The Herpetological Bulletin 173, 2025: 14–23
RESEARCH ARTICLE

https://doi.org/10.33256/hb173.1423

The roar of the tiger stream treefrog *Hyloscirtus tigrinus*: advertisement call, distribution update, novel colouration patterns and comments on natural history

ANDRÉS FELIPE LIÉVANO-BONILLA[1*], GUSTAVO ADOLFO PISSO-FLOREZ[2], JONH JAIRO MUESES-CISNEROS[3], SANTIAGO RON[4] & HÉCTOR E. RAMÍREZ-CHAVES[5]

[1]Fundación Ecohabitats, Calle 78N # 19–157 Casa 24 Portería 1, Popayán, Cauca, Colombia
[2]Parques Nacionales Naturales de Colombia, Carrera 9 # 25N–06, Popayán, Cauca, Colombia
[3]Wildlife Conservation Society Colombia, Mocoa, Putumayo, Colombia
[4]Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Roca, Apartado 17-01-2184, Quito, Ecuador
[5]Facultad de Ciencias Exactas y Naturales, Departamento de Ciencias Biológicas, Universidad de Caldas, and Centro de Museos, Museo de Historia Natural, Universidad de Caldas, Colombia

[*]Corresponding author e-mail: aflb73@gmail.com

ABSTRACT – *Hyloscirtus tigrinus* is a rare, Endangered and colourful stream treefrog inhabiting the paramos and high Andean forest of south-western Colombia and northern Ecuador. During 2021–2023, we undertook fieldwork in Colombia at the Puracé National Park and surrounding areas, which yielded observations on the distribution, behaviour, advertisement call, colour pattern variations and phylogeny of this species. We report two new localities for *H. tigrinus*, which extends the north-eastern margin of its range by 18 km and gives a total of 10 known localities; these are restricted to between 2,638–3,470 m a.s.l.. Recordings of the male advertisement call indicate a single low-pitched pulsed note without frequency modulation, composed of two to six pulses. The call is emitted at a rate of 37.8–48.3 calls/min, with a dominant frequency between 1,636.52–1,894.92 Hz, and resembles that of *Hyloscirtus larinopygion* and *Hyloscirtus antioquia*, but *H. tigrinus* shows a lower number of pulses per call. Like other *Hyloscirtus* spp., the passive defensive behaviour of the species may include immobility, crouching and contraction. The populations from the new localities exhibit a light brown to reddish-brown dorsal colouration with black reticulate pattern or scattered blotches, which represents the most divergent colouration observed in the species. Phylogenetic and morphological analyses provide evidence that the most northerly populations, which exhibit divergent colouration, belong to *H. tigrinus*. Genetic distances for gene 16S (0.293–0.298%), indicate they are closely related to populations from Ecuador but remain distinguishable from them.

INTRODUCTION

The *Hyloscirtus larinopygion* (Amphibia, Hylidae) species group is a clade that comprises 19 Neotropical anurans species (Rivera-Correa et al., 2024), distributed in Andean ecosystems of Colombia, Ecuador and Peru, which are confined strictly to Andean creeks and streams in restricted elevational ranges (Coloma et al., 2012; Ron et al., 2018; Frost, 2024). The species within the group are relatively large (SVL >60 mm) and are colourful, ranging from dark to grey/brown tones contrasting with bright marks. They are nocturnal, with apparently seasonal activity and are remarkably elusive (Duellman & Hills, 1990; Coloma et al., 2012). Knowledge of the diversity and systematics of this group has increased in the last two decades (e.g. Coloma et al., 2012; Almendariz et al., 2014; Ron et al., 2018; Reyes-Puig et al., 2022; Rivera-Correa et al., 2024) but certain species still lack comprehensive information regarding their natural history.

Hyloscirtus tigrinus Mueses-Cisneros & Anganoy-Criollo 2008, is an Endangered, rare and colourful frog, part of the northern clade of *H. larinopygion* group (Coloma et al., 2012; IUCN SSC Amphibian Specialist Group, 2018; Ron et al., 2018). This medium-sized (SVL ~ 57 mm) frog is distinguished from its congeners by having a distinctive colour pattern: the dorsal region is yellow-green or yellowish-brown with transverse black stripes, sometimes reticulated, with or without mid-dorsal stripe; the flanks are yellow-green with black reticulation, and the iris may be coloured light grey or yellow with black reticulation (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros & Perdomo-Castillo, 2011). This species is known from eight localities in high Andean ecosystems of central and centro-oriental cordilleras in the departments of Cauca, Huila, Putumayo and Nariño in south-western Colombia, and the province of Sucumbíos in the cordillera oriental of northern Andes of Ecuador, at altitudes of 2,640–3,470 m a.s.l. (Mueses-Cisneros & Anganoy-Criollo, 2008; Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011;

Coloma et al., 2012). In Colombia, *H. tigrinus* has been associated with the riparian vegetation of streams and lakes in high Andean and subparamo ecosystems (Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011). The potential threats to *H. tigrinus* are logging, burning, unregulated use of land for cattle raising and agricultural activities, pesticide use, invasive species (i.e. *Oncorhynchus mykiss*), unregulated tourism, and chytrid fungus (Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011; Coloma et al., 2012).

The available information about the species includes adults and tadpole morphology, conservation status, geographical and ecological distribution and systematics (Mueses-Cisneros & Anganoy-Criollo, 2008; Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011; Coloma et al., 2012; Rivera-Correa & Faivovich, 2013; Rivera-Correa et al., 2016; Ron et al., 2018; Reyes-Puig et al., 2022; Sánchez-Nivicela et al., 2023). Herein, we contribute the first information on the advertisement call and the defensive behaviour of the species and provide an updated phylogeny for the *H. larinopygion* species group. We also present new records of *H. tigrinus*, updating its geographical distribution and describing the external morphology and additional colour patterns of the specimens collected.

MATERIALS & METHODS

New records and defensive behaviour

Our field work was undertaken during three visits to the Guanacas-Puracé-Coconucos and the Sotará paramo complexes in the Andean region of south-western Colombia. In the Guanacas paramo, we visited two localities - in February 2021 La Sabana (2° 25'53.06'' N, 76° 14'00.75'' W; 3,090 m a.s.l.) and in February 2023 Quebrada La Marquesa (2° 28'00.73'' N, 76° 12'56.14'' W; 3,180 m a.s.l.). Both are located in the village of Río Sucio, municipality of Inzá in the department of Cauca. In December 2021, in the Sotará complex we visited the Laguna La Magdalena, located in the Puracé National Park, municipality of San Agustín, department of Huila (1° 56'10.55'' N, 76° 36'22.64'' W; 3,460 m a.s.l.). At each site, we conducted visual and auditory encounter surveys (Crump & Scott, 1994) along the streams, creeks and lakes of Andean forest and subparamo ecosystems (Cuatrecasas, 1958). Geographical co-ordinates and altitude at survey sites were obtained with a Garmin GPSMAP 64s (map datum WGS 84). We described the defensive behaviour of the species based on observations during visual encounters and handling of specimens.

We photographed the captured individuals with a Nikon D610 with AF-S VR Micro-Nikkor 105mm f/2.8G IF-ED and external flash. Collected specimens were euthanised using benzocaine (Chen & Combs, 1999), fixed in 10% formalin, preserved in 70% ethanol (Cortez et al., 2006), and stored at the herpetological collection of the Museo de Historia Natural de la Universidad del Cauca (MHNUC-HE-AN) and the Amphibians Collection of the Museo de Historia Natural de la Universidad de Caldas (MHN-UCa-Am). These voucher specimens are listed in the Supplementary Material.

External morphology

We examined the external traits and took morphometric measurements for six collected specimens according to the definitions described by Duellman (1970) and Heyer et al. (1990). All measurements were taken with a digital caliper and rounded to the nearest 0.1 mm. The description of nuptial pads follows the terminology of Luna et al. (2012). Sex was determined by direct examination of gonads through a dorsolateral incision, while colour patterns were described from photographs of live specimens. We compare these morphological traits with those described for *H. tigrinus* by Mueses-Cisneros & Anganoy-Criollo (2008) as well as variations reported subsequently by other authors.

Phylogenetic analyses and genetic distances

Some individuals from the studied populations showed markedly different colour patterns relative to the known variation in *H. tigrinus*. To confirm their identification and update the phylogeny of the *H. larinopygion* group, we sequenced the mitochondrial genes 12S rRNA, tRNA-Val and 16S rRNA of two individuals we collected from Río Sucio, Colombia (MHN-UCa-Am 1869–1870). We also included one specimen from Santa Bárbara, Ecuador (QCAZ 38421). Tissues obtained from the specimens collected were deposited in the collection of the Museum of Zoology QCAZ at Pontificia Universidad Católica del Ecuador. Our phylogenetic analyses are based on the same matrix used by Ron et al. (2018), including the recently described *Hyloscirtus sethmacfarlanei* and *Hyloscirtus arcanus* (Reyes-Puig et al., 2022; Rivera-Correa et al., 2024; sequences from GenBank, https://www.ncbi.nlm.nih.gov/genbank). The sequences were aligned with MAFFT, E-INS-i algorithm (Katoh & Standley, 2013). Phylogenetic relationships were inferred under maximum likelihood (ML) with software IQ-TREE 2.2.0.7 (Nguyen et al., 2015; Minh et al., 2020) under default settings. We partitioned the matrix by gene and each partition was analysed under model GTR + R + I. To estimate branch support, we made 1,000 ultrafast non-parametric bootstrap searches (-bb 1000 command; Hoang et al., 2018) and 1,000 replicates for the SH-like approximate likelihood ratio test with the -alrt 1000 command (Guindon et al., 2010). We considered that branches with bootstrap values > 94 and SH-aLRT values > 79 had strong support. Uncorrected genetic distances for the gene 16S were calculated with Mesquite 3.7 (Maddison & Maddison, 2021).

Advertisement call description

We obtained call recordings of adult males from La Sabana and Laguna La Magdalena populations following the standard procedures described by Arcila-Pérez et al. (2024). All recordings were digitised in WAV format at a sampling frequency of 44.1 kHz and 16 bits amplitude resolution using a Tascam DR-40X handheld digital recorder connected to a Rode NTG1 unidirectional condenser microphone positioned about 1–2 m from the calling males. We recorded the air temperature (T) and humidity (RH) with an HTC-2 digital thermohygrometer (accuracy ± 0.1 °C and 1%), while the body size (SVL) of the captured individuals was measured with a SPI super polymid dial caliper (± 0.1 mm). Calls were

analysed using the software Raven Pro 1.6.5 (K. Lisa Yang Center for Conservation Bioacoustics, 2024); temporal parameters were measured from the waveform (in ms) and spectral parameters in spectrograms generated from the Fourier transformation with a Blackman algorithm, window length of 256 samples, overlap of 80% and a DFT of 1,024. We elaborated calls figures of waveforms, spectrograms and power spectra with Seewave v.2.1.8 package (Sueur et al., 2008) in the R environment using the RStudio interface (R Core Team, 2023; Posit Team, 2024).

We followed the note centred approach and terminology suggested by Köhler et al. (2017) and Emmrich et al. (2020) for the classification and description of the advertisement call. The following quantitative parameters were measured: call duration (= note duration), intercall silent interval, call repetition rate (calls per minute), number of pulses per call, pulse duration and pulse repetition rate (pulses per second), dominant frequency, low frequency (= 5% frequency), high frequency (= 95% frequency) and frequency bandwidth (= difference between high and low frequencies). We deposited the call recordings at the Colección de Sonidos Ambientales, Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia (IAvH-CSA; listed in the Supplementary Material). We reviewed published information about the advertisement calls described for the stream treefrogs of the *H. larinopygion* group and compiled a comparative table showing the variation of commonly measured temporal and spectral traits.

Geographic distribution update

We compiled the historical localities of *H. tigrinus* from the literature (Mueses-Cisneros & Anganoy-Criollo, 2008; Montezuma & Mueses-Cisneros, 2009; Mueses-Cisneros & Perdomo-Castillo, 2011; Coloma et al., 2012) and web portals such as Anfibios del Ecuador and the Global Biodiversity Information Facility (Ortiz & Páez-Rosales, 2022; GBIF.org, 2024). We generated an updated map and estimate of the extent of occurrence for the species using the geometric convex hull polygon, calculated from Minimum Bounding Geometry algorithm in QGIS 3.34.3-Prizren (IUCN SSC Red List Technical Working Group, 2024; QGIS.org, 2024).

RESULTS

New records and defensive behaviour

We documented 11 individuals of *H. tigrinus* at La Sabana from 10–18 February 2021. The vegetation in these areas is characterised by shrubs of *Baccharis* spp., *Blechnum loxense*, *Clethra* spp., *Diplostephium* spp., *Espeletia hartwegiana*, and *Pentacalia* sp., with emergent trees of *Clusia multiflora*, *Hedyosmum* spp. and *Weinmannia* spp. in a subparamo ecosystem. Two individuals were encountered on the ground in gallery forests along streams at least 5 m wide. The first individual was observed at 22:30 h near a small puddle beside the Río Negro (Fig. 1A). Upon capture, this individual exhibited defensive behaviour, bending its limbs close to the body, and remaining immobile for ten minutes until placed in a plastic bag (Fig. 1B). The second individual was vocalising at sunset (18:20 h) in the shrubs of a Río Negro tributary, approximately 1 km away from the first, but eluded capture.

Figure 1. *Hyloscirtus tigrinus* in situ - **A.** Male from La Sabana (left) and its Río Negro gallery forest habitat (right), **B.** Defensive behaviour of the same individual from La Sabana, **C.** Male from La Magdalena (left) and its subparamo ecosystem habitat (right).

The remaining nine individuals were detected near to streams and creeks in the Andean forest. One of these individuals was found on the ground about 2 m from the Quebrada San Antonio, another tributary of the Río Negro. Other individuals were detected near to creeks perched between 1.5–5.0 m high in the branches of shrubs or bamboo (*Chusquea* sp.). On 12 February 2023, in Quebrada La Marquesa, one individual was detected vocalising and captured on branches 1.5–2.0 m above the ground in the Andean forest, and a second was observed on nearby branches.

In the northern part of the Laguna La Magdalena wetland, several males were heard vocalising on 7 December 2021, near a border of subparamo vegetation, predominantly *Chusquea tessellata*, *Baccharis* spp., *Blechnum loxense*, *Clethra* spp. and *Diplostephium* spp. However, dense vegetation impeded observation. We located one male vocalising within mosses covering a shrub, about 1.40 m above the ground (Fig. 1C). The first detection occurred around 19:30 h, and the individual was recorded and

Figure 2. Colour pattern variation in *Hyloscirtus tigrinus* from Laguna La Magdalena- **A.** MHNUC-HE-AN 1410, and La Sabana, **B.** MHNUC-HE-AN 1351, **C.** MHNUC-HE-AN 1352, **D.** MHN-UCa-Am 1870. For a detailed description of these specimens see Supplementary Material.

captured at 23:48 h. On 8 December 2021, another individual was observed walking on the ground under shrub vegetation, using natural tunnels formed by the vegetation as a refuge.

External morphology

The six individuals collected, four from La Sabana (MHN-UCa-Am 1869 –1870, MHNUC-HE-AN 1351–1352), one from Quebrada La Marquesa (MHN-UCa-Am 1868) and one from Laguna La Magdalena (MHNUC-HE-AN 1410), were adult males with an SVL between 55.6–56.9 mm. They exhibited the diagnostic characters reported in the original description of the species (for details see Supplementary Material Tables 1S & 2S). Dorsally, their skin texture was shagreen while ventrally it was finely areolate but less so towards the throat; the surface of hind limbs was strongly areolate. The snout was rounded in dorsal view and truncate in lateral view. The tympanum and tympanic annulus were visible, with a prominent supratympanic fold extending from below eye across upper and posterior margins of tympanum towards posterior end of mouth and down to the arm insertion. Forearms were robust, axillar membrane absent, ulnar tubercles and folds absent, nuptial pads absent, fingers and toes with lateral fringes, disks on fingers and toes rounded, disks colouration black with cream or brownish spots, tarsal tubercles absent, webbing membrane on hands absent, calcar tubercle present, webbing membrane on feet present (I 2+-2- II1- -3 III 2+ 3IV 3--2V).

We observed four different colour patterns in life on the individuals collected (Fig. 2). Detailed descriptions of these colour patterns are presented in the Supplementary Material.

Phylogeny and genetic distances

Hyloscirtus tigrinus is sister to a clade comprising *Hyloscirtus psarolaimus, Hyloscirtus criptico, Hyloscirtus pacha* and *Hyloscirtus staufferorum*. Within *H. tigrinus*, the Colombian samples are sister to each other, and the resulting clade is sister to a clade of the Ecuadorian population (Fig. 3). Branch lengths are extremely short, suggesting low genetic differentiation between populations. Branch support for *H. tigrinus* is strong (ultrafast bootstrap = 100, SH-aLRT = 100). Genetic distances between the Colombian and Ecuadorian populations range from 0.293 to 0.298%. Intrapopulation distances range from 0 to 0.145%.

Advertisement call

We analysed 306 calls from four male *H. tigrinus*, two from La Sabana (102 calls) and two from Laguna La Magdalena (204 calls). The advertisement call consists of a single low-pitched pulsed note without frequency modulation (= non-frequency modulated pulsed call), with a duration of 142.88 ± 28.38 ms (61.9–216.6 ms) and composed of two to six pulses (188 of the 306 calls were composed of four pulses). The call is emitted at a rate of 37.8–48.3 calls/min with inter-call silent intervals of 1290.52 ± 293.18 ms (669.0–3024.2 ms). The pulses had a duration of 14.59 ± 4.32 ms (3.2–44.8 ms) and a repetition rate of 25.39 ± 1.91 pulses/s (17.34–31.77 pulses/s). The call had a dominant frequency of 1702.95 ± 68.34 Hz (1636.52–1894.92 Hz) and a bandwidth of 234.75 ± 45.45 Hz (172.26–344.53 Hz). Commonly, the highest energy is found in the second and third pulses. However, the first pulse may exhibit varying amplitudes, ranging from high to low. The final pulse consistently presents the lowest amplitude (Fig. 4). Similar to *Hyloscirtus antioquia* and *Hyloscirtus larinopygion*, the advertisement call of *H. tigrinus* is audible to the human ear as a faint melodious ‘brrrrr’ and resembles the sound of a stridulating cricket.

The males from Laguna La Magdalena were recorded at air temperature of 9.0 °C in the subparamo ecosystem and presented calls commonly composed for four to five pulses of longer duration and lower repetition rate (3.2–44.8 ms and 17.3–31.8 pulses/s) when compared with those from La Sabana (5.2–25.6 ms and 21.0–29.7 pulses/s; Fig. 4A & B). The recordings from La Sabana were obtained in the Andean forest ecosystem at air temperatures of 12.1–13.4 °C and showed calls without strong amplitude modulation between pulses, so the silent inter-pulse intervals were not always distinguishable. These calls had a shorter duration (76.2–154.2 ms) when compared with the calls from Laguna La Magdalena (61.9–216.6 ms) and were usually composed for three to four pulses; a male from this population showed the highest values of dominant frequency for the species, 1,808.8–1,894.9 Hz (Fig. 4C & D). Call variation in temporal and spectral traits of the four recorded males is summarised in the Supplementary Material Table 3S.

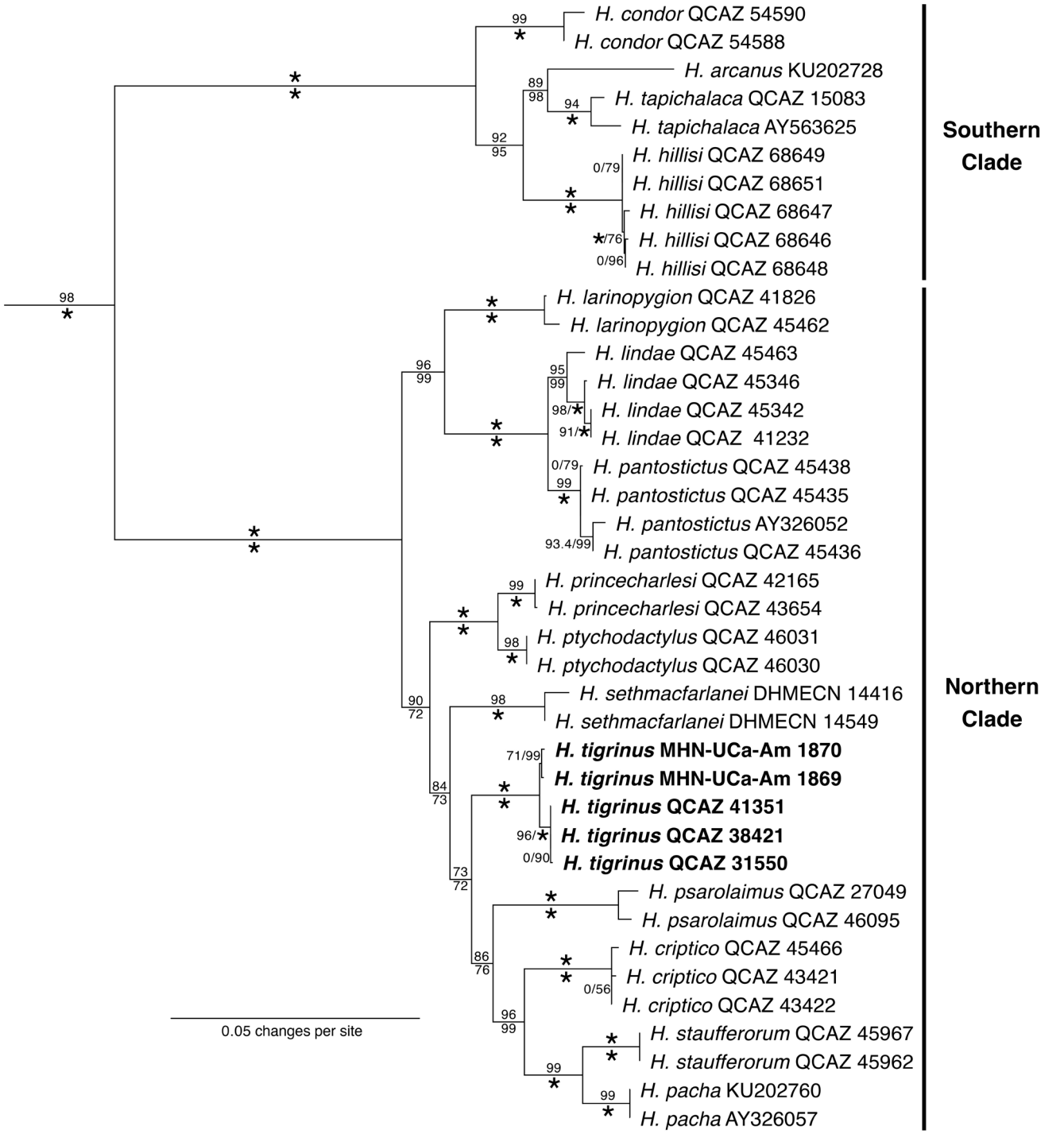

Figure 3. Phylogenetic relationships of species in the *Hyloscirtus larinopygion* group (updating Ron et al., 2018), inferred from the mitochondrial genes 12S rRNA, tRNA-Val, and 16S rRNA under maximum likelihood. Bootstrap values are shown above the branches and SH-aLRT values below. Values of 100% are represented by an asterisk.

Geographic distribution update

Based on our fieldwork and the geographical data associated with historical records, *H. tigrinus* is present in ten known localities in the Andes of south-western Colombia (departments of Cauca, Huila, Nariño and Putumayo) and northern Ecuador (province of Sucumbíos). The populations reported in the village of Río Sucio correspond to the northern most distribution of *H. tigrinus*, with Quebrada La Marquesa being the most northerly locality known for the species. The lowest altitudinal records of *H. tigrinus* have been from Santa Bárbara (Sucumbíos; 2,638 m), while Laguna La Magdalena corresponds to the highest altitudinal record (Huila; 3,467 m). The Minimum Bounding Geometry algorithm applied to these localities estimated the extent of occurrence of *H. tigrinus* to be 2,119.94 km^2 (Fig. 5).

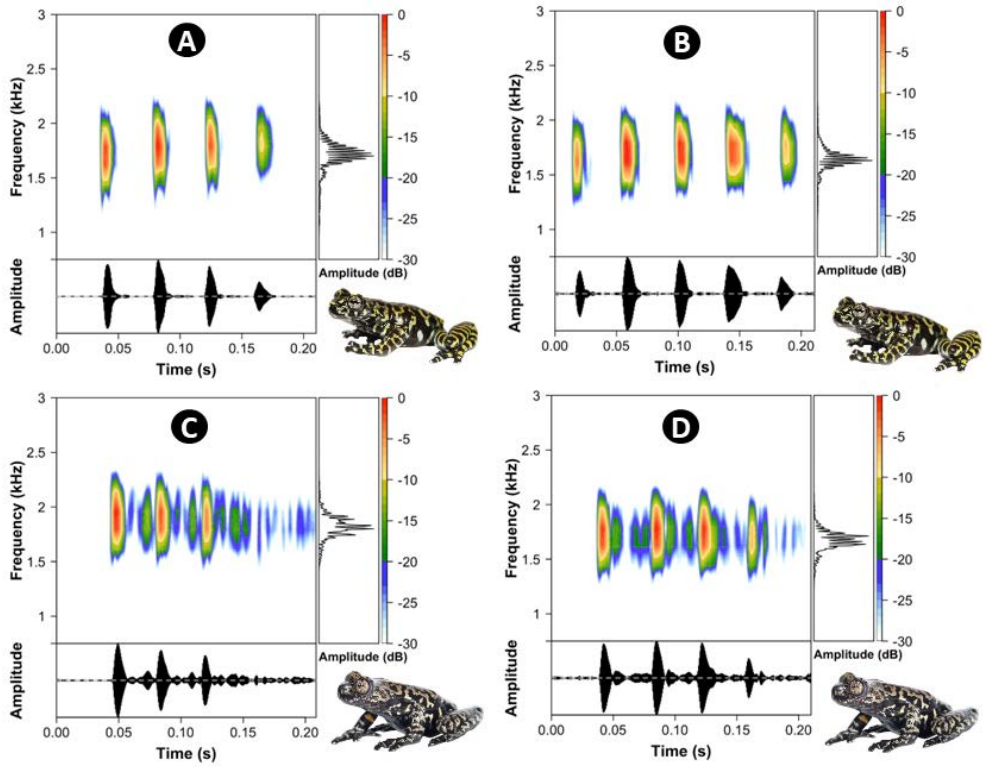

Figure 4. The advertisement calls for *Hyloscirtus tigrinus* from Laguna La Magdalena- **A.** IAvH-CSA-39385, **B.** IAvH-CSA-39386 and from La Sabana, **C.** IAvH-CSA-39383, **D.** IAvH-CSA-39384). For each male the spectrogram (top), oscillogram (bottom) and power spectrum (right) are shown.

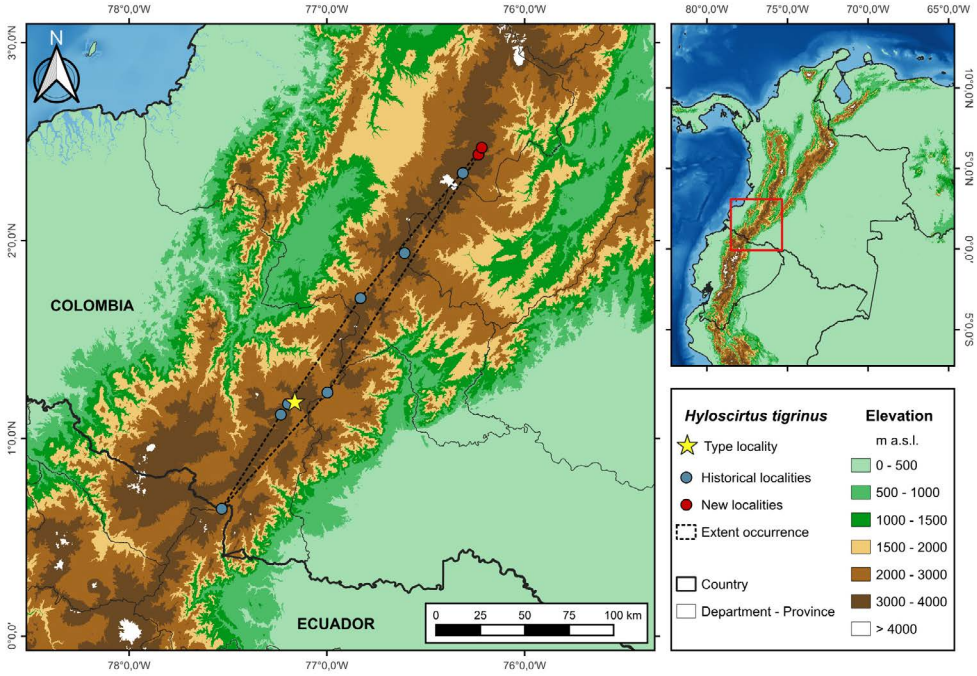

Figure 5. Locality records of *Hyloscirtus tigrinus* and the estimated extent of the species occurrence

DISCUSSION

As with other species of the *H. larinopygion* group, the individuals of *H. tigrinus* that we observed were associated with undisturbed montane streams and exhibited nocturnal activity (Coloma et al., 2012). They used dense vegetation as refuges and perches, where most individuals were observed in areas with dense branches at various heights, ranging from 0.5 m to 3–4 m above the ground. Based on our records from village of Río Sucio, we have extended the distribution range of *H. tigrinus* by 18 km in a straight line from the previous most northerly locality (Termales de San Juan, Puracé National Park, Cauca; Fig. 5). Likewise, this area is dominated by Andean forests and subparamo ecosystems, as has been

previously documented for the species (Mueses-Cisneros & Perdomo-Castillo, 2011).

The records from Laguna La Magdalena suggest that, among the species of the *H. larinopygion* group, *H. tigrinus* reaches the highest altitude (3,467 m), followed only by *H. larinopygion, H. antioquia* and *Hyloscirtus tolkieni* which are found up to 3,200 m (Rivera-Correa & Faivovich, 2013; Sánchez-Nivicela et al., 2023). Additionally, the Santa Bárbara locality in Sucumbíos represents the lowest recorded altitude for the species at 2,638 m, where *H. tigrinus* occurs in sympatry with *Hyloscirtus pantostictus, Hyloscirtus psarolaimus* and *Hyloscirtus lindae* (Coloma et al., 2012). Similarly, certain populations from Nariño and Putumayo, may coexist with *H. lindae* (Mueses-Cisneros & Perdomo-Castillo, 2011). In contrast, *H. tigrinus* has not been found in sympatry with other members of the *H. larinopygion* group at the known localities from its northern distribution (Cauca and Huila) (Mueses-Cisneros & Perdomo-Castillo, 2011; this study). The village of Río Sucio also corresponds to a historical locality for *Hyloscirtus caucanus* (km 66–67 road Popayán - Inzá; 2,660–2,700 m), where the paratypes and tadpoles referred in the species description were collected (Ardila-Robayo et al., 1993). However, during our fieldwork in La Sabana and La Marquesa, restricted to elevations above 3,000 m, we did not record *H. caucanus* or any other species of the *H. larinopygion* group coexisting with *H. tigrinus*. Further expeditions are needed to identify additional populations of this rare and endangered species, as well as to understand the distribution patterns of the species of *H. larinopygion* group that inhabit the high Andean ecosystems of south-western Colombia and northern Ecuador.

Defensive behaviour

The defensive behaviour observed in the new record of an individual from the village of Río Sucio corresponds to a passive strategy to avoid detection by predators (Toledo et al., 2010; 2011), previously documented for some species of the northern clade of *H. larinopygion* group (Rivera-Correa & Faivovich, 2013; Bejarano et al., 2015; Duarte-Marin et al., 2019; Reyes-Puig et al., 2022). Other species such as *H. psarolaimus* and *H. lindae* also display immobility, crouching and contracting (Bejarano et al., 2015), while *H. larinopygion*, *H. antioquia* and *H. sethmacfarlanei* may enhance these behaviours by emitting distress calls (as documented in *H. larinopygion*) and producing odoriferous and noxious secretions (Rivera-Correa & Faivovich, 2013; Duarte-Marin et al., 2019; Reyes-Puig et al., 2022). Some defensive behaviours such as body-rise, contracting and production of secretions, have also been reported in members of the southern clade like *Hyloscirtus diabolus* and *Hyloscirtus tapichalaca* (Kizirian et al., 2003; Rivera-Correa et al., 2016), and particularly *Hyloscirtus condor* exhibits additional defensive strategies such as biting and spine aggression (Almendariz et al., 2014).

External morphology

The morphological characters that we examined in the specimens from Río Sucio and La Magdalena mostly match those described for the type series and other additional specimens (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros & Perdomo-Castillo, 2011). However, our findings regarding nuptial pads and calcar tubercle align with those reported by Rivera-Correa & Faivovich (2013), suggesting that *H. tigrinus* lacks nuptial pads and may have a fleshy calcar tubercle that is poorly developed.

The variation in dorsal colouration of the type series of *H. tigrinus* and certain individuals from nearby localities in Nariño and Putumayo, include patterns ranging from yellow to green tones and presence of transverse black stripes or reticulate blotches (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros & Perdomo-Castillo, 2011). A subtle variation is reported in a female from Laguna La Magdalena (Montezuma & Mueses-Cisneros, 2009), which shows an intense yellow hue and more intricate reticulated black pattern (Mueses-Cisneros & Perdomo-Castillo, 2011). The male (MHNUC-HE-AN 1410) registered at the same locality during our fieldwork shares a similar colour pattern, but the flanks and some upper areas of the forelimbs and hind limbs show a smooth transition from dark yellow to a green-yellowish hue. Additional variation corresponds to the individual from Termales de San Juan (Puracé National Park, Cauca), which exhibits a darker brown dorsal tone with a yellow mid-dorsal stripe and irregular black spots, although it maintains yellow colourations on the forelimbs and hind limbs (Mueses-Cisneros & Perdomo-Castillo, 2011).

The specimens from the village of Río Sucio show ventral colouration and conspicuous orange markings similar to that of individuals reported in the type locality (Castelví Natural Reserve; Mueses-Cisneros & Perdomo-Castillo, 2011). With our observations, the *H. tigrinus* populations located in the northern part of the distribution limits represent the most divergent colouration observed in the species. The colouration patterns of these individuals resemble that described for *H. caucanus*, however, they can be differentiated from *H. caucanus* by external ulnar tubercles and yellow discs on the fingers and toes (Ardila-Robayo et al., 1993). A recent taxonomic key to identify species within the *H. larinopygion* group (Sánchez-Nivicela et al., 2023), uses the yellow, green or greyish cream colour with thick black reticulum or stripes to identify *H. tigrinus*, but our findings suggest the need to include brown to reddish-brown colourations to account for the extensive colour variability of this species.

Phylogeny and genetic distances

The phylogeny we describe is generally consistent with previous publications (e.g. Coloma et al., 2012; Ron et al., 2018; Reyes-Puig et al., 2022; Rivera-Correa et al., 2024). However, there are differences in some relationships within the northern clade. In our phylogeny, as well as those of Ron et al. (2018) and Coloma et al. (2012), *H. tigrinus* is sister to the clade *H. psarolaimus* + *H. criptico* + *H. pacha* + *H. staufferorum* while in Reyes-Puig et al. (2022) it is only sister to *H. criptico* + *H. pacha* + *H. staufferorum*. However, support in Reyes-Puig et al. (2022) is much weaker than in Ron et al. (2018) or Coloma et al. (2012), suggesting a less reliable topology. Despite the remarkable geographic variation described in the colouration patterns of *H. tigrinus* (Mueses-Cisneros & Anganoy-Criollo, 2008; Mueses-Cisneros

& Perdomo-Castillo, 2011; this study), populations from marginal localities exhibit very low genetic distances for gen 16S (0.293–0.298%). These values align with the divergence range (0.2–0.9%) observed for the same gene among conspecific populations of sequenced members within the *H. larinopygion* species group (Coloma et al., 2012). Although the population distances of the 16S gene in *H. tigrinus* are comparable to those observed in *H. criptico* (0.2%; Coloma et al., 2012), the geographic separation between *H. tigrinus* marginal populations is significantly greater (250 km). This geographic distance is even greater than that of *H. lindae* populations (122 km), which display the highest genetic divergence within the group (0.9%; Coloma et al., 2012). Likewise, the intrapopulation distances observed for the gen 16S in *H. trigrinus* (0–0.145%) are considerably lower than those reported for *H. sethmacfarlanei* (0.4%; Reyes-Puig et al., 2022). Therefore, the most northerly populations reported in this study, despite exhibiting divergent colouration, correspond to *H. tigrinus*, and the genetic results support and corroborate the morphological findings, confirming that these populations belong to the same species.

Advertisement call

The description of the advertisement call of *H. tigrinus* increases to 11 the number of species with documented acoustic data in the *H. larinopygion* species group (Table 4S in Supplementary Material). The integration of this evidence within recent phylogenetic hypotheses (Fig. 3; Rojas-Runjaic et al., 2018; Ron et al., 2018; Rivera-Correa et al., 2024), shows a pattern consistent with previous findings (i.e. Rivera-Correa et al., 2017), where non-pulsed calls are suggested as the ancestral character of *Hyloscirtus*, while pulsed calls have several independent origins in the northern clade of the *H. larinopygion* group: *H. tigrinus, H. psarolaimus* and the clade *H. lindae* + *H. pantostictus* + *H. larinopygion* + *H. antioquia*. However, for a comprehensive understanding of the evolutionary trajectories of these acoustic traits in the northern clade, acoustic data needs to be collected for *Hyloscirtus princecharlesi, Hyloscirtus pychodactylus* and *H. sethmacfarlanei*, in addition the phylogenetic positions and advertisement calls of *H. caucanus, Hyloscirtus sarampiona* and *H. tolkieni* should be addressed.

The detailed analysis of the advertisement call of *H. tigrinus* shows similarities in call duration with *H. psarolaimus* (Coloma et al., 2012), but the lowest number of pulses per call in *H. tigrinus* and the higher dominant frequency in *H. psarolaimus* allows the pulsed calls of these closely related species to be distinguished. The advertisement call of *H. tigrinus* resembles the call duration, calls per minute and dominant frequencies of *H. larinopygion* and *H. antioquia* (Rivera-Correa et al., 2017), which share similar altitudinal distribution and habitat conditions in the Andean cloud forest and subparamos (Rivera-Correa & Faivovich, 2013). However, *H. tigrinus* exhibit the lowest number of pulses per call reported in the northern clade of the *H. larinopygion* group, and despite its wide range of variation in pulse duration, the mean values suggest that *H. tigrinus* tends to produce shorter pulses than *H. antioquia* and *H. larinopyion* (Tables 3S & 4S in Supplementary Material).

The acoustic variation reported here for *H. tigrinus* and its 'basal' position relative to the clade *H. criptico* + *H. pacha* + *H. staufferorum*, might offer insights into the evolution of call structure among these related species. The individuals recorded in Andean forest near to streams (La Sabana), emitted calls of shorter duration with fewer pulses, weak amplitude modulation between pulses and slightly higher dominant frequency, in contrast to those recorded near to lakes in subparamo (La Magdalena) where dense vegetation and flowing water noise are less prevalent (Table 3S in Supplementary Material). The abiotic noise produced by streams constitute the main directional selective force of spectral components of the advertisement calls in *Hyloscirtus* spp. (Vargas-Salinas et al., 2024), whereas the temporal components, particularly in allopatric distribution patterns, seems to be shaped by genetic drift and weak selective pressures related to species recognition and reproductive isolation (Rivera-Correa et al., 2017; Vargas-Salinas et al., 2024). However, other selective forces, such as the attractiveness of calls to females, territorial signalling to other males, detectability by predators or level of abiotic noise, need to be considered as additional factors that may shape the evolution of advertisement calls in stream treefrogs (Vargas-Salinas & Amezquita, 2014; Röhr et al., 2016; Vargas-Salinas et al., 2024), and need to be addressed to better understand the evolutionary history of acoustic signals in the *H. larinopygion* group.

ACKNOWLEDGEMENTS

We thank Jhoana Saavedra, Doris Rojas, Gustavo Papamija, Karen Matabanchoy and Isaac Bedoya Dorado for providing support visiting the localities around the Puracé National Park and Parques Nacionales Naturales de Colombia through research permit 20192000006553 authorising the collection of *H. tigrinus* specimens. AFLB and GAPF thank the support of The Mohamed Bin Zayed (Grant 192521814).

REFERENCES

Almendáriz, A., Brito, J., Batallas, D. & Ron, S. (2014). Una especie nueva de rana arbórea del género *Hyloscirtus* (Amphibia: Anura: Hylidae) de la Cordillera del Cóndor. *Papéis Avulsos de Zoologia* 54: 33–49. https://doi.org/10.1590/0031-1049.2014.54.04.

Arcila-Pérez, L.F., Barona-Cortés, E., Marín-Zuñiga, D., Martínez-Medina, D., David-Gutiérrez, D., Mendoza-Henao, A. & Pantoja-Sánchez, H. (2024). Protocolo de grabación, procesamiento y depósito de especímenes de audio. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, D.C., Colombia. 33 pp.

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, Física y Naturales* 18(71): 559–566.

Bejarano-Muñoz, P., Pérez Lara, M.B. & Brito, M.J. (2015). Ranas Terrestres Endémicas y otros Anuros Emblemáticos de la vía Papallacta–El Reventador. Serie de Publicaciones

Museo Ecuatoriano de Ciencias Naturales del Instituto Nacional de Biodiversidad (INB-MECN). Guías Rápidas de Campo Nro. 2. Quito, Ecuador. 68 pp.

Chen, M. & Combs, C. (1999). An alternative anesthesia for amphibians: ventral application of benzocaine. *Herpetological Review* 30: 34.

Coloma, L.A., Carvajal-Endara, S.J., Dueñas, F., Paredes-Recalde, A., et al. (2012). Molecular phylogenetics of stream treefrogs of the *Hyloscirtus larinopygion* group (Anura: Hylidae), and description of two new species from Ecuador. *Zootaxa* 3364: 1–78. https://doi.org/10.11646/zootaxa.3364.1.1.

Crump M.L. & Scott, N.J. (1994). Visual encounter surveys. In: *Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians*. 84–92 pp. Heyer, R.W., Donnelly, M.A., McDiarmid, R.W., Hayek, L.A. & Foster, M.S. (Eds.). Smithsonian Institution Press, Washington and London.

Cuatrecasas, J. (1958). Aspectos de la vegetación natural de Colombia. *Revista de la Academia de Ciencias Exactas, Físicas y Naturales* 10(40): 221–264.

Duarte-Marín, S., López-Molina, K.J. & Arcila-Pérez, L.F. (2019). Defensive behavior in *Hyloscirtus larinopygion* (Duellman, 1973) (Anura: Hylidae). *Herpetology Notes* 12: 509–511.

Duellman, W.E. (1970). The Hylid frogs of Middle America. *Monographs of the Museum of Natural History, University of Kansas* 1–2: 1–753.

Duellman, W.E. & Hills, 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.

Emmrich, M., Vences, M., Ernst, R., Köhler, J., Barej, M.F., Glaw, F., Jansen, M. & Rödel, M.O. (2020). A guild classification system proposed for anuran advertisement calls. *Zoosystematics and Evolution* 96(2): 515–525.

Frost, D.R. (2024). Amphibian Species of the World: an Online Reference. Version 6.2. Accessed on 26 September 2024. Electronic American Museum of Natural History, New York, USA. https://doi.org/10.5531/db.vz.0001.

GBIF.org (2024). *Hyloscirtus tigrinus* GBIF Occurrence Download. Downloaded on 26 September 2024. https://doi.org/10.15468/dl.nx8tpy.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59(3): 307–321. https://doi.org/10.1093/sysbio/syq010.

Heyer, W.R., Rand, A.S., Cruz, C.A., Peixoto, O.L. & Nelson, C.E. (1990). Frogs of Boracéia. *Arquivos de Zoologia* 31: 231–410.

Hoang, D.T., Chernomor, O., von Haeseler, A. & Minh, B.Q. (2018). UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518–522. https://doi.org/10.1093/molbev/msx281.

IUCN SSC Amphibian Specialist Group (2018). *Hyloscirtus tigrinus* (errata version published in 2018). The IUCN Red List of Threatened Species 2018: e.T158615A136514530. Downloaded on 26 September 2024. https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T158615A779423.en.

IUCN SSC Red List Technical Working Group (2024). Mapping Standards and Data Quality for the IUCN Red List Spatial Data. Version 1.20. 2024. https://www.iucnredlist.org/resources/mappingstandards .

K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology (2024). Raven Pro: Interactive Sound Analysis Software (Version 1.6.5). Ithaca, NY: The Cornell Lab of Ornithology. https://www.ravensoundsoftware.com/.

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(3): 339–349. https://doi.org/10.1655/02-41.

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.

Köhler, J., Jansen, M., Rodríguez, A., Kok, P.J.R., Toledo, L.F., Emmrich, M., Glaw, F., Haddad, C.F.B., Mark-Oliver, R. & Vences, M. (2017). The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251: 1–124.

Maddison, W.P. & Maddison, D.R. (2021). Mesquite: a modular system for evolutionary analysis. Version 3.7. http://mesquiteproject.org.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A. & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37: 1530–1534. https://doi.org/10.1093/molbev/msaa015.

Montezuma, M.F. & Mueses-Cisneros, J.J. (2009). Amphibia, Anura, Hylidae, *Hyloscirtus tigrinus*: Distribution extension, first department record, Cauca and Huila, Colombia. *Check List. A Journal of Species Lists and Distribution* 5(2): 243–245. https://doi.org/10.15560/5.2.243.

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.

Mueses-Cisneros, J.J. & Perdomo-Castillo, I.V. (2011). *Hyoloscirtus tigrinus* Mueses-Cisneros y Anganoy-Criollo, 2008: una especie amenazada, con comentarios sobre su distribución geográfica e historia natural. *Herpetotropicos* 5: 93–103.

Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–275. https://doi.org/10.1093/molbev/msu300.

Posit Team (2024). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. http://www.posit.co/.

Ortiz, D.A. & Páez-Rosales, N. (2022). *Hyloscirtus tigrinus*. In: *Anfibios del Ecuador*. Ron, S.R., Merino-Viteri, A. &. Ortiz, D.A. (Eds.). Version 2022.0. Museo de Zoología,

Pontificia Universidad Católica del Ecuador. Downloaded on 28 February 2024. https://bioweb.bio/faunaweb/amphibiaweb/FichaEspecie/Hyloscirtus%20tigrinus.

QGIS.org (2024). QGIS Geographic Information System. QGIS Association. http://www.qgis.org.

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

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 rediagnosis of *Hyloascirtus 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: 29–40. https://doi.org/10.11646/zootaxa.4061.1.3.

Rivera-Correa, M., Vargas-Salinas, F. & Grant, T. (2017). Statistical differences and biological implications: a comparative analysis of the advertisement calls of two Andean stream treefrog (Hylidae: *Hyloscirtus*) and the evolution of acoustic characters. *Salamandra* 53: 237–244.

Rivera-Correa, M., Ron, S., Nunes, I., Araujo-Vieira, K., Pinherio, P.D.P. & Grant, T. (2024). 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. *Zootaxa* 5474(2): 101–126. https://doi.org/10.11646/zootaxa.5474.2.1.

Röhr, D.L., Paterno, G.B., Camurugi, F., Juncá, F.A. & Garda, A.A. (2016). Background noise as a selective pressure: stream-breeding anurans call at higher frequencies. *Organisms Diversity & Evolution* 16: 269–273. https://doi.org/10.1007/s13127-015-0256-0.

Rojas-Runjaic, F., Infante-Rivero, E., Salerno, P. & 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: 121–146. https://doi.org/10.11646/zootaxa.4382.1.4.

Ron, S.R., Caminer, M., 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.

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.

Sueur, J., Aubin, T. & Simonis, C. (2008). Seewave: A free modular tool for sound analysis and synthesis. *Bioacoustics* 18: 213−226. https://doi.org/10.1080/09524622008.9753600.

Toledo, L.F., Sazima, I. & Haddad, C.F.B. (2010). Is it all death feigning? Case in anurans. *Journal of Natural History* 44: 1979–1988. https://doi.org/10.1080/00222931003624804.

Toledo, L.F., Sazima, I. & Haddad, C.F.B. (2011). Behavioral defenses of anurans: an overview. *Ethology Ecology & Evolution* 23: 1–25. https://doi.org/10.1080/03949370.2010.534321.

Vargas-Salinas, F. & Amezquita, A. (2014). Abiotic noise, call frequency and stream-breeding anuran assemblages. *Evolutionary Ecology* 28: 341–359. https://doi.org/10.1007/s10682-013-9675-6.

Vargas-Salinas, F., Londoño-Guarnizo, C.A., Duarte-Marín, S., Torres-Suárez, O.L. & Amézquita, A. (2024). Uncoupled evolutionary patterns in spectral and temporal components of acoustic signals in anurans associated with streams. *Ethology* 130: e13510. https://doi.org/10.1111/eth.13510.

Accepted: 24 January 2025

Please note that the Supplementary Material for this article is available online via the Herpetological Bulletin website: https://thebhs.org/publications/the-herpetological-bulletin/issue-number-173-summer-2025