Honoring the Afro-Colombian musical culture with the naming of *Epipedobates [to be revealed]* sp. nov. (Anura: Dendrobatidae), a frog from the Pacific rainforests

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Abstract

The number of amphibian species described yearly shows no signs of slowing down, especially in tropical regions, implying that the biodiversity of amphibians remains woefully underestimated. We describe a new species of poison frog from the Pacific lowlands of southwestern Colombia: *Epipedobates [to be revealed]* sp. nov., named for the Pacific music and dance genre known as *bambuco viejo* or *[to be revealed]*. This species inhabits lowland forests from 0–260 m. This taxon differs from congeners by having a combination of yellow blotches in the dorsal anterior region of the thigh and upper arm, homogenous dark-brown dorsal coloration, and advertisement calls of long duration and many pulses. We also describe the courtship call of *E. [to be revealed]*, which is lower in frequency and shorter in duration than the advertisement call. Molecular phylogenetic analyses confirm the monophyly of the populations sampled and its position as the sister-group of *Epipedobates narinensis*, which occurs in southwestern Colombia. Among species of *Epipedobates*, the new species has been previously confused with *E. boulengeri*, but we find that the two species are allopatric and represent two divergent clades (1.77% divergent for *12S–16S* and 5.39% for *CYTB*). These species can be distinguished by the presence of a bright yellow blotch in the dorsal anterior region of the thigh in *E. [to be revealed]*, which is absent or diffuse and pale or cream in *E. boulengeri*. *Epipedobates [to be revealed]* is the most northern species of *Epipedobates*, which extends southwards along the western edge of the Andes. Known as the Chocó, this biogeographic region has been largely converted to agriculture in Ecuador and is experiencing widespread transformation in Colombia, which may endanger *E. [to be revealed]* and biodiversity in the region. This version is a preprint, and we only intend to name the species in the final published version.

Keywords: bioacoustics, Chocó, music, megadiverse, poison frogs, new species, alpha taxonomy, DNA barcoding.

Resumen

El número de especies de anfibios descritas cada año continúa aumentando, especialmente en las regiones tropicales, lo que implica que la biodiversidad de anfibios sigue siendo subestimada. Describimos una nueva especie de rana venenosa de las tierras bajas del Pacífico del suroccidente de Colombia: *Epipedobates [to be revealed]* sp. nov., nombrada así por el género de música y danza del Pacífico conocido como *bambuco viejo* o *[to be revealed]*. Las ranas de esta especie habitan en bosques de tierras bajas desde el nivel del mar hasta los 260 m. Este taxón se diferencia de sus

congéneres por tener una combinación de manchas amarillas en la región dorsal anterior del muslo y los brazos, una coloración dorsal homogénea marrón oscuro y cantos de advertencia más largos y en consecuencia con mayor número de pulsos. También describimos la llamada de cortejo de *E. [to be revealed],* con menor frecuencia pico y duración que la llamada de advertencia. Los análisis filogenéticos confirman la monofilia de la especie y su posición como hermana de *Epipedobates narinensis*, la cual se distribuye en el suroccidente de Colombia. Entre las especies de Epipedobates, la nueva especie ha sido previamente asignada a *E. boulengeri,* pero las dos especies son alopátricas y representan dos clados filogenéticamente divergentes (1.77% divergentes para *12S–16S* y 5.39% para *CYTB*). Estas especies se pueden distinguir fenotípicamente por la presencia de una mancha amarilla brillante en la región dorsal anterior del muslo en *E. [to be revealed]*, que está ausente o difusa y pálida o crema en *E. boulengeri. Epipedobates [to be revealed]* es la especie distribuida más al norte del género *Epipedobates*, el cual se extiende hacia el sur a lo largo del flanco occidental de la cordillera de los Andes. Esta región conocida como el Chocó biogeográfico, ha sido fuertemente transformada por agricultura en Ecuador y está experimentando una transformación generalizada de sus bosques en Colombia, lo cual pone en peligro a *E. [to be revealed]* y toda su biodiversidad en un futuro cercano.

INTRODUCTION

Epipedobates sensu is a taxon of neotropical poison frogs (Anura: Dendrobatidae) with nine putative species (Grant et al. (2006, 2017) and López-Hervas et al. (2023): *Epipedobates* aff. *espinosai* (see López-Hervas et al. 2023)*, E. anthonyi* (Noble 1921), *E. boulengeri* (Barbour 1909), *E.* sp. 1 (see López-Hervas et al. 2023)*, E. espinosai* (Funkhouser 1956), *E. machalilla* (Coloma 1995), *E. maculatus* (Peters 1873)*, E. narinensis* Mueses-Cisneros et al. 2008, and *E. tricolor* (Boulenger 1899). Frogs of the genus *Epipedobates* inhabit dry and humid tropical forests from 0 to 1800 m across the Pacific lowlands and foothills of the western side of the Andes of Colombia, Ecuador, and northern Peru (Grant et al., 2006, 2017). A recent assessment of genetic and phenotypic diversity in *Epipedobates* found several distinct evolutionary lineages with similar morphology, i.e., cryptic species (López-Hervas et al. 2023). It is thus no surprise that prior phylogenetic studies, which sampled different, deeply diverged cryptic species that were thought to belong to a single species at the time, failed to resolve phylogenetic relationships at the species level (Clough & Summers, 2000; Grant et al., 2017; Santos et al., 2014; Tarvin et al., 2017; Vences et al., 2003).
Previous studies of *Epipedobates boulengeri* found high genetic diversity and interpopulation variation in acoustic traits and larval morphology, indicating that *Epipedobates boulengeri* was likely a species complex (López-Hervas et al., 2023; Lötters et al., 2003; Santos et al., 2009; Tarvin et al., 2017). The most recent phylogenetic assessment (López-Hervas et al., 2023) found that *Epipedobates boulengeri sensu lato* contained representatives of four genetically distinct lineages: *E. boulengeri* (*sensu stricto*), distributed in southwestern Colombia and the northwestern edge of Ecuador; *E. espinosai,* distributed in central-western Ecuador; *E.* sp. 1, distributed in northwestern Ecuador and up into the southern end of the Andean foothills of Colombia; and *E.* aff. *espinosai*, distributed in central-western Ecuador (López-Hervas et al., 2023). In prior assessments of phenotypic diversity of this species, Lötters et al (2003) found differences in temporal characteristics of the advertisement call between specimens referred to as *E. boulengeri* from Colombia (Anchicayá, Valle del Cauca, corresponding to *E.* sp. 1) and *E. boulengeri* from Ecuador (Lita, Imbabura, corresponding to *E.* aff. *espinosai*), while Anganoy-Criollo and Cepeda-Quilindo (2017) described the external morphology of the tadpoles of *E. boulengeri* from Colombia (Tumaco, Nariño, possibly representing *E. boulengeri sensu stricto*) and from Ecuador (Carchi, Esmeraldas, Imbabura, and

Santo Domingo provinces, identification uncertain, possibly corresponding to *E.* aff. *espinosai*, *E. boulengeri sensu stricto*, or *E. espinosai*). Although they only examined tadpoles from the southern half of the species distribution area of *E. boulengeri* (i.e., excluding the lineage described as *E.* sp. 1 by López-Hervas et al bioRxiv), they found that the Ecuadorian populations differed from those of Colombia in body shape, tips of marginal papillae, and pattern of tail coloration. Thus, previous evidence pointed strongly to *E. boulengeri* as a species complex.

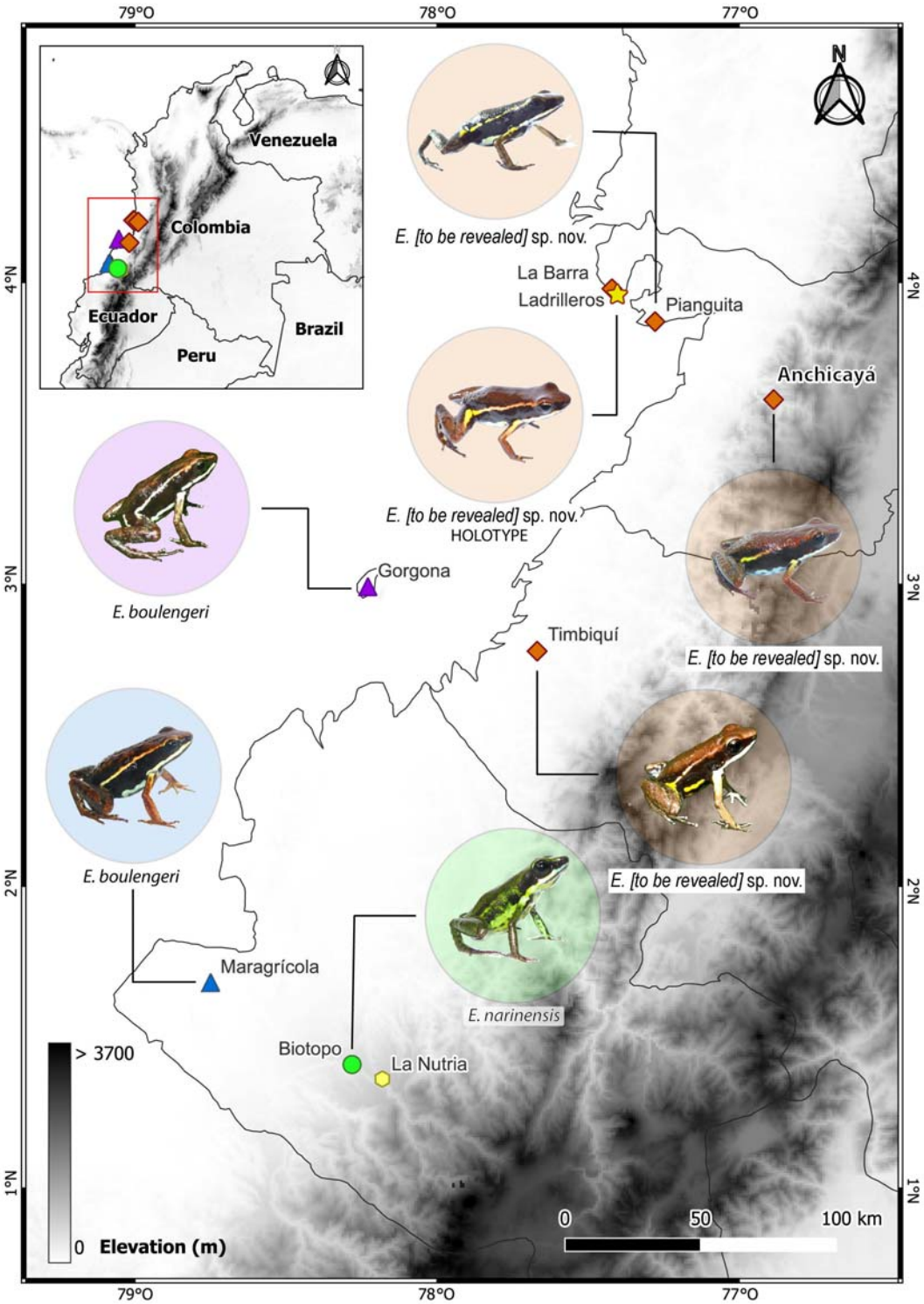

Figure 1. Map of research area. The southern half of the Pacific lowlands of Colombia, part of the Chocó biogeographical region, hosts at least three species of *Epipedobates*. Photograph of the frog from Timbiquí (Reserva Ranita terribilis, Fundación ProAves de Colombia) was obtained from iNaturalist (observation No. 135253843 by Juan Carlos Luna); other images were taken by the authors.

In this study, we describe a new species of *Epipedobates* from the Colombian Pacific lowlands corresponding to *E.* sp. 1 as assigned by López-Hervas et al. (bioRxiv). Using a combination of acoustic, morphological, and molecular analyses, we verify that this frog is a distinct and previously unrecognized species. We name the new species *Epipedobates [to be revealed]* sp. nov. and provide a detailed description of its calls, morphology, behavior, and natural history. As with many other amphibian species, *Epipedobates [to be revealed]* sp. nov. is under threat from habitat loss, pollution, and climate change (Myers et al., 2000; Powers & Jetz, 2019; Warren et al., 2013). By identifying and describing this new species, we can better understand the diversity, evolution, geographic distribution, habitat requirements, and conservation needs of *Epipedobates* species in Colombia. We hope that our findings will stimulate further research and conservation efforts to protect the unique and irreplaceable amphibian biodiversity of the Chocó biogeographical region.

METHODS

Ethics statement

Scientific collecting procedures involving live animals followed protocols approved by the IACUC at the University of Texas at Austin (AUC-2012-00032), the University of California, Berkeley (AUP-2019-08-12457), and *CICUAL* of the Universidad de los Andes (POE 18–003). Research and field collection of samples were authorized by the Autoridad Nacional de Licencias Ambientales (ANLA) of Colombia under the *permiso marco resolución* No. 1177 to the Universidad de los Andes. Specimens of *E. boulengeri* from the type locality were collected under Res. 061-2016 by Parques Nacionales Naturales de Colombia. Samples were exported under the following permits: CITES No. CO39282, CO41443, CO46948, and ANLA No. 00561.

Scientific collecting

We collected genetic, morphological, and acoustic data, as well as whole specimens, of different species and populations of *Epipedobates* in Colombia (Fig. 1; see also López-Hervas et al. 2023) during 2014, 2016 and 2022. Animals were euthanized by an overdose of lidocaine. Sex for most of the specimens was determined at the time of collection based on whether individuals were calling or not and was confirmed by direct examination of the gonads after euthanasia. Before fixation, liver and muscle samples were taken for molecular genetic analyses and stored in 95% ethanol. In some cases, skin was removed for quantification of defensive alkaloid content (see Suppl. material 1). Voucher specimens were fixed in 10% neutral buffered formalin and transferred directly to 70% ethanol for long-term storage in the amphibian collection of the Museo de Historia Natural C.J. Marinkelle at the Universidad de los Andes (ANDES:A) and the Herpetology Collection at the Museum of Vertebrate Zoology at the University of California, Berkeley (MVZ:Herp). As holotype and paratypes we designate individuals that were collected in August 2022 from Ladrilleros, Buenaventura, Valle de Cauca, Colombia (see Table 1).

Morphology

Following previous descriptions of new *Epipedobates* species (Cisneros-Heredia & Yánez-Muñoz, 2010; Mueses-Cisneros et al., 2008), we took 13 standardized morphometric measurements after fixation (Watters et al., 2016): Snout-vent-length (SVL); forearm length (FAL), taken from tip of the flexed elbow and the proximal edge of the palmar tubercle; hand length (HaL), measured from the proximal edge of the large medial palmar tubercle to the tip of the longest (third) finger; tibia length (TL), taken from the outer surface of the flexed knee to the heel inflection; foot length (FL), taken from

the proximal edge of the external metatarsal tubercle to the end of the fourth toe; head width (HW), taken between the angles of the jaws; head length (HL), distance from the tip of the snout to the angle of the jaw; distance from the center of one nares to the anterior edge of the eye (NED); eye width (EW); eye-nares distance (END); internarial distance (IND); interocular distance (IOD); and horizontal diameter of the tympanum (TD). All measurements were taken under a dissecting microscope using a digital caliper at a resolution of 0.01 mm. We conducted a Kruskal-Wallis test to compare body size (snout-vent-length) across species and a post-hoc paired comparison using a Wilcoxon rank-sum test. We also conducted an ANOVA to assess sexual dimorphism in body size, previously checked its assumptions.

We also reviewed and summarized species-level phenotypic variation as described in López-Hervas et al. (2023) to provide a species-level assessment of color pattern variation that can be used to distinguish species.

Molecular and phylogenetic analyses

DNA was extracted from liver and muscle tissue using the Qiagen DNeasy Blood & Tissue kit (Valencia, CA) following the manufacturer's protocol. We sequenced two mitochondrial gene fragments from 5 individuals of *Epipedobates [to be revealed]* sp. nov. collected from the type locality: a fragment including parts of the 12S and 16S mitochondrial rRNA genes (692 bp) including the intervening valine tRNA gene (*12S–16S*), and cytochrome b (*CYTB*; 659 bp). We used the same primers and protocols as described in López-Hervas et al. (bioRxiv). Briefly, the PCR protocols followed Goebel et al., (1999), Santos and Cannatella (2011), and Santos et al. (2003). PCR products were sequenced in both directions at the Functional Genomics Laboratory at the University of California, Berkeley. The chromatograms were trimmed and consensus contigs were obtained using *sangeranalyseR* (Chao et al., 2021) in *R* v.4.3.1 (R Core Team, 2023). New sequences were deposited in GenBank (accession numbers OR789875–OR789884).

We aligned the five new sequences using MUSCLE v3.8.31 (Edgar, 2004) in AliView (Larsson, 2014) with existing *12S–16S* and *CYTB* data for 108 other *Epipedobates* individuals, including one outgroup (*Silverstoneia nubicola*) and 4 individuals assigned to *Epipedobates* sp. 1 by López-Hervas et al. (2023), which correspond here to *Epipedobates [to be revealed]* sp. nov. Using the resulting alignment (Data S1), we calculated mean pairwise p-distances (uncorrected) between species for each gene with the R script provided by López-Hervas et al. (2023) with *ape* v5.7.1 (Paradis & Schliep, 2019), using pairwise deletion for missing data. Following López-Hervas et al. (2023), we excluded the individuals from the *E.* aff. *espinosai* Bilsa and La Tortuga localities, which show evidence of introgression with *E. machalilla*.

We obtained additional sequences of three nuclear markers (total length of 2227 bp), namely histone H3 (*H3*), bone morphogenetic protein 2 (*BMP2*), and voltage-gated potassium channel 1.3 ($K_V1.3$), as well as the mitochondrial control region (*CR*; 1031 bp) from previously published sequences of the same 108 individuals referenced above (Suppl. material 1). A maximum likelihood phylogeny was estimated using this expanded alignment in IQ-TREE v2.0.3 (Kalyaanamoorthy et al., 2017; Minh et al., 2020) and parameters described in López-Hervas et al. (bioRxiv). Briefly, we partitioned protein-coding genes by gene and by codon position. We excluded the first and second codon positions of *BMP* and *H3*, which were invariant across all samples. We selected the model of evolution and data partitions under the Bayesian Information Criterion using option -m TESTMERGE. To assess branch

support, we performed 10,000 ultrafast bootstrap replicates (Minh et al., 2013) and plotted them onto the optimal likelihood tree in Suppl. material S2.

Call recording and bioacoustic analyses

We recorded the advertisement calls of twelve male frogs of *Epipedobates [to be revealed]* sp. nov. from three Valle de Cauca localities under natural conditions: Anchicayá (four individuals), Pianguita (six), and from the type locality, Ladrilleros (two). We also recorded five males of *E. boulengeri* from the type locality (Isla Gorgona, Cauca, Colombia), five males of *E. boulengeri* from Maragrícola (Tumaco, Nariño, Colombia) and six males of *E. narinensis* from its type locality (Biotopo Natural Reserve, Nariño, Colombia). All recordings were deposited in Fonozoo (FZ-SOUND-CODE 14657-14685). Recordings were taken during the morning (7–13 h) using a unidirectional microphone (Sennheiser K6/ME66) connected to a digital recorder (Marantz PMD660/Zoom H4n Pro) and positioned 50–150 cm in front of a calling male. Immediately after recording, we measured the temperature of the substrate using an infrared thermometer (Oakton model 35629). We digitized the recordings at a minimum of 16-bit resolution and 44.1 kHz sampling rate in RAVEN PRO 1.6 (Bioacoustics Research Program, 2014). For the terminology and procedures for measuring call traits we follow Köhler et al. (2017). The spectrograms and oscillograms were graphed using Seewave R package (Sueur et al., 2008) with an FFT window using the Blackman algorithm, window length of 256 samples and overlap of 90%.

We defined a call as the sound produced during a single abdominal muscular contraction (Erdtmann & Amézquita, 2009; Köhler et al., 2017). In *Epipedobates*, such contractions produce a single "buzz" composed of several pulses (Brown et al., 2011; Erdtmann & Amézquita, 2009; Myers & Daly, 1976). We analyzed five consecutive calls per individual by measuring their peak frequency (the frequency with the highest amplitude), low frequency (the lower frequency of the call), high frequency (the upper frequency of the call) and 90% bandwidth (-10 dB threshold; containing the frequency range that encompasses 90% of the sound energy in each call). High and low frequencies of the calls were measured at 20 dB (re 20 mPA) below the peak intensity of the peak frequency. At this value the signal energy could still be clearly distinguished from the background noise. We also measured the following temporal variables: call duration, number of pulses per call and inter-call interval. For *E. [to be revealed]* sp. nov., we also measured the pulse rate, pulse duration, and inter-pulse interval. Call parameters for the calls of each individual were averaged to statistically compare among individuals. To avoid redundancy among the acoustic variables, we used a Principal Component Analysis (PCA) to reduce the number of parameters implemented in the *'PCA'* function of the 'FactoMineR' R package (Lê et al., 2008). In order to identify whether *E. [to be revealed]* sp. nov. could be differentiated based on advertisement calls, we ran a LDA on the first 5 PCs using the *lda'* function of the MASS R package (Venables & Ripley, 2002). We utilized an Kruskal-Wallis test to examine the relevant call traits (peak frequency, call duration and number of pulses per call) that differentiate *E. [to be revealed]* sp. nov. from other species and a post-hoc paired comparison using a Wilcoxon rank-sum test.

Repositories, Institutional acronyms, and Institutional abbreviations

Throughout our paper we use globally unique identifiers (GUIDs, Globally Unique Identifiers Task Group, 2011) to refer to the voucher specimens at QCAZ (QCAZ:A:XXXX, Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito, Ecuador), the MVZ (MVZ:Herp:XXXX, herpetological collections of the Museum of Vertebrate Zoology, University of California, Berkeley, USA), and the amphibian collection at the Museo de Historia Natural C.J. Marinkelle at the

Universidad de los Andes, Bogotá, Colombia (ANDES:A:XXXX), in an effort to facilitate future machine-readability of this text.

RESULTS

Systematics

Epipedobates [to be revealed] sp. nov.

Proposed English common name: [to be revealed] Nurse Frog.

Proposed Spanish common name: Rana nodriza de [to be revealed].

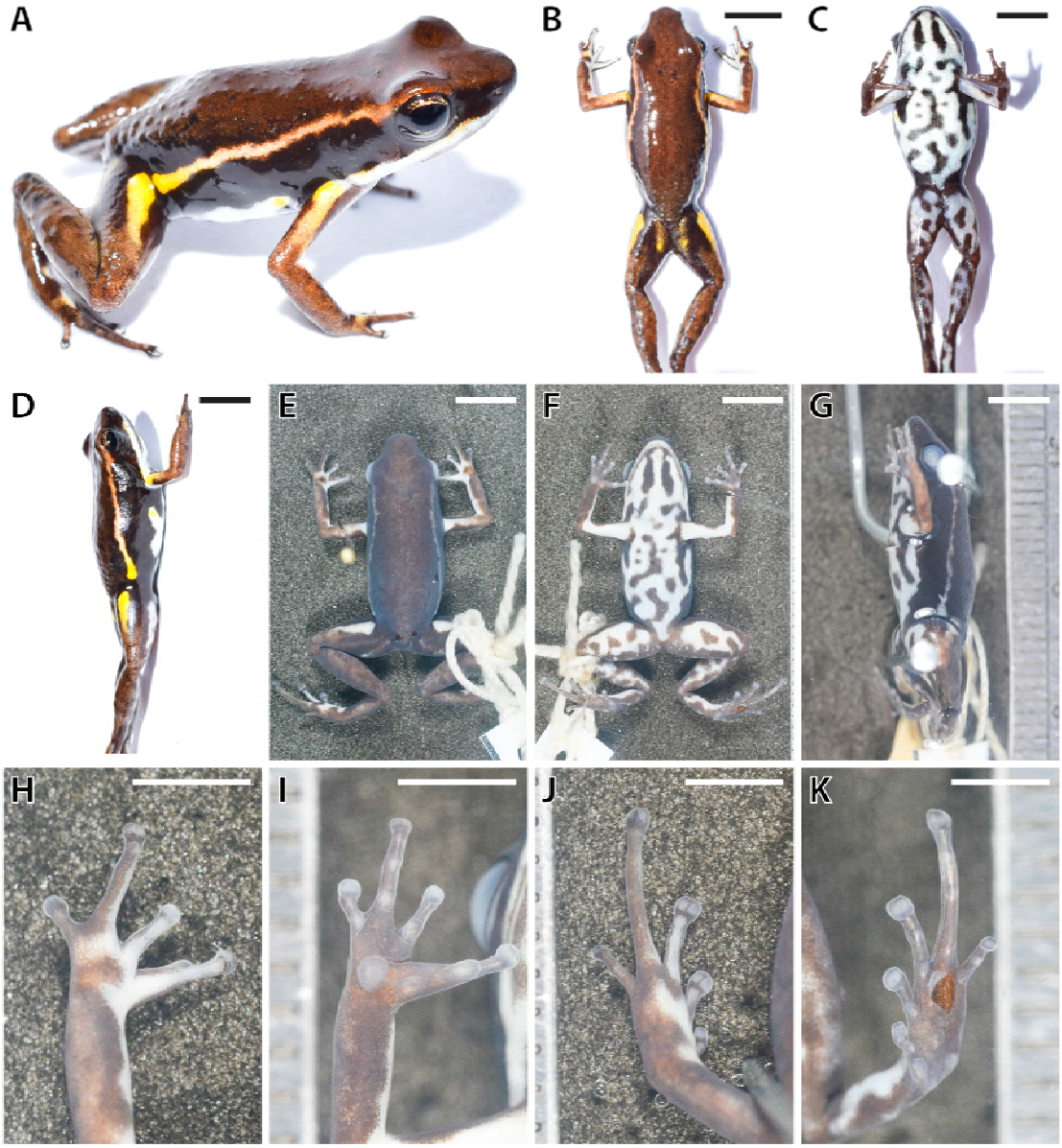

Figure 2. Images in life and in preservative of the holotype of *Epipedobates [to be revealed]* sp. nov.. **A** Full specimen in life; **B** Dorsal view in life; **C** Ventral view in life; **D** Lateral view in life; **E** Dorsal view in preservative (70% ethanol); **F** Ventral view in preservative; **G** Lateral view in

preservative; **H** Dorsal hand in preservative; **I** Ventral hand in preservative; **J** Dorsal foot in preservative; **K** Ventral foot in preservative. Scale bars represent 5 mm (B to G) or 2.5 mm (H to K).

Table 1. Morphometric characters and summary statistics of the males of type series of *Epipedobates [to be revealed]* sp. nov. Measurements (in mm) of adult females and males; values are given as mean, SD, and range. See Methods section for explanation of abbreviations. *Holotype.

Museum Code	Colector Code	Sex	SVL	FAL	HaL	TL	FL	HW	HL	NED	EW	END	IND	IOD	TD
ANDES-A 5254	MBC753	Male	17.2	4.3	3.7	7.7	7	5.4	6.3	2.5	2.1	1.6	2.1	2.9	0.9
ANDES-A 5256	AJC07731	Male	17.20	4.10	3.90	7.80	6.90	5.80	6.90	2.90	2.10	1.60	2.20	3.30	1.00
ANDES-A 5260	AJC07735	Male	17.40	4.40	3.70	7.90	7.00	5.60	6.40	3.00	2.50	1.60	2.50	3.80	1.00
ANDES-A 5261	AJC07736	Male	18.70	4.50	4.30	8.50	7.30	5.90	7.00	3.30	2.70	1.70	2.50	3.80	1.00
ANDES-A 5263	AJC07738	Male	17.50	4.60	3.80	7.70	6.60	6.00	7.30	3.00	2.40	1.60	2.30	3.50	0.90
MVZ:Herp:295434	AJC07742	Male	16.70	4.00	3.60	7.50	6.70	4.90	6.70	2.80	1.90	1.70	2.30	3.20	0.90
MVZ:Herp:295436	AJC07744	Male	16.70	3.80	3.70	7.10	6.70	5.70	6.90	2.70	2.40	1.70	2.10	3.40	1.00
ANDES-A 5259	RDT0935	Male	16.90	4.40	3.80	7.90	7.20	5.30	6.60	3.60	2.40	1.80	2.30	3.80	0.90
Mean			17.29	4.26	3.81	7.76	6.93	5.58	6.76	2.98	2.31	1.66	2.29	3.46	0.95
SD			0.64	0.27	0.22	0.40	0.25	0.36	0.33	0.35	0.26	0.07	0.16	0.33	0.05
Min			16.70	3.80	3.60	7.10	6.60	4.90	6.30	2.50	1.90	1.60	2.00	2.90	0.90
Max			18.70	4.60	4.50	8.70	7.60	6.20	7.80	3.60	2.70	2.00	2.50	4.20	1.20

Table 2. Morphometric characters and summary statistics of the females of type series of *Epipedobates [to be revealed]* sp. nov. Measurements (in mm) of adult females and males; values are given as mean, SD, and range. See Methods section for explanation of abbreviations. *Holotype.

Museum Code	Colector Code	Sex	SVL	FAL	HaL	TL	FL	HW	HL	NED	EW	END	IND	IOD	TD
ANDES-A 5255*	MBC754	Female	18.50	4.60	3.90	8.10	6.90	5.70	6.80	3.00	2.30	1.70	2.30	3.70	0.90
ANDES-A 5257	AJC07733	Female	19.50	4.50	4.50	8.70	7.20	6.00	6.70	3.20	2.60	2.00	2.40	4.20	1.10
ANDES-A 5262	AJC07737	Female	18.30	4.40	3.90	7.70	6.70	6.10	7.00	3.20	2.40	1.80	2.00	3.60	1.10
ANDES-A 5264	AJC07739	Female	17.50	4.40	3.90	8.20	7.20	5.70	7.00	2.90	2.40	1.90	2.40	3.60	0.90
MVZ:Herp:295433	AJC07740	Female	19.10	4.30	4.00	8.00	7.20	6.20	7.10	3.10	2.30	1.90	2.40	3.40	1.20
MVZ:Herp:295435	AJC07743	Female	18.90	4.60	4.20	8.60	7.60	5.80	7.10	2.90	2.20	1.70	2.10	3.40	1.10
MVZ:Herp:295432	RDT0934	Female	19.10	4.30	4.00	8.00	7.00	5.90	7.80	2.90	2.40	1.60	2.40	3.50	0.90
ANDES-A 5258	AJC07734	NA	18.60	4.40	3.60	7.30	6.60	6.00	6.50	3.40	2.30	1.90	2.30	3.90	1.00
Mean			18.70	4.44	4.06	8.19	7.11	5.91	7.07	3.03	2.37	1.80	2.29	3.63	1.03
SD			0.66	0.13	0.22	0.35	0.29	0.20	0.35	0.14	0.13	0.14	0.17	0.28	0.13
Min			17.50	4.30	3.90	7.70	6.70	5.70	6.70	2.90	2.20	1.60	2.00	3.40	0.90
Max			19.50	4.60	4.50	8.70	7.60	6.20	7.80	3.20	2.60	2.00	2.40	4.20	1.20

Material examined

Holotype

COLOMBIA • ♀; Ladrilleros, Buenaventura, Valle del Cauca; 3.945221, −77.364993; 28 m. a.s.l.; 6 Aug. 2022; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar, Juan Camilo Ríos-Orjuela; ANDES:A:5255.

Paratypes

COLOMBIA • 4 ♀♀, 6 ♂♂, 1 ND; same data as for holotype; ANDES:A:5254, 5256–5265 • 3 ♀♀, 2 ♂♂; same data as for holotype; Genbank: OR789880–84 and OR789875–79; MVZ:Herp:295432–295436.

Other material

COLOMBIA

• *Epipedobates [to be revealed]* 2 ♀♀, 6 ND; Type locality, Ladrilleros, Buenaventura, Valle del Cauca; 3.950882, −77.358293; 53 m a.s.l.; 26 Nov. 2014; collectors Rebecca D. Tarvin and Fray Arriaga; GenBank: OR489011–12, OR179790–91, OR734703–04, OR179835–36, OR179875, OR179880; ANDES:A:2458–2465. The skin was removed prior to preservation for evaluation of alkaloid content from these individuals, with the exception of ANDES:A:2464. • *E. [to be revealed]* 1 ♀; La Barra, Buenaventura, Valle del Cauca; 3.985064, -77.376723; 15 m a.s.l.; 26 Nov. 2014; collectors Rebecca D. Tarvin and Fray Arriaga; GenBank: OR489010, OR179789, OR734702, OR179834, OR179851; ANDES:A:2455. • *E. [to be revealed]* 5 ♂♂; Pianguita, Buenaventura, Valle del Cauca; 3.841954, -77.198718; 17 m.a.s.l.; 12 Sep. 2016; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar, Sandra V. Flechas; ANDES:A:3690-94. • *E. [to be revealed]* 4 ♀♀, 3 ♂♂, Danubio, Dagua, Valle del Cauca; 3.611528, -76.885194; 705 m a.s.l.; 5 Nov. 2016; collectors Mileidy Betancourth-Cundar, Adolfo Amézquita, and Ivan Beltrán; GenBank: OR489013, OR179792, OR734705, OR179837; ANDES:A:3714–20.

• *E. boulengeri* 3 ♀♀, 4 ♂♂, 1 ND; Isla Gorgona, Guapi, Cauca; 2.96465, -78.173685; 20 m a.s.l.; 12 Sep. 2016; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar and Sandra V. Flechas; GenBank: OR OR488992, OR179771, OR734681, OR179812; ANDES:A:3695-3702. • 1 ND; Isla Gorgona, Guapi, Cauca; 08 Sep. 2005; ANDES:A:0560; Collector unknown . • *E. boulengeri* 3 ♀♀, 4 ♂♂, 1 ND; Isla Gorgona, Guapi, Cauca; 2.96465, -78.173685; 20 m a.s.l.; 12 Sep. 2016; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar and Sandra V. Flechas; GenBank: OR OR488992, OR179771, OR734681, OR179812; ANDES:A:3695-3702. • *E. boulengeri* 8 ♀♀; Maragrícola, Tumaco, Nariño; 1.68084, -78.74924; 7 m a.s.l.; 09 Dec. 2014; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar, Cristian Flórez; GenBank: OR488990, OR179769, OR734679, OR179810, OR179862, OR488991, OR179770, OR734680, OR179811, OR179863; ANDES:A:2468–75. • *E. boulengeri* 1 ND; La Nutria, El Diviso, Barbacoas, Nariño; 1.36083, -78.18076; 762 m a.s.l.; 10 Dec. 2014; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar, Cristian Flórez; GenBank: OR488977, OR179756, OR734663, OR179794, OR179840; ANDES:A:2476.

• *E. narinensis* 3 ♀♀, 5 ♂♂, 1 ND; Reserva Natural Biotopo, Berlín, Barbacoas, Nariño; 1.408999, -78.281246; 518 m a.s.l.; 25 Sep. 2016; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar and Cristian Flórez; GenBank: OR489008, OR179787, OR734700, OR179832, OR489009, OR179788, OR734701, OR179833; ANDES:A:3703–3711. • *E. narinensis* 10 ♂♂; Reserva Natural Biotopo, Berlín, Barbacoas, Nariño; 1.411263, -78.285099; 600 m a.s.l.; 22 Jul. 2006; collectors

Viviana Moreno-Quintero, Jonh Jairo Mueses-Cisneros, Luisa Mercedes Bravo, Carol Narváez, and Bienvenido Cortés; ICN 53344 (holotype), ICN:53336–53340, 53342–53346 (paratypes). • *Andinobates minutus* 4ND; La Barra, Buenaventura, Valle del Cauca; 3.985064, -77.376723; 15 m a.s.l.; 25 Nov. 2014; collectors Rebecca D. Tarvin and Fray Arriaga; ANDES:A 2451–54. • *A. minutus* 1ND; Ladrilleros, Buenaventura, Valle del Cauca; 3.945221, −77.364993; 28 m a.s.l; 6 Aug. 2022; collectors Rebecca D. Tarvin, Mileidy Betancourth-Cundar, Juan Camilo Ríos-Orjuela; ANDES:A:5266.

ECUADOR

• *Epipedobates* aff. *espinosai* 1ND; Lita, Carchi; 12 Aug. 1992; collector Bueno M.; ICN:32504.

Diagnosis

Epipedobates [to be revealed] is a small dendrobatid frog (SVL mean = 17.99 mm and SD = 0.95 mm, N = 16 frogs; Table 2) characterized by having a uniformly brown dorsal coloration extending throughout the limbs, and a bright yellow blotch in the concealed region of the groin. For a detailed comparison of the coloration of *E. [to be revealed]* sp. nov. with other species of the genus see suppl. material S3. The first finger is longer than the second and the third finger is swollen in males. In our revision of the morphology, we noted that fingers II and IV are reduced in length in *E. [to be revealed]* (Finger II/SVL: mean = 16.1, SD = 1.59%; Finger IV/SVL: mean = 15.1, SD = 1.42%, N = 37 frogs) (Fig. 2H–I) and *E. narinensis* (Finger IV/SVL: mean = 16.9, SD = 0.85%; Finger IV/SVL: mean = 16.2, SD = 1.08%, N=8 frogs) relative to *E. boulengeri*-Gorgona (Finger IV/SVL: mean = 19.2%, SD = 0.67%; Finger IV/SVL: mean = 18.2, SD = 1.12%, N=9 frogs), *E. boulengeri*-Nariño (Finger IV/SVL: mean = 17.5, SD = 0.99%; Finger IV/SVL: mean =17.7, SD = 2.14%, N=9 frogs) or *E.* aff. *espinosai* (Table 3, suppl. material S3). Besides its coloration, *E. [to be revealed]* sp. nov. differs in the structure and composition of its advertisement calls, with longer calls and in turn a greater number of pulses than other *Epipedobates* species, but in series of only one call. A summary of the morphological characters of *E. [to be revealed]* sp. nov. compared to other *Epipedobates* species in Colombia is shown in Table 3. We describe below in detail the advertisement calls and morphological traits of *E. [to be revealed]* sp. nov. in comparison with its congeners, and an assessment of its genetic divergence from other species in this genus.

Table 3. Characteristics of morphology, coloration, and calls of *E. [to be revealed]* sp. nov. compared to other species of *Epipedobates*. For comparison of morphological structures, we used specimens deposited at the Instituto de Ciencias Naturales of the Universidad Nacional de Colombia. For *E. narinensis* we reviewed the holotype (ICN:53344) and type series (ICN:53336–53340, 53342–53346). For *E.* aff. *espinosai,* we reviewed only one specimen present in this collection (ICN:32504) and the body size data were provided by López-Hervas et al. (2023). The table is presented in parts across the next several pages for readability.

Species	Snout-Vent Length (mm) (mean ± SD)	Finger Length I>II	Fingers II and IV reduced	Finger III swelling in adult males	Webbing: Toe II Postaxial	Webbing: Toe III Preaxial	Webbing: Toe III Postaxial	Webbing: Toe IV Preaxial	Webbing: Toe IV Postaxial

E. [to be revealed]	16.92 ± 0.47, N = 9	Yes	Yes	Yes	Absent	Absent	Present	Present	Present, extends beyond the basal subarticular tubercle on toe IV
E. narinensis	19.92 ± 0.47, n=8	Yes	Yes	Yes	Present, reduced	Absent	Present	Present	Present, extends to or just below the basal subarticular tubercle on toe IV
E. boulengeri - Gorgona	17.69 ± 0.11, n=39	Yes	No	Yes	Absent	Absent	Present	Present	Present, extends beyond the basal subarticular tubercle on toe IV
E. boulengeri - Nariño	17.37 ± 0.67, n=9	Yes	No	Yes	Absent	Absent	Present	Present	Present, extends beyond the basal subarticular tubercle on toe IV
E. aff. *espinosai*	17.11 ± 1.69, n=28	Yes	No	Yes	Absent	Absent	Absent	Absent	Present, extends beyond the basal subarticular tubercle on toe IV

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Species	**Relative Finger Lengths**	**Relative Toe Lengths**	**Metatarsal Fold**	**Dorsal skin texture**	**Doral color**	**Oblique Lateral Stripe color**	**Oblique Lateral Stripe morphology**	**Ventrolateral stripe**
E. [to be revealed]	III>I>II>IV	IV>III>V>II>I	Absent	Finely granulated	Dark brown, reddish brown	Present, yellow posteriorly and copper anteriorly	Complete	Absent

E. narinensis	III>I>II>IV	IV>III>V>II>I	Absent	Finely granulated	Dark green	Present, light green	Only reaches up to 1/4 of the body length from the groin	Present, light green
E. boulengeri - Gorgona	III>I>II>IV	IV>III>V>II>I	Absent	Finely granulated	Brown, reddish brown	Present, cream, narrows anteriorly	Complete	Present, white
E. boulengeri - Nariño	III>I>II>IV	IV>III>V>II>I	Absent	Finely granulated	Reddish brown with dark spots	Present, cream posteriorly and copper towards the anterior region	Complete	Present
E. aff. *espinosai*	III>I>II>IV	IV>III>V>II>I	Absent	Finely granulated	Dark brown, reddish brown	Present, cream posteriorly and copper towards the anterior region	Complete	Present

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Species	**Abdomen coloration**	**Abdomen pattern**	**Lateral shape of snout**	**Dorsal shape of snout**	**Tympanum/Eye (%)**	**HW/SVL (%)**	**HW/HL (%)**	**Spot on anteriodorsal side of thigh**
E. [to be revealed]	Turquoise blue with black spots	Heavily mottled	Projecting, protruding	Slightly rounded	33.3–66.0	27.7–36.3	68.4–103.7	Present, conspicuous, and intense yellow
E. narinensis	Light olive green with black spots	Speckled or slightly mottled	Projecting	Slightly rounded	53.8–58.9	29.8–33.7	79.3–87.6	Absent, yellow, or diffuse
E. boulengeri - Gorgona	Creamy white with black spots	Mottled	Projecting, protruding	Slightly rounded	44.8–59.4	28.7–33.2	80.4–103.2	Present, cream, and extended along the thigh
E. boulengeri - Nariño	Bluish white with black spots	Mottled or with isolated spots	Projecting, protruding	Slightly rounded	45.0–64.0	25.7–32.2	76.4–86.6	Absent

E. aff. *espinosai*	White or cream with large black spots	Mottled or with large spots	Projecting, protruding	Slightly rounded	NA	NA	NA	Absent or diffuse cream-colored

Species	**Peak frequency** (mean ± SD, in kHz)	**Number of pulses** (mean ± SD)	**Call duration (s)** (mean ± SD, in s)	**Number of calls in call series**
E. [to be revealed] (N = 15 frogs)	5.23 ± 0.11	73.94 ± 18.78	2.21 ± 0.54	1
E. narinensis (N = 6)	5.64 ± 0.21	8.98 ± 2.45	0.28 ± 0.070	5–14
E. boulengeri - Gorgona (N = 6)	4.76 ±0.11	14.48 ± 2.19	0.30 ± 0.036	2–3
E. boulengeri - Nariño (N = 5)	5.51 ± 0.11	13.09 ± 1.75	0.28 ± 0.042	3–6
E. aff. *espinosai*	NA	NA	NA	NA

At the type locality the new species occurs in sympatry with *Andinobates minutus* but differs in body size and coloration (Fig. 3). *Andinobates minutus* has orange rather than yellow spots on the groin and dorsal side of the arms and its supralabial band is dark orange rather than white as in *E. [to be revealed]*. Ventrally in *A. minutus* the proportion of black and turquoise blue is similar. In contrast, in *E. [to be revealed]* the coloration is mainly turquoise blue with some black blotches (Fig. 2C). In addition, adults of *A. minutus* are much smaller (mean = 12.00 mm, SD = 0.91 mm, N = 5) than adults of *E. [to be revealed]* (17.99 mm, SD = 0.95, N = 16), but *A. minutus* can be confused with *E. [to be revealed]* juveniles. The coloration of juvenile *E. [to be revealed]* is very similar to *E. [to be revealed]* adults, so color can still be used to differentiate the two species.

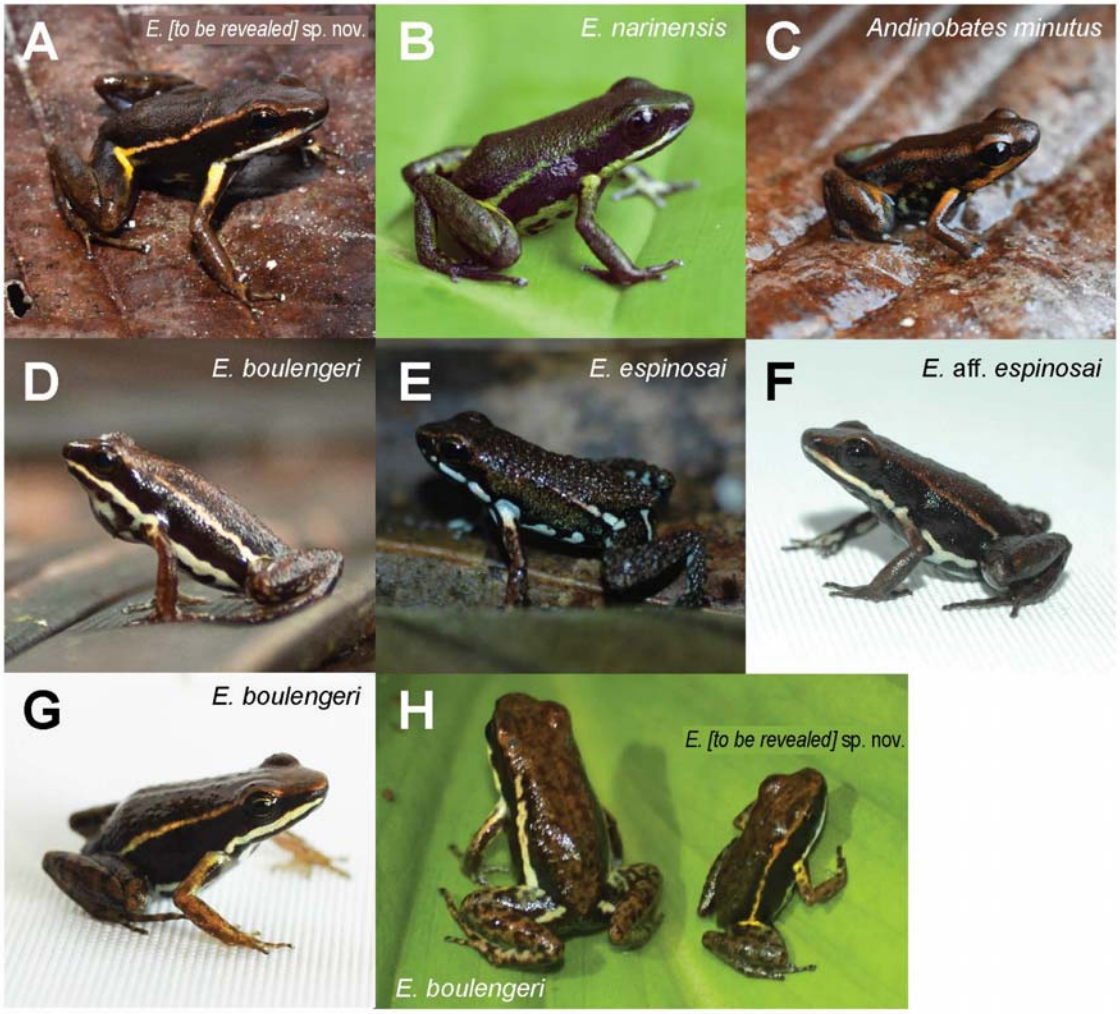

Figure 3. Images in life of *Epipedobates [to be revealed]* sp. nov. in comparison with close congeners and sympatric species. A. *E. [to be revealed]* sp. nov. from the type locality of Ladrilleros, Valle de Cauca, Colombia (ANDES:A:5261; SVL = 20.0 mm; adult male; paratype); **B.** *E. narinensis* from Biotopo, Nariño, Colombia (ANDES:A:3704; 16.39 mm; adult male); **C.** *Andinobates minutus* from Ladrilleros, Valle de Cauca, Colombia (ANDES:A:5266; 13 mm; sex not determined); **D.** *E. boulengeri* from Isla Gorgona, Cauca, Colombia (individual not captured); **E.** *E. espinosai* from Río Palenque, Santo Domingo de los Tsáchilas, Ecuador (individual not captured); **F.** *E.* aff. *espinosai* from La Nutria, Nariño, Colombia (ANDES:A:2476; 17.77 mm; sex not determined); **G.** *E. boulengeri* from Maragrícola, Nariño, Colombia (ANDES:A:2472; 18.96 mm; adult female); **H.** A side-by-side image of *E. boulengeri* from Isla Gorgona (ANDES:A:3695; 20.65 mm; adult female) and *E. [to be revealed]* sp. nov. from Pianguita, Valle de Cauca, Colombia (ANDES:A:3690; 16.42 mm; adult female) demonstrating the large size difference between the two species. All images were taken by RDT except for A and C, which were taken by JCR. Photos are not to scale.

Molecular and phylogenetic analyses

We estimated a phylogeny using existing data (López-Hervas et al. 2023) and 10 new sequences from five *E. [to be revealed]* from the type locality. The resulting phylogeny contains strong support

for the monophyly of *E. [to be revealed]* and recovers known phylogenetic relationships among species in this genus (Figs. 4 and S2; López-Hervas et al. 2023). The sister species to *E. [to be revealed]* is *E. narinensis*, which occurs in the south-central part of the Pacific coast of Colombia. Together, these two species constitute a clade of *Epipedobates* that is highly divergent from the rest of the genus.

The average p-distances among clades of *Epipedobates* species, including two clades within the nominal species, *E. boulengeri* (from the type-locality of Isla Gorgona and from the mainland) show that *E. [to be revealed]* is genetically most similar to *E. narinensis* (1.77% for *12S–16S* and 5.39% for *CYTB*), as expected given the molecular phylogeny.

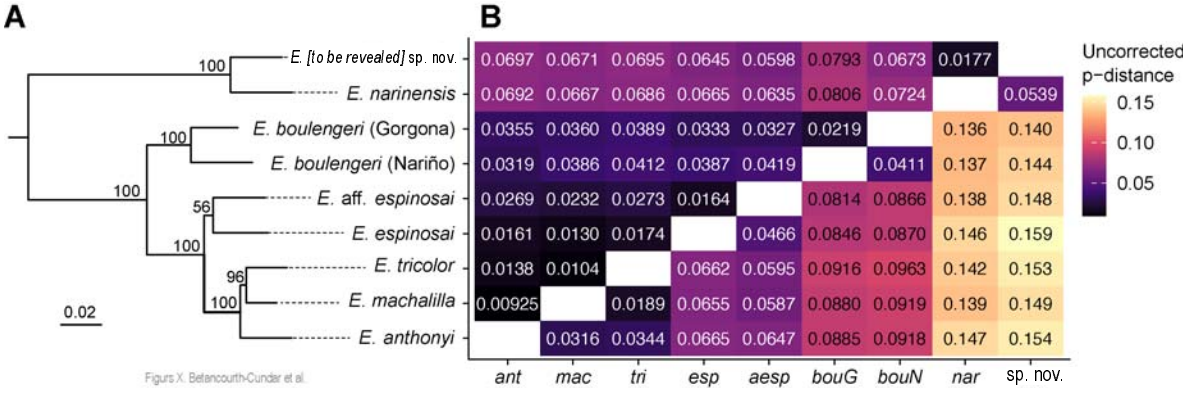

Figure 4. Phylogenetic position and genetic distances of *E. [to be revealed]* sp. nov. and other species of *Epipedobates*. A A species-level maximum-likelihood phylogeny of *Epipedobates* estimated in IQ-TREE using two nuclear genes (*BMP2, H3*) and three mitochondrial gene fragments (*CYTB, 12S–16S, CR*) (see Fig. Supp. material S2 for full phylogeny). Node support values from 10,000 ultrafast bootstrap replicates indicated strong support of *E. [to be revealed]* sp. nov. as the sister-group of *E. narinensis*. **B** Matrix of mean pairwise p-distances among species of *Epipedobates*. The lower triangle distances are based on *CYTB* and the upper triangle distances are from *12S–16S*. *Epipedobates [to be revealed]* is genetically most similar to *E. narinensis* (1.77% for *12S–16S* and 5.39% for *CYTB*). Abbreviations: cur, *E. [to be revealed]* sp. nov; nar, *E. narinensis*; bouN, *E. boulengeri* (Nariño, Colombia); bouG, *E. boulengeri* (Isla Gorgona, Cauca, Colombia); aesp, *E.* aff. *espinosai*; esp, *E. espinosai*; tri, *E. tricolor*; mac, *E. machalilla*; ant, *E. anthonyi*. See Supp. material S2 for full phylogeny.

There are significant differences in body size between *Epipedobates [to be revealed]* sp. nov., *E. narinensis*, and *E. boulengeri* (Gorgona and Nariño) (Kruskal-Wallis test: $H_3 = 19.058$, $P < 0.001$; Fig. 5A). *Epipedobates boulengeri*-Gorgona exhibits significantly larger mean body size than the other populations analyzed. A Wilcoxon test showed that *E. [to be revealed]* sp. nov. is larger than *E. narinensis* ($P = 0.05$, $N = 33$) and smaller than *E. boulengeri*-Gorgona ($P < 0.006$, $N = 32$). We found no significant differences in body size between *E. [to be revealed]* sp. nov. and the populations of Nariño assigned to *E. boulengeri* ($P = 0.462$, $N = 33$). We found sexual dimorphism in body size (SVL) for *E. [to be revealed]* sp. nov. (ANOVA: $F_{1,46} = 6.269$, $P < 0.01$), where mean SVL in females was larger (mean = 18.24, SD = 1.09 mm, N = 28, Table 2) than the mean in males (mean = 17.45, SD = 1.07 mm, N = 20, Table 1).

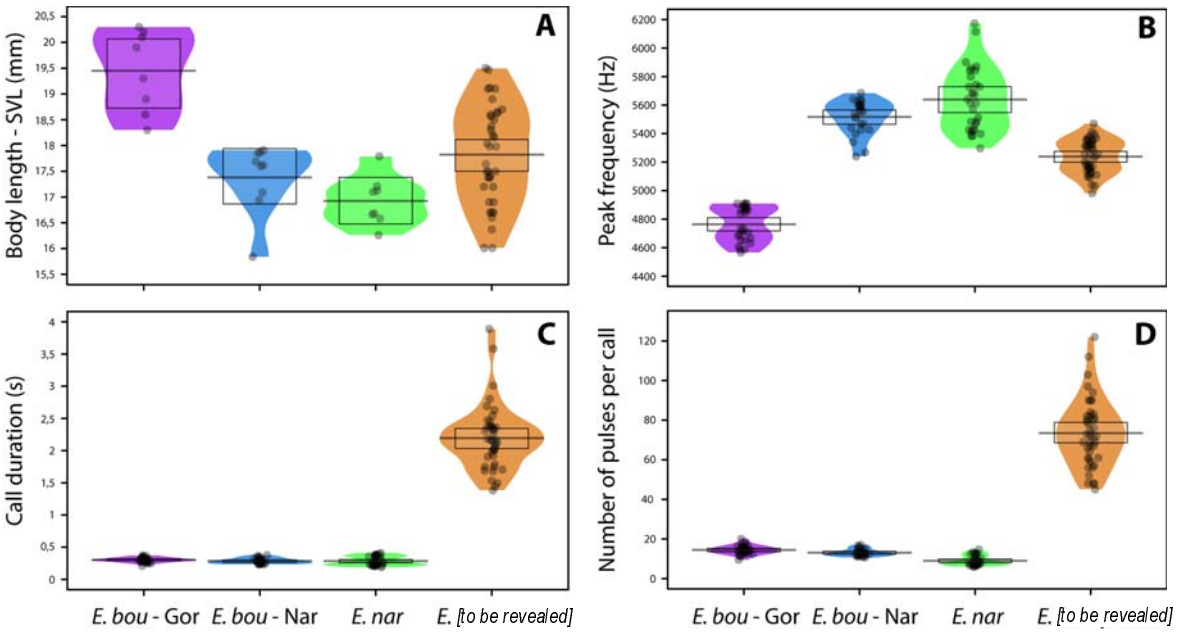

Figure 5. Comparisons of SVL and advertisement call traits between *E. [to be revealed]* sp. nov. and other species of *Epipedobates*. **A** Snout-vent length (SVL) **B** Peak frequency **C** Call duration and **D** Number of pulses per call. Abbreviations include: E. bou - Gor, *E. boulengeri* - Gorgona; E. bou - Nar, *E. boulengeri* - Nariño; *E. nar, E. narinensis;* and *E. cur* sp. nov., *E. [to be revealed]* sp. nov. Black points represent raw data, the horizontal bar shows the mean, the shaded diagram is a smoothed density curve showing the full data distribution, and the rectangle represents the uncertainty around the mean using a 95% Bayesian highest density interval.

Epipedobates [to be revealed] sp. nov., *E. narinensis*, and *E. boulengeri* (from Gorgona and Nariño) display noteworthy distinctions in call parameters. We found significant differences in the peak frequency of advertisement calls among all species and populations tested (Kruskal-Wallis test: H_3 = 109.25, P < 0.001; Fig. 5B). Wilcoxon test showed that *Epipedobates [to be revealed]* sp. nov. calls with a lower peak frequency than *E. narinensis* (P < 0.001, N = 81) and *E. boulengeri*-Nariño (P < 0.001, N = 76), but its peak frequency is higher than for *E. boulengeri*-Gorgona (P < 0.001, N = 79). We found statistically significant differences in call duration (Kruskal-Wallis test: H_3 = 95.302, P < 0.001; Fig. 5C). *Epipedobates [to be revealed]* sp. nov. exhibits longer calls than *E. narinensis* (P < 0.001, N = 81), *E. boulengeri*-Nariño (P < 0.001, N = 76) and *E. boulengeri*-Gorgona (P < 0.001, N = 79). Similar results were found for the number of pulses per call (Kruskal-Wallis test: H_3 = 107.52, P < 0.001, Fig. 5D). *Epipedobates [to be revealed]* sp. nov. has calls with more pulses than *E. narinensis* (P < 0.001, N = 81), *E. boulengeri*-Nariño (P < 0.001, N = 76), and *E. boulengeri*-Gorgona (P < 0.001, N = 79).

Description

Coloration of holotype in life (Fig. 2A–D). Dorsal surfaces are dark brown with an oblique lateral stripe extending from the posterior region of the eye to the groin, with metallic orange-yellow coloration in the anterior region becoming more yellow towards the groin. Groin region is dark brownish black with a distinct yellow blotch continuing to the anterior concealed surface of the thigh. Flanks are black. Forelimbs and hind limbs are dark brown. Anterior region of the upper arm has a

yellow blotch similar in color to that of the thigh. Supralabial stripe is creamy white, extending from the nares to the axilla. Ventral surfaces are turquoise blue with irregular black spots. Iris is copper.

Coloration of holotype in preservative (Fig. 2E–K; 70% ethanol). Dorsally black to dark brown, hind limbs dark brown. Lighter brown forelimbs with some dark brown blotches. Yellow blotches in life on forelimbs as well as inner and dorsal thighs are white in preservation. Ventrally pale white to dark gray with rounded black to dark brown spots. Groin black. Oblique lateral line is dark gray extending from the posterior region of the eye to the groin and can be very faint. Light gray supralabial band running from the tip of the face to the axilla. Two elongated black spots in the gular region (Fig. 2F).

Coloration of type series and other populations in life. All individuals in the type series exhibit a uniform dark brown color on the dorsum and dorsal surfaces of limbs (Suppl. material S3). The dorsum, head, thigh, and shanks feature a skin texture covered with fine, evenly distributed tubercles. Flanks and concealed surfaces of the forelimbs and hindlimbs are smooth and solid black. All individuals show an oblique lateral stripe that stretches from the posterior region of the eye to the groin. It appears metallic orange-yellow in the anterior region and gradually transitions to a more yellow color as it reaches the groin. The oblique lateral stripe differs slightly among populations. In the Ladrilleros population (type locality), the oblique lateral stripe displays an orange-yellow color with indistinct edges and stretches from the front part of the groin to the eye, gradually changing from yellow to a more brownish-orange hue. The stripe continues onto the upper eyelid and fades as it reaches the canthus rostralis. In the Anchicayá population, the oblique lateral stripe takes on a yellowish hue and, in certain specimens, it may taper, fragment into patches, and gradually disappear before reaching the eye. In some individuals from Pianguita, the oblique lateral stripe exhibits regular interruptions along its entire length.

In all individuals of *E. [to be revealed]* sp. nov., the groin region is characterized by a dark brown-black color, with a noticeable yellow blotch that in some individuals extends to the front inner thigh (Suppl. material S3). Most individuals have a paracloacal spot similar in coloration, but smaller and more elongated, on the posterior dorsal surface of the thigh. In the Anchicayá population, these spots are present in only some individuals. Most individuals have a yellow patch on the dorsal region of the arm that matches the color of the concealed thigh patch. All individuals exhibit a creamy white to pale turquoise blue upper labial stripe that is notably lighter than the oblique lateral stripe. In some cases, this stripe has a slight iridescent quality and extends from below the nostril to the axilla. Also, in certain individuals, it continues posteriorly as a vaguely defined ventrolateral stripe. The ventral surfaces of the throat, belly, and thighs exhibit a pale turquoise blue to white color with irregular black spots and patterns resembling worm-like lines (vermiculations). In males, this coloration may be partially obscured or interrupted by a diffuse dark gray pigment located just anterior to the pectoral region and the vocal sac. A few individuals display a diffuse yellowish coloration towards the outer edges of the belly.

Vocalizations. *Epipedobates* species have pulsed calls. Advertisement calls are composed of groups of calls, one call for *E. [to be revealed]* sp. nov., 2–3 for *E. boulengeri*-Gorgona, 3–6 for *E. boulengeri*-Nariño and 5–14 for *E. narinensis* (Fig. 6). Calls of *E. [to be revealed]* sp. nov consisted of 22–122 pulses per call (mean = 73.98, SD = 18.77, N = 15), with a call duration of 0.67–3.88 s (mean = 2.21, SD = 0.54 s, N = 15). The peak frequency ranged between 4.98 and 5.46 kHz (mean = 5.23, SD = 0.11 kHz), and the frequency interquartile bandwidth between 0.21 and 0.67 kHz (mean = 0.35, SD = 0.87 kHz) (Table 4). We recorded two males close to a female with vocalizations different from

their advertisement call (Fig. 6E). These vocalizations, most likely a courtship call, ranged from 0.21–0.31 s in length (mean = 0.25, SD = 0.03 s, N = 2), and included 7–9 pulses per call (mean 8.25 ± SD 0.83). The peak frequency ranged between 4.78–5.17 kHz (mean = 4.96, SD = 0.13 kHz).

Table 4. Summary statistics of spectral and temporal characteristics of the advertisement calls of *Epipedobates [to be revealed]* sp. nov.

Parameters	*Call Duration (s)*	*Number of pulses*	*Pulse rate*	*Intercall interval (s)*	*Pulse duration (s)*	*Interpulse interval (s)*	*Low Frequency (kHz)*	*High Frequency (kHz)*	*Peak Frequency (kHz)*	*Bandwidth 90% (kHz)*
Mean	2.213	73.938	34.894	62.675	0.017	0.013	4.845	5.586	5.237	0.354
SD	0.541	18.786	2.077	60.762	0.004	0.009	0.222	0.210	0.115	0.087
Min	0.674	22.000	26.000	15.120	0.009	0.001	4.236	5.221	4.981	0.215
Max	3.888	122.000	39.000	315.012	0.028	0.068	5.287	6.093	5.469	0.675

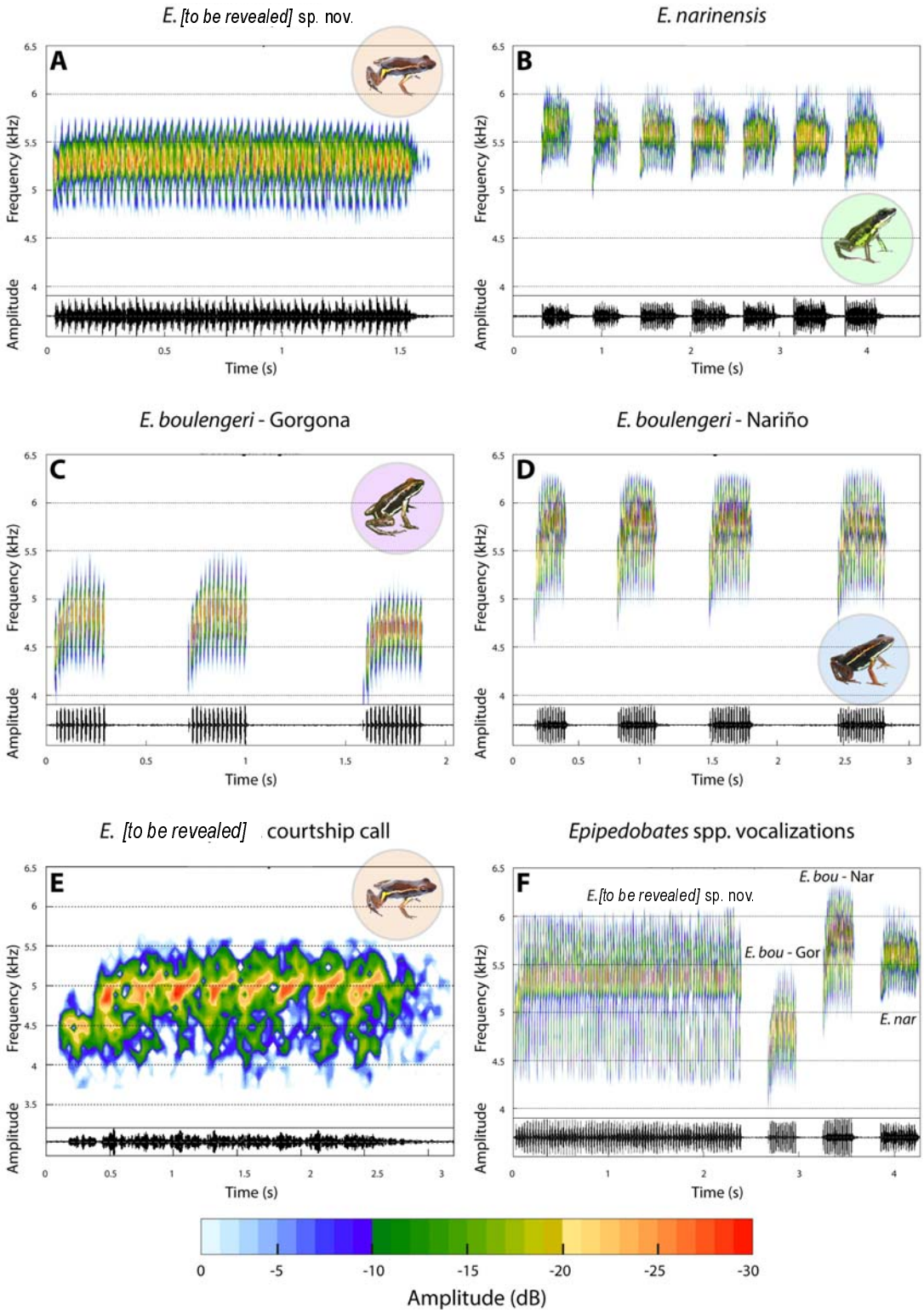

Figure 6. **Vocalizations in *E. [to be revealed]* sp. nov. and other species of *Epipedobates*.** Spectrograms (above) and oscillograms (below) showing general aspects of the structure of one spontaneous advertisement call for one male of **A** *E. [to be revealed]* sp. nov. of Ladrilleros, Valle del Cauca, **B** *E. narinensis* of Biotopo, Nariño, **C** *E. boulengeri*-Gorgona (Type locality) and **D** *E. boulengeri*-Nariño of Maragrícola, Nariño. Advertisement calls consist of call groupings, comprising a

single call for *Epipedobates [to be revealed]* sp. nov., 2–3 calls for *E. boulengeri*-Gorgona, 3–6 calls for *E. boulengeri*-Nariño, and 5–14 calls for *E. narinensis*. **E** Spectrograms and oscillograms showing general aspects of the structure of one courtship call of *E. [to be revealed]* sp. nov. of Ladrilleros, Valle del Cauca. **F** Comparison of one advertisment call between *E. [to be revealed]* sp. nov. and other species of *Epipedobates*. *E. bou* - Gor: *E. boulengeri* - Gorgona, *E. bou* - Nar: *E. boulengeri* - Nariño, E. nar: *E. narinensis* and *E. cur* sp. nov.: *E. [to be revealed]* sp. nov. Fast Fourier Transform-FFT = 256, overlap = 90%.

A principal component analysis (PCA) indicated that the first three principal components explain 88% of the variation in calls (Fig. 7A). PC1 explained 45% of the variation and was positively associated with call duration, number of pulses, and intercall interval. PC1 was also negatively related to high frequency and 90% bandwidth (Suppl. material S4). PC2 explained an additional 33% of the variation in call parameters. It was positively related to low frequency and peak frequency. PC3 explained 10% of the variation and was positively associated with frequency interquartile bandwidth 90% (Fig. 7A, Suppl. material S4). In summary, PC1 was mainly associated with temporal characteristics of the advertisement call and PC2 with spectral characteristics. The linear discriminant analysis (LDA) (Fig. 7B) function using five PCs showed that *E. [to be revealed]* can be easily differentiated from other species based on their advertisement calls. LD1 was positively associated with PC1 (temporal characteristics) (LD coef. = 1.71), and LD2 was negatively associated with PC2 (spectral characteristics) (LD coef. = -1.47). This means *E. [to be revealed]* is distinguished by having longer calls and a greater number of pulses (Figs 7B and 5C-D). With respect to low and peak frequency, *E. [to be revealed]* calls are characterized by intermediate values compared to the other species analyzed (peak frequency: 4.84 ± 0.22 kHz, N=15). *Epipedobates boulengeri*-Gorgona has the lowest peak frequency (4.76 ± 0.11 kHz, N=5) and *E. narinensis* has the highest peak frequency (5.64 ± 0.21 kHz, N=6) (Figs 7B and 5B).

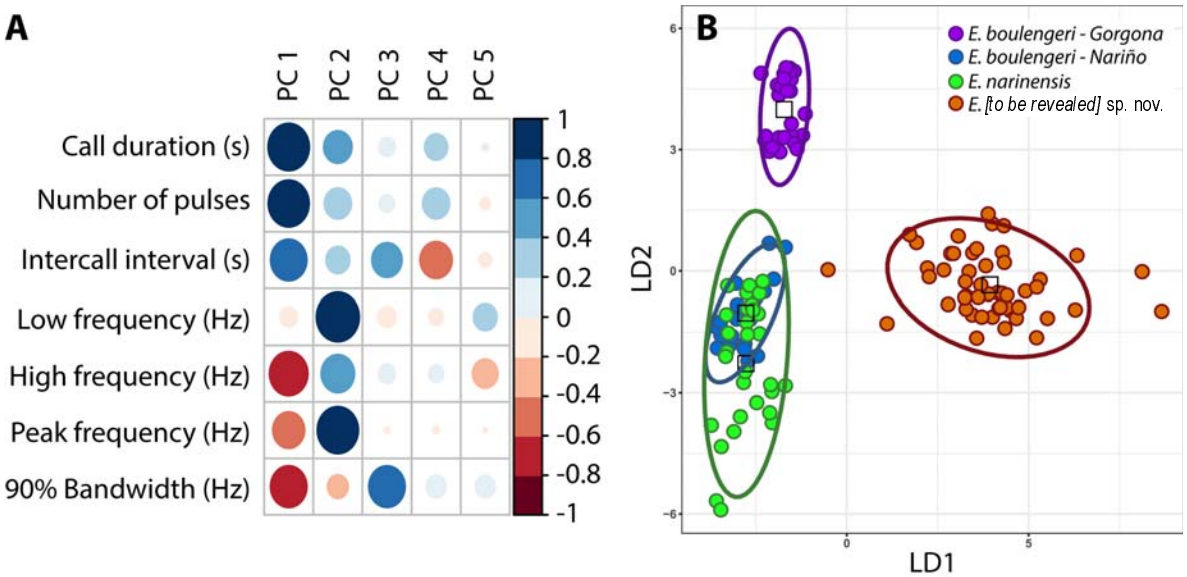

Figure 7. Differences in the acoustic parameters of *E. [to be revealed]* sp. nov. compared to other species of *Epipedobates*. **A** The relative contribution of each acoustic variable to the first five principal components obtained in the Principal Component Analysis. The absolute value of each contribution is represented according to the size of the circle, while blue and red colors show positive and negative contributions, respectively. Call duration, number of pulses and intercall interval are

strongly and positively associated with PC1 while high frequency and 90% bandwidth are strongly and negatively associated with PC1. PC2 is strongly and positively related to low frequency and peak frequency, while the frequency interquartile bandwidth 90% is negatively associated with PC2. **B** A Linear Discriminant Analysis using PCs as input indicates *E. [to be revealed]* can be differentiated from other species based on their advertisement calls, particularly regarding call duration and number of pulses (PC1 loads positively on LD1). Dots indicate the individuals, color indicates the species, and ellipses indicate the 95% confidence intervals for LDA data points.

Etymology

The specific epithet of this new species refers to the musical genre that originated on the southern Pacific coast of Colombia and Ecuador, where *E. [to be revealed]* occurs and also contributes to the local soundscape. *[to be revealed]*, also known as *bambuco viejo*, is an Afro-Colombian sounded practice that inspires dancing and transmits the happiness and cultural tradition of this region and is a symbol of resilience in the face of racial and regional oppression (Abadía, 1973; Aristizabal, 2002; Birenbaum Quintero, 2006, 2019). We named this species in honor of, and as an homage to, this musical genre that represents the culture of the southern Colombian Pacific because: *"la música, como la vida, no se pueden dejar perder"*, which translates to "music, like life, cannot be allowed to be lost" (Cruz Hoyos, 2016). It is a noun in apposition of masculine gender.

Distribution

Epipedobates [to be revealed] sp. nov. occurs in the Department of Valle del Cauca in the Pacific lowlands of southwestern Colombia. These frogs inhabit lowland forests from 0–260 m. The type locality is Ladrilleros, Buenaventura, Valle del Cauca, Colombia. We also observed the species in areas close to the type locality including Corregimiento Pianguita and Corregimiento Juan Chaco (La Barra beach) in the municipality of Buenaventura. The distribution towards the western flank of the western mountain range is in the Vereda El Danubio, the upper basin of the Anchicayá River, Dagua, Valle del Cauca. If we assume that the coloration traits of the new species are consistent for all populations of this species, records from the iNaturalist Platform would extend the distribution of *E. [to be revealed]* 194 km (132 km) straight line south to the municipality of Timbiquí, Cauca (see iNaturalist observations No. 135253843 and 85214439 and Fig. 1). Because individuals of this species were previously assigned to *E. boulengeri*, we recommend further exploration and inspection of museum specimens to better understand the geographic distribution of this species.

Ecology

Epipedobates [to be revealed] sp. nov. is a terrestrial species found on the ground during the day in agroforestry areas, on the edges of secondary forests, or in small patches of disturbed secondary forest always near or within marshes and/or slow-flowing streams (Fig. 8). The species is also likely present in primary forest in the region. We observed individuals actively moving among the grass and leaf litter, or actively calling at the edges of water bodies. The type locality (Ladrilleros, Valle de Cauca, Colombia) includes tiny forest fragments among human dwellings. Usually, these areas are contaminated with garbage or agricultural residues (Fig. 8A–F). In Anchicayá, populations of *E. [to be revealed]* are usually found along roadsides and forest edges, whenever small streams and leaf litter are present (Fig. 8G–J). We observed greater vocal activity during the morning and in the late afternoon than during warmer or sunnier parts of the day.

The density of *Epipedobates* varies across species and sites in Colombia. During our fieldwork, we noticed that the populations of *E. boulengeri* at Isla Gorgona and Maragrícola are quite abundant. For

example, more than five individuals can be heard calling at the same time, so it was very difficult to record a single male. For *E. narinensis* at Biotopo natural reserve individuals were calling less densely but were still abundant, with 8-10 m distance between calling males. But in other nearby locations we have heard only two or three individuals in a 3–4-hour walk. The individuals of *E. [to be revealed]* sp. nov. are more spread out and males were observed to call at approximately 4 to 5 m distance from each other at type locality (Ladrilleros and La Barra). At Anchicaya, males call every 3-4 m and are associated with small streams at the edge of the unpaved road. We do not know the reason for differences in density, as the pattern does not necessarily correspond to differences in habitat quality. While Isla Gorgona is a national park, *E. boulengeri* appears to be more abundant in the disturbed areas adjacent to field station buildings. The Maragrícola site where we sampled *E. boulengeri* in Nariño is an experimental cacao plantation. Ladrilleros and La Barra are disturbed sites similar to where *E. boulengeri* was abundant at Isla Gorgona, yet *E. [to be revealed]* did not occur at such high densities. It is plausible that the *E. [to be revealed]* is more vulnerable to habitat transformation than *E. boulengeri*, but additional research is necessary to understand what drives differences in density across sites and species.

Unfortunately, we did not collect larval stages of *E. [to be revealed]*. Larval morphology of Dendrobatoidea has contributed to understanding of the phylogenetic relationships within the superfamily (Anganoy-Criollo & Cepeda-Quilindo, 2017). It would be interesting to collect tadpoles of the new species for comparison with other *Epipedobates* species. Comparisons of larval stages made by Anganoy-Criollo and Cepeda-Quilindo (2017) detected some differences between the populations of Ecuador and southwest of Colombia in body shape, tips of marginal papillae, and pattern of tail coloration. However, the specimens from Colombia used in their study likely correspond to *E. boulengeri*-Nariño (*E. boulengeri sensu stricto*) and we are not certain of the identification of the specimens from Ecuador, which could correspond to *E.* aff. *espinosai*, *E. boulengeri sensu stricto*, or *E. espinosai*, which all occur in the region (López-Hervas et al., 2023).

Figure 8: Habitat structure of *Epipedobates [to be revealed]* sp. nov. at two localities. A–C Images of the type locality at Ladrilleros, Buenaventura, Valle del Cauca, Colombia. Usually, this species is found on roadsides near streams formed by rainfall. **D–F** At the type locality, we observed frogs in habitats contaminated with garbage or agricultural waste. Note the frog in F (red circle). **G–J** Habitat characteristics in Anchicayá, Dagua, Valle del Cauca, Colombia. Images B, C and E were taken by RDT, all others by MBC.

Conservation status

The populations observed in the four localities (Ladrilleros, Pianguita, La Barra, and Anchicayá) are probably affected by housing construction, garbage, or agricultural waste as well as forest fragmentation and loss of habitat. We do not know if these populations have adapted to human disturbance or if they are remnants of the original distribution of the species, but congeners in Ecuador are abundant in highly modified habitats such as cacao and banana populations (though they are notably absent from African palm plantations; RDT pers. obs.). Although we consider that *E. [to be revealed]* sp. nov. is abundant at the type locality, as three of us (RDT, MBC, JCR) captured 17 individuals in an approximate area of 30 m^2 in four hours, we do not know their abundance in less disturbed habitats. Improving our understanding of its conservation status will require monitoring and explorations in potential localities in Cauca and Valle del Cauca, especially in protected areas close to the localities reported in this study.

Despite the lack of certainty regarding the distribution of *E. [to be revealed]* sp. nov., its extent of occurrence is likely below 20,000 km^2. We know the species occurs in at least four localities (Ladrilleros, Anchicayá, Pianguita, and La Barra), with one more pending genetic validation (Timbiquí). The forests in the range of this species have been, and probably will continue to be,

subject to strong deforestation pressures that reduce the quantity and quality of available habitat and increase its fragmentation. Thus, we would recommend categorizing *E. [to be revealed]* sp. nov. as Vulnerable (VU: B1a, biii; IUCN, 2019). The distribution of *E. [to be revealed]* sp. nov. is very close to the Parque Nacional Natural Uramba Bahía Málaga, but we have only recorded the species in areas close to human settlements. We are not aware of the presence of *E. [to be revealed]* sp. nov. within the park. However, this conservation area protects more than 47,000 ha of marine and coastal areas, so it is very likely that *E. curralao* sp. nov. is found within the park. More research about this species' distribution, ecological requirements, and population dynamics will help confidently assign it to a threat category.

DISCUSSION

We describe *Epipedobates [to be revealed]* sp. nov. as a new species found along the Pacific chocoan rainforest of Colombia, which was previously considered to be part of the *E. boulengeri* species complex (López-Hervas et al. 2023). We describe this species as new based on its distinct advertisement call, coloration, and divergence from its sister species, *E. narinensis*. More information will be necessary to evaluate the conservation status of *E. [to be revealed]*, but we propose that it may be considered as Vulnerable under IUCN categorization given its relatively small range. Although *E. [to be revealed]* is not known to be sympatric with other species of *Epipedobates*, regions in southwestern Colombia (especially east of Guapi) have not been extensively surveyed and may contain sites with sympatry between *E. [to be revealed]*, *E. boulengeri*, and/or *E.* aff. *espinosai*. *Epipedobates [to be revealed]* does occur in sympatry and can be confused with *Andinobates minutus* unless color patterns are carefully distinguished (see Diagnosis section above). The other species of the *Epipedobates* genus are found along the western coast and Andean foothills in Ecuador and Peru. Although one species (*Epipedobates maculatus*) is thought to occur in western Panama (Grant et al., 2017; Jungfer, 2017), its assignment to this genus has been brought into question (López-Hervas et al. bioRxiv).

We find that the advertisement call of *E. [to be revealed]* is unique compared to other *Epipedobates* distributed in Colombia. Previously, Lötters et al., (2003) reported advertisement call differences between *Epipedobates boulengeri* recorded at Anchicayá (now *E. [to be revealed]*) and at Lita (now *E.* aff. *espinosai*), suggesting at the time that the populations may belong to a species complex. In our recordings, we found that *E. [to be revealed]* makes only one call per bout, although Lötters et al. (2003) describe calls with up to three notes; however, they recorded only one male. In the oscillogram and spectrogram of the advertisement call reported in their study (Fig 4, Lötters et al 2003), the internal intervals are so short that it is difficult to define when one note ends and the next begins, thus we consider that these calls correspond to one call.

We provide data on the courtship call of *E. [to be revealed]*, which to our knowledge is the first courtship call reported for *Epipedobates*. Like courtship calls of other neotropical poison frogs, it is lower frequency than the advertisement call of *E. [to be revealed]* (Caldeira Costa et al., 2006; González-Santoro et al., 2021; Marques Correia da Rocha et al., 2018; Moss et al., 2023). It was also shorter and had fewer pulses (7.9 vs 22-122 per call).

Epipedobates [to be revealed] is named in honor of the Afro-Colombian *[to be revealed]* musical genre and traditions that arose from generations of multicultural practices in the Pacific region, starting with African slaves brought to work in the gold mines, and continuing today with infusion of

new ideas and interpretations (Birenbaum Quintero, 2019; Guevara Calderón & Godoy Acosta, 2015). As part of the cultural heritage of Black communities in the Pacific region, *[to be revealed]* music, also known as *bambuco viejo*, helped bolster arguments that led to the recognition of the land rights and cultural identity of Afro-Colombians through Law 70 of 1993 (Birenbaum Quintero, 2019).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Funding acquisition: RDT, DCC. Conceptualization: MBC, RDT. Investigation: MBC, RDT, JCR. Methodology: MBC, RDT, JCR, AJC. Formal analysis: MBC. Writing – original draft: MBC, RDT. Writing – review and editing: MBC, RDT, DCC, JCR, AJC.

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Data availability

All of the data that support the findings of this study are available in the main text and were uploaded to public databases.

REFERENCES

Abadía G (1973) La música folklórica colombiana. Universidad Nacional de Colombia, Dirección de Divulgación Cultural, Bogota D.C.- Colombia, 158 pp.

Anganoy-Criollo M, Cepeda-Quilindo B (2017) Redescription of the tadpoles of *Epipedobates narinensis* and *E. boulengeri* (Anura: Dendrobatidae). Phyllomedusa, 16(2), 155–182. https://doi.org/10.11606/issn.2316-9079.v16i2p155-182

Aristizabal M (2002) El festival del [to be revealed] en Tumaco: Dinámicas culturales y construcción de identidad étnica en el litoral pacífico colombiano. Master Thesis, Universidad del Valle, Valle del Cauca, Colombia. https://www.humanas.unal.edu.co/colantropos/files/2914/5615/3098/aristizabal_[to be revealed].pdf

Barbour T (1909) Corrections regarding the names of two recently described Amphibia Salientia. Proceedings of the Biological Society of Washington, 22, 89.

Bioacoustics Research Program (2014) Raven Pro: Interactive Sound Analysis Software (Version 1.5) [Computer software] (1.5). The Cornell Lab of Ornithology. http://www.birds.cornell.edu/raven

Birenbaum Quintero M (2006) "La música pacífica" al Pacífico violento: Música, multiculturalismo y marginalización en el Pacífico negro colombiano. Trans. Revista Transcultural de Música, 10, 1–32. https://www.redalyc.org/pdf/822/82201002.pdf

Birenbaum Quintero M (2019) Rites, Rights & Rhythms A Genealogy of Musical Meaning in Colombia's Black Pacific. Oxford University Press, Canada, 342 pp. https://www.google.com/books/edition/Rites_Rights_and_Rhythms/YtB0DwAAQBAJ

Boulenger GA (1899) Descriptions of new reptiles and batrachians collected by Mr. P. O. Simons in the Andes of Ecuador. Annals and Magazine of Natural History, Series 7, 4, 454–457.

Brown J, Twomey E, Amézquita A, Barbosa De Souza M, Caldwell J, Lötters S, Von May R, Melo-Sampaio P, Mejía-Vargas D, Perez-Peña P, Pepper M, Poelman E, Sanchez-Rodriguez M, & Summers K (2011) A taxonomic revision of the Neotropical poison frog genus Ranitomeya (Amphibia: Dendrobatidae). Zootaxa, 120(3083), 1–120. https://doi.org/10.11646/zootaxa.3083.1.1

Caldeira Costa R, Gomes Facure K, Giaretta A (2006) Courtship, vocalization, and tadpole description of *Epipedobates flavopictus* (Anura: Dendrobatidae) in southern Goiás, Brazil. Biota Neotropica, 6(1). https://doi.org/10.1590/S1676-06032006000100006

Chao K-H, Barton K, Palmer S, Lanfear R (2021) sangeranalyseR: Simple and Interactive Processing of Sanger Sequencing Data in R. Genome Biology and Evolution, 13(3), 1–7. https://doi.org/10.1093/gbe/evab028

Cisneros-Heredia D, Yánez-Muñoz M (2010) A new poison frog of the genus Epipedobates (Dendrobatoidea: Dendrobatidae) from the north-western Andes of Ecuador. Avances, 2(3), B83–86. http://www.cisneros-heredia.org/pdfs/2010_Epipedobatesdarwinwallacei.pdf

Clough M, Summers K (2000) Phylogenetic systematics and biogeography of the poison frogs: evidence from mitochondrial DNA sequences. Biological Journal of the Linnean Society, 70(3), 515–540. https://doi.org/10.1006/bijl.1999.0418

Coloma L (1995) Ecuadorian Frogs of the Genus Colostethus (Anura: Dendrobatidae). Miscellaneous Publication. Museum of Natural History, University of Kansas, 87, 1–72. https://doi.org/10.5962/bhl.title.16171

Cruz Hoyos S (2016) “Samuelito”, el hombre que hizo del [to be revealed] una danza universal. Https://www.Elpais.Com.Co/Entretenimiento/Cultura/Samuelito-El-Hombre-Que-Hizo-Del-[to be revealed]-Una-Danza-Universal.Html.

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32(5), 1792–1797. https://doi.org/10.1093/nar/gkh340

Erdtmann L, Amézquita A (2009) Differential evolution of advertisement call traits in dart-poison frogs (Anura: Dendrobatidae). Ethology, 115(9), 801–811. https://doi.org/10.1111/j.1439-0310.2009.01673.x

Funkhouser JW (1956) New frogs from Ecuador and southwestern Colombia. Zoologica. New York, 41, 73–80.

Globally Unique Identifiers Task Group (2011) GUID and Life Sciences Identifiers Applicability Statements. Biodiversity Information Standards (TDWG). http://www.tdwg.org/standards/150

Goebel AM, Donnelly JM, Atz ME (1999) PCR Primers and Amplification Methods for 12S Ribosomal DNA, the Control Region, Cytochrome Oxidase I, and Cytochromebin Bufonids and other Frogs, and an overview of PCR Primers which have amplified DNA in amphibians auccessfully. Molecular Phylogenetics and Evolution, 11(1), 163–199. https://doi.org/10.1006/MPEV.1998.0538

González-Santoro M, Hernández-Restrepo J, Palacios-Rodríguez P (2021) Aggressive behaviour, courtship and mating call description of the neotropical poison frog *Phyllobates aurotaenia* (Anura: Dendrobatidae). Herpetology Notes, 14, 1145–1149. https://www.biotaxa.org/hn/article/view/60982

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. https://doi.org/dx.doi.org/10.1206/0003-0090(2006)299[1:PSODFA]2.0.CO;2

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. https://doi.org/10.2994/SAJH-D-17-00017.1

Guevara Calderón A, Godoy Acosta C (2015) El [to be revealed]: una propuesta de exploración, interpretación y creación, a través de la batería y la guitarra eléctrica. Master Thesis, Universidad del Bosque, Bogotá D.C. - Colombia.

Jungfer K-H (2017) On Warszewicz’s trail: The identity of *Hyla molitor* O. SCHMIDT, 1857. Salamandra, 53(1), 18–24. https://www.researchgate.net/publication/316542564

Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS (2017) modelfinder: fast model selection for accurate phylogenetic estimates. 14(6). https://doi.org/10.1038/nmeth.4285

Köhler J, Jansen M, Rodriguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel M-O, Vences M (2017) The use of bioacoustics in anuran taxonomy: Theory, terminology, methods and recommendations for best practice. Zootaxa, 4251(1), 1–124. https://doi.org/https://doi.org/10.11646/zootaxa.4251.1.1

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics, 30(22), 3276–3278. https://doi.org/10.1093/BIOINFORMATICS/BTU531

Lê S, Josse J, Husson F (2008) FactoMineR: An R Package for multivariate analysis. Journal of Statistical Software, 25(1), 1–18. https://doi.org/10.18637/JSS.V025.I01

López-Hervas K, Santos J, Ron S, Betancourth-Cundar M, Cannatella D, Tarvin R (2023) Deep divergences among cryptic clades of Epipedobates poison frogs. bioRxiv, 547117. https://doi.org/10.1101/2023.06.29.547117

Lötters S, Reichle S, Jungfer K-H (2003) Advertisement calls of Neotropical poison frogs (Amphibia: Dendrobatidae) of the genera Colostethus, Dendrobates and Epipedobates, with notes on dendrobatid call classification. Journal of Natural History, 37(15), 1899–1911. https://doi.org/10.1080/00222930110089157

Marques Correia da Rocha S, Pimentel Lima A, Kaefer IL (2018) Reproductive Behavior of the Amazonian Nurse-Frog *Allobates paleovarzensis* (Dendrobatoidea, Aromobatidae). South American Journal of Herpetology, 13(3), 260–270. https://doi.org/10.2994/SAJH-D-17-00076.1

McLachlan GJ (2004). Discriminant Analysis and Statistical Pattern Recognition | Wiley. Wiley-Interscience. https://www.wiley.com/en-us/Discriminant+Analysis+and+Statistical+Pattern+Recognition-p-9780471691150

Minh BQ, Nguyen MAT, Von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution, 30(5), 1188–1195. https://doi.org/10.1093/MOLBEV/MST024

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R, Teeling E (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. Molecular Biology and Evolution, 37(5), 1530–1534. https://doi.org/10.1093/MOLBEV/MSAA015

Moss JB, Tumulty JP, Fischer EK (2023) Evolution of acoustic signals associated with cooperative parental behavior in a poison frog. Proceedings of the National Academy of Sciences of the United States of America, 120(17), 1–7. https://doi.org/10.1073/PNAS.2218956120/-/DCSUPPLEMENTAL

Mueses-Cisneros JJ, Cepeda-Quilindo B, Moreno-Quintero V (2008) Una nueva especie de Epipedobates (Anura: Dendrobatidae) del suroccidente de Colombia. Papéis Avulsos de Zoologia (São Paulo), 48(1), 1–10. https://doi.org/10.1590/S0031-10492008000100001

Myers CW, Daly JW (1976) Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison-dart frogs (Dendrobatidae). Bulletin of the American Museum of Natural History, 157(3), 173–262.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature, 403, 853–858. https://doi.org/10.1038/35002501

Noble GK (1921) Five new species of Salientia from South America. American Museum Novitates, 29, 1–7. http://hdl.handle.net/2246/4615

Paradis E, Schliep K (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics, 35(3), 526–528. https://doi.org/10.1093/BIOINFORMATICS/BTY633

Peters WCH (1873) Über eine neue Schildrötenart, Cinosternon Effeldtii und einige andere neue oder weniger bekannte Amphibien. Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin, 603–618.

Powers RP, Jetz W (2019) Global habitat loss and extinction risk of terrestrial vertebrates under future land-use-change scenarios. Nature Climate Change, 9(4), 323–329. https://doi.org/10.1038/s41558-019-0406-z

R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://www.R-project.org/.

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, 281, 20141761. https://doi.org/10.1098/rspb.2014.1761

Santos JC, Cannatella DC (2011) Phenotypic integration emerges from aposematism and scale in poison frogs. Proceedings of the National Academy of Sciences of the United States of America, 108(15), 6175–6180. https://doi.org/10.1073/pnas.1010952108

Santos JC, Coloma LA, Cannatella DC (2003) Multiple, recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences of the United States of America, 100(22), 12792–12797. https://doi.org/10.1073/pnas.2133521100

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 e1000056(3), 0448–0461. https://doi.org/10.1371/journal.pbio.1000056

Sueur J, Aubin T, Simonis C (2008) Seewave, a free modular tool for sound analysis and synthesis. Bioacoustics, 18, 213–226. http://cran.r-project.org/src/contrib/Descriptions/

Tarvin RD, Powell EA, Santos JC, Ron SR, Cannatella DC (2017) The birth of aposematism: High phenotypic divergence and low genetic diversity in a young clade of poison frogs. Molecular Phylogenetics and Evolution, 109, 283–295. https://doi.org/10.1016/j.ympev.2016.12.035

Venables W, Ripley B (2002) Modern Applied Statistics with S (Fourth Edition). Springer. https://www.stats.ox.ac.uk/pub/MASS4/.

Vences M, Kosuch J, Boistel R, Haddad CFB, La Marca E, Lötters S, Veith M (2003) Convergent evolution of aposematic coloration in Neotropical poison frogs: a molecular phylogenetic perspective. Organisms Diversity & Evolution, 3, 215–226. http://www.urbanfischer.de/journals/ode

Warren R, Vanderwal J, Price J, Welbergen JA, Atkinson I, Ramirez-Villegas J, Osborn TJ, Jarvis A, Shoo LP, Williams SE, Lowe J (2013) Quantifying the benefit of early climate change mitigation in avoiding biodiversity loss. Nature Climate Change, 3(7), 678–682. https://doi.org/10.1038/nclimate1887

Watters JL, Cummings ST, Flanagan RL, Siler CD (2016) Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. Zootaxa, 4072(4), 477–495. https://doi.org/10.11646/zootaxa.4072.4.6

Figure legends.

Figure 1. Map of research area. The southern half of the Pacific lowlands of Colombia, part of the Chocó biogeographical region, hosts at least three species of *Epipedobates*. Photograph of the frog from Timbiquí (Reserva Ranita terribilis, Fundación ProAves de Colombia) was obtained from iNaturalist (observation No. 135253843 by Juan Carlos Luna); other images were taken by the authors.

Figure 2. Images in life and in preservative of the holotype of *Epipedobates [to be revealed]* sp. nov.. **A** Full specimen in life; **B** Dorsal view in life; **C** Ventral view in life; **D** Lateral view in life; **E** Dorsal view in preservative (70% ethanol); **F** Ventral view in preservative; **G** Lateral view in preservative; **H** Dorsal hand in preservative; **I** Ventral hand in preservative; **J** Dorsal foot in preservative; **K** Ventral foot in preservative. Scale bars represent 5 mm (B to G) or 2.5 mm (H to K).

Figure 3. Images in life of *Epipedobates [to be revealed]* sp. nov. in comparison with close congeners and sympatric species. A. *E. [to be revealed]* sp. nov. from the type locality of Ladrilleros, Valle de Cauca, Colombia (ANDES:A:5261; SVL = 20.0 mm; adult male; paratype); **B.** *E. narinensis* from Biotopo, Nariño, Colombia (ANDES:A:3704; 16.39 mm; adult male); **C.** *Andinobates minutus* from Ladrilleros, Valle de Cauca, Colombia (ANDES:A:5266; 13 mm; sex not determined); **D.** *E. boulengeri* from Isla Gorgona, Cauca, Colombia (individual not captured); **E.** *E. espinosai* from Río Palenque, Santo Domingo de los Tsáchilas, Ecuador (individual not captured); **F.** *E.* aff. *espinosai* from La Nutria, Nariño, Colombia (ANDES:A:2476; 17.77 mm; sex not determined); **G.** *E. boulengeri* from Maragrícola, Nariño, Colombia (ANDES:A:2472; 18.96 mm; adult female); **H.** A side-by-side image of *E. boulengeri* from Isla Gorgona (ANDES:A:3695; 20.65 mm; adult female) and *E. [to be revealed]* sp. nov. from Pianguita, Valle de Cauca, Colombia (ANDES:A:3690; 16.42 mm; adult female) demonstrating the large size difference between the two species. All images were taken by RDT except for A and C, which were taken by JCR. Photos are not to scale.

Figure 4. Phylogenetic position and genetic distances of *E. [to be revealed]* sp. nov. and other species of *Epipedobates*. A A species-level maximum-likelihood phylogeny of *Epipedobates* estimated in IQ-TREE using two nuclear genes (*BMP2, H3*) and three mitochondrial gene fragments (*CYTB, 12S–16S, CR*) (see Fig. Supp. material S2 for full phylogeny). Node support values from 10,000 ultrafast bootstrap replicates indicated strong support of *E. [to be revealed]* sp. nov. as the sister-group of *E. narinensis*. **B** Matrix of mean pairwise p-distances among species of *Epipedobates*. The lower triangle distances are based on *CYTB* and the upper triangle distances are from *12S–16S*. *Epipedobates [to be revealed]* is genetically most similar to *E. narinensis* (1.77% for *12S–16S* and 5.39% for *CYTB*). Abbreviations: cur, *E. [to be revealed]* sp. nov; nar, *E. narinensis*; bouN, *E. boulengeri* (Nariño, Colombia); bouG, *E. boulengeri* (Isla Gorgona, Cauca, Colombia); aesp, *E.* aff. *espinosai*; esp, *E. espinosai*; tri, *E. tricolor*; mac, *E. machalilla*; ant, *E. anthonyi*. See Supp. material S2 for full phylogeny.

Figure 5. Comparisons of SVL and advertisement call traits between *E. [to be revealed]* sp. nov. and other species of *Epipedobates*. A Snout-vent length (SVL) **B** Peak frequency **C** Call duration and **D** Number of pulses per call. Abbreviations include: E. bou - Gor, *E. boulengeri* - Gorgona; E. bou -
Nar, *E. boulengeri* - Nariño; *E. nar, E. narinensis;* and *E. cur* sp. nov., *E. [to be revealed]* sp. nov. Black points represent raw data, the horizontal bar shows the mean, the shaded diagram is a

smoothed density curve showing the full data distribution, and the rectangle represents the uncertainty around the mean using a 95% Bayesian highest density interval.

Figure 6. **Vocalizations in *E. [to be revealed]* sp. nov. and other species of *Epipedobates*.** Spectrograms (above) and oscillograms (below) showing general aspects of the structure of one spontaneous advertisement call for one male of **A** *E. [to be revealed]* sp. nov. of Ladrilleros, Valle del Cauca, **B** *E. narinensis* of Biotopo, Nariño, **C** *E. boulengeri*-Gorgona (Type locality) and **D** *E. boulengeri*-Nariño of Maragrícola, Nariño. Advertisement calls consist of call groupings, comprising a single call for *Epipedobates [to be revealed]* sp. nov., 2–3 calls for *E. boulengeri*-Gorgona, 3–6 calls for *E. boulengeri*-Nariño, and 5–14 calls for *E. narinensis*. **E** Spectrograms and oscillograms showing general aspects of the structure of one courtship call of *E. [to be revealed]* sp. nov. of Ladrilleros, Valle del Cauca. **F** Comparison of one advertisment call between *E. [to be revealed]* sp. nov. and other species of *Epipedobates*. *E. bou* - Gor: *E. boulengeri* - Gorgona, *E. bou* - Nar: *E. boulengeri* - Nariño, E. nar: *E. narinensis* and *E. cur* sp. nov.: *E. [to be revealed]* sp. nov. Fast Fourier Transform-FFT = 256, overlap = 90%.

Figure 7. Differences in the acoustic parameters of *E. [to be revealed]* sp. nov. compared to other species of *Epipedobates*. A The relative contribution of each acoustic variable to the first five principal components obtained in the Principal Component Analysis. The absolute value of each contribution is represented according to the size of the circle, while blue and red colors show positive and negative contributions, respectively. Call duration, number of pulses and intercall interval are strongly and positively associated with PC1 while high frequency and 90% bandwidth are strongly and negatively associated with PC1. PC2 is strongly and positively related to low frequency and peak frequency, while the frequency interquartile bandwidth 90% is negatively associated with PC2. **B** A Linear Discriminant Analysis using PCs as input indicates *E. [to be revealed]* can be differentiated from other species based on their advertisement calls, particularly regarding call duration and number of pulses (PC1 loads positively on LD1). Dots indicate the individuals, color indicates the species, and ellipses indicate the 95% confidence intervals for LDA data points.

Figure 8: Habitat structure of *Epipedobates [to be revealed]* sp. nov. at two localities. A–C Images of the type locality at Ladrilleros, Buenaventura, Valle del Cauca, Colombia. Usually, this species is found on roadsides near streams formed by rainfall. **D–F** At the type locality, we observed frogs in habitats contaminated with garbage or agricultural waste. Note the frog in F (red circle). **G–J** Habitat characteristics in Anchicayá, Dagua, Valle del Cauca, Colombia. Images B, C and E were taken by RDT, all others by MBC.