A spectacular new species of *Hyloscirtus* (Anura: Hylidae) from the Cordillera de Los Llanganates in the eastern Andes of Ecuador

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We have discovered a spectacular new frog in the genus *Hyloscirtus,* belonging to the *H. larinopygion* group, characterized adult females mostly black with large red spots on the whole body and on the toe pads, while the juveniles are black heavily mottled on the dorsal surface with mustard-yellow bands and blotches, and yellow toe pads; fleshy calcar tubercle extending from the ankle, and cloacal ornamentation with two parallel canals, with supracloacal fold present and well defined, reaching the vent. The new species is known only from Cerro Mayordomo, in Fundacion EcoMinga´s Machay Reserve at 2900m, in the eastern Andes of Tungurahua province, Ecuador, near the southern edge of Los Llanganates National Park.

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Abstract

We have discovered a spectacular new frog in the genus *Hyloscirtus,* belonging to the *H. larinopygion* group, characterized adult females mostly black with large red spots on the whole body and on the toe pads, while the juveniles are black heavily mottled on the dorsal surface with mustard-yellow bands and blotches, and yellow toe pads; fleshy calcar tubercle extending from the ankle, and cloacal ornamentation with two parallel canals, with supracloacal fold present and well defined, reaching the vent. The new species is known only from Cerro Mayordomo, in Fundacion EcoMinga´s Machay Reserve at 2900m, in the eastern Andes of Tungurahua province, Ecuador, near the southern edge of Los Llanganates National Park.

KEY WORDS:
Hyloscirtus larinopygion group, Llanganates mountains, Upper Rio Pastaza watershed, phylogenetic position.

Introduction

The upper Rio Pastaza watershed was identified as a center of endemism for amphibians by Lynch and Duellman (1980), and subsequent investigations have tripled the number of species apparently endemic to this region, known as the Sangay–Llanganates Ecological Corridor (Reyes Puig et al., 2010, 2014, 2015, 2019a; Reyes-Puig & Yánez-Muñoz, 2012; Reyes-Puig, 2013; Reyes-Puig et al., 2019b; Franco-Mena et al., 2019).

The Machay Reserve is a private reserve owned by the Ecuadorian NGO Fundación EcoMinga on Cerro Mayordomo in the upper Rio Pastaza watershed, within the buffer zone of the Los Llanganates National Park. Investigators from Fundación EcoMinga and Instituto Nacional de Biodiversidad (INABIO) have been conducting botanical and herpetological expeditions there for two decades, which have led to the discovery of more than a dozen new species of plants, especially orchids (Jost, 2004) and several new amphibian and reptile species (Reyes Puig et al., 2010, 2014, 2015, 2019a; Reyes-Puig & Yánez-Muñoz, 2012; Reyes-Puig, 2013; Sheehy et al. 2014, Reyes-Puig et al., 2019b). During a botanical expedition in March 2018, one of the participants, Darwin Recalde, fortuitously encountered a striking black and red frog hiding in a leaf axil of a bromeliad at eye level; it was a new Stream Frog belonging to the genus *Hyloscirtus* Peters 1882, of the *H. larinopygion* group. During the following year, herpetologists from Fundación EcoMinga and INABIO conducted additional expeditions to the site, and found two juveniles of the same species just a few meters from the spot where the original female had been found.

The genus *Hyloscirtus,* in the family Hylidae, contains 38 species of arboreal frogs (Frost, 2021; Yánez-Muñoz et al. 2021). The genus is distributed from Costa Rica to the Andes of Venezuela, Colombia, Ecuador, Peru and Bolivia (Faivovich et al. 2005, Coloma et al., 2012; Frost, 2021). The genus is characterized mainly by the synapomorphy of a well-developed lateral membrane on the fingers of the hands and feet. At the time of the last revision, all known species were thought to reproduce alongside rushing streams.

The *Hyloscirtus larinopygion* group is composed of 19 species (Frost 2021), of which 13 are reported from Ecuador (Ron et al. 2021). The group consists of two clades which correlate with latitude, with a small area of overlap in central Ecuador (Almendariz et al. 2014, Ron et al. 2018). Adults of this group are characterized by a snout vent length > 60mm and dark overall skin color contrasting with bright patterns, especially on the tips of the digits. Species in this group "could be considered as some of the most colorful and beautiful frogs on earth" according to Coloma et al. (2012). The new species described here is perhaps the most striking of all *Hyloscirtus*.

Materials and Methods

Ethics statement. Our study was authorized under research permits MAE-DNB-CM-2016-0045 and NºMAE-DNB-CM-2019-0120, issued by the Ministerio del Ambiente del Ecuador. We followed the guidelines for use of live amphibians and reptiles in field research (Beaupre et al., 2004), compiled by the American Society of Ichthyologists and Herpetologists, the Herpetologists' League, and the Society for the Study of Amphibians and Reptiles.

Taxon sampling. We examined specimens deposited in the herpetological collections of the Instituto Nacional de Biodiversidad, Quito (DHMECN) and Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá (ICN) (Appendix 1). All museum acronyms follow Sabaj (2016). Our taxonomic description employs several lines of evidence, including external morphological characters, genetic divergence, monophyly and geographic data. Similar approaches have been useful in recognizing and identifying closely related species of anurans in the eastern Andes of Ecuador (Páez-Moscoso et al. 2011, Reyes-Puig et al., 2019a, b).

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Field work. All of the specimens were found fortuitously during day walks in botanical expeditions to the summit of Cerro Mayordomo (-1.370204 S; -78.267943 W; 2,972 m) on 16–20 March 2018 and 18–19 October 2018.

Laboratory work. The female and one juvenile were collected during the day in plastic containers and then taken alive to the INABIO, where they were photographed in life and sampled for DNA. They were subsequently fixed in 10% formalin for twelve hours, and then preserved as voucher specimens in 70% alcohol following the recommendations of Heyer et al. (1994), and deposited in the herpetological collection of INABIO as holotype (female) and paratype (juvenile). A third juvenile was photographed in situ and not collected.

External morphological data. Character descriptions were made according to the specialized literature treating the *H. larinopygion* group (Coloma et al., 2012; Almendáriz et al., 2014; Ron et al., 2018).

Description of webbing formulae of the hands and feet follow Savage and Heyer (1967), as modified by Myers and Duellman (1982). Morphological measurements were taken with digital calipers (± 0.01 mm) from the specimens preserved in 70% ethanol according to the methodology described in Duellman (1970). The following measurements were taken: snout-vent length (SVL), head length (HL), head width (HW), upper eyelid width (EW), interorbital distance (IOD), inter-nostril distance (IND), nostril-eye distance (NED), eye diameter (ED), tympanum diameter (TD), hand length (HaL), tibia length (TL), and foot length (FL). Sex was determined by direct examination of gonads.

We also compared qualitative morphological characters between the new species and its closest relatives. Six characters were evaluated: (1) dorsal coloration; (2) ventral coloration; (3) marks on flanks and hidden surfaces of thighs; (4) iris coloration; (5) prepollex condition; and (6) in life, webbing coloration. Life coloration was obtained from color photographs.

Osteological data and analysis: The holotype of the new species and a specimen of each of five closely related species were scanned by use of a high-resolution micro-computed tomography (micro-CT) desktop device (Bruker SkyScan 1173, Kontich, Belgium) at the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK, Bonn, Germany). To avoid movements during scanning, the specimens were placed in a small plastic container and mounted with styrofoam. The scans were conducted over 180 degrees with rotation steps of 0.3-0.4 degrees, with a source voltage of 35 kV and source current of 150 µA, without the use of a filter, at an image resolution of 39.3-50.0 µm. Scan duration was 30:01-45:37 min with an exposure time of 280 ms and average framing of 5. The CT-datasets were reconstructed using N-Recon software (Bruker MicroCT, Kontich, Belgium) and rendered in three dimensions through the aid of CTVox for Windows 64 bits version 2.6 (Bruker MicroCT, Kontich, Belgium). Osteological terminology follows Trueb (1973), Duellman & Trueb (1994) and Coloma et al. (2002). Cartilage structures were omitted from the osteological descriptions, because micro-CT does not render cartilage.

Genetic sampling. We generated eight new sequences for the mitochondrial 16S gene (see Figure 1), following the primers and protocols described in Guayasamin et al. (2015). The new sequences were aligned with all sequences available for *Hyloscirtus* in GenBank (http://www.ncbi.nlm. nih.gov/genbank), originally published by Faivovich et al. (2005), Coloma et al. (2012), Almendáriz et al. (2014), Guayasamin et al. (2015) and Ron et al. (2018).

Phylogenetic analysis. Sequences were aligned using MAFFT v. 7 (Katoh & Standley, 2013) with the Q-INS-i strategy. Maximum likelihood (ML) trees were estimated using GARLI 2.01 (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006). GARLI uses a genetic algorithm that finds the tree topology, branch lengths and model parameters that maximize ln(L) simultaneously (Zwickl, 2006). Individual solutions were selected after 10,000 generations with

no significant improvement in likelihood, with the significant topological improvement level set at 0.01. The final solution was selected when the total improvement in likelihood score was lower than 0.05, compared to the last solution obtained. Default values were used for other GARLI settings, as per recommendations of the developer (Zwickl, 2006). Bootstrap support was assessed via 1000 pseudoreplicates under the same settings used in tree search. Genetic distances (uncorrected *p*) between the new species and its closest relatives were calculated using PAUP v.4.0a (Swofford, 2002).

Ecological niche modelling. We use Maxent (version 3.4.2) to obtain a model of the ecological niche of the northern clade of the H. larynopigion group. Localities for all species of the group were obtained from literature and museum collections. Recommended default values were used for convergence threshold, maximum number of iterations, maximum background points; 25% of localities were randomly set aside as test points; regularization was set to 1. Selected format for representation of probabilities for models was logistic. Parametrization was based on WorldClim (version 2.1, Fick & Hijmans 2017). Statistical analyses of variable contributions for data layers, including jackknife tests and correlation tests, were used to obtained more informative and less correlated variables. Models were evaluated through quantitative and qualitative tests, including threshold-independent test, threshold-dependent test, visual evaluations, and evaluation of variable importance and response curves. A geographical information system was developed based on grids from Maxent with ArcGis Desktop to analyze data and produce relevant maps.

Results

Phylogenetic relationships. Our phylogenetic analysis (Fig. 1) shows that the new species is sister to a clade consisting of *Hyloscirtus criptico* Coloma et al. (2012), *H. larinopygion* (Duellman, 1973), *H. lindae* (Duellman & Altig,1978), *H. pacha* (Duellman & Hillis, 1990), *H. pantostictus* (Duellman & Berger, 1982), *H. princecharlesi* Coloma et al. (2012), *H. psarolaimus* (Duellmn & Hillis, 1990), *H. ptychodactylus* (Duellman & Hillis, 1990), *H. staufferroum* (Duellman & Coloma, 1993) and , *H. tigrinus* Mueses-Cisneros & Anganoy-Criollo (2008). Genetic distances (mitochondrial 16S percent differences calculated from uncorrected p values between the new species and the most closely related *Hyloscirtus* are given in Table 1; genetic distances between the new species and its closest relative were 2.2-2.9% to *H. tigrinus* and 2.6-2.8% to *H. ptychodactylus*.

Systematic accounts

Hyloscirtus sethmacfarlanei sp. nov.

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Proposed standard Spanish name: Rana de torrente de Seth MacFarlane
Proposed standard English name: Seth MacFarlane's torrent frog

Holotype (Fig. 2, 3, 4, 5, 6). DHMECN 14416 adult female, collected in the Machay Reserve of Fundacion EcoMinga, Cerro Mayordomo (-1.370204 S; -78.267943 W; 2,972 m), , Tungurahua province, Republic of Ecuador, on 17 March 2018, by Darwin Recalde, Fausto Recalde, Santiago Recalde and Jordy Salazar.

Paratype (Fig. 4, 5, 6). DHMECN 14549, juvenile, collected in the same locality and location the holotype on 19 October 2018, by Fausto Recalde, Santiago Recalde, Jesús Recalde and Jordy Salazar.

Generic placement. We assign the new species to the genus *Hyloscirtus* Peters, 1882, defined according to Faivovich et al. (2008) and Rojas-Runjaic et al. (2015), and as a member of the *Hyloscirtus larinopygion* group (sensu Duellman & Hillis, 1990; Faivovich et al., 2005) according to its phylogenetic position and phenotypic characteristics such as wide dermal fringes on fingers and toes, hands and legs with large terminal discs and a reduced membrane, adults characterized by a snout vent length > 60mm and dark overall skin color contrasting with bright patterns, especially on the tips of the digits.

Diagnosis. *Hyloscirtus sethmacfarlanei* sp. nov. is characterized by the following combination of characters: SVL of 72mm in the female and 46.5mm in the juvenile. Discs of digits narrow; fleshy calcar present; supracloacal fold present extending and reaching to the vent; cloacal ornamentation with two parallel grooves; a unique color pattern, with black ground color covered with large bright orange-red spots on both the dorsal and ventral surfaces, and red tips on all digits in the female, and black ground color heavily stippled and mottled mustard yellow, especially on the upper body and head, with flanks black covered with broad yellow-orange bars in juveniles; cloacal ornamentation composed of a well defined supracloacal fold reaching to the vent; skin strongly areolate and granular surrounding vent; and thick well-defined paracloacal folds.

Comparison with similar species (Figs. 7, 8) The black and orange-red pattern of the female of the new species is most similar to the patterns of *Hyloscirtus pantostictus* (Duellman & Berger, 1982) from extreme northeastern Ecuador and *princecharlesi* Coloma et al. (2012) from the Pacific slopes of the Andes of northwestern Ecuador. The new species differs from these in

having both the dorsal and ventral surfaces spotted with orange-red (versus ventral surface without red spots in *H. pantostictus* and *H. princecharlesi*, Fig. 7) and a well-defined supracloacal fold reaching next to the vent (reduced supracloacal fold without contacting the side of the vent in *H. pantostictus;* defined and reaching the border of the vent on *H. princecharlesi*). The female of the new species also differs from these two species in having orange-red discs on the tips of all digits (versus yellow discs in *H. pantostictus* and grayish discs in *H. princecharlesi*). The new species' orange-red discs are shared with *Hyloscirtus lindae* (Duellman & Altig, 1978) from the eastern Andes, but *H. lindae* does not have red spots on its dorsal surface and a thick supra cloacal fold close to the side of the vent (Fig. 8). The juvenile of the new species has a pattern similar to that of *Hyloscirtus princecharlesi* and *H. larinopygion* (Duellman, 1973) from northwestern slopes of the Andes. It differs from both species in having the dorsum mottled and stippled mustard-yellow and black (versus dorsum densely spotted orange-red in *H. princecharlesi,* and yellowish-brown with distinctive cream bars with black interspaces in *H. larinopygion*). The supracloacal fold is faintly defined in *H. larinopygion* and is distant to the side of the vent (well-defined reaching next to the vent in the new species).

Hyloscirtus sarampiona (Ruiz-Carranza & Lynch, 1982) from western slopes of the Colombian Andes has dorsal surfaces orange spotted with pale olive, but differs from the new species by having hidden areas of the limbs, flanks, palmar, plantar surfaces and discs black.

Description of holotype (Figs. 2, 3). Adult female, 72.0mm SVL. Body slender, head rounded in dorsal view, longer than wide (head length 113.4% of head width); width of upper eyelid 72.6% of the interorbital distance; texture of the dorsal surface of the head rough, including the eyelids; snout truncate in dorsal and lateral views; eye- nostril distance slightly less than the diameter of the eye; canthus rostralis short and slightly rounded, loreal region slightly concave; internarial region flat and slightly depressed; top of head slightly concave; nostrils oval and slightly protuberant, directed laterally; eyes large and protuberant, 24.6% of head length; interorbital region concave; eye diameter 1.76 times larger than the diameter of the tympanic ring; supratympanic fold well-defined, directed obliquely from the posterior border of the eye, covering the dorsal edge of the tympanum, extending back to the upper shoulder; tympanum and tympanic ring evident and round, 56.6% of eye diameter, separated from the eye by a distance 1.62 times greater than the diameter of the tympanum.

Body large, anterior and posterior extremities slim. Forearms robust compared to the arms; axillar membrane present; ulnar tubercles absent; relative length of fingers I < II < IV < III; fingers with large oval disks, slightly wider than finger; subarticular tubercles simple and enlarged, round and prominent; multiple round and oval supernumerary tubercles present; thenar tubercle large and flat, oval and elongated, palmar tubercle asymmetric with a slightly heart-shaped outline; prepollex absent; glandular nuptial pad covering the outer margin of Finger I; fingers long and slightly wide with interdigital webbing basally and with fleshy lateral fringes on all fingers.

Hind limbs long and slender, tibia length 46.3% of SVL; foot length 46.4% of SVL; heel tubercles large and round in outline; inner tarsal fold absent; large rounded to slightly oval subarticular tubercles in all fingers, supernumerary foot tubercles rounded, low; toes long, narrower than the hand, discs not expanded; relative lengths of toes I < II < III >V < IV ; inner metatarsal tubercle large, oval; outer metatarsal tubercle absent; toes with interdigital membrane, toe membrane formula: I 2-3II1- 2 ½ III 2-3 IV 2- ½ V (Fig. 3).

Body skin finely areolate, especially on flanks; inguinal glands absent; ventral skin densely granular, less so towards throat. Supracloacal flap transversal, well-defined, with supracloacal fold present, reaching the level of the vent, with two paracloacal folds; skin around the cloaca strong areolate and granular surrounding it. (Table 2, Fig. 7).

Choana large and oval, notably separated from each other and perpendicular to the floor of the mouth; dentigerous processes of vomers transverse, with vomerine teeth numbering 9-10; tongue wide and rugose, slightly rounded, partially attached to the floor of the mouth.

Coloration of holotype in preservative (~70% ethanol) (Fig. 2). All dorsal surfaces of the head, body, and extremities are black with round, oval, or irregular yellowish-white spots 3-4mm in diameter dispersed over the whole body; in the extremities the spots can be slightly elongated; the tips of the digits are marked with white spots. Ventral surfaces and throat grayish black with scattered irregular white elongated spots 5-10mm long, with palms of hands and feet grayish.

Coloration of holotype in life (Figs. 4, 5, 6, 7, 9): Entire dorsal and ventral surfaces of the body black with large bright orange-red round to oval spots scattered over the whole body, including the tips of the digits; spots more elongated on the extremities and flanks. Iris light gray with fine dark reticulations, while the nictitating membrane, revealed in defense and at rest, is well-developed, black in color, with irregular orange-red reticulations.

Measurements of the holotype (in mm). SVL= 72.0; HL=22.9; HW =20.2; EW =6.0; IOD =8.3; IND=5.2; NED= 5.4 ; ED=5.7; TD=3.2; HL=25.2; TL=33.3; FEL= 26.1; FL= 33.38.

Variation (Figs 4, 5, 9). The juvenile paratype DHMECN 14549 differs from the female holotype as follows. In life the dorsal surface of this juvenile was blackish, heavily stippled with mustard yellow especially near the head; nictitating membrane dotted with mustard yellow on a gray ground. Extremities banded orange on a grayish black ground; flanks black with orange reticulations. Throat marbled with irregular yellowish patches with orange tones, on a grayish black ground. Belly and ventral surfaces of the extremities grayish black with irregular sparse diffuse whitish-yellow patches; palms of hands and feet black with diffuse light orange spots. In addition to the juvenile paratype, an uncollected juvenile was photographed on December 2019

(Figure 7), within a few meters of the collection locality of the holotype and paratype. This individual had a mainly yellow dorsal coloration, with diffuse blackish spots scattered on the flanks and hidden surfaces of the arms and between the fingers, whose tips are yellow. The belly is light cream with diffuse blackish spots.

Measurements of the Paratype (in mm). SVL= 46.54; HL= 16.08; HW= 15.63; EW= 4.6; IOD= 5.17; IND= 3.68; NED= 3.5; ED= 4.15; TD= 1.98; HL=16.68; TL= 24.32; FEL= 20.8; FL= 22.71.

Osteology:

Skull. (Figs. 10, 11). The skull is generally consistent with those of the other species of the *H. larinopygion* group (Coloma et al.2012). In the new species, main differences are observed in the texture and shape of the sphenoid, and in frontoparietals, that are comparatively more rugose than other species of the group; additionally the frontoparietal fontanelle is more elongate and thinner. Other relevant differences can be observed in the shape of vomers that are paired, denticulate, and not in contact medially; the prechoanal rami are triangular and prominent; postchoanal rami are spine-like in form, with anterior border horizontal and posterior border obliquely oriented. The premaxillary alary process is posterodorsally projected forming an acute angle with the pars facialis; the alary process of the premaxilla has a round profile anteriorly and it is longer than the pars dentalis relative to other species of the *H. larinopygion* group.

There are no relevant differences in forelimb bones of the new species relative to other species of the group, although the prepolex appears wider, with acute termination (Fig. 12).

Posteromedial processes of the hyobranchium (Fig. 13). The posteromedial processes of the hyobranchium are paired ossified structures, longer than broad, with an anterior portion with "head-like shape" and a posterior elongate part. In the new species these paired structures possess a triangular shaped anterior portion, and a shorter posterior portion compared with other species in the *Hyloscirtus larinopygion* group, which have an external round border and internal spine-like border. In *H. lindae, H. psarolaimus* and *H. pacha*, the anterior portions have rounded external and internal borders. In *H. tapichalaca* it is broad and "shell-like" in its anterior border.

Tadpole: Not known.

Distribution (Fig.14**).** *Hyloscirtus sethmacfarlanei* sp. nov. is known only from the type locality in Fundación EcoMinga's Machay Reserve, Cerro Mayordomo, 2,972 m of altitude, in the eastern cordillera of the central Ecuadorian Andes, in the northern side of the upper Rio Pastaza near the southern border of Llanganates National Park.

Natural history. The type locality consists of dwarf open mossy forest, covered with bryophytes and epiphytes, and saturated with humidity. All three known individuals of this species were

found on a narrow mountain ridge, in bromeliads of the genus *Guzmania,* growing within 60-90cm above the ground (Fig. 15).

The windward (east-facing) forest elevation band of 2900-3000m is continuous from the type locality on Cerro Mayordomo to the Cerro Hermoso massif in the center of Los Llanganates National Park,17km to the north of the type locality. The new species probably occupies at least this range. During Holocene glacial maxima this forest community would probably have moved down the mountains by 1000m (Dodson 2003), potentially connecting this population to other nearby mountains such as the Cordillera Abitagua.

The striking coloration of the adult female of the new species is aposematic. The live frog transferred toxins to the frog discoverer´s uninjured skin by passive contact after briefly handling the frog. He noticed an unpleasant tingling sensation down his arm, that was not restricted to the area where he had come into contact with the frog. The only uncollected subadult was found on the same square meter as the holotype and paratype. It slept during the day, and when disturbed, it adopted a defensive ball-like position, as observed in other species of the *larinopygion* group. (Kizirian et al., 2003; Bejarano-Muñoz et al., 2015).

Sympatric anuran species recorded near the habitat of *Hyloscirtus sethmacfalanei* sp. nov. are two undescribed *Pristimantis* species and one species of the *Pristimantis buckleyi complex.*

Conservation Status. Since only one location is known for this species, and since the area is so poorly studied and inaccessible, we suggest the IUCN category Data Deficient (DD) for this species.

Etymology. The specific epithet *sethmacfarlanei* is a patronym in honor of Seth MacFarlane, American writer, director, producer, actor, artist, musician and conservationist, whose passion for science, biodiversity and the natural world is beyond compare.

Discussion

Phylogenetically the closest species to *H. sethmacfarlanei* are *H. tigrinus* (genetic distance 2.2-2.9%) from the northeastern Andes of Ecuador and adjacent Colombia, and *H. ptychodactylus* (genetic distance 2.6-2.8%) from the west-central Andes of Ecuador. Morphologically these two species are extremely different from *H. sethmacfarlanei* sp. nov.; the dorsal surface of *H. tigrinus* is black with strong whitish marbling/banding (not spots), and the dorsal surface of H. *ptychodactylus* is black with large dull poorly-defined reddish-brown blotches (again, no spots). Neither of these species has been found close to the type locality of *H. sethmacfarlanei* sp. nov. These genetic distances between *H. sethmacfarlanei* sp.nov. and its closest relatives are

considerably greater than the genetic distances between some other clearly-defined species in the *H. larinopygion* group, such as the distance between *H. ptychodactlyus* and *H. princecharlesi* (1.3%).

The divergence times between some of the species in the *H. larinopygion* group were estimated by Coloma et al (2012). While *H. sethmacfarlanei* sp.nov. was not known at the time of that study, our Table 1 allows us to identify other species pairs which were analyzed by Coloma et al (2012) and whose genetic distances are similar to the genetic distances between *H. sethmacfarlanei* sp. nov. and its closest relatives. An example is the pair *H tigrinus and H. ptychodactylus* with a genetic distance of 2.6%, which is similar to the genetic distances between the new species and its closest relatives (2.2-2.9%). Coloma et al (2012) estimated the divergence time between *H tigrinus and H. ptychodactylus* at about 9-19Mya. Therefore the divergence time between *H. sethmacfarlanei* sp.nov. and either of its closest relatives, *H. tigrinus* and *H. ptychodactylus*, should also be on the order of at least several million years. This shows that *Hyloscirtus sethmacfarlanei* is a very old species, not a product of Quaternary isolating mechanisms. This age predates the final rapid rise of the northern Andes to their current height. This could explain the relatively close relationship between eastern Andean *H. sethmacfarlanei* sp.nov. and western Andean *H. ptychodactylus*. The most recent common ancestor of both species may have comprised a continuous population on both slopes when the Andes were not so high. Later uplift of the Andes may then have led to the split of the population.

The two specimens of *H. sethmacfarlanei* sp. nov. collected show a moderate genetic distance of 0.4%, although they come from exactly the same location. With only two specimens, our conclusions from this are necessarily very limited, but this suggests a moderate genetic diversity within the population, implying that the actual population is not small. This degree of divergence within a population is about average for members of the *H. larinopygion* group (0.2–0.9%; Coloma et al 2012).

The *Hyloscirtus larinopygion* group is characterized by overlapping morphological and morphometric characters. In many cases, the preserved and living coloration patterns continue to be the first source to discriminate externally the limits of species in this group (Duellman & Hillis, 1990; Duellman & Coloma, 1993; Coloma et al. 2012; Rivera-Correa & Faivovich, 2013; Rivera-Correa et al., 2016; Ron et al., 2018). Our study shows the need to continue incorporating and exploring new evidence (e.g. CTscan osteology, DNA and supracloacal folds) to help delimit cryptic and conspicuous lineages of the group, whose adaptive radiation in the Ecuadorian Andes is apparently still underestimated. Finally the Maxent model shows that the type locality of *H. sethmacfarlanei* is within the predicted niche for the northern clade of the *H. larynopygion* group. Many areas across the Andes of Colombia and Ecuador show high probabilty of occupation according to the model, but no species records, e.g., Cordillera Oriental of Colombia,

southern Cordillera Occidental of Colombia, and the extreme northern and central Cordillera Oriental of Ecuador. Interestingly, two undescribed species recently collected and deposited at Ecuadorian museums come from these areas. This model suggest that the species richness of the group is still underestimated (Fig. 14-15)

Conclusions

It is remarkable that despite intensive research work in the upper Rio Pastaza watershed, we still continue discovering new amphibian species. We present different lines of evidence to define a new species of *Hyloscirtus* and assess its phylogenetic position inside the *Hyloscirtus larinopygion* species group. Our study also highlights the importance of the Llanganates – Sangay Ecological Corridor, outside of Ecuador´s national park system, as a center of endemism and diversity. Additionally distribution model for *Hyloscirtus larinipygion* species group reflects many potential areas of occurrence along northern andes.

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Figure 1

Evolutionary relationships of species in the *Hyloscirtus larinopygion* group, based on the mitochondrial gene 16S under ML criterion.

Clade support (bootstrap %) are in blue. The new species is in red.

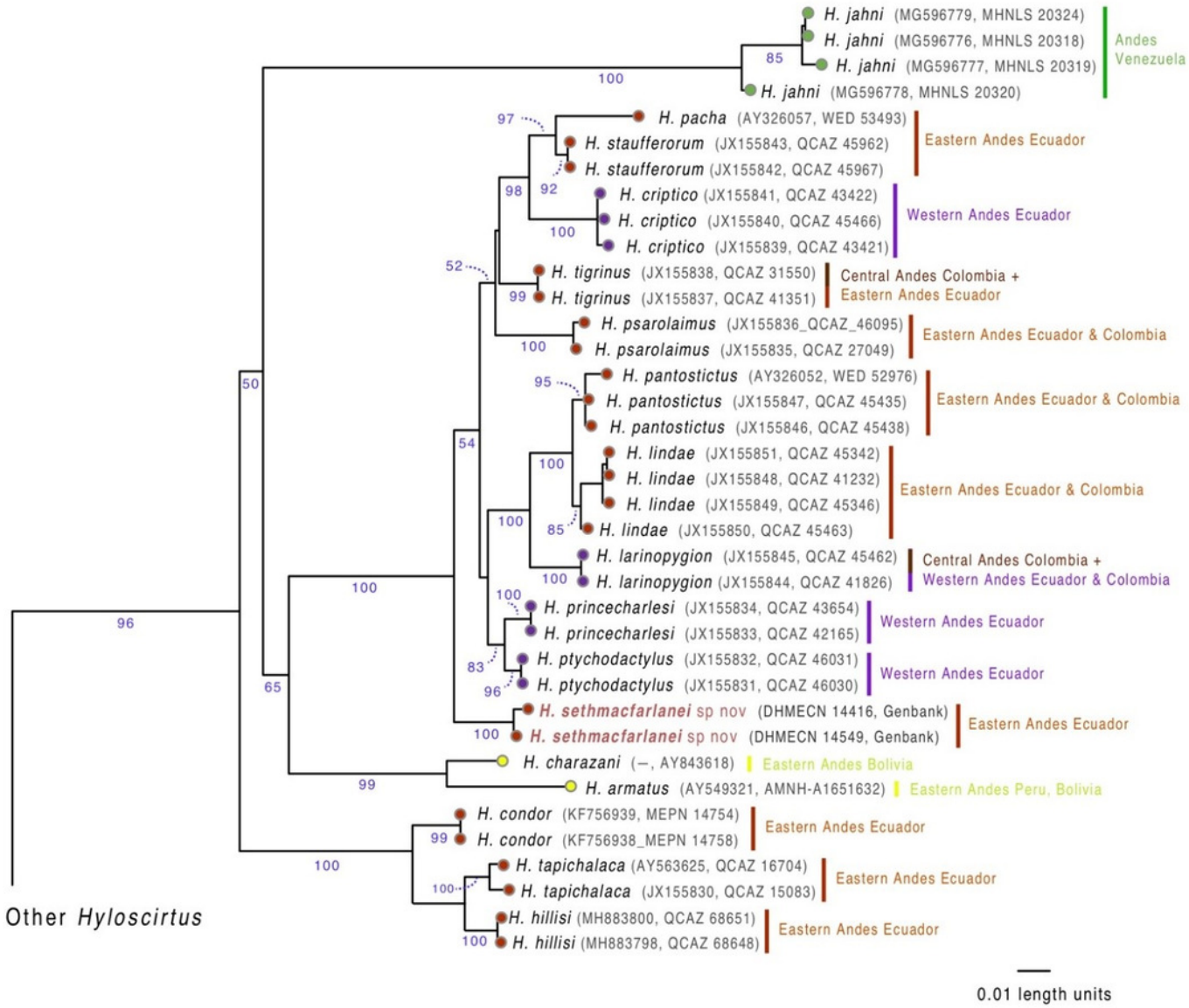

Figure 2

Dorsal, lateral, and ventral views of the holotype DHMECN 14416 of *Hyloscirtus sethmacfarlanei*.

Photographs: MYM


10 mm
10 mm

Figure 3

Details of the hand and foot of the holotype of *Hyloscirtus sethmacfarlanei*, DHMECN 14416

Photographs: MYM

Figure 4

Dorsal coloration of *Hyloscirtus sethmacfarlanei*

Top: Female holotype (DHMECN 14416), Bottom: male (paratype DHMECN 14549).

Photographs: MYM.

Figure 5

Ventral coloration of *Hyloscirtus sethmacfarlanei*

Left: Female holotype (DHMECN 14416), Right: male paratype (DHMECN 14549).

Photographs: MYM

Figure 6

Lateral detail of head in life of the type series of *Hyloscirtus sethmacfarlanei*

Top: Female holotype (DHMECN 14416), Bottom: male paratype (DHMECN 14549), note colouration in nictitating membrane . Photographs: MYM

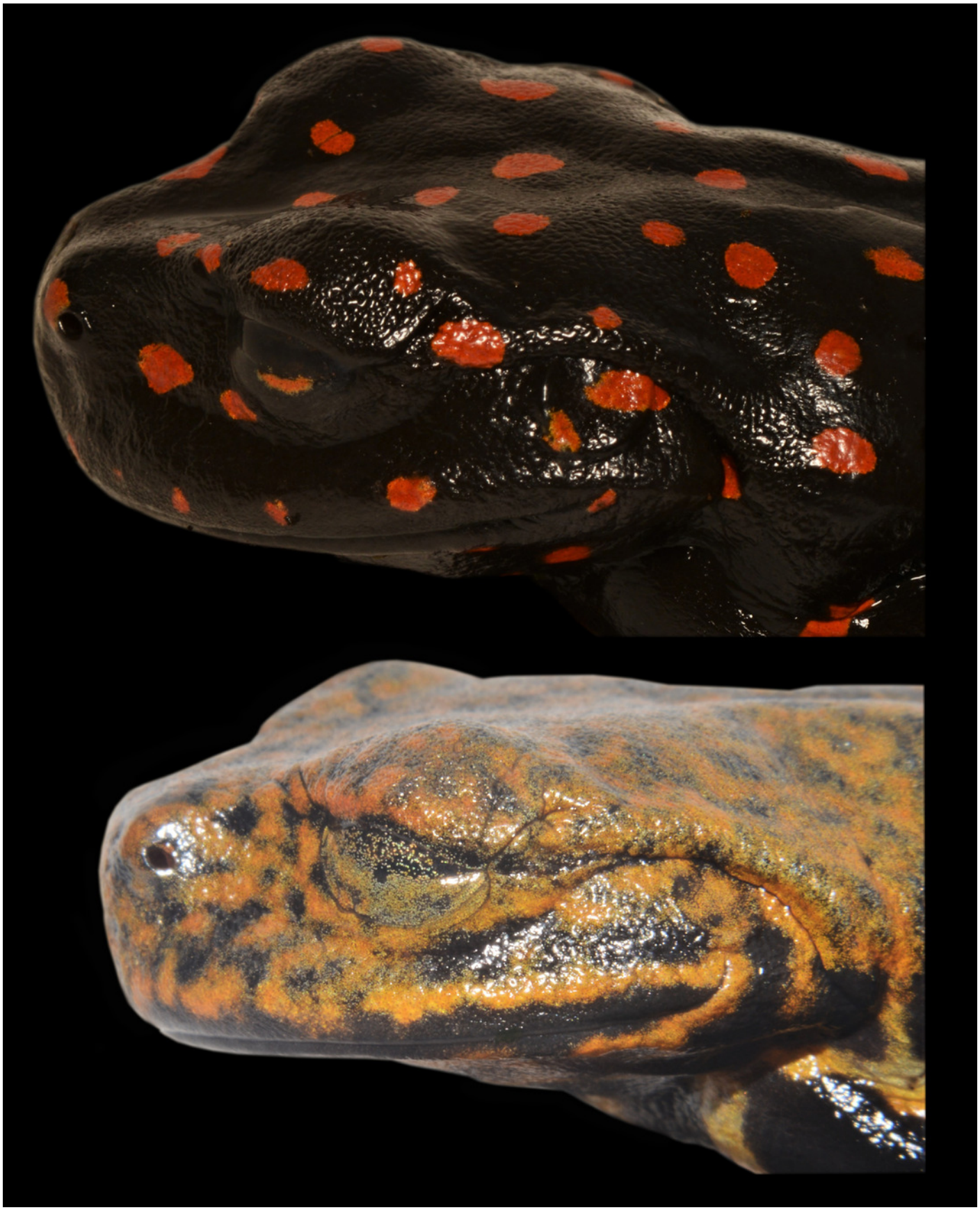

Figure 7

Life Comparison of *Hyloscirtus sethmacfarlanei* with six species of *Hyloscirtus* from the *H. larinopygion* group from the Andes of Ecuador

(A) *H. sethmacfarlanei,* female holotype, DHMECN 14416; (B) *H. sethmacfarlanei,* male paratype, DHMECN 14549, (C) *H. princecharlsi* photographic record QCAZ; (D). *H. larinopygion* photographic record QCAZ ; (E) *H. lindae* DHMECN; (F) *H. pantostictus* photographic record QCAZ; (G) *H. psarolaimus* DHMECN ; (H) *H. pacha* DHMECN. Photographs JPRP, MYM, Santiago Ron QCAZ, Jorge Brito

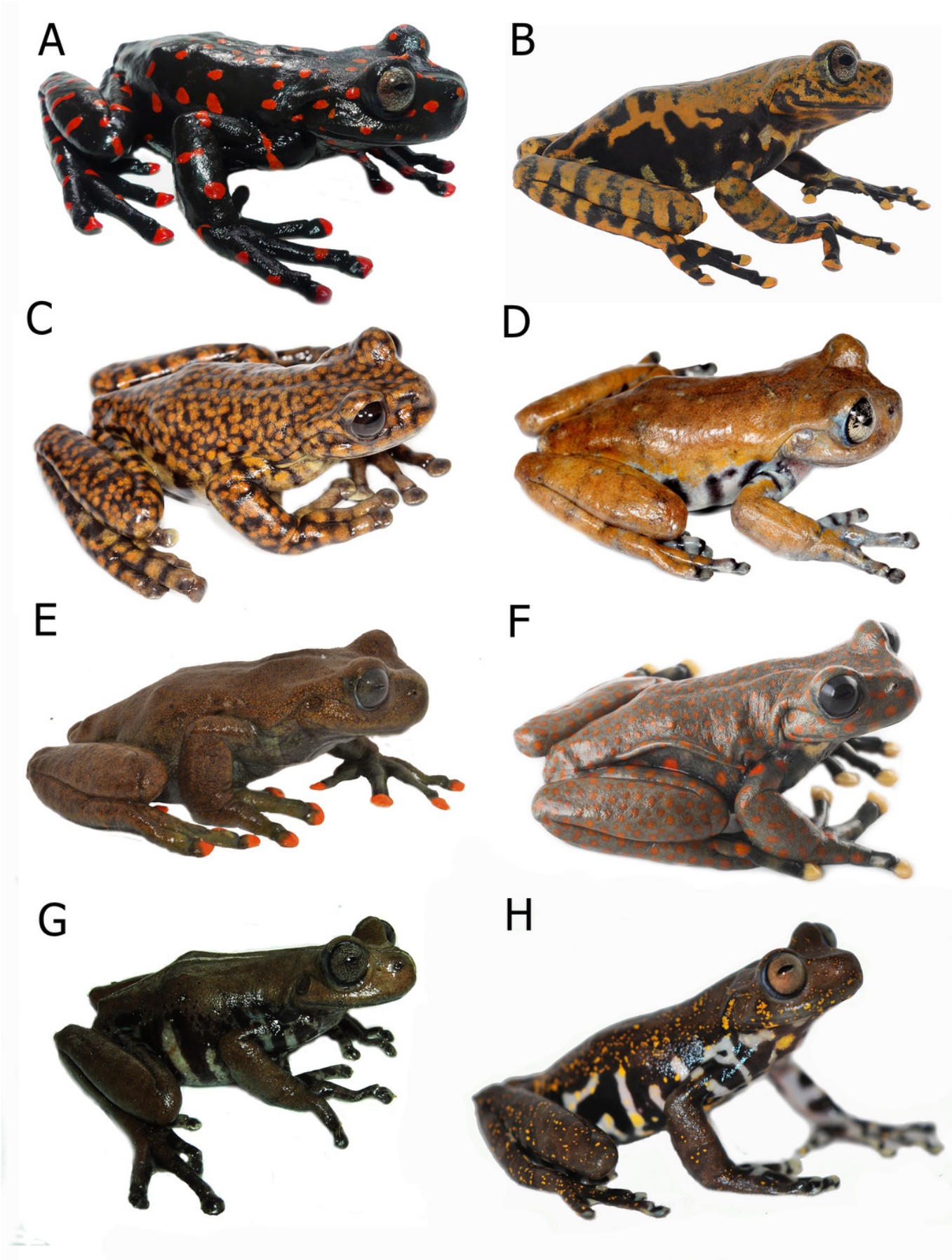
A
B
C
D
E
F
G
H

Figure 8

Vent condition in six species of *Hyloscirtus* of the *H. larynopygion* group

A) Hyloscirtus larynopigyin (DHMECN 3799); B) *H. psarolaimus* (DHMECN 6493); C) *H. sethmacfalanei sp nov* (DH MECN 14416) D); D) *H. pacha* (DHMECN 12111); E) *H. lindae* (DHMECN 12483); F) *H. tapichalaca* (DHMECN 9686). Photographs MYM

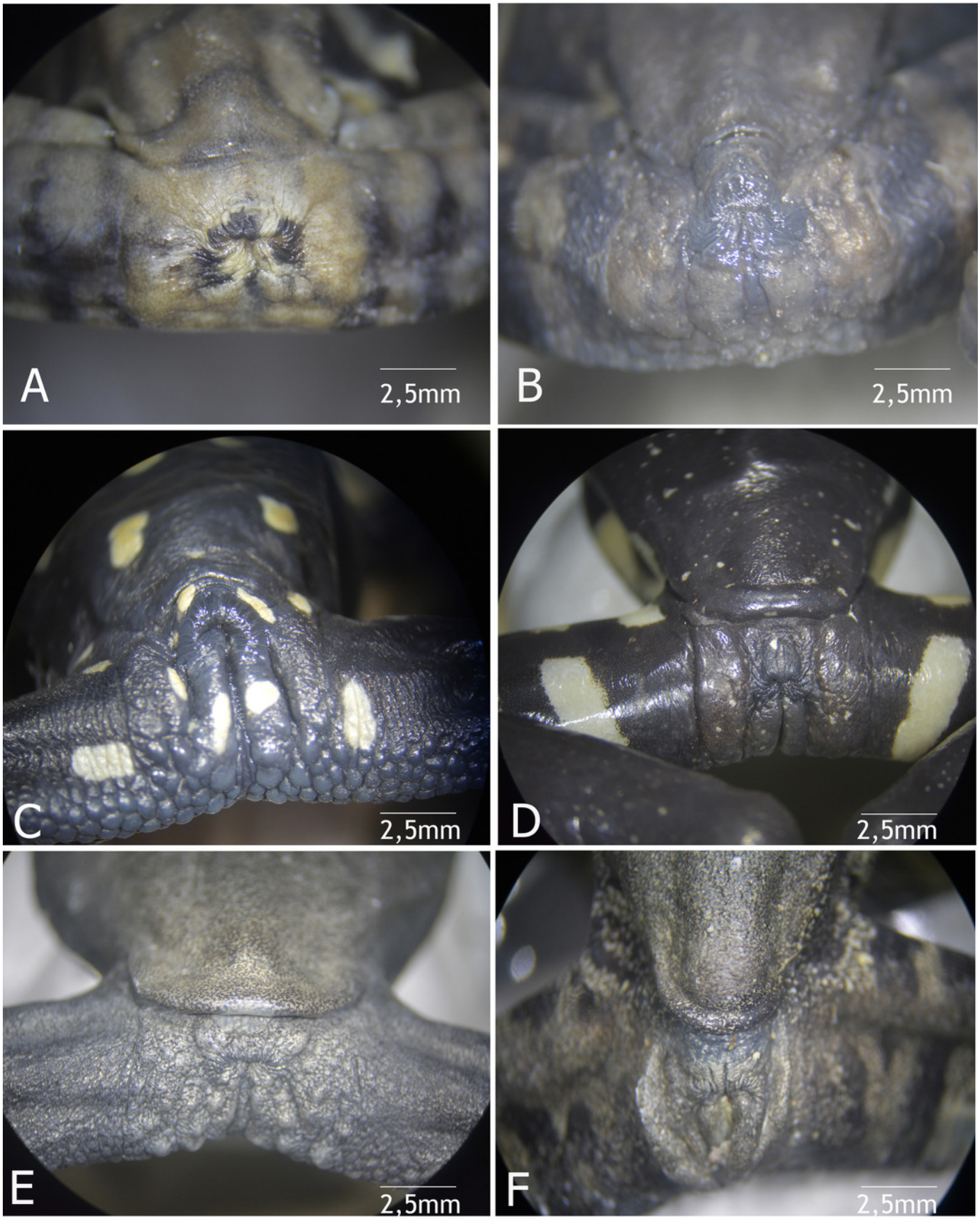
A
2,5mm
B
2,5mm
C
2,5mm
D
2,5mm
E
2,5mm
F
2,5mm

Figure 9

Live specimens of *Hyloscirtus sethmacfarlanei* in situ

(A) Female DHMECN 14416, holotype; (B) Juvenile paratype (DHMECN 14549). (C) juvenile not collected on its natural habitat. (D) Juvenile adopting defensive behavior.

Photographs: LJ, Fausto Recalde, JPRP.

Figure 10

Osteological details of the cranium of the adult female holotype (DHMECN 14416) *Hyloscirtus sethmacfarlanei*

Individual skull diagnostic bones in colors: blue: premaxilla alary process; brown:maxillae; purple :sphenethmoid ; orange: vomer; white: parasphenoid; light blue anterior :neopalatine; light blue posterior :pterigoides; reddish anterior: frontoparietals; posterior double red dotted lines: conjunction with exooccipital; anterior red dotted outline outline: frontoparietal fontanelle; yellow :prootic; green:squamosal; pink: quadratojugal.

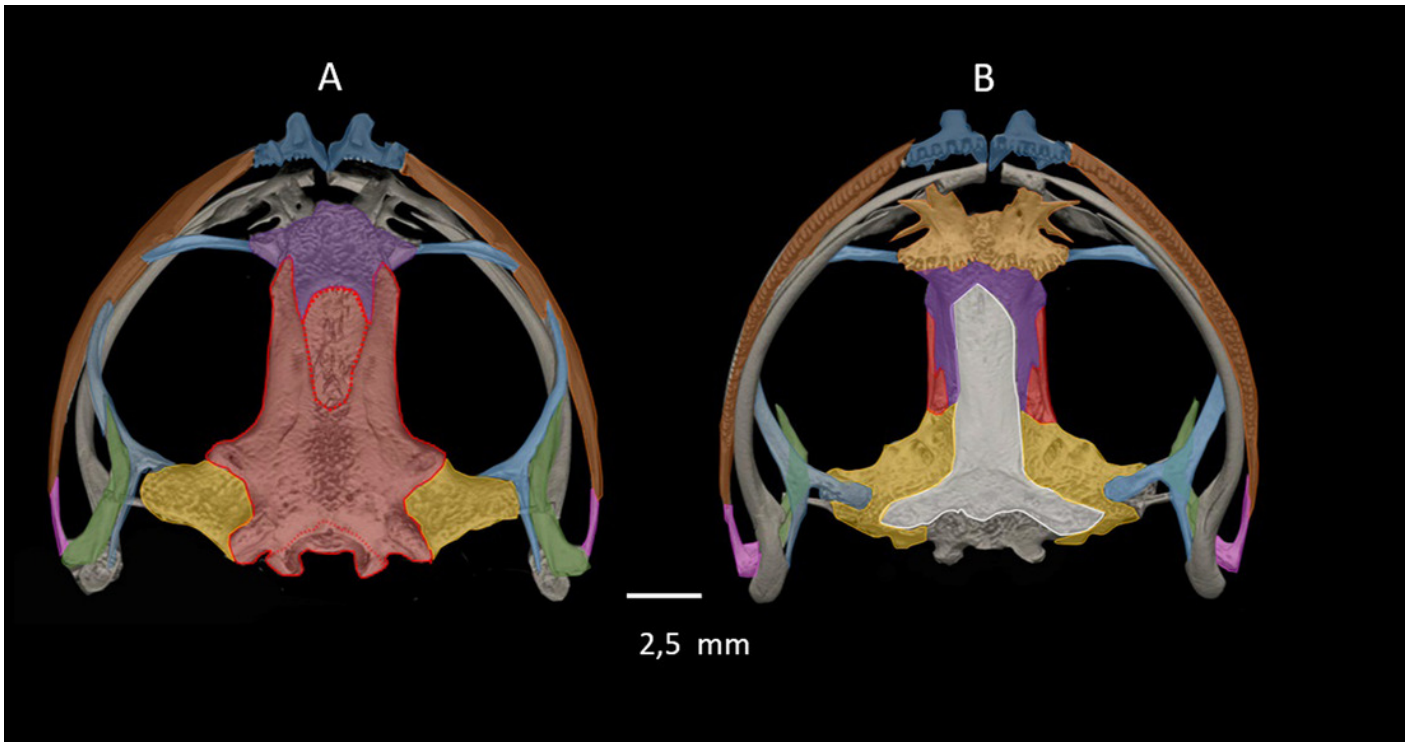

Figure 11

CT scan of the forelimb bones of the holotype of *Hyloscirtus sethmacfarlanei sp. nov.* DHMECN 14416

From the top to the bottom: Light blue: distal clawed phalanges; dark blue: medial phalanges; red: proximal phalanges; Light green: prepolex; Purple:distal carpal II; Pink: fused distal Carpal 3+4+5; Orange: Radial; Yellow: Ulnar; Dark green: Radioulna; brown; humerus.

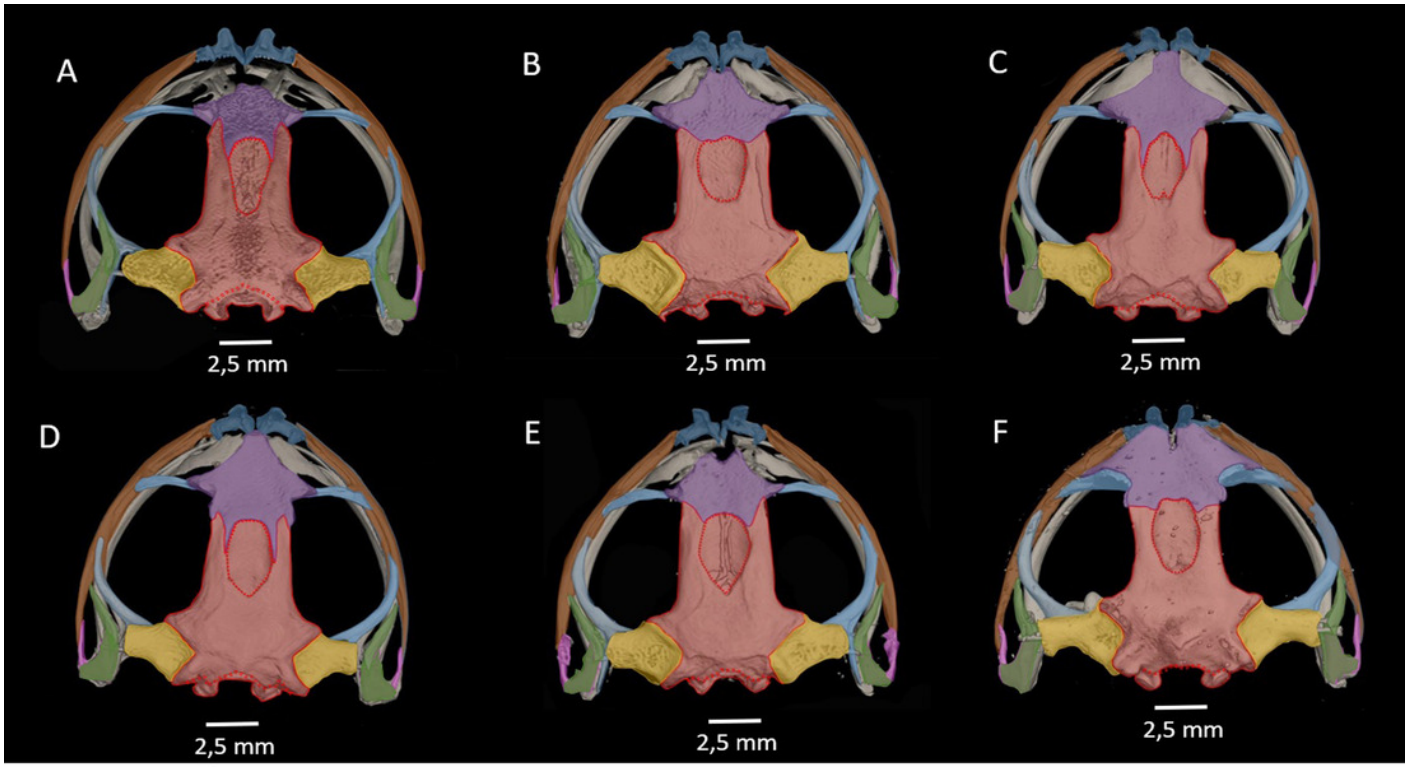

Figure 12

CT scan of the forelimb bones of the holotype of *Hyloscirtus sethmacfarlanei sp. nov.* DHMECN 14416

From the top to the bottom: Light blue: distal clawed phalanges; dark blue: medial phalanges; red: proximal phalanges; Light green: prepolex; Purple:distal carpal II; Pink: fused distal Carpal 3+4+5; Orange: Radial; Yellow: Ulnar; Dark green: Radioulna; brown; humerus.

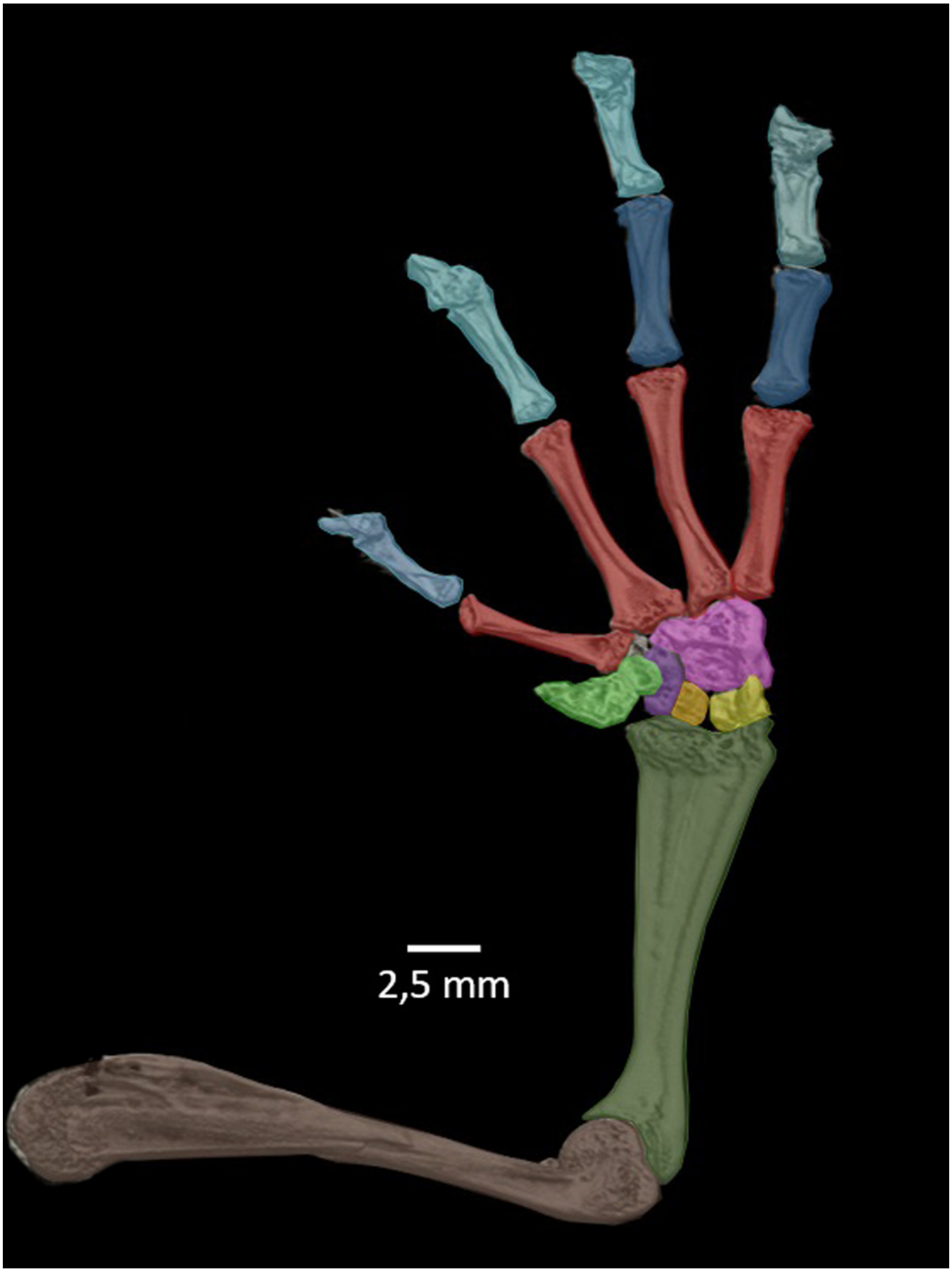
2,5 mm

Figure 13

Comparison of the CT scans of the posteromedials of the hyobranchium in different species of the *H. larinopygion* group.

A) *Hyloscirtus sethmacfarlanei* holotype (DHMECN 14416); B) *H. larinopygion*, (DHMECN 3799); C) *H. lindae* (DHMECN 12483); D) *H. psarolaimus* (DHMECN 6493); E) H. pacha (DHMECN 12111); F) *H. tapichalaca* (DHMECN 9686).


A
B
C
D
E
F
5 mm

Figure 14

Maps of northwestern South America showing the ecological niche modeling for all species of the northern clade of Hyloscirtus larinopygion species group

(yellow to red shadows). Both maps show the same ecological niche model but species were divided into two maps to allow locality points for known all species of Hyloscirtus larinopygion species group to be included. Type locality of H. sethmacfarlanei sp. nov. indicated by a white triangle in left map.

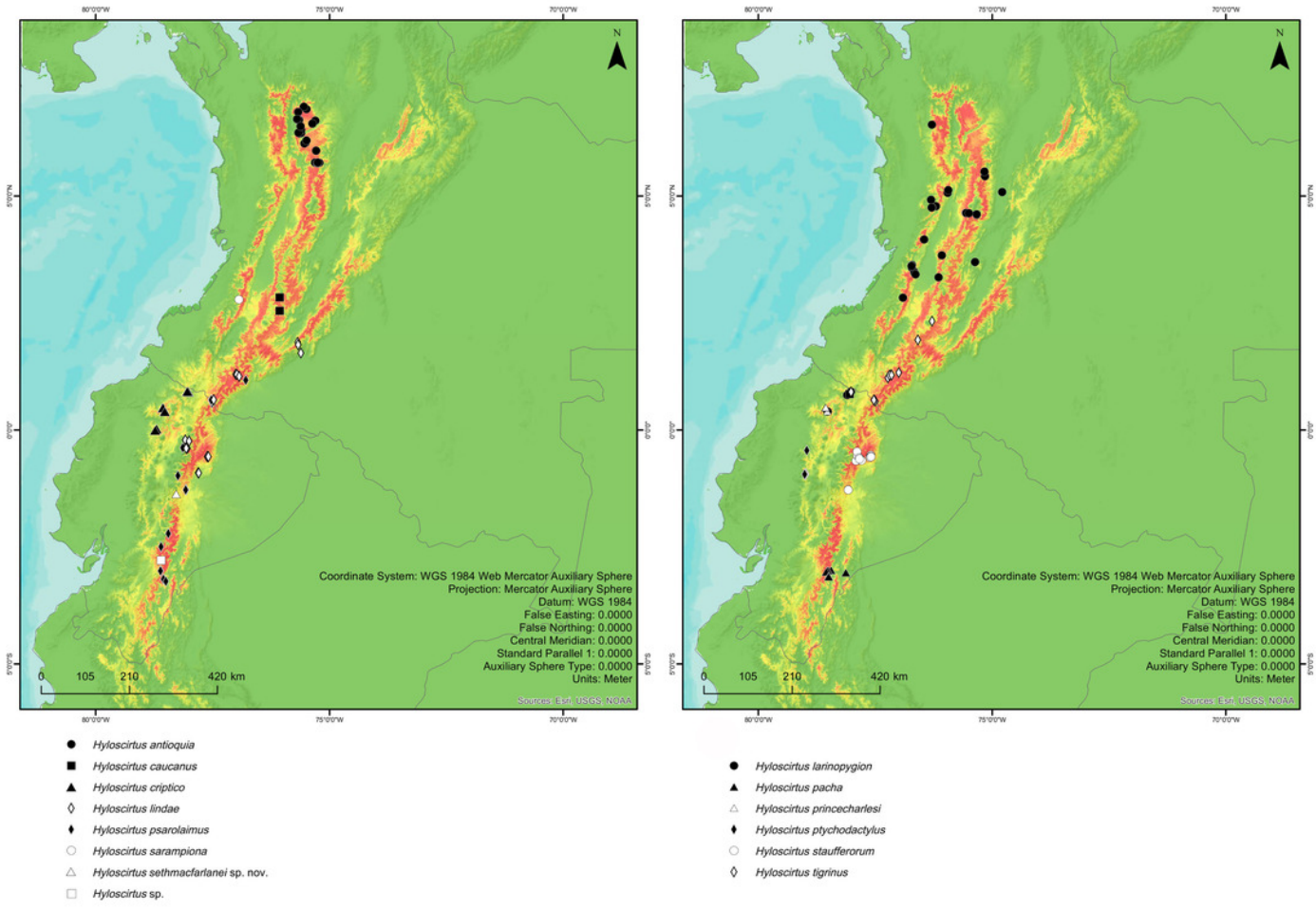

Figure 15

Habitat and *Hyloscirtus sethmacfarlanei* at the collection site

(A) humid cloud forest at type locality. (B) Holotype female(DHMECN 14416). (C) Paratype juvenile (DHMECN 14549) . (C) uncollected juvenile. Photographs: Fausto Recalde, JPRP

Table 1(on next page)

Genetic distances (mitochondrial 16S) between *Hyloscirtus sethmacfarlanei sp. nov*. and its most closely related congeners.

Values are presented as percent distances calculated from uncorrected *p* values.

1

	H. condor (n = 2)	*H. hillisi* (n = 5)	*H. pacha* (n = 1)	*H. larinopygion* (n = 2)	*H. lindae* (n = 4)	*H. pantostictus* (n = 3)	*H. tapichalaca* (n = 2)	*H. ptychodactylus* (n = 2)	*H. princecharlesi* (n = 2)	*H. psarolaimus* (n = 2)	*H. tigrinus* (n = 2)	*H. staufferorum* (n = 2)	*H. sethmacfarlanei* sp nov (n = 2)
H. condor	0.0												
H. hillisi	3.8–4.1	0.0–0.1											
H. pacha	10.2–10.5	11.3–11.4	0.0										
H. larinopygion	9.6–9.8	10.2–10.4	4.6	0.0									
H. lindae	10.1–10.8	11.0–11.7	4.6–5.3	2.6–3.1	0.0–0.5								
H. pantostictus	10.4–10.8	11.1–11.5	4.7–4.8	2.7–2.8	6.0–14.0	0.0–0.1							
H. tapichalaca	3.7–4.0	2.7–3.1	11.0–11.1	10.1–10.4	10.9–11.4	11.1–11.4	0.6						
H. ptychodactylus	9.5–9.6	10.2–10.5	3.8	2.9–3.0	2.9–3.6	3.1–3.2	10.1–10.2	0.0					
H. princecharlesi	9.7–9.9	10.8–11.0	4.3	3.2	3.3–3.6	3.4–3.6	10.6–10.7	1.3	0.0				
H. psarolaimus	10.8–11.1	11.3–11.7	4.2–4.5	4.8–5.0	4.5–5.4	4.8–5.2	11.2–11.4	3.6–3.8	3.8–4.1	0.3			
H. tigrinus	9.8–10.1	10.5–10.9	3.1–3.2	3.9–4.0	3.9–4.6	4.0–4.2	10.5–10.8	2.6	2.8–2.9	3.0–3.3	0.0		
H. staufferorum	9.6–10.1	10.6–11.1	1.8	4.2–4.4	4.0–4.7	4.1–4.3	10.6–10.8	2.8–2.9	3.3–3.4	3.4–3.7	2.7–2.8	0.0	
H. sethmacfarlanei sp nov	9.3–9.5	9.2–9.6	3.7–3.9	2.9–3.5	3.7–4.6	3.5–3.7	9.4–9.9	2.6–2.8	3.1–3.3	3.1–3.3	2.2–2.9	3.5–4.0	0.4

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Table 2(on next page)

Cloacal ornamentation and presence of calcar on the heel in Ecuadorian species of the *H. larynopigyon* group.

Museum Number	Species	Supracloacal fold	Vent-supracloacal fold relation	Paracloacal fold	Vent texture	Calcar on the heel
DHMECN 14416	*H. sethmacfarlanei*	well-defined	reaching the vent	thick, well defined	strong, areolate and granular	present
DHMECN 12483	*H. lindae*	thick, we---developed, tongue-like shape	reaching the border of the vent	weakly defined	densely tuberculate	absent
DHMECN 12113	*H. pacha*	thick	separated from the vent	Thick, well-defined	smooth	present
DHMECN 3799	*H. larinopgygion*	well-defined	separated from the vent	absent	smooth	present
DHMECN 6493	*H.psarolaimus*	weakly defined	separated from the vent	absent	little tubercles	absent
DHMECN MYM, 4590	*H. criptico*	well-defined	separated from the vent	weakly defined	strongly granular	absent
KU 217695	*H.staufferorum*	weakly defined	separated from the vent	absent	smooth	absent
KU190000	*H. pantostictus*	well-defined	reaching the border of the vent	absent	densely tuberculate	absent
QCAZ 44893	*H.princecharlesi*	defined	reaching the border of the vent	Present, thick	smooth	absent
DHMECN 9686	*H. tigrinus*	thick	separated from the vent	present	smooth with granular tubercles	present

KU 209780	*H. ptychodactylus*	well-defined	reaching the border of the vent	present	smooth	present
QCAZ 65235	*H. tapichalaca*	well-defined	reaching the border of the vent	present, thick	smooth	absent
QCAZ 68646	*H.condor*	thick	reaching the border of the vent	absent	smooth	present
QCAZA40331	*H. hillisi*	well-defined	separated from the vent	Present, thin	areolate	absent

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