

Reproductive ecology, free-swimming tadpoles, and natural history of the Santa Marta Rocket Frog “*Colostethus*” *ruthveni* Kaplan, 1997 (Anura: Dendrobatidae), with a review of the distribution of larval and reproductive characters in Dendrobatoidea

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Abstract.—This study focuses on various biological aspect of the Santa Marta Rocket Frog, “*Colostethus*” *ruthveni*, from the Sierra Nevada de Santa Marta, in the Colombian Caribbean, and provides quantitative data on relative abundance and habitat use, and a comprehensive description of the free-swimming tadpoles and reproductive strategies. Additionally, the distribution of larval and reproductive characters across Dendrobatoidea was examined aiming to enhance the diagnostic criteria for the “C.” *ruthveni* group. A total of 853 specimens of “C.” *ruthveni* were recorded in six localities, indicating a relatively high abundance at each site. Leaf-litter and rocks were the most predominant substrates, although their proportions of use varied across the sites. The external morphology of free-swimming tadpoles of “C.” *ruthveni* resembles the former members of the *Colostethus sensu lato*. Courtship is a complex interaction of acoustic, visual, and tactile displays, which leads to cephalic amplexus. “*Colostethus*” *ruthveni* showed wide variation in oviposition sites, including both natural and artificial substrates at ground level, as well as creeping vegetation above the ground or water. This variability suggests the presence of multiple reproductive modes, one of them previously unknown for anurans. Several larval and reproductive characters of “C.” *ruthveni* consist of a compendium of ancestral states. However, some characters are of special interest because they could be considered reversions to ancestral states or apomorphies, which could constitute synapomorphies of the “C.” *ruthveni* group if they can be demonstrated to occur in the other undescribed members of the complex. Moreover, this study highlighted a distinctive trait of “C.” *ruthveni* as the only Dendrobatinae with tadpoles exhibiting a considerably narrow A-2 gap. The morphological and natural history information in this study provides insights into the evolutionary processes of poison frogs and basic information necessary for the management of this potentially endangered species.

Keywords. Character mapping, courtship, larval morphology, microhabitat uses, relative abundance, reproductive mode

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Resumen.—Investigamos varios aspectos de la biología de la rana cohete de Santa Marta “*Colostethus*” *ruthveni* de la Sierra Nevada de Santa Marta, Caribe colombiano. Se cuantificó la abundancia relativa y el uso del hábitat. Igualmente, se describieron los renacuajos de natación libre y las estrategias reproductivas. Además, se examinó la distribución de caracteres larvales y reproductivos a través de Dendrobatoidea como una aproximación a su evolución y para identificar si estos pueden complementar el diagnóstico del grupo “C.” *ruthveni*. Registramos 853 especímenes de “C.” *ruthveni* en seis localidades, lo que resultó en una abundancia

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relativa bastante alta en cada sitio. La hojarasca y las rocas fueron los sustratos más utilizados, aunque su proporción de uso varió según los lugares. La morfología externa de los renacuajos de “C.” *ruthveni* que nadan libremente se asemeja a la de los antiguos miembros de *Colostethus sensu lato*. El cortejo es una compleja interacción de exhibiciones acústicas, visuales y táctiles, que conducen al amplexo cefálico. “*Colostethus*” *ruthveni* mostró una amplia variación en los lugares de ovoposición, incluidos sustratos naturales y artificiales a nivel del suelo, así como vegetación rastrera sobre el suelo o el agua. Por lo tanto, consideramos que la especie exhibe múltiples modos reproductivos, uno de ellos desconocido hasta ahora para los anuros. Varios caracteres larvarios y reproductivos de “C.” *ruthveni* consisten en un compendio de estados ancestrales. Sin embargo, algunos caracteres son de especial interés porque podrían considerarse reversiones a estados ancestrales o apomorfías, que podrían constituir sinapomorfías del grupo “C.” *ruthveni* si se demuestra que ocurren en los otros miembros no descritos del complejo. Además, evidenciamos que los “C.” *ruthveni* es el único Dendrobatinae cuyos renacuajos poseen un espacio A-2 considerablemente estrecho. Toda esta información sobre morfología e historia natural permite comprender mejor los procesos evolutivos de las ranas venenosas y proporciona información básica necesaria para la gestión de esta especie potencialmente amenazada.

Palabras Claves. Abundancia relativa, cortejo, mapeo de caracteres, modo reproductivo, morfología larval

Introduction

Poison frogs of the superfamily Dendrobatoidea are one of the most representative groups of Neotropical amphibian fauna. These frogs have a series of highly diverse and particular characteristics including their color patterns, the presence or absence of toxicity, elaborate displays of visual and acoustic signals, and reproductive modes (Bourne et al. 2001; Caldwell and Lima 2003; Hill et al. 2011; Juncá et al. 1994; Lima and Keller 2003; Summers and Tumulty 2014; Wells 1980a,b,c; Weygoldt 1987). Within this family is the non-monophyletic genus *Colostethus sensu lato*, and while multiple efforts have been made to order it in recent decades (Grant 1998; Grant et al. 2006, 2017; Marin et al. 2018; Santos et al. 2009), this genus remains polyphyletic (Grant et al. 2017; Portik et al. 2023). The “*Colostethus*” *ruthveni* group *sensu* Grant et al. (2017) is more closely related to the aposematic dendrobatids (subfamily Dendrobatinae) than to *Colostethus sensu stricto* (ss; subfamily Colostethinae). Therefore, the “C.” *ruthveni* group constitutes an undescribed genus endemic to the Sierra Nevada de Santa Marta (SNSM), an isolated massif of the northeastern Caribbean coast of Colombia (Grant et al. 2017). Currently, the “C.” *ruthveni* group comprises the nominal species (“*Colostethus*” *ruthveni* Kaplan 1997 ss) and at least two undescribed species (Grant et al. 2017; Jiménez-Bolaño et al. 2019). “*Colostethus*” *ruthveni* ss was defined by Jiménez-Bolaño et al. (2019) as a population of the complex distributed in the northwestern sector of the SNSM, based on the bioacoustic variation, external morphology, and geographic location of the type locality.

In the last decade, our knowledge of the biology of the “C.” *ruthveni* group has increased considerably, including data on its distribution, feeding ecology, phylogenetic relationships, and acoustic repertoire (Blanco-Torres et al. 2014; González-Maya et al. 2011; Granda-Rodríguez et al. 2014; Grant et al. 2017; Jiménez-Bolaño et al. 2019; Meza-Joya et al. 2019).

However, multiple topics related to its reproductive mode are still unknown. Understanding the reproductive modes is of great interest, because natural and sexual selection favor reproductive mechanisms that maximize the probability of successful mating and the survival of offspring, thereby directly affecting fitness and biological performance in response to different selective pressures (Carvajal-Castro et al. 2020). In this sense, the reproductive mode can be defined as a combination of ecological, physiological, developmental, and behavioral traits such as the oviposition site, ovule morphology, clutch size, and the presence or absence of different types of parental care (Duellman and Trueb 1994). In the Dendrobatoidea, the study of the reproductive modes is particularly interesting due to their great diversity (Carvajal-Castro et al. 2020; Vitt and Caldwell 2014). However, the only information available on this aspect for the group “C.” *ruthveni* was published more than a century ago by Ruthven and Gage (1915), who briefly described the reproductive habitat and microhabitat, and the male transport of larvae.

Tadpoles of “C.” *ruthveni* present the typical morphology of *Colostethus sensu lato* larvae. The first tadpole description was based on back-riding larvae removed from the male dorsum and free-swimming specimens (Ruthven and Gage 1915). However, this description is too limited by current standards and describes the free-swimming and back-riding tadpoles together. This approach is not recommended, as it makes comparisons difficult due to the ontogenetic variations between the different larval stages (Anganoy-Criollo 2013). More recent research has been limited to describing characters, with the aim of inferring phylogenetic relationships based on larval morphology and the evolution of larval characters (Anganoy-Criollo and Cepeda-Quilindo 2017; Sánchez 2013). Such studies revealed that “C.” *ruthveni* apparently possesses multiple ancestral character states (e.g., the presence of a notch in the upper jaw sheath, the long gut sinistrally coiled, and the inner margin of the nasal rim projected), especially

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considering the phylogenetic relationships of the group *sensu* Grant et al. (2017). Given our limited knowledge of the external morphology of free-swimming tadpoles of “*C.*” *ruthveni* and the potential phylogenetic implications obtained from this line of evidence, a redescription of the tadpoles of the species following the standards of current descriptions is necessary.

This species is currently categorized by the IUCN as Near Threatened (NT- B1a; IUCN SSC Amphibian Specialist Group 2018) due to habitat loss because of agricultural activities (including pollution) and livestock, particularly in the southeastern sector of the SNSM (Granda-Rodríguez et al. 2020). Despite its relatively small range (EOO < 11,000 km²), the populations appear to be stable and somewhat tolerant to disturbance. Although the IUCN categorization states that populations are stable and abundant, that statement is based on anecdotal observations that do not account for sampling effort. Consequently, acquiring information on the basic ecological dimensions of “*C.*” *ruthveni*, its relative abundance, and habitat use will provide more appropriate tools to identify priority areas for the management and conservation of this species. Thus, according to the multiple knowledge gaps that exist on the biology of the “*C.*” *ruthveni* group, this study was developed with the following objectives: (1) quantifying the relative abundance and use of microhabitats; (2) redescriving the external morphology of free-swimming tadpoles; (3) documenting some aspects of the reproductive mode, such as amplexus and laying site, in a population of *Colostethus ruthveni* ss; and (4) exploring from a phylogenetic perspective the distribution of external larval and reproductive characters and how these can potentially facilitate the diagnosis of the group “*C.*”

ruthveni based on the most recent available topology of Dendrobatoidea.

Materials and Methods

Study Area

This study was carried out at 10 sites distributed in four pericontinental basins in the distribution area of “*C.*” *ruthveni* ss, the northwestern sector of the Sierra Nevada de Santa Marta, district of Santa Marta, department of Magdalena, Colombian Caribbean (Fig. 1, Table 1). The sites occupy the altitudinal strip from the foothills to the low mountains, at altitudes between 400 and 1,521 m, which cover almost the entire altitudinal distribution known for “*C.*” *ruthveni* (Granda-Rodríguez et al. 2014; Jiménez-Bolaño et al. 2019). In its lower sector, this altitudinal strip includes the tropical dry biomes that succeed one another with the sub-Andean Forest biome at approximately 700 m (Fundación Pro-Sierra Nevada 1998), except for the Los Rodríguez stream basin, where the limit could be at around 550 m asl. Its presence at the lower altitude is due to the fog, a product of the condensation due to the humid coastal winds (Fundación Pro-Sierra Nevada 1998). The area’s water regimes vary between unimodal and bimodal, with one or two annual rainfall peaks. Both climatic modes converge from the driest period, extending from December to March, and the rainiest period, extending from August to November. There may or may not be a drop in rainfall in July. Mountain lotic ecosystems show rapid flow due to the steep slope of the mountain, and have abundant boulders and sandy or pebble-covered bottoms (Manjarrés-García and Manjarrés-Pinzón 2004).

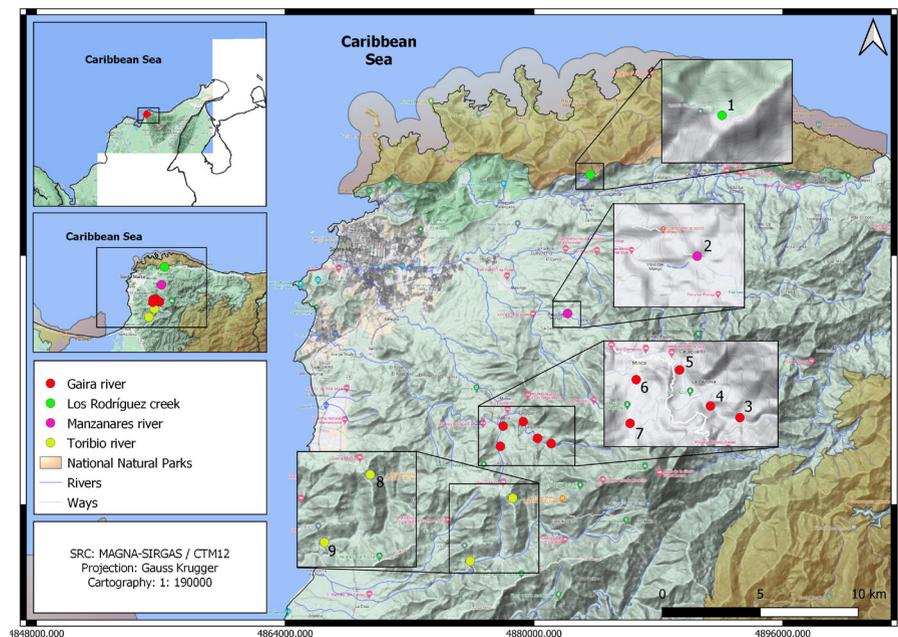


Fig. 1. Localization of the study area. 1. Las Tinajas, 2. Paso El Mango, 3. Sierra Minca, 4. La Victoria, 5. Pozo Azul, 6. Arimaca, 7. Marinca, 8. Bella Vista, 9. Central Córdoba. Data of reproductive biology were from 2, 4, 5–9. Data of relative abundance and microhabitat were from 1–4. Tadpole batch CBUMAG:ANF:00662 from 4. *Developed by Juan David Jiménez-Bolaño.*

Relative Abundance and Microhabitat Use

To estimate the relative abundance, 21 sampling events were carried out at six locations during the rainy and dry seasons between 2005 and 2021 (Tables 1 and 2). Two observers searched randomly, carefully inspecting the

microhabitats to find specimens of “*C.*” *ruthveni* using a visual encounter survey method (Crump and Scott 1994). The sampling effort varied between 2–6 hours (h) per event, for a total of 105 h (Table 1). Based on the total counts of individuals, a relative abundance index (RAI) was estimated, defined as the number of individuals (n)

Table 1. List of localities where “*Colostethus*” *ruthveni sensu stricto* was recorded, with date of observation, sampling effort, total count per sampling event, and relative abundance (n/h/obs) per sampling event.

Basin and site	Sampling date	Sampling effort (h × obs)	n	RAI (n/h/obs)
Gaira river				
La Victoria	7/11/2005	5 × 2	37	3.7
	12/11/2005	5 × 2	43	4.3
	21/03/2010	5 × 2	17	1.7
	22/03/2010	5 × 2	20	2.0
	06/06/2010	5 × 2	30	3.0
	07/06/2010	5 × 2	38	3.8
	06/07/2011	5 × 2	24	2.4
Sierra Minca	20/10/2020	5 × 2	35	3.5
	21/10/2020	5 × 2	44	4.4
	22/10/2020	5 × 2	46	4.6
	24/01/2021	4 × 2	80	10.0
Manzanares river				
Paso el Mango	15/04/2011	2 × 2	10	2.5
Los Rodríguez Creek				
Las Tinajas	10/02/2006	6 × 2	42	3.5
	20/02/2007	6 × 2	55	4.6
	20/02/2010	6 × 2	68	5.7
	06/07/2011	6 × 2	28	2.3
	18/08/2014	2 × 2	20	5.0
	17/01/2021	4 × 2	45	5.6
Toribio river				
Bella Vista Central Córdoba	5/10/2014	2 × 2	11	2.8
	26/11/2020	5 × 2	63	6.3
	27/11/2020	5 × 2	35	3.5
	28/11/2020	5 × 2	62	6.2

Table 2. Microhabitat use of “*Colostethus*” *ruthveni sensu stricto* in four localities of the northwestern sector of the Sierra Nevada de Santa Marta. χ^2 = Chi square test, df = degrees of freedom.

Site	Bare floor	Boulders	Decayed logs	Leaf-litter	Leaves	Total	χ^2
Las Tinajas	14	52	4	11	4	11	96 $\chi^2 = 102.38$, df = 5, p-value < 0.001
La Victoria	3	9	4	13	3	5	37 $\chi^2 = 13.1$, df = 5, p-value = 0.022
Sierra Minca	2	7	2	22	2	15	50 $\chi^2 = 42.4$, df = 5, p-value < 0.001
Central Córdoba	1	9	5	11	1	3	30 $\chi^2 = 17.6$, df = 5, p-value < 0.001
Total	20	77	15	57	10	34	213 $\chi^2 = 54.5$, df = 15, p-value < 0.001

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Table 3. Summary of morphometric characters of free-swimming tadpoles of “*Colostethus*” *ruthveni sensu stricto* through the Gosner (1960) stages of s25 through s38. Measurements are expressed in mm, with range (average ± standard deviation). Abbreviations: total length (TL), body length (BL), tail length (TAL), body height (BH), maximum tail height (MTH), tail muscle height (TMH), nostril-snout distance (NSD), nostril-eye distance (NED), nostril length (NL), eye diameter (ED), spiracle length (SH), spiracle length (SL), spiracle-snout distance (SSD), eye-snout distance (ESD), body width (BW), interorbital distance (IND), interorbital distance (IOD), tail muscle width (TMW), oral disc width (ODW), anterior lip gap width (AL-gap), width of medial gap in second anterior tooth row (A-2 gap), upper jaw sheath width (UJS-W), lower jaw sheath width (LJS-W), papillae in the anterior per mm (P-AL/mm), and papillae in the posterior lip per mm (P-PL/mm).

Character	s25 (n=15)	s26 (n=13)	s27 (n=8)	s28 (n=5)	s29 (n=2)	s30–s31 (n=2)	s33 (n=1)	s34 (n=3)	s35 (n=3)	s36 (n=2)	s37 (n=1)	s38 (n=2)
TL	12.9–17.6 (15.6±1.5)	16.7–23.3 (18.8±1.8)	23.8–27.7 (25.8±1.6)	27.2–29.2 (28.1±0.1)	28.6–29.6 (29.1±0.7)	28.5–29.4 (29.0±0.6)	28.5	29.6–30.5 (30.2±0.5)	29.3–31.5 (30.5±1.1)	31.8–32.3 (32.0±0.3)	30.6	32.5–33.8 (33.1±0.9)
BL	5.0–6.7 (6.0±0.6)	6.5–8.4 (7.1±0.7)	9.0–10.2 (9.6±0.5)	10.0–10.9 (10.3±0.4)	10.3–10.6 (10.4±0.2)	10.2–10.3 (10.3±0.1)	10.4	10.6–11.0 (10.8±0.2)	10.0–11.1 (10.7±0.6)	10.7–11.1 (10.9±0.3)	10.7	11.1–11.6 (11.3±0.3)
TAL	7.9–10.9 (9.7±0.9)	10.1–14.9 (11.6±1.7)	14.8–17.5 (16.3±1.2)	17.1–18.7 (17.8±0.7)	18.0–19.3 (18.6±0.9)	18.3–19.1 (18.7±0.6)	18.1	19.0–19.5 (19.3±0.3)	19.3–20.4 (19.8±0.5)	21.1–21.2 (21.1±0.1)	19.9	21.4–22.2 (21.8±0.6)
BH	2.8–3.7 (3.3±0.3)	3.6–4.6 (4.0±0.4)	4.2–5.3 (4.9±0.4)	4.9–5.9 (5.3±0.4)	5.2–5.4 (5.3±0.1)	3.3–5.2 (4.3±1.3)	5.3	5.4–5.7 (5.6±0.2)	5.4–5.5 (5.5±0.1)	5.5–5.6 (5.5±0.1)	5.4	5.2–5.5 (5.3±0.2)
MTH	2.4–3.2 (2.8±0.2)	3.0–4.1 (3.4±0.3)	4.0–4.6 (4.4±0.2)	4.5–4.9 (4.7±0.1)	4.6–4.7 (4.6±0.1)	4.3–4.5 (4.4±0.1)	4.9	4.6–5.1 (4.9±0.2)	4.5–5.0 (4.7±0.3)	4.9–5.1 (5.0±0.1)	4.7	4.9–5.0 (4.9±0.1)
TMH	1.3–1.9 (1.7±0.2)	1.8–2.4 (2.0±0.9)	2.8–4.5 (3.7±0.7)	3.1–3.6 (3.3±0.2)	3.3–3.5 (3.4±0.1)	3.0–3.3 (3.2±0.2)	3.4	3.2–3.7 (3.5±0.3)	3.3–3.6 (3.5±0.1)	3.3–3.5 (3.4±0.1)	3.2	3.0–3.2 (3.1±0.1)
NSD	0.5–0.7 (0.6±0.1)	0.6–0.9 (0.7±0.1)	0.9–1.2 (1.0±0.1)	1.1–1.3 (1.2±0.1)	1.0–1.1 (1.0±0.1)	1.0–1.1 (1.1±0.1)	1.1	0.9–1.1 (1.0±0.1)	1.0–1.2 (1.1±0.1)	0.9–1.0 (0.9±0.1)	0.9	0.9–1.1 (1.0±0.1)
NED	0.4–0.7 (0.6±0.1)	0.6–0.9 (0.7±0.1)	0.9–1.0 (1.0±0.05)	0.9–1.0 (1.0±0.05)	1.0–1.0 (1.0±0.0)	0.9–1.0 (1.0±0.1)	1.0	0.9–1.0 (1.0±0.1)	0.8–1.0 (0.9±0.1)	0.9–0.9 (0.9±0.0)	0.8	1.0–1.0 (1.0±0.0)
NL	0.1–0.2 (0.1±0.05)	0.2–0.3 (0.2±0.03)	0.2–0.4 (0.3±0.05)	0.3–0.4 (0.3±0.05)	0.3–0.3 (0.3±0.0)	0.3–0.4 (0.4±0.1)	0.2	0.4–0.5 (0.4±0.1)	0.3–0.4 (0.4±0.1)	0.4–0.4 (0.4±0.0)	0.3	0.3–0.4 (0.3±0.1)
ED	12.9–17.6 (15.6±1.5)	0.6–0.9 (0.8±0.1)	0.8–1.1 (0.9±0.1)	1.0–1.1 (1.0±0.05)	1.1–1.2 (1.1±0.1)	1.1–1.1 (1.1±0.0)	1.2	1.2–1.3 (1.3±0.1)	1.3–1.5 (1.4±0.1)	1.3–1.3 (1.3±0.0)	1.4	1.4–1.5 (1.4±0.1)
SH	0.4–0.7 (0.5±0.09)	0.6–0.8 (0.6±0.1)	0.6–1.2 (0.9±0.2)	0.9–1.2 (1.0±0.1)	1.3–1.4 (1.3±0.1)	1.0–1.3 (1.2±0.2)	1.3	1.0–1.3 (1.2±0.1)	1.0–1.3 (1.1±0.2)	1.2–1.3 (1.2±0.1)	1	0.9–1.1 (1.0±0.1)
SL	0.8–1.3 (1.1±0.1)	1.1–1.6 (1.3±0.1)	1.4–2.7 (2.0±0.4)	1.6–2.5 (2.1±0.3)	2.3–2.7 (2.5±0.3)	1.5–2.7 (2.1±0.8)	2.7	2.1–2.5 (2.3±0.2)	2.1–2.4 (2.3±0.1)	2.2–2.3 (2.2±0.1)	1.9	2.0–2.4 (2.2±0.2)
SSD	3.2–4.3 (3.9±0.4)	4.2–5.1 (4.5±0.3)	5.7–6.6 (6.1±0.3)	6.5–6.8 (6.6±0.1)	6.4–6.5 (6.4±0.1)	6.4–6.4 (6.4±0.0)	6.8	6.6–6.7 (6.6±0.1)	6.6–7.0 (6.8±0.2)	6.6–6.8 (6.7±0.1)	6.7	6.5–7.1 (6.8±0.4)
ESD	1.2–1.6 (1.4±0.2)	1.5–1.9 (1.7±0.1)	2.2–2.5 (2.3±0.1)	2.5–2.6 (2.5±0.05)	2.2–2.5 (2.3±0.2)	2.3–2.3 (2.3±0.0)	2.2	2.2–2.3 (2.2±0.1)	2.1–2.6 (2.3±0.3)	2.1–2.2 (2.1±0.1)	2	2.3–2.5 (2.4±0.1)
BW	3.8–5.6 (4.6±0.6)	4.6–6.4 (5.4±0.5)	5.6–6.4 (6.0±0.3)	6.0–6.5 (6.4±0.2)	6.3–6.5 (6.4±0.1)	6.0–6.4 (6.2±0.3)	6.5	6.8–7.1 (6.9±0.1)	6.4–6.9 (6.7±0.2)	6.9–6.9 (6.9±0.0)	6.8	6.6–6.8 (6.7±0.1)
IND	1.1–1.6 (1.4±0.1)	1.3–1.8 (1.6±0.1)	1.8–2.0 (1.9±0.05)	1.9–2.0 (1.9±0.05)	1.9–2.1 (2.0±0.1)	1.9–2.0 (2.0±0.1)	2.1	2.0–2.1 (2.0±0.1)	2.0–2.1 (2.1±0.03)	2.0–2.1 (2.0±0.1)	2.1	2.1–2.1 (2.1±0.0)
IOD	1.6–2.2 (1.9±0.2)	2.0–2.5 (2.2±0.1)	2.5–2.8 (2.7±0.1)	2.8–2.9 (2.8±0.04)	2.9–2.9 (2.9±0.0)	2.9–2.9 (2.9±0.0)	3.1	3.2–3.3 (3.3±0.1)	3.1–3.3 (3.2±0.1)	3.3–3.3 (3.3±0.0)	3.4	3.5–3.5 (3.5±0.0)
TMW	1.3–2.1 (1.7±0.2)	1.7–2.7 (2.1±0.3)	2.2–3.2 (2.8±0.3)	2.8–3.3 (3.1±0.1)	3.0–3.2 (3.1±0.1)	3.0–3.1 (3.1±0.1)	3.1	3.2–3.5 (3.4±0.1)	3.4–4.0 (3.6±0.4)	3.6–3.7 (3.6±0.1)	3.4	3.5–3.6 (3.0±0.1)
ODW	1.7–2.9 (2.2±0.4)	2.1–3.0 (2.5±0.2)	2.7–3.0 (2.9±0.1)	2.9–3.4 (3.1±0.2)	3.0–3.8 (3.4±0.6)	2.7–2.9 (2.8±0.1)	3.2	3.2–3.5 (3.3±0.2)	2.9–3.1 (3.0±0.1)	3.1–3.2 (3.1±0.1)	3.3	3.0–3.3 (3.1±0.2)
AL gap	0.9–1.4 (1.1±0.6)	1.1–1.6 (1.3±0.1)	1.4–1.7 (1.5±0.1)	1.3–1.7 (1.5±0.1)	1.5–1.6 (1.5±0.1)	1.4–1.8 (1.6±0.3)	1.7	1.5–1.8 (1.7±0.1)	1.6–2.1 (1.8±0.3)	1.4–1.7 (1.5±0.2)	1.8	1.6–1.7 (1.6±0.1)
UJS-W	0.6–1.0 (0.9±0.1)	0.8–1.2 (1.0±0.1)	1.2–1.5 (1.3±0.1)	1.3–1.5 (1.4±0.1)	1.3–1.4 (1.3±0.1)	1.3–1.4 (1.4±0.1)	1.4	1.3–1.5 (1.4±0.1)	1.4–1.6 (1.5±0.1)	1.3–1.5 (1.4±0.1)	1.5	1.3–1.4 (1.3±0.1)
LJS-W	0.4–0.7 (0.5±0.1)	0.4–0.7 (0.6±0.1)	0.8–1.0 (0.9±0.1)	0.9–1.1 (1.0±0.1)	0.9–1.0 (0.9±0.1)	0.8–0.8 (0.8±0.0)	1.0	0.9–1.1 (1.0±0.1)	1.1–1.2 (1.1±0.1)	1.0–1.1 (1.0±0.1)	1	1.0–1.0 (1.0±0.0)
P-AL/mm	11–17 (14.3±1.8)	15–16 (15.5±0.5)	15–18 (17.3±1.0)	18–19 (18.6±0.5)	14–15 (14.5±0.7)	14–20 (17.0±4.2)	17	19–19 (19.0±0.0)	16–17 (16.7±0.6)	16–20 (18.0±2.8)	23	15–18 (16.5±2.1)
P-PL/mm	12–21 (16.6±2.2)	14–18 (15.8±1.5)	13–16 (14.61±1.3)	13–20 (15.0±3.1)	12–13 (12.5±0.7)	11–14 (12.5±2.1)	20	15–16 (15.3±0.6)	13–18 (15.0±2.6)	14–17 (15.5±2.1)	21	13–15 (14.0±1.4)
A-2 gap	0.07–0.1 (0.1±0.02)	0.0–0.2 (0.1±0.04)	0.09–0.2 (0.1±0.05)	0.09–0.2 (1.0±0.05)	0.1–0.1 (0.1±0.0)	0.1–0.1 (0.1±0.0)	0.1	0.09–0.1 (0.1±0.01)	0.1–0.2 (0.2±0.1)	0.09–0.1 (0.1±0.01)	0.1	0.1–0.1 (0.1±0.0)

per capture effort (hours × observers) and denoted as n/h/obs (Lips 1999). The evaluation of microhabitat use followed Johnson (1980), and Krausman (1999), which identifies the specific components of habitat utilized and their respective proportions of use. The substrate

directly beneath each specimen was documented and the frequency of observations for each substrate type was calculated following Inger (1994) and Vargas-Salinas and Castro (1999). A Chi-square test of homogeneity (χ^2) was employed to assess potential variations in

substrate (microhabitat) usage. The null hypothesis states no significant differences among the observed categories. To ensure accuracy, the expected frequencies for each microhabitat category observed in the field were calculated, assuming equal probabilities of occupancy across microhabitats.

Free-swimming Tadpoles

This study followed the nomenclature of the Dendrobatoidea superfamily proposed by Grant et al. (2017), with the arrangements of Marin et al. (2018). The description of the tadpole was based on a group of 57 tadpoles deposited in the amphibian collection at the Centro de Colecciones Científicas de la Universidad del Magdalena (CBUMAG:ANF:00662), that had been collected by J. M. Renjifo at the La Victoria farm, Minca village, District of Santa Marta, department of Magdalena, Colombia (see Fig. 1). Additionally, some observations on the natural history were obtained in the localities of Las Tinajas, Bella Vista, Pozo Azul, and Arimaca. Tadpoles were determined to the species level as "*Colostethus*" *ruthveni sensu stricto* by the following three characteristics: (1) the presence of a golden band on the body of the tadpole described by Ruthven and Gage (1915); (2) the geographic distribution of the complex proposed by Jiménez-Bolaño et al. (2019); and (3) the presence of three metamorphics in the group whose morphological characters correspond to those described by Kaplan (1997). A Leica M205A stereomicroscope with a Leica DFC450 camera was used for the examination and photography of the specimens. The morphometric characters (expressed in mm) were characterized with the Leica Application Suite software (Version 4.6.0), with a precision of ± 0.01 .

The tadpole description was based on the external morphology of three tadpoles at stage 35 of Gosner (1960), since this stage represents the midpoint of larval development (Suarez-Mayorga and Lynch 2001). The other stages were used to describe the ontogenetic variation of the morphological and morphometric characters. The terminology for morphometric characterization proposed by Altig and McDiarmid (1999) and Anganoy-Criollo (2013) was followed for evaluating the total length (TL), body length (BL), body width (BW), tail length (TAL), maximum tail height (MTH), tail muscle height (TMH), tail muscle width (TMW), internarial distance (IND), interorbital distance (IOD), oral disc width (ODW), anterior lip gap width (AL-gap), width of medial gap in second anterior tooth row (A-2 gap), nostril length (NL), nostril-snout distance (NSD), nostril-eye distance (NED) spiracle length (SL), spiracle height (SH), spiracle -snout distance (SSD), eye diameter (ED) eye-snout distance (ESD), upper jaw sheath width (UJS-W), and lower jaw sheath width (LJS-W). Additionally, the arrangement of the rows of papillae in the anterior (PAL) and posterior (PPL) lips were examined and described, following the

terminology of Sánchez (2010). The number of papillae on each lip was counted in the space of 1 mm (Anganoy-Criollo 2013). For metamorphic tadpoles, total length, tail length, and body width were measured.

Reproductive Ecology

To identify egg-laying sites, seven monthly fieldtrips lasting two days each were conducted in Las Tinajas from June to December 2015, as well as three additional fieldtrips during August 2015 (two days), December 2016 (one day), and January 2021 (one day) in Bella Vista, Arimaca, and Sierra Minca, respectively. At each site, two observers explored the watercourses by proceeding upstream and using the visual encounter survey method (Crump and Scott 1994). The searches lasted from 0600 to 1800 h for a total effort of 210 h \times 2 observers (168 h in Los Rodríguez ravine, 24 h in Bella Vista, 12 h in Marınca, and 6 h in Sierra Minca). At each egg laying site, the number of eggs, egg diameter, location, and distance from the water source were recorded. Information on courtship and type of amplexus was obtained through direct observations. Aspects of courtship such as color change, jumps, circle jumps, body elevation, and others, were described following the terminology of Hödl and Amézquita (2001). The reproductive mode was established from the proposal of Nunes-De-Almeida et al. (2021, see Supplemental Information S2), where 11 reproductive and larval characters were examined using a dichotomous key.

Distribution of Larval and Reproductive Characters

A matrix coding 35 phenotypic characters (larval and reproductive morphology) for each genus of the Dendrobatoidea superfamily (22 genera) was built. Thirty-two characters were extracted from Appendix 1 of Grant et al. (2017) (characters 94-126). The coding of these characters was extracted via an extensive literature review on the free-swimming tadpole morphology and reproductive strategies of Dendrobatoidea (Almendáriz et al. 2012; Anganoy-Criollo 2013; Anganoy-Criollo and Cepeda-Quilindo 2017; Barrio-Amorós et al. 2004; Breder 1946; Brown et al. 2008; Caldwell 2005; Caldwell and Myers 1990; Caldwell et al. 2002; Carvajal-Castro et al. 2020; Castillo-Trenn 2004; Dias et al. 2018a,b, 2021; Donnelly et al. 1990; Downie and Nokhbatolfoghahai 2014; Duarte-Marín et al. 2020; Duellman 2004; Edwards 1971, 1974; Fouquet et al. 2019; French et al. 2019; González-Maya et al. 2011; Grant 1998; Grant et al. 2006, 2007, 2017; Granda-Rodríguez et al. 2014; Granda-Rodríguez et al. 2018; Hill et al. 2011; Jiménez-Bolaño et al. 2019; Klein et al. 2020; Kok et al. 2006a,b, 2010, 2013; La Marca 1985, 1989, 1993, 1994; La Marca and Mijares-Urrutia 1988; Lehtinen and Halley 2008; Lescure 1976; Lima et al. 2014, 2015; Lüddecke 1999; Lynch 1982; Marin et al. 2018; Masche et al. 2010; Menin

et al. 2017; Mijares-Urrutia 1991; Mijares-Urrutia and La Marca 1997; Moraes et al. 2019; Myers and Donnelly 1997, 2001; Myers et al. 1984; Pezzuti et al. 2022; Páez-Vacas et al. 2010; Perez-Peña et al. 2010; Pisso-Florez et al. 2023; Poelman et al. 2010; Regnet et al. 2023; Rojas-Morales et al. 2021; Rodriguez and Myers 1993; Ruthven and Gaige 1915; Sánchez 2013; Savage 1968, 2002; Schulze et al. 2015; Schulte 1990; Serrano-Rojas et al. 2017; Silverstone 1975; Simões et al. 2010, 2013; Simões and Lima 2012; Summers and Tumulty 2014; Twomey and Brown 2008, 2009; van Wijngaarden and Bolaños 1992; Vitt and Caldwell 2014; von May et al. 2008; Wells 1980a,b; Weygoldt 1987).

One of the characters from Grant et al. (2006, 2017) was reinterpreted as follows:

119. Oviposition site: aquatic = 0; terrestrial: at ground level = 1; terrestrial: phytoteltama = 2; terrestrial: in leaves above ground or water level = 3.

This character will be considered as additive for describing the degree of association with the ground level or the distance from the water (Grant et al. 2006). State 1 includes all microhabitats at ground level such as leaf litter or bare soil, as well as on and under fallen objects such as rocks, logs, and even artificial objects. State 2 is restricted to cavities with liquid water in terrestrial plants. State 3 consists of species that lay their eggs on the upper surface or underside of the leaves, which had been considered as part of state 2 by Grant et al. (2006, 2017). Additionally, two new characters were declared, following the argument of Sereno (2007), that were enumerated following the consecutive numbering of the character list of Grant et al. (2017):

189. Second keratodon row of anterior lip, medial gap (A-2 gap): absent = 0; present = 1.

190. First keratodon row of posterior lip, medial gap (P-1 gap): absent = 0; present = 1.

Subsequently, these 35 characters were mapped by hand on the topology that summarizes the generic relationships of Dendrobatoidea proposed by Grant et al. (2017, modified from figure 47), with the goal of identifying how the larval morphological plans and the reproductive modes are distributed throughout this hypothesis of the evolutionary relationships of the Dendrobatoidea. In addition, we explored the intra- and intergeneric variation of a continuous character: the ratio of the width of the medial gap of the second row of keratodon teeth of the anterior lip by the width of the oral disc (A-2 gap/ODW %), since it has recently been proposed as potentially useful for diagnosing some taxa within Dendrobatoidea (Granda-Rodríguez et al. 2018).

Results

Relative Abundance and Microhabitat Use

A total of 853 individuals of “*C.*” *ruthveni* ss were observed in six study locations. The species was very abundant in all samplings and locations (Table 1), since the RAI in all cases reached values greater than 1, i.e., between 1.7–6.3 n/h/obs, with an exceptional observation of 10 n/h/obs. This means that the species maintained an approximate encounter rate of two to six individuals per hour throughout the entire time window studied. Although the largest numbers of individuals were detected in Las Tinajas (n = 258) and La Victoria (n = 209), the highest RAI values were obtained in Sierra Minca (10 n/h/obs) and Central Córdoba (6.2 n/h/obs), since less sampling effort was required to record considerable numbers of specimens at these sites. Microhabitat use data were obtained from 213 specimens. Six categories of substrate were used by the species: bare floor, boulders, decayed logs, leaf-litter, leaves, and stream, where the most heavily used substrates were boulders (77 individuals, 36.2%) and leaf-litter (57 individuals, 26.8%). Differential use of microhabitats was found at all sites, and the proportion of use of each microhabitat also varied between them (Table 2). In the locality of Las Tinajas, most individuals were found associated with boulders, demonstrating a statistically significant difference ($\chi^2 = 102.38$, df = 5, p-value < 0.001). In La Victoria, a greater use of leaf litter and boulders was found ($\chi^2 = 13.1$, df = 5, p-value = 0.022). In Sierra Minca, the highest usage occurred in leaf litter and individuals in streams ($\chi^2 = 42.4$, df = 5, p-value < 0.001). Finally, in Central Córdoba, a greater usage of leaf litter was found ($\chi^2 = 17.6$, df = 5, p-value < 0.001).

Redescription of Free-swimming Tadpoles

Table 3 summarizes the morphometric characters analyzed, with the individual data for each tadpole presented in Supplementary Material 1: Table S1. Stage 35 tadpoles have the following characteristics: body ovoid in dorsal view and compressed in lateral view (BW/BL = 60–64%, BH/BW = 78–86%, Fig. 2), reaching just over one-third of the total length (BL/TL = 34–36%); snout rounded in dorsal and lateral views; nostrils rounded in dorsal view, directed dorsolaterally, with gently projecting margins, equidistant between eyes and tip of snout, occupying almost one-third of eye diameter (NL/ED = 21–31%); internarial distance approximately two-thirds of the interorbital distance (IND/IOD = 62–67%); and the interorbital distance one-half the body width (IOD/BW = 47–48%). Eyes located dorsally and directed dorsolaterally, with a diameter less than half the interorbital distance (ED/IOD = 39%). Sinister spiracle, as long as high (SH/SL = 48–54%), visible in lateral, dorsal, and ventral views, directed

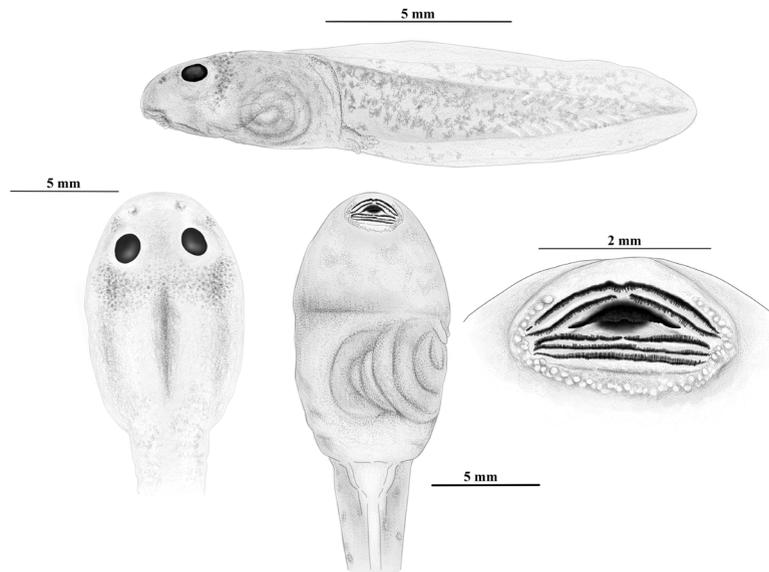


Fig. 2. Detail of larval morphology of a free-swimming tadpole of “*Colostethus ruthveni*” *sensu stricto* at stage 35 (*sensu* Gosner 1960) from the batch CBUMAG:ANF:00662. Drawings by Jhuliyana Lopez-Caro.

posteriorodorsally, opening posterior to midpoint of body, exceeding half the body length ($SSD/TL = 61\text{--}66\%$).

Vent opening dexterous, tail musculature as long as the fin, fin tip rounded. The tail exceeds half the total length ($TAL/TL = 64\text{--}66\%$) and its maximum height is 15–16% of the total length, but 82–93% of the body height. Caudal myotomes visible, tail axis straight. Robust caudal musculature in the front part of the body, tapering gradually toward the end of the tail. The caudal musculature is half as wide and about one-third shorter than the body ($TMW/BW = 51\text{--}58\%$, $TMH/BH = 61\text{--}65\%$). The dorsal fin extends only as a slight ridge along the body at the body-tail junction and is narrower than the tail muscle. In the middle of the tail, the dorsal fin is slightly higher than the ventral fin. The ventral fin originates from the body and is narrower than the tail muscle. The point of origin of the hind legs is parallel to the cloacal tube.

The oral disc is emarginate, directed anteroventrally, surrounded by marginal papillae, (except in the middle of the anterior lip, $AL\text{-}gap/ODW = 53\text{--}68\%$) occupying just under half the width of the body ($ODW/BW = 43\text{--}48\%$). Papillae are abundant on both lips, where the right lateral margin of the anterior lip has 16–17 papillae/mm that are blunt and smaller than those of the posterior lip. On the posterior lip, the papillae in the central part are conical, elongated, and blunt (13–16 papillae/mm), while on the lateral margins they are conical and pointed. Dental formula 2(2)/3(1) with a very small A-2 gap ($A\text{-}2\text{ gap}/ODW = 3\text{--}7\%$). The first row of teeth of the posterior lip (P-1) is briefly interrupted (P-1 gap), while P-2 and P-3 are continuous. Rows P-1 and P-2 are of equal length, while P-3 is slightly shorter. The teeth in P-3 are slightly smaller and less keratinized than those in the other rows. The upper jaw sheath is half the width of the oral disc ($UJS/ODW = 48\text{--}52\%$) with thin, arch-shaped, medially

notched (W-shaped) lateral processes. On the other hand, the lower jaw sheath is narrower ($LJS/ODW = 37\text{--}39\%$), thinner, and broadly V-shaped; both with small, blunt striae.

The general morphology of the free-swimming tadpoles did not present abrupt changes between the different stages analyzed (Fig. 3a), although slight variations were observed in some characters of the configuration of the oral disc. In stages 25–38, only one row of papillae was observed on the anterior lip and the number of these varied in a range of 11–23 papillae/mm. On the posterior lip, a simple row, a biseriata row, and two rows of papillae were observed, with 11–21 papillae/

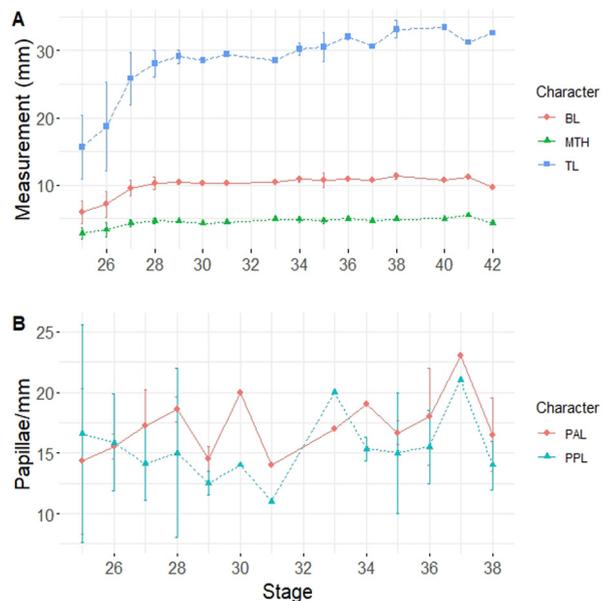


Fig. 3. Ontogenetic variation of body: some morphometric characters (a) and papillae per millimeter counts (b) through Gosner (1960) stages. BL = body length, MTH = maximum tail height, TL = tail length, PAL = number of papillae/mm on anterior lip, PPL = number of papillae/mm on posterior lip.

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mm (Fig. 3b). Regarding the length of the rows of teeth on the posterior lip, they could either all have the same length ($P1 = P2 = P3$) or two equal and one shorter ($P1 = P2 > P3$ or $P2 > P1 = P3$). Metamorphics of stages 42 ($n = 1$; TL = 18.2 mm, TAL = 12.5 mm, BW = 4.5 mm), 43 ($n = 2$, TL = 17.7 mm, TAL = 9.0 mm, BW = 4.0 mm) and 45 ($n = 1$, TL = 11.4 mm, BW = 4.2) showed reductions in total length, maximum tail height, and tail length. In addition, other structures such as the oral disc, keratinized ornamentation (UPJ, LJS, and tooth rows), blowhole, and cloacal tube disappear.

Tadpoles are generally uniformly dark-backed, although some specimens have minute golden spots toward the front of the body. Free-swimming tadpoles have a gold stripe toward the back of the eyes, which have tiny gold dots (Fig. 4a–b). Between the eyes and the tip

of the snout, golden spots are directed laterally, passing under the nostrils. The back of the body is dark brown with golden spots (in some cases evenly brown). The tail in lateral view presents dark brown spots, encompassing the dorsal and ventral fins. The caudal musculature is greenish yellow. In ventral view, it is whitish to greyish, and slightly translucent. When preserved, the body is light brown in dorsal view, but completely black in some cases. The golden stripe and spots disappear, being replaced by brown or black spots on the tail. In the distal region, the tail becomes translucent. Ventrally, the intestine is visible under the skin, showing a dark brown coloration.

The free-swimming tadpoles of “*C.*” *ruthveni* ss generally inhabit pools in small mountain lotic systems that are located in the shade under large trees and surrounded by medium-sized vegetation. These sites present benthic and hidden habitats. In pools with a lot of sun exposure, individuals hide under rocks, marginal vegetation, or the bottom leaf litter, or are partially buried in the sand. The tadpoles have an apparently omnivorous diet, since some individuals were found feeding on leaves and flowers that fell into the ponds as well as earthworm remains and a dead conspecific (Fig. 4c–d).

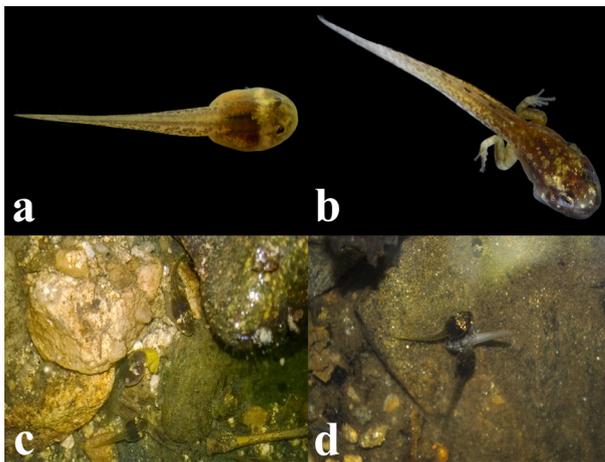


Fig. 4. Color in life of uncollected free-swimming tadpoles of “*Colostethus*” *ruthveni sensu stricto* in stage 28 (a) and 37 (b) from La Victoria; and records *in situ* of the consumption of plant material (c) and conspecific larvae (d). Photos by Andrés Camilo Montes-Correa (a), Juan Manuel Renjifo (b), and Fredy Polo-Córdoba (c, d).

Courtship and Reproductive Mode

The observations of four pairs involved in courtship in Las Tinajas and Pozo Azul were used to build a general scheme of courtship behavior that had been previously unknown (Fig. 5). It is worth mentioning that the courtship of “*C.*” *ruthveni* ss exhibits some slight variations among the partial observations made in this study. All the courtship events began with the issuance of the mating call by the male from various

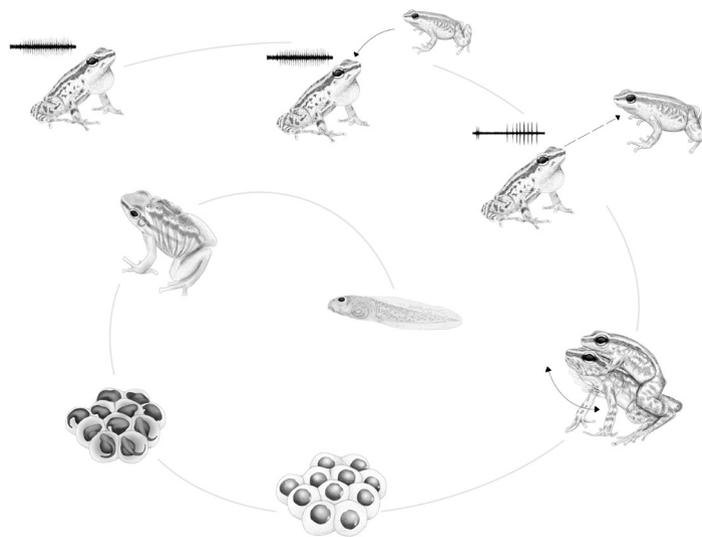


Fig. 5. Simplified schematic representation of courtship behavior and reproductive mode known up to now for “*Colostethus*” *ruthveni sensu stricto*, with the following steps: male issues advertisement call, female approaches, male issues courtship call, amplexus occurs from axilla to head, laying and development of terrestrial eggs, transport of larvae, and laying of free-swimming tadpoles in a pool or stream. Drawings by Jhuliyana Lopez-Caro.

substrates (e.g., cavities between the boulders, on fallen logs on the edge of the ravine, or on the broad leaves of grazing vegetation). The males darken in color during vocalization (color change). This results in positive phonotaxis by the females that approach the vocalization sites. In most of the observations, during the first phase of the close encounter between the male and the female, the male began to “circle,” circling the female with small jumps while she issued the mating call. Later, the male exhibited “body raising,” a raised posture stretching all four legs, followed by a “throat display” of inflating the vocal sac. In other cases, there were tactile interactions that included the stealthy approach of the male to the female, who then rubbed his forelimbs on her back, like a “massage.” This was followed by the emission of the courtship or advertisement call and cephalic amplexus, which always occurred in hidden spaces, such as the spaces between boulders or in cavities of fallen logs. On one occasion for a few seconds, pseudo positions or variants ranging from axillary to cephalic grasping were observed, ending in the latter. Only in one pair during cephalic amplexus was the male observed to make “toe trembling” behavior (see Supplementary Material 2: video).

The positions of nine egg masses were observed, five of which were deposited at the bases of the upper leaves (Fig. 6a) of low-growing plants hidden by the foliage. Four egg masses (spawn) were laid on leaves just above the water surface. The remaining spawn was on a plant 0.57 m horizontally from the water and 14 cm high. However, it is possible that this spawn was originally on the surface of the water, but probably moved away from it due to the shrinking volume of water in the creek. In

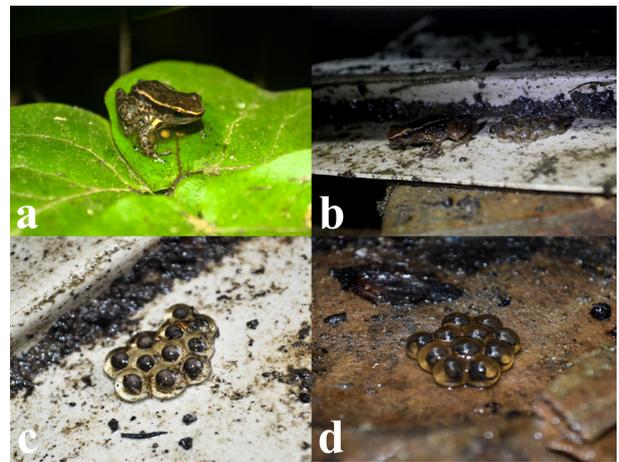


Fig. 6. Parental care and oviposition substrates of “*Colostethus*” *ruthveni sensu stricto*. Male attending to a posture on the upper side of a leaf (a) and under a plastic sheet (b). Detail of the posture on the plastic sheet (c) and leaf litter (d). Photos by Liliiana Saboyá-Acosta (a) and Fredy Polo-Córdoba (b, c, d).

three of these positions, parental care by the males was detected. In addition, the use of artificial substrates was identified (Fig. 6b–c), as egg masses were deposited in a pile of damp cardboard and plastic sheets surrounded by leaf litter, both about 50 m from the nearest watercourse. Parental care was observed in both sexes. In addition, two egg masses were found at ground level. One of them was deposited in a hole in a rock covered by leaf litter, approximately two horizontal meters from the watercourse. The other was deposited directly on wet leaf litter (Fig. 6d) about four meters from the ravine. In neither of the two cases was parental care observed, but males were vocalizing around the spawn. Finally, in Sierra Minca a spawn was oviposited directly on the

Table 4. Summary of features of the nests of “*Colostethus*” *ruthveni sensu stricto*. Egg diameter is expressed as range (average ± standard deviation).

Site and month	Nest location	Nest height (cm)	Nest water distance (m)	Substrata	Number of eggs	Egg diameter (mm)	Parental care
Las Tinajas, Jun 2015							
1	Above the water	47	0	Leaf base	15	3.2–5.2 (4.2±0.6)	X
2	Above the water	31	0	Leaf base	15	2.5–4.5 (3.9±0.4)	X
3	Above the water	25	0	Leaf base	7	4.4–6.9 (5.3±0.8)	X
4	Above the water	20	0	Leaf base	2	2.7–3.9 (3.3±0.8)	
5	On the floor	14	0.57	Leaf base	6	1.8–2.8 (2.4±0.3)	
Bella Vista, Aug 2015							
6	On the floor	0	50	Wet cardboard and leaf-litter	11	4.1–5.8 (4.5±0.5)	X
7	On the floor	0	53	Plastic lamina and leaf-litter	11	4.2–6.0 (4.9±0.6)	X
Arimaca, Dec 2016							
8	On the floor	0	2	Rock hole and leaf-litter	11	3.5–7.3 (5.5±1.0)	
9	On the floor	0	4	Leaf-litter	10	4.1–5.8 (4.7±0.6)	
Sierra Minca, Jan 2021							
24	On the floor	0	3	Rock	3	3.7–4.1 (3.9±0.2)	X

Reproductive ecology and larval morphology of “*Colostethus*” *ruthveni*

surface of a rock next to the creek flow. Although it was not possible for us to photograph this record, photographs taken by other researchers in the region that document it are available on social networks (https://www.instagram.com/p/BIBQrNqhLDI/?igshid=MzRlODBiNWFlZA%3D%3D&fbclid=IwAR1F3LUaS7Y9XIUhZOhQ6uqUbhK3cRYSkZ5FRjVNHbhncKp-m32OJ9n3y74&img_index=2). The data from the spawn of “*C.*” *ruthveni* ss reported in this study suggests that this species produces a small number of eggs (Table 4, range = 2–15 eggs; mean \pm standard deviation = 9.2 ± 4.4 eggs) that are small in size (1.8–7.3 mm, 4.4 ± 1).

Distribution of Larval and Reproductive Characters

New information was obtained for 12 of the 35 characters examined: 10 reproductive characters and two larval characters (Fig. 7, see Supplementary Material 1: Tables S2 and S3; and Supplementary Material 3). Under the hypothesis of the relationships of the Dendrobatoidea genera of Grant et al. (2017, Appendix 1), 27 characters

(17 larval, 10 reproductive) can be considered symplesiomorphic for the group “*C.*” *ruthveni*. The caudal coloration of the larvae is arranged in dispersed melanophores that are grouped in diffuse spots [93(0); a character number in Appendix 1 of Grant et al. (2017, state of character)], occurrence of reproductive amplexus [115(1)], and larval habitat in wells and streams [123(0)] can indicate reversions to ancestral states. Likewise, the presence of lateral line stitches [106(1), character examined by Grant et al. 2017] could be considered as an apomorphy. Two polymorphic characters were observed. In the case of the laying site, terrestrial clutches at ground level [118(1)] are a symplesiomorphic state, while terrestrial clutches above the ground or water level [118(3)] are an apomorphy. On the diet of exotrophic tadpoles, predatory habits [125(1)] consist of a symplesiomorphy, while detritivorous habits [125(2)] consist of a reversal.

Regarding the continuous character of A-2 gap/ODW (%), the ranges were so wide among most of the genera that it was not possible to classify them

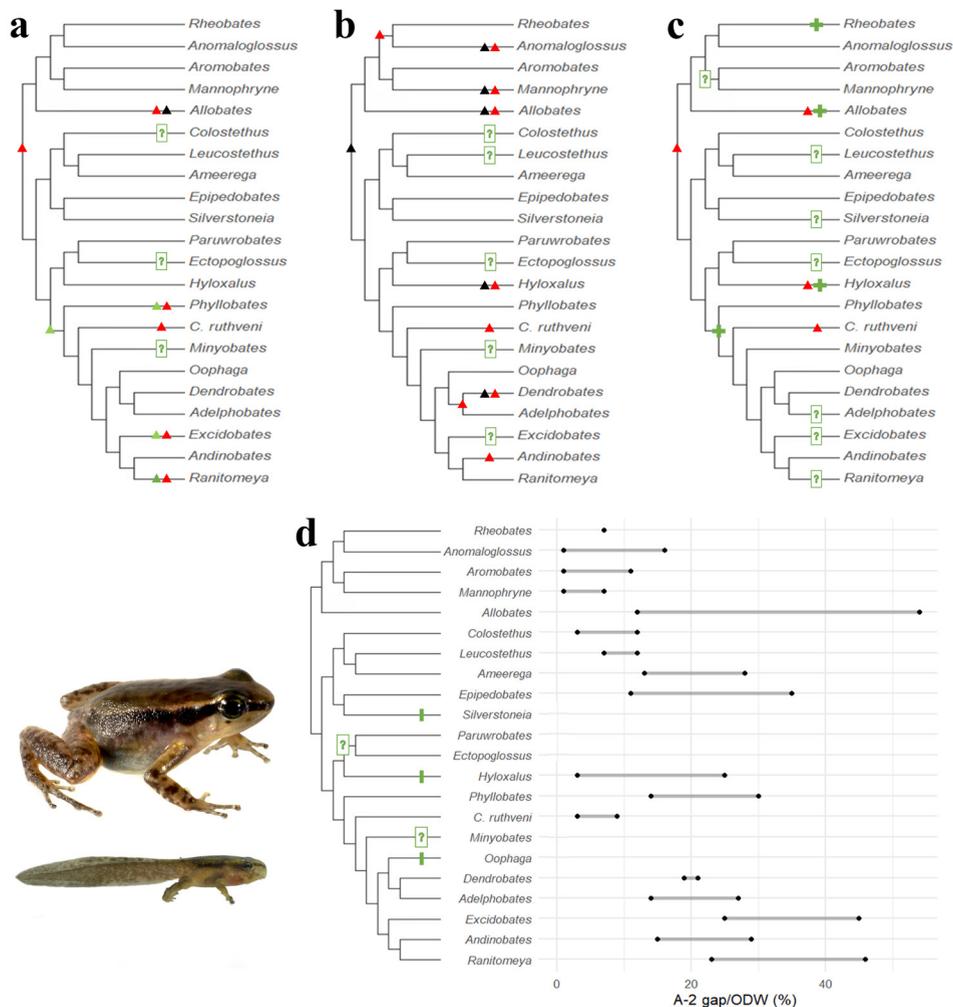


Fig. 7. Mapping of selected larval and reproductive character on the topology with summary of phylogenetic relationships of genera across Dendrobatoidea by Grant et al. 2017. 93. Larval caudal coloration (a): 0. vertically striped (\blacktriangle); 1. scattered melanophores clumped to form diffuse blotches (\blacktriangle); 2. evenly pigmented (\blacktriangle). 106. Lateral line stitches (b): 0. absent (\blacktriangle); 1. present (\blacktriangle). 116. Reproductive amplexus position (c): 0. absent (\blacktriangle); 1. present (\blacktriangle); not applicable ($+$). Range of A-2 gap/oral disc ratio in percentage (d). Adult male and metamorphic tadpole (stage 45) of “*Colostethus*” *ruthveni* ss from Minca, Santa Marta, Magdalena. Photographs are not scaled.

in a single category (see Supplementary Material 1: Table S4). Only the “C.” *ruthveni* group, *Rheobates*, and *Mannophryne* showed strictly narrow A-2 gaps. Likewise, the genera *Anomalogluss*, *Aromobates*, *Colostethus*, and *Leucostethus* had narrow to moderate A-2 gaps (in all cases the A-2 gap/ODW \leq 16%). Something similar occurred with *Hyloxalus*, where most of the species with the presence of an A-2 gap (absent in eight species), showed gaps falling between narrow and moderate (A-2 gap/ODW = 3–13%). The exception was *H. sauli*, which had a considerably wider A-2 gap (A-2 gap/ODW = 25%). The genera *Allobates*, *Amereega*, *Epipedobates*, *Phyllobates*, *Dendrobates*, *Adelphobates*, and *Andinobates* all had moderate to wide A-2 gaps. Only the genera *Excidobates* and *Ranitomeya* had strictly wide A-2 gaps. These findings indicated to us that “C.” *ruthveni* is the only Dendrobatinae with a narrow A-2 gap.

Discussion

Relative Abundance and Microhabitat Use

“*Colostethus*” *ruthveni* ss is an abundant species, at least during the periods and at the sites sampled in this study. Although these data do not allow us to assume that the relative abundance is constantly high, they suggest that the populations can remain very conspicuous, despite the marked water deficit in the lower range of the SNSM (< 1000 m asl) during the dry season (Fundación Pro-Sierra Nevada 1998). The variations of the relative abundance in each sampling event could be the product of multiple factors inherent to the biology of “C.” *ruthveni* ss, such as the occupancy and detection probabilities, as well as those related to the characteristics of the sampling, such as the capture effort and the accessibility of the microhabitats for the observer (MacKenzie et al. 2002). Regarding the latter, the localities where the greatest abundance was recorded (Sierra Minca, Central Córdoba, and Las Tinajas) have easily accessible ravines, gentler slopes, and longer walkable stretches, where observers would have fewer physical difficulties in finding specimens.

The apparently stable population status of “C.” *ruthveni* ss in the study area may be related to the fact that the northwestern sector of the SNSM is one of the best-preserved areas of the entire massif since it has had the least loss of natural vegetation cover in the past two decades (Granda-Rodríguez et al. 2020). Previous studies of the other endemic amphibians of the SNSM in the genera *Atelopus*, *Tachiramantis*, *Serranobatrachus*, *Geobatrachus*, and *Ikakogi* (Granda Rodríguez et al. 2012; Granda-Rodríguez et al. 2020; Martínez-Baños et al. 2011; Roach et al. 2021) have shown high relative abundances, despite the presence of chytridiomycosis in the region (Flechas et al. 2017). This contrasts with the general condition of the conservation status of amphibians in the North-Andean region, where multiple

population declines have been documented (Womack et al. 2022). Although measuring population attributes such as population density is necessary, we suggest calculating relative abundance as it is a cost-effective and comparable technique that provides at least one population attribute which can serve as an early warning in case of declines in amphibian populations. As in many parts of Latin America, population information has never been documented for many species, and currently, the population status of many amphibians in Colombia remains unknown (Young et al., 2001; IUCN SSC Amphibian Specialist Group, 2018).

Despite the apparent stability in the relative abundance of amphibians in the northwestern SNSM, multiple potential threats must be addressed. With the recent “Peace Process” in Colombia and the dismantling of some of the insurgent armed groups, areas that were previously protected indirectly by the Colombian armed conflict are now available for use. Due to the increase in tourism and agricultural activity in the area, demographic growth in the distributional area of “C.” *ruthveni* has recently accelerated (Carvajalino-Slaghekke 2015; Guardiola 2019). This leads to an increase in infrastructure and water demand, as well as environmental disturbances that, together with poor wastewater management, can synergistically affect the population status of this and other amphibian species in the area. Therefore, the implementation of environmental management measures and territorial planning in this region should be encouraged. Although, “C.” *ruthveni* is currently considered a Near Threatened species, the recent discovery of cryptic diversity within “C.” *ruthveni* (Grant et al. 2017; Jiménez-Bolaño et al. 2019) will generate substantial changes in the interpretation of the conservation status, because the area of distribution and threats will be fragmented according to the number of species that make up this group and how they are distributed throughout the SNSM.

The microhabitat use data obtained in this study show that the greatest activity of “C.” *ruthveni* occurs in the lowest strata of the forest, below approximately 50 cm. Like other dendrobatoids from northern Colombia, “C.” *ruthveni* finds shelter and food in microhabitats on the forest floor such as rocks and leaf litter (Blanco-Torres et al. 2013, 2014; Granda-Rodríguez et al. 2018; Posso-Peláez et al. 2017). In addition, all the processes involved in reproductive biology known up to now, such as singing, courtship, amplexus, laying site, and others, also occur at the ground level or in the lowest strata of the forest.

Free-swimming Tadpoles

The tadpoles of “C.” *ruthveni* have the typical morphology of the larvae that were in the genus *Colostethus sensu lato* (Anganoy-Criollo 2013; Coloma 1995), but are currently included in various genera of the families

Aromobatidae and Dendrobatidae (Colostethinae and Hyloxalinae). Several character states of external larval morphology, such as the UJS notch, sinister gut, and projecting nostril rim, are ancestral (Sánchez 2013), and symplesiomorphically shared with Aromobatidae, Colostethinae, Hyloxalinae, and *Phylllobates*. On the other hand, the narrow A-2 gap and the moderately sized nostrils could constitute putative synapomorphies of the “C.” group *ruthveni*, if they are verified to be also present in group members that are not yet described (*sensu* Grant et al. 2017; Jiménez-Bolaño et al. 2019).

The external morphology of the free-swimming tadpoles of “C.” *ruthveni* from La Victoria agrees with the character states described by Anganoy-Criollo (2013), Sánchez (2013), and Anganoy-Criollo and Cepeda-Quilindo (2017). However, part of the material examined and determined by Sánchez (2013) does not correspond to “C.” *ruthveni* ss. One batch of specimens (ICN 35773) came from the southwestern sector of the SNSM, where “C.” sp. *ruthveni*-like is distributed (Grant et al. 2017; Jiménez-Bolaño et al. 2019). In addition, the ICN 35779 batch of specimens came from the Serranía de Perijá, a region part of the Andean mountain range that is not connected to SNSM (Granda-Rodríguez et al. 2014; Jiménez-Bolaño et al. 2019; Meza-Joya et al. 2019). These tadpoles may correspond to two aromobatids from the western foothills of the Serranía de Perijá, i.e., *Rheobates palmatus* Werner, 1899 or *Allobates ignotus* Anganoy-Criollo, 2012 (Anganoy-Criollo 2012; Granda-Rodríguez et al. 2018; Moreno-Arias et al. 2009; Romero-Martínez and Lynch 2012).

The states of some tadpole characters of “C.” *ruthveni* determined in this study showed strong variation with respect to the data reported by Ruthven and Gaige (1915). The illustrations provided in that study of back-riding and free-swimming tadpoles show the A-2 gap to be very wide (i.e., back-riding tadpole: A-2 gap/ODW = 30%, free-swimming tadpole: A-2 gap/ODW = 25%, calculated qualitatively from Ruthven and Gaige (1915, Figs. 1 and 3)). Furthermore, referring to the same free-swimming tadpole with a 20 mm TL, they suggested that “the upper second row of teeth is not always interrupted.” Although it was not possible to determine the larval stage (according to the tadpoles illustrated by them), our larvae of similar size (Stage 26, n = 13, TL = 16.7–23.3 mm) have a dramatically reduced A-2 gap on average (A-2 gap/ODW = 5.1%), but it is never absent. One possibility is that the marked differences in the A-2 gap ratio of Ruthven and Gaige (1915) tadpoles with respect to the recent literature (Anganoy-Criollo and Cepeda-Quilindo 2017) is due to ontogenic variation; however, this variation would far exceed that detected in our material, since the highest A-2 gap/ODW ratio was <8% at stage 26

Another contrasting detail of the Ruthven and Gaige (1915) illustrations is that they suggest the UJS notch is absent, a condition contrary to the reports of recent

studies (UJS notch present, UJS “W-shaped;” Sánchez 2013; Anganoy-Criollo and Cepeda-Quilindo 2017). The presence of the UJS notch is a character with little variation, and it is easily distinguishable in free-swimming tadpoles (from stage 25; Sánchez 2013; MA Anganoy-Criollo, pers. comm.). Thus, the A-2 gap/ODW ratio and the absence of the UJS notch in the free-swimming tadpole (20 mm TL) illustrated by Ruthven and Gaige (1915) could be considered as atypical character states considering the recent evidence.

Courtship and Reproductive Mode

Like other reproductive behavior traits, courtship is undoubtedly one of the most conspicuous and fascinating features of dendrobatoids (Wells 2007; Summers and Tumulty 2014). Thus, the partial observations on courtship in “C.” *ruthveni* are similar to those reported in other dendrobatoid species such as *Mannophryne trinitatis* Garman, 1888, *Mannophryne collaris* Boulenger, 1912, *Anomaloglossus stepheni* Martins, 1989, *Allobates marchesianus* Melin, 1941, *Allobates femoralis* Boulenger, 1884, *Allobates paleovarzensis* Lima, Caldwell, Biavati, and Montanarin, 2010, *Allobates velocicantus* Souza, Ferrão, Hanken, and Lima, 2020, *R. palmatus*, *Colostethus panamansis* Dunn, 1933, *Hyloxalus toachi* Coloma, 1995, *Dendrobates auratus* Girard, 1855, and *Dendrobates tinctorius* Cuvier, 1797, where behaviors such as color changes, body raising, circling, throat displays, and tactile interactions have been observed (Coloma and Quiguango-Ubillús 2008; Dole and Durant 1974; Duellman 1966; Hödl and Amézquita 2001; Juncá 1998; Juncá and Rodrigues 2006; Lima et al. 2010; Lüddecke 1976; Montanarin et al. 2011; Pašukonis and Rojas 2019; Rocha et al. 2018; Rojas and Pašukonis 2019; Wells 1978, 1980a,b). The tactile interactions of “C.” *ruthveni* are similar to those described in *A. stepheni*, *Allobates subfolionidificans* Lima, Sánchez, and Souza 2007, and *D. tinctorius*, where the male approaches the female and places his front legs on her back, perhaps a stimulant prior to oviposition (Juncá and Rodrigues 2006; Rojas and Pašukonis 2019; Souza et al. 2017).

Of all the possible courtship variations within the Dendrobatoidea clade, some behaviors contrast with our observations. For example, something very particular that differs notably from the behavior of “C.” *ruthveni* ss is the upright posture on the hind legs observed during courtship only in *M. collaris*, which the authors called a “toe-dance” (Dole and Durant 1974). Likewise, in aposematic species such as *Ameerega braccata* Steindachner, 1864, *Ameerega flavopicta* Lutz, 1925, and *Oophaga sylvatica* Funkhouser, 1956, courtship is accompanied by the display of conspicuous coloration on the hidden surfaces through visual cues involving the movement of the limbs (Costa et al. 2006; Forti et al. 2013; Summers 1992). “Toe-trembling” is a very

common visual signal in anurans (Sloggett and Zeilstra 2008). However, exhibiting this signal in “*C.*” *ruthveni* ss during amplexus differs from that reported in species such as *Oophaga histrionica* Berthold, 1845, *D. auratus*, and *D. tinctorius*, where it has been observed during courtship, foraging, and agonistic interactions (Rojas and Pašukonis 2019; Silverstone 1973; Wells 1978). All of this variation in the courtship behavioral displays raises two questions that could be addressed from an evolutionary perspective in future research: Does aposematism have a direct relationship with the use of visual, acoustic, or tactile displays? Does aposematism somehow favor greater diversity of displays during courtship? The answers to these questions could provide an interesting evolutionary context, considering that most aposematic groups have a cryptic ancestor, whereas so far, the “*C.*” *ruthveni* group is the only cryptic coloration lineage with an aposematic ancestor.

Cephalic amplexus is one of the most striking characteristics of dendrobatoid courtship. It is present in at least 22 species and is strongly associated with terrestrial habits, representing 6.5% of the known diversity of the clade (Carvajal-Castro et al. 2020; Frost 2023). This behavior is quite complex, and the establishment of its homology depends largely on the reproductive context, where certain pre-ovipositional variants are not part of the cephalic grasp in a strict sense (Castillo-Trenn and Coloma 2008). In accordance with the above observations, “*C.*” *ruthveni* ss exhibits various forms of grasping in the nuptial embrace, resulting in intermediate points between the axillary position and the final cephalic position. This is similar to observations in *A. flavopicta*, where the axillary amplexus was initially reported (Costa et al. 2006). However, later observations determined intraspecific variation in the position of the embrace, with intermediate positions between axillary and cephalic amplexus, with the latter being the predominant variation (Magrini et al. 2010). However, this differs from what was reported in *Hyloxalus azureiventris* (Kneller and Henle 1985) and some cryptic species that were part of the extensive group *Colostethus sensu lato*, such as *Allobates caeruleodactylus* Lima and Caldwell, 2001, and *A. subfolionidificans*, in which this type of amplexus has not been recorded (Lima et al. 2002; Quiguango-Ubillús and Coloma 2008; Souza et al. 2017).

Several authors have considered parental care and larval transport as reproductive mode traits (Crump 2015; Duellman and Trueb 1994; Haddad and Prado 2005; Wells 2007). However, in their redefinition of the reproductive mode, Nunes-De-Almeida et al. (2021) excluded parental care as it is a difficult characteristic to identify, except for those cases where care involves feeding and incubation. This is interesting when compared with our observations. Considering the proposal of Haddad and Prado (2005), the annotations for the transport of larvae made by Ruthven and Gaige (1915) complemented with the masses of eggs at ground

level found in “*C.*” *ruthveni* ss in localities of the SNSM coincide with Mode 20 (eggs that hatch into exotrophic tadpoles that are transported to the water by the adult), which agrees with that exhibited by *C. panamansis* and other dendrobatoids of the *Ameerega*, *Silverstoneia*, *Epipedobates*, *Hyloxalus*, and *Allobates* genera (Wells 1980b; Lima et al. 2010; Summers and Tumulty 2014; Crump 2015).

Regarding the spawn positions above the ground level found for “*C.*” *ruthveni* in the Las Tinajas, these are similar to previous observations in *Allobates brunneus* (Cope, 1887), *Ameerega bilinguis* (Jungfer, 1989), *Ameerega hahneli* (Boulenger, 1884), *Allobates carajas* Simões, Rojas, and Lima, 2019, *Leucostethus fraterdanieli* (Silverstone, 1971), and *A. subfolionidificans* (Beirne and Whitworth 2011; Brown et al. 2019; Lima et al. 2009; Rojas-Morales et al. 2021; Simões et al. 2019). However, *A. subfolionidificans* differs from the remaining species because it deposits its eggs on the undersides of leaves (Lima et al. 2007; Souza et al. 2017). Unlike the species mentioned, the transport of larvae by “*C.*” *ruthveni* in this area was not observed, so we have elaborated two hypotheses to explain this difference: a) the transport and deposition of larvae in the pond is done immediately after hatching, and/or b) there is a mode without the transport of larvae in which they hatch and fall directly into the water (Mode 24, Haddad and Prado 2005). The latter hypothesis and the location of egg masses of “*C.*” *ruthveni* at and above ground level suggest some degree of phenotypic plasticity in the reproductive mode, as has been shown in the species *Dendropsophus ebraccatus* Cope, 1874 (Hylidae), which alternates between aquatic and terrestrial locations (Touchon and Warkentin 2008). The “*C.*” *ruthveni* ss egg masses in vegetation above ground level, as well as those deposited at ground level, correspond to modes 22 and 32 (Nunes-De-Almeida et al. 2021). Curiously, there is no reproductive mode in the scheme of Nunes-De-Almeida et al. (2021) that includes clutches deposited on rocky substrates that derive from tadpoles (indirect development) from exotrophic nutrition and lotic habitats. So far, only rocks have been documented as the ovipositing substrates in salamanders that produce direct developing offspring (Mode 35). Therefore, we propose an additional reproductive mode that continues the list of Nunes-De-Almeida et al. (2021): *Mode “75”: Terrestrial non-froth eggs laid on rock. Offspring with indirect development, lecithotrophic nutrition, exotrophic, and without parental feeding. Known only for anurans.* This reproductive mode variation is probably the product of phenotypic plasticity in this species against environmental conditions, although this must be tested experimentally both *in situ* and *ex situ* (Barboza 2014).

Distribution of Larval and Reproductive Characters

The hypothesis regarding the phylogenetic relationships

of the group “*C.*” *ruthveni* by Grant et al. (2017) could be considered a surprising result, since phylogenetic logistic models indicate that the probability of gaining the ability to sequester alkaloids or going from a cryptic to aposematic phenotype is considerably higher than that of a reversion (Santos et al. 2014). In fact, a study recently determined that group “*C.*” *ruthveni* lacks the ability to sequester epibatidines, the most widely distributed alkaloid of dendrobatoid frogs (Waters et al. 2023). However, this hypothesis has the robustness conferred by the Goodman-Bremer values and the YBIRÁ procedure that tests the stability of nodes through the removal of clades or “wildcard” terminals (Grant and Kluge 2008; Machado 2015). While our objective is not evaluating the plausibility the phylogenetic relationships of group “*C.*” *ruthveni* proposed by Grant et al. (2017), according to the new evidence, we believe that the sampling and refinement of certain characters could provide greater clarity about their relationships. First, how does it affect the analysis that “*C.*” *ruthveni* does not present the only phenotypic synapomorphy of the subfamily Dendrobatinae [160(1); the ability to sequester lipophilic alkaloids present]? However, we detected that at least 27 characters (Supplementary Table 5) were declared as a mixture of neomorphic and transformational characters. This character set includes the three synapomorphies of the clade group “*C.*” *ruthveni* + Dendrobatini (characters 71, 72, and 156). Although the declarations of these characters undoubtedly follow a logical sequence (Serenó 2007), examining the appearance/loss and the transformation of the states in the same character is inappropriate because that would violate the precept of the independence of characters. Including these considerations and the additional characters presented here could at best reinforce the Grant et al. (2017) hypothesis, or perhaps provide a different perspective of the relationships of the group “*C.*” *ruthveni* and even Dendrobatoidea.

Considering these aspects of Grant et al. (2017) on the relationships of Dendrobatoidea, this is the most comprehensive and complete approach to the topic, and we will base our hypotheses on the evolution of larval and reproductive characters on this approach. In general, the larval morphology of Dendrobatoidea is conservative, where there is a generalized ancestral morphological pattern symplesiomorphically shared by Aromobatidae, Colostethinae, Hyloxalinae, and some members of Dendrobatinae such as *Phyllobates* and the group “*C.*” *ruthveni* (Sánchez 2013). Compiling the data obtained by Sánchez (2013), Grant et al. (2017), and this study, this ancestral body plan can be characterized by the caudal coloration of the tadpole with scattered melanophores clumped to form diffuse blotches [93(1)], the presence of a notch in the upper jaw sheath [103(0)], the cloacal tube in dextral position [104 (0)], the long gut concealing other organs [107 (0)], and the presence of a projection of the external sagittal edge of the nostril [108 (1)].

Although the larval characters of “*C.*” *ruthveni* mostly correspond to a compendium of symplesiomorphies, some provide an interesting perspective. For example, the caudal coloration in “*C.*” *ruthveni* as clusters of melanophores forming diffuse spots [93(1)] corresponds to the loss of an acquired novelty in the Dendrobatinae subfamily clade [93(2)]. Both this reversal and the presence of lateral line stitches [106 (1)] could constitute a synapomorphy of the “*C.*” *ruthveni* if they are shown to be present in the undescribed members of the complex. Likewise, the presence of lateral line stitches could constitute a synapomorphy of the clade group “*C.*” *ruthveni* + Dendrobatini, if the presence of this character state is demonstrated in *Minyobates steyermarki* (Rivero, 1971). The A-2gap/ODW ratio also proved to be valuable for the diagnosis of the genera, despite the strong intrageneric variation detected. Intergeneric variation in A-2 gap width in the phylogenetic context of Grant et al. (2017) suggests that narrow to moderate gaps could be the ancestral condition and that moderate to wide gaps evolved independently in *Allobates*, *Amerega*, *Epipedobates*, a species of *Hyloxalus*, and the subfamily Dendrobatinae. In that sense, the presence of a narrow A-2 gap in “*C.*” *ruthveni* could be a reversal, and a synapomorphy in the case that its presence is verified in the other members of the group.

Of the reproductive characters, the occurrence of amplexus [115(1)] and of cephalic grasping [116(1)] are quite informative. For decades, cephalic amplexus was a dendrobatoid synapomorphy with multiple subsequent losses (Myers et al. 1991). However, from total evidence-based phylogenies, Grant et al. (2006) noted that cephalic amplexus has arisen in three independent lineages within the clade (*Anomaloglossus beebei* Noble 1923, Colostethinae, and *M. steyermarki*). Nevertheless, the occurrence of this character is known to be much more widely distributed throughout the Dendrobatoidea, being present in some *Anomaloglossus*, *Allobates*, *Colostethinae*, *Paruwrobates*, *Hyloxalus* and the group “*C.*” *ruthveni* (Bourne et al. 2001; Carvajal-Castro et al. 2020; Castillo-Trenn and Coloma 2008; Forti et al. 2013; Grant and Castro 1998; Grant et al. 2017 [see Supplementary Material S4]; Jungfer 1989; Juncá 1998; Lima et al. 2010; Magrini et al. 2010; Montanarin et al. 2011; Myers and Burrowes 1987; Myers et al. 1978; Quiguango-Ubillús and Coloma 2008; Rocha et al. 2018; Roithmair 1994; Souza et al. 2020; Wells 1980c). So far, this is the only member of the subfamily Dendrobatinae that still retains some form of amplexus, as the observations of Myers (1987) on *M. steyermarki* may be related to aggressive behavior, i.e., possible male-fighting (López-López et al. 2016), and for this reason it must be verified (Castillo-Trenn and Coloma 2008). All the above observations suggest that the larval and reproductive characters are a good source of information for understanding the relationships between the genera of Dendrobatoidea, and also that some characteristics

such as free-swimming tadpoles with a lateral line and a narrow A-2 gap, as well as the occurrence of cephalic amplexus, are useful for diagnosing the group “C.” *ruthveni*.

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Literature Cited

- Almendáriz CA, Ron SR, Brito MJ. 2012. Una especie nueva de rana venenosa de altura del género *Excidobates* (Dendrobatoidea: Dendrobatidae) de la Cordillera del Cóndor. *Papéis Avulsos de Zoología* 52(32): 387–399.
- Altig R, McDiarmid RW. 1999. Body plan, development, and morphology. Pp. 24–51 In: *Tadpoles. The Biology of Anuran Larvae*. Editors, McDiarmid RW, Altig R. University of Chicago Press, Chicago, Illinois, USA. 454 p.
- Anganoy-Criollo M. 2012. A new species of *Allobates* (Anura, Dendrobatidae) from the western flank of the Serranía de Perijá, Colombia. *Zootaxa* 3308(1): 49–62.
- Anganoy-Criollo M. 2013. Tadpoles of the High-Andean *Hyloxalus subpunctatus* (Anura: Dendrobatidae) with description of larval variation and species distinction by larval morphology. *Papeis Avulsos de Zoología* 53(15): 211–224.
- Anganoy-Criollo M, Cepeda-Quilindo B. 2017. Redescription of the tadpoles of *Epipedobates narinensis* and *E. boulengeri* (Anura: Dendrobatidae). *Phyllomedusa* 16(2): 155–182.
- Barboza FR. 2014. Retomando el concepto de plasticidad fenotípica en el estudio de los modos reproductivos de anfibios anuros. *Boletín de la Sociedad Zoológica del Uruguay* 23(1): 1–14.
- Beirne C, Whitworth A. 2011. *Frogs of the Yachana Reserve*. Global Vision International, Exeter, United Kingdom. 109 p.
- Blanco-Torres A, Navarro-Gutiérrez K, Gutiérrez-Moreno LC. 2013. *Colostethus inguinalis* (Common Rocket Frog) diet. *Herpetological Review* 44(3): 493–494.
- Blanco-Torres A, Ordóñez-Pachón MP, Franco-Rozo MC. 2014. *Colostethus ruthveni* (Santa Marta poison arrow frog) diet. *Herpetological Review* 45(3): 476.
- Bourne GR, Collins AC, Holder AM, McCarthy CL. 2001. Vocal communication and reproductive behavior of the frog *Colostethus beebei* in Guyana. *Journal of Herpetology* 35(2): 272–281.
- Breder CM. 1946. Amphibians and reptiles of the Rio Chucunaque drainage, Darien, Panama, with notes on their life histories and habitats. *Bulletin of the American Museum of Natural History* 86(8): 375–346.
- Brown JL, Siu-Ting K, Von May R, Twomey E, Guillory WX, Deutsch MS, Chávez G. 2019. Systematics of the *Ameerega rubriventris* complex (Anura: Dendrobatidae) with descriptions of two new cryptic species from the East-Andean versant of Peru. *Zootaxa* 4712(2): 211–235.
- Brown JL, Twomey E, Morales V, Summers K. 2008. Phytotelm size in relation to parental care and mating strategies in two species of Peruvian poison frogs. *Behaviour* 145(9): 1,139–1,165.
- Caldwell JP. 2005. A new Amazonian species of *Cryptophyllobates* (Anura: Dendrobatidae). *Herpetologica* 61(4): 449–461.
- Caldwell JP, Myers CW. 1990. A new poison frog from Amazonian Brazil, with further revision of the *quinquevittatus* group of *Dendrobates*. *American Museum Novitates* 2988: 1–21.
- Caldwell JP, Lima AP. 2003. A new amazonian species of *Colostethus* (Anura: Dendrobatidae) with a nidicolous tadpole. *Herpetologica* 59(2): 219–234.
- Caldwell JP, Lima AP, Biavati GM. 2002. Descriptions of tadpoles of *Colostethus marchesianus* and *Colostethus caeruleodactylus* (Anura: Dendrobatidae) from their type localities. *Copeia* 2002(1): 166–172.
- Carvajal-Castro JD, López-Aguirre Y, Ospina-L AM, Santos JC, Rojas B, Vargas-Salinas F. 2020. Much more than a clasp: evolutionary patterns of amplexus diversity in anurans. *Biological Journal of the Linnean Society* 129(3): 652–663.
- Carvajalino-Slaghekke A. 2015. Territorio e identidad en la Sierra Nevada de Santa Marta (Colombia) Territory. *Revista Latina de Sociología* 8(3): 94–112.
- Castillo-Trenn P. 2004. Description of the tadpole of *Colostethus kingsburyi* (Anura: Dendrobatidae) from Ecuador. *Journal of Herpetology* 38(4): 600–606.
- Castillo-Trenn P, Coloma LA. 2008. Notes on behavior and reproduction in captive *Allobates kingsburyi* (Anura: Dendrobatidae), with comments on the evolution of reproductive amplexus. *International Zoo Yearbook* 42(1): 58–70.

Reproductive ecology and larval morphology of “*Colostethus*” *ruthveni*

- Coloma LA. 1995. Ecuadorian frogs of the genus *Colostethus* (Anura: Dendrobatidae). *The University of Kansas Natural History Museum Miscellaneous Publications* 87: 1–72.
- Costa RC, Facure KG, Giaretta AA. 2006. Courtship, vocalization, and tadpole description of *Epipedobates flavopictus* (Anura: Dendrobatidae) in southern Goiás, Brazil. *Biota Neotropica* 6(1): [no pagination].
- Crump ML. 2015. Anuran reproductive modes: evolving perspectives. *Journal of Herpetology* 49(1): 1–16.
- Crump ML, Scott NJJ. 1994. Visual encounter survey. Pp 84–91 In: *Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians*. Editors, Heyer WR, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 384 p.
- Dias PHDS, Anganoy-Criollo M, Guayasamin JM, Grant T. 2018a. The tadpole of *Epipedobates darwinwallacei* Cisneros-Heredia and Yáñez-Muñoz, 2011 (Dendrobatidae: Colostethinae), with new synapomorphies for *Epipedobates*. *South American Journal of Herpetology* 13(1): 54–63.
- Dias PHDS, Brandão AP, Grant T. 2018b. The buccopharyngeal morphology of the tadpole of *Ameerega flavopicta* (Anura: Dendrobatidae: Colostethinae), with a redescription of its external morphology. *Herpetologica* 74(4): 323–328.
- Dias PHDS, Anganoy-Criollo M, Rada M, Grant T. 2021. The tadpoles of the funnel-mouthed dendrobatids (Anura: Dendrobatidae: Colostethinae: *Silverstoneia*): external morphology, musculoskeletal anatomy, buccopharyngeal cavity, and new synapomorphies. *Journal of Zoological Systematics and Evolutionary Research* 59(2): 691–717.
- Dole JW, Durant P. 1974. Courtship behavior in *Colostethus collaris* (Dendrobatidae). *Copeia* 1974(4): 988–990.
- Donnelly MA, Guyer C, de Sá RO. 1990. The tadpole of a dart-poison frog *Phylllobates lugubris* (Anura: Dendrobatidae). *Proceedings of the Biological Society of Washington* 103(2): 427–431.
- Duarte-Marín S, González-Acosta CC, Dias PHDS, Arias-Álvarez GA, Vargas-Salinas F. 2020. Advertisement call, tadpole morphology, and other natural history aspects of the threatened poison frog *Andinobates daleswansoni* (Dendrobatidae). *Journal of Natural History* 54(45–46): 3,005–3,030.
- Duellman WE. 1966. Aggressive behavior in dendrobatid frogs. *Herpetologica* 22(3): 217–221.
- Duellman WE. 2004. Frogs of the genus *Colostethus* (Anura: Dendrobatidae) in the Andes of Northern Peru. *Scientific Papers - Natural History Museum, The University of Kansas* 35: 1–49.
- Duellman WE, Trueb L. 1994. *Biology of Amphibians*. Johns Hopkins University Press, Baltimore, Maryland, USA. 670 p.
- Edwards SR. 1971. Taxonomic notes on South American *Colostethus* with descriptions of two new species (Amphibia, Dendrobatidae). *Proceedings of the Biological Society of Washington* 84(18): 147–162.
- Edwards SR. 1974. Taxonomic notes on South American dendrobatid frogs of the genus *Colostethus*. *Occasional Papers of the Museum of Natural History, The University of Kansas*. 30: 1–14.
- Flechas SV, Paz A, Crawford AJ, Sarmiento C, Acevedo A, Arboleda A, Bolívar-García W, Echeverry-Sandoval CL, Franco R, Mojica C, et al. 2017. Current and predicted distribution of the pathogenic fungus *Batrachochytrium dendrobatidis* in Colombia, a hotspot of amphibian biodiversity. *Biotropica* 49(5): 685–694.
- Forti LR, Mott T, Strüssmann C. 2013. Breeding biology of *Ameerega braccata* (Steindachner, 1864) (Anura: Dendrobatidae) in the Cerrado of Brazil. *Journal of Natural History* 47(35–36): 2,363–2,371.
- Fouquet A, Vacher JP, Courtois EA, Deschamps C, Ouboter P, Jairam R, Gaucher P, Dubois A, Kok PJR. 2019. A new species of *Anomaloglossus* (Anura: Aromobatidae) of the *stepheni* group with the redescription of *A. baeobatrachus* (Boistel and de Massary, 1999), and an amended definition of *A. leopardus* Ouboter and Jairam, 2012. *Zootaxa* 4576(3): 439–460.
- French CM, Burkette C, Reichle S, Brown JL. 2019. The tadpole of *Ameerega boehmei* in southeastern Bolivia. *Zootaxa* 4661(1): 197–200.
- Frost D. 2023. Amphibian species of the world: an online reference. Version 6.2. American Museum of Natural History, New York, USA. Available: <https://amphibiansoftheworld.amnh.org/index.php> [Accessed: 5 October 2023].
- Fundación Pro-Sierra Nevada. 1998. *Evaluación Ecológica Rápida. Definición de Áreas Críticas para la Conservación en la Sierra Nevada de Santa Marta, Colombia*. Editores, Fundación Pro-Sierra Nevada de Santa Marta, Ministerio del Ambiente - Unidad Administrativa Especial del Sistema de Parques Nacionales Naturales (UAESPNN), The Nature Conservancy, USAID, Embajada de Japón. Santa Marta, Magdalena, Colombia. 134 p.
- Funkhouser JW. 1956. New frogs from Ecuador and southwestern Colombia. *Zoologica: Scientific Contributions of the New York Zoological Society* 41(9): 73–80.
- González-Maya JF, González M, Zárrate-Charry D, Charry F, Cepeda AA, Balaguera-Reina SA. 2011. A new population record and conservation assessment of the Santa Marta Poison Arrow Frog *Colostethus ruthveni* Kaplan, 1997 (Anura: Dendrobatidae) from Sierra Nevada de Santa Marta, Colombia. *Journal of Threatened Taxa* 3(3): 1,633–1,636.
- Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16(3): 183–190.

- Granda-Rodríguez HD, Mozo A, Renjifo JM, Bolaños F. 2012. ¿Están declinando todos los *Atelopus* de alta montaña? El caso de las ranas arlequín de la Sierra Nevada de Santa Marta, Colombia. *Herpetotropicos* 7: 21–30.
- Granda-Rodríguez HD, Saboyá-Acosta LP, del Portillo-Mozo A, Renjifo JM. 2014. Range extension of dendrobatid frog *Colostethus ruthveni* Kaplan, 1997 (Anura: Dendrobatidae) in the Sierra Nevada de Santa Marta, Colombia. *Check List* 10(3): 674–676.
- Granda-Rodríguez HD, Montes-Correa AC, Jiménez-Bolaño JD, Anganoy-Criollo M. 2018. Natural history and conservation of the Nurse Frog of the Serranía del Perijá *Allobates ignotus* (Dendrobatoidea: Aromobatidae) in northeastern Colombia. *Acta Herpetologica* 13(1): 51–64.
- Granda-Rodríguez HD, Montes-Correa AC, Jiménez-Bolaño JD, Alaniz AJ, Cattán PE, Hernández P. 2021. Insights into the natural history of the endemic Harlequin Toad, *Atelopus laetissimus* Ruiz-Carranza, Ardila-Robayo, and Hernández-Camacho, 1994 (Anura: Bufonidae), in the Sierra Nevada de Santa Marta, Colombia. *Amphibian & Reptile Conservation* 14(1) [General Section]: 29–42 (e221).
- Grant T. 1998. Una nueva especie de *Colostethus* del grupo *edwardsi* de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 22(84): 423–428.
- Grant T, Castro F. 1998. The cloud forest *Colostethus* (Anura, Dendrobatidae) of a region of the Cordillera Occidental of Colombia. *Journal of Herpetology* 32(3): 378–392.
- Grant T, Kluge AG. 2008. Clade support measures and their adequacy. *Cladistics* 24(6): 1,051–1,064.
- Grant T, Frost DR, Caldwell JP, Gagliardo R, Haddad CFB, Kok PJR, Means DB, Noonan BP, Schargel WE, Wheeler WC. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History* 299: 1–262.
- Grant T, Acosta A, Rada M. 2007. A name for the species of *Allobates* (Anura: Dendrobatoidea: Aromobatidae) from the Magdalena Valley of Colombia. *Copeia* 2007(4): 844–854.
- Grant T, Rada M, Anganoy-Criollo M, Batista A, Dias PH, Jeckel AM, Machado DJ, Rueda-Almonacid JV. 2017. Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology* 12(s1): S1–S90.
- Guardiola ADR. 2019. Potencial turístico de Santa Marta (Colombia): Una revisión de su desarrollo, crecimiento y barreras. *Espacios* 40(6): 22.
- Haddad CFB, Prado CPA. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience* 55(3): 207–217.
- Hills RL, Kaylock JB, Gagliardo RW, Timpe EK, Griffith E, Ross H. 2011. Observations on the captive reproduction of *Colostethus pratti* (Boulenger, 1899). *Herpetological Review* 42(3): 365–367.
- Hödl W, Amézquita A. 2001. Visual signaling in anuran amphibians. Pp. 121–141 In: *Anuran Communication*. Editor, Ryan MJ. Smithsonian Institution Press, Washington, DC, USA. 252 p.
- Inger RF. 1994. Distribution of microhabitat. Pp. 60–66 In: *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians*. Editors, Heyer RW, Donnelly MA, McDiarmid RW, Hayek LA, Foster MS. Smithsonian Institution Press, Washington, DC, USA. 384 p.
- IUCN SSC Amphibian Specialist Group. 2018. *Colostethus ruthveni*. Available: <https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T55144A85891000.en> [Accessed: 5 May 2023].
- Jiménez-Bolaño JD, Montes-Correa AC, Polo-Córdoba F, Linares-Vargas K, Vergara-Gil D, Barrio-Amorós CL, Koch C. 2019. Acoustic repertory of the “*Colostethus*” *ruthveni* group (Anura: Dendrobatidae) and comments on their distribution in the Sierra Nevada de Santa Marta, Colombia. *Salamandra* 55(1): 27–38.
- Johnson DH. 1980. The comparison of usage and availability measurements for evaluating resource preference. *Ecology* 61(1): 65–71.
- Juncá FA. 1998. Reproductive biology of *Colostethus stepheni* and *Colostethus marchesianus* (Dendrobatidae), with the description of a new anuran mating behavior. *Herpetologica* 54(3): 377–387.
- Juncá FA, Rodrigues M. 2006. The reproductive success of *Colostethus stepheni* (Anura: Dendrobatidae). *Studies on Neotropical Fauna and Environment* 41(1): 9–17.
- Juncá FA, Altig R, Gascon C. 1994. Breeding biology of *Colostethus stepheni*, a dendrobatid frog with a nontransported nidicolous tadpole. *Copeia* 1994(3): 747–750.
- Jungfer K. 1989. Pfeilgiftfrösche der Gattung *Epipedobates* mit rot granuliertem Rücken aus dem Oriente von Ecuador und Peru. *Salamandra* 25(2): 81–98.
- Kaplan M. 1997. A new species of *Colostethus* from the Sierra Nevada de Santa Marta (Colombia) with comments on intergeneric relationships within the Dendrobatidae. *Journal of Herpetology* 31(3): 369–375.
- Klein B, Regnet RA, Krings M, Rödder D. 2020. Larval development and morphology of six Neotropical poison-dart frogs of the genus *Ranitomeya* (Anura: Dendrobatidae) based on captive-raised specimens. *Bonn Zoological Bulletin* 69(2): 191–223.
- Kok PJR, MacCulloch RD, Gaucher P, Poelman EH, Bourne GR, Lathrop A, Lenglet GL. 2006a. A new species of *Colostethus* (Anura, Dendrobatidae) from French Guiana with a redescription of *Colostethus beebei* (Noble, 1923) from its type locality.

Reproductive ecology and larval morphology of “*Colostethus*” *ruthveni*

- Phyllomedusa* 5(1): 43–66.
- Kok PJR, Sambhu H, Roopsind I, Lenglet GL, Bourne GR. 2006b. A new species of *Colostethus* (Anura: Dendrobatidae) with maternal care from Kaieteur National Park, Guyana. *Zootaxa* 1238(1): 35–61.
- Kok PJR, MacCulloch RD, Lathrop A, Willaert B, Bossuyt F. 2010. A new species of *Anomaloglossus* (Anura: Aromobatidae) from the Pakaraima Mountains of Guyana. *Zootaxa* 2660(1): 18–32.
- Kok PJR, Willaert B, Means DB. 2013. A new diagnosis and description of *Anomaloglossus roraima* (La Marca, 1998) (Anura: Aromobatidae: Anomaloglossinae), with description of its tadpole and call. *South American Journal of Herpetology* 8(1): 29–45.
- Krausman PR. 1999. Some basic principles of habitat use. Grazing behavior of livestock and wildlife. *Idaho Forest, Wildlife and Range Experiment Station Bulletin* 70: 85–90.
- La Marca E. 1985. A new species of *Colostethus* (Anura: Dendrobatidae) from the Cordillera de Merida, Northern Andes, South America. *Occasional Papers of the Museum of Zoology, University of Michigan* 710: 1–10.
- La Marca E. 1989. A new species of collared frog (Anura: Dendrobatidae: *Colostethus*) from Serrania de Portuguesa, Andes of Estado Lara, Venezuela. *Amphibia-Reptilia* 10(2): 175–183.
- La Marca E. 1993. Phylogenetic relationships and taxonomy of *Colostethus mandelorum* (Anura: Dendrobatidae), with notes on coloration, natural history, and description of the tadpole. *Bulletin of the Maryland Herpetological Society* 29(1): 4–19.
- La Marca E. 1994. Taxonomy of the frogs of the genus *Mannophryne* (Amphibia; Anura; Dendrobatidae). *Publicaciones de la Asociación de Amigos de Doñana* 4: 1–75.
- La Marca E, Mijares-Urrutia A. 1988. Description of the tadpole of *Colostethus mayorgai* (Anura: Dendrobatidae) with preliminary data on reproductive biology of the species. *Bulletin of the Maryland Herpetological Society* 24(3): 47–57.
- Lehtinen RM, Halley A. 2008. A description of the tadpole of *Mannophryne olmonae* (Anura: Aromobatidae). *Caribbean Journal of Science* 44(2): 260–264.
- Lescure J. 1976. Étude de deux têtards de *Phyllobates* (Dendrobatidae): *P. femoralis* (Boulenger) et *P. pictus* (Bibron). *Bulletin de la Société Zoologique de France* 101(2): 299–306.
- Lima AP, Caldwell JP. 2001. A new Amazonian species of *Colostethus* with sky blue digits. *Herpetologica* 57(2): 133–138.
- Lima AP, Keller C. 2003. Reproductive characteristics of *Colostethus marchesianus* from its type locality in Amazonas, Brazil. *Journal of Herpetology*. 37(4): 754–757.
- Lima AP, Caldwell JP, Biavati GM. 2002. Territorial and reproductive behavior of an Amazonian dendrobatid frog, *Colostethus caeruleodactylus*. *Copeia* 2002(1): 44–51.
- Lima AP, Sanchez DEA, Souza JRD. 2007. A new Amazonian species of the frog genus *Colostethus* (Dendrobatidae) that lays its eggs on the underside of leaves. *Copeia* 2007(1): 114–122.
- Lima AP, Caldwell JP, Biavati G, Montanarin A. 2010. A new species of *Allobates* (Anura: Aromobatidae) from Paleovárzea Forest in Amazonas, Brazil. *Zootaxa* 2337(1): 1–17.
- Lima AP, Caldwell JP, Strussmann C. 2009. Redescription of *Allobates brunneus* (Cope) 1887 (Anura: Aromobatidae: Allobatinae), with a description of the tadpole, call, and reproductive behavior. *Zootaxa* 1988(1): 1–16.
- Lima AP, Simões PI, Kaefer IL. 2014. A new species of *Allobates* (Anura: Aromobatidae) from the Tapajós River basin, Pará State, Brazil. *Zootaxa* 3889(3): 355–387.
- Lima AP, Simões PI, Kaefer IL. 2015. A new species of *Allobates* (Anura: Aromobatidae) from Parque Nacional da Amazônia, Pará State, Brazil. *Zootaxa* 3980(4): 501–525.
- Lips K. 1999. Mass mortality and population declines of anurans at an upland site in Western Panama. *Conservation Biology* 13(1): 117–125.
- López-López FJ, Rada M, Kahn TR. 2016. La Planada Poison Frog *Amerega andina* (Myers and Burrowes 1987). Pp. 116–120 In: *A. Aposematic Poison Frogs (Dendrobatidae) of the Andean Countries: Bolivia, Colombia, Perú, and Venezuela. Tropical Fields Guides Series*. Editors, Kahn TR, La Marca E, Lötters S, Brown JL, Twomey E, Amézquita A. Conservation International, Arlington, Virginia, USA. 588 p.
- Lüddecke H. 1976. Einige Ergebnisse aus Feldbeobachtungen an *Phyllobates palmatus* (Amphibia, Ranidae) in Kolumbien. *Mitteilungen aus dem Institut Colombo Alemán de Investigaciones Científicas* 8: 157–163.
- Lüddecke H. 1999. Behavioral aspects of the reproductive biology of the Andean frog *Colostethus palmatus* (Amphibia: Dendrobatidae). *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales* 23(Suplemento Especial): 303–316.
- Lynch JD. 1982. Two new species of poison-dart frogs (*Colostethus*) from Colombia. *Herpetologica* 38(3): 366–374.
- Machado DJ. 2015. YBYRÁ facilitates comparison of large phylogenetic trees. *BMC Bioinformatics* 16(204): 2–5.
- MacKenzie DI, Nichols JD, Lachman GB, Droege S, Royle JA, Langtimm CA. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83(8): 2,248–2,255.
- Magrini L, Facure KG, Giaretta AA, da Silva WR, Costa RC. 2010. Geographic call variation and further notes on habitat of *Ameerega flavopicta* (Anura,

- Dendrobatidae). *Studies on Neotropical Fauna and Environment* 45(2): 89–94.
- Manjarrés-García G, Manjarrés-Pinzón G. 2004. Contribución al conocimiento hidrobiológico de la parte baja de los ríos de la vertiente noroccidental de la Sierra Nevada de Santa Marta, Colombia. *Revista Intropica* 1: 39–50.
- Marín CM, Molina-Zuluaga C, Restrepo A, Cano E, Daza JM. 2018. A new species of *Leucostethus* (Anura: Dendrobatidae) from the eastern versant of the Central Cordillera of Colombia and the phylogenetic status of *Colostethus fraterdanieli*. *Zootaxa* 4461: 359–380.
- Martínez-Baños V, Pacheco V, Ramírez-Pinilla MP. 2011. Abundancia relativa y uso de microhábitat de la rana *Geobatrachus walkeri* (Anura: Strabomantidae) en dos hábitats en Sierra Nevada de Santa Marta, Colombia. *Revista de Biología Tropical* 59(2): 907–920.
- Masche S, Zimmermann H, Pröhl H. 2010. Description and ecological observations of the tadpole of *Ranitomeya variabilis* (Anura: Dendrobatidae). *South American Journal of Herpetology* 5(3): 207–211.
- Menin M, Pinto RMC, Pegorini RJ, Da Silva MR. 2017. Redescription of the tadpole of *Ameerega hahneli* (Boulenger, 1884) (Anura: Dendrobatidae) with notes on ontogenetic variations and development habitats. *South American Journal of Herpetology* 12(3): 236–243.
- Meza-Joya FL, Chinchilla-Lemus W, Ramos E, Armesto O, Acevedo AA. 2019. New and noteworthy locality records of anurans from northeastern Andes of Colombia. *Herpetology Notes* 12: 61–69.
- Mijares-Urrutia A. 1991. Descripción del renacuajo de *Colostethus leopardalis* Rivero con algunos comentarios sobre su historia natural. *Amphibia-Reptilia* 12: 145–152.
- Mijares-Urrutia A, La Marca E. 1997. Tadpoles of the genus *Nephelobates* La Marca 1994 (Amphibia, Anura, Dendrobatidae), from Venezuela. *Tropical Zoology* 10(1): 133–142.
- Montanari A, Kaefer IL, Lima AP. 2011. Courtship and mating behavior of the Brilliant-thighed Frog *Allobates femoralis* from Central Amazonia: implications for the study of a species complex. *Ethology, Ecology, & Evolution* 23(2): 141–150.
- Moraes LJCL, Pavan D, Lima AP. 2019. A new nurse frog of *Allobates masniger-nidicola* complex (Anura, Aromobatidae) from the east bank of Tapajós River, eastern Amazonia. *Zootaxa* 4648(3): 401–434.
- Moreno-Arias RA, Medina-Rangel GF, Carvajal-Cogollo JE, Castaño-Mora OV. 2009. Herpetofauna de La Serranía de Perijá. Pp. 449–470 In: *Colombia Diversidad Biológica VIII: La Alta Montaña de la Serranía del Perijá, Colombia*. Editor, Rangel-Ch JO. Instituto de Ciencias Naturales-Universidad Nacional de Colombia – CORPOCESAR, Bogotá DC, Colombia. 708 p.
- Myers CW. 1987. New generic names for some neotropical poison frogs (Dendrobatidae). *Papéis Avulsos de Zoologia* 36(25): 301–306.
- Myers CW, Burrows PA. 1987. A new poison frog (*Dendrobates*) from Andean Colombia, with notes on a lowland relative. *American Museum Novitates* 2899: 1–17.
- Myers CW, Donnelly MA. 1997. A tepui herpetofauna on a granitic mountain (Tamacuari) in the borderland between Venezuela and Brazil: report from the Phipps Tapirapecó Expedition. *American Museum Novitates* 3213: 1–71.
- Myers CW, Donnelly MA. 2001. Herpetofauna of the Yutajé-Corocoro massif, Venezuela. Second report from the Robert G. Goellet American Museum–Terrarum Expedition to the northwestern Tepuis. *Bulletin of the American Museum of Natural History* 261: 1–85.
- Myers CW, Daly JW, Malkin B. 1978. A dangerously toxic new frog (*Phyllobates*) used by Emberá Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. *Bulletin of the American Museum of Natural History* 161(2): 307–366.
- Myers CW, Daly JW, Martínez V. 1984. An arboreal poison frog (*Dendrobates*) from Western Panama. *American Museum Novitates* 2783: 1–20.
- Myers CW, Paolillo OA, Daly JW. 1991. Discovery of a defensively malodorous and nocturnal frog in the family Dendrobatidae: phylogenetic significance of a new genus and species from the Venezuelan Andes. *American Museum Novitates* 3002: 1–33.
- Nunes-De-Almeida CHL, Haddad CFB, Toledo LF. 2021. A revised classification of the amphibian reproductive modes. *Salamandra* 57(3): 413–427.
- Páez-Vacas MI, Coloma LA, Santos JC. 2010. Systematics of the *Hyloxalus bocagei* complex (Anura: Dendrobatidae), description of two new cryptic species, and recognition of *H. maculosus*. *Zootaxa* 2711(1): 1–75.
- Pérez-Peña PE, Chávez G, Twomey E, Brown JL. 2010. Two new species of *Ranitomeya* (Anura: Dendrobatidae) from eastern Amazonian Peru. *Zootaxa* 2439(1): 1–23.
- Pezzuti TL, Araújo RB, Sanches PR, Santos FP, Dias-Souza MR, Costa-Campos CE. 2022. The tadpole of the endemic poison frog *Ameerega pulchripecta* (Silverstone, 1976) with the description of its chondrocranium (Anura: Dendrobatidae: Colostethinae). *Zootaxa* 5115(2): 295–300.
- Pisso-Florez GA, Liévano-Bonilla AC, Mazepa G, Mulder KP, Ramírez-Chaves HE. 2023. Distribution update, advertisement call, and phylogenetic position of the Malvasá Rocket Frog, *Hyloxalus pinguis* (Anura: Dendrobatidae). *South American Journal of Herpetology* 28(1): 78–88.
- Poelman EH, Verkade JC, van Wijngaarden RPA, Félix-

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- Novoa C. 2010. Descriptions of the tadpoles of two poison frogs, *Ameerega parvula* and *Ameerega bilinguis* (Anura: Dendrobatidae) from Ecuador. *Journal of Herpetology* 44(3): 409–417.
- Portik DM, Streicher JW, Wiens JJ. 2023. Frog phylogeny: a time-calibrated, species-level tree based on hundreds of loci and 5,242 species. *Molecular Phylogenetics and Evolution* 188: 107907.
- Posso-Peláez C, Blanco-Torres A, Gutierrez-Moreno LC. 2017. Uso de microhábitats, actividad diaria y dieta de *Dendrobates truncatus* (Cope, 1861) (Anura: Dendrobatidae) en bosque seco tropical del norte de Colombia. *Acta Zoológica Mexicana* (Nueva Serie) 33(3): 490–502.
- Quiguango-Ubillús A, Coloma LA. 2008. Notes on behavior, communication, and reproduction in captive *Hyloxalus toachi* (Anura: Dendrobatidae), an endangered Ecuadorian frog. *International Zoo Yearbook* 42: 78–89.
- Regnet RA, Lukas P, Rödder D, Wipfler B, Solé M. 2023. Tadpole morphological characterization of *Ranitomeya variabilis* (Zimmermann and Zimmermann, 1988) (Anura: Dendrobatidae), with skeleton, muscle system and inner organs. *Zoomorphology* 142: 477–486.
- Rivero JA. 1971. Un nuevo e interesante *Dendrobates* (Amphibia, Salientia) del Cerro Yapacana de Venezuela. *Kasmera* 3: 389–396.
- Roach NS, Acosta D, Lacher TE. 2021. Shade coffee and amphibian conservation, a sustainable way forward? Understanding the perceptions and management strategies of coffee growers in Colombia. *Ecology & Society* 26(2): 33.
- Rocha SMC, Lima AP, Kaefer IL. 2018. Reproductive behavior of the amazonian nurse-frog *Allobates paleovarzensis* (Dendrobatoidea, Aromobatidae). *South American Journal of Herpetology* 13(3): 260–270.
- Rodriguez L, Myers CW. 1993. A new poison frog from Manu National Park, southeastern Peru (Dendrobatidae, *Epipedobates*). *American Museum Novitates* 3068: 1–16.
- Roithmair ME. 1994. Field studies of reproductive behavior in two dart-poison frog species (*Epipedobates femoralis*, *Epipedobates trivittatus*) in Amazonian Peru. *Herpetological Journal* 4(3): 77–85.
- Rojas B, Pašukonis A. 2019. From habitat use to social behavior: natural history of a voiceless poison frog, *Dendrobates tinctorius*. *PeerJ* 7(1) 1–30 (e7648).
- Rojas-Morales JA, La Marca E, Ramírez-Chaves HE. 2021. Advertisement call of a population of the nurse frog *Leucostethus fraterdanieli* (Anura: Dendrobatidae), with notes on its natural history. *Biota Colombiana* 22(1): 122–132.
- Romero-Martínez HJ, Lynch JD. 2012. Anfíbios de la Región Caribe. Pp. 677–701 In: *Colombia Diversidad Biológica XII. La Región Caribe de Colombia*. Editor, Rangel-Ch JO. Instituto de Ciencias Naturales-Universidad Nacional de Colombia, Bogotá DC, Colombia. 1,018 p.
- Ruiz-Carranza P, Ramírez-Pinilla MP. 1992. Una nueva especie de *Minyobates* (Anura: Dendrobatidae) de Colombia. *Lozania* 61: 1–16.
- Ruthven AG, Gaige HT. 1915. The breeding habitats of *Prostherapis subpunctatus* Cope. *Occasional Papers of the Museum of Zoology – University of Michigan* 11: 1–7.
- Sánchez DA. 2010. Larval development and synapomorphies for species groups of *Hyloscirtus* Peters, 1882 (Anura: Hylidae: Cophomantini). *Copeia* 2010(3): 351–363.
- Sánchez DA. 2013. Larval morphology of dart-poison frogs (Anura: Dendrobatoidea: Aromobatidae and Dendrobatidae). *Zootaxa* 3637(5): 569–591.
- Santos JC, Coloma LA, Summers K, Caldwell JP, Ree R, Cannatella DC. 2009. Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. *PLoS Biology* 7(3): 448–461 (e1000056).
- Santos JC, Baquero M, Barrio-Amorós C, Coloma LA, Erdtmann LK, Lima AP, Cannatella DC. 2014. Aposematism increases acoustic diversification and speciation in poison frogs. *Proceedings of the Royal Society B: Biological Sciences* 281(1796): 1–9.
- Savage JM. 1968. The Dendrobatid frogs of Central America. *Copeia* 1968(4): 745–776.
- Savage JM. 2002. *The Amphibians and Reptiles of Costa Rica: a Herpetofauna between Two Continents, between Two Seas*. University of Chicago Press, Chicago, Illinois, USA. 954 p.
- Schulte R. 1990. Redescubrimiento y redefinición de *Dendrobates mysteriosus* (Myers 1982) de la Cordillera del Cóndor. *Boletín de Lima* 70: 57–68.
- Schulze A, Jansen M, Köhler G. 2015. Tadpole diversity of Bolivia’s lowland anuran communities: molecular identification, morphological characterization, and ecological assignment. *Zootaxa* 4016(1): 1–111.
- Sereno PC. 2007. Logical basis for morphological characters in phylogenetics. *Cladistics* 23(6): 565–587.
- Serrano-Rojas SJ, Whitworth A, Villacampa J, von May R, Gutiérrez RC, Padial JM, Chaparro JC. 2017. A new species of poison-dart frog (Anura: Dendrobatidae) from Manu Province, Amazon region of southeastern Peru, with notes on its natural history, bioacoustics, phylogenetics, and recommended conservation status. *Zootaxa* 4221(1): 71–94.
- Silverstone PA. 1971. Status of certain frogs of the genus *Colostethus*, with descriptions of new species. *Contributions in Science. Natural History Museum of Los Angeles County* 215: 1–8.
- Silverstone PA. 1973. Observations on the behavior and ecology of a Colombian poison-arrow frog, the Kokoe-Pa (*Dendrobates histrionicus* Berthold). *Herpetologica* 29(4): 295–301.
- Silverstone PA. 1975. Two new species of *Colostethus*

- (Amphibia: Anura: Dendrobatidae) from Colombia. *Natural History Museum of Los Angeles County – Contributions in Science* 268: 1–10.
- Simões PI, Lima AP, Farias IP. 2010. The description of a cryptic species related to the pan-Amazonian frog *Allobates femoralis* (Boulenger 1883) (Anura: Aromobatidae). *Zootaxa* 2406(1): 1–28.
- Simões PI, Lima AP. 2012. The tadpole of *Allobates sumtuosus* (Morales, “2000” 2002) (Anura: Aromobatidae) from its type locality at Reserva Biológica do Rio Trombetas, Pará, Brazil. *Zootaxa* 3499(1): 86–88.
- Simões PI, Sturaro MJ, Peloso PLV, Lima AP. 2013. A new diminutive species of *Allobates* Zimmermann and Zimmermann, 1988 (Anura, Aromobatidae) from the northwestern Rio Madeira-Rio Tapajós interfluvio, Amazonas, Brazil. *Zootaxa* 3609(3): 251–273.
- Sloggett JJ, Zeilstra I. 2008. Waving or tapping? Vibrational stimuli and the general function of toe twitching in frogs and toads (Amphibia: Anura). *Animal Behaviour* 76:1–4.
- Simões PI, Rojas D, Lima AP. 2019. A name for the nurse-frog (*Allobates*, Aromobatidae) of Floresta Nacional de Carajás, Eastern Brazilian Amazonia. *Zootaxa* 4550(1): 71–100.
- Souza JRD, Ferrão M, Hanken J, Lima AP. 2020. A new nurse frog (Anura: *Allobates*) from Brazilian Amazonia with a remarkably fast multi-noted advertisement call. *PeerJ* 8(2): 1–31.
- Souza JRD, Kaefer IL, Lima AP. 2017. The peculiar breeding biology of the Amazonian frog *Allobates subfolionidificans* (Aromobatidae). *Anais da Academia Brasileira de Ciências* 89(2): 885–893.
- Suarez-Mayorga AM, Lynch JD. 2001. Redescription of the tadpole of *Hyla vigilans* (Anura: Hylidae) and notes about possible taxonomic relationships. *Caribbean Journal of Science* 37(1–2): 116–119.
- Summers K. 1992. Mating strategies in two species of dart-poison frogs: a comparative study. *Animal Behaviour* 43: 907–919.
- Summers K, Tumulty J. 2014. Parental care, sexual selection, and mating systems in neotropical poison frogs. Pp. 289–320 In: *Sexual Selection: Perspectives and Models from the Neotropics*. Editors, Macedo RH, Machado G. Academic Press, Cambridge, Massachusetts, USA. 466 p.
- Touchon JC, Warkentin KM. 2008. Reproductive mode plasticity: aquatic and terrestrial oviposition in a treefrog. *Proceedings of the National Academy of Sciences of the United States of America* 105(21): 7,495–7,499.
- Twomey E, Brown JL. 2008. Spotted poison frogs: rediscovery of a lost species and a new genus (Anura: Dendrobatidae) from Northwestern Peru. *Herpetologica* 64(1): 121–137.
- Twomey E, Brown JL. 2009. Another new species of *Ranitomeya* (Anura: Dendrobatidae) from Amazonian Colombia. *Zootaxa* 2302(1): 48–60.
- van Wijngaarden R, Bolaños F. 1992. Parental care in *Dendrobates granuliferus* (Anura: Dendrobatidae), with a description of the tadpole. *Journal of Herpetology* 26(1): 102–105.
- Vargas-Salinas F, Castro-Herrera F. 1999. Distribución y preferencias de microhábitat en anuros (Amphibia) en bosque maduro y áreas perturbadas en Anchicayá, Pacífico Colombiano. *Caldasia* 21(1): 95–109.
- Vitt LJ, Caldwell JP. 2014. *Herpetology: An Introductory Biology of Amphibians and Reptiles*. Fourth Edition. Academic Press, London, United Kingdom. 776 p.
- von May R, Medina-Müller M, Donnelly MA, Summers K. 2008. The tadpole of the bamboo-breeding poison frog *Ranitomeya biolat* (Anura: Dendrobatidae). *Zootaxa* 1857(1): 66–68.
- Waters KR, Dugas MB, Grant T, Saporito RA. 2023. The ability to sequester the alkaloid epibatidine is widespread among dendrobatid poison frogs. *Evolutionary Ecology* 2023: 10260.
- Wells KD. 1978. Courtship and parental behavior in a Panamanian poison-arrow frog (*Dendrobates auratus*). *Herpetologica* 34(2): 148–155.
- Wells KD. 1980a. Behavioral ecology and social organization of a dendrobatid frog (*Colostethus inguinalis*). *Behavioral Ecology and Sociobiology* 6: 199–209.
- Wells KD. 1980b. Evidence for growth of tadpoles during parental transport in *Colostethus inguinalis*. *Journal of Herpetology* 14(4): 428–430.
- Wells KD. 1980c. Social behavior and communication of a dendrobatid frog (*Colostethus trinitatis*). *Herpetologica* 36(2): 189–199.
- Wells KD. 2007. *The Ecology and Behavior of Amphibians*. University of Chicago Press, Chicago, Illinois, USA. 1,400 p.
- Weygoldt P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 25: 51–67.
- Womack MC, Steigerwald E, Blackburn DC, Cannatella DC, Catenazzi A, Che J, Koo MS, McGuire JA, Ron SR, Tarvin RD, et al. 2022. State of the Amphibia 2020: a review of five years of amphibian research and existing resources. *Ichthyology and Herpetology* 110(4): 638–661.
- Young BE, Lips KR, Reaser JK, Ibáñez R, Salas AW, Rogelio Cedeño J, Coloma LA, Ron S, La Marca E, Meyer JR, et al. 2001. Population declines and priorities for amphibian conservation in Latin America. *Conservation Biology* 15: 1,213–1,223.

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