Ichthyology & Herpetology 113, No. 4, 2025, 625–637

The Tadpole, Bioacoustics, Natural History, Morphological Variation, and Conservation of *Isthmohyla debilis* (Hylidae: Hylinae: Hylini)

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The description of the tadpole of *Isthmohyla debilis* by Duellman (1970) revealed a distinctive morphology that deviated from closely related stream-breeding species within the *I. tica* group in several important aspects, including the size of the oral disc, configuration of submarginal papillae on both labia, body shape, and jaw sheath morphology. These character states complicated the identification of phenotypic synapomorphies for the clade composed by *I. debilis*, *I. rivularis*, *I. tica*, and tentatively, *I. pictipes* and *I. xanthosticta*. We collected adults and tadpoles of *I. debilis* in the Reserva Forestal Fortuna, western Panama. Our findings indicate that the previously described tadpoles assigned to *I. debilis* were misidentified and likely correspond to the Panamanian lineage associated with *I. lancasteri*. We show that the larvae of *I. debilis* indeed share all the character states present in their closely related species, including a depressed body with a sloping snout and an enlarged oral disc that is nearly as wide as the body, deep angular folds, complete marginal papillae, three or more rows of submarginal papillae in the upper and lower labia, an M-shaped upper jaw sheath and LTRF 2/3, with complete tooth rows. Moreover, we provide information on vocalizations, natural history, and adult coloration patterns across the known geographic range of *I. debilis*. Additionally, we report a currently extant population of *I. debilis* from Boquete, western Panama, complementing a previous, tentative record of the species from the same area.

THE hylid frog genus *Isthmohyla* Faivovich et al. (2005) includes 13 species distributed across the isthmian highlands of lower Central America in Costa Rica and western Panama (Faivovich et al., 2018; Chaves-Acuña et al., 2024; Frost, 2025). Based on the phylogenetic hypothesis of Faivovich et al. (2018) as further refined by Chaves-Acuña et al. (2024), the genus is divided into three species groups: the pond-breeding *I. pseudopuma* group (*I. infucata* and *I. pseudopuma*), the bromeliad-breeding *I. zeteki* group (*I. picadoi* and *I. zeteki*), and the *I. tica* group (*I. angustilineata*, *I. calypsa*, *I. debilis*, *I. graceae*, *I. lancasteri*, *I. pictipes*, *I. rivularis*, *I. tica*, and *I. xanthosticta*). Four species (*I. angustilineata*, *I. calypsa*, *I. pictipes*, and *I. xanthosticta*) are tentatively included in the *I. tica* group based on previous studies considering phenotypic similarities with the other species in this group (Duellman, 1970, 2001; Myers and Duellman, 1982; Lips, 1996; Faivovich et al., 2018).

Within the *Isthmohyla tica* group, there is a clade comprised by *I. debilis*, *I. rivularis*, *I. tica*, and tentatively *I. pictipes* and *I. xanthosticta* that includes species from a variety of riparian environments (Duellman, 1970; Faivovich et al., 2018). With the exception of *I. debilis* and *I. xanthosticta* (larvae unknown for the latter; see Duellman, 1970), these stream-breeding species show several similarities in their larval morphology, including a (1) depressed body with a sloping snout in profile, (2) enlarged oral disc, (3) numerous rows of submarginal papillae on the upper and lower labia, (4) M-shaped upper jaw sheath, and (5) complete tooth rows (Duellman, 1970, 2001). In contrast, the tadpole of *I. debilis* was originally described by Duellman (1970) with distinctive morphological characters from those of closely related species. These include a globular body with a rounded snout in profile, an oral disc approximately half the width of the body, few rows of submarginal papillae in both labia, a broad arched upper jaw sheath, and the second upper row of teeth interrupted medially, and slightly shorter than the first (Duellman, 1970, 2001). Some of these differences (2, 3, and 4) between the tadpole of *I. debilis* and those of *I. pictipes*, *I. rivularis*, and *I. tica* were noticed by Faivovich et al. (2018). These authors added that the different character states in the tadpole of *I. debilis* resulted in ambiguities in the inference of phenotypic synapomorphies for the clade including all these riparian treefrogs and suggested the need of corroborating the identification of the tadpoles of *I. debilis* described by Duellman (1970).

Isthmohyla debilis is a little-known hylid found in mountain streams within cloud forests of central Costa Rica

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Submitted: 30 December 2024. Accepted: 27 May 2025. Associate Editor: T. Grande.

DOI: 10.1643/h2024113

(Heredia and Cartago Provinces) and western Panama (Chiriquí and Bocas del Toro Provinces and the Comarca Ngäbe-Buglé), ranging from 1,160 to 1,643 m.a.s.l. (Duellman, 1970, 2001; Savage, 2002; Hertz et al., 2012). The species is characterized by its small size (SVL in males = 25–29.5 mm; females = 27–32 mm), smooth dorsum, immaculate venter, light suborbital spot, uniformly dull yellow posterior surfaces of thighs, and a pulsed call emitted at high frequencies (4,800–5,360 Hz) with a duration of 100 to 160 ms (Duellman, 1970).

In the mid-1960s, W. E. Duellman, L. Trueb, and C. W. Myers collected adults and larvae and made call recordings of *Isthmohyla debilis* at intermediate elevations in the north slope of Cerro Pando, western Panama (hereafter referred to as Cerro Pando). The call of *I. debilis* was described as a series of weak "cricket-like chirps" with multiple single-pulsed notes (Duellman, 1970). Subsequently, Myers and Duellman (1982) documented the species around the Reserva Forestal Fortuna, western Panama (hereafter referred to as Fortuna). These authors reported color variation among populations, with specimens from Fortuna differing from those in Cerro Pando and central Costa Rica (Myers and Duellman, 1982). Jungfer (1988) also assessed the amphibian diversity of Fortuna, documenting ontogenetic color variation and acoustic activity in individuals of *I. debilis* kept in captivity. The author reported raising larvae of *I. debilis* to metamorphosis, noting that their oral discs resembled those of closely related species, specifically *I. rivularis* as described and illustrated by Duellman (1970). This work was not cited in subsequent studies (Duellman, 2001; Savage, 2002; Faivovich et al., 2018).

Based on recent collections of both adults and larvae in western Panama, we show that the tadpoles originally assigned to *Isthmohyla debilis* by Duellman (1970) were misidentified. In this study, we describe the larvae of *I. debilis* and show that the tadpoles described by Duellman (1970) likely correspond to *I. lancasteri*. In addition, we comment on the vocal repertoire of adults, provide notes on natural history and color variation, and discuss the value of our findings in the context of current knowledge on phylogenetic relationships of the *I. tica* group. Finally, we assess the conservation status of the species and its implications for future management efforts.

MATERIALS AND METHODS

Study site and fieldwork.—Data was collected in rocky-bottom streams across the Chiriquí Province, western Panama. Field surveys were conducted in June 2018, between July and September 2022, and in October 2024 in two streams at Fortuna: the Alemán stream, located adjacent to a road (8°42′36″N, 82°13′58″W, 1,282 m.a.s.l.); and the Tube Stream, located within the grounds of the Smithsonian field station 'Jorge L. Araúz' (8°43′20″N, 82°14′14″W, 1,220 m.a.s.l.). Fortuna is located on the continental divide between the Chiriquí and Bocas del Toro Provinces, delimiting the eastern end of the Cordillera de Talamanca and the western end of the Serranía de Tabasará (see Myers and Duellman, 1982). It is located approximately 272 km SE of the type locality of *Isthmohyla debilis* in Isla Bonita, Alajuela Province, central Costa Rica (Taylor, 1952). Between June and July 2024, and in October 2024, we conducted fieldwork on a second population in the Pata Macho stream, at Palo Alto, Boquete District (08°48′48″N, 82°23′54″W, 1,679 m.a.s.l.). Palo Alto connects to the Parque Internacional La Amistad, a biogeographical corridor with exceptional diversity within the Cordillera de Talamanca that spans Panama and Costa Rica. It is located 256 km SE from the type locality (Taylor, 1952). The vegetation of the study sites is mainly composed of mature secondary forest, with continuous open canopy areas above the streams. We obtained temperature and relative humidity measurements using a digital thermo-hygrometer.

Twelve adults (11 males, 1 female) and nine tadpoles of *Isthmohyla debilis* were collected. All specimens were euthanized using 5% lidocaine. Adults were fixed in 10% formalin and stored in 70% ethanol. Tadpoles were preserved in 5% formalin. We preserved tissue samples in 96% ethanol. Sex was determined by examination of secondary sexual characters (nuptial pads and expansion of the vocal sac) or, when in doubt, by examination of gonads. Specimens were deposited at the Museo de Vertebrados, Universidad de Panamá (MVUP), Museo Herpetológico de Chiriquí, Universidad Autónoma de Chiriquí (MHCH), and the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN). To corroborate the identity of the tadpoles, we sequenced a 537 base pair segment of the 16S rRNA mitochonrial gene of one tadpole (MACN 62276, GenBank accession number PX458084) and one adult (MHCH 5059, GenBank accession number PX458082). The sequences were identical (uncorrected p-distance = 0%), confirming conspecificity.

Tadpole morphology.—Morphological terminology and characterization follow Duellman (1970), Altig and McDiarmid (1999a), and Pezzuti et al. (2021). Developmental stages were determined according to Gosner (1960). A commercial solution of methylene blue was applied topically to enhance contrast in oral structures. We took 22 measurements (in millimeters, rounded to the nearest 0.1 mm) following Altig and McDiarmid (1999b) for total length (TL), body length (BL), tail length (TAL), maximum tail height (MTH), internostril distance (IND), interorbital distance (IOD), tail muscle height (TMH), and tail muscle width (TMW); Lavilla and Scrocchi (1986) for body width (BW), body width at level of nostrils (BWN), body width at eye level (BWE), body height (BH), eye–nostril distance (END), nostril–snout distance (NSD), eye diameter (ED), nostril diameter (ND), snout–spiracle distance (SSD), and oral disc width (ODW); Grosjean (2005) for dorsal fin height (DFH) and ventral fin height (VFH); and Lins et al. (2018) for spiracle length (SL) and spiracle–venter distance (SVD). Measurements were taken from nine specimens (stages 25 to 42; lots MACN 62276 and MHCH 5074) using a digital caliper under a dissecting microscope, and from two additional specimens (stages 27 and 36; lot AMNH 94902) from photographs analyzed through ImageJ (Abramoff and Magalhaes, 2004).

We also examined the tadpole lot used by Duellman (1970) to describe the larvae of *Isthmohyla debilis* from the north slope of Cerro Pando (KU 104239) and a second lot assigned to the species collected in Fortuna (AMNH A-94902). We complemented the comparisons of the tadpole of *I. debilis* by examining tadpoles of other species in the *I. tica* group, including *I. angustilineata* (KU 104120–1), *I. calypsa* (LACM 180863; UCR 16113–4), *I. lancasteri* (KU 104254; UCR 15406, 23180, 23214–15, 24372), *I. pictipes* (LACM 178504, 180436, 180438; UCR 23382), *I. rivularis* (KU 68444, 86447; UCR 23178, 24347), and *I. tica* (KU 104127).

Vocalizations.—Recordings were done with a Tascam DR-40x recorder and unidirectional microphone Sennheiser ME 66 positioned at about 1–3 m from the calling individuals. Calls were recorded at a sampling rate of 44.1 kHz and a 16-bit resolution. We measured spectral and temporal parameters using Raven Pro 1.6.1 (Cornell Lab of Ornithology) with the following settings: window type Hann, window size of 512 samples, 3 dB filter bandwidth of 124 Hz, 50% overlap, hop size of 256 samples, DFT size of 512 samples, and grid spacing at 86.1 Hz. In total, we examined ten recordings with a total of 39 minutes and 650 calls from eight males. Figures were produced with seewave v. 1.7.6 (Sueur et al., 2008) in R 4.0.2 (R Core Team, 2020), using the following settings: window = Hanning, FFT overlap = 90%, FFT size = 512 points. Spectrograms were produced with a relative amplitude color scale of 30 dB (red = maximum amplitude). Calls were described using the terminology of Köhler et al. (2017), considering the following parameters: call duration, intercall duration, call envelope, minimum and maximum frequency, dominant frequency, and amplitude and frequency modulation. We used the definition of 'call' as a unit of vocalization, which is separated from other calls by an intercall interval (silent period) that is longer than the call duration (Köhler et al., 2017). Oscillograms were used to manually obtain the number of calls per call series and the number of pulses per call. Oscillograms were also used to calculate the call duration (ms) and the intercall interval within call series (ms) using the "Delta time" function of Raven. We also described the amplitude modulation of calls considering the presence of amplitude peaks throughout the call duration. We estimated the call rate within a call series as the number of calls divided by the call series duration. Spectrograms were used to obtain dominant frequency (Hz), minimum frequency (Hz), and maximum frequency (Hz) values using the "Peak frequency," "Frequency 5%," and "Frequency 95%" functions, respectively. We deposited digital copies of audio files in Fonoteca Neotropical Jacques Vielliard (https://www2.ib.unicamp.br/fnjv/; Universidade Estadual de Campinas, Campinas, Sao Paulo, Brazil; FNJV 0124935–0124943).

RESULTS

One tadpole lot, two species.—Duellman (1970) reported four tadpole lots assigned to *Isthmohyla debilis* (Duellman, 1970: appendix 1). He presented measurements from 20 specimens in stages 25–28, noting that some tadpoles were particularly larger than others. Specifically, he used a specimen from lot KU 104239 to illustrate the body in lateral view and the oral disc. This lot contains tadpoles from two different species: 22 specimens in stages 25–29 correspond to what we identify in the present study as *I. debilis*, and three specimens in stages 28–30 belong to a different species that lacks an enlarged oral disc and an M-shaped upper jaw sheath. Tadpoles of this second species have a globular body with a rounded snout in profile; a thin nasal rim; a spiracle opening located at the second third of the body with the inner wall fused to body; an oral disc that is about half the body width, with no angular folds, one or two rows of submarginal papillae, and an arch-shaped upper jaw sheath with a LTRF 2/3, with A-2 incomplete; and a dorsal fin that is parallel to the longitudinal axis of the tail (see Fig. 1). The description provided by Duellman (1970) was based on these misidentified specimens, which likely correspond to *I. lancasteri* (see Discussion).

***The tadpole of* Isthmohyla debilis.**—Measurements and descriptions were based on 11 individuals at stages 25–42 (lots AMNH A-94902 [2 tadpoles], MACN 62276 [3], MHCH 5074 [6]). See Table 1 for individual measurements of larvae. Tadpoles belong to Guild II (exotrophic), C (lotic), and 11 (suctorial) as per McDiarmid and Altig's (1999) classification. Body depressed (BH/BW = 0.79–0.93); elliptical in dorsal view; in lateral view, ventral contour of body flat in the peribranchial region, convex in the abdominal region; 29–38% total length (Fig. 2A). Tail long, 61–70% total length. Snout rounded in dorsal view (BWN/BWE = 0.77–1) and sloping in profile. Eyes small (ED/BWE = 0.13–0.26), located dorsally (IOD/BWE = 0.70–0.89), directed dorsolaterally (Fig. 2B). Nostrils rounded, small (ND/ED = 0.15–0.21), directed anterolaterally, and with a thick fleshy rim that is notably protruded (Fig. 2E); nostril–snout distance 51–70% of eye–snout distance. Spiracle sinistral, lateral (SVH/BH = 0.17–0.28), directed posteriorly, short (SL/BL = 0.07–0.12), opening at the posterior third of the body (SSD/BL = 0.74–0.88; Fig. 2F); inner wall completely free from body. Neuromasts not forming stitches. Intestinal tube circularly coiled, switchback point located at the center of abdominal region. Vent tube dextral, directed posteriorly, laterally fused to margin of ventral fin (Fig. 2G); ventral and dorsal walls of same length.

Oral disc large (ODW/BW = 0.73–0.88), positioned ventrally (Fig. 2C); bordered by a complete row of single or biserial marginal papillae, with a small medial emargination. Upper labium with 3–4 rows of small, conical or triangular submarginal papillae above the angular area, extending medially to the central region of the labium; the lower row continues medially as a single, continuous row of enlarged, quadrangular papillae, located above A-1. Lower labium with 3–4 rows of small, conical or triangular submarginal papillae proximal to the angular area, extending medially to the central region of the labium and expanding to form 5–6 irregular rows; the uppermost submarginal papillae row continues medially as quadrangular or conical papillae; submarginal papillae larger than marginal papillae; deep angular folds with few submarginal papillae in the labial margin. LTRF 2/3, all tooth rows with the same length. The labial tooth size varies, with those in the central portions of P1 > A2 > A1 ≈ P2 > P3 and all are proportionally larger than teeth on the sides of the rows. Jaw sheaths narrow, finely serrated upper jaw sheath, M-shaped; lower jaw sheath with concave free margin, V-shaped; upper jaw sheath wider than lower (Fig. 2D).

Tail musculature robust (TMH/BH = 0.58–0.88), almost reaching tail tip; tail tip rounded. Dorsal fin low (DFH/TAL = 0.06–0.10), higher than ventral fin (DFH/VFH = 1.1–1.4) and originating on the posterior third of body; free margin convex. Ventral fin low (VFH/TAL = 0.05–0.07), free margin slightly convex, originating at level of vent tube. Maximum height of dorsal and ventral fin at the last third of the tail.

Coloration in life.—Body and caudal musculature light brown, with irregular dark blotches and scattered iridophores. A dark epithelium covers the lateral and ventrolateral surfaces of the visceral mass, visible through the transparent body wall, with scattered iridophores contrasting against the dark background. Iris black, speckled with iridophores, and surrounded by a golden rim around the pupil. Fins translucent, bearing irregular blotches or a reticulate pattern formed by scattered melanophores. Ventrally, the branchial region and intestinal mass are visible through the body wall.

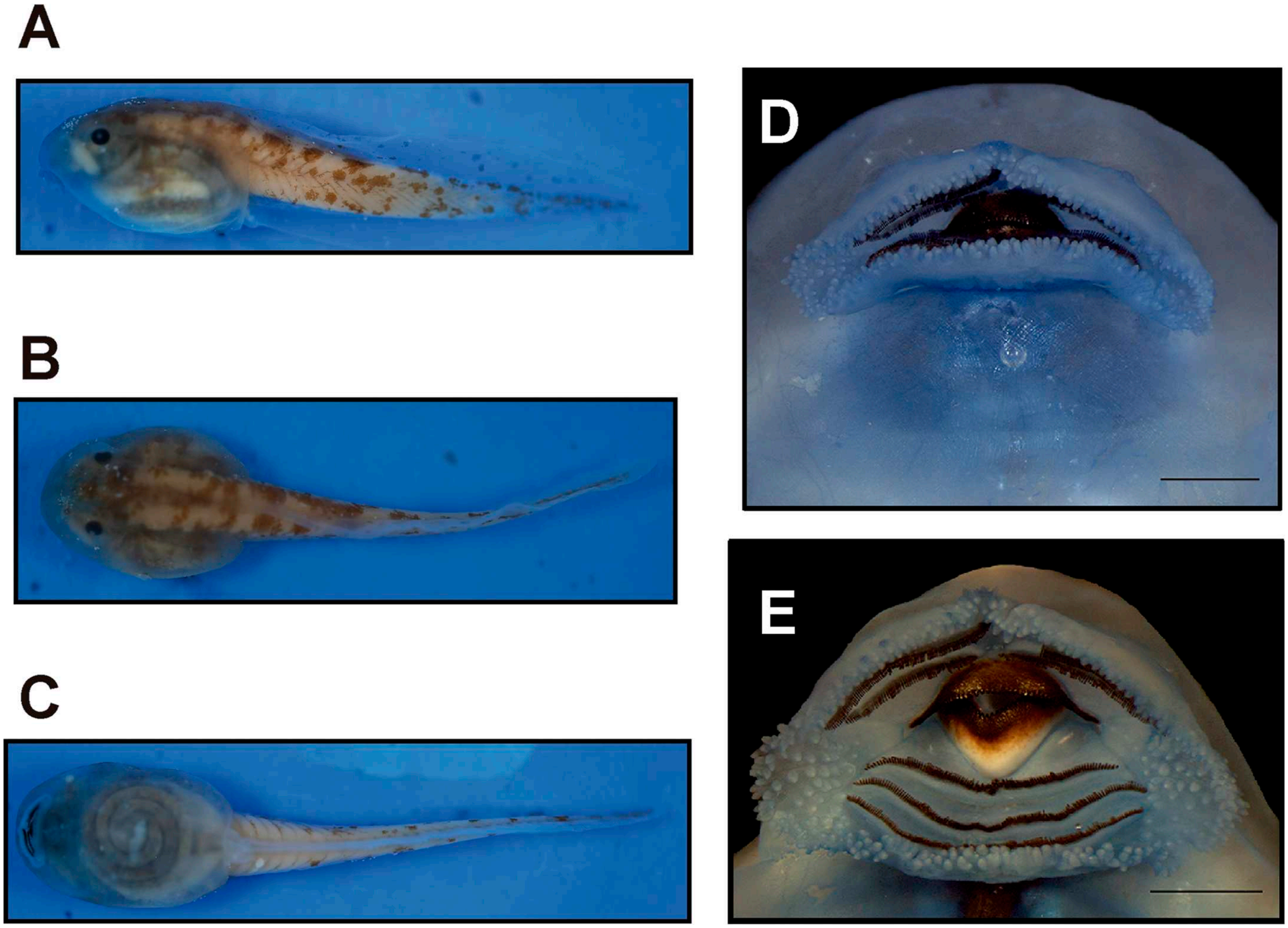

Fig. 1. Tadpole (stage 28) belonging to one of the two species included in lot KU 104239, and corresponding to the one illustrated in Duellman (1970: figs. 128C and 129C), here reidentified as likely corresponding to the Panamanian lineage of *Isthmohyla lancasteri* (see Discussion). (A) Lateral view. (B) Dorsal view. (C) Ventral view. (D) Oral disc, folded. (E) Oral disc, open. Scale bars for A–C = 5 mm. Scale bars for D–E = 1 mm. Photos courtesy of Ana Motta.

Coloration in preservative.—Body mostly unpigmented, with dark blotches made up of relatively sparce melanophores; proximal half of caudal musculature light brown, distal part translucent; dark epithelium covering the visceral mass laterally and ventrolaterally evident through the translucent skin. Iris black.

Comparison with tadpoles of other species in the* Isthmohyla tica *group.—The larvae of *Isthmohyla debilis* shares several character states with its closely related stream-breeding species in the *I. tica* group: *I. pictipes*, *I. rivularis*, and *I. tica*. These characters include a depressed body with a sloping snout; a thick, protruded nasal rim surrounding the nostril; a spiracle with its opening located on the posterior third of the body and with the inner wall completely free from body; and an enlarged oral disc that is nearly as wide as the body, with deep angular folds, complete marginal papillae, three or more rows of submarginal papillae in the upper and lower labia, an M-shaped upper jaw sheath and LTRF 2/3, with complete tooth rows. The tadpole of *I. debilis* is distinguished from other tadpoles in this clade because the angular areas of the oral disc have few submarginal papillae (many in *I. rivularis*, absent in *I. pictipes* and *I. tica*).

Vocalizations.—We identified two types of calls in *Isthmohyla debilis*, which we designated as calls A and B (Fig. 3). Call A is the advertisement call, characterized by a single, high-frequency call with non-concatenated pulses, typically emitted in call series by adult males from vegetation overhanging streams (Fig. 3A–B). Call B, the release call, is a single, tonal call composed of two co-dominant frequency bands, emitted during handling (Fig. 3D). The recordings were conducted at air temperatures of 20–22°C and air humidity percentages of 92–100%. See Table 2 for values of spectral and temporal parameters of the calling repertoire of *I. debilis*.

Call A (Fig. 3A–C, E, G) consists of a single call composed by 5–10 concatenated pulses (Fig. 3C). Calls are typically produced in call series that contain between 3–112 calls/series, emitted at intercall intervals of 260–1,030 ms (Fig. 3A–B). The duration of call series ranged from 540–3,345 ms, and the call rate within the series varied from 1–5 calls/s. Call A is amplitude modulated, with the amplitude peak occurring in the last pulses of the call, resulting in a triangular call envelope (pointing left; Fig. 3E). Pulses are amplitude modulated, with the amplitude peak occurring at the beginning or middle of the pulse durations, depicting either a triangular (pointing right) or diamond-shaped call envelope. Call duration ranges from

Table 1. Some measurements (in mm) of tadpoles of *Isthmohyla debilis* (n = 11). See Materials and Methods for abbreviations.

	Stage (n)							
Variable	25 (2)	27	29	35	36 (2)	37	41	42 (2)
TL	19.2–22.4	34	34	32.4	36–37	36.2	37.9	38.4–42
BL	5.2–7.3	9.2	9.8	10.5	9.9–10.2	9.5	10.7	10.3–12
TAL	12–13.1	22.1	22.2	19.9	23.8–25.1	24.7	25.2	26.1–29
MTH	2.9–3.4	4.2	4.3	5.4	5.1–6.1	5.9	5.4	5.7–6.6
IND	2.09–2.18	2.2	2.2	3.2	2–3.3	3.1	2.4	2.8–3.6
IOD	2.65–2.91	4.1	4.2	5.2	4.4–5.7	5.1	5.2	5.3–5.8
TMH	2–2.4	3.7	3.5	4.5	4.6–5.2	4.1	3.7	5.1–6.1
TMW	1.5–1.8	3.2	3.3	3.1	4.1–4.6	3.8	3.8	4.1–4.2
BW	3.73–4.39	5.8	5.9	6.9	6.9–7.6	6.5	7.8	7.5–7.8
BWN	3.2–4.01	5.2	5.6	5.3	5.3–6.1	5.3	4.9	6.2
BWE	3.7–4.1	5.4	5.7	5.9	6.3–6.4	5.9	6.3	6.8–7.3
BH	3.2–3.7	5.4	5.5	5.5	5.9–6.3	5.9	6.3	6.7–6.9
END	0.92–1.01	1.9	1.6	1.8	2	1.8	1.9	2–2.5
NSD	1.9–2.1	3	3	3.2	2.3	2.4	2.6	2.8–3.3
ED	0.5–0.7	1.1	1.3	1.4	1.3–1.5	1.5	1.6	1.6–1.8
ND	0.19–0.2	0.2	0.2	0.3	0.2–0.3	0.3	0.3	0.3
ODW	3.1–3.8	5.9	5.8	5.1	5.4–6	5.5	5	5.8–6
SSD	4.7–6.05	8.7	8.8	8.7	7.6–8.1	7.9	8	8.3–9.2
DFH	0.8–1.3	1.3	1.4	1.6	1.2–1.5	1.8	1.9	1.7–2.4
VFH	0.6–0.9	0.5	0.7	1.4	0.7–1.3	1.6	1.6	1.5–1.8
SL	0.6–0.7	1.4	1.2	1.2	1.6–1.2	1.1	0.6	1.4–1.6
SVD	0.8–1.5	2.5	1.2	1.4	1.1–1.5	1.3	1.1	1.6–2

61–173 ms, and pulse duration varies from 10–20 ms. Pulse rate varies from 39–74 pulses/s. The frequency band has a general upward frequency modulation throughout the call, with the first pulses emitted at a slightly lower frequency than the last ones. However, some calls have no clear general frequency modulation. The minimum frequency of the call is 4,909–5,598 Hz, the dominant frequency is 5,167–5,857 Hz (Fig. 3G), and the maximum frequency is 5,340–5,943 Hz.

Call B (Fig. 3D, F, H) consists of a single, tonal call (Fig. 3D). The call is amplitude modulated, with the amplitude peak occurring at the beginning of the call duration, depicting a call envelope with a triangular shape (pointing right; Fig. 3F). Call duration ranges from 3.3–13.9 ms. The call is composed of two co-dominant frequencies (Fig. 3H): the lowest frequency band ranges from 2,325–2,860 Hz and has a dominant frequency of 2,411–2,497 Hz, while the highest frequency band ranges from 4,392–5,426 Hz, with a dominant frequency of 4,823–4,995 Hz. This call was unintentionally elicited by gently pressing the frogs' flanks and dorsum during handling. We documented the release call during daytime, without inflation of the vocal sac during sound emission.

Natural history.—Calling males of *Isthmohyla debilis* aggregate in vegetation patches near waterfalls and/or within the river, where they call intermittently at a constant rate, often moving short distances between call series (ca. 10–40 cm). Adults of *I. debilis* were heard calling in areas with low water flow and in regions with fast-flowing water. Several observations included groups of multiple calling individuals (2–5) using different leaves and branches at heights exceeding 2 meters above the stream in areas ranging from 1.5–2 m^2. Adults were also found active inside the forest, away from the stream. On 27 June and 12 July 2024, we observed one adult individual 300 meters from the nearest stream at Palo Alto, calling on a leaf at a height of 1.6 m along a small ephemeral streambed, which only holds water after heavy rainfall. The two observations were made approximately 1 meter apart, although it is unclear whether they involved the same individual.

On 17 August 2022, we estimated hearing over 70 calling individuals of *Isthmohyla debilis* throughout the Alemán stream between 1830–2300 h. During this period, 20 males and one gravid female were directly observed (collected males MHCH 3203, 5059, 5061–3; female MHCH 5060). The female was found 1.5 m away from a group of four males, using one vegetation patch near the river's headwaters within a 2 m^2 area at heights of 2–3 m. The female was perched on a rock at stream level, near a pool that had formed in the stream. We monitored the female for two hours, documenting interactions between her and the males. While the males continued calling and moving through the vegetation, we did not observe any movements by the female, nor did we witness any approaches between her and the males. The female contained unpigmented ovarian eggs, as evidenced by dissection.

We found tadpoles of *Isthmohyla debilis* in pools (1 m deep and 1.5 m wide) formed between large rocks and cascades, downstream from the point of our primary observations of the adult frogs at Fortuna. The larvae were mostly active during nighttime when they were observed in groups of several individuals clinging to large rocks against the current using their oral discs. During the day, the tadpoles were found sheltering beneath large rocks and rarely clung to exposed surfaces unless disturbed. On 5 June 2018 and 28 March 2024, we observed recently metamorphosed *I. debilis* froglets, still with tail, on the margins of the Alemán stream, climbing over rocks.

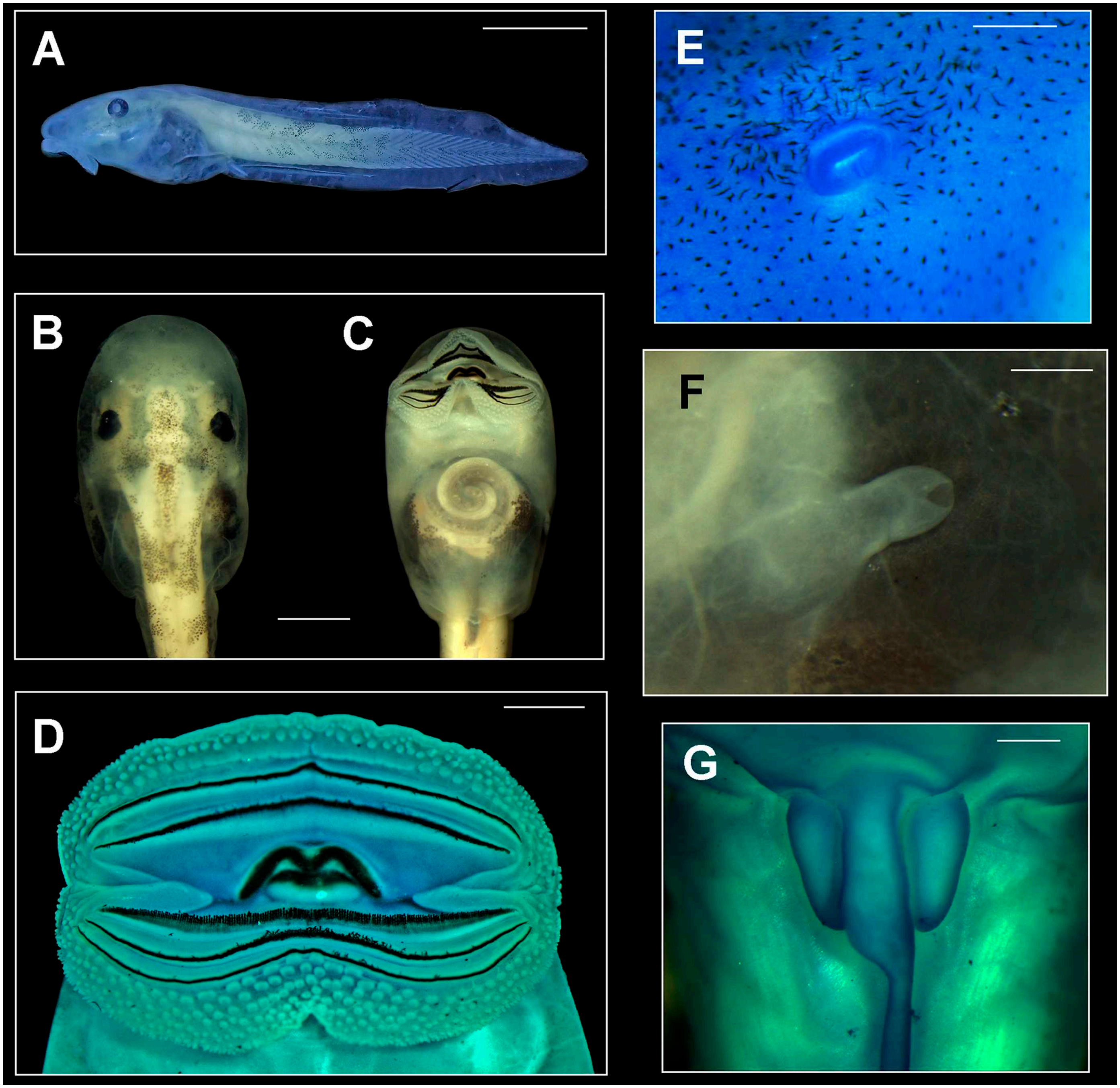

Fig. 2. Tadpole of *Isthmohyla debilis* (MACN 62276). (A–F) Stage 25. (G) Stage 35. (A) Lateral view. (B) Dorsal view of body. (C) Ventral view of body. (D) Oral disc. (E) Nostril. (F) Spiracle. (G) Vent tube (stage 35).

Color variation.—In adult males, the dorsal coloration varies significantly, ranging from lighter and darker shades of green and blue to olive tan and dark grayish or light brown (Figs. 4–5). Some individuals have scattered flecks on the dorsum, which may be uniformly grayish or green-colored or show darker patterns of brown or black. During the day, the dorsal color shows metachrosis, presenting lighter tones compared to the darker hues observed at nighttime. The snout is typically colored like the dorsum and usually exhibits a brown canthal stripe, though in a few individuals, the stripe is light colored and almost matches the dorsal color. A continuous white or creamy yellow labial stripe is present, but in some individuals it is incomplete. The suborbital spot varies in coloration, size, and position, ranging from creamy yellow to white, from wide to discrete, and starting from either the anterior or posterior part of the eye. In the hands, the dorsal surface of digits III and IV is brown colored, while digits I and II are not. In the feet, the dorsal surface of the first three toes is yellow, while digits IV and V are not. The flanks may be outlined medially by a white or yellow stripe that extends from the posterior part of the eye or posteriorly to the supratympanic membrane and onto the inner part of the groin, but in some individuals the flanks are colored like the dorsum. Most specimens show a row of discrete white tubercles on the outer edge of the forearm and a white stripe on the outer edge of the tarsus and above the cloacal opening, but in

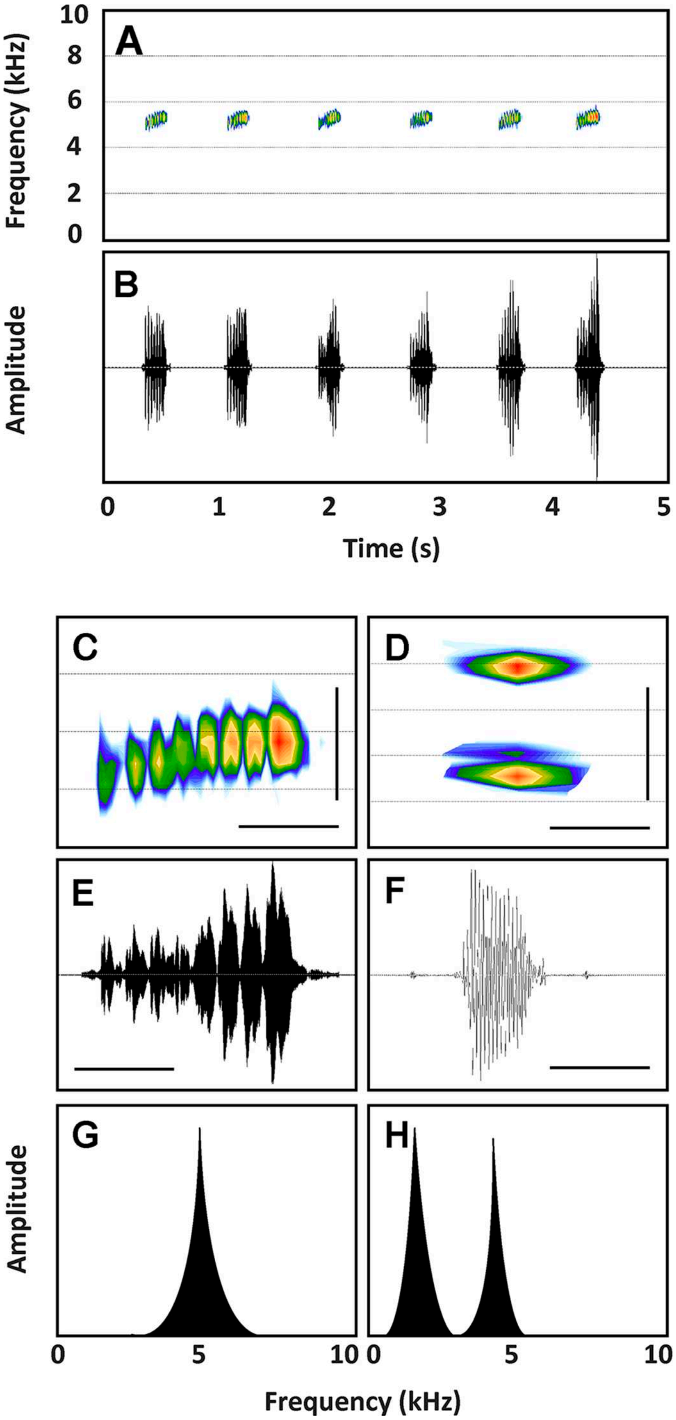

Fig. 3. Vocalizations of *Isthmohyla debilis* (MHCH 5058) recorded at Fortuna, Panama. (A, C, D) Spectrograms. (B, E, F) Oscillograms. (G, H) Power spectra. (A, B) Series with six advertisement calls (type A) emitted in five seconds. (C, E, G) Detail of advertisement call (type A; recording FNJV 0124935). (D, F, H) Detail of release call (type B; recording FNJV 0124943). Scale bar = 100 ms (horizontal), 1,000 Hz (vertical).

some individuals these markings were not evident. Cloacal tubercles vary from being white to yellow. The posterior and anterior parts of the legs are uniformly dull yellow or orange. Flanks are adorned with distinct, bright yellow blotches in the groin. The venter is uniformly white or creamy white, and the vocal sac is generally translucent in males, although some individuals may have a less translucent whitish appearance (Fig. 4H–I). In females, the throat is colored as the venter. Recently metamorphosed specimens have a dark greenish-brown dorsum with a yellowish dorsolateral stripe starting at the nostril, extending over the eye, and continuing along the arm to cover the entire whitish-colored flank (Fig. 4K–L).

DISCUSSION

Confusion.—Our results indicate that the larvae described by Duellman (1970) as *Isthmohyla debilis* were misidentified; they were included in a tadpole lot that contained larvae of both this species and a different species. For the description and illustration, the larvae of the second species were selected (see figs. 128C and 129C in Duellman, 1970). Consequently, the description of the tadpole attributed to *I. debilis* actually corresponds to that second species, which does not include the diagnostic characters of stream-breeding species with enlarged oral discs in the *I. tica* group. Interestingly, in 1976 in Fortuna, C. W. Myers collected four larvae in stages 26–35 that he identified as *I. debilis* (AMNH A-94902; D. Dickey, pers. comm.), which exhibit an overall morphology consistent with the tadpole described here (see Fig. 6), contrasting with the description provided by Duellman (1970). Similarly, Jungfer (1988) provided additional observations on the species' larvae, noting similarities with those of torrent-dwelling species like *I. rivularis*. However, these findings were overlooked in later studies (Duellman, 2001; Savage, 2002; Faivovich et al., 2018).

The larvae identified as *Isthmohyla debilis* by Duellman (1970) share similarities with those collected at Cerro Pando and identified as *I. lancasteri* (KU 104254) in the same work. These include a small oral disc whose width is almost half the maximum body width, abundant submarginal papillae in angular areas, one or two distinct rows of large submarginal papillae in both labia that extend throughout the angular area and onto the end of labial teeth rows, A-1 medially interrupted, an arch-shaped and serrated upper jaw sheath, and a spiracle with its opening located on the second third of the body and with the inner wall fused to the body.

Larvae of *Isthmohyla lancasteri* collected in Costa Rica and studied here (see Materials and Methods) show a similar configuration in terms of the submarginal papillae to that described for lots KU 104239 and KU 104254 (excluding larvae of *I. debilis*), although with generally smaller papillae and shorter rows of papillae in both labia, which are concentrated proximal to the angular area. Additionally, the larva of *I. lancasteri* has lateral processes of the upper jaw sheath that extend posteriorly onto the lower jaw sheath and which are oriented toward the lower tooth rows. However, in larvae of the aforementioned lots, the lateral processes of the upper jaw sheath extend posteriorly onto the lower jaw sheath and are oriented toward the angular areas. Moreover, the interruption of both upper tooth rows was only noted in lot KU 104254 and, coincidently, in some specimens of lot KU 104239 (see Duellman, 1970). Duellman (2001) suggested that larvae from lot KU 104254 are more similar to the description of *I. calypsa* provided by Lips (1996) rather than *I. lancasteri* (see also Starrett, 1960). We find it possible, however, that these larvae and those misidentified as *I. debilis* from lot KU 104239 correspond to the Panamanian lineage of *I. lancasteri* identified by Faivovich et al. (2018).

Table 2. Acoustic parameters of the vocalizations of *Isthmohyla debilis* recorded at Fortuna, Panama. Air temperature and air humidity percentage were 20–22°C and 92–100%, respectively. Values are reported as mean ± SD (range). Advertisement calls are deposited in the FNJV under catalog numbers 0124935–0124942; the release call is archived under FNJV 0124943.

Call parameter	Call A (advertisement)	Call B (release)
Males recorded	8	2
Analyzed calls	650	6
Call duration (ms)	120 ± 10 (61–173)	10 ± 6 (3.3–13.9)
Pulse duration (ms)	12 ± 2 (10–20)	–
Minimum frequency (Hz)	5,353 ± 118 (4,909–5,598)	2,368 ± 50 (2,325–2,860)
Maximum frequency (Hz)	5,690 ± 108 (5,340–5,943)	5,060 ± 108 (4,392–5,426)
Dominant frequency (Hz)	5,562 ± 113 (5,167–5,857)	3,079 ± 1,162 (2,411–4,995)
Number of pulses	7 ± 1 (5–10)	–
Intercall within series (ms)	470 ± 150 (260–1,030)	–
Pulse rate (pulses/s)	59 ± 7 (39–74)	–
Call series duration (ms)	10,170 ± 9,030 (540–33,450)	–
Number of calls per series	21 ± 22 (3–112)	–
Call rate within series (calls/s)	2 ± 1 (1–5)	–

A clade of suctorial larvae in the* Isthmohyla tica *group.—Our findings clarify and resolve the ambiguities noted by Faivovich et al. (2018) regarding the putative larval phenotypic synapomorphies of the clade composed by stream-breeding species with enlarged oral discs within the *Isthmohyla tica* group. Specifically, we corroborate that this clade is supported by the following putative synapomorphies: depressed body with a sloping snout; a thick, protruded nasal rim surrounding the nostril; a spiracle with its opening located on the posterior third of the body and with the inner wall completely free from body; and an enlarged oral disc that is nearly as wide as the body, with deep angular folds, three or more rows of submarginal papillae in the upper and lower labia, an M-shaped upper jaw sheath and LTRF 2/3 (see also Faivovich et al., 2005, 2018).

In the context of the phylogenetic relationships of *Isthmohyla* (Faivovich et al., 2018; Chaves-Acuña et al., 2024), another character state, the complete marginal papillae, optimizes ambiguously. This occurs as well in larvae of the presumably closely related *I. calypsa* and *I. lancasteri* (Lips, 1996) and in the species of the *I. zeteki* group, *I. picadoi* (Robinson, 1977; Savage, 2002; but see Duellman, 2001) and *I. zeteki* (Dunn, 1937; Starrett, 1960; Duellman, 1970); there is an anterior gap in the marginal papillae in larvae of *I. angustilineata* and *I. graceae* (Duellman, 1970; Myers and Duellman, 1982), and in the *I. pseudopuma* group (Duellman, 1970).

Most of the putative synapomorphies involve larval character states associated with riparian habitats. Submarginal papillae are also present in both labia in tadpoles of many of the genera of the Hylini that have complete marginal papillae, but in larvae of the clade of the *Isthmohyla tica* group with enlarged oral discs, the submarginal papillae in both labia are arranged in three or more rows, and in the upper labia, the lower row has enlarged, quadrangular papillae above A-1 (Duellman, 1970; see also fig. 3D in Chaves-Acuña et al., 2020). Similar submarginal papillae are illustrated for other species of the Hylini in Duellman (1970), but a more comprehensive morphological assessment is needed to better understand the variation of these character states in a taxonomic context.

In the clade of the *Isthmohyla tica* group with enlarged oral discs, the upper jaw sheath is particularly distinctive, featuring a highly pronounced M-shaped configuration that is unique within Hylini. This sheath displays a central ridge that extends upward and then bends downward toward the lateral edges of the oral disc, forming a sharply defined M-shape. Altig and Johnston (1989), using *I. pictipes* and *I. tica* as an example, hypothesized that the M-shaped upper jaw sheath acts as a working surface that adds strength, retards warping, and increases working surface length in slower and faster water conditions, potentially aiding in feeding by plowing material toward the mouth. A similar M-shaped upper jaw sheath, or with some differences in the level of keratinization or shape, has also been documented in lotic larvae of a few anuran clades in the Bufonidae, Odontobatrachidae, pelodryadine hylids, and Ranidae (Channing et al., 2012; Hirschfeld et al., 2012; Anstis, 2013; Doumbia et al., 2018; Arifin et al., 2021). The upper jaw sheath of some mantellids could also possibly be described as M-shaped, but these are also notably slender and occur in tadpoles that are not associated with high gradient steams but live among dead leaves in the bottom of slow-flowing streams (Randrianiaina et al., 2011).

In this paper, we employed the term angular fold to describe the deep folds that delimit a raised area in the angular region of the oral disc that extends medially from the lateral margins of A-2 and P-1 to shortly before the lateral margins of the upper jaw sheath. These structures were also clearly illustrated in tadpoles of *Isthmohyla pictipes*, *I. rivularis*, and *I. tica* by Duellman (1970). Similarly, among other larvae with 2/3 LTRF, they occur in the enlarged oral discs of *Plectrohyla hartwegi* and *P. teuchestes*, as evidenced in the figures provided by Duellman and Campbell (1992). The angular folds need further study to clarify their structure and potential contribution in the adherent process of the enlarged oral disc.

The thick, protruded nasal rim surrounding the small nostril that we describe in the larvae of *Isthmohyla debilis* deserves comment. Although elevated rims or papillae are relatively common in adherent and suctorial tadpoles (e.g., Noble, 1927; Channing et al., 1988; Altig and McDiarmid, 1999a; Sánchez, 2010), these have not been noticed in previous descriptions of larvae of *Isthmohyla* (Dunn, 1937; Starrett, 1960, 1966; Duellman, 1970, 2001; Robinson, 1977;

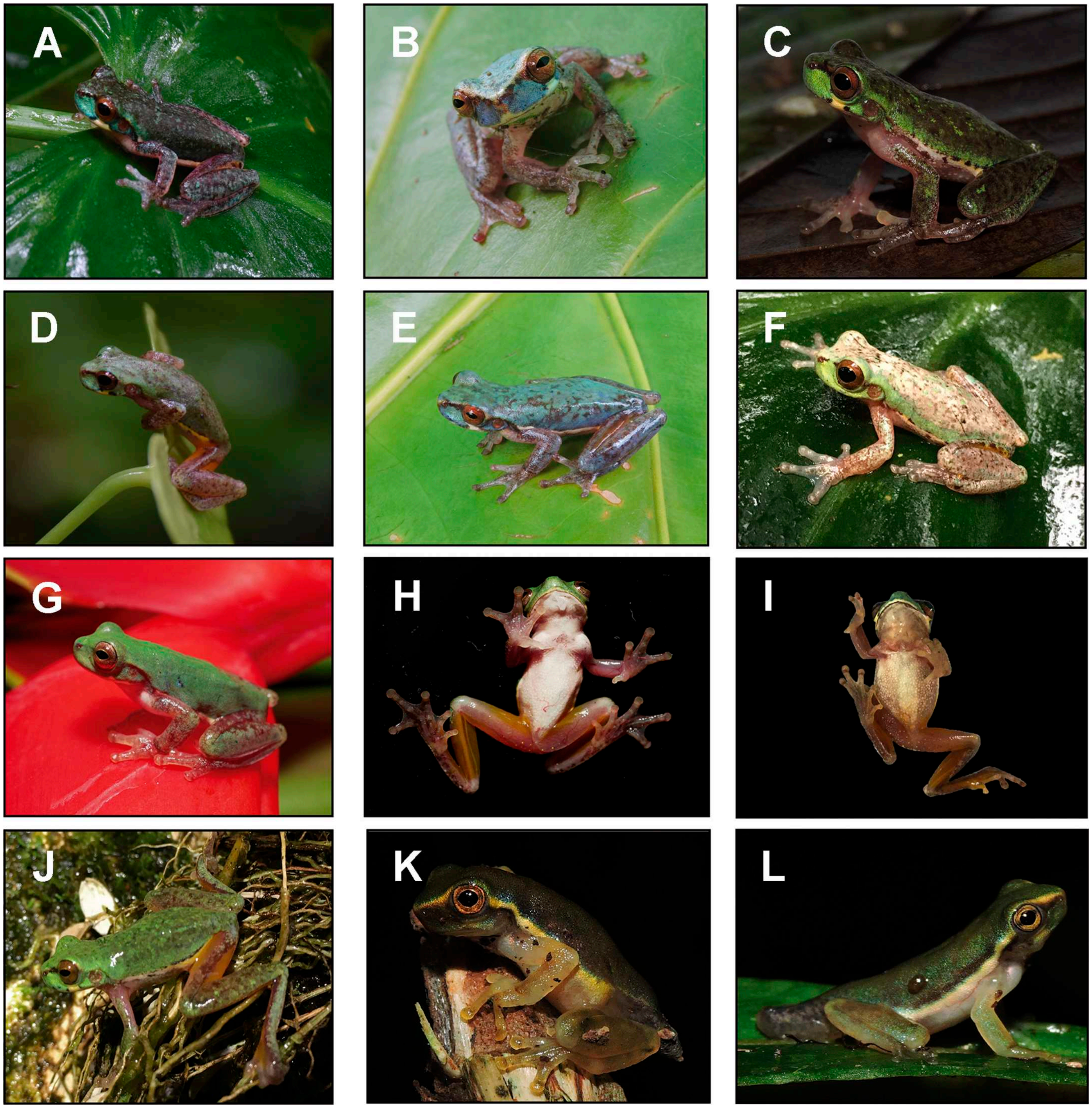

Fig. 4. *Isthmohyla debilis*, color variation in adults and recently metamorphosed individuals from Fortuna, western Panama. All adults are males, except for MHCH 5060 (female). (A) MHCH 5058. (B) MHCH 5067. (C) MHCH 5065. (D) MVUP 2740. (E) MHCH 5066. (F) MHCH 5062. (G–H) MHCH 3203. (I) MHCH 5061. (J) MHCH 5060. (K–L) Recently metamorphosed individuals (not collected).

Myers and Duellman, 1982; Lips, 1996). Our observations on larvae of *I. pictipes*, *I. rivularis*, and *I. tica* indicate that these have the same nostril morphology that we describe for *I. debilis*. Functional studies considering the elevated rims in larval nostrils are very limited (Gradwell, 1971, 1973) and pertain specifically to *Ascaphus*, which also has a peculiar tubular rim (Noble, 1927). The functional role, if any, of elevated rims and papillae in the nostrils remains uncertain in most tadpoles.

***Acoustic variation in* Isthmohyla debilis.**—Duellman (1970) described the mating call of *Isthmohyla debilis* as a single-pulse, "cricket-like" chirp composed of 5–7 notes in a call group, with durations ranging from 100 to 160 ms and a dominant frequency around 5,235 Hz. We have redefined this call as Call A, the advertisement call in our study. Using a call-centered approach (Köhler et al., 2017), we find that Call A comprises 5–10 concatenated pulses per call, with a call duration of 61–173 ms and a dominant frequency range

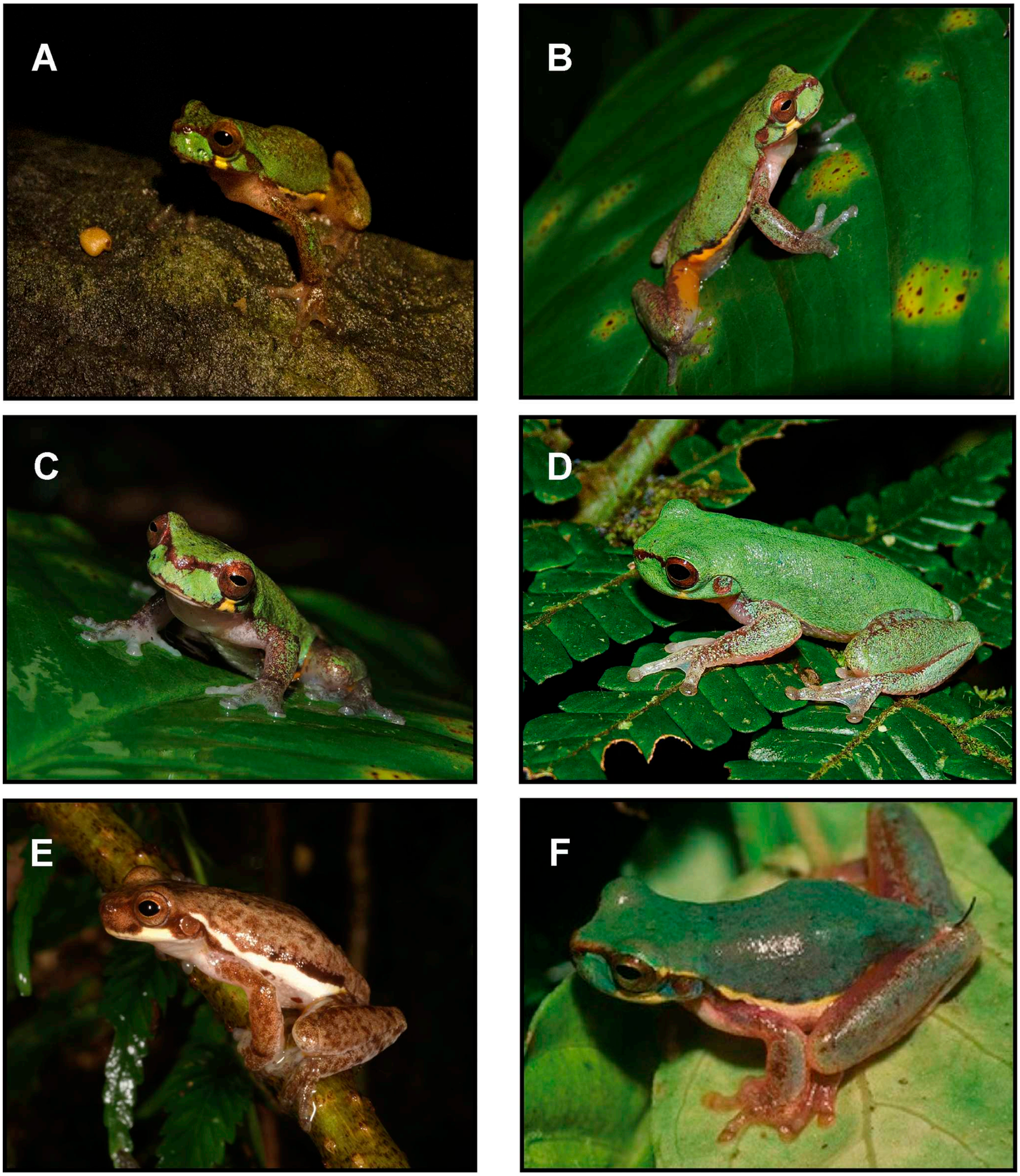

Fig. 5. Color variation in adults of *Isthmohyla debilis* from other populations. (A–C) Palo Alto, Boquete District, western Panama. (D–E) Comarca Ngäbe-Buglé, Llano Tugri District, western Panama. (F) Cerro Chompipe, Heredia Province, central Costa Rica. (A) MHCH 5075. (B–C) Adult male (not collected). (D) Adult male (not collected). (E) Adult male, SMF 89835. (F) Unsexed individual (not collected), observed by a third party. Photo provided by Alejandro Acuña; photographer unknown.

of 5,167–5,857 Hz. This represents a broader and more variable frequency range than Duellman's (1970) earlier measurements. Additionally, we describe the release call (Call B) of *I. debilis*, characterized by a tonal emission with two co-dominant frequency bands (2,411–2,497 Hz and 4,823–4,995 Hz) and duration of 3.3–13.9 ms. In the majority of our findings of *I. debilis*, individuals were calling from vegetation at heights exceeding 2 meters (see also Jungfer, 1988), differing from the lower heights (10 cm to 1 meter) reported by Duellman (1970). Based on the observations of Jungfer

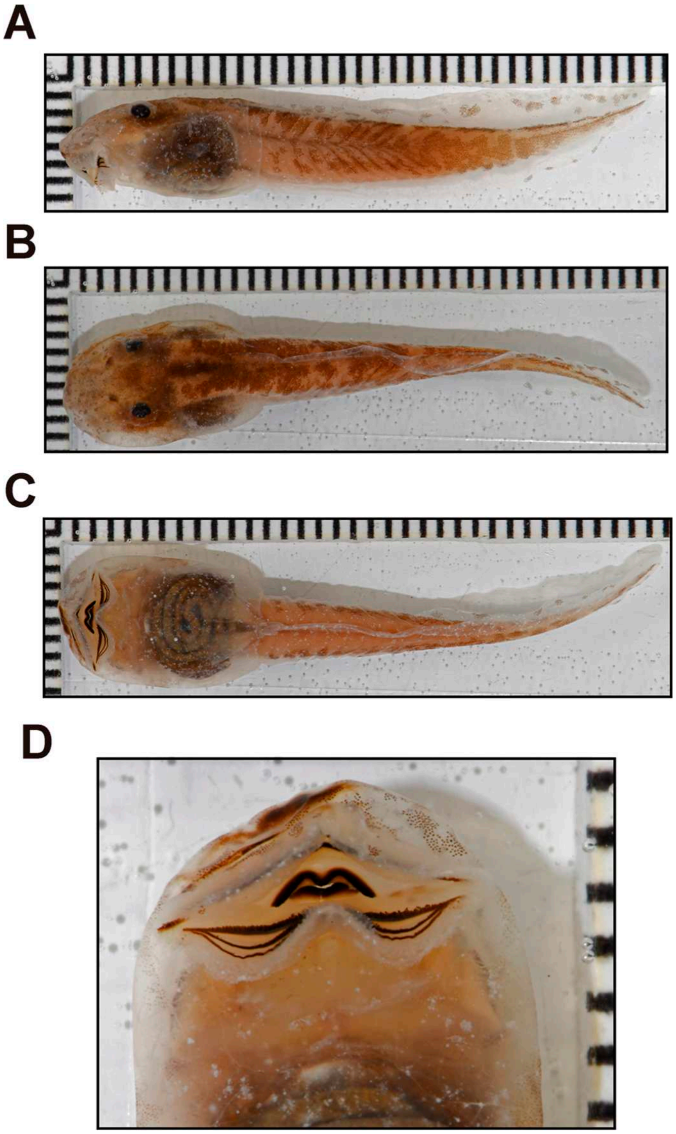

Fig. 6. Larvae of *Isthmohyla debilis* (AMNH A-94902), collected in Fortuna by Charles W. Myers in 1976. (A–D) Tadpole in stage 27. (A) Lateral view. (B) Dorsal view. (C) Ventral view. (D) Oral disc. Scale bars are divided by mm. Photos by Lauren Vonnahme, copyright American Museum of Natural History (used with permission).

(1988) under captive conditions, the species can also vocalize during the day.

***Polymorphism and color patterns in* Isthmohyla debilis.**—The species of *Isthmohyla* have been historically differentiated on the basis of coloration patterns (Duellman, 1970, 2001; Savage, 2002). However, polymorphism is common among species of the genus, and certain color patterns, as currently understood, may not have taxonomic significance in some species (Duellman, 1970, 2001; Myers and Duellman, 1982; Chaves-Acuña et al., 2020).

Despite the range of color variation noted in *Isthmohyla debilis* (Savage and Heyer, 1969; Duellman, 1970; Myers and Duellman, 1982; Hertz et al., 2012; see Figs. 4–5), certain color patterns were consistently observed across all adult individuals. These include a white or yellow suborbital spot (also present in *I. calypsa* and *I. lancasteri*) that varies in size and position, ranging from wide to discrete, and originating from either the anterior or posterior part of the eye; brown canthal stripe (shared with *I. xanthosticta* and some specimens of *I. pictipes*); brown-colored tympanum (diagnostic of *I. debilis*); translucent vocal sac (shared with *I. tica*); flanks medially demarcated by a light-colored line (diagnostic); bright yellow blotch present on the inner side of the groin (shared with *I. tica* and *I. lancasteri*, but with black blotches in the latter); uniformly light venter (occurring in some specimens of *I. pictipes*, and in *I. graceae* and *I. xanthosticta*, as well as those species in the *I. pseudopuma* and *I. zeteki* groups); and uniformly yellowish or orange hidden surfaces of the legs (shared with *I. rivularis* and *I. tica*).

Conservation and distribution.—In the mid 1990s, Lips (1999) documented amphibian declines at Fortuna and noted that numerous riparian species, including *Isthmohyla debilis*, were being affected by chytridiomycosis. This fungal disease was found to kill adults through skin infections and cause mouth deformities in larvae (Berger et al., 1998; Lips, 1999). Around the same period, reports of dead amphibians and low-amphibian detectability emerged from Cerro Pando (D. Green and E. Lindquist, pers. comm. in Lips, 1999) and adjacent areas (Lips, 1998). *Isthmohyla debilis* was considered as possibly extinct in the 2004 assessment of the International Union for the Conservation of Nature (Solís et al., 2004). However, Hertz et al. (2012) noted the presence of the species on the western and eastern slopes of Cerro Santiago, Comarca Ngäbe-Buglé in the Serranía de Tabasará, western Panama (see Fig. 4K). Despite intensive fieldwork efforts, *I. debilis* was not observed at Fortuna for many years. The first verbal reports among scientists of sightings at Fortuna emerged in 2016 (IUCN, 2025). Since then, the number of observed individuals at the site has been increasing, suggesting a recovery of the species. A mucosome growth inhibition assay on *Batrachochytrium dendrobatidis* (Bd) zoospores (Woodhams et al., 2014) was performed using mucosome samples from two individuals collected at Fortuna in 2016. Both individuals were Bd-negative, and their mucosome exhibited a slight inhibitory effect on Bd growth (AH, unpubl. data). Further tests with additional individuals are needed to better understand the species' recovery and the dynamics of the disease.

In this paper, we report another extant population of *Isthmohyla debilis* in the area of the Boquete District (MHCH 5075, adult male collected), complementing a previous tentative record of the species from the same area (UMMZ 69496), which could not be confirmed by Duellman (1970) because most of the pigment of this specimen was faded. Additionally, this record complements recent observations documented through community science platforms, such as the iNaturalist platform (https://www.inaturalist.org), further supporting the presence of this species in the Boquete area. Despite its vulnerability, *I. debilis* continues to be underreported, likely due to its low detectability (i.e., small size and barely audible call) and thus field reports may underestimate its abundance. The species has not been reported in central Costa Rica since the 1960s (Savage and Heyer, 1969; IUCN, 2025; VertNet, 2025), although an individual was registered in 2011 by a group of naturalist guides in Cerro Chompipe, Heredia Province, central Costa Rica (A. Acuña, pers. comm., see Fig. 4L). Given the ongoing environmental pressures, including climate change, habitat destruction, and diseases, regular field surveys are essential for understanding the full distribution of the species and to better assess its needs for conservation.

DATA ACCESSIBILITY

AI STATEMENT

The authors declare that no AI-assisted technologies were used in the design and generation of this article and its figures.

ACKNOWLEDGMENTS

We thank Florencia Vera-Candioti, Pedro H. Dias, Tiago Pezzuti, and Martín O. Pereyra for comments and discussion. Evidelio García, Carlos Espinoza, and Ignacio Del Cid provided assistance and help during fieldwork. Rafe Brown, Richard Glor, Ana Motta, and Camila Meneses (University of Kansas), David Kizirian, David Dickey, and Lauren Vonnahme (Amerian Museum of Natural History), Katyuscia Araujo-Vieira (Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"), and Gerardo Chaves and Federico Bolaños (Universidad de Costa Rica) kindly shared photographs of museum specimens. Lucas A. Minino shared with us his database of anuran larval morphology, which greatly assisted in the discussion. The Cornell Lab of Ornithology kindly provided a free license of Raven Pro software.

For funding, we thank ANPCYT (PICT 346/2019, 59/2021), CONICET (PIP2800), FAPESP (Grants # 2018/15425-0, 2021/10639-5), the Mohamed Bin Zayed Species Conservation Fund (Projects 210526640, 242534066), the Smithsonian Tropical Research Institute Short Term Fellowship (Project 4948), and the Theodore Roosevelt Memorial Fund of the American Museum of Natural History. We thank IDEA WILD for supporting this research. AH was financially supported by a German Science Foundation (DFG) research grant (HE 7562/1-1) and the UMass Boston office of Global Programs. This study was conducted under collecting permits ARB-032-2021 and ARB-092-2024, approved by the STRI Animal Care and Use Committee (ACUC; protocol SI-20003), and complied with the bioethics protocol approved by the Ethics and Animal Welfare Committee of the University of Panama (protocol CEIBA-UP-047-2024).

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