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A new species of Scinax (Anura: Hylidae) from the Ecuadorian Amazon

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A la Ciencia

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A new species of *Scinax* (Anura: Hylidae) from the Ecuadorian Amazon

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Abstract

We describe a new species of frog of the genus *Scinax* (Hylidae) from the Ecuadorian Amazon, provincia de Orellana. The new species is the sister species of *Scinax chiquitanus* and is part of the *Scinax funereus* species group. *Scinax wekemo* sp. nov. is characterized by its yellow, beige, or dark brown dorsum with scattered dark brown spots and a characteristic golden iris. The new species differs from *Scinax chiquitanus* in texture of the dorsum: shagreen in *Scinax wekemo* sp. nov. vs. smooth in *S. chiquitanus* and by the presence of spots on the dorsum (absent in *S. chiquitanus*).

Keywords: Amazon, Ecuador, Diversity, Scinax, Phylogeny, *Scinax wekemo.*

Introduction

Currently the anuran family Hylidae is considered the richest in diversity of arboreal anurans species with 1036 species, divided into 3 subfamilies Hylinae (747 sp.), Pelodryadinae (222 sp.), Phyllomedusinae (67 sp.) (Frost 2022). The genus *Scinax*, part of the subfamily Hylinae, is a group of neotropical frogs belonging to the tribe Scinaxinae (Duellman, Marion & Hedges 2016). Currently 129 species are known (Frost 2021) of which only seven species occur in Ecuador (Ron, Merino & Ortiz 2021). *Scinax* is the most species-rich genus of Neotropical treefrogs (Novaes et al. 2021). The genus *Scinax* is distributed from southeastern Mexico to Argentina (Faivovich 2002), with its highest species richness in Amazonian forests in Brazil (Ferrão et al. 2017).

The systematics of the genus *Scinax* has changed several times since it was described in the XIX century (Wagler 1830). Species of the genus *Scinax* are distinguished from other hylids mainly by their depressed head and acuminate or subacuminate snout (Acosta-Galvis 2018). It is estimated that a high percentage of *Scinax* species in the tropical region remain undescribed (Ferrão et al. 2016). Araujo-Vieira et al. (submitted) estimate that there are 57 undescribed species belonging to this genus.

Araujo-Vieira et al. (submitted) partitioned *Scinax* into three genera (*Scinax, Julianus* & *Ololygon*). The genus *Scinax* is composed by the majority species of the former clade *S. ruber* is divided into 13 groups: *S. auratus, S. boesemani, S. cruentomma, S. danae, S. elaeochroa, S. eurydice, S. funereus, S. fuscomarginatus, S. fuscovarius, S. granulatus, S. nasicus, S. rostratus and S. squalirostris.*

The *Scinax funereus* group does not have synapomorphies but can be characterized by SVL males 27.9−37.5 mm, SVL females 30.2−39.3 mm and the combination of a rounded snout in dorsal view, pectoral fold present, tubercles in the lower lip absent, lack of spicules in nuptial pads, epidermal projections in the nuptial pad are present, pectoral glands absent or present in males, single or weakly bilobate vocal sac, dorsal skin smooth, shagreen, or densely covered with tubercles, inguinal glands absent, iris orange, bronze, golden, or bicolor silvery gray and bronze, irregular spots or blotches on the dorsum, posterior margin of the folded oral disc straight , oral disc subterminal or ventral, horizontal or irregular spots on hidden surfaces of the uniform black to light brown thighs,

physiological chlorosis absent or present, eyes of the tadpole visible or not visible ventrally, keratinized dark-colored plates on the sides of the lower jaw-sheath absent, vent tube above the margin of the ventral fin, oviposition in ponds, advertisement call with 1 note/call, 16–42 pulses/note and note duration 70–338 ms (Araujo Viera et al. submitted).

In this article we describe a new species of *Scinax* of the *S. funereus* group discovered in 2009 during amphibian surveys along the Napo River in Ecuador carried out by researchers from Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Materials and methods

Study site

Four samplings areas in the NE of the country in the Amazon region of Ecuador near the border with Peru. All sampling localities were close to the Napo River in the province of Orellana. (Fig.1).

Sample preparation

All specimens were euthanized with a topic solution of 20% benzocaine, fixed in 10% formaldehyde solution, then placed in water for 1 to 4 hours and preserved in 75% ethanol. Tissue samples were obtained from adult specimens before fixation and stored in 96% ethanol at laboratories of the Museum of Zoology, Pontificia Universidad Católica del Ecuador. Measurements were taken with a digital calliper. Sex and reproductive condition of specimens were determined by observing secondary sexual characters as nuptial pads & vocal slits, the absence of these characters were used as an indicator of females.

Morphological analysis

For the morphological analysis, qualitative characters used in the taxonomy of *Scinax* were examined (Faivovich 2005). The characters were coloration of inguinal areas, lateral and dorsal shape of the snout, relative length of the fingers, presence of nuptial pads,

presence of interdigital membranes in fingers I-II and presence of spots in hidden areas of flanks and thighs. These characters have been used in different publications of morphological descriptions in species of the genus *Scinax* (Lourenço et al. 2020).

Eight measurements (in mm) were taken using a digital caliper following Duellman (1970): (1) Snout Vent Length (SVL), (2) Foot Length (FL), (3) Head Width (HW), (4) Eye Diameter (ED), (5) Tympanum Diameter (TD), (6) Tibia Length (TL), (7) Femur Length (FemurL), and (8) Disc width of the finger III (DHIII). Color images of specimens were used for the description of coloration in life. All collected specimens were deposited in the Museum of Zoology, Pontificia Universidad Católica del Ecuador.

DNA Extraction and sequencing

DNA samples were extract from liver or muscle tissue from 13 individuals of the new species and preserved in 95% ethanol, the tissue was obtained from the genome bank of the Zoology Museum, Pontificia Universidad Católica del Ecuador (QCAZ). The PCR amplification was performed under standard protocols and amplicons were sequenced by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea). We used DNA sequences of the nuclear genes of the new species Tyrosinase and POMC and sequences of the mitochondrial genes ND1, 12S rRNA, and 16S rRNA.

We combined our sequences with those analyzed by Araujo et al. (submitted). The alignment of the sequences was performed with the extension MAFFT version 7 Katoh and Standley (2013) in program Geneious Pro 9.1.8 (Kearse et al. 2012). Our final matrix consisted of 79 terminals for six nuclear genes: RAG1(428 bp), TYR (532 bp), CXCR4 (675 bp), POMC (502 bp), RHOD (316 bp) and SIA (532 bp). We also analyzed mitochondrial genes $tRNA^{Leu}$, NADH1 (1093 bp), Cytb (385 bp), COI (646 bp), 12S and 16S rRNA (2887 bp).

Phylogenetic analyses

Maximum likelihood (ML) was used as the optimality criterion for the phylogenetic search. We partitioned the data by codon position and by gene to obtain the best

evolutionary model for each partition, using IQtree version 2.2 (Nguyen et al. 2015) with the -m MPF command (Chernomor et al. 2016, Kalyaanamoorthy et al. 2017). To assess branch support, we used 100 non-parametric bootstrap searches (-b 100 command) and 1000 replicates for the SH-like approximate likelihood ratio test (-alrt 1000 command; Guindon et al. 2010). We considered that branches with bootstrap values > 70 and SH-aLRT values > 80 had strong support.

Bioacoustic samples

Call recordings from three males (QCAZ 4644 and two males not collected) were recorded

from 4km NW of parroquia Nuevo Rocafuerte, 200 meters from the north bank of Río Napo, near a pond between bushes in a grassland area in a flooded forest of *Mauritia flexuosa.*

Recordings were made at night on 09 July 2009, between 21:00 and 22:00 h by SRR. The body temperature of male QCAZ 4644 was 21.8 °C. Calls were analyzed using Raven pro 1.6.3 software K. Lisa Yang Center for Conservation Bioacoustics (2022). The following call parameters were measured: number of analyzed calls, call duration, pulses per note, pulses per second, dominant frequency, and fundamental frequency. These measurements follow De la Riva, Marquez & Bosh (1994).

Results

Phylogeny and genetic variation

Our maximum likelihood phylogeny shows a strong support for a clade composed of *Scinax wekemo* sp.nov. and *S. chiquitanus*.

The average uncorrected p-genetic distances (gene 16S) between *Scinax wekemo* sp. nov. and its sister species, *S. chiquitanus*, is 5.1% (range 4.4 to 5.8%). These genetic distances suggest that *S. wekemo* sp. nov. is a species distinct from *S. chiquitanus* (Following the threshold value for uncorrected genetic distances proposed by Fouquet et al. (2007), of 3% for 16S gene).

Bioacoustic analyses

We analyzed 36 advertising calls with an average song duration of 0.27(0.19±0.34s) with an average rhythm of 96.57 (71.29±140.8) pulses per second, an average dominant frequency of 1907. 03 (2062± 1781 Hz), an average fundamental frequency of 1907.03 (2062± 1781 Hz), obtained from the first of two harmonics because it had more energy. The spectrogram and oscillogram data are in Fig.8.

In Table 2 we can observe similarities in call duration between *Scinax wekemo* sp.nov. and its sister *S. chiquitanus. Scinax wekemo* sp.nov. had lower values in dominant and fundamental frequency compared to *S. chiquitanus.* De la Riva, Marquez & Bosh (1994) describe the dominant and fundamental frequencies of *S. chiquitanus* with an average of 2172.4 Hz.

We found differences between the songs of both species considering the values of pulses per second, with a significant difference between *Scinax wekemo* sp.nov. and *S. chiquitanus* (BOL) (96.57 vs 151.17 pulses per second). De la Riva, Marquez & Bosh (1994) describes *S. chiquitanus* with an average of 122.1 pulses per second. The values of *Scinax wekemo* sp.nov. and *S. chiquitanus* (PER) were more similar (96.57 vs. 109.69 pulses per second), Also, De la Riva (2000) suggests that the populations *of S. chiquitanus* (PER) may not be conspecific to the Bolivian populations due to the presence of differences in the songs and coloration of males. *S. chiquitanus* (BRA) are described as similar to those of *S. chiquitanus* (BOL) (Ferrão et al. 2016).

Systematic account

***Scinax wekemo* sp. nov.**

Holotype

QCAZ 43681(Fig. 2), adult female, from provincia de Orellana, Puerto Francisco de Orellana (El Coca), on the north bank of Río Napo (0.477820 °S, 76.98981 °W, 267m a.s.l, Fig. 1) collected at 16:00 on 26 June 2009, by Santiago Ron, Eduardo Toral and Italo Tapia.

Paratypes

All specimens collected in Ecuador in provincia de Orellana near the Napo River. One female (QCAZ 43685) and three males (QCAZ 43695, 43700, 43702) collected at the same locality of the holotype (0.477820 °S, 76.98981 °W, 267m a.s.l: Fig. 1); one male (QCAZ 43823) from cantón Orellana, parroquia Taracoa, El Descanso community, La primavera, on the south bank of Río Napo (0.44427°S, 76. 78685 °W, 244m a.s.l: Fig. 1) collected on 29 June 2009, by Santiago Ron, Eduardo Toral and Italo Tapia; two males (QCAZ 44353,44381) from cantón Aguarico, parroquia Augusto Rivadeneira, in the community Chiru Isla on the south bank of Río Napo (0.57997°S, 75.91769 °W 207m a.s.l: Fig. 1) collected on 07 May 2009, by Santiago Ron, Eduardo Toral and Italo Tapia; four females (QCAZ 44746, 44749, 44755, 44756) and six males (QCAZ 44644, 44747, 44748, 44750, 44751, 44754) from cantón Aguarico 4km NW of parroquia Nuevo Rocafuerte in the community Santa Teresita on the north bank of Río Napo (0.90087°S, 75.41357 °W, 186m a.s.l: Fig. 1) collected on 07 September 2009 by Santiago Ron, Eduardo Toral and Italo Tapia.

Generic placement

The new species is assigned to the genus *Scinax* based on the phylogeny and the condition of reduced webbing between toes I and II, a morphological synapomorphy of *Scinax*.

The new species is assigned to the *Scinax funereus* group based on the phylogeny and by having a bluntly rounded snout in dorsal view, single vocal sac, absence of spicules on nuptial pads, dorsal pattern with dark spots or blotches, and hidden surfaces of thighs dark with irregular blotches.

Diagnosis

In this section, coloration pertains to live specimens unless otherwise noticed. The new species is characterized by: (1) SVL 28.8–33.6mm (n = 12) in males and SVL 28.2–38.5mm (n = 6) in females; (2) Bluntly rounded snout in dorsal view and in profile; skin on dorsum shagreen; (3) canthus rostralis slightly concave; (4) dorsum brown to beige with scattered dark brown spots, larger brown blotches near the base of forelimbs and hindlimbs; (5) poorly developed nuptial pads (see Fig. 3); (6) tubercles absent on lower jaw; (7) rounded tongue; (8) simple external vocal sac; (9) supernumerary tubercles on hands and feet (see Fig. 3); (10) length of toes from smallest to largest 1-2-3-5-4; (11) enlarged heel tubercles absent; (12) ulnar and tarsal tubercles absent; (13) creamy flanks, usually with a dark brown spots; (14) belly and throat cream to greenish yellow with brown spots on the edges; (15) reddish golden iris with irregular dark reticulation; (16) no sexual dimorphism except for size, females are larger than males (Table 1).

Comparisons

We compare the new species to other species of the *Scinax funereus* group. Morphologically, *Scinax wekemo* sp. nov. can be distinguished from other species by having reddish golden iris and by the following combination of characters (characters of other species in parentheses). The new species is most similar to *Scinax chiquitanus*

(Fig.4). *Scinax wekemo* sp. nov. differs by a reddish golden iris (light or pale golden; Duellman and Wiens 1993). The calls from *Scinax wekemo* sp. nov. (Fig. 8) differ from those of *S. chiquitanus* in pulse rate 71.29 pulses/s ±140.8 (107.8 ± 189.8), De la Riva, Marquez & Bosh (1994) describe *Scinax chiquitanus* with an average of 122.1 pulses per second (Table 2).

The new species differs from *S. iquitorum* by shagreen skin (smooth or slightly to coarsely shagreen), brown or beige dorsum coloration (light olive green to brown), reddish golden iris (gold to bronze with irregular dark reticulation), presence or not of brown spots in hidden areas of flanks (black spots present, becoming gradually larger from axillae to groin; Moravec et al. 2009).

Scinax wekemo sp. nov. can be distinguished from *S. oreites* by having a bluntly rounded snout in dorsal view (acutely rounded), internal and external metatarsal tubercle (absent), shagreen skin (smooth), golden iris (bronze), cream or brown thighs with or without brown spots (brown with unpigmented spots), brown spots or stripes on dorsum (usually absent), absence of dorsolateral stripes extending from eye to groin (present and creamy white; Duellman and Wiens 1993).

Scinax wekemo sp.nov. differs from *S. onca* by having a bluntly rounded snout in dorsal view (truncated), anterior and posterior surfaces of thighs yellow to brown (black), pink or cream groin without spots (white with black irregular spots), cream to greenish yellow belly without spots (white to yellow with rounded dark spots; Ferrão et al. 2017).

Scinax wekemo sp. nov. differs from *S. ictericus* by having brown to beige dorsum (green to yellowish) and ulnar and tarsal tubercles absent (present; Duellman and Wiens 1993).

The new species differs from *S. funereus* by having a bluntly rounded snout in dorsal view (acutely rounded; Duellman 1978), shagreen skin (tuberculated, can be finely shagreened in some specimens), brown or beige dorsum coloration (pale green or greenish brown), golden iris (greenish bronze), enlarged tubercle on the heel absent (present), ulnar and tarsal tubercles absent (present; Duellman and Wiens 1993).

Description of Holotype

Adult female 33.52 mm SVL. Head wider than body, slightly slender than long (HL/HW = 1.17, HL = 31.56% of SVL, HW = 26.9% of SVL); bluntly rounded snout in dorsal and lateral view; nostrils marked; eye-nostril distance larger than ED; eyelid width 91.3% of interorbital width; canthus rostralis slightly concave; loreal region slightly concave; tympanic membrane not evident; tympanic length 55% of ED; rounded tongue.

Forearm slightly wider than arm, axillary membrane absent; pectoral fold present; length of hand 27.3% of SVL; slender fingers with horizontally expanded disks; diameter of finger III of disk 44% of ED; length of fingers from smallest to largest 1-2-4-3; bifid palmar tubercle, longer than wide; thenar tubercle elongate; distal subarticular tubercles on finger 1-2-3-4; supernumerary tubercles on fingers 1-2-4.

Tibia longer than femur, Tibia length = 52.5% of SVL, Femur length = 43.5% of SVL; Tarsus length = 29% of SVL; Foot length = 41. 82% of SVL; length of toes from smallest to largest 1-2-3-5-4; oval-shaped internal metatarsal; small and round external metatarsal tubercle; basal subarticular tubercles on toes 1-2-3-4-5; penultimate subarticular tubercles on toes 3-4-5; distal subarticular tubercles on toe 4; membranes present on all toes; heel tubercles absent.

Shagreen dorsum skin, areolate skin on throat and belly; smooth skin on forelimbs and hindlimbs.

Measurements of the Holotype

Snout Vent Length 33.52 mm, Foot Length 14.02 mm, Head Length 10.58 mm, Head Width 9.02 mm, Eye Diameter 3.79 mm, Tympanum Diameter 2.09 mm, Tibia Length 17.61 mm, Femur Length 14.61mm.

Coloration of holotype in life

Coloration of body, head, limbs and dorsum reddish brown; a red stripe on dorsolateral region between eyes and nostrils; small brown stripe extending from corner of

eye to the beginning of forelimb in lateral dorsal view; interorbital region with few brown spots; canthus rostralis with few small brown spots; numerous brown spots distributed over the entire dorsum; greenish yellow belly without spots; pink groin without spots; cream thighs without spots; yellow flanks with greenish spots; scattered brown spots on forearms; small brown spots on thighs and hindlimbs; yellow toes and fingers disks in dorsal view; cream toes and fingers disks in ventral view; yellow interdigital membranes with gray spots; pink throat with few unpigmented spots on edges; yellow anterior surfaces of thighs with pink spots; pink posterior surfaces of thighs without spots; greenish yellow eyelid; yellow tympanum with red borders; golden iris with black reticulation and black border extending to the middle of the eye.

Coloration of holotype in alcohol

Light brown dorsal surfaces of body, head and limbs; cream throat, belly and groin; small brown spots distributed throughout dorsum, head and limbs, as in life; black eyelids, see fig 5 & 6.

Variation

Measurements of 18 specimens (12 males and 6 females) are presented in Table 1. Females are larger than males, male size represents 91% of females SVL size. Variation of color in life and preservative is shown in Figs.5, 6 and 7.

Etymology

The term wekemo in the name refers to the word frog in the Wao language, a language spoken in Waorani and Kichwa communities near the locality where the holotype of the species was collected, it has been decided to use this term in honor of the native communities of areas near Río Napo (Trujillo 2011).

Ecology and distribution

Scinax wekemo sp.nov. was found artificial open areas near the Napo River. It is active by night or evenings and males were calling between bushes near a pond in a grassland area.

It is known from four localities along the Napo River in the Ecuadorian Amazon (Fig.1). Habitat type is Amazonian Tropical Rainforest (as defined by Ron et al. 2021).

Two individuals found in amplexus at 20:00 near the Napo River in cantón Aguarico 4km NW of parroquia Nuevo Rocafuerte in the community Santa Teresita on the north bank of Río Napo.

Conservation status

We know that this species is found in at least four localities from the Ecuadorian Amazon, provincia de Orellana, however, we do not have updated data on the growth or decline of populations of *Scinax wekemo* sp.nov. so it cannot be possible for us to give a status of conservation for this species.

Discussion

Scinax wekemo sp.nov. currently only has reports from Ecuador; however, we consider that it may be present in Peru because of the proximity of the localities where the specimens were found to the border with this country.

Analyses of frog advertisement calls were considered in order to give a clear differentiation between the new species *Scinax wekemo* sp. nov. and its sister *Scinax chiquitanus*. These analyses were used because they serve as unique characteristics of each species to study communication behaviors between individuals of the same species (Cocroft & Ryan 1995). In frogs, males use their vocal sacs to produce courtship calls that can be identified by females of the same species (Gerhardt & Huber 2002).

Our analyses helped us to clarify certain differences between calls of *Scinax wekemo* sp.nov. and songs of two populations of *Scinax chiquitanus*.

In conclusion, we can determine based on bioacoustics and phylogenetic analyses that *Scinax wekemo* sp.nov. is a species that has not been described before, belonging to the *Scinax funereus group* and related to *Scinax chiquitanus*. Our description allows us to differentiate *Scinax wekemo* sp.nov. from other species of the genus *Scinax* by morphological and acoustic characters.

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Literature Cited

Acosta-Galvis AR (2018) Una nueva rana de huesos verdes del género *Scinax* (Anura: Hylidae) asociada a los bosques subandinos de la cuenca del río Magdalena, Colombia. Biota Colombiana, 19(s1), 129–157. https://doi.org/10.21068/c2018.v19s1a11

Araujo-Vieira K, Lourenço AC, Lacerda JV, Lyra ML, Blotto BL, Ron SR, Baldo D, Pereyra MO, Suárez-Mayorga A, Baêta D, Ferreira RB, Barrio-Amorós CL, Borteiro C, Brandão RA, Brasileiro CA, Donelly MA, Dubeux MJ, Köhler J, Kolenc F, Fortes Leite FS, Maciel NM, Nunes I, Orrico VG, Peloso P, Pezzuti TL, Reichle S, Rojas-Runjaic FJ, Da Silva HR, Sturaro M, Langone JA, Garcia P, Trefaut-Rodrigues M, Frost DR, Wheeler WC, Grant T, Pombal Jr JP, Haddad C, Faivovich J (submitted) Treefrog Diversity in the Neotropics: Phylogenetic Relationships of Scinaxini (Anura: Hylidae: Hylinae), South American Journal of Herpetology.

Baldo D, Araujo-Vieira K, Cardozo D, Borteiro C, Leal F, et al. (2019) A review of the elusive bicolored iris Snouted Treefrogs (Anura: Hylidae:*Scinax uruguayus* group). PLOS ONE 14(9): e0222131. https://doi.org/10.1371/journal.pone.0222131

Chernomor O, von Haeseler A, Minh BQ (2016) Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. Systematic Biology 65: 997–1008. doi:10.1093/sysbio/syw037

Cocroft RB, Ryan MJ (1995) Patterns of advertisement call evolution in toads and chorus frogs. Animal Behaviour, 49, 283–303.

De la Riva I (1990) Una especie nueva de *Ololygon* (Anura: Hylidae) procedente de Bolivia. Revista Española de Herpetología 4: 81–86.

De la Riva I, J. Köhler, S. Lötters and Reichle S (2000) Ten years of research on Bolivian amphibians: updated checklist, distribution, taxonomic problems, literature and iconography. Revista Española de Herpetología 14: 19–164

De la Riva I, R. Marquez, and Bosch J (1994) Advertisement calls of Bolivian species of *Scinax* (Amphibia, Anura, Hylidae). Bijdragen tot de Dierkunde 64: 75–85.

Duellman WE (1978) The biology of an equatorial herpetofauna in Amazonian Ecuador. Miscellaneous Publications of the University of Kansas Museum of Natural History 65:1–352

Duellman WE (1970) The hylid frogs of Middle America 1. Monograph of the Museum of Natural History, The University of Kansas 1: 1−428. https://doi.org/10.5962/bhl.title.2835

Duellman WE, AB Marion, and Hedges SB (2016) Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). In Zootaxa (Vol. 4104, Issue 1). https://doi.org/10.11646/zootaxa.4104.1.1

Duellman WE, Wiens JJ (1993) Hylid frogs of the genus *Scinax* Wagler, 1830, in Amazonian Ecuador and Peru. Occasional Papers of the Museum of Natural History University of Kansas 153: 1–57.

Faivovich J (2002) A cladistic analysis of *Scinax* (Anura: Hylidae). Cladistics, 18(4), 367–393. https://doi.org/10.1111/j.1096-0031.2002.tb00157.x

Faivovich J, Haddad CFB, Garcia PCA, Frost DR, Campbell JA, Weeler WC (2005) Systematic review of the frog family Hylidae, with special reference to Hylinae: 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

Ferrão M, Colatreli O, De Fraga R, Kaefer IL, Moravec J & Lima A P (2016) High species richness of *Scinax* treefrogs (hylidae) in a threatened amazonian landscape revealed by an integrative approach. PLoS ONE, 11(11), 1–16. https://doi.org/10.1371/journal.pone.0165679

Ferrão M, Moravec J, Fraga R, Almeida AP, Kaefer IL, Lima AP (2017) A new species of *Scinax* from the Purus-Madeira interfluve, Brazilian Amazonia (Anura, Hylidae). ZooKeys 706: 137–162. https://doi.org/10.3897/zookeys.706.14691

Frost DR (2021) Amphibian Species of the World: An Online Reference. Version 6.1 (20 October 2022). Electronic Database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. doi.org/10.5531/db.vz.0001

Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans. Chicago: University of Chicago Press

Guindon S, Dufayard JF, 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: 307-321. doi:10.1093/sysbio/syq010

K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology (2022) Raven Pro: Interactive Sound Analysis Software (Version 1.6.3) [Computer software]. Ithaca, NY: The Cornell Lab of Ornithology. Available from https://ravensoundsoftware.com/.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14: 587–589

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution. 30, 772–780

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and

491 analysis of sequence data. Bioinformatics 28: 1647–1649.
492 doi:10.1093/bioinformatics/bts199
493
494 Lourenço ACC, Lacerda JVA, Cruz CAG, Nascimento L B, Pombal J P (2020) A new species
495 of the Scinax catharinae species group (Anura: Hylidae) from the Atlantic rainforest of
496 northeastern Minas Gerais, southeastern Brazil. Zootaxa, 4878(2), 305–321.
497 https://doi.org/10.11646/zootaxa.4878.2.5
498
499 Maddison WP, Maddison DR (2021) Mesquite: a modular system for evolutionary analysis.
500 Version 3.70. http://www.mesquiteproject.org.
501
502 Moravec J, Tuanama IA, Pérez PE, Lehr E (2009) A new species of Scinax (Anura: Hylidae)
503 from the area of Iquitos, Amazonian Peru. South American Journal of Herpetology 4: 9–16
504
505 Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective
506 stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular biology and
507 evolution, 32(1), 268-274
508
509 Novaes-E-Fagundes G, Araujo-Vieira K, Entiauspe-Neto OM, Roberto I J, Orrico VGD,
510 Solé M., Haddad CFB, Loebmann D (2021) A new species of *Scinax* Wagler (Hylidae:
511 Scinaxini) from the tropical forests of Northeastern Brazil. Zootaxa, 4903(1), 1–41.
512 https://doi.org/10.11646/zootaxa.4903.1
513
514 Ron SR, A Merino-Viteri, and Ortiz D (2021) Anfibios del Ecuador. Version 2021.0. (20
515 October 2021) Museo de Zoología, Pontificia Universidad Católica del Ecuador. <
516 https://bioweb.bio/faunaweb/amphibiaweb>
517
518 Trujillo P (2011) BOTO WAORANI, BITO COWURI La fascinante historia de los wao.
519 Fundacion FIAAM.
520

Wagler J. (1830). Natürliches System der Amphibien, mit vorangehender Classification der Säugthiere und Vogel. Ein Beitrag zur vergleichenden Zoologie. J.G. Cotta, München, Stuttgart and Tübingen. https://doi.org/10.5962/bhl.title.108661

552 **Table 1.** Measurements (in mm) of 18 individuals of *Scinax wekemo* sp.nov. 6 females and
553 12 males. See Materials and methods section for the abbreviations of measurements
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	Female (N = 6)	**Male (N = 12)**	**Overall (N = 18)**
SVL			
Mean (SD)	34.4 (3.49)	31.4 (1.32)	32.4 (2.63)
Median [Min, Max]	35.0 [28.2, 38.5]	31.8 [28.8, 33.6]	32.0 [28.2, 38.5]
FL			
Mean (SD)	13.8 (1.56)	12.7 (0.872)	13.1 (1.21)
Median [Min, Max]	13.9 [12.1, 16.5]	13.0 [10.9, 14.4]	13.1 [10.9, 16.5]
HW			
Mean (SD)	9.09 (0.907)	8.67 (0.546)	8.81 (0.689)
Median [Min, Max]	9.26 [7.40, 9.89]	8.66 [7.83, 10.1]	8.69 [7.40, 10.1]
ED			
Mean (SD)	3.92 (0.335)	3.65 (0.399)	3.74 (0.392)
Median [Min, Max]	3.81 [3.58, 4.43]	3.82 [2.95, 4.04]	3.82 [2.95, 4.43]
TD			
Mean (SD)	2.14 (0.178)	2.07 (0.344)	2.09 (0.295)
Median [Min, Max]	2.15 [1.85, 2.37]	1.98 [1.76, 2.71]	2.10 [1.76, 2.71]
TL			
Mean (SD)	18.0 (1.41)	16.3 (0.577)	16.9 (1.19)
Median [Min, Max]	18.3 [15.4, 19.6]	16.3 [15.5, 17.5]	16.4 [15.4, 19.6]
FemurL			
Mean (SD)	15.5 (1.78)	13.7 (0.700)	14.3 (1.40)
Median [Min, Max]	15.6 [12.9, 17.4]	13.8 [12.3, 14.7]	14.0 [12.3, 17.4]
DHIII			
Mean (SD)	1.66 (0.173)	1.54 (0.222)	1.58 (0.210)
Median [Min, Max]	1.67 [1.42, 1.90]	1.53 [1.04, 1.93]	1.59 [1.04, 1.93]

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560 **Table 2.** Comparison of bioacustic analysis of *Scinax wekemo* sp. nov. and *Scinax*
561 *chiquitnaus.*

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	Number of individuals	Calls analyzed	Calls duration (s)	Pulses per note	Pulses per second	Dominant frequency (Hz)	Fundamental frequency (Hz)
Scinax wekemo sp.nov.	3	36	0.27	26.25	96.57(71.29±140.8)	1907.03(1781.25±2062.5)	1907.03(1781.25±2062.5)
Scinax chiquitanus (BOL)	1	10	0.22	32.50	151.17(115±187)	2080.10 (2067.18±2153.32)	2080.10 (2067.18±2153.32)
Scinax chiquitanus (PER)	2	16	0.26	27.86	109.69(107.8±189.8)	1948.14(1894±92-2056.4)	1948.14(1894±92-2056.4)

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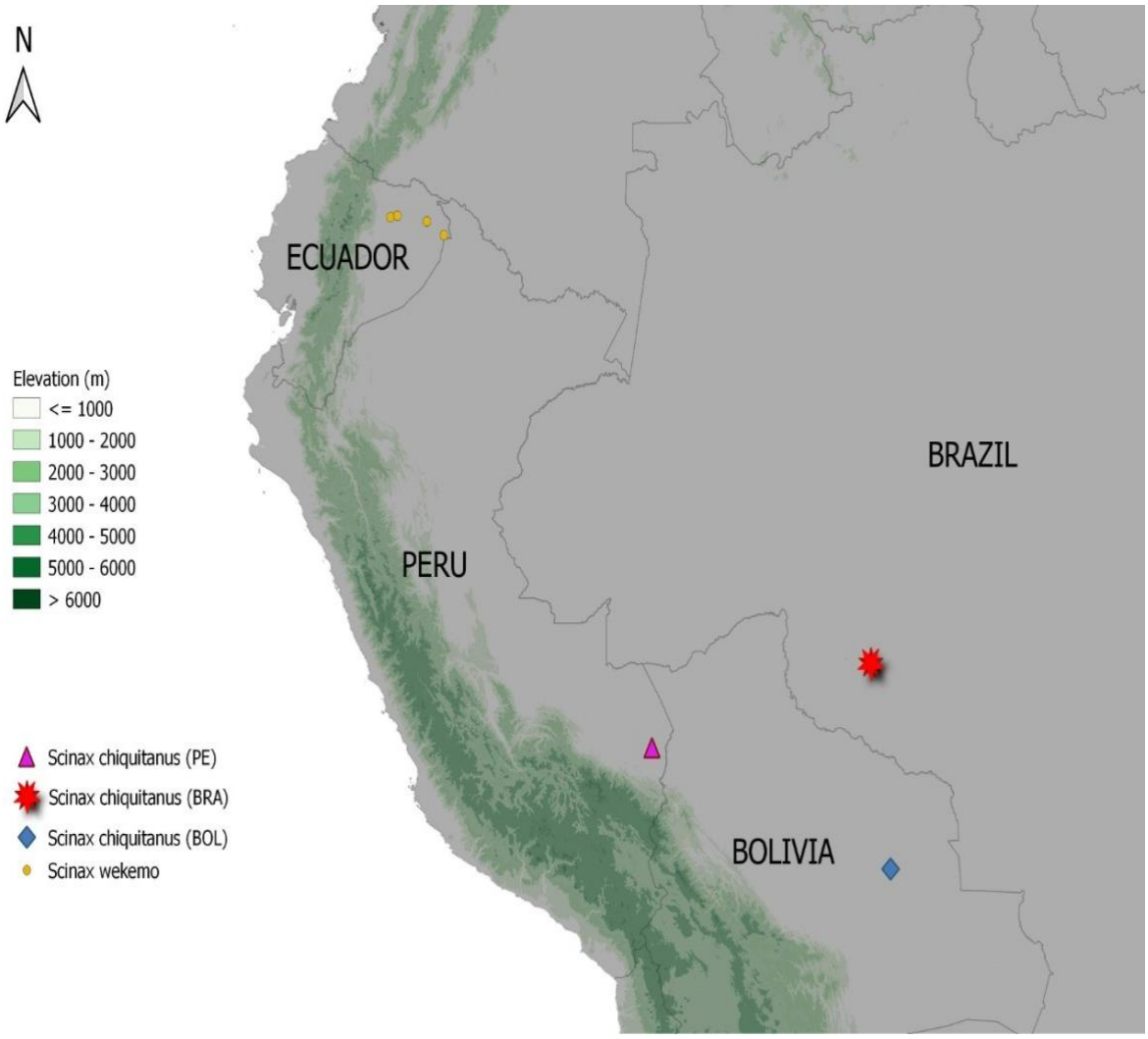

576

577 **Figure 1.** Geographic distribution of *Scinax wekemo* sp.nov. (From left to right) • Puerto
578 Francisco de Orellana (El Coca), (0.477820°S, 76.98981°W, 267m a.s.l), • El Descanso
579 community, (0.44427°S, 76. 78685 °W, 244m a.s.l), • Chiru Isla community (0.57997°S,
580 75.91769 °W, 207m a.s. l) • Santa Teresita community (0.90087°S, 75.41357 °W, 186m
581 a.s.l). *Scinax chiquitanus* (PE) • Puerto Maldonado, Reserva Tambopata (12.920555 °S,
582 69.281944 °W); *Scinax chiquitanus* (BOL) •Puerto Almacén, Río Negro, 70 Km NW of
583 Concepción, Departamento de Santa Cruz, Bolivia (15.766666 °S, 62.25 °W); *Scinax*
584 *chiquitanus* (BRA) • Rondonia (10.942777° S, 62.8275°W).

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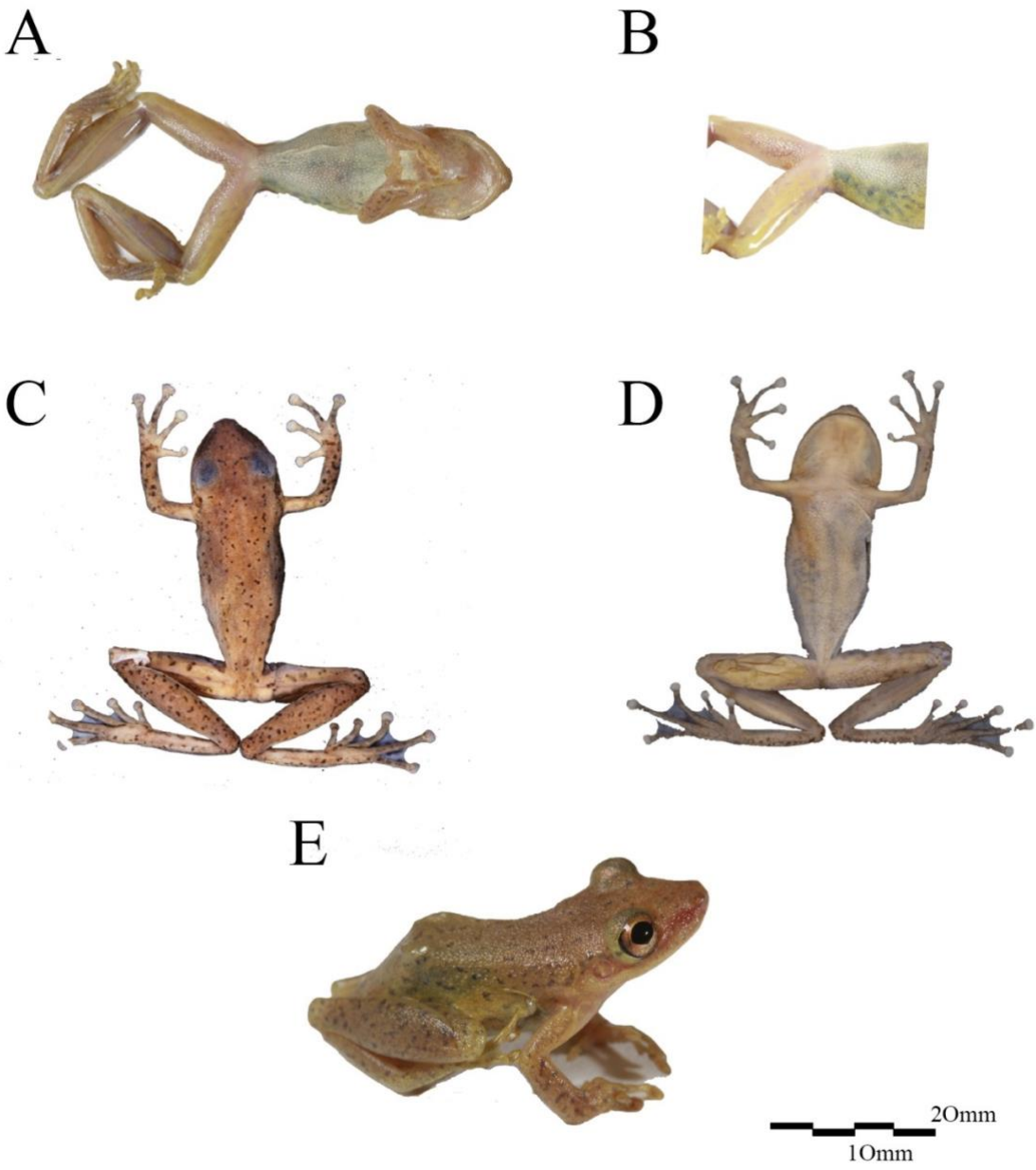

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587 **Figure 2.** Holotype of *Scinax wekemo* sp.nov. QCAZ 43681adult female, SVL = 33.53 mm
588 from provincia de Orellana, Puerto Francisco de Orellana (El Coca), on the north bank of Río
589 Napo (0.477820 °S, 76.98981 °W, 267m a.s.l) collected on 26 June 2009, by Santiago Ron,
590 Eduardo Toral, and Italo Tapia. **A** ventral view of the individual**, B** flanks of the individual**,**
591 **C** dorsal view of preserved individual**, D** ventral view of preserved individual, **E** a view of
592 life individual.

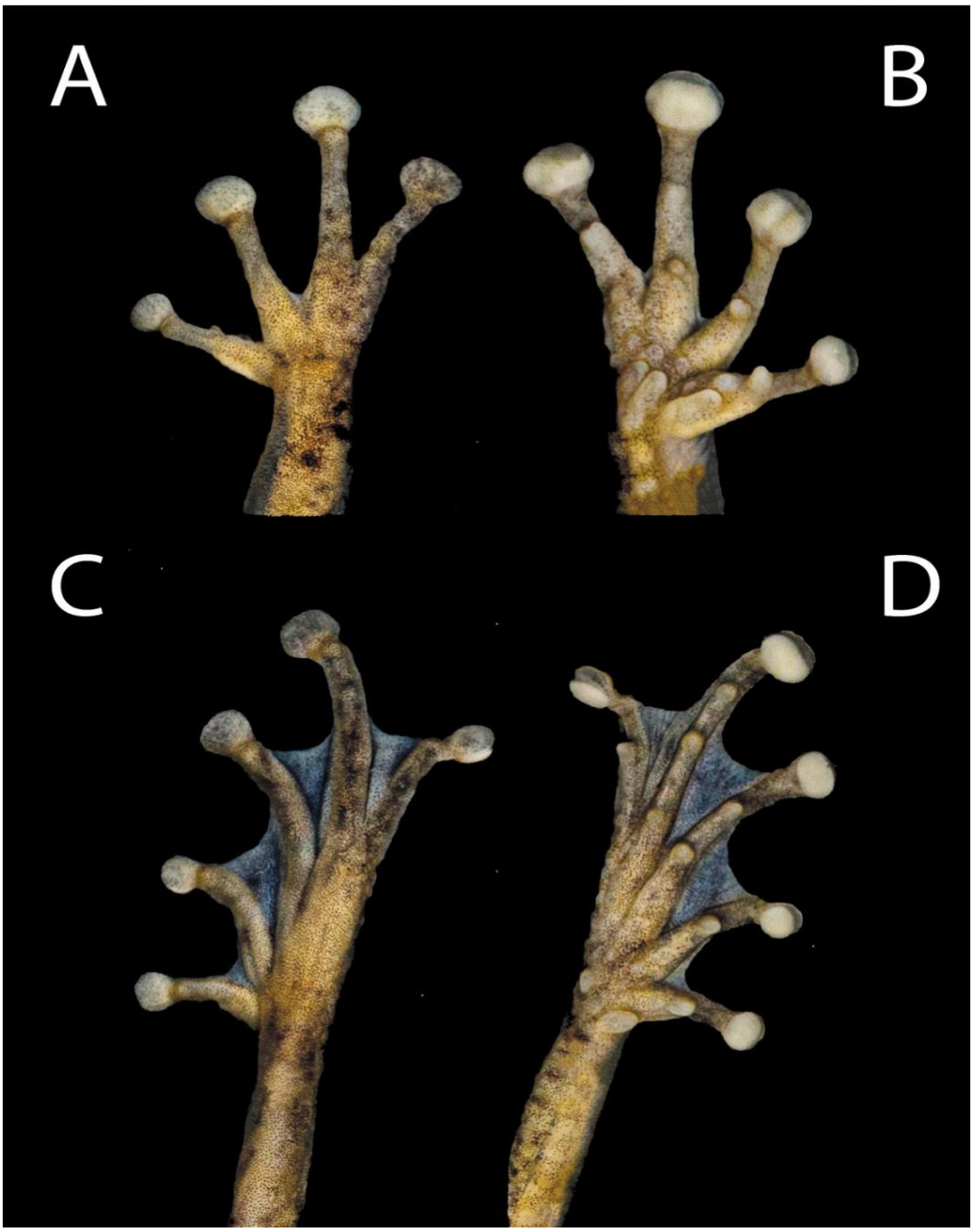
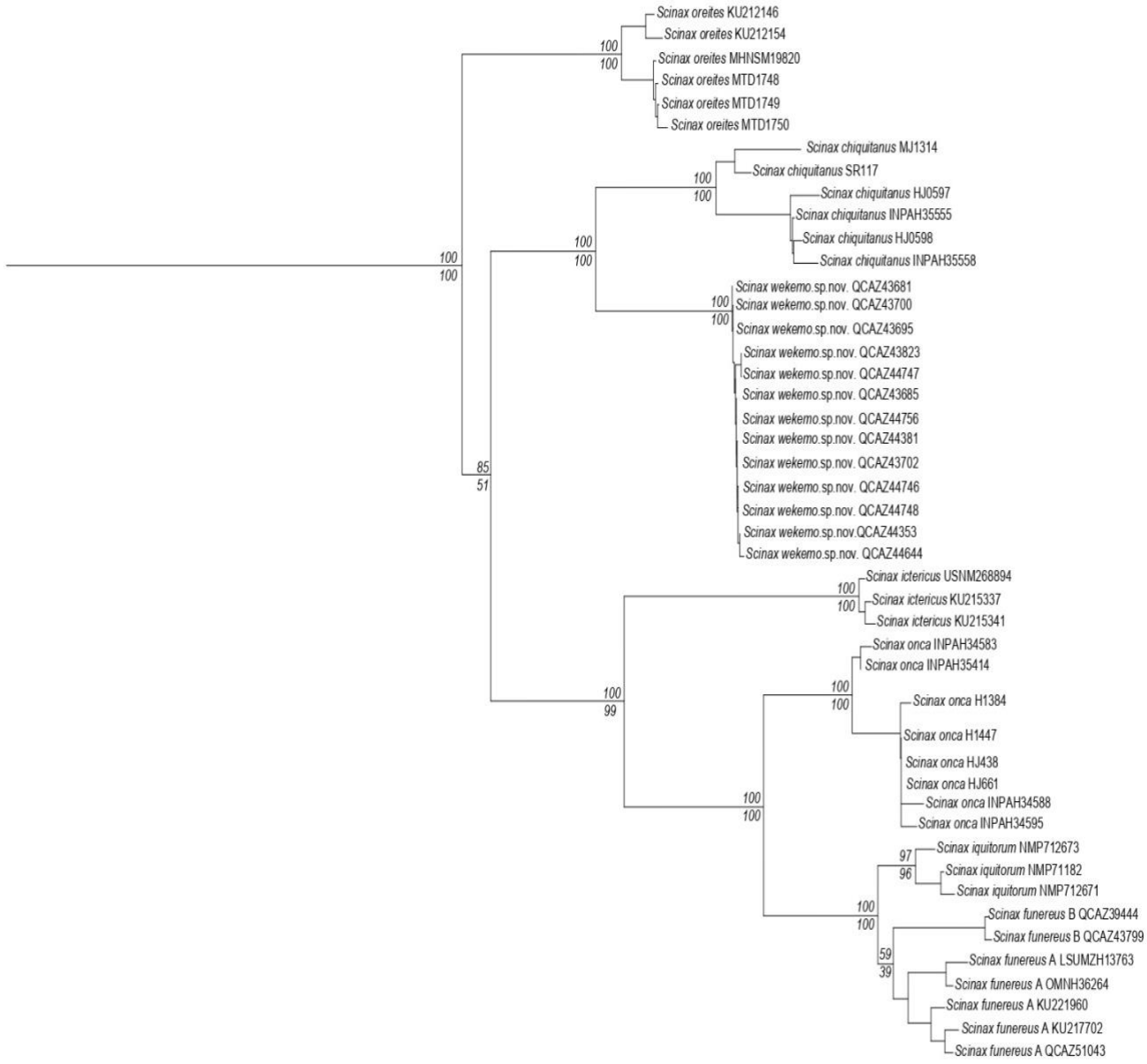


Figure 3. Hand and foot of *Scinax wekemo* sp.nov. A) dorsal view of hand B) ventral view of hand, C) dorsal view of foot, D) ventral view of foot.



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599 **Figure** 4. Phylogenetic tree of species related to *Scinax wekemo* sp. nov. belonging to Scinax
600 funereus group values of bootstrap of a Maximum Likelihood analysis (up the branch) and
601 values of Bayesian probability (down the branch).

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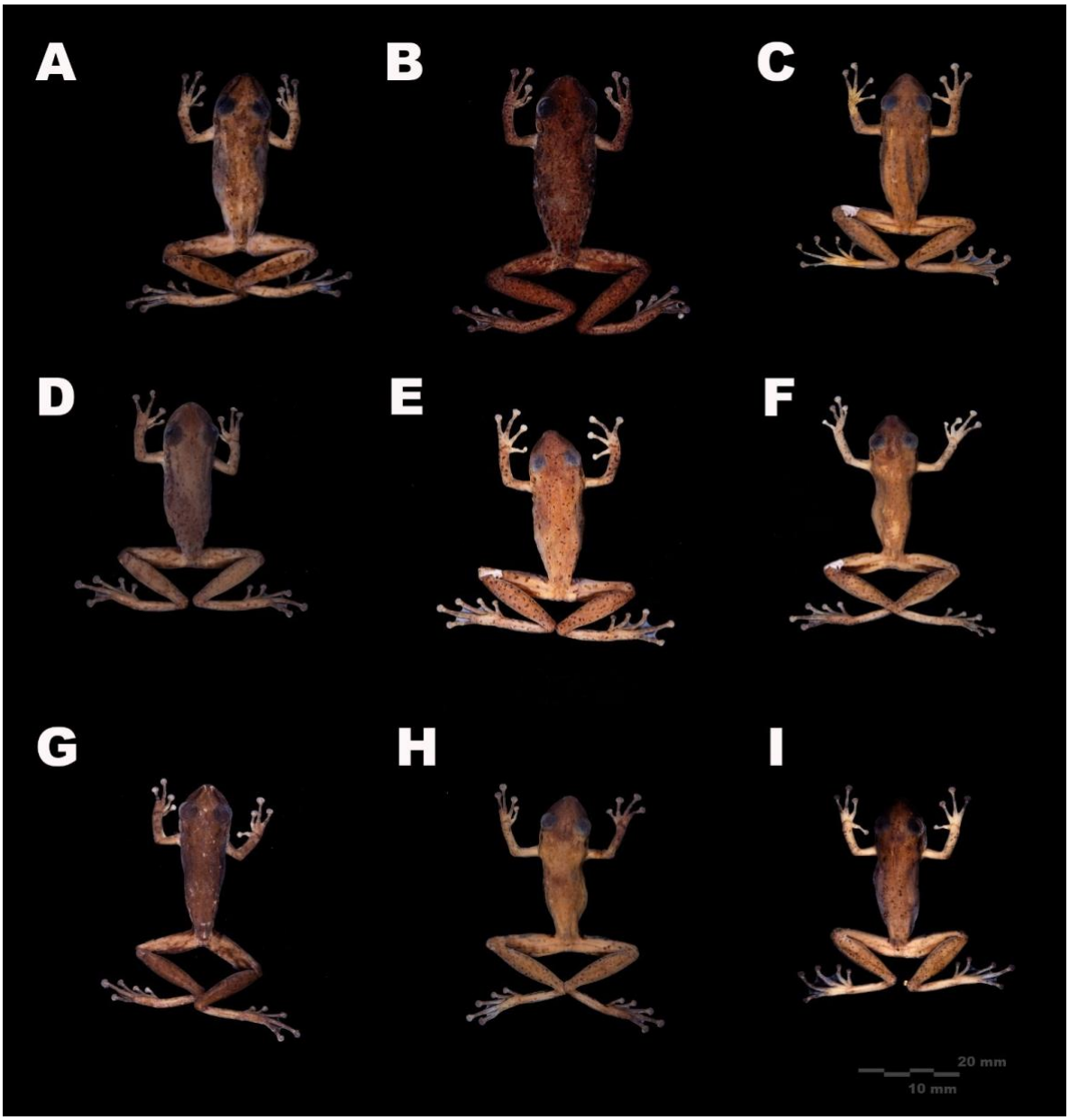
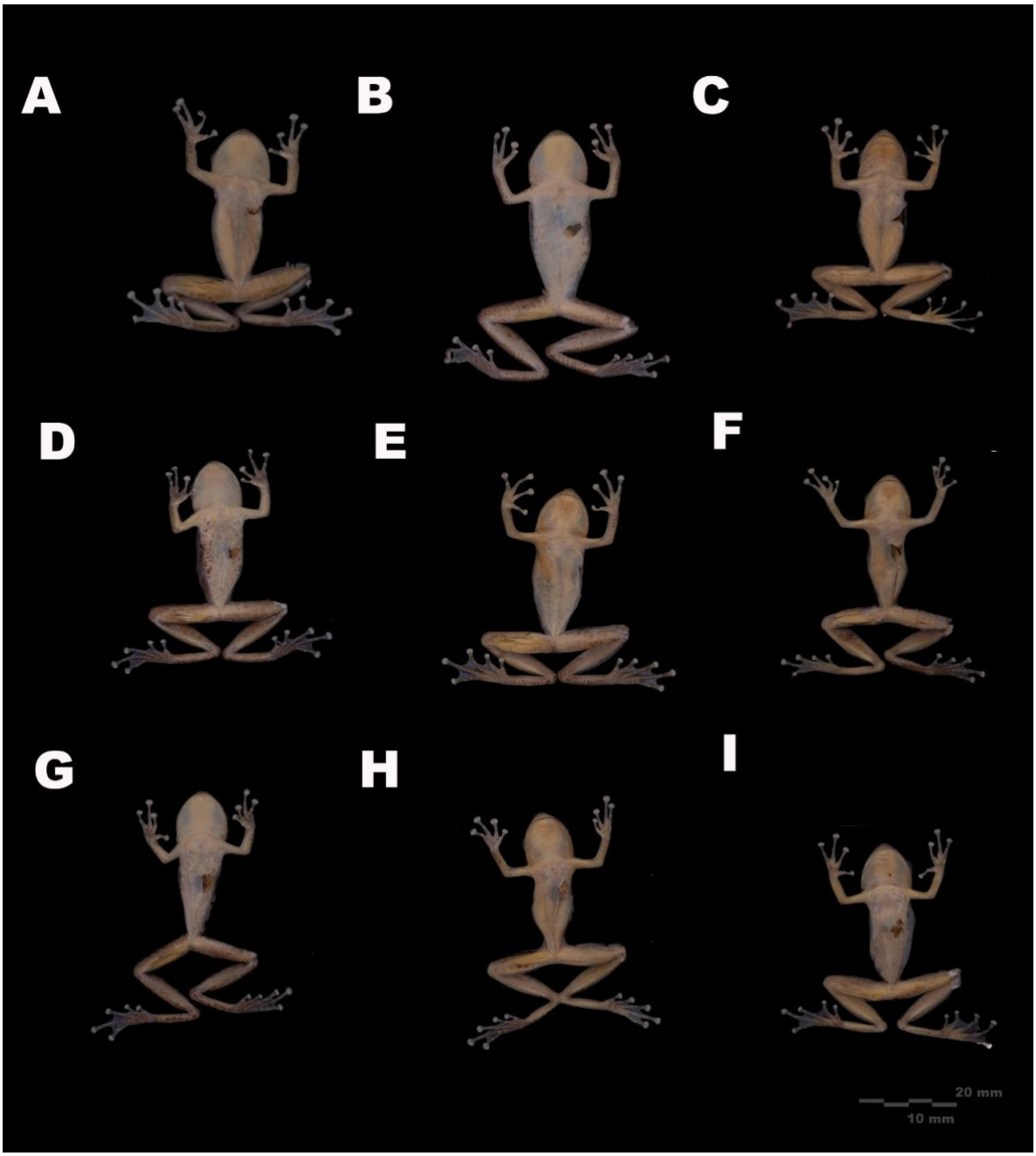


Figure 5. Color variation in preserved individuals of *Scinax wekemo* sp.nov.**A** Dorsal view: QCAZ 44746 (SVL 35.14mm), **B** Dorsal view: QCAZ 44749 (SVL 38.47 mm), **C** Dorsal view: QCAZ 43695 (SVL 31.78 mm), **D** Dorsal view: QCAZ 44751 (32.67 mm), **E** Dorsal view: QCAZ 43681 (SVL 33.52 mm), **F** Dorsal view: QCAZ 43700 (SVL 30.14 mm), **G** Dorsal view: QCAZ 44747 (SVL 32.22 mm), **H** Dorsal view: QCAZ 43702 (SVL 30.82 mm), **I** Dorsal view: QCAZ 44644 (SVL 31.81 mm).



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612 **Figure 6.** Color variation in preserved individuals of *Scinax wekemo* sp.nov. **A** Ventral view:
613 QCAZ 44746 (SVL 35.14mm), **B** Ventral view: QCAZ 44749 (SVL 38.47 mm), **C** Ventral
614 view: QCAZ 43695 (SVL 31.78 mm), **D** Ventral view: QCAZ 44751 (32.67 mm), **E** Ventral
615 view: QCAZ 43681 (SVL 33.52 mm), **F** Ventral view: QCAZ 43700 (SVL 30.14 mm), **G**
616 Ventral view: QCAZ 44747 (SVL 32.22 mm), **H** Ventral view: QCAZ 43702 (SVL 30.82
617 mm), **I** Ventral view: QCAZ 44644 (SVL 31.81 mm).

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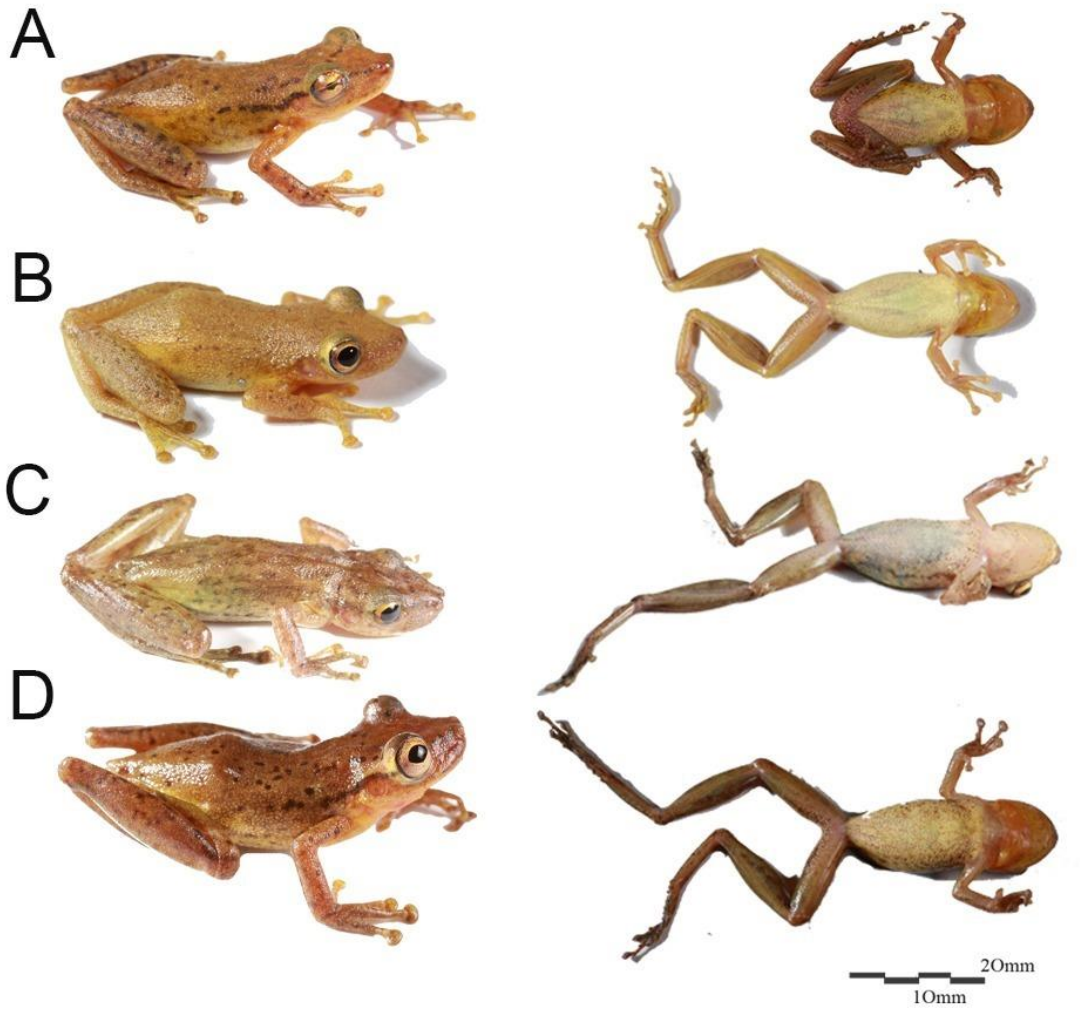

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620 **Figure 7**.Paratypes of *Scinax wekemo* sp. nov. **A** QCAZ 43823 adult male (SVL 30.37
621 mm) **B** QCAZ 43695 adult male (SVL 31.78 mm) **C** QCAZ 44756 adult female (SVL
622 36.39 mm) **D** QCAZ adult female (SVL 34.88mm).

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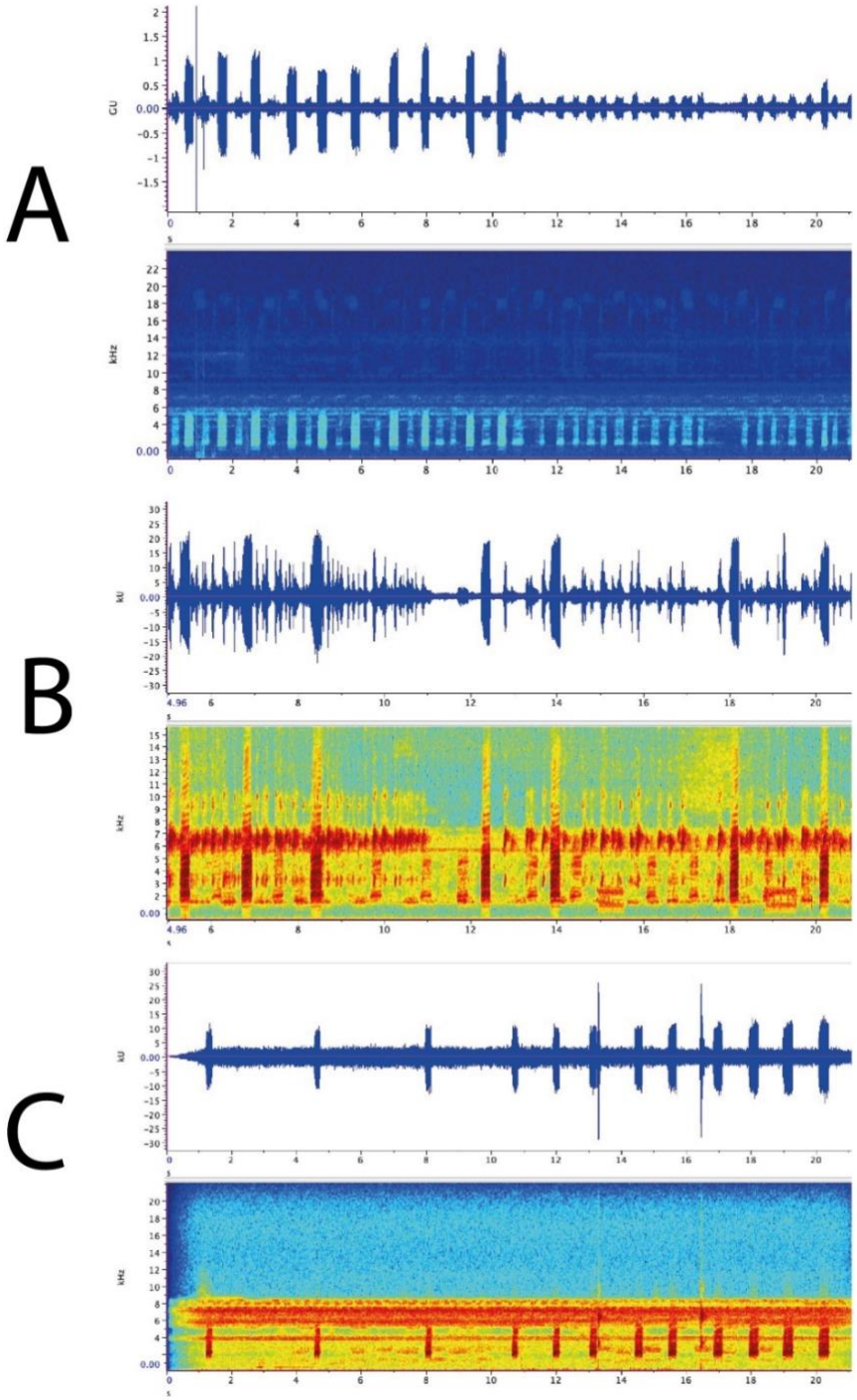

625

626 **Figure 8.** A) Advertisement calls of *Scinax wekemo* sp.nov. showing oscillogram (top), and
627 power spectrum (bottom) (B) Advertisement calls of *Scinax chiquitanus* (PER) showing
628 oscillogram (top), and power spectrum (bottom). (C) Advertisement calls of *Scinax*
629 *chiquitanus* (BOL) showing oscillogram (top), and power spectrum (bottom).