Article

Forty years later: a new Andean stream treefrog of the genus *Hyloscirtus* (Anura: Hylidae) from Ecuador, with comments on arm hypertrophy in the *H. larinopygion* group

MAURICIO RIVERA-CORREA[1,*], SANTIAGO RON[2], IVAN NUNES[3], KATYUSCIA ARAUJO-VIEIRA[4], PAULO D. P. PINHEIRO[5] & TARAN GRANT[6]

[1]*Grupo Herpetológico de Antioquia, Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.*

[2]*Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Roca, Apartado 17-01-2184, Quito, Ecuador.*
✉ *santiago.r.ron@gmail.com;* ⓘ *https://orcid.org/0000-0001-6300-9350*

[3]*Laboratório de Herpetologia (LHERP), Instituto de Biociências, Campus do Litoral Paulista, UNESP, São Vicente, São Paulo, Brazil.*
✉ *ivan.nunes@unesp.br;* ⓘ *https://orcid.org/0000-0001-7985-2836*

[4]*Laboratório de Herpetologia, Departamento de Biodiversidade and Centro de Aquicultura (CAUNESP), Universidade Estadual Paulista-UNESP, Av. 24 A, 1515, Bela Vista, 13506-970, Rio Claro, São Paulo, Brazil.*
✉ *katy.vieira@gmail.com;* ⓘ *https://orcid.org/0000-0002-8193-2736*

[5]*Division of Amphibians & Reptiles, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, D.C., 20560, U.S.A.*
✉ *PinheiroP@si.edu;* ⓘ *https://orcid.org/0000-0003-4398-4380*

[6]*Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Trav. 14, no. 101, Cidade Universitária, 05508-090, São Paulo, SP, Brazil.*
✉ *taran.grant@ib.usp.br;* ⓘ *https://orcid.org/0000-0003-1726-999X*

Corresponding author: ✉ *mauricio.rivera1@udea.edu.co;* ⓘ *https://orcid.org/0000-0001-5033-5480*

Abstract

We present the description of a novel treefrog species inhabiting the Andean streams in southeastern Ecuador that has been erroneously identified as *Hyloscirtus lindae* for four decades. The new species is closely related to *H. tapichalaca* and is part of the southern clade of the *H. larinopygion* group, which comprises five species confined to the southeastern Andes of Ecuador to the northeastern Andes of Peru. It is diagnosed from its close relatives by a unique combination of characteristics, including hypertrophied forelimbs in males, a pronounced, curved, and protruding spine-shaped prepollex, a substantial supracloacal flap, supratympanic fold, digital discs colored in shades of orange-red or bright-red, and the concealed surfaces of limbs displaying a bluish-gray hue and dorsal spicules in males. We briefly explore the implications of this discovery for the evolution of arm morphology in the *H. larinopygion* group. Our findings underscore the continued importance of periodically reviewing historical specimens, leading to unexpected discoveries; once again confirming the importance of natural history museums and their custodian biological collections.

Key words: Andes, Biological Collections, Biodiversity, Morphology, Systematics, Taxonomy

Introduction

Hyloscirtus Peters, 1882 is a stream-dwelling genus that occurs from Costa Rica in Central America to Bolivia in western South America and Venezuela and currently includes 40 species (Frost 2024). The species are divided among four species groups: the *H. armatus*, *H. bogotensis*, *H. larinopygion*, and *H. jahni* groups (Faivovich *et al.* 2005; Sánchez 2010; Rojas-Runjaic *et al.* 2018; Yánez-Muñoz *et al.* 2021; Reyes-Puig *et al.* 2022; Sánchez-Nivicela *et al.* 2023).

Knowledge of the diversity and systematics of the charismatic Andean *H. larinopygion* group has increased recently thanks to studies of their taxonomy, adult and tadpole morphology, bioacoustics, and phylogeny (Sánchez 2010; Coloma *et al.* 2012; Rivera-Correa & Faivovich 2013; Almendáriz *et al.* 2014; Rivera-Correa *et al.* 2016;

Ron *et al.* 2018; Reyes-Puig *et al.* 2022; Sánchez-Nivicela *et al.* 2023). Among the 19 species currently recognized in the *H. larinopygion* group, 14 are distributed in Ecuador (Frost 2024), making it the most diverse country for this group.

The *Hyloscirtus larinopygion* group is composed of two clades with well-structured geographic patterns (Almendáriz *et al.* 2014; Ron *et al.* 2018; Reyes-Puig *et al.* 2022). The northern clade is distributed from the northern Andes of Colombia to the southeastern Andes of Ecuador, and the southern clade is distributed from the southeastern Andes of Ecuador to the northeastern Andes of Peru (Rivera-Correa & Faivovich 2013; Almendáriz *et al.* 2014; Ron *et al.* 2018). The southern clade is composed by *H. condor* Almendáriz, Brito, Batallas, and Ron, 2014, *H. diabolus* Rivera-Correa, García-Burneo, and Grant, 2016, *H. hillisi* Ron, Caminer, Varela-Jaramillo, and Almeida-Reinoso, 2018, and *H. tapichalaca* (Kizirian, Coloma, and Paredes-Recalde, 2003), which share morphological traits (see Rivera-Correa *et al.* 2016 for details). These taxa are distinctive in having hypertrophied forelimbs with enlarged muscles and a spine-shaped prepollex (Pinheiro *et al.* 2022). While hypertrophied forelimbs are symplesiomorphic [shared with *H. armatus* (Boulenger, 1902) and *H. charazani* (Vellard, 1970)], the spine-shaped prepollex appears to be a synapomorphy for the southern clade of the *H. larinopygion* group (Pinheiro *et al.* 2022).

Previous phylogenetic analyses have included a sequence of a specimen with hypertrophied forelimbs and a spine-shaped prepollex, identified as *Hyla lindae* Duellman & Altig, 1978 (Duellman & Hillis 1990), *Hyloscirtus lindae* (Wiens *et al.* 2006, 2010; Pyron & Wiens 2011), *Hyloscirtus "lindae"* (Almendáriz *et al.* 2014), and, most recently, *Hyloscirtus* sp. (Ron *et al.* 2018). However, hypertrophied forelimbs with enlarged muscles and a spine-shaped prepollex are absent in *H. lindae*, as well as in all species of the northern clade of the *H. larinopygion* group. The inclusion of samples and sequences from the type locality of *H. lindae* by Coloma *et al.* (2012) were able to establish that the sample provided by Duellman & Hillis (1990) did not correspond to *H. lindae*, being misidentified over four decades from the time of its collection (probably due to the red coloration of fingers and toes discs). Given the context provided above, specimens deposited at KU (Biodiversity Institute, University of Kansas) Were reviewed, and their phenotypic characteristics also support the hypothesis that they correspond to an undescribed species. Thus, the objectives of this paper are to (1) formally describe this new species, (2) briefly discuss the potential reasons for its previous misidentification, and (3) explore the implications of this discovery on the evolution of arm morphology within the *H. larinopygion* group.

Materials and Methods

Morphological data. Measurements follow Rivera-Correa & Faivovich (2013) and include some recommendations from Watters *et al.* (2016). All measurements were taken using digital calipers to the nearest 0.1 mm (Table 1). Abbreviations are: SVL (snout-vent length: direct line distance from tip of snout to posterior margin of vent), HL (head length: from the posterior of the jaws to the tip of the snout), HW (head width: at the widest point; angle at the jaws), ED (eye diameter), EN (eye-nostril distance: horizontally from the anterior to posterior corner of the eye), NS (snout-nostril distance: distance from the center of the external nares to the tip of the snout), IND (internarial distance: shortest distance between the inner margins of the nostrils), AMD (distance between anterior margins of eyes), TD (tympanum diameter: greatest horizontal width of the tympanum), FAL (forearm length: from the flexed elbow to the base of the outer palmar tubercle), FAW (forearm width: greatest horizontal width of the forearm), HAL (hand length: from the base of the outer palmar tubercle to the tip of Finger IV), THL (thigh length: distance from the vent to the knee), TL (tibia length: distance from the outer surface of the flexed knee to the heel/tibiotarsal inflection), TAL (tarsal length: distance from the tibiotarsal inflection to the base of the inner metatarsal tubercle), FL (foot length: from the base of the inner metatarsal tubercle to the tip of Toe IV), TFD (measure at the widest point on the Finger III disk), and FTD (measure at the widest point on the Toe IV disk). Webbing formulae follow the terminology of Savage & Heyer (1967) as modified by Myers & Duellman (1982). Description of prepollical morphology follows Pinheiro *et al.* (2022).

Sex was determined by examination of secondary sexual characters (hypertrophied forelimbs, nuptial pads, vocal slits, expansion of the vocal sac) and presence of oocytes. Regarding forelimb hypertrophy, Pinheiro *et al.* (2022) provide a brief discussion on the definition of hypertrophy in anurans and the difficulties in defining it. We employ the term 'hypertrophy' to refer to forelimbs that are proportionally wider in males than females. Coloration in life and natural history data were obtained from the field notes of William E. Duellman and David

M. Hillis deposited at KU. Information on other species was obtained from preserved specimens (Appendix I), field notes, photos of live specimens, and the literature (i.e., original species descriptions). Unless otherwise noted, color descriptions refer to live specimens. Coordinates for the type locality were georeferenced based on locality verbal descriptions from Duellman & Hillis (1990) and the University of Kansas, Biodiversity Institute database (available at https://biodiversity.ku.edu/herpetology/collection-search). We assumed that distances from Plan de Milagro were measured along the road. This assumption was corroborated by the coincidence between the locality elevation reported by Duellman & Hillis (1990) and the elevation estimated from digital elevation maps of the region.

Institutional abbreviations used throughout this paper are (following Sabaj 2023): CORBIDI (División de Herpetología, Centro de Ornitología y Biodiversidad, Lima, Peru), DHMECN (División de Herpetología, Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador), EPN (Museo de Historia Natural Gustavo Orcés de la Escuela Politécnica Nacional, Quito, Ecuador), ICN (Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia), KU (Biodiversity Institute, The University of Kansas, Lawrence, USA), MHUA (Museo de Herpetología, Universidad de Antioquia, Medellín, Colombia), and QCAZ (Museo de Zoología, Pontificia Universidad Católica del Ecuador, Ecuador).

Molecular sampling and analysis. We produced mitochondrial sequences for three adult specimens (KU 202728, 202729, and 202731; see Appendix II for the GenBank accession numbers) of the new species collected at the type locality. The sequences produced include a fragment including the ribosomal 12S rRNA, the intervening tRNAVal, and the 16S rRNA ($\approx$ 1,770 bp). The cellular DNA was extracted from ethanol-preserved tissues (liver or muscle) using the Qiagen DNeasy® Blood & Tissue kit (QIAGEN, Valencia, CA). Primers used in PCR amplification are MVZ59 (ATAGCACTGAAAAYGCTDAGATG; Graybeal 1997), 12sF-H (CTTGGCTCGTAGTTCCCTGGCG; Goebel *et al.* 1999), 12sA-L (AAACTGGGATTAGATACCCCACTAT; Goebel *et al.* 1999), tRNA^{VAL}-H (GGTGTAAGCGARAGGCTTTKGTTAAG; Goebel *et al.* 1999), 16L2a (CCAAACGAGCCTAGTGATAGCTGGTT; Hedges 1994), 16H10 (TGATTACGCTACCTTTGCACGGT; Hedges 1994), 16SAR (CGCCTGTTTATCAAAAACAT; Palumbi *et al.* 1991), and 16SBR (CCGGTCTGAACTCAGATCACGT; Palumbi *et al.* 1991).

PCR amplification was carried out in 25 μl reaction using 12.5 μl of Taq DNA polymerase Master Mix (Ampliqon A/S, Denmark), 10.5 μl of nucleotide free H$_2$0, 1 μl of genomic DNA, and 0.5 μl for each primer. The PCR protocol consisted of an initial denaturation step of 3 min at 95°C, 36 cycles of 20 s at 95°C, 20 s at 50°C, 50 s at 68°C, and a final extension step of 5 min at 68°C. The PCR products were cleaned with 0.4 μl of ExoSAP-IT express (Thermo Fisher Scientific, Massachusetts, USA) plus 1.6 μl of nuclease-free water per 5 μl of reaction and incubated for 4 min at 37°C and 1 min at 80°C. Sequencing was done using fluorescent-dye labels terminators (ABI Prism Big Dye Terminators v. 3.1 Cycle Sequencing kit; Applied Biosystems, Foster City, CA) on an automatic sequencer ABI 3730XL (Applied Biosystems, Foster City, CA) in both directions to check for potential errors and polymorphisms. The chromatograms obtained from the automated sequencer were trimmed for quality and the contigs for each gene fragment were assembled using Sequencher 5.4.6 (Gene Codes, Ann Arbor, MI).

We complemented our new data with the corresponding 12S and 16S rRNA sequences of species in the *H. armatus*, *H. bogotensis*, and *H. larinopygion*, and *H. jahni* groups available on GenBank produced by Darst & Cannatella (2004), Faivovich *et al.* (2005), Wiens *et al.* (2006), Coloma *et al.* (2012), Almendáriz *et al.* (2014), Guayasamin *et al.* (2015), Rojas-Runjaic *et al.* (2018), Ron *et al.* (2018), Yánez-Muñoz *et al.* (2021), and Reyes-Puig *et al.* (2022).

We aligned the 12S and 16S rRNA gene fragments using MAFFT version 7 (Katoh & Standley 2013) with the Q-INS-i strategy (secondary structure of RNA is considered). The alignments were visualized in BioEdit (Hall 1999). The phylogenetic analyses, including the two gene fragments, were performed under the maximum parsimony criterion because it offers the greatest explanatory power by minimizing both the number of evolutionary events and the assumptions about the process of character evolution required to explain the observed variation (Farris 1983; Goloboff 2003; Goloboff & Pol 2005; Kluge & Grant 2006; Grant & Kluge 2009). For the analysis we employed TNT v1.6 (Goloboff & Morales 2023). Searches used the command "Traditional Search" with 100 rounds of random addition sequences (RAS) + TBR branch swapping, saving 10 trees per round. The resulting trees were submitted to a round of TBR branch swapping. Gaps were treated as fifth state and all transformations were weighted equally.

Our outgroup sample included *Aplastodiscus perviridis* Lutz, 1950, *Boana faber* (Wied-Neuwied, 1821), *Bokermannohyla circumdata* (Cope, 1871), and *Nesorohyla kanaima* (Goin and Woodley, 1969), the latter used to root the tree (Faivovich *et al.* 2005; Pinheiro *et al.* 2019). Parsimony jackknife absolute frequencies (36 removal

probability; Farris *et al*. 1996) were calculated in TNT v1.6 using traditional search requesting 100 rounds of TBR branch swapping, for a total of 1000 replicates. The resulting tree was read and edited with FigTree v1.4.3 (Rambaut 2014).

Uncorrected pairwise distances (UPDs) of the fragment 12S (≈857 bp) and 16S rRNA genes (AR-BR, ≈585 bp) between the new species and the remaining species of the *Hyloscirtus larinopygion* group were calculated in PAUP* (Swofford 2002). To minimize missing data, we excluded short 12S rRNA sequences of the new species KU 202731 (≈400 bp) and *H. staufferorum* (Duellman and Coloma, 1993) QCZA 45967 (≈499 bp) from the UPDs calculations.

Results

Phylogenetic relationships and UPDs. The parsimony analyses resulted in 92 most parsimonious trees (mpts) of 2,790 steps. One of the most parsimonious trees is shown in Fig. 1. The analysis recovered *Hyloscirtus* as monophyletic (93% jackknife). The *H. armatus, H. bogotensis, H. larinopygion*, and *H. jahni* groups are also monophyletic (88–100% jackknife), but the relationships between them are poorly supported (≤ 60% jackknife). The conflicts among mpts within *Hyloscirtus* are restricted to: (i) the position of *H. jahni* group; (ii) the interspecific relationships in the northern clade of the *H. larinopygion* group; (iii) the relationships between *H. alytolylax* (Duellman, 1972), *H. conscientia* Yánez-Muñoz, Reyes-Puig, Batallas-R., Broaddus, Urgilés-Merchán, Cisneros-Heredia, and Guayasamin, 2021, *H. mashpi* Guayasamin, Rivera-Correa, Arteaga-Navarro, Culebras, Bustamante, Pyron, Peñafiel, Morochz, and Hutter, 2015, *H. simmonsi* (Duellman, 1989), *Hyloscirtus* sp. within the *H. bogotensis* group; (iv) and the position of *H. palmeri* (Boulenger, 1908) + *H. phyllognathus* (Melin, 1941) within the *H. bogotensis* group. The *H. larinopygion* group is moderately supported, with 88% jackknife support. Within this group, *H. arcanus* **sp. nov.** is the moderately supported sister taxon of *H. tapichalaca* (79% jackknife); these species are sister of *H. hillisi* (94% jackknife), which together form a well-supported clade (100% jackknife) with *H. condor*—the southern clade. This clade is the sister taxon of all remaining species of the *H. larinopygion* group—the northern clade. Although the interspecific relationships within the northern clade are generally poorly supported (< 50% jackknife), some internal clades with 2–3 species are moderately or well supported (80–100% jackknife), such as the clade including *H. larinopygion* (Duellman, 1973), *H. lindae*, and *H. pantostictus* (Duellman and Berger, 1982).

The UPDs between the new species and its closest relatives (*H. condor, H. hillisi*, and *H. tapichalaca*; see Almendáriz *et al.* 2014; Ron *et al.* 2018; this study) are 2.6–3.8% (12S rRNA, ≈857 bp) and 1.7–2.6% (16S rRNA, ≈595 bp; see Appendices III–IV).

Species Account

Hyloscirtus arcanus sp. nov.
(Figs. 2–4)

Hyla lindae, Duellman & Hillis (1990: 16; table 3–4; fig. 7; appendix I; part)
Hyloscirtus lindae, Wiens *et al.* (2006: supp. mat. fig A2: 16; 2010: 877); Pyron & Wiens (2011: 570)
Hyloscirtus "*lindae*", Almendáriz *et al.* (2014: 37; table 1; fig. 2; part)
Hyloscirtus sp., Ron *et al.* (2018: 101; table 1; fig. 1)

Holotype. KU 202730, adult male, Ecuador, Provincia de Morona-Santiago, 23.3 km WSW Plan de Milagro, (-3.2075, -78.5450; 2350 m a.s.l), collected on 12 March 1984 by David M. Hillis and Patricia A. Burrowes.

Paratypes. KU 202731, adult male, collected with holotype. KU 202728, adult male; KU 202729, adult female, Ecuador, Provincia de Morona-Santiago, 21.6 km SW Plan de Milagro, (-3.1907, -78.5414; 2425 m a.s.l), collected on 4 March 1984 by William E. Duellman and David M. Hillis.

Etymology. The specific epithet is derived from the Latin adjective *arcanus*, meaning "hidden" or "secret," in reference to the fact that this species remained concealed in a museum and literature under an erroneous taxonomic identity for forty years.
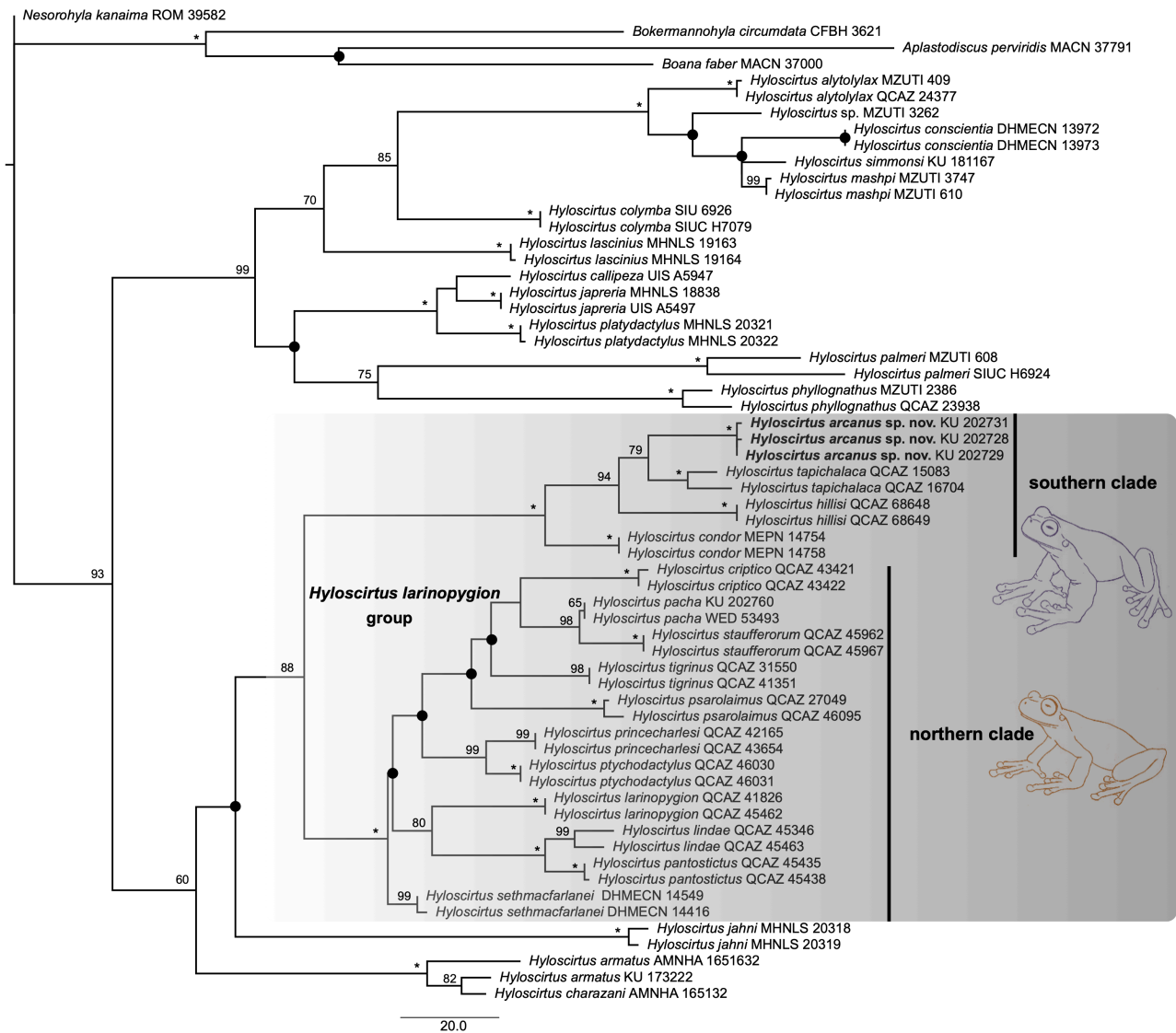
FIGURE 1. Phylogenetic relationships of *Hyloscirtus* as recovered in one of the 92 most parsimonious trees obtained from the analysis of 12S, tRNAVal, and 16S rRNA gene fragments with gaps as fifth state, equal weights for all transformations, and zero-length branches collapsed. Black circles indicate nodes that collapse in the strict consensus. Numbers above nodes are parsimony jackknife support values. An asterisk (*) indicates groups with 100% for parsimony jackknife frequencies. Nodes lacking values have < 50% jackknife support; all are retained in the strict consensus.

Definition. We assign the new species to *Hyloscirtus* and the *H. larinopygion* group based on its phylogenetic placement (Wiens *et al.* 2006, 2010; Pyron & Wiens 2011; Almendáriz *et al.* 2014; Ron *et al.* 2018), corroborated by our own phylogenetic analysis (Fig. 1). *Hyloscirtus arcanus* **sp. nov.** can be distinguished from other species of the *H. larinopygion* group by the following combination of characters: dorsum and venter pale to dark gray, concealed surfaces of limbs bluish gray (in life); digital discs orange-red to bright-red (in life); iris dull olive bronze (in life); large, curved, spine-shaped prepollex; strongly hypertrophied forelimbs in males; large, thick, supracloacal flap and supratympanic fold; nuptial pad absent; vomerine odontophore includes two small processes in contact with each other and carrying four teeth each; finger webbing formula: II $1^{2/3}$–$2^{2/3}$ III 2^{+}–2 IV, toe webbing formula: I $1^{1/2}$ –2^{-} II 1^{-}–2 III $1^{1/2}$ –$2^{1/2}$ IV $2^{1/2}$–1^{-}V, and spicules on snout, *canthus rostralis*, eyelid, supratympanic fold, and shank in adult males.

Diagnosis. In the *Hyloscirtus larinopygion* group, only four species possess highly hypertrophied forelimbs in adult males and enlarged, curved, spine-shaped prepollex. These species are *H. tapichalaca* (Kizirian *et al.* 2003), *H. condor* (Almendáriz *et al.* 2014), *H. diabolus* (Rivera-Correa *et al.* 2016), and *H. hillisi* (Ron *et al.* 2018). The

remaining species in the *H. larinopygion* group, including *H. lindae*, lack these characters, exhibiting, instead, a blade-shaped prepollex and non-hypertrophied forelimbs (Kizirian *et al.* 2003; Rivera-Correa & Faivovich 2013; Rivera-Correa *et al.* 2016; Pinheiro *et al.* 2022; Reyes-Puig *et al.* 2022; Sánchez-Nivicela *et al.* 2023). *Hyloscirtus arcanus* **sp. nov.** resembles *H. lindae* in having red disks on fingers and toes. In addition to forearm morphology, males of both species differ in shank dorsal texture, being smooth in *H. lindae* and spiculate in *H. arcanus* **sp. nov.** (Fig. 2). *Hyloscirtus arcanus* **sp. nov.** differs from species of the southern clade in the following characteristics described below.
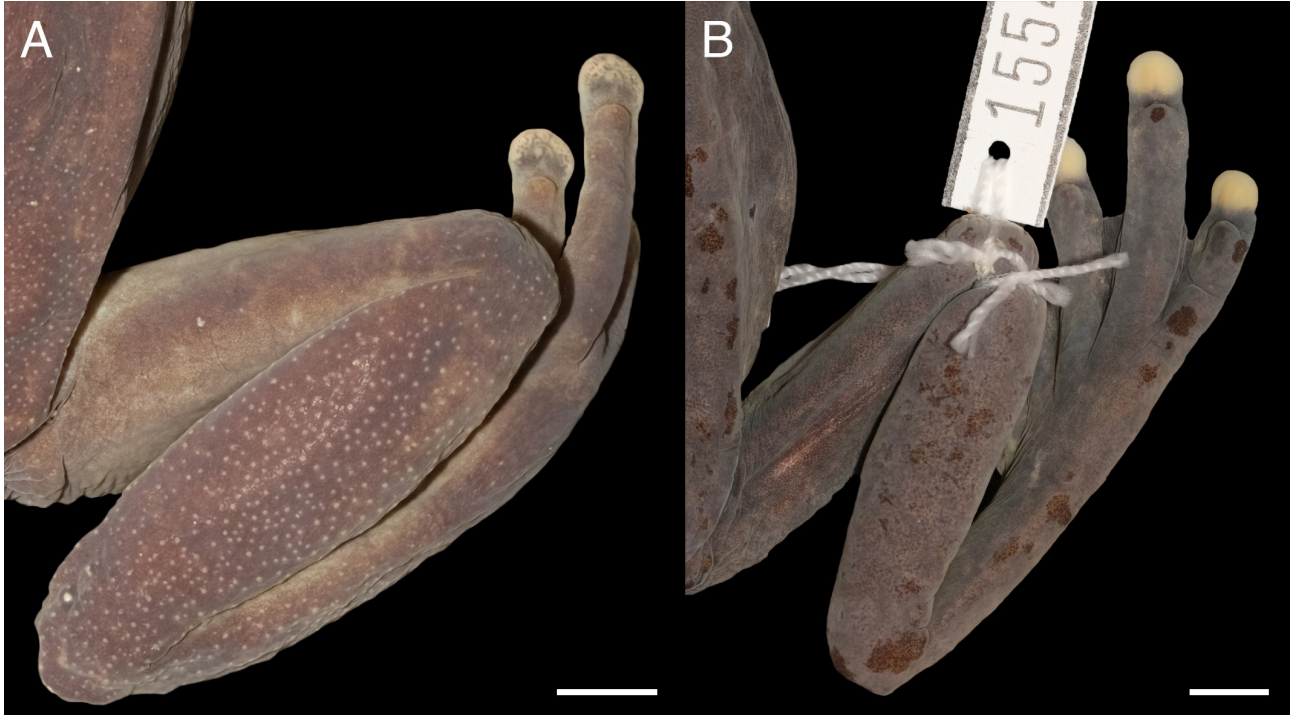

FIGURE 2. Dorsal view of right leg of (A) holotype of *Hyloscirtus arcanus* **sp. nov.** (KU 202730) and (B) paratopotype of *H. lindae* (KU 155475), both males. Notice the presence of scattered spiculae on the shank in (A), absent in (B). Scale bars = 5 mm.

FIGURE 3. Dorsal and ventral view of the holotype of *Hyloscirtus arcanus* **sp. nov.** (KU 202730; adult male, SVL 64.9 mm).
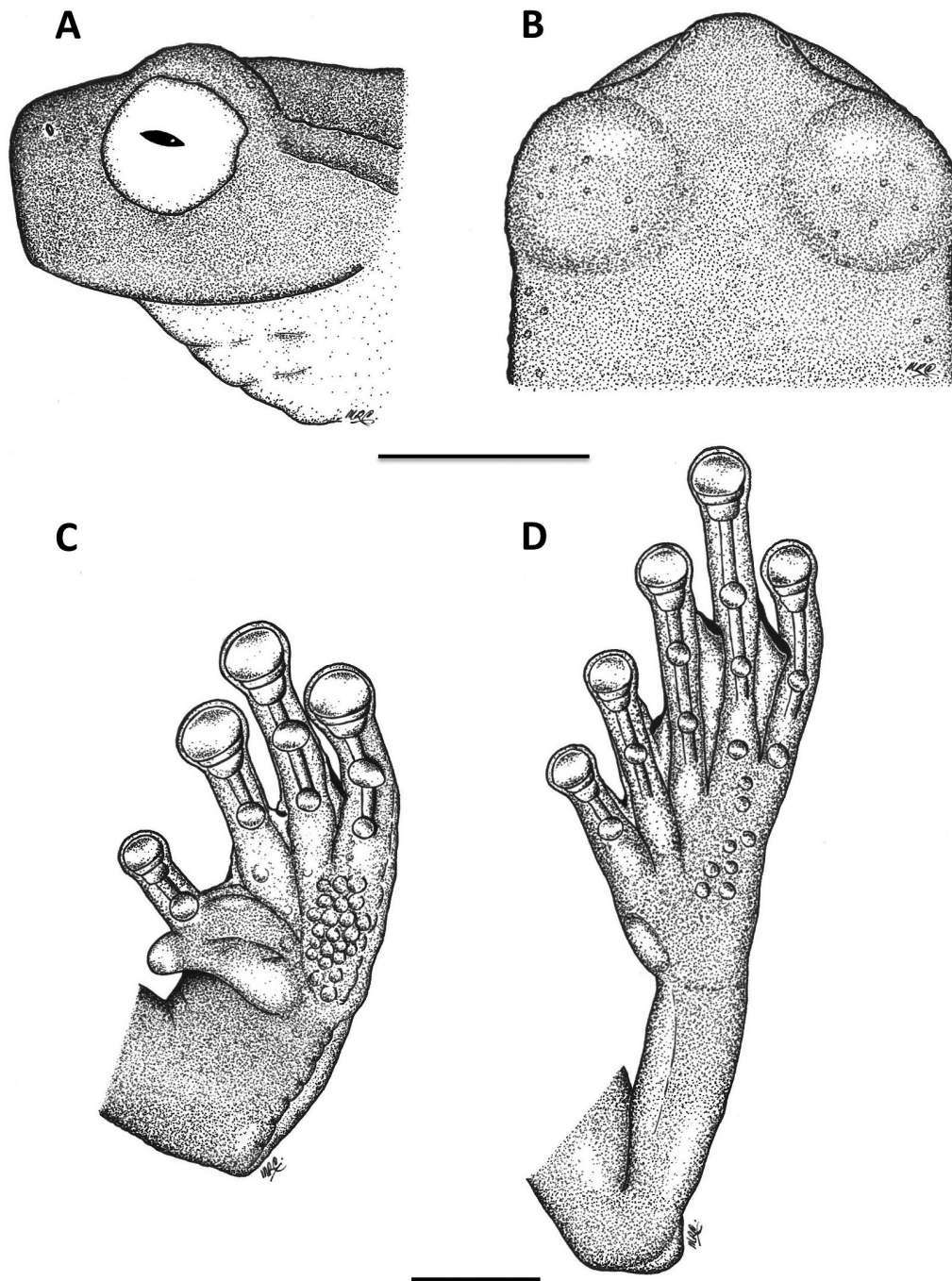
FIGURE 4. Drawings of *Hyloscirtus arcanus* **sp. nov.** (KU 202730, holotype). (A) Head in lateral view (B) head in dorsal view; (C) left hand in ventral view; (D) left foot in ventral view. Scale bars = 10 mm.

Hyloscirtus tapichalaca: axillary and inguinal regions with pink-orange marks (absent in *H. arcanus* **sp. nov.**), thighs and shanks with brown transverse bands (absent in *H. arcanus* **sp. nov.**), concealed surfaces of limbs brown (concealed surfaces of limbs bluish gray in *H. arcanus* **sp. nov.**), lateral and lower margin of cloaca knee, elbow, and outer margin of forearm white (white absent on cloaca and limbs in *H. arcanus* **sp. nov.**), digital discs white (orange-red to bright-red digital discs in life or greyish-orange in preserved specimens of *H. arcanus* **sp. nov.**), iris yellow-gold with fine black reticulations (dull olive-bronze in *H. arcanus* **sp. nov.**), vomerine odontophore processes separate (contacting medially in *H. arcanus* **sp. nov.**), dorsal spicules absent (present in *H. arcanus* **sp. nov.**), gular and abdominal region dark grey (pale grey in *H. arcanus* **sp. nov.**), and calcar short and triangular (transverse fringe in *H. arcanus* **sp. nov.**).

TABLE 1. Measurements (mm) of the type series of *Hyloscirtus arcanus* **sp. nov.** See Materials and Methods for abbreviations.

Measurements	Males (*n* = 3)				Female (*n* = 1)
	Mean	SD	Min	Max	
SVL	66.5	1.5	64.9	67.8	70.8
HL	18.6	0.8	17.7	19.3	19.6
HW	21.6	0.7	21.0	22.4	22.7
ED	7.4	0.2	7.2	7.5	7.6
EN	4.2	0.2	4.0	4.3	3.8
NSD	3.3	0.3	3.1	3.6	3.6
IND	5.1	0.5	4.7	5.7	5.1
AMD	11.2	0.8	10.5	12.1	11.6
TD	-	-	-	-	3.4
FAL	10.9	0.5	10.5	11.6	11.4
FAW	9.8	0.2	9.6	10.0	6.0
HAL	22.4	1.0	21.4	23.4	22.9
THL	31.1	0.2	31.0	31.3	34.8
TL	30.7	0.7	30.1	31.4	34.0
TAL	16.6	0.1	16.5	16.7	20.0
FL	28.6	1.1	27.4	29.4	32.3
TFD	3.6	0.5	3.1	4.0	3.4
FFD	3.5	0.1	3.4	3.6	3.2

Hyloscirtus condor: dorsum dark brown with contrasting yellow spots (gray without spots in *H. arcanus* **sp. nov.**), digital discs light brown, yellow-brown, or brown with abundant yellow blotches (orange-red to bright-red digital in *H. arcanus* **sp. nov.**), iris golden with fine tan reticulations (dull olive-bronze in *H. arcanus* **sp. nov.**), vomerine odontophore processes separate (contacting medially in *H. arcanus* **sp. nov.**), dorsal spicules in males absent (present in *H. arcanus* **sp. nov.**).

Hyloscirtus diabolus: dorsum dark brown with abundant small yellow marks or yellow blotches (gray without spots in *H. arcanus* **sp. nov.**), concealed surfaces of thighs and shanks dark brown (bluish-gray in *H. arcanus* **sp. nov.**), digital discs dark brown, with or without yellow spots (orange-red to bright-red digital discs of *H. arcanus* **sp. nov.**), iris dark red (dull olive-bronze in *H. arcanus* **sp. nov.**), vomerine odontophore processes separate (contacting medially in *H. arcanus* **sp. nov.**), and dorsal spicules in males absent (present in *H. arcanus* **sp. nov.**).

Hyloscirtus hillisi: dorsum dark brown with contrasting round orange marks (gray without spots in *H. arcanus* **sp. nov.**), digital discs dark brown (orange-red to bright-red in *H. arcanus* **sp. nov.**), iris bronze or yellowish with brown reticulations (dull olive-bronze in *H. arcanus* **sp. nov.**), fleshy calcar absent (present in *H. arcanus* **sp. nov.**).

Description of holotype. Adult male, 64.9 mm SVL (Fig. 3). Body robust. Head 16% wider than long, as wide as body; head width 35% of SVL; head length 27% of SVL. Snout rounded in dorsal view, truncate in profile (Fig. 4A–B); *canthus rostralis* rounded, indistinct; loreal region concave; lips rounded, faintly flared; nostrils weakly protuberant, directed anterolaterad, positioned at the level of anterior margin of the lower jaw. Dorsal surface of internarial region concave. Interorbital distance slightly larger than upper eyelid. Eye prominent, diameter larger than eye-nostril distance. Tympanum and tympanic annulus inconspicuous. Supratympanic fold prominent, extending from behind eye and to posterior edge of arm insertion. Region between head and suprascapulae depressed. Vomerine teeth in two short and massive processes, in contact medially, located behind choanae; each series with four prominent teeth. Choanae small, ovoid, separated by more than 3.5 times their maximum diameter. Tongue cordiform, attached along entire length except narrow free area around postero-lateral margin. Vocal slits longitudinal, originating lateral to tongue, extending to the corner of the mouth. Vocal sac large, single, median, subgular, evident externally. Mental gland undetectable.

Upper arm and forearm hypertrophied; axillary membrane absent; thick ulnar fold present. Fingers short, thick, bearing large, ovoid discs with circumferential grooves clearly defined by the difference in size between disc and pad; width of finger III disc 53% of eye diameter. Relative lengths of fingers 1 < 2 < 4 < 3. Fingers with dermal fringes, webbing thick, conspicuous; webbing formula II 2^-–$2^{2/3}$ III $2^{1/3}$–2 IV (Fig. 4C). Distal subarticular tubercles large, single, rounded in ventral view, conical in profile. Palmar surfaces with deep folds. Supernumerary tubercle at the base of palm large, high, and round. Outer metacarpal tubercle well defined, thick, elliptical, flat. Inner metacarpal tubercle large, following outline of underlying enlarged, protruding spine-shaped, distal prepollex. Nuptial pad absent.

Hindlimbs robust; tibia length 48% of SVL; foot length 45% of SVL. Calcar forming transverse fringe; tarsal fold absent, but tubercles extending along the outer edge of tarsus; inner tarsal fold present. Inner metatarsal tubercle large, ovoid; outer metatarsal inconspicuous. Toes short, lacking lateral fringes, toe discs smaller than those of fingers, slightly wider than digit shaft. Relative length of toes 1 < 2 < 5 < 3 < 4; toe webbing formula: I $1^{1/2}$–2 II $1^{1/2}$–2^+ III $1^{1/2}$–$2^{1/2}$ IV $2^{1/2}$–$2^·$ V (Fig. 4D). Subarticular tubercles large, rounded in ventral view, conical in profile; single rows of smaller supernumerary tubercles along axis of each toe. Cloacal opening directed posteriorly at upper level of thighs; supracloacal flap large, thick; edges of vent with numerous small folds; cloacal sheath short. Dorsal skin, gular region, pectoral region, and flanks smooth except for abundant spicules on the loreal region, eyelids, posterior dorsum, and tibia; belly and proximal portion of ventral surface of inner thigh granular.

Color of holotype in life. Dorsal surfaces and flanks dark gray, ventral surfaces pale gray. Concealed surfaces of limbs bluish gray; digital discs orange-red. Iris dull olive-bronze.

Color of holotype in preservative. Dorsum and flanks grayish purple, ventral surfaces pale gray. Concealed surfaces of limbs gray; toe webbing cream gray, digital discs orange pink.

Measurements of the holotype (mm). SVL 64.9; HL 17.7; HW 21.0; ED 7.4; EN 4.0; NSD 3.0; IND 4.7; AMD 10.5; FAL 10.7; FAW 9.6; HAL 22.4; THL 31.0; TL 31.4; TAL 16.7; FL 29.4; TFD 4.0; FTD 3.6.

Variation and sexual dimorphism. The female (KU 202729) is slightly larger than the males, and although her forelimbs are robust, they do not present the remarkable hypertrophy observed in the three males (FAW/FAL 0.45 in female, 0.78–0.88 in the three males; Fig. 5). The prepollex is an osseous spine-shaped element in both sexes, although it is slightly smaller in the female (inner metacarpal tubercle length/FAL 0.59 in female, 0.70–0.72 in the three males). The tympanum is partially visible in the female, being covered dorsally by the supra-tympanic fold, and is rounded and inclined medially towards the transversal body axis such that it is almost visible from above; its diameter is 44% of eye diameter; in males, the tympanum is inconspicuous. In life, the dorsum of males was dark gray and dull grayish brown in female. The belly of the female was dark gray, light gray in males. The color on the tips of the digits varied from reddish-orange to bright red (tomato red), covering almost the entire disc (see male KU 202731; Fig. 6). The males have keratinized spicules on the snout, *canthus rostralis*, eyelid, supratympanic fold, dorsum, and near the cloaca and shanks; spicules are absent in the only female. The males have subtly more extensive webbing than the female; the webbing formula varies as follows: II (2–2^-)–($2^{1/2}$–$2^{2/3}$) III ($2^{1/3}$–$2^{1/2}$)–(2^+–2^-) IV / I (2–2^+)–(2^+–2^-) II $1^{1/2}$–(2^+–2^-) III ($1^{1/2}$–2^-)–(2–$2^{1/2}$) IV (2–$2^{1/2}$)–($1^{1/2}$–2^-) V. Other secondary sexual characters (i.e., vocal slits, vocal sac) are absent in the female. Morphometric measurements of the paratypes are given in Table 1.

Prepollex anatomy. The prepollex of adult male KU 202728 is entirely osseous and consists of two elements, a short, cuboid, proximal element and a distal, enlarged, spine-shaped element (Fig. 7A). The distal element has a small dorsomedial crest that anchors one of the insertions of the m. abductor pollicis longus and lacks a post-articular process. Metacarpal II articulates with both proximal and distal elements of the prepollex without a medial expansion of its proximal epiphysis.

Ventrally, the prepollex serves as point of insertion for three muscles: the m. adductor pollicis, m. flexor indicis brevis profundus, and m. pronator quadratus. The m. adductor pollicis originates on the ventral surface of distal carpal 3-4-5 and inserts ventrally via a fleshy attachment on the distal prepollex. The m. flexor indicis brevis profundus also originates on distal carpal 3-4-5, with most fibers inserted ventromedially on metacarpal II and some fibers inserting laterally on the distal prepollex. The m. pronator quadratus has two origins, one on the ulnar side of the radioulna, distally and ventrally, and the other on the ulnare. Some of the fibers from both origins converge to insert ventrally via a tendon on the proximal portion of metacarpal II. The remaining radioulna fibers insert directly (ventrally and proximally) on the prepollical elements and deeply on element Y; the ulnare fibers insert proximally on the proximal portion of the prepollex. Thick, white connective tissue binds both the m. adductor pollicis and m. pronator quadratus together ventrally near the prepollex.

FIGURE 5. Paratype series of *Hyloscirtus arcanus* **sp. nov.**. On top dorsal view, on the bottom ventral view. From left to right: female KU 202729, males KU 202728 and KU 202731. Scale bars = 20 mm.

FIGURE 6. *Hyloscirtus arcanus* **sp. nov.** in life. (KU 202731, paratype, adult male, SVL 67.8 mm). Photo: D. Hillis (1984).
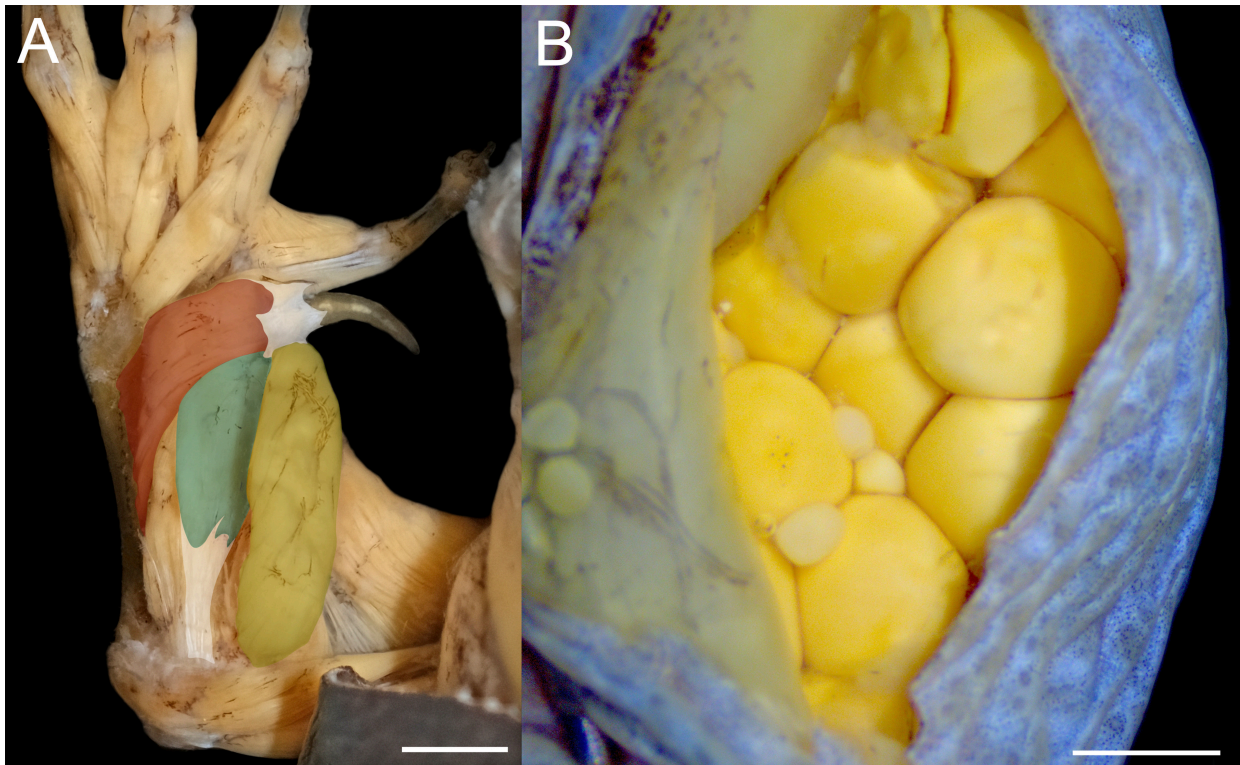
FIGURE 7. A. Dorsal view of left forearm of *Hyloscirtus arcanus* **sp. nov.** adult male KU 202728 with the three slips of the m. abductor pollicis longus highlighted. White highlights tendons; maroon highlights the slip originating via fleshy attachments on the radioulna; teal highlights the slip originating via a tendon on the distal epicondyle of humerus; olive highlights the slip originating via a fleshy attachment on the cristal lateralis of humerus. The following muscles were previously removed: m. extensor communis, m. extensor carpi ulnaris, m. epicondylo-cubitalis, and m. epitrochleo-cubitalis. Scale bar = 3 mm. B. Ventral cut on abdomen of *Hyloscirtus arcanus* **sp. nov.** female specimen (KU 202729) revealing the large and mature oviducal oocytes, with unpigmented animal pole. Scale bar = 2 mm.

Dorsally, the prepollex also interacts with three muscles: the m. abductor pollicis longus, m. extensor indicis brevis medius, and m. abductor indicis brevis dorsalis. The m. abductor pollicis longus has three slips, one with a broad, fleshy origin from across almost the entire length of radioulna, another, narrower, originating distally from the distal epicondyle of the humerus via a broad but thin tendon, and a third robust slip with a fleshy origin from the lateral crest of the humerus, proximal to the second slip's origin. The slip from the radioulna inserts on both metacarpal II, at its midlength dorsomedially, and dorsally on the portion of the prepollex; both slips insert via a common, broad tendon. The two slips from the humerus join the same tendon of insertion on the portion that attaches to the prepollex (Fig. 7A). The m. extensor indicis brevis medius originates on the radiale and inserts dorsomedially on metacarpal II, proximal to the m. abductor pollicis longus insertion, dorsolaterally on the distal prepollex. The m. abductor indicis brevis dorsalis originates on element Y and inserts dorsally on the distal prepollex via a fleshy attachment between the insertions of the m. abductor pollicis longus and m. extensor indicis brevis medius. The m. dorsometacarpalis indicis proximalis lacks the slip originating from the distal prepollex.

Distribution and natural history. *Hyloscirtus arcanus* **sp. nov.** is known from two nearby localities on the eastern Andean slopes of southern Ecuador (Provincia Morona Santiago; Fig. 8; airline distance between localities < 2 km). The elevation is 2,350–2,425 m a.s.l. Specimens KU 202728–29 were found at night on 4 March 1984 on tree branches in the spray zone of a waterfall. The male was calling from a cavity beneath moss; the call was a whistle with a three-note pattern and a low-pitched. The female was on the branch of a bush. An additional calling male (not collected) was observed higher up the same waterfall. The vegetation type (according to the classification of Sierra *et al.* 1999) is Cloud Montane Forest of the eastern Andes. The tadpole is unknown. The new species occurs less than 10 km from populations of *H. pacha* (Duellman and Hillis, 1990), a species of the northern clade (Duellman & Hillis 1990).

The skin on the tip of the prepollex presents scars in the males but not in the female, suggesting its effective usage only by males. Probably, during combats and/or amplexus the prepollex pierces the encapsulating skin that covers it. However, as observed in the males KU 202728 and KU 202731, the female KU 202729 also has scars on the dorsum, suggesting either combat or a complex breeding behavior. The oviducts of the female are full of large, unpigmented oocytes (oocyte diameter 2.28–2.97 mm; *n* = 3; Fig. 7B).
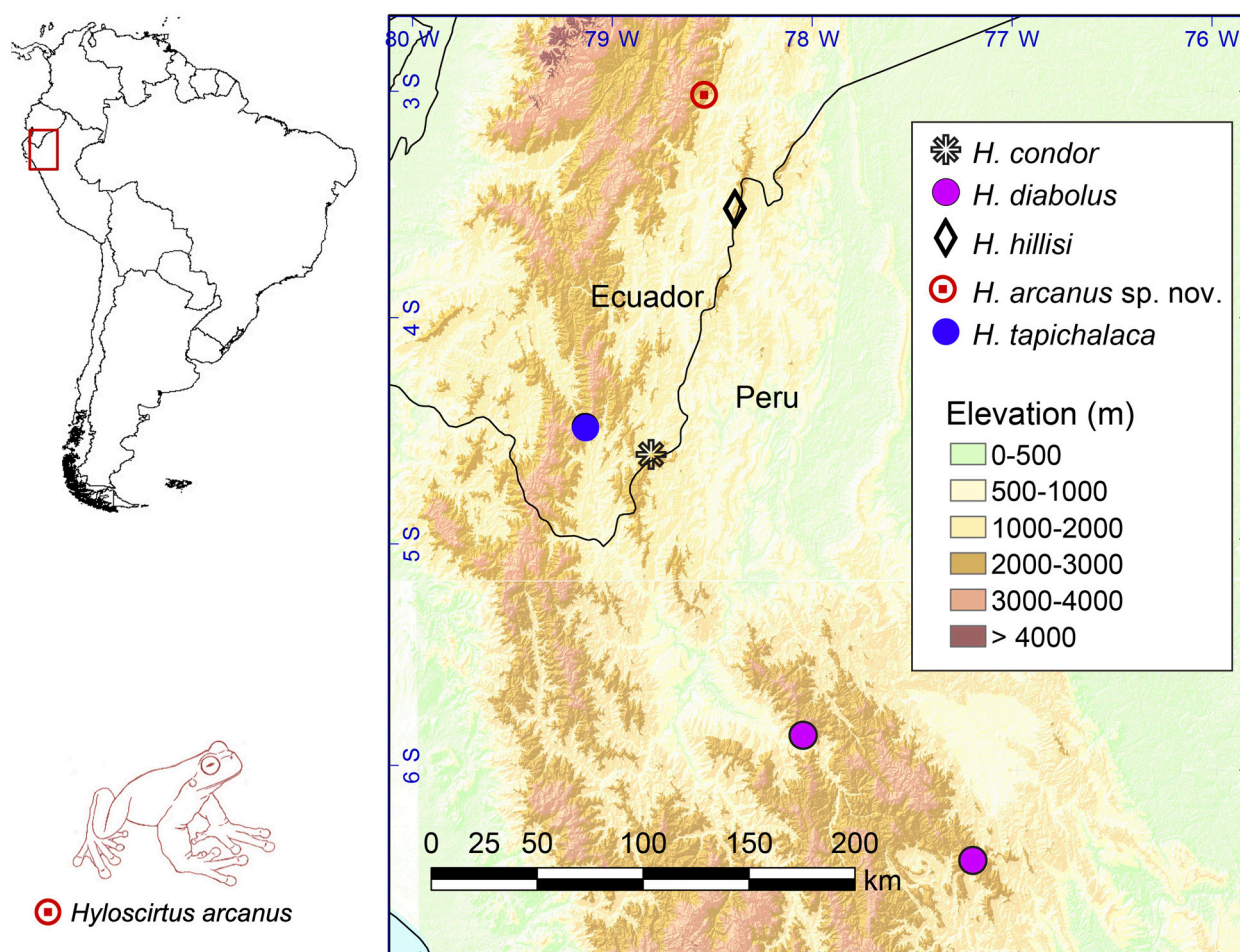

FIGURE 8. Map showing currently known localities for species of the southern clade of the *Hyloscirtus larinopygion* group, including *Hyloscirtus arcanus* **sp. nov.** (the species with the northernmost distribution of the clade).

Discussion

The phylogenetic trees presented by Duellman & Hillis (1990), Wiens *et al.* (2006, 2010), and Pyron & Wiens (2011) differ from the phylogeny put forth by Coloma *et al.* (2012). These differences stem from the use of a specimen historically misidentified as '*Hyloscirtus lindae*' (or '*Hyla lindae*'; KU 202728) in the studies of Duellman & Hillis (1990), Wiens *et al.* (2006, 2010), and Pyron & Wiens (2011). Consequently, their results placed '*H. lindae*' within the southern clade of the *H. larinopygion* group. In contrast, Coloma *et al.* (2012) employed correctly identified samples of *H. lindae*, placing it in the northern clade of the group. With the recognition of *H. arcanus* **sp. nov.** for the "*H. lindae*" from the southern clade and updated identification of KU 202728, all four previous phylogenetic analyses converge, aligning with our findings.

Duellman & Hillis (1990) overlooked the presence of a prominent spine-shaped prepollex and enlarged forelimbs in the specimens described here as *H. arcanus* **sp. nov.** These features, absent in *H. lindae* and all the other species within the *H. larinopygion* group known at that time, were first recognized by Kizirian *et al.* (2003) when describing *H. tapichalaca*. One potential reason for misidentifying *H. arcanus* **sp. nov.** as *H. lindae* may be their shared characteristic of having orange or red discs on their digits (in life). In *Hyloscirtus*, the coloration on digit discs is

unusual, showing reddish colors only in *H. lindae*, *H. pantostictus*, and the species we have described here (i.e., *H. arcanus* **sp. nov.**). Therefore, within the broader context of the known phylogeny of the *H. larinopygion* group, we deduce that coloration on digit discs has a complex evolutionary history. The reddish coloration evolved at least twice (*H. lindae* and *H. arcanus* **sp. nov.**), but other colors also appeared in other species, such as yellow (once, in *H. pantostictus*; Duellman & Berger 1982) and white [twice, in *H. tapichalaca* and *H. caucanus* (Ardila-Robayo, Ruiz-Carranza, and Roa-Trujillo, 1993); Ardila-Robayo *et al.* 1993; Kizirian *et al.* 2003]. The evolutionary history of these traits depends on establishing the phylogenetic position of *H. caucanus* and understanding the homology between those different coloration patterns.

The description of *H. arcanus* **sp. nov.** raises to 19 the number of known species in the *H. larinopygion* group. Of these, ten are endemic to Ecuador [i.e., *H. arcanus* **sp. nov.**, *H. condor*, *H. criptico* Coloma, Carvajal-Endara, Dueñas, Paredes-Recalde, Morales-Mite, Almeida-Reinoso, Tapia, Hutter, Toral-Contreras, and Guayasamin, 2012, *H. pacha*, *H. princecharlesi* Coloma, Carvajal-Endara, Dueñas, Paredes-Recalde, Morales-Mite, Almeida-Reinoso, Tapia, Hutter, Toral-Contreras, and Guayasamin, 2012, *H. ptychodactylus* (Duellman and Hills, 1990), *H. sethmacfarlanei* Reyes-Puig, Recalde, Recalde, Koch, Guayasamin, Cisneros-Heredia, Jost, and Yánez-Muñoz, 2022, *H. staufferorum*, *H. tapichalaca*, and *H. tolkieni* Sánchez-Nivicela, Falcón-Reibán, and Cisneros-Heredia, 2023], five species are distributed in both Ecuador and Colombia [i.e., *H. larinopygion*, *H. lindae*, *H. pantostictus*, *H. psarolaimus* (Duellman and Hills, 1990), and *H. tigrinus* Mueses-Cisneros and Anganoy-Criollo, 2008], three species are endemic to Colombia [*H. antioquia* Rivera-Correa and Faivovich, 2013, *H. caucanus*, *H. sarampiona* (Ruiz-Carranza and Lynch, 1982)], and one to Peru (*H. diabolus*). Consequently, Ecuador maintains the highest species diversity of the *H. larinopygion* group with highly restricted distribution ranges, which are reflected in the high levels of endemism in the country (Coloma *et al.* 2012; Frost 2024; see also Ron *et al.* 2022).

Our phylogenetic results corroborated the phylogenies shown by Almendáriz *et al.* (2014), Ron *et al.* (2018) and Reyes-Puig *et al.* (2022), giving strong support to two clades within the group, a northern clade, and a southern clade (Fig. 1). The northern clade is more diverse and putatively includes the 15 species (we are tentatively including the Colombian species *H. antioquia*, *H. caucanus*, *H. sarampiona*, and *H. tolkieni*, based on phenotypical data; Sánchez-Nivicela *et al.* 2023; MRC unpublished data). The southern clade is less specious, with the remaining five species (i.e., *H. arcanus* **sp. nov.**, *H. condor*, *H. hillisi*, *H. tapichalaca* and, putatively, *H. diabolus*, which has not yet been included in phylogenetic analyzes but has the same character states associated with this clade, such as the hypertrophy of the arms and the presence of a spine-shaped prepollex; see also Sanchéz-Nivicela *et al.* 2023). Given the limited field research conducted in the Andean highlands of southern Ecuador and northern Peru, and the differences in species diversity between both clades of the *H. larinopygion* group, it would not be a surprise if the alpha diversity of the southern clade is underestimated.

Pinheiro *et al.* (2022) conducted an in-depth examination of prepollex diversity, related traits, and historical context within the clades of the tribe Cophomantini, showing a notable evolutionary plasticity. However, the authors stressed that the absence of natural history information for several of the internal groups of the tribe (*Hyloscirtus* is a group with an important gap in this knowledge) precludes the advance in understanding potential selective pressures acting on those traits. In this tribe, numerous groups of species have a spine-shaped distal prepollex, and it is frequently noted that males exhibit hypertrophied forelimbs (e.g., Duellman *et al.* 1997; Rivera-Correa *et al.* 2016; Ron *et al.* 2018; Pinheiro *et al.* 2022). The authors optimized the differences in forearm size between sexes, finding that the presence of sexual dimorphism in forelimbs size is plesiomorphic for *Hyloscirtus*, with a further transformation to no sexual dimorphism in the northern clade of the *H. larinopygion* group (Pinheiro *et al.* 2022: supp. info. S7). Research involving androgens has demonstrated a positive correlation of these hormones with the enlargement of male musculature (Müller *et al.* 1969; Regnier & Herrea 1993), as well as with male behavior directly linked to muscular systems (Mangiamele *et al.* 2016).

Overall, the skeleton structure and muscles associated with the prepollex in *H. arcanus* **sp. nov.** agree with the general pattern described for Cophomantini (Pinheiro *et al.* 2022). But three muscular traits deserve attention. *Hyloscirtus arcanus* **sp. nov.** is the first species with a spine-shaped prepollex to have its muscles studied for the genus. Differently from other groups of Cophomantini with a spine-shaped prepollex, the new species does not exhibit the slip of the m. dorsometacarpalis indicis proximalis originating from the prepollex. This slip is present in almost all species of *Bokermannohyla* Faivovich, Haddad, Garcia, Frost, Campbell, and Wheeler, 2005 and *Boana* Gray, 1825 with a spine-shaped prepollex so far studied, the only exceptions being *Boana pombali* (Caramaschi, Pimenta, and Feio, 2004) and *B. tepuiana* Barrio-Amorós and Brewer-Carias, 2008 (Pinheiro *et al.* 2022). The m.

flexor indicis brevis profundus, besides inserting ventrally on metacarpal II, as usually observed in Cophomantini, in *H. arcanus* **sp. nov.** it also inserts through few fibers laterally on distal prepollex. Finally, in the context of Hylidae, *H. arcanus* **sp. nov.** is the single known species to have the m. abductor pollicis longus presenting three slips and originating from both radioulna and humerus. All other hylid species studied so far exhibit this muscle originating only from radioulna (see Pinheiro *et al.* 2022). The insertion of the m. flexor indicis brevis profundus on the distal prepollex and the m. abductor pollicis longus presenting three slips originating from both the humerus and radioulna are putative synapomorphies of the southern clade of the *H. larinopygion* group, pending their scoring for the other species of this clade.

In the Neotropics, hypertrophied forelimbs are characteristic of males in several groups of stream-breeding hylids, including *Bokermannohyla circumdata*, *B. martinsi*, *B. pseudopseudis*, *Hyloscirtus armatus*, *H. larinopygion*, and *Plectrohyla guatemalensis* species groups (Duellman 1970, 2001; Faivovich *et al.* 2005, 2009; Kizirian *et al.* 2003; Lugli & Haddad 2006; Rivera-Correa *et al.* 2016; Pinheiro *et al.* 2022). These stream-breeding species are not necessarily closely related. Therefore, the enlarged forelimbs of males are thought to have evolved several times (Pinheiro *et al.* 2022). Within *Hyloscirtus*, although not sister to each other, males of the *H. armatus* group and the southern clade of the *H. larinopygion* group are remarkable for the extreme development of their arms, which are associated with clusters of keratinous spines dorsomedially to the prepollex in the *H. armatus* group (Duellman *et al.* 1997; Faivovich & De la Riva 2006; Rivera-Correa *et al.* 2016), while in the southern clade of the *H. larinopygion* group the well-developed arms are associated with an enlarged, curved and protuberant prepolical spine (Pinheiro *et al.* 2022).

The behavior of most species within the *H. larinopygion* group remains enigmatic, leading to a limited understanding of the purpose behind their enlarged forelimbs. Nonetheless, considering that this feature is absent in females, it could be strongly influenced by sexual selection. Thus, it appears plausible that males employ these enlarged forelimbs for intraspecific combat or to grasp females during mating in these species (if amplexus occurs). Both hypotheses will necessitate further observations of the species' behaviors to elucidate the evolutionary significance of these characters. However, in *H. tapichalaca* anti-predatory behavior consisting of the use of the arms to cover the snout and eyes is reported (Kizirian *et al.* 2003). It has also been reported that the prepollical spine could scratch human skin during manipulation (Kizirian *et al.* 2003; Almendáriz *et al.* 2014). The development of the forelimbs of *Hyloscirtus* is variable; therefore, the taxonomic distribution of the relative development of the radioulna and lateral and medial humeral crests needs to be assessed to establish whether the level of development appreciated in the southern clade of the *H. larinopygion* group is a putative synapomorphy.

Pinheiro *et al.* (2022) found that the female of *H. tapichalaca*, as the female of *H. arcanus* **sp. nov.**, have the prepollex ossified, as in males, while the female of *H. hillisi* has a cartilaginous element. Females of *H. condor* and *H. diabolus* remain unknown. The scars found on the female dorsum of the species described herein suggest elaborated social interactions not known in other species with a spine-shaped prepollex. Alternatively, this female could be biasing our observations and represent a rare condition (i.e., females' fight).

The large and unpigmented eggs in *H. arcanus* **sp. nov.** are in accordance with what is known for the genus (see a list of species with eggs known in Faivovich *et al*. 2013). Lyra *et al.* (2020) optimized oocytes pigmentation and found it to be a synapomorphy for Cophomantini, being plesiomorphic for *Hyloscirtus*.

Finally, this work underscores the significance of biological collections, encompassing not only the acquisition of new specimens but also their preservation in natural history museums, along with the wealth of data linked to each individual. The specific case presented here involved the extraction of tissues for potential DNA studies, even when molecular techniques had not yet been fully developed. These efforts led to the surprising revelation of a new species forty years after researchers collected the specimens. This reiterates the crucial value of delving into the archives of such repositories, where a rich tapestry of biodiversity awaits comprehensive documentation and discoveries.

References

Almendaìriz, A., Brito M.J., Batallas, R.D. & Ron, S.R. (2014) Una especie nueva de rana arboìrea del geìnero *Hyloscirtus* (Amphibia: Anura: Hylidae) de la Cordillera del Coìndor. *Papeis Avulsos de Zoologia*, 54, 33–49.
https://doi.org/10.1590/0031-1049.2014.54.04

Ardila-Robayo, M.C., Ruiz-Carranza P.M. & Roa-Trujillo, S.H. (1993) Una nueva especie de *Hyla* del grupo *larinopygion* (Amphibia: Anura: Hylidae) del sur de la Cordillera Central de Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 18, 559–565.

Barrio-Amorós, C.L. & Brewer-Carias, C. (2008) Herpetological results of the 2002 expedition to Sarisariñama, a tepui in Venezuelan Guayana, with the description of five new species. *Zootaxa*, 1942 (1), 1–68.
https://doi.org/10.11646/zootaxa.1942.1.1

Boulenger, G.A. (1902) Descriptions of new batrachians and reptiles from the Andes of Peru and Bolivia. *Annals and Magazine of Natural History*, Series 7, 10, 394–402.
https://doi.org/10.1080/00222930208678691

Boulenger, G.A. (1908) Descriptions of new batrachians and reptiles discoverd by Mr. M.G. Palmer in south-western Colombia. *Annals and Magazine of Natural History*, Series 8, 2, 515–522.
https://doi.org/10.1080/00222930808692531

Caramaschi, U., Pimenta, B.V.S. & Feio, R.N. (2004) Nova especie do grupo de *Hyla geographica* Spix, 1824 da floresta Atlântica, Brasil (Amphibia, Anura, Hylidae). *Boletim do Museu Nacional*, Nova Serie, Zoologia, 518, 1–14.

Coloma, L.A., Carvajal-Endara, S., Dueñas, J.F., Paredes-Recalde, A., Morales-Mite, M., Almeida-Reinoso, D., Tapia, E.E., Hutter, C.R., Toral, E. & Guayasamin, J.M. (2012) Molecular Phylogenetics of stream treefrogs of the *Hyloscirtus larinopygion* group (Anura: Hylidae), and description of two new species from Ecuador. *Zootaxa*, 3364 (1), 1–78.
https://doi.org/10.11646/zootaxa.3364.1.1

Cope, E.D. (1871) "1870". Eighth contribution to the herpetology of tropical America. *Proceedings of the American Philosophical Society*, 11, 553–559.

Darst, C.R. & Cannatella, D.C. (2004) Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 31, 462–475.
https://doi.org/10.1016/j.ympev.2003.09.003

Duellman, W.E. (1970) Hylid frogs of Middle America. *Monographs of the Museum of Natural History, University of Kansas*, 1–2, 1–753.
https://doi.org/10.5962/bhl.title.2835

Duellman, W.E. (1972) A review of the neotropical frogs of the *Hyla bogotensis* group. *Occasional Papers of the Museum of Natural History, University of Kansas*, 11, 1–31.
https://doi.org/10.5962/bhl.part.15140

Duellman, W.E. (1973) Descriptions of new hylid frogs from Colombia and Ecuador. *Herpetologica*, 29, 219–227.

Duellman, W.E. (1989) New species of hylid frogs from the Andes of Colombia and Venezuela. *Occasional Papers of the Museum of Natural History, University of Kansas*, 131, 1–12.

Duellman, W.E. (2001) *Hylid frogs of Middle America*. Society for the Study of Amphibians and Reptiles, Ithaca, New York, 1159 pp.

Duellman, W.E. & Altig, R. (1978) New species of tree frogs (family Hylidae) from the Andes of Colombia and Ecuador. *Herpetologica*, 34 (2), 177–185.

Duellman, W.E. & Berger, T.J. (1982) A new species of Andean treefrog (Hylidae). *Herpetologica*, 38, 456–460.

Duellman, W.E. & Hillis, D.M. (1990) Systematics of frogs of the *Hyla larinopygion* group. *Occasional Papers of the Museum of Natural History, The University of Kansas*, 134, 1–23.

Duellman, W.E. & Coloma, L.A. (1993) *Hyla staufferorum*, a new species of treefrog in the *Hyla larinopygion* group from the cloud forest of Ecuador. *Occasional Papers of the Museum of Natural History, University of Kansas*, 161, 1–11.
https://doi.org/10.5962/bhl.part.15140

Duellman, W.E., De la Riva, I. & Wild, E.R. (1997) Frogs of the *Hyla armata* and *Hyla pulchella* groups in the Andes of South America, with definitions and analyses of phylogenetic relationships of Andean groups of *Hyla. Scientific Papers of the Natural History Museum, The University of Kansas*, 3, 1–41.
https://doi.org/10.5962/bhl.title.48689

Faivovich, J. & De la Riva, I. (2006) On*"Hyla" chlorostea* Reynolds and Foster, 1992, a hylid of uncertain relationships, with some comments on *Hyloscirtus* (Anura: Hylidae). *Copeia*, 2006, 785–791.
https://doi.org/10.1643/0045-8511(2006)6[785:OHCRAF]2.0.CO;2

Faivovich, J., Haddad, C.F.B., Garcia, P.C. de A., Frost, D.R., Campbell, J.A. & Wheeler, W.C. (2005) Systematic review of the frog family Hylidae, with special reference to Hylinae: a phylogenetic analysis and taxonomic revision. *Bulletin of the American Museum of Natural History*, 294, 1–240.
https://doi.org/10.1206/0003-0090(2005)294[0001:SROTFF]2.0.CO;2

Faivovich, J., Lugli, L., Lourenço, A.C.C. & Haddad, C.F.B. (2009) A new species of the *Bokermannohyla martinsi* group from central Bahia, Brazil with comments on *Bokermannohyla* (Anura: Hylidae). *Herpetologica*, 65, 303–310.
https://doi.org/10.1655/0018-0831-65.3.303

Faivovich, J., McDiarmid, R.W. & Myers, C.W. (2013) Two new species of *Myersiohyla* (Anura: Hylidae) from Cerro de la Neblina, Venezuela, with comments on other species of the genus. *American Museum Novitates*, 3792, 1–63.
https://doi.org/10.1206/3792.1

Farris, J.S. (1983) The logical basis of phylogenetic analysis. *In*: Platnick, N.I. & Funk V.A. (Eds.), *Advances in Cladistics: Proceedings of the Third Meeting of the Willi Hennig Society*. Columbia University Press, New York, New York, pp. 7–36,

Farris, J.S., Albert, V.A., Källersjo, M., Lipscomb, D. & Kluge, A.G. (1996) Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, 12, 99–124.
https://doi.org/10.1111/j.1096-0031.1996.tb00196.x

Frost, D.R. (2024) Amphibian Species of the World: an Online Reference. Version 6.2 (13 April 2024). Electronic Database. American Museum of Natural History, New York, New York. Available from: http://research.amnh.org/herpetology/amphibia/index.html (accessed 4 June 2024)

Goebel, A.M., Donnelly, J.M. & Atz, M.E. (1999) PCR Primers and amplification methods for 12S Ribosomal DNA, the Control Region, Cytochrome Oxidase I, and Cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. *Molecular Phylogenetics and Evolution*, 11, 163–199.
https://doi.org/10.1006/mpev.1998.0538

Goloboff, P.A. (2003) Parsimony, likelihood, and simplicity. *Cladistics*, 19, 91–103.
https://doi.org/10.1111/j.1096-0031.2003.tb00297.x

Goloboff, P.A. & Pol, D. (2005) Parsimony and Bayesian phylogenetics. *In*: Albert, V.A. (Ed.), *Parsimony, Phylogeny, and Genomics*. Oxford University Press, London, pp. 148–159.
https://doi.org/10.1093/acprof:oso/9780199297306.003.0008

Goloboff, P.A. & Morales, M.E. (2023) TNT. Version 1.6, with a graphical interface for MacOS and Linux, including new routines in parallel. *Cladistics*, 39, 144–153.
https://doi.org/10.1111/cla.12524

Goin, C.J. & Woodley, J.D. (1969) A new tree-frog from Guyana. *Zoological Journal of the Linnean Society*, 48, 135–140.
https://doi.org/10.1111/j.1096-3642.1969.tb00709.x

Grant, T. & Kluge, A.G. (2009) Perspective: Parsimony, explanatory power, and dynamic homology testing. *Systematics and Biodiversity*, 7, 357–363.
https://doi.org/10.1017/S147720000999017X

Gray, J.E. (1825) A synopsis of the genera of reptiles and Amphibia, with a description of some new species. *Annals of Philosophy*, Series 2, 10, 193–217.

Graybeal, A. (1997) Phylogenetic relationships of bufonid frogs and tests of alternate macroevolutionary hypotheses characterizing their radiation. *Zoological Journal of the Linnean Society*, 119, 297–338.
https://doi.org/10.1111/j.1096-3642.1997.tb00139.x

Guayasamin, J.M., Rivera-Correa, M., Arteaga, A., Culebras, J., Bustamante, L., Pyron, R.A., Peñafiel, N., Morochze, C. & Hutter, C.R. (2015) Molecular phylogeny of stream treefrogs (Hylidae: *Hyloscirtus bogotensis* Group), with a new species from the Andes of Ecuador. *Neotropical Biodiversity*, 1, 2–21.
https://doi.org/10.1080/23766808.2015.1074407

Hall, T.A. (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Hedges, S.B. (1994) Molecular evidence for the origin of birds. *Proceedings of the National Academy of Sciences*, 91, 2621–2624.
https://doi.org/10.1073/pnas.91.7.2621

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780.
https://doi.org/10.1093/molbev/mst010

Kluge, A.G. & Grant, T. (2006) From conviction to anti-superfluity: old and new justifications of parsimony in phylogenetic inference. *Cladistics*, 22, 276–288.
https://doi.org/10.1111/j.1096-0031.2006.00100.x

Kizirian, D., Coloma, L.A. & Paredes-Recalde, A. (2003) A new treefrog (Hylidae: *Hyla*) from southern Ecuador and a description of its antipredator behavior. *Herpetologica*, 59, 339–349.
https://doi.org/10.1655/02-41

Lugli, L. & Haddad, C.F.B. (2006) A new species of the *Bokermannohyla pseudopseudis* group from central Bahia, Brazil (Amphibia, Hylidae). *Herpetologica*, 62, 453–465.
https://doi.org/10.1655/0018-0831(2006)62[453:ANSOTB]2.0.CO;2

Lutz, B. (1950) Anfíbios anuros da coleção Adolpho Lutz do Instituto Oswaldo Cruz. V/ Frogs in the Adolpho Lutz collection of the Instituto Oswaldo Cruz. V. *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro*, 48, 599–637.
https://doi.org/10.1590/S0074-02761950000100022

Lyra, M.L., Lourenço, A.C.C., Pinheiro, P.D.P., Pezzuti, T.L., Baêta, D., Barlow, A., Hofreiter, M., Pombal, J.P. Jr., Haddad, C.F.B. & Faivovich, J. (2020) High-throughput DNA sequencing of museum specimens sheds light on the long-missing species of the *Bokermannohyla claresignata* group (Anura: Hylidae: Cophomantini). *Zoological Journal of the Linnean Society*, 190, 1235–1255.
https://doi.org/10.1093/zoolinnean/zlaa033

Mangiamele, L.A., Fuxjager, M.J., Schuppe, E.R., Taylor, R.S., Hödl, W. & Preininger, D. (2016) Increased androgenic sensitivity in the hind limb muscular system marks the evolution of a derived gestural display. *Proceedings of the National Academy of Sciences of the USA*, 113, 5664–5669.
https://doi.org/10.1073/pnas.1603329113

Melin, D.E. (1941) Contributions to the knowledge of the Amphibia of South America. Göteborgs Kungl. Vetenskaps-och Vitterhets-samhälles. Handlingar. *Serien B, Matematiska och Naturvetenskapliga Skrifter*, 1, 1–71.

Müller, E.R., Galavazi, G. & Szirmai, J.A. (1969) Effect of castration and testosterone treatment on fiber width of the flexor carpi radialis muscle in the male frog (*Rana temporaria*). *General and Comparative Endocrinology*, 13, 275–284.
https://doi.org/10.1016/0016-6480(69)90249-4

Mueses-Cisneros, J.J. & Anganoy-Criollo, M.A. (2008) Una nueva especie del grupo *Hyloscirtus larinopygion* (Amphibia: Anura: Hylidae) del suroccidente de Colombia. *Papéis Avulsos de Zoologia*, 48, 129–138.
https://doi.org/10.1590/S0031-10492008001500001

Myers, C.W. & Duellman, W.E. (1982) A new species of *Hyla* from Cerro Colorado, and other tree frog records and geographical notes from western Panama. *American Museum Novitates*, 2752, 1–32.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabawski, G. (1991) *The Simple Fool's Guide to PCR. 2nd Edition*. University of Hawaii, Honolulu, 45 pp.

Peters, W.C.H. (1882) Der namen der Batrachiergattung *Hylonomus* in *Hyloscirtus* zu Ändern und legte zwei neue Arten von Schlangen, *Microsoma notatum* und *Liophis Ygraecum*. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin*, 1882, 127–129.
https://doi.org/10.5962/bhl.part.15223

Pinheiro, P.D.P., Kok, P.J.R., Noonan, B.P., Means, D.B., Haddad, C.F.B. & Faivovich, J. (2019) A new genus of Cophomantini, with comments on the taxonomic status of *Boana liliae* (Anura: Hylidae). *Zoological Journal of the Linnean Society*, 185, 226–245.
https://doi.org/10.1093/zoolinnean/zly030

Pinheiro, P.D.P., Blotto, B.L., Ron, S.R., Stanley, E.L., Garcia, P.C., Haddad, C.F., Grant, T. & Faivovich, J. (2022) Prepollex diversity and evolution in Cophomantini (Anura: Hylidae: Hylinae). *Zoological Journal of the Linnean Society*, 195 (3), 995–1021.
https://doi.org/10.1093/zoolinnean/zlab079

Pyron, A. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61, 543–583.
https://doi.org/10.1016/j.ympev.2011.06.012

Rambaut, A. (2014) FigTree. Tree figure drawing tool. Version 1.4.2. Available from: http://tree.bio.ed.ac.uk/software/figtree (accessed 28 May 2024)

Regnier, M. & Herrera, A.A. (1993) Changes in contractile properties by androgen hormones in sexually dimorphic muscles of male frogs (*Xenopus laevis*). *Journal of Physiology*, 461, 565–581.
https://doi.org/10.1113/jphysiol.1993.sp019529

Reyes-Puig, J.P., Recalde, D., Recalde, F., Koch, C., Guayasamin, J.M., Cisneros-Heredia, D.F., Jost, L. & Yánez-Muñoz, M.H. (2022) A spectacular new species of *Hyloscirtus* (Anura: Hylidae) from the Cordillera de Los Llanganates in the eastern Andes of Ecuador. *PeerJ*, 10, e14066.
https://doi.org/10.7717/peerj.14066

Rivera-Correa, M. & Faivovich, J. (2013) A new species of *Hyloscirtus* (Anura: Hylidae) from Colombia with a rediagnosis of *Hyloscirtus larinopygion* (Duellman, 1973). *Herpetologica*, 69, 298–313.
https://doi.org/10.1655/HERPETOLOGICA-D-12-00059

Rivera-Correa, M., García-Burneo, K. & Grant, T. (2016) A new red-eyed of stream treefrog of *Hyloscirtus* (Anura: Hylidae) from Peru, with comments on the taxonomy of the genus. *Zootaxa*, 4061 (1), 29–40.
https://doi.org/10.11646/zootaxa.4061.1.3

Rojas-Runjaic, F.J.M., Infante-Rivero, E.E., Salerno, P.E. & Meza-Joya, F.L. (2018) A new species of *Hyloscirtus* (Anura, Hylidae) from the Colombian and Venezuelan slopes of Sierra de Perijá, and the phylogenetic position of *Hyloscirtus jahni* (Rivero, 1961). *Zootaxa*, 4382 (1), 121–146.
https://doi.org/10.11646/zootaxa.4382.1.4

Ron, S.R., Caminer, M.A., Varela-Jaramillo, A. & Almeida-Reinoso, D. (2018) A new treefrog from Cordillera del Cóndor with comments on the biogeographic affinity between Cordillera del Cóndor and the Guianan Tepuis (Anura, Hylidae, *Hyloscirtus*). *ZooKeys*, 809, 97–124.
https://doi.org/10.3897/zookeys.809.25207

Ron, S.R., Merino-Viteri, A. & Ortiz, D.A. (2022) Anfibios del Ecuador. Version 2022.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. Available from https://bioweb.bio/faunaweb/amphibiaweb (accessed 13 May 2024)

Sánchez, D.A. (2010) Larval development and synapomorphies for species groups of *Hyloscirtus* Peters, 1882 (Anura: Hylidae: Cophomantini). *Copeia*, 2010, 351–363.
https://doi.org/10.1643/CH-10-010

Sánchez-Nivicela, J.C., Falcón-Reibán, J.M. & Cisneros-Heredia, D.F. (2023) A new stream treefrog of the genus *Hyloscirtus* (Amphibia, Hylidae) from the Río Negro-Sopladora National Park, Ecuador. *ZooKeys*, 1141, 75–92.
https://doi.org/10.3897/zookeys.1141.90290

Sabaj, M.H. (2023) Codes for Natural History Collections in Ichthyology and Herpetology. Online Supplement. Version 9.5. 10 November 2023. Available from: https://asih.org (accessed 17 February 2024)

Savage, J.M. & Heyer, R.W. (1967) Variation and distribution of the tree-frog genus *Phyllomedusa* in Costa Rica. *Beiträge zur Neotropical Fauna*, 5, 111–131.
https://doi.org/10.1080/01650526709360400

Sierra, R., Cerón, C., Palacios, W. & Valencia, R. (1999) *Mapa de vegetación del Ecuador Continental 1:1'000.000*. Proyecto INEFAN/GEF-BIRF, Wildlife Conservation Society y Ecociencia, Quito. [map]

Swofford, D.L. (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods)*. Sinauer Associates, Sunderland, Massachusetts. [program]

Vellard, J. (1970) Contribución al estudio de los batracios Andinos. *Revista del Museo Argentino de Ciencias Naturales «Bernardino Rivadavia» Zoología*, 10, 1–21.

Yánez-Muñoz, M.H., Reyes-Puig, J.P., Batallas-Revelo, D., Broaddus, C., Urgilés-Merchán, M., Cisneros-Heredia, D.F. & Guayasamin, J.M. (2021) A new Andean treefrog (Amphibia: *Hyloscirtus bogotensis* group) from Ecuador: An example of community involvement for conservation. *PeerJ*, 9 (e11914), 1–33.
https://doi.org/10.7717/peerj.11914

Watters, J.L., Cummings, S.T., Flanagan, R.L. & Siler, C.D. (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

Wied-Neuwied, M.A.P. zu (1821) *Reise nach Brasilien in den Jahren 1815 bis 1817. Vol. 2*. Henrich Ludwig Brönner, Franfurt a.M, 345 pp.
https://doi.org/10.5962/bhl.title.85967

Wiens, J.J., Fetzner, J.W. Jr., Parkinson, C.L. & Reeder, T.W. (2005) Hylid frog phylogeny and sampling strategies for speciose clades. *Systematic Biology*, 54, 719–748.
https://doi.org/10.1080/10635150500234625

Wiens, J.J., Graham, C.H., Moen, D.S., Smith, S.A. & Reeder, T.W. (2006) Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. *The American Naturalist*, 168, 579–596.
https://doi.org/10.1086/507882

Wiens, J.J., Kuczynski, C.A., Hua, X. & Moen, D.S. (2010) An expanded phylogeny of treefrogs (Hylidae) based on nuclear and mitochondrial sequence data. *Molecular Phylogenetics and Evolution*, 55 (3), 871–882.
https://doi.org/10.1016/j.ympev.2010.03.013

APPENDICES

APPENDIX I. Additional specimens examined.

Hyloscirtus antioquia: COLOMBIA, Antioquia, Bello, San Félix, Serranía Las Baldías, Corregimiento de San Félix, Vereda Las Huertas, Quebrada El Hato, 2660 m, Km. 5 San Félix road—Truchera San Félix., MHUA-A 7227 (holotype), 7228–29 (paratypes)

Hyloscirtus caucanus: COLOMBIA, Cauca, Páez, Hacienda Montenegro, Km. 34.5 Belalcazar–Tacueyo, 2400 m., ICN 7071 (holotype), 7002, 7056, 7072–76, 7238, 7241–48, 7250–52, 7055, 7239, 7253.

Hyloscirtus condor: ECUADOR, Zamora Chinchipe, Cantón Nangaritza, Parroquia Nuevo Paraíso, Reserva Biológica Cerro Plateado, 2317 m., EPN 14758 (holotype), EPN 14754, 14755, 14756, 14757, 14759, 14760 (paratypes).

Hyloscirtus criptico: ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2760–2885 m., QCAZ 43516–18, 43528 (paratypes).

Hyloscirtus diabolus: PERU, Amazonas, Chachapoyas, La Jalca, 2300 m., CORBIDI 12885 (holotype).

Hyloscirtus hillisi: ECUADOR, Morona Santiago, Reserva Biológica El Quimi, Río Quimi valley (3.5190S, 78.3788W), 2128 m, QCAZ 68649 (holotype).

Hyloscirtus larinopygion: COLOMBIA, Caldas, Villa Maria, Km. 7 Villa Maria–Mariquita, ICN 34433; Caldas, Pensilvania, Km. 24 Pensilvania-Arboleda, 2000 m., ICN 36518–19; Cauca, Popayan, Santa Teresa stream, 2200 m., KU 144127 (holotype); Huila, Belalcazar, Parque Nacional Natural Nevado del Huila, 2900 m., ICN 41880; Quindío, Salento, Hacienda La Caleria, 2300 m., ICN 15626–27; Risaralda, Pereira, Parque Regional Ucumarí, Las Delicias stream, 2340 m., ICN 36133–38, 34970–72; Tolima, Ibague, Juntas, El Silencio, 2600–2820 m., ICN 9380–82, 9670; Valle del Cauca, El Cairo, Las Amarillas, 2140–2200 m., ICN 28926, 28828–29, 42779; Valle del Cauca, Farallones de Cali, Campamento Corea, 2600 m., ICN 13595. ECUADOR, Carchi, Espejo, Morán, 2452 m., QCAZ 41826.

Hyloscirtus lindae: ECUADOR, Napo, Papallacta, 2.660 m., KU 164402 (holotype), 155475–155476 (paratypes); Pacto Sumaco, Parque Nacional Sumaco, Refugio La Laguna, 2476–2775 m., QCAZ 41232, 41294–98, 45345–47.

Hyloscirtus pacha: ECUADOR, Morona Santiago, Plan de Milagro, 2350 m., KU 202762 (holotype); Plan de Milagro, Km 8 Plan de Milagro–Cuenca, 2150–2300 m., QCAZ 48237–41.

Hyloscirtus pantostictus: ECUADOR, Sucumbios, km 5 Santa Bárbara–La Bonita, 2650 m., KU 190000 (holotype); Santa Barbara, 2590–2800 m., QCAZ 10488,11660–67, 12171, 12174, 14084, 30529–31, 38421, 40331, 41393, 41412, 42350, 45434–38, 45443–44, 45446, 45450–53.

Hyloscirtus princecharlesi: ECUADOR, Imbabura, Cuellaje, Reserva Cotacachi–Cayapas, 2720–2794 m., QCAZ 43654, 44893 (paratypes).

Hyloscirtus psarolaimus: ECUADOR, Napo Papallacta, Km 11 Papallacta–Baeza, 2660 m., KU 164313 (holotype), QCAZ 13252, 23070; Carchi, Tulcán–Santa Bárbara road, QCAZ 15366; Morona Santiago, San Vicente, Parque Nacional Sangay, 15 km to road Lagunas de Atillo, 2815 m., QCAZ 31671; Sucumbios, La Sofía, Campamento Río Verde, 2726 m., DHMECN 6493–94.

Hyloscirtus ptychodactylus: ECUADOR, Cotopaxi, Pilalo, 2320 m., KU 209780 (holotype).

Hyloscirtus sarampiona: COLOMBIA, Cauca, Parque Nacional Natural Munchique, Sopladero stream, 33 Km., 2190 m., ICN 7440 (holotype), 7441 (paratype).

Hyloscirtus sethmacfarlanei: ECUADOR, Tungurahua, Machay Reserve of Fundación EcoMinga, Cerro Mayordomo, Río Verde, 2,970 m, DHMECN 14416, (holotype).

Hyloscirtus staufferorum: ECUADOR, Napo, 27 Km N Jondachi, 2040 m., KU 217695 (holotype); Pacto Sumaco, Lago Sumaco, 2500 m., QCAZ 3701–03; Pastaza, Santa Clara, Puyo–Tena road, Comunidad San Rafael–Chonta Yaku, 2250 m., QCAZ 45962–63, 45965–67.

Hyloscirtus tapichalaca: ECUADOR, Zamora Chinchipe, Reserva Tapichalaca, 2667 m., QCAZ 15083–85, 16704–06, 17776–77, KU 291362 (paratypes).

Hyloscirtus tigrinus: COLOMBIA, Nariño, Pasto, El Encano, Reserva Natural Privada Castelví, 3060 m., ICN 53804 (holotype), ICN 53805–06 (paratypes).

APPENDIX II. GenBank accession numbers for DNA sequences included in the phylogenetic analysis. ***Short sequences excluded from the 12S rRNA UPDs calculations.**

Collection number	Species group	Species	GenBank accession numbers		Source
			12S rRNA	16S rRNA	
KU 202728	*H. larinopygion*	*H. arcanus* sp. nov.	**PP736392**	**PP736395**	**This study**
KU 202729	*H. larinopygion*	*H. arcanus* sp. nov.	**PP736393**	**PP736396**	**This study**
KU 202731	*H. larinopygion*	*H. arcanus* sp. nov.	**PP736394***	**PP736397**	**This study**
MEPN 14754	*H. larinopygion*	*H. condor*	KF756939	KF756939	Almendáriz *et al.* (2014)
MEPN 14758	*H. larinopygion*	*H. condor*	KF756938	KF756938	Almendáriz *et al.* (2014)
QCAZ 43421	*H. larinopygion*	*H. criptico*	JX155812	JX155839	Coloma *et al.* (2012)
QCAZ 43422	*H. larinopygion*	*H. criptico*	JX155814	JX155841	Coloma *et al.* (2012)
QCAZ68648	*H. larinopygion*	*H. hillisi*	MH883793	MH883798	Ron *et al.* (2018)
QCAZ68649	*H. larinopygion*	*H. hillisi*	MH883794	MH883799	Ron *et al.* (2018)

Collection number	Species group	Species	GenBank accession numbers		Source
			12S rRNA	16S rRNA	
QCAZ 41826	*H. larinopygion*	*H. larinopygion*	JX155817	JX155844	Coloma *et al.* (2012)
QCAZ 45462	*H. larinopygion*	*H. larinopygion*	JX155818	JX155845	Coloma *et al.* (2012)
QCAZ 45346	*H. larinopygion*	*H. lindae*	JX155822	JX155849	Coloma *et al.* (2012)
QCAZ 45463	*H. larinopygion*	*H. lindae*	JX155823	JX155850	Coloma *et al.* (2012)
WED 53493	*H. larinopygion*	*H. pacha*	AY326057	AY326057	Darst & Cannatella (2004)
KU 202760	*H. larinopygion*	*H. pacha*	DQ380364	-	Wiens *et al.* (2006)
QCAZ 45438	*H. larinopygion*	*H. pantostictus*	JX155819	JX155846	Coloma *et al.* (2012)
QCAZ 45435	*H. larinopygion*	*H. pantostictus*	JX155820	JX155847	Coloma *et al.* (2012)
QCAZ 42165	*H. larinopygion*	*H. princecharlesi*	JX155806	JX155833	Coloma *et al.* (2012)
QCAZ 43654	*H. larinopygion*	*H. princecharlesi*	JX155807	JX155834	Coloma *et al.* (2012)
QCAZ 27049	*H. larinopygion*	*H. psarolaimus*	JX155808	JX155835	Coloma *et al.* (2012)
QCAZ 46095	*H. larinopygion*	*H. psarolaimus*	JX155809	JX155836	Coloma *et al.* (2012)
QCAZ 46030	*H. larinopygion*	*H. ptychodactylus*	JX155804	JX155831	Coloma *et al.* (2012)
QCAZ 46031	*H. larinopygion*	*H. ptychodactylus*	JX155805	JX155832	Coloma *et al.* (2012)
DHMECN 14549	*H. larinopygion*	*H. sethmacfarlanei*	-	OM293946	Reyes-Puig *et al.* (2022)
DHMECN 14416	*H. larinopygion*	*H. sethmacfarlanei*	-	OM293945	Reyes-Puig *et al.* (2022)
QCAZ 45967	*H. larinopygion*	*H. staufferorum*	JX155815*	JX155842	Coloma *et al.* (2012)
QCAZ 45962	*H. larinopygion*	*H. staufferorum*	JX155816	JX155843	Coloma *et al.* (2012)
QCAZ 15083	*H. larinopygion*	*H. tapichalaca*	JX155830	JX155830	Coloma *et al.* (2012)
QCAZ 16704	*H. larinopygion*	*H. tapichalaca*	AY563625	AY563625	Faivovich *et al.* (2005)
QCAZ 41351	*H. larinopygion*	*H. tigrinus*	JX155810	JX155837	Coloma *et al.* (2012)
QCAZ 31550	*H. larinopygion*	*H. tigrinus*	JX155811	JX155838	Coloma *et al.* (2012)

Collection number	Species group	Species	GenBank accession numbers		Source
			12S rRNA	16S rRNA	
AMNH-A 1651632	*H. armatus*	*H. armatus*	AY549321	AY549321	Faivovich *et al.* (2005)
KU 173222	*H. armatus*	*H. armatus*	AY819423	-	Wiens *et al.* (2005)
AMNH-A 165132	*H. armatus*	*H. charazani*	AY843618	AY843618	Faivovich *et al.* (2005)
MZUTI 409	*H. bogotensis*	*H. alytolylax*	KT279504	KT279543	Guayasamin *et al.* 2015
QCAZ 24377	*H. bogotensis*	*H. alytolylax*	JX155798	JX155825	Coloma *et al.* (2012)
UIS-A 5947	*H. bogotensis*	*H. callipeza*	MG596780	MG596780	Rojas-Runjaic *et al.* (2018)
SIU 6926	*H. bogotensis*	*H. colymba*	DQ380353	-	Wiens *et al.* (2006)
SIUC H-7079	*H. bogotensis*	*H. colymba*	AY843620	AY843620	Faivovich *et al.* (2005)
DHMECN 13972	*H. bogotensis*	*H. conscientia*	-	MW692910	Yánez-Muñoz *et al.* (2021)
DHMECN 13973	*H. bogotensis*	*H. conscientia*	-	MW692911	Yánez-Muñoz *et al.* (2021)
MHNLS 18838	*H. bogotensis*	*H. japreria*	MG596764	MG596764	Rojas-Runjaic *et al.* (2018)
UIS-A 5497	*H. bogotensis*	*H. japreria*	MG596771	MG596771	Rojas-Runjaic *et al.* (2018)
MHNLS 19163	*H. bogotensis*	*H. lascinius*	MG596762	MG596762	Rojas-Runjaic *et al.* (2018)
MHNLS 19164	*H. bogotensis*	*H. lascinius*	MG596763	MG596763	Rojas-Runjaic *et al.* (2018)
MZUTI 3747	*H. bogotensis*	*H. mashpi*	KT279512	KT279532	Guayasamin *et al.* (2015)
MZUTI 610	*H. bogotensis*	*H. mashpi*	KT279510	KT279533	Guayasamin *et al.* (2015)
MZUTI 608	*H. bogotensis*	*H. palmeri*	KT279520	KT279549	Guayasamin *et al.* (2015)
SIUC H-6924	*H. bogotensis*	*H. palmeri*	AY843650	AY843650	Faivovich *et al.* (2005)
MZUTI 2386	*H. bogotensis*	*H. phyllognathus*	KT279516	KT279548	Guayasamin *et al.* (2015)
QCAZ 23938	*H. bogotensis*	*H. phyllognathus*	JX155800	JX155827	Coloma *et al.* (2012)
MHNLS 20321	*H. bogotensis*	*H. platydactylus*	MG596772	MG596772	Rojas-Runjaic *et al.* (2018)
MHNLS 20322	*H. bogotensis*	*H. platydactylus*	MG596773	MG596773	Rojas-Runjaic *et al.* (2018)
KU 181167	*H. bogotensis*	*H. simmonsi*	DQ380376	-	Wiens *et al.* (2006)

Collection number	Species group	Species	GenBank accession numbers		Source
			12S rRNA	16S rRNA	
MZUTI 3262	*H. bogotensis*	*H.* sp.	KT279503	KT279544	Guayasamin *et al.* (2015)
MHNLS 20318	*H. jahni*	*H. jahni*	MG596776	MG596776	Rojas-Runjaic *et al.* (2018)
MHNLS 20319	*H. jahni*	*H. jahni*	MG596777	MG596777	Rojas-Runjaic *et al.* (2018)
MACN 37791		*Aplastodiscus perviridis*	AY843569	AY843569	Faivovich *et al.* (2005)
MACN 37000		*Boana faber*	AY549334	AY549334	Faivovich *et al.* (2005)
CFBH 3621		*Bokermannohyla circumdata*	AY549328	AY549328	Faivovich *et al.* (2005)
ROM 39582		*Nesorohyla kanaima*	AY843634	AY843634	Faivovich *et al.* (2005)

APPENDIX III. Uncorrected p-distances (%) between the 12S rRNA gene (MVZ59-Tval, ≈857 bp) fragment sequences for the *Hyloscirtus larinopygion* group. See Appendix II for the list of GenBank accession numbers.

	Voucher	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	**H. arcanus sp. nov.** KU202728	-																										
2	**H. arcanus sp. nov.** KU202729	0.1	-																									
3	*H. condor* MEPN14754	3.4	3.3	-																								
4	*H. condor* MEPN14758	3.3	3.2	0.0	-																							
5	*H. criptico* QCAZ43421	8.5	8.7	8.3	8.5	-																						
6	*H. criptico* QCAZ43422	9.1	9.2	8.8	8.9	0.0	-																					
7	*H. hillisi* QCAZ68648	3.6	3.8	3.8	3.7	9.1	9.6	-																				
8	*H. hillisi* QCAZ68649	3.6	3.7	3.9	3.9	9.2	9.7	0.1	-																			
9	*H. larinopygion* QCAZ41826	9.5	9.6	9.1	9.3	3.8	4.0	10.1	10.0	-																		
10	*H. larinopygion* QCAZ45462	9.4	9.6	9.0	9.2	3.8	4.0	10.1	9.9	0.0	-																	
11	*H. lindae* QCAZ45346	8.8	9.0	9.4	9.6	4.0	4.0	9.9	10.0	4.3	4.3	-																
12	*H. lindae* QCAZ45463	10.1	10.2	9.7	9.8	5.0	5.2	10.7	10.8	5.1	5.1	1.0	-															
13	*H. pacha* KU202760	8.9	9.1	8.8	8.8	1.6	1.6	9.8	9.9	3.7	3.8	4.2	4.9	-														
14	*H. pacha* WED53493	9.6	9.8	9.1	9.2	2.4	2.5	10.3	10.4	3.8	3.8	3.7	4.3	0.0	-													
15	*H. pantostictus* QCAZ45435	9.8	9.9	9.8	9.9	4.8	4.8	10.6	10.7	4.8	4.8	1.4	1.8	4.6	4.1	-												

APPENDIX III. (Continued)

	Voucher	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
16	*H. pantostictus* QCAZ45438	**9.7**	**9.8**	9.7	9.8	4.7	4.9	10.5	10.6	4.8	4.8	1.4	1.8	4.6	4.2	0.0	-											
17	*H. princecharlesi* QCAZ42165	**8.9**	**9.0**	7.9	8.2	3.4	3.5	9.0	9.1	3.5	3.5	4.8	5.4	3.5	3.9	4.9	4.8	-										
18	*H. princecharlesi* QCAZ43654	**9.6**	**9.7**	8.5	8.7	3.4	3.6	9.7	9.8	3.7	3.7	4.8	5.3	3.5	3.9	4.9	4.9	0.0	-									
19	*H. psarolaimus* QCAZ27049	**9.7**	**9.9**	9.0	9.1	2.6	2.6	10.2	10.3	3.4	3.4	4.5	5.1	2.7	2.8	5.1	5.2	3.4	3.5	-								
20	*H. psarolaimus* QCAZ46095	**9.9**	**10.0**	9.1	9.2	2.6	2.7	10.5	10.7	3.5	3.5	4.4	5.2	2.7	2.9	5.3	5.3	3.5	3.6	0.4	-							
21	*H. ptychodactylus* QCAZ46030	**9.6**	**9.7**	8.5	8.8	3.2	3.4	9.7	9.9	3.6	3.6	4.8	5.6	3.2	3.8	5.0	5.1	0.4	0.5	3.6	3.7	-						
22	*H. ptychodactylus* QCAZ46031	**9.6**	**9.7**	8.5	8.8	3.2	3.4	9.7	9.9	3.6	3.6	4.8	5.6	3.2	3.8	5.0	5.1	0.4	0.5	3.6	3.7	0.0	-					
23	*H. staufferorum* QCAZ45962	**9.6**	**9.7**	9.9	9.9	2.3	2.3	10.4	10.5	4.4	4.4	5.2	5.8	1.4	1.5	5.3	5.3	4.3	4.3	3.5	3.5	4.1	4.1	-				
24	*H. tapichalaca* QCAZ15083	**2.7**	**2.6**	3.7	3.6	9.4	10.2	3.6	3.5	10.4	10.4	9.4	10.4	9.6	10.4	10.2	10.2	9.2	10.1	10.7	10.7	10.1	10.1	10.6	-			
25	*H. tapichalaca* QCAZ16704	**3.1**	**3.0**	3.7	3.6	9.8	10.5	4.0	4.0	10.6	10.6	9.9	10.7	10.0	10.6	10.6	10.5	9.7	10.5	10.9	10.9	10.5	10.5	11.0	0.9	-		
26	*H. tigrinus* QCAZ31550	**9.5**	**9.6**	8.7	8.8	2.4	2.5	10.2	10.3	3.6	3.6	4.5	4.7	2.0	2.1	4.5	4.5	3.7	3.6	2.8	3.0	3.3	3.3	2.9	10.2	10.4	-	
27	*H. tigrinus* QCAZ41351	**9.2**	**9.3**	8.4	8.5	2.4	2.5	9.8	10.0	3.5	3.5	4.5	4.7	2.0	2.0	4.5	4.5	3.7	3.7	2.8	2.9	3.6	3.6	2.9	10.2	10.1	0.0	-

APPENDIX IV. Uncorrected p-distances (%) between the 16S rRNA gene (AR-BR, ≈595 bp) fragment sequences for the *Hyloscirtus larinopygion* group. See Appendix II for the list of GenBank accession numbers.

	Voucher	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	*H. arcanus* sp. nov. KU202728																														
2	*H. arcanus* sp. nov. KU202729	0.0	-																												
3	*H. arcanus* sp. nov. KU202731	0.2	0.2	-																											
4	*H. condor* MEPN14754	2.4	2.4	2.6	-																										
5	*H. condor* MEPN14758	2.4	2.4	2.6	0.0	-																									
6	*H. criptico* QCAZ43421	10.0	10.0	10.2	9.5	9.5	-																								
7	*H. criptico* QCAZ43422	9.7	9.7	9.9	9.2	9.1	0.2	-																							
8	*H. hillisi* QCAZ68648	2.2	2.2	2.4	1.9	1.9	9.5	9.1	-																						
9	*H. hillisi* QCAZ68649	2.2	2.2	2.4	1.9	1.9	9.5	9.1	0.0	-																					
10	*H. larinopygion* QCAZ41826	8.8	8.8	9.0	8.5	8.3	5.1	4.9	8.3	8.3	-																				
11	*H. larinopygion* QCAZ45462	8.8	8.8	9.0	8.4	8.2	5.1	4.9	8.2	8.2	0.0	-																			
12	*H. lindae* QCAZ45346	8.2	8.2	8.4	8.2	8.0	5.7	5.5	8.0	8.0	2.7	2.7	-																		
13	*H. lindae* QCAZ45463	8.4	8.4	8.6	8.4	8.2	5.3	5.0	8.2	8.2	2.3	2.3	0.7	-																	
14	*H. pacha* WED53493	8.4	8.4	8.6	8.7	8.4	4.4	4.3	8.7	8.7	4.5	4.7	5.3	5.0	-																
15	*H. pantostictus* QCAZ45435	8.6	8.6	8.7	8.6	8.4	4.7	4.6	8.4	8.4	2.0	2.0	1.6	1.3	4.0	-															
16	*H. pantostictus* QCAZ45438	8.4	8.4	8.6	8.5	8.2	4.7	4.5	8.2	8.2	2.0	2.0	1.6	1.3	4.1	0.0	-														

APPENDIX IV. (Continued)

	Voucher	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
17	*H. princecharlesi* QCAZ42165	**8.2**	**8.2**	**8.4**	8.3	8.1	5.5	5.2	8.1	8.1	3.4	3.4	4.3	3.9	5.4	3.6	3.6	-													
18	*H. princecharlesi* QCAZ43654	**8.3**	**8.3**	**8.4**	8.3	8.1	5.5	5.2	8.1	8.1	3.4	3.4	4.4	3.9	5.2	3.6	3.6	0.0	-												
19	*H. psarolaimus* QCAZ27049	**10.4**	**10.4**	**10.4**	10.3	10.0	4.9	4.9	10.0	10.0	5.5	5.5	6.6	6.2	5.3	5.6	5.6	5.7	5.7	-											
20	*H. psarolaimus* QCAZ46095	**10.4**	**10.4**	**10.4**	10.3	10.0	5.1	5.1	10.0	10.0	5.4	5.4	6.6	6.2	5.4	5.6	5.6	5.6	5.7	0.2	-										
21	*H. ptychodactylus* QCAZ46030	**7.9**	**7.9**	**8.1**	7.9	7.7	5.1	4.8	7.7	7.7	3.6	3.6	4.2	3.8	5.2	3.4	3.4	1.4	1.4	5.6	5.6	-									
22	*H. ptychodactylus* QCAZ46031	**7.9**	**7.9**	**8.1**	7.9	7.7	5.1	4.8	7.7	7.7	3.6	3.6	4.2	3.8	5.2	3.4	3.4	1.4	1.4	5.6	5.6	0.0	-								
23	*H. sethmacfarlanei* DHMECN14549	**5.6**	**5.6**	**5.8**	5.8	5.8	3.1	2.9	5.4	5.4	1.7	1.7	3.0	2.5	2.9	2.3	2.3	2.1	2.1	2.5	2.5	1.9	1.9	-							
24	*H. sethmacfarlanei* DHMECN14416	**6.7**	**6.7**	**6.7**	6.9	6.9	3.7	3.7	6.4	6.4	2.3	2.3	3.7	3.3	3.7	3.0	3.0	2.8	2.8	3.2	3.2	2.6	2.6	0.5	-						
25	*H. staufferorum* QCAZ45962	**8.7**	**8.7**	**8.7**	8.5	8.1	3.4	3.4	8.5	8.5	3.8	3.8	5.1	4.7	1.7	3.9	4.0	4.1	4.2	4.1	4.3	3.9	3.9	2.6	3.3	-					
26	*H. staufferorum* QCAZ45967	**8.5**	**8.5**	**8.6**	8.3	7.9	3.5	3.2	8.3	8.3	3.6	3.6	4.9	4.5	1.6	3.8	3.8	3.9	3.9	4.1	4.3	3.8	3.8	2.5	3.3	0.0	-				
27	*H. tapichalaca* QCAZ15083	**2.3**	**2.3**	**2.2**	2.2	2.1	10.2	9.9	2.0	2.0	9.0	8.9	8.9	9.1	9.1	9.1	8.9	8.9	9.0	10.6	10.6	8.6	8.6	5.6	6.4	9.0	9.0	-			
28	*H. tapichalaca* QCAZ16704	**1.9**	**1.9**	**1.7**	1.7	1.7	9.5	9.1	1.5	1.5	8.3	8.2	8.2	8.4	8.7	8.4	8.2	8.2	8.3	10.2	10.2	7.9	7.9	5.7	6.6	8.7	8.6	0.7	-		
29	*H. tigrinus* QCAZ31550	**9.3**	**9.3**	**9.3**	9.1	8.9	4.3	4.3	8.7	8.7	3.8	3.8	4.9	4.5	4.3	4.5	4.5	4.0	4.0	4.7	4.7	3.8	3.8	2.2	2.8	2.8	2.8	9.5	8.7	-	
30	*H. tigrinus* QCAZ41351	**9.1**	**9.1**	**9.3**	9.0	8.7	4.4	4.2	8.5	8.5	3.6	3.6	4.7	4.4	4.2	4.4	4.4	3.8	3.8	4.7	4.7	3.6	3.6	2.1	2.8	2.8	2.7	9.5	8.7	0.0	-