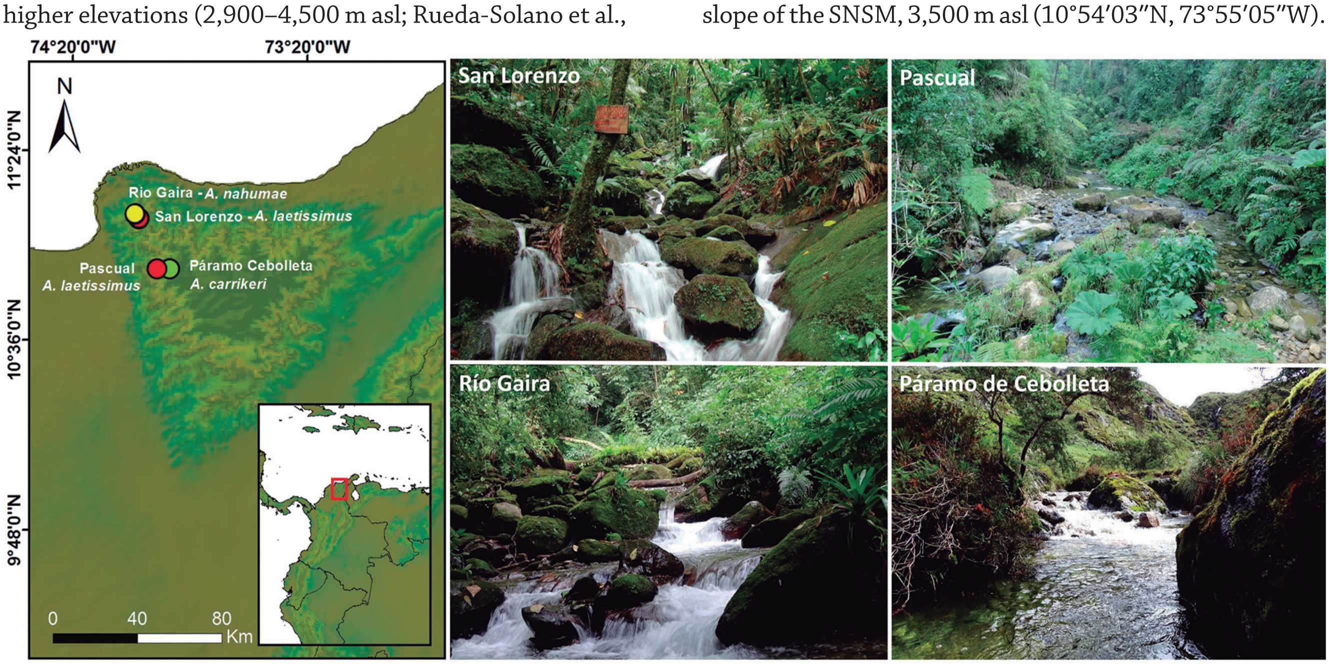

Figure 1. Geographic location and images of the study areas at Sierra Nevada de Santa Marta, northern Colombia, South America. The *Atelopus* species in each of the four study areas are indicated on the map.

Field sampling, identifications, and comparisons

We performed visual encounter surveys along four streams in the Sierra Nevada de Santa Marta to record *Atelopus* larvae (Fig. 1). The collected tadpoles were euthanized in lidocaine, fixed and preserved in 10% formaldehyde, and deposited in the herpetological collection of the Universidad del Magdalena CBUMAG, Santa Marta, Colombia (*Atelopus laetissimus:* CBUMAG-ANF: 0963 and CBUMAG-ANF: 0971; *A. nahumae:* CBUMAG-ANF: 0961; *A. carrikeri* CBUMAG-ANF: 0892).

Tadpole characters follow the terminology and definitions of McDiarmid and Altig (1999) and Mijares-Urrutia (1998) and the developmental stages were defined according to Gosner (1960). We used the following variables to describe the morphology of tadpoles for *Atelopus nahumae* and *A. laetissimus:* total length (TL), body length (BL), tail length (TAL), internarial distance (IND), interorbital distance (IOD, taken from the medial edges of the corneas), maximum tail height (MTH), maximum tail muscle height (TMH), and tail muscle width (TMW). Measurements were taken from a series of standardized (dorsal, lateral, and ventral) photographs obtained from each individual and species using a Sony Camera (DSC-H400) and a stereoscope (Nikon H550S) with an Axion cam ERc 5s Camera (Carl Zeiss Gmbh, Göttingen, Germany) and are reported as $\bar{x}$ ± SD. The tadpoles of *A. nahumae* and *A. laetissimus* were compared to tadpoles of 17 species of the genus deposited in the amphibian collection of the Instituto de Ciencias Naturales of the National University of Colombia in Bogotá (ICN; Appendix S1): *A. angelito* Ardila-Robayo and Ruiz-Carranza, 1998, *A. elegans* Boulenger, 1882, *A. eusebianus* Rivero and Granados-Díaz, 1993, *A. famelicus* Rivero and Morales, 1995, *A. farci* Lynch, 1993, *A. lozanoi* Osorno-Muñoz et al., 2001, *A. mandingues* Osorno-Muñoz et al., 2001, *A. marinkellei* Cochran and Goin, 1970, *A. minutulus* Ruiz-Carranza et al.,1988, *A. mittermeieri, A. monohernandezii* Ardila-Robayo et al., 2002, *A. muisca* Rueda-Almonacid and Hoyos, 1994, *A. nicefori* Rivero, 1963, *A. quimbaya* Ruiz-Carranza and Osorno-Muñoz, 1994, *A. sernai* Ruiz-Carranza and Osorno-Muñoz, 1994, *A. simulatus* Ruiz-Carranza and Osorno-Muñoz, 1994, *A. sonsonensis* Vélez-Rodriguez and Ruiz-Carranza, 1997.

Tadpole identification was based on the presence of adults occurring on the same area or stream as larvae, so we also collected postmetamorphic juveniles and adults of *Atelopus laetissimus* (Fig. 2A–D) and *A. nahumae*

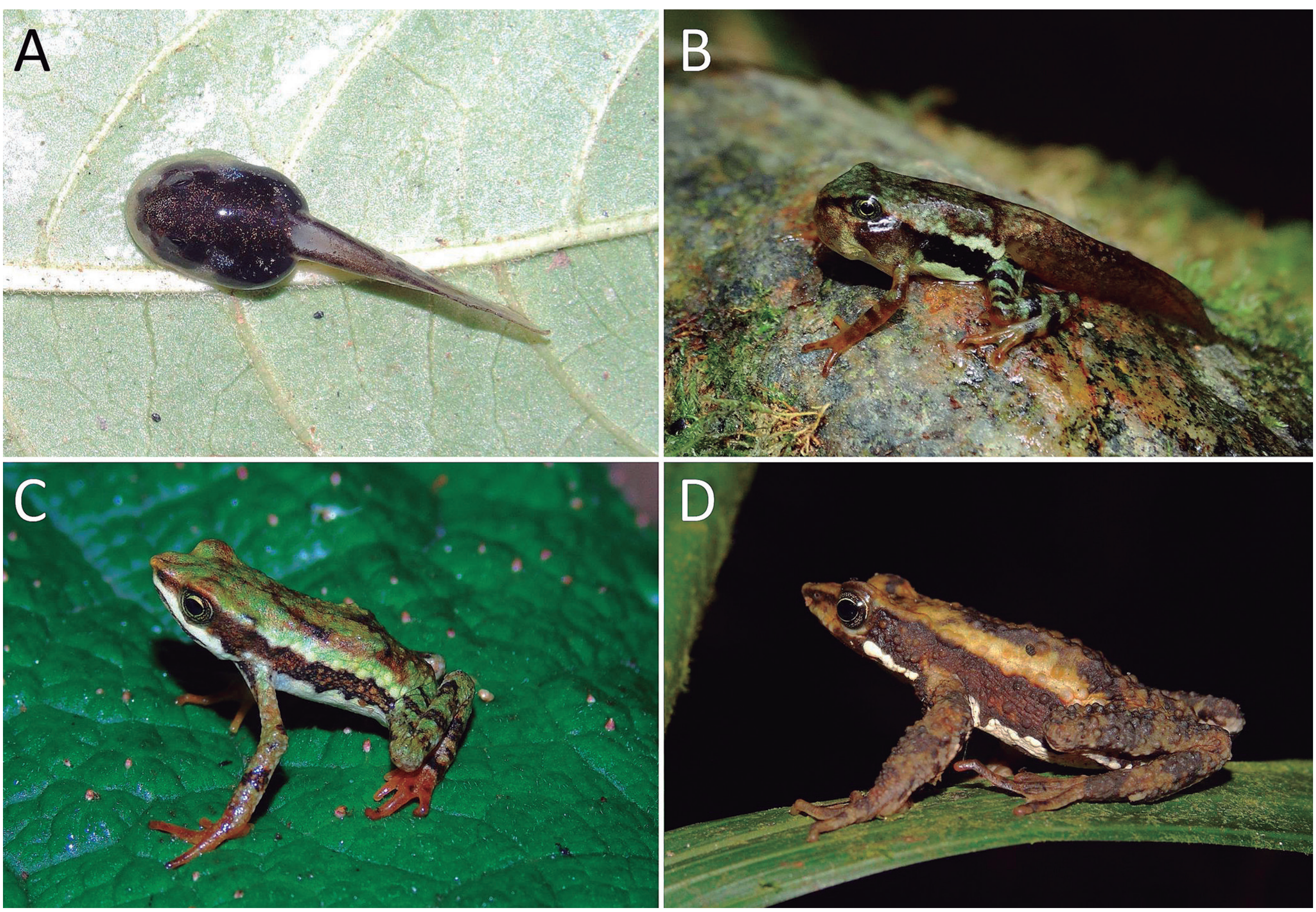

Figure 2. Individuals of *Atelopus laetissimus*. **(A)** Tadpole, CBUMAG: ANF 0963, stage 28–30 (sensu Gosner, 1960), **(B)** froglet, **(C)** juvenile, and **(D)** adult male (not collected).

(Fig. 3A–D), which allowed us to assign the tadpoles to the corresponding species on the basis of external morphology.

Microhabitat

We recorded microhabitat preferences of tadpoles of *Atelopus laetissimus* and *A. nahumae,* using the methodology proposed by Rueda-Solano et al. (2015), but with the following modifications: we selected four streams in the Serranía de San Lorenzo, Quebradas San Lorenzo, Betoma 1, Betoma 2, and Cascada Río Gaira (Fig. 1), separated from each other by more than 300 m; in each stream, we established a transect 50 m long by 2 m wide. We divided each transect into 100 quadrants of one 1 m^2 each and then sampled half of them. In each sampled quadrant, we determined presence/absence of tadpoles, depth of the water column, level of O_2 dissolved (mg/L), and water temperature with a multiparameter sensor (3420 set G WTM 2FD46G). Water temperature in San Lorenzo Creek was also monitored with a HOBO Pro V.2 data logger U23–004 from 2014–2016, including a season of extreme drought in 2015.

Data analysis

Photographs used to obtain the morphometric variables of tadpoles were processed using ImageJ (Schneider et al., 2012). Two discriminant analyzes were performed to establish which variables have the highest weight in differentiating the tadpoles of *Atelopus carrikeri, A. laetissimus,* and *A. nahumae.* The first analysis included all tadpoles; the second analysis included only the individuals in developmental stages ≥ 29. This latter analysis was performed to control for the possible effect of small and morphologically undifferentiated tadpoles. In both analyses, we standardized morphometric variables by dividing them by TL. To determine interspecific differences in body size at the end of metamorphosis (Stage 46) for *Atelopus laetissimus, A. nahumae,* and *A. carrikeri,* we performed an analysis of variance (ANOVA). Finally, to evaluate microhabitat preferences for *A. nahumae* and *A. laetissimus* (using tadpole presence/quadrant), a binary logistic regression was performed using the environmental parameters (water depth, O_2 dissolved, and water temperature) as the independent variables and the presence/absence of tadpoles as the dependent variable. All analyses were done using the statistical software IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp, 2015).

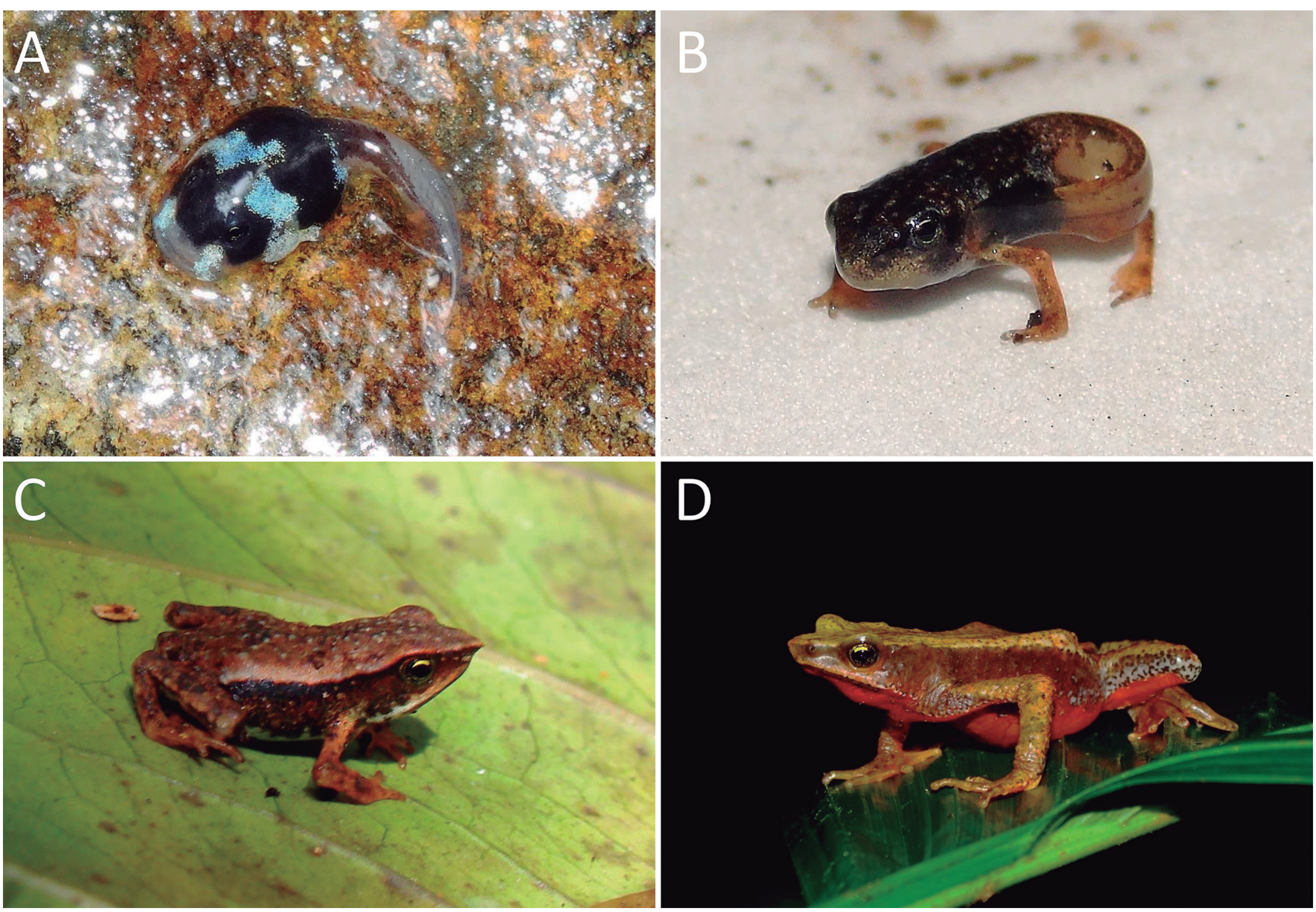

Figure 3. Individuals of *Atelopus nahumae.* **(A)** Tadpole, CBUMAG: ANF 0961, stage 33–35 (sensu Gosner, 1960), **(B)** froglet, **(C)** juvenile, and **(D)** adult (not collected).

Table 1. Measurements (in mm) of the tadpoles of *Atelopus carrikeri, A. laetissimus,* and *A. nahumae* ($\bar{x}$ ± SD; range in parentheses). BL = body length; TAL = tail length; TL = total length; IND = internarial distance; IOD = interorbital distance; MTH = maximum tail height; TMH = maximum tail muscle height; TMW = tail muscle width. Individuals in stage 46 (sensu Gosner, 1960) not included.

Species	Stage	*n*	BL	TAL	TL	IND	IOD	MTH	TMH	TMW
A. carrikeri	25–28	41	9.0 ± 0.16	15.0 ± 2.6	24.0 ± 3.9	1.8 ± 0.4	2.2 ± 0.5	3.2 ± 0.8	1.7 ± 0.4	1.7 ± 0.3
			(6.5–12.7)	(8.3–20.2)	(16.2–31.6)	(1.3–2.9)	(1.6–3.5)	(1.7–4.8)	(1.0–2.5)	(1.0–2.3)
	29–30	4	9.4 ± 0.9	20.0 ± 0.9	29.4 ± 0.4	1.7 ± 0.3	2.3 ± 0.3	3.4 ± 0.3	1.8 ± 0.1	1.6 ± 0.3
			(8.6–10.6)	(18.7–20.8)	(28.9–29.9)	(1.2–2.0)	(2.0–2.7)	(3.0–3.8)	(1.7–1.9)	(1.3–2.1)
	33–34	2	11.9 ± 1.0	21.6 ± 2.4	33.5 ± 3.3	2.5 ± 0.0	3.5 ± 0.2	4.8 ± 0.5	4.1 ± 0.9	2.4 ± 0.3
			(11.2–12.6)	(19.9–23.3)	(31.1–35.9)	(2.6–2.5)	(3.4–3.7)	(4.4–5.2)	(4.7–3.5)	(2.2–2.7)
	35–36	3	12.4 ± 1.1	21.3 ± 1.8	33.7 ± 0.7	2.5 ± 0.4	3.2 ± 0.3	4.6 ± 0.6	2.9 ± 0.1	2.1 ± 0.2
			(11.2–13.2)	(19.8–23.3)	(32.9–34.4)	(2.0–2.8)	(2.9–3.5)	(3.9–4.9)	(2.8–3.0)	(2.0–2.3)
	42	3	13.3 ± 1.7	24.2 ± 2.7	37.4 ± 1.7	2.0 ± 0.1	3.3 ± 0.5	4.2 ± 0.7	2.3 ± 0.4	2.5 ± 0.7
			(11.4–14.6)	(21.0–26.0)	(35.7–39.1)	(1.9–2.2)	(2.8–3.8)	(3.4–4.7)	(1.9–2.7)	(2.1–3.3)
A. laetissimus	25–28	75	6.2 ± 0.9	10.0 ± 1.7	16.2 ± 2.5	1.2 ± 0.5	1.5 ± 0.5	2.5 ± 0.6	1.4 ± 0.4	1.2 ± 0.4
			(3.9–8.4)	(6.2–13.9)	(10.7–20.9)	(0.3–2.0)	(0.7–2.9)	(1.1–3.9)	(0.5–2.3)	(0.6–2.0)
	29–30	3	7.8 ± 0.2	13.1 ± 0.8	20.9 ± 0.1	1.6 ± 0.3	1.5 ± 0.4	3.0 ± 0.2	1.8 ± 0.2	1.9 ± 0.3
			(7.6–8.0)	(12.5–14.0	(20.2–22.0)	(1.3–1.9)	(1.2–1.9)	(2.8–3.2)	(1.6–2.0)	(1.7–2.2)
	35–37	6	8.6 ± 0.4	15.6 ± 0.8	24.2 ± 1.0	2.0 ± 0.3	2.0 ± 0.6	3.7 ± 0.3	2.4 ± 0.2	2.1 ± 0.3
			(8.1–8.9)	(14.8–16.6)	(22.9–25.3)	(1.7–2.4)	(1.4–2.7)	(3.5–4.1)	(2.2–2.5)	(1.7–2.4)
	41–42	13	9.8 ± 0.9	17.3 ± 1.6	27.2 ± 2.0	2.1 ± 0.3	3.5 ± 0.9	4.4 ± 0.6	2.7 ± 0.3	2.6 ± 0.5
			(8.6–11.2)	(15.2–20.8)	(24.8–31.4)	(1.4–2.6)	(1.6–4.6)	(3.3–5.5)	(2.2–3.4)	(1.7–3.5)
A. nahumae	26–28	14	4.2 ± 0.3	7.6 ± 0.5	11.8 ± 0.6	0.9 ± 0.2	0.8 ± 0.0	1.8 ± 0.3	1.1 ± 0.1	0.9 ± 0.1
			(3.6–4.8)	(6.9–8.6)	(11.0–13.2)	(0.7–0.2)	(0.7–0.8)	(1.3–2.3)	(0.9–1.3)	(0.8–1.0)
	29–30	13	5.1 ± 0.3	8.4 ± 0.3	13.5 ± 0.5	0.9 ± 0.1	0.9 ± 0.1	2.1 ± 0.2	1.3 ± 0.2	1.0 ± 0.1
			(4.5–5.5)	(8.0–8.9)	(12.6–14.1)	(0.8–1.0)	(0.8–1.1)	(1.7–2.5)	(1.0–1.6)	(0.9–1.2)
	31–34	26	5.5 ± 0.5	9.1 ± 0.6	14.6 ± 1.0	1.0 ± 0.1	1.0 ± 0.1	2.5 ± 0.3	1.4 ± 0.2	1.1 ± 0.1
			(4.9–6.6)	(8.2–10.4)	(13.1–16.6)	(0.7–1.1)	(0.8–1.3)	(2.1–3.0)	(1.0–1.9)	(0.9–1.5)
	35–37	27	5.7 ± 0.5	9.8 ± 0.7	15.5 ± 1.0	1.1 ± 0.1	1.2 ± 0.3	2.7 ± 0.2	1.5 ± 0.2	1.2 ± 0.2
			(4.8–6.9)	(8.8–11.4)	(14.1–17.6)	(0.8–1.4)	(0.9–1.8)	(2.2–3.2)	(1.2–2.0)	(0.9–1.6)
	39–45	5	7.2 ± 0.9	8.2 ± 4.8	15.3 ± 4.5	1.2 ± 0.2	2.2 ± 0.6	2.5 ± 0.1	1.7 ± 0.4	1.5 ± 0.6
			(5.5–7.9)	(1.8–12.0)	(9.6–19.6)	(0.9–1.4)	(1.2–2.6)	(2.4–2.5)	(1.3–2.2)	(0.9–2.3)

RESULTS

We collected 298 specimens, including 235 tadpoles and 63 froglets, divided among *Atelopus carrikeri* (n = 60), *A. laetissimus* (n = 103), and *A. nahumae* (n = 135). The tadpoles for the former two species represented 12 stages of development, while tadpoles of the latter species represented 17 developmental stages (Table 1, Appendix S2).

The tadpole of *Atelopus laetissimus*

The following description is based on three individuals in stage 35. Measurements (in mm) for *Atelopus laetissimus* are as follows (see also Appendix S2): TL = 23.9 ± 0.9; BL = 8.6 ± 0.5; TAL = 15.3 ± 0.4); IND = 1.8 ± 0.2); IOD = 1.7 ± 0.6; MTH = 3.7 ± 0.3); TMH = 2.4 ± 0.9; TMW = 2.0 ± 0.2. Body ovoid, elongate in dorsal view, depressed in lateral view; snout broadly rounded in dorsal view, rounded in lateral profile, chondrocranial elements not visible; eyes located dorsally; nostrils small, semicircular, with flat, non-protrusive margin, directed dorsolaterally, approximately equidistant between eyes and tip of snout. Spiracle small, single, sinistral, 1/2 free, originating at midpoint of body, inconspicuous in dorsal and ventral views; opening of spiracle directed posterolaterally, diameter less than 1/2 of length of free tube. Vent tube short, medial; caudal musculature robust anteriorly, narrowing abruptly just posterior to midlength of tail. Dorsal and ventral fin originating on the tail, narrower than caudal musculature; tip of tail rounded. Mouth ventral, surrounded by well-developed labia forming complete oral disc; upper lip with bilateral anterior projections forming conspicuous M-shaped structure. Marginal papillae short, acuminate; one row of complete marginal papillae anteriorly; few submarginal papilla present, located on the medial and lateral regions of oral disc; posterior papillae absent. Labial tooth row formula 2/3, length of rows subequal. Upper jaw sheath strongly keratinized, arch-shaped; lower jaw sheath V-shaped. Both jaws slightly serrate. Abdominal sucker large, extending from posterior labium to midbody, forming complete, round structure (diameter 6.9 mm) with raised edge; abdominal sucker moderately extended (Fig. 4A–C).

In life, the tadpoles of *Atelopus laetissimus* (Fig. 2A) are almost uniformly black with very minute metallic blue or golden dorsal spots and a translucent to whitish border around the snout. In ventral view, the oral and suctorial discs are translucent. Tail musculature is cream with scattered black spots in dorsal and ventral view; some individuals have concentrated black spots in the dorsolateral area close to the body junction and towards the end of the tail in lateral view. The dorsal and ventral fins are translucent with very minute dark spots. Changes in coloration were observed during ontogenetic development: in early stages the individuals have uniform white coloration and the presence of light and dark bands interspersed with light brown or light green lines on the dorsum and limbs when the larva reaches stage 42 (Fig. 2B). The same color pattern was observed on forelimbs, once they had emerged in stage 43. In preservative, the color pattern is similar to that of living tadpoles, but it fades and loses its golden and blue/white iridescent tones. The tail musculature becomes light cream (Fig. 4A).

Natural history

Adult male *Atelopus laetissimus* were observed during the day and night when calling, perched on vegetation at the border of streams, at a height of 20–100 cm from the water surface. Amplexus is axillary, and the unpigmented eggs are deposited beneath stones and in leaf litter accumulated in the backwater areas of streams. The egg string and jelly capsules are not attached to the substrate. The string is indented and each capsule clearly differentiated. Empty capsules occur at various points along the string, similar to those reported in *A. subornatus* by Lynch (1986). Tadpoles of *A. laetissimus* were abundant in San Lorenzo Creek, and they exhibited nocturnal habits (JLP-G, pers. obs, April 2015). Tadpoles developed at an

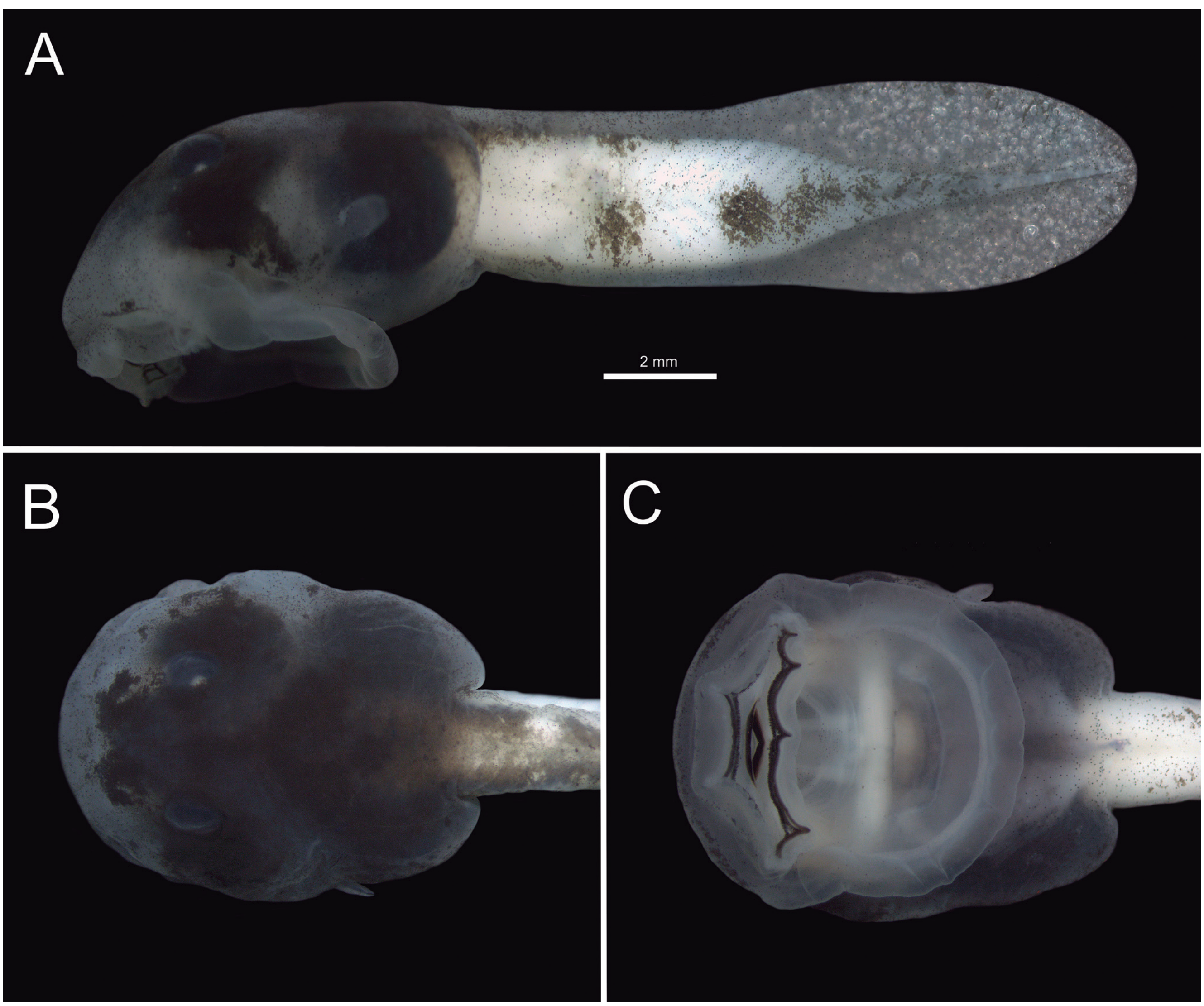

Figure 4. Tadpole of *Atelopus laetissimus* in stage 27 (sensu Gosner 1960). **(A)** Lateral, **(B)** dorsal, and **(C)** ventral views. CBUMAG: ANF 0963.

average water temperature of 14.22°C ± 1.04 (*n* = 4,527, range = 11–29°C) and hatch in stage 19. Postmetamorphs and juveniles were found on vegetation bordering the ravines at a height of 40–100 cm from the ground level, after 4–5 months.

The tadpole of *Atelopus nahumae*

This description is based on 14 individuals in stage 35 (Fig. 5A–C). Measurements (in mm) for *Atelopus nahumae* are as follows (see also Appendix S2): TL = 14.9 ± 0.8; BL = 5.6 ± 0.4; TAL = 9.8 ± 0.5; IND = 1.1 ± 0.1; IOD = 1.1 ± 0.2; MTH = 2.6 ± 0.2; TMH = 1.6 ± 0.2; TMW = 1.2 ± 0.1. Body ovoid, elongate in dorsal view, depressed in lateral view; snout broadly rounded in dorsal view, rounded in profile; chondrocranial elements not detectable; eyes located dorsally; nostrils, small semicircular, with a flat, non-protrusive margin, directed dorsolaterally, equidistant between eyes and tip of snout. Spiracle, small, single, sinistral, 1/3 free, originating at midpoint of body, visible in dorsal and ventral views; spiracle opening directed dorsoventrally, diameter less than 1/4 length of free tube. Vent tube short, medial; caudal musculature robust anteriorly, narrowing abruptly just posterior to midlength of tail, terminating just anterior to end of tail. Dorsal fin originating on body; ventral fin originating on tail. Both fins narrower than caudal musculature, tip of tail rounded. Mouth situated ventrally, surrounded by well-developed labia forming complete oral disc; upper lip with bilateral anterior projections forming conspicuous M-shaped structure. Marginal papillae short, acuminate; one row of marginal papillae anteriorly; submarginal papillae present. Labial row formula 2/3, length of rows subequal. Upper jaw partially keratinized, arch-shaped; lower jaw sheath V-shaped. Abdominal sucker small, extend-

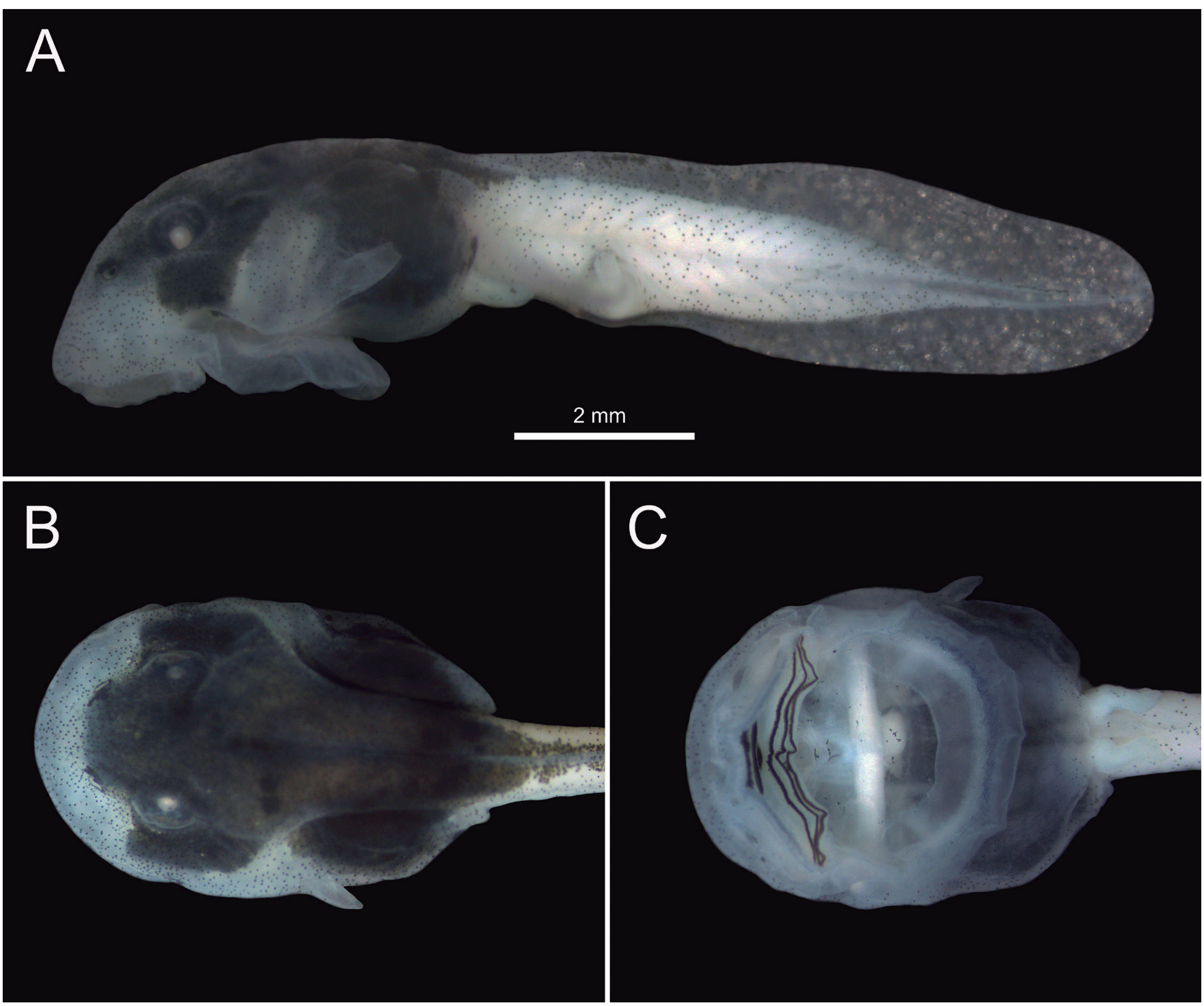

Figure 5. Tadpole of *Atelopus nahumae* in stage 35 (sensu Gosner 1960). **(A)** Lateral, **(B)** dorsal, and **(C)** ventral views. CBUMAG: ANF 0961.

ing from posterior labium to midbody, forming complete round structure (4.7 mm in diameter) with raised edge; abdominal sucker slightly extended.

In life, the body is black with a conspicuous metallic blue transverse band behind the eyes and two bluish blotches between the nostrils and the snout tip. The snout is translucent with very small dark spots, clearing gradually towards the tip. The tail is black with dark brown spots and the distal-most portion is translucent (Fig. 3A); in ventral view, the oral and suctorial discs are translucent. The ventral and dorsal fins are translucent with tiny dark spots. In lateral view, the tail musculature is cream with scattered, minute, dark spots. During ontogeny, the dorsum becomes dark brown with dark brown blotches and small cream spots. Also, the hindlimbs are yellowish in early and middle stages (30–39) and become light brown by stage 42. The same color pattern was observed on forelimbs, once they had emerged (Stage 43). In preservative, the color pattern is similar to that of living tadpoles but fades and loses the bluish and iridescent tones. The tail musculature becomes light cream (Fig. 5A).

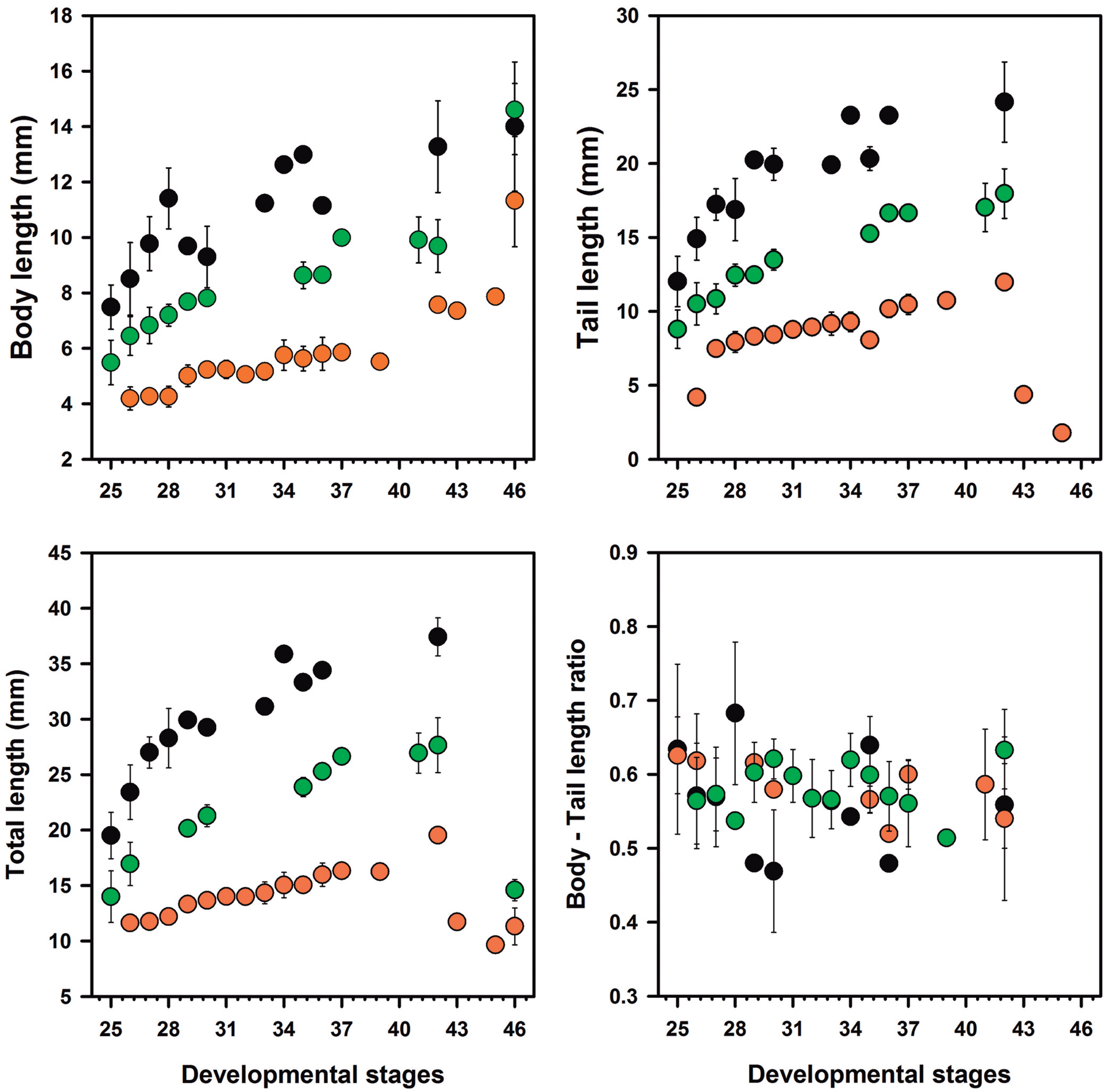

Figure 6. Morphological measurements according to the developmental stage (sensu Gosner 1960) of tadpoles of *Atelopus carrikeri* (black dots), *A. laetissimus* (green dots), and *A. nahumae* (orange dots). Some dots overlap. Dots indicate mean values, error bars indicate standard deviation.

Natural history

Breeding activity of *Atelopus nahumae* was observed from April to November at the headwaters of Gaira River. Adult males were detected in both day and night on rocks or vegetation beside the streams, ca. 0–50 cm from the water. Amplexus was axillary and tadpole and juveniles were observed over the entire year. The tadpoles were attached to rocks at the margins of the medium-sized streams (ca. 3–4 m wide), where the water flow was weaker. Unlike *A. laetissimus,* juveniles of *A. nahumae* were found on the ground on litter and rocks accumulated at the river edges. Juveniles and adults were abundant (JLP-G, pers. obs, April 2015).

Comparison with other *Atelopus* tadpoles of SNSM

In *Atelopus nahumae,* the abdominal sucker is short relative to body length (7.6%), whereas in *A. laetissimus* it is moderate (12.2%) and in *A. carrikeri* it is long (25%). In *A. laetissimus* (Fig. 4A, C) and *A. carrikeri* (Rueda-Solano et al., 2015), the spiracle is small and less visible in ventral and dorsal views than the large, conspicuous spiracle of *A. nahumae* (Fig. 5A, C). In all the stages of development, the dorsal fins of *A. laetissimus* and *A. carrikeri* originate at base of the tail, whereas the dorsal fin of *A. nahumae* originates on the posterior part of the body. In life, the dorsal coloration of *A. laetissimus* is mostly black with minute metallic blue or golden dorsal spots (Fig. 2A); however, they have a translucent or slightly whitish border around the snout that is maintained throughout larval development. The tadpole of *A. nahumae* is also black, but they have metallic blue dots and one transverse band behind the eyes; they are usually more colorful than their congeners found at SNSM and the translucent snout border observed in *A. laetissimus* is less evident. Additionally, *A. nahumae* has white spots of variable size distributed on the body, accompanied by small golden blotches (Fig. 3A). The tadpole of *A. carrikeri* is also uniformly black or dark brown with golden dots in all the stages of development (Rueda-Solano et al., 2015).

Among these three *Atelopus* species, *A. carrikeri* had the longest BL, TAL, and, therefore, TL in all stages of development (Fig. 6). However, TL for all three species was different in stage 46 ($n = 63$; F = 15.852; df = 2; $P < 0.001$). TL in *A. carrikeri* ($n = 7$; 14.0 ± 2.3 mm) and *A. laetissimus* ($n = 6$; 14.6 ± 0.9 mm) was similar (HSD Tukey $P < 0.801$), while TL in *A. nahumae* ($n = 50$; 11.3 ± 1.6 mm) was shorter than *A. laetissimus* (HSD Tukey $P < 0.001$) and *A. carrikeri* (HSD Tukey $P < 0.001$). Tadpole measurements and comparative data of *Atelopus* species from the literature are shown in Tables 1 and 2, respectively.

The first discriminant analysis performed with morphometric measurements of the entire sample (i.e., including all developmental stages) showed differences between *Atelopus laetissimus, A. nahumae,* and *A. carrikeri* (Fig. 7A). This analysis correctly separated 85.9% of the tadpoles measured for the predicted groups. Function 1 accounted for 92.1% of the variance, with TL having the

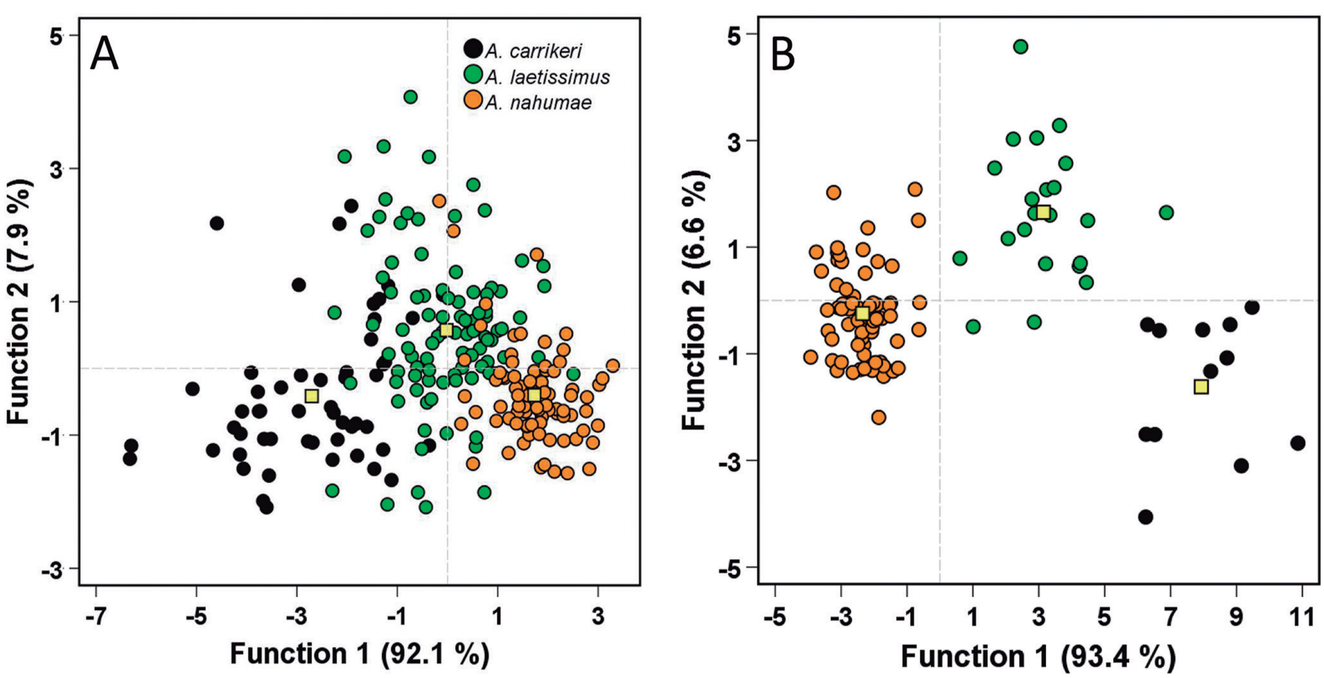

Figure 7. Discriminant analysis based on morphometric measurements of the tadpoles of *Atelopus carrikeri* (black dots), *A. laetissimus* (green dots), and *A. nahumae* (orange dots). **(A)** tadpoles between developmental stages 25–45. **(B)** Tadpoles between developmental stages 29–45. Yellow squares indicate the centroid value for each species.

Table 2. Summary of external morphological characters scored in tadpoles of *Atelopus*. Species in **bold** are those with published descriptions and/or illustration occurring in Colombia. * = data inferred from the tadpole's illustration but not stated in the description by the author (pending corroboration); ** = new information; *** = modification from the source.

Species	Gosner stage	Labial tooth row formula	Submarginal papillae	Spiracle position	Vent tube	Dorsal fin origin	Dorsal color pattern (ETOH)	Caudal band	Reference
Atelopus balios	20–42	2/3	Absent*	2/3	Short	Tail***	Reticulated, black, and cream	Absent	Coloma and Lötters, 1996
Atelopus carbonerensis	34, 37, and 38	2/3	Absent*	Posterior (62.5%)*	Short	Tail	Reticulated, black, and cream	Absent	Mijarres-Urrutia and La Marca, 2005
Atelopus carrikeri	34	2/3	Present**	Posterior	Short	Tail	Uniformly pigmented, black	Absent	Rueda-Solano et al., 2015
Atelopus certus	25–38	2/3*	Absent*	3/5	Short	Tail***	Uniformly pigmented, black	Absent	Duellman and Lynch, 1969
	42	2/0		3/6					
	44	0/0		3/7					
Atelopus cruciger	–	2/3	Absent*	3/5	Short	Tail	Uniformly pigmented, black	Absent	Mebs, 1980
Atelopus exiguus	31	2/3	Absent*	Posterior	Short	Tail***	Uniformly pigmented, black	Absent	Coloma et al., 2000
Atelopus flavescens	28	2/3	Present	Posterior	Median	Tail***	Uniformly pigmented, black	Absent	Lescure, 1981
	25–28								Boistel et al., 2005
	25–44			Posterior*					Gawor et al., 2012
Atelopus franciscus	27, 30, and 34	2/3	Present	–	Short	Tail***	Reticulated, black, and cream	Absent	Boistel et al., 2005
Atelopus ardila	18–29	2/3*	Absent*	–	Short	–	Uniformly pigmented, black	Absent	Gómez Castillo, 1982, 1993
Atelopus laetissimus	35	2/3	Present	Posterior	Short	Tail	Uniformly pigmented, black	Absent	This study
Atelopus mindoensis	36	2/3	Absent*	Posterior	Short	Tail***	Uniformly pigmented, black	Absent	Lötters, 2001
Atelopus mittermeieri	27–34	2/3	Present**	Posterior*	–	Tail	Reticulated, black, and cream	Present	Acosta-Galvis et al., 2006
Atelopus mucubajiensis	32, 34, and 36	2/3	Absent*	Posterior (73%)*	Short	Tail	Uniformly pigmented, black	Absent	Mijarres-Urrutia and La Marca, 2005
Atelopus nahumae	35	2/3	Present	Posterior	Short	Body	Reticulated, black, and cream	Absent	This study
Atelopus nanay	28	2/3	Absent*	Posterior	Short	Tail ***	Uniformly pigmented, black	Absent	Coloma, 2002
Atelopus peruensis	25–32	2/3**	Absent*	Posterior**	–	Tail **	Uniformly pigmented, black	Absent	Gray and Cannatella, 1985
Atelopus cf. *pulcher*	25–42	2/3	Absent*	3/5	Median	Tail ***	Reticulated, black, and cream	Absent	Gascon, 1989; Lötters et al., 2002; Boistel et al., 2005
Atelopus sorianoi	26	2/3	Absent*	Posterior (69.2%)*	Median	Tail	Reticulated, black, and cream	Absent	Mijarres-Urrutia and La Marca, 2005
Atelopus spumarius	37	2/3	Absent*	3/5	Short	Tail ***	Uniformly pigmented, black	Absent	Duellman and Lynch, 1969; Lötters et al., 2002
Atelopus subornatus	26	2/3	Present**	Posterior**	–	Tail ***	Reticulated, black, and cream	Present	Lynch, 1986
Atelopus tamaense	27 and 36	2/3	Absent*	Posterior (66%)*	Short	Tail	Reticulated, black, and cream	Absent	Mijarres-Urrutia and La Marca, 2005
Atelopus tricolor	33–34	2/3	Absent*	Posterior	Short	Tail ***	Reticulated, black, and cream	Absent	Lavilla et al., 1997
Atelopus varius		2/3	Absent*	2/3	Short	Tail ***	Reticulated, black, and cream	Absent	Starrett, 1967
Atelopus zeteki	36	2/3	Absent*	Posterior	Median*	Tail ***	Uniformly pigmented, black	Absent	Lindquist and Hetherington, 1998

Table 3. Summary of microhabitat variables (*n* = 104) for tadpoles of *Atelopus nahumae* in streams of the Serranía San Lorenzo, Sierra Nevada de Santa Marta, Colombia. * = *A. nahumae* tadpoles present; ⁺ = *A. laetissimus* tadpoles present.

Locality	Variable	Mean	SD	CV	Range
Quebrada San Lorenzo⁺ 2,200 m asl	Water temperature (°C)	13.8	0.0	0.0	13.7–13.8
	Dissolved O_2 (mg/L)	8.1	0.1	0.0	7.9–8.2
	Water depth (cm)	17.6	9.2	0.5	3.9–37.3
Betoma 1 2,100 m asl		14.8	0.3	0.0	14.5–15.3
		7.3	0.4	0.0	6.6–7.7
		4.6	1.2	0.3	2.5–6.4
Betoma 2 2,100 m asl		14.3	0.1	0.0	14.2–14.5
		8.0	0.1	0.0	7.2–8.0
		4.8	1.0	0.2	2.5–7.6
Cascada Rio Gaira* 1,560 m asl		16.8	0.2	0.0	16.4–17.5
		8.2	0.1	0.0	8.1–8.4
		14.1	6.8	0.5	5.9–30.5

greatest weight in this Function. Function 2 accounted for 7.9%, with IOD having the greatest weight. The second discriminant analysis, performed using only tadpoles at developmental stages 29–45 (Fig. 7B), clearly shows the differences between the tadpoles of the three species. This latter analysis correctly separated 99% of the tadpoles. The main source of variance was TL (Function 1; 93.4% of variance), while tail width (TMW; Function 2) represented the remaining 6.6% of the variance.

Microhabitat preference

The tadpoles of *Atelopus laetissimus* and *A. nahumae* were collected in medium-sized, fast-flowing streams (Table 3). For *A. nahumae,* the logistic regression model

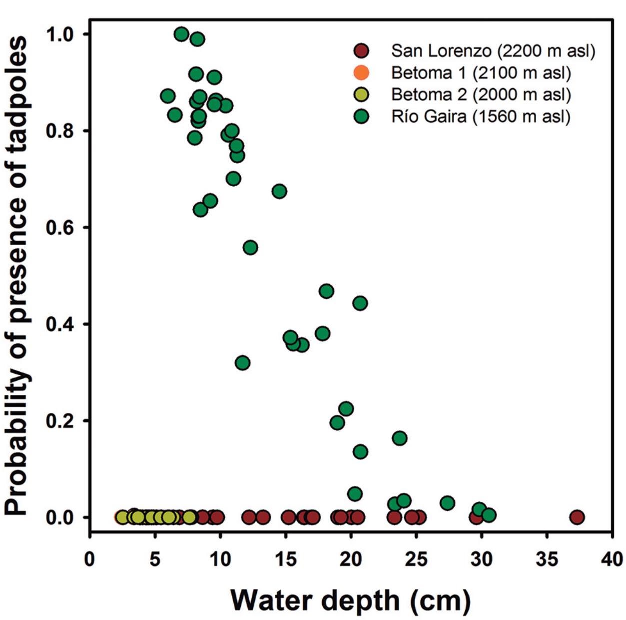

Figure 8. Logistic regression analysis showing the relationship between water depth and the presence of *Atelopus nahumae* tadpoles in streams of four localities at Sierra Nevada de Santa Marta, Colombia.

accounted for 50–78% of the probability of finding tadpoles (Cox and Snell R^2 = 0.50; Nagelkerke R^2 = 0.781; Fig. 8). Water depth best predicted the presence of tadpoles, with the greatest probability of detection at 10 cm depth (*n* = 107; Wald = 5.23; $P < 0.022$), but this probability varies among streams. Water temperature (*n* = 107, $\bar{x}$ = 16.8°C, Wald = 1.65, *P* = 0.19) and dissolved oxygen (*n* = 107, $\bar{x}$ = 8.2 mg/L, Wald = 2.88, *P* = 0.090) had no predictive power (Table 3). Data were insufficient to perform this analysis for *A. laetissimus.*

DISCUSSION

Our study increases to seven the number of *Atelopus* species in Colombia with described tadpoles (Table 2). Generally, *Atelopus* tadpoles are quite similar. For example, some of the most distinctive features observed in these gastromyzophorous tadpoles are a depressed body, robust caudal musculature, low fins, and a ventral mouth surrounded by well-developed labia that form a large and complete abdominal sucker (Lötters, 1996, 2001; Altig and McDiarmid, 1999; see Table 2). Despite these similarities, several authors have suggested that certain morphological traits, such as the relative size of the abdominal sucker, the relative size of the tail, the presence of submarginal papillae, and, in particular, the dorsal color pattern, are useful in distinguishing among species (Duellman and Lynch, 1969; Ruiz-Carranza and Osorno-Muñoz, 1994; Ruiz-Carranza et al., 1994; Coloma and Lötters, 1996; Coloma et al., 2000; Boistel et al., 2005).

The dorsal color pattern of *Atelopus laetissimus, A. nahumae,* and *A. carrikeri* is constant throughout stages 25–42. The black dorsal color pattern with large white or bluish-white spots, bands, or blotches in the tadpoles of *A. nahumae* is also known for tadpoles of other species of *Atelopus,* such as *A. certus* Barbour, 1923, *A. carbonerensis* Rivero, 1974, *A. subornatus, A. mittermeieri,* and *A. sorianoi* La Marca, 1983 (Duellman and Lynch, 1969; Lynch, 1986; Mijares-Urrutia and La Marca, 2005; Acosta-Galvis et al., 2006). These tadpoles are usually found in low and mid-elevation mountain ecosystems. In contrast, a color pattern of black coloration or uniformly dark brown with minute golden spots is characteristic of tadpoles that inhabit high-elevation ecosystems like paramos (e.g., *A. mucubajiensis* Rivero, 1974, *A. peruensis* Gray and Cannatella, 1985, *A. exiguus* Boettger, 1892, *A. tamaense* La Marca et al.,1990, *A. nanay* Coloma, 2002). The biological role of this phenomenon is unknown and has not been tested experimentally in *Atelopus,* but several authors have hypothesized that dark coloration has a thermoregulatory function, increasing absorbance of solar radiation in the cold environments that predominate at high elevations (Duellman and Lynch, 1969; Gray and Cannatella, 1985; Coloma et al., 2000; Coloma, 2002; Mijares-Urrutia and La Marca, 2005; Rueda-Solano et al., 2015).

Whereas most *Atelopus* tadpoles have been described as black (e.g., Duellman and Lynch, 1969; Coloma et al., 2002; Rueda-Solano et al., 2015; see above), the presence of a large, dark band on the fins and caudal musculature has been widely overlooked. A large, black, vertical, caudal band has been reported throughout all the stages of *A. subornatus* (Fig. 9) and *A. mittermeieri* (Lynch, 1986; Acosta-Galvis et al., 2006) but is also present in several Colombian species from the Cordillera Oriental (*A. farci, A. muisca, A. minutulus,* and *A. monohernandezi;* absent in *A. lozanoi, A. mandingues,* and *A. marinkellei;* Appendix S3), Cordillera Central (*A. angelito, A. sonsonensis, A. eusebianus, A. quimbaya,* and *A. sernai);* absent in *A. simulatus*), Cordillera Occidental (*A. famelicus* and *A. nicefori*), and Gorgona Island (*A. elegans*), but absent in SNSM (*A. carrikeri, A. laetissimus,* and *A. nahumae;* Appendix S3). Finally, of the remaining 18 *Atelopus* species with published descriptions (see Table 2), none have a large black band on the fins and caudal musculature.

Another characteristic that has gone largely unnoticed in tadpoles of the genus *Atelopus* is the presence of submarginal papillae on the oral disc, described for *A. franciscus* Lescure, 1974 and *A. flavescens* Duméril and Bibron, 1841 by Boistel et al. (2005) and observed by us in *A. laetissimus* and *A. nahumae* (see Table 2). Boistel et al. (2005) discussed its presence/absence in *A.* cf. *pulcher* and *A.* sp. from Central Amazonia, suggesting that the apparent absence might be an observational artifact (probably related to preservation artifacts) and predicted that submarginal papillae will be discovered when some described tadpoles are reexamined. Here, we report for the first time the presence of small, usually 2–4 submarginal papillae in the previously described tadpoles of *A. carrikeri* and *A. mittermeieri,* and in the undescribed tadpoles of *A. angelito, A. elegans, A. eusebianus, A. lozanoi, A. famelicus, A. farci, A. quimbaya, A. mandingues, A. marinkellei, A. minutulus, A. monohernandezi, A. muisca, A. nicefori, A. sernai, A. sonsonesis,* and *A. simulatus* (Appendix S4). Our observations corroborate Boistel et al.'s (2005) prediction.

Tadpoles of mid-elevation species like *Atelopus laetissimus* and *A. subornatus* tend to be larger than their lowland counterparts (Lynch, 1986; Gascon, 1989; Lavilla et al., 1997; Acosta-Galvis et al., 2006). Similarly, the tadpoles of high-elevation species (e.g., *A. carrikeri* and *A. ardila*) are larger than those of low and middle elevations (see Rueda-Solano et al., 2015). Among phylogenetically related species distributed on an altitudinal gradient, those that inhabit higher altitudes are usually larger (Ashton, 2002). According to our results and reports on *A. flavescens, A. balios* Peters, 1973, and *A. carrikeri,* larval growth, measured as TL, ceases at stage 42 in *A. balios* (Coloma and Lötters, 1996) and stage 43 in *A. carrikeri, A. laetissimus, A. nahumae,* (Rueda-Solano et al., 2015) and *A. flavescens* (Gawor et al., 2012). Size then gradually decreases, so that by stage 46 larval length reaches the minimum values (14.5 mm in *A. laetissimus* and 11.3 mm in *A. nahumae*). According to published records, the values of *A. laetissimus* are equivalent to those reported for *A. carrikeri* (14.0 mm; Rueda-Solano et al., 2015) and *A. zeteki* Dunn, 1933 (14.0 mm; Lindquist and Hetherington, 1998). These differences between species/stages might have a genetic basis, but they might also be affected by biotic or abiotic pressures, such as predators, food sources, or temperature changes that cause development to vary (Dahl et al., 2012).

Water depth had the greatest predictive power when the microhabitat preferences were evaluated in tadpoles of *Atelopus nahumae* (Fig. 8). This depth might be correlated with other factors like low speed of the current in which the tadpoles develop (Boistel et al., 2005). Likewise, a certain depth could increase the survival of tadpoles; however, this does not explain the absence of tadpoles in streams with similar depths (see Rueda-Solano et al., 2015). Alternatively, reproductive behavior could determine the presence/absence of the tadpoles (Rocha Usuga et al., 2017). Field observations suggest that *A. laetissimus, A. nahumae,* and *A. zeteki* (see Karraker, 2006; Rocha Usuga et al., 2017) use certain streams exclusively during the dry season for reproduction and oviposition. This behavior and the seasonality of the rain could determine the probability of detecting tadpoles of these species in the streams. In *A. balios, A. flavescens,* and *A. zeteki,* the variable that best predicts the presence of tadpoles is dissolved oxygen, because they require high oxygen concentrations for their development and survival (see Lescure, 1981; Coloma and Lötters, 1996; Lindquist and Hetherington, 1998). However, for *A. carrikeri* (Rueda-Solano et al., 2015) and *A. nahumae,* and perhaps *A. laetissimus* as well, dissolved oxygen has no predictive power to determine the presence of tadpoles in streams of the study area, because the concentration of dissolved oxygen in the water is always high and varies little.

The description of the tadpoles of *Atelopus laetissimus* and *A. nahumae* from the SNSM offers information for the taxonomic delimitation of these two species, serves as a basis for future comparative studies that seek to elucidate the diversity of morphological characters within *Atelopus*, and enables knowledge about their ecology and reproductive behavior to be improved. To date, almost half of the known diversity of *Atelopus* (46 species) occurs in Colombia (Frost, 2018); however, the tadpoles of only seven species (including the present work) have been described or illustrated. Finally, in terms of future studies on *Atelopus* tadpoles, comparative revisions of internal morphology are needed; these studies would ideally include data on buccopharyngeal cavity structures, cranial muscles, and chondrocranium.

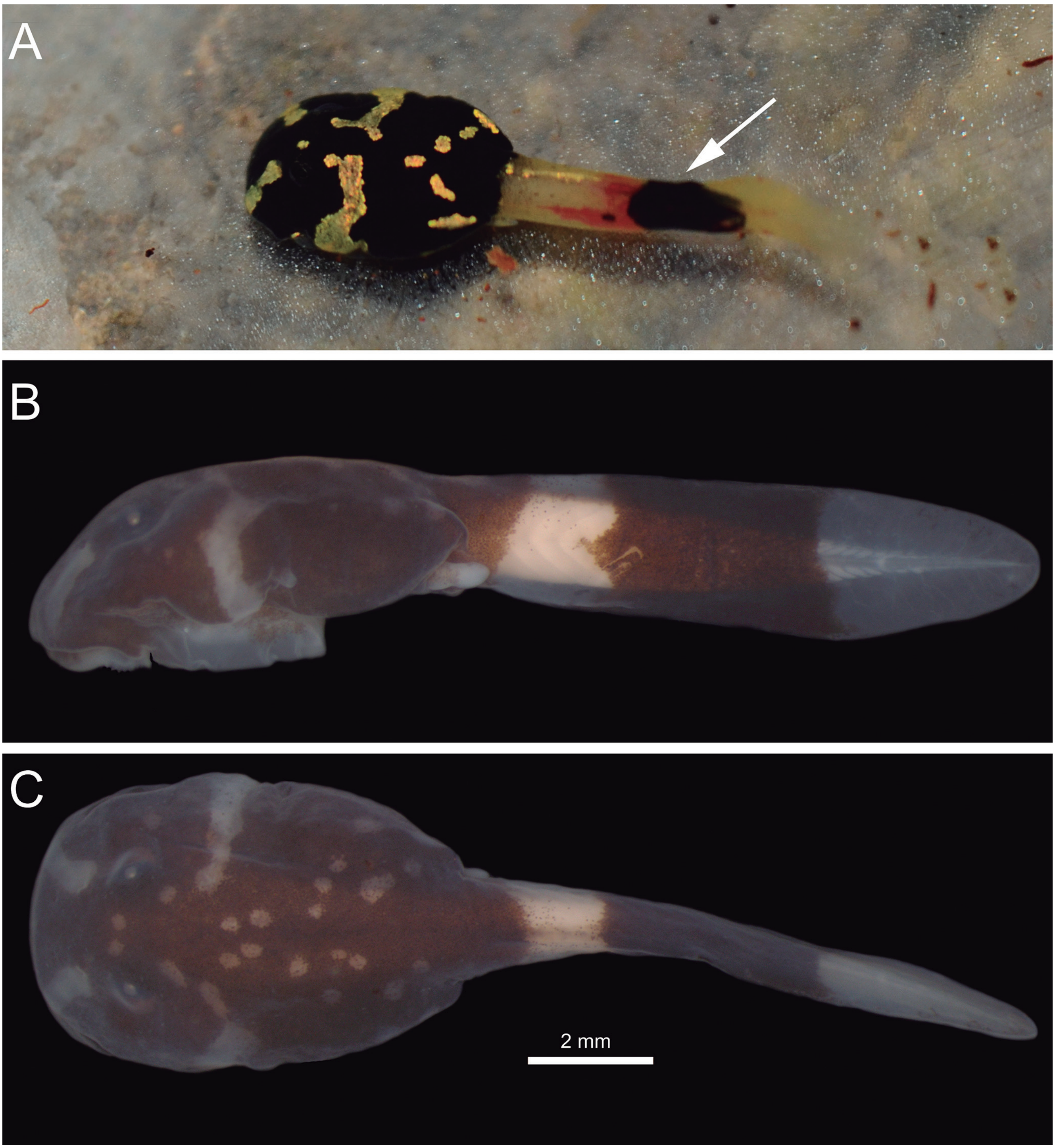

Figure 9. Presence of dark band on the fins and caudal musculature in *Atelopus subornatus* (arrow). **(A)** CZUT-A: 2256-4 in life, stage 36, (not to scale; photo: Marvin Anganoy Criollo); **(B–C)** ICN 31435, stage 31.

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ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

Appendix S1. Specimens examined for comparisons (tadpole lot).

Appendix S2. Morphological measurements (mm) and developmental stages (Gosner, 1960) for 60 tadpoles of *Atelopus carrikeri*, 103 of *A. laetissimus*, and 135 of *A. nahumae*, including larvae in stage 46.

Appendix S3. Species/specimens examined for occurrence of large dark band on the fins and caudal musculature.

Appendix S4. Species/specimens examined for the occurrence of submarginal papillae on the oral disc.