







First records of *Scinax ruberoculatus* Ferrão, Fraga, Moravec, Kaefer & Lima, 2018 (Anura, Hylidae) in the state of Amapá, eastern Amazon, Brazil, with comments on its vocalization and distribution


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Abstract

We report the first records of *Scinax ruberoculatus* Ferrão, Fraga, Moravec, Kaefer & Lima, 2018 in the state of Amapá, Brazil. We provide an updated distribution map of this species, comment on its occurrence in French Guiana and Suriname, and provide morphometric and bioacoustic data for a population from the municipality of Porto Grande, Amapá, including the first description of the species' territorial call. This record from Porto Grande extends the distribution of *S. ruberoculatus* approximately 1430 km northeast from its type locality and helps to better understand its actual distribution.

Keywords

Advertisement call, Amazonia, bioacoustics, Guiana Shield, morphometric measurements, range extension, territorial call

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Introduction

The genus *Scinax* Wagler, 1830 currently comprises 128 species of small to medium-sized treefrogs distributed from southern Mexico to central-eastern Argentina, including the Caribbean islands of Trinidad and Tobago, and Santa Lucia (Frost 2021). These species form two major clades, the *S. ruber* and the *S. catharinae* clades (Faivovich 2002; Faivovich et al. 2005), with 31 species of the *S. ruber* clade occurring in Amazonia *sensu lato* (Ferrão et al. 2018b).

The species richness of *Scinax* is currently considered to be highly underestimated (Fouquet et al. 2007b; Ferrão et al. 2016; Menezes et al. 2016). Some factors contributing to this scenario are the high morphological conservatism and the complex taxonomic history of many species (e.g., Nunes et al. 2012; Brusquetti et al. 2014; Araujo-Vieira et al. 2020), the insufficient sampling of some areas (Ferrão et al. 2016), and the continual detection of potentially cryptic lineages within nominal species (e.g., Fouquet et al. 2007b; Ferrão et al. 2016; Vacher et al. 2020).

Scinax ruberoculatus Ferrão, Fraga, Moravec, Kaefer & Lima, 2018 is a small treefrog (snout vent-length of 22.6–25.9 mm in males and 25.4–27.5 mm in females) recently described from the Brazilian Amazonia and

assigned to the *S. ruber* clade. This species was known to occur in primary and old-growth secondary lowland rainforests in five municipalities along the Purus-Madeira interfluvium, state of Amazonas (Ferrão et al. 2018a), and at three localities within and nearby the “Arc of Deforestation” in the central and western portions of the state of Mato Grosso (Camera et al. 2019). More recently, Vacher et al. (2020) reported populations of *S. ruberoculatus* in French Guiana and Suriname, based on 16S mitochondrial DNA sequences (see Discussion for details).

Herein, we report the first records of *S. ruberoculatus* from the state of Amapá, eastern Amazon, and provide an updated map of its distribution. We also provide morphometric and acoustic data for one of the documented populations, including comments on its advertisement call and the first description of the species’ territorial call.

Methods

We conducted visual and auditory surveys during faunal inventories and monitoring in the municipality of Porto Grande, state of Amapá, Brazil (00.7150°N, 051.4718°W) (Fig. 1), between 15 and 17 March 2021, during twilight from 5:30 h to 7:00 h and from 17:30 h to 18:30 h. The specimens were collected in the Module IV of the fauna

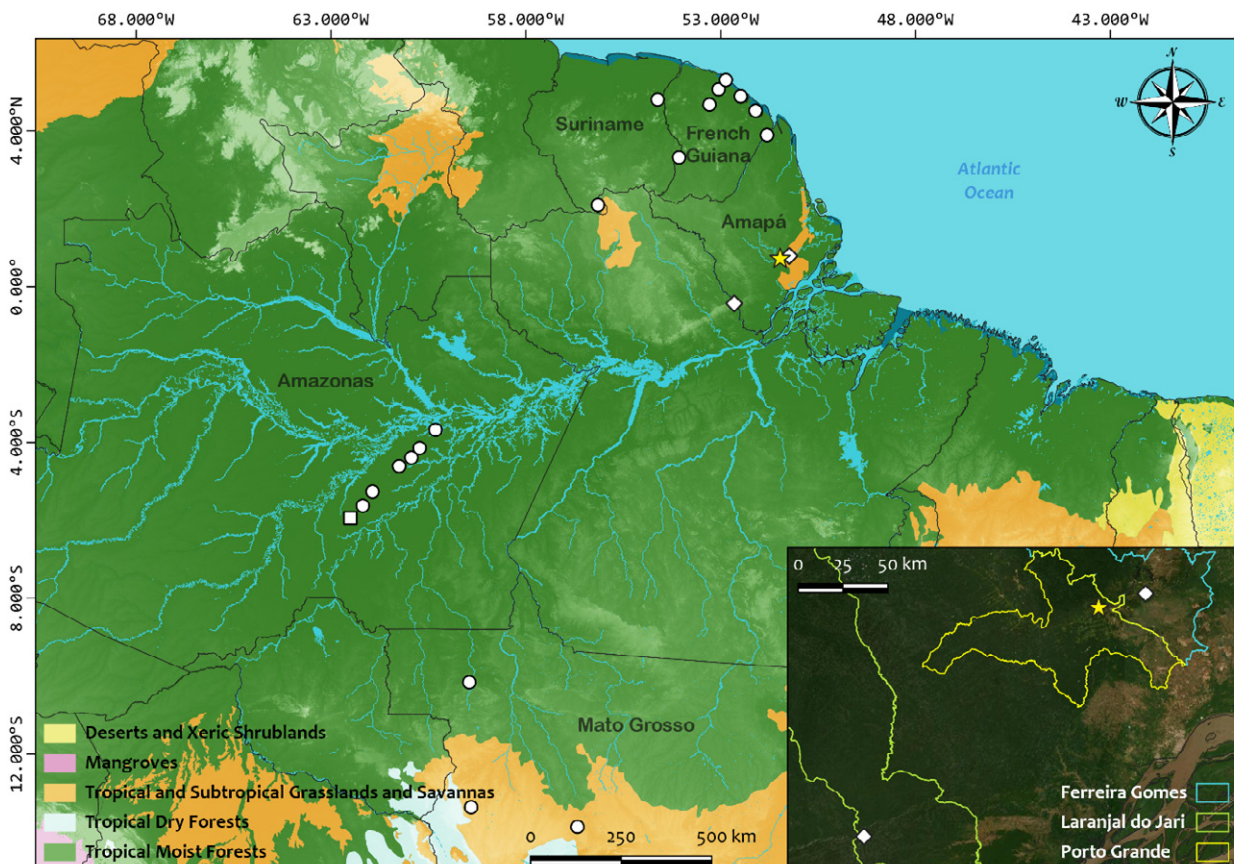


Figure 1. Map of the northern portion of South America, with the previous known records of *Scinax ruberoculatus* represented by white square (type locality; Ferrão et al. 2018a) and white circles (other previous records: Ferrão et al. 2018a; Camera et al. 2019; Vacher et al. 2020). The new records in the state of Amapá reported here are represented by a yellow star (collected and recorded individuals) and white lozenges [unvouchered individuals (only photographed)]. See Discussion for comments on the occurrences in French Guiana and Suriname.

monitoring campaign of the Cachoeira Caldeirão Hydroelectric Power Plant. The voucher specimens were euthanized using 5% lidocaine, fixed in 10% formalin solution, preserved in 70% ethanol, and deposited in the Herpetological Collection of the Universidade Federal do Amapá (CECC). The specimens were identified as *Scinax ruberoculatus* considering both morphological and advertisement call data, based on comparisons with the species' original description (Ferrão et al. 2018a). We also report here uncollected and not recorded specimens (only photographed) from surveys carried out in the municipalities of Laranjal do Jari (00.4318°S, 052.6486°W) and Ferreira Gomes (00.7851°N, 051.2368°W), both also in the state of Amapá (Fig. 1). In order to provide a general update on the distribution of this species, we produced a distribution map using Google Earth and QGIS software (QGIS Development Team 2017) based on geographic coordinates obtained from our herpetological surveys and the literature (see Ferrão et al. 2018a; Camera et al. 2019; Vacher et al. 2020).

Bioacoustics. Vocalizations of two males (one voucher specimen: CECC 3612; SVL 23.9 mm) were recorded on 14 March 2021 at Porto Grande with a Shotgun CSR YOGA HT-81 directional microphone coupled to a Zoom H1N digital recorder (sampling rates of 44.1 or 48.0 kHz; 16 bits resolution). The microphone was positioned at a distance of ca. 0.5 m from the calling individuals. A 600-Hz high-pass filter was applied to the sound files prior to the acoustic analyses in order to reduce background noise. Analyses were conducted in Raven Pro v. 1.5 software (Center for Conservation Bioacoustics 2014) with the following settings (some values vary according to the sampling rate): window size = 256 samples; 3dB filter bandwidth = 248 or 270 Hz; window type = Hann; overlap = 86.3% (locked); hop size = 0.729 or 0.794 ms; DFT size = 1024 samples; grid spacing = 43.1 or 46.9 Hz. Acoustic terminology and definitions followed Köhler et al. (2017). Dominant, maximum, and minimum frequency values were respectively obtained through the “Peak Frequency”, “Frequency 95%”, and “Frequency 5%” functions. Pulse rate (pulses/s) was calculated as “the number of pulses of a call / call duration”. The within-pulse amplitude modulations, referred here as to pulse sub-units (*sensu* Bang et al. 2017), were quantified from three pulses at the central portion of each call. Sound figures were generated in R platform v. 3.6.2 (R Core Team 2019) using seewave v. 2.1.6 (Sueur et al. 2008) and tuneR v. 1.3.3 (Ligges et al. 2018) packages with the following settings: window = Hanning; overlap = 85%; FFT = 256. The analyzed sound files (labels: *Scinax ruberoculatus*_CECC_3612_14_03_2021; *Scinax ruberoculatus*_CECC_3614_14_03_2021) are deposited in the Herpetological Collection of the Universidade Federal do Amapá (CECC).

Two types of calls were recorded. One type corresponds to the advertisement call, which is the most common and emitted repeatedly in a stereotyped way (Köhler

et al. 2017). The other type was classified as the territorial call (*sensu* Toledo et al. 2015), as it was emitted by the foreground recorded individual only in response to other males, when they started vocalizing close to it.

Morphometric data. All measurements were performed using digital calipers (precision 0.01 mm) and a stereomicroscope in the laboratory. We measured the following 16 morphometric traits in six individuals: eye–nostril distance (END), foot length (FL), fourth toe disk diameter (4TD), hand length (HAL), head length (HL), head width (HW), horizontal eye diameter (ED), horizontal tympanum diameter (TD), internarial distance (IND), interorbital distance (IOD), length of the tarsus (TAL), snout–vent length (SVL), thigh length (THL), third finger disk diameter (3FD), tibia length (TL) and upper eyelid width (UEW). Terminology and definitions of these traits follow Duellman (1970), Heyer et al. (1990), and Napoli (2005).

Results

New records. BRAZIL – Amapá • municipality of Porto Grande, Cachoeira Caldeirão Hydroelectric Power Plant; 00.7150°N, 051.4718°W; 108 m a.s.l.; 14.III.2021; Jackson C. Souza leg.; CECC 3612–3617, six adults ♂ (Fig. 2), SVL 23.8–25.7 mm • municipality of Laranjal do Jari, Iratapuru River Sustainable Development Reserve; 00.4318°S, 052.6486°W; 226 m a.s.l.; 06.IV.2019; Vinicius A.M.B. de Figueiredo obs.; 1 adult ♂ (Fig. 4A) • municipality of Ferreira Gomes, Cachoeira Caldeirão hydroelectric power plant; 00.7851°N, 051.2368°W; 65 m a.s.l.; 25.VI.2016; Jucivaldo D. Lima obs.; 1 adult ♂ (Fig. 4B).

The collected specimens were found in Dense Ombrophylous Forest, about 4 km northwest of the Araguari River. They were active, found perched on the leaves and branches of the vegetation in the humid forest understory. The site had small streams with sections of temporary flooding (Fig. 2F).

Identification. The specimens (Figs. 2, 4) were identified as *Scinax ruberoculatus* according to the diagnosis provided in its original description by Ferrão et al. (2018a), based on the following morphological/chromatic traits: SVL of 23.8–25.7 mm in males; dorsal ground colour varying from light grey to brown, with a conspicuous dark grey moth-shaped blotch on the interorbital and scapular regions, and a dark grey chevron-shaped blotch on the sacral region; iris bicolored, with upper half reddish and lower half grey, and both parts being separated by a narrow central red streak; dorsal surface of the arm cream to yellowish cream; anterior and posterior surfaces of thigh uniformly brown, and dorsal surface yellowish cream to light brown; broad dark grey stripes on the dorsal surface of tibia; a whitish cream stripe in the lower portion of the flanks; ventral surface of the hand light grey and ventral surface of foot light grey to brown; semi translucent white vocal sac; white belly, with an areolate skin texture; dark grey webbing between toes.

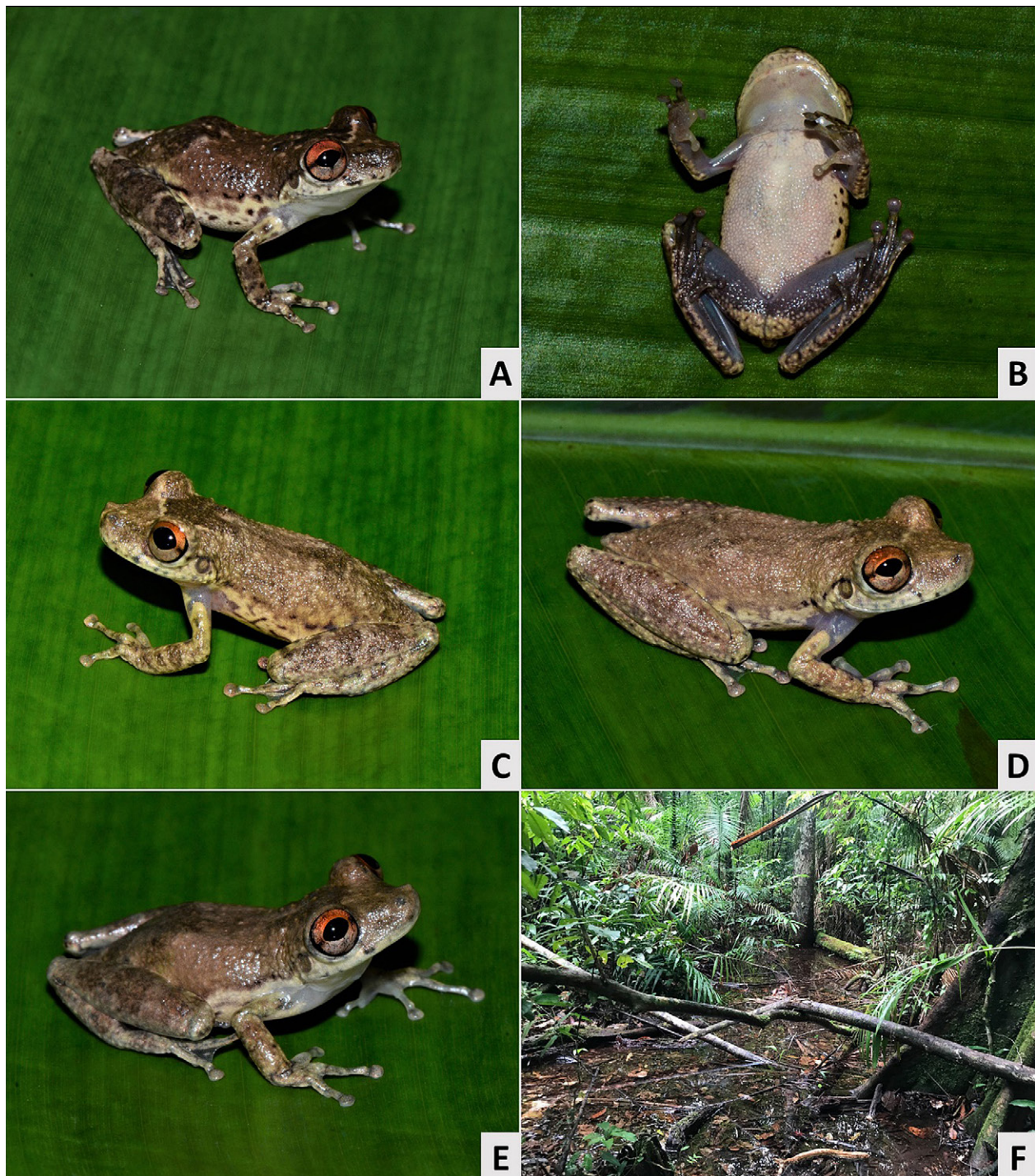


Figure 2. Color pattern variation in life of adult males of *Scinax ruberoculatus* from the municipality of Porto Grande, state of Amapá, Brazil. **A, B.** Dorsal and ventral views; CECC 3612, SVL 23.9 mm. **C.** CECC 3614, SVL 24.9 mm. **D.** CECC 3615, SVL 24.1 mm. **E.** CECC 3616, SVL 25.7 mm. **F.** Breeding habitat used by *S. ruberoculatus* in Porto Grande.

In addition, almost all morphometric traits measured from the males of our sample are within the range values presented by Ferrão et al. (2018a) for males of the species' type series, with the exception of the slightly longer head of males of the type series (Table 1).

The advertisement call of the population from Porto Grande matched the call descriptions reported for *S. ruberoculatus* by Ferrão et al. (2018a) from the type locality, and by Camera et al. (2019) from two populations of the state of Mato Grosso.

Advertisement call. This call [n = two males (one voucher: CECC 3612); 13 calls] (Fig. 3A) consists of a single multipulsed note which have an ascending amplitude modulation from its first to its third pulse, then reaching an amplitude level that is sustained with minimal variation over the remaining duration. The call duration ranges from 0.112 to 0.281 s (mean = 0.141, SD = 0.040, n = 13), and calls contain 8–19 pulses (mean = 10, SD = 3). Pulses last from 0.008 to 0.014 s (mean = 0.010, SD = 0.0002, n = 148), are spaced by intervals of

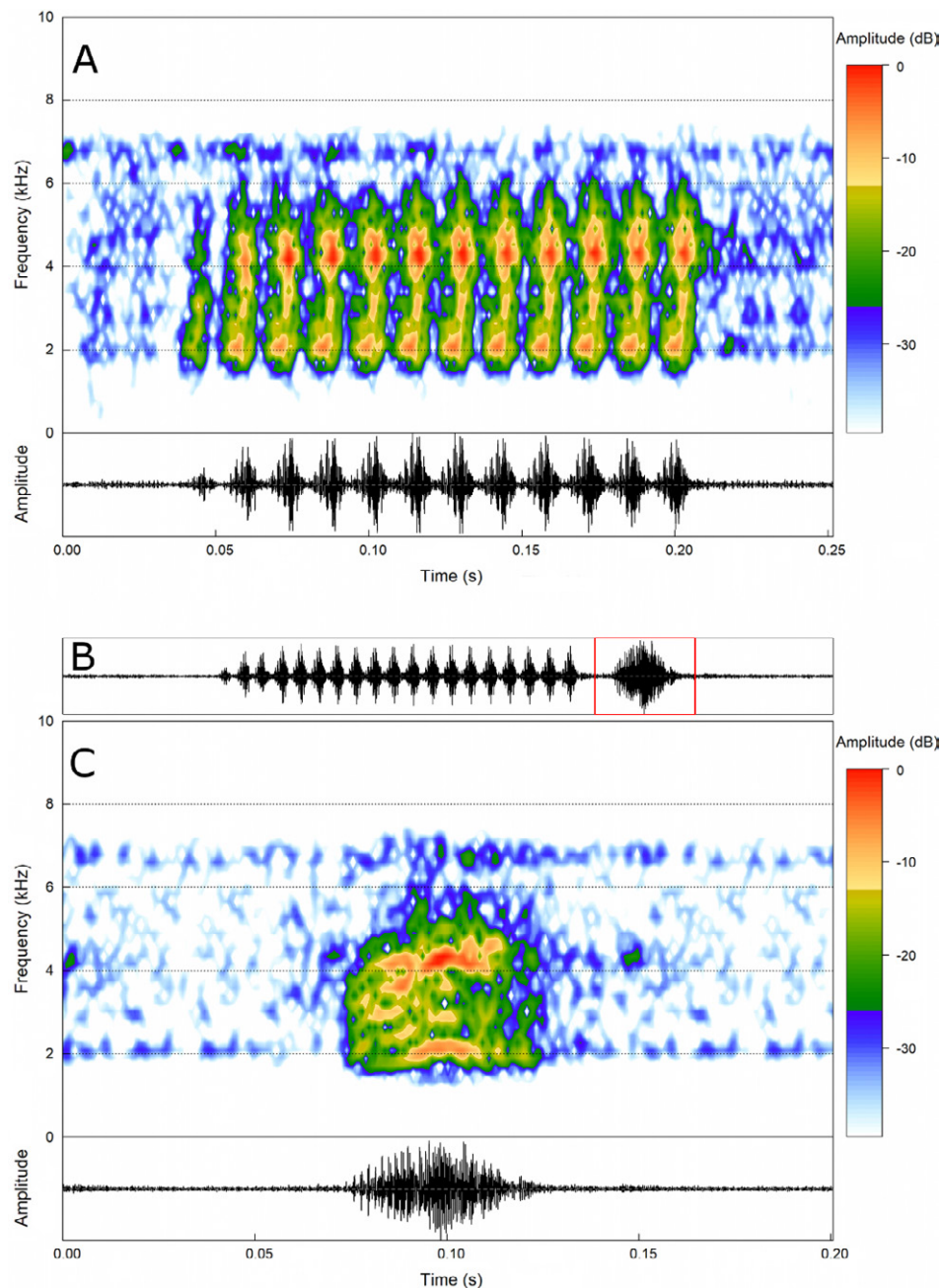


Figure 3. Vocalizations of a voucher specimen (CECC 3612) of *Scinax ruberoculatus* from the municipality of Porto Grande, state of Amapá, Brazil. **A.** Audiospectrogram (top) and respective oscillogram (bottom) of the advertisement call. **B.** Oscillogram section (ca. 0.6 s) depicting an advertisement call followed by a territorial call (the latter highlighted by a red rectangle). **C.** Audiospectrogram (top) and respective oscillogram (bottom) detailing the territorial call highlighted in B. Sound file: *Scinax_ruberoculatus_CECC_3612_14_03_2021*. FFT of 256 samples; 3dB filter bandwidth of 270 Hz.



Figure 4. Unvouchered specimens of *Scinax ruberoculatus* (collected only for photographic records and subsequently released in the same place), both from the state of Amapá, Brazil. **A.** Specimen from Iratapuru River Sustainable Development Reserve, municipality of Laranjal do Jari. **B.** Specimen from Cachoeira Caldeirão hydroelectric power plant, municipality of Ferreira Gomes.

Table 1. Morphometric measurements (in mm) of six males of *Scinax ruberoculatus* from the municipality of Porto Grande, state of Amapá, Brazil (CECC 3612-3617). The "Overall" column correspond to the range values (minimum–maximum) of our sample; range values of the 28 males of the species' type series (taken from Ferrão et al. 2018a) are presented in the last column for comparison. See Methods for the terminology of the abbreviations of the measurements.

Measurements	3612	3613	3614	3615	3616	3617	Overall	Type series
SVL	23.9	23.8	24.9	24.1	25.7	24.5	23.8–25.7	22.6–25.9
HL	7.1	7.1	8.1	7.4	7.4	7.7	7.1–8.1	8.3–9.8
HW	8.2	7.1	8.3	7.0	8.0	7.8	7.0–8.3	7.9–8.9
ED	2.7	2.2	3.1	2.9	3.0	3.1	2.2–3.1	2.7–3.4
TD	1.0	0.9	1.1	1.4	1.5	1.2	0.9–1.5	1.1–1.6
UEW	1.8	2.1	1.7	1.7	1.9	2.0	1.7–2.1	1.8–2.6
IOD	2.7	2.5	2.0	2.6	2.4	2.4	2.0–2.7	2.2–2.7
IND	2.0	2.1	1.6	1.7	1.8	1.8	1.6–2.1	1.5–2.0
TAL	6.4	5.7	6.0	6.3	6.7	6.4	5.7–6.7	6.0–7.2
FL	9.6	9.8	9.6	9.7	9.6	9.2	9.2–9.8	8.6–10.3
HAL	6.5	7.1	6.8	7.6	6.7	6.3	6.3–7.6	5.9–7.6
3FD	1.1	0.9	1.3	1.1	1.2	1.0	0.9–1.3	0.7–1.2
4TD	1.1	1.0	1.0	0.9	0.9	1.2	0.9–1.2	0.7–1.3
END	2.7	2.8	2.9	3.1	2.9	2.4	2.4–3.1	2.4–3.1
TL	12.6	11.8	13.0	12.1	12.6	12.3	11.8–13.0	11.2–13.1
THL	11.9	11.1	11.4	11.5	11.3	11.7	11.1–11.9	10.2–12.1

0.003–0.007 s (mean = 0.004, SD = 0.0003, $n = 135$), and are emitted at rates of 68–73 pulses/s (mean = 71, SD = 0.03, $n = 13$). Pulses have a gradual ascending amplitude modulation up to reaching their peak in about half or somewhat more than half of their duration, and then gradually descend towards their end. The first pulse has notably lower amplitude than the others (Fig. 3A). With the exception of the first pulse, the others clearly present regular internal amplitude modulations (i.e., pulse sub-units *sensu* Bang et al. 2017), with the pulses from the central portion of the call presenting 4–5 pulse sub-units. The call has two emphasized frequency bands (non-harmonically related), hereinafter referred to as the lower (LFB) and the higher (HFB) frequency bands. The LFB peaks from 2016 to 2063 Hz (mean = 2035, SD = 15, $n = 13$) and the HFB peaks from 3230 to 4266 Hz (mean = 3783, SD = 599, $n = 13$); the dominant frequency corresponded to the LFB in the calls of one individual, and to the HFB in the calls of the other one. The minimum frequency ranges from 1875 to 1969 Hz (mean = 1919, SD = 34, $n = 13$) and the maximum frequency ranges from 4393 to 5109 Hz (mean = 4696, SD = 337, $n = 13$).

Territorial call. This call ($n =$ one voucher male: CECC 3612; 2 calls) (Fig. 3B, C) was emitted shortly after the advertisement call, and consists of a single note with incomplete internal amplitude modulations (i.e., there were no silent intervals between pulse units, so that these could not be quantified). The call duration ranges from 0.049 to 0.051 s (mean = 0.050, SD = 0.001, $n = 2$), and it is emitted 0.025–0.035 s (mean = 0.030, SD = 0.007, $n = 2$) after the end of an advertisement call. Similar to the advertisement call, the territorial call has two main portions with greater energy, with the LFB peaking at 2109 Hz ($n = 2$) and the HFB peaking at 4219–4266 Hz (mean = 4242, SD = 33, $n = 2$); dominant frequency

corresponded to the HFB in all calls. Both frequency bands have a slight upward modulation at the call onset. The minimum frequency is of 1969 Hz ($n = 2$) and the maximum frequency is of 4594 Hz ($n = 2$).

Discussion

The advertisement calls analyzed here have the same structural pattern (i.e., a single multipulsed note) and also are in general agreement with the trait values previously reported for *Scinax ruberoculatus* by Ferrão et al. (2018a) and Camera et al. (2019), thus corroborating the identification of the specimens reported here. Although none of these previously mentioned studies drew attention to the presence of two emphasized frequency bands, it is noticeable in their spectrograms that these bands are also evident in their calls (see Ferrão et al. 2018a: fig. 7; Camera et al. 2019: fig. 3). It is important to provide dominant frequency values separately for each band in call descriptions, as it is very common in calls of *Scinax* species for the dominant frequency to alternate its correspondence between bands along call emissions (e.g., Magrini et al. 2011; Novaes and Zina 2016; A.G. Lopes pers. obs.). Although the values reported by Ferrão et al. (2018a) for maximum frequency (2378–2579 Hz) are substantially lower than those reported here (4393–5109 Hz) and by Camera et al. (2019) (4458.3–5047.1 Hz), it can be noticed in their spectrogram that their call has substantial energy up to ca. 4500 Hz (see Ferrão et al. 2018a: fig. 7). Considering that Ferrão et al. (2018a) did not specify exactly how spectral traits were measured, it cannot be stated for sure whether this difference is due to the use of different measurement methods or to some error by them when making the call selection in the spectrogram.

The territorial call of *S. ruberoculatus* was herein described for the first time. The foreground recorded individual emitted two territorial calls in a situation

where other males started calling close to it; these other males in the proximities also emitted territorial calls amid this context. In both call figures provided by Camera et al. (2019) there is a note shortly after the advertisement call (see Camera et al. 2019: fig. 3), which is extremely similar to the note described here as the territorial call. Although Camera et al. depicted this note in their advertisement call figures, they did not mention it in the text, which indicates that they probably misinterpreted it as being part of the advertisement call. Some studies have also reported other species of *Scinax* which may emit aggressive calls shortly after the advertisement call (e.g., Drummond et al. 2007; Bang et al. 2017; Lopes et al. 2020).

Scinax ruberoculatus was known to occur in the Brazilian states of Amazonas and Mato Grosso based on solid evidence (Ferrão et al. 2018a; Camera et al. 2019) (Fig. 1). Recently, Vacher et al. (2020) carried out an extensive molecular analysis of 16S mitochondrial sequences of Amazonian anurans and recovered a clade they identified as *S. ruberoculatus*, which comprises sequences referred to as the Operational Taxonomic Units (OTUs) *Scinax* 51 (from French Guiana) and *Scinax* 73 (from French Guiana and Suriname) (Fig. 1). Although assigned as two OTUs, *Scinax* 51 and *Scinax* 73 correspond to a single clade; that is, they do not constitute distinct subclades within *S. ruberoculatus*. Besides the sequences specifically produced for the study, other sequences from French Guiana which have been historically referred to as *Scinax cruentommus* (Duellman, 1972) (e.g., Salducci et al. 2005; Fouquet et al. 2007a, 2007b; Duellman et al. 2016) were also nested within this clade. Recently, Ferrão et al. (2016) had already demonstrated that these sequences from French Guiana previously referred to as *S. cruentommus* are distantly related to this taxon and actually constitute the sister clade of *S. ruberoculatus* (as *Scinax* sp. 7 there). Salducci et al. (2005: fig. 5) provided a small photograph of a specimen associated with this clade, which shows a dorsal coloration pattern similar to that of *S. ruberoculatus* (i.e., a moth-shaped blotch and a chevron-shaped blotch on the dorsum, and dark grey strips on the dorsal surface of tibia). However, considering this intricate historical context involving these sequences from French Guiana, and the fact that Ferrão et al. (2016) reported a substantial genetic divergence ($\geq 7\%$) for the 16S mitochondrial gene between these sequences and those of *S. ruberoculatus*, these occurrences of *S. ruberoculatus* in French Guiana and Suriname should be reassessed for confirmation using detailed morphological data and, especially, advertisement call data.

Our novel records of *S. ruberoculatus* from the state of Amapá extends the distribution of this species by approximately 352 km south from the nearest locality at Saint Georges, French Guiana (03.8882°N, 051.8078°W), and about 1430 km northeast from the species' type locality at Nascentes do Lago Jari National Park (05.9444°N, 062.5011°W) (Fig. 1). We have also observed this species in two other localities in the state of Amapá: in the

municipality of Laranjal do Jari at the Iratapuru River Sustainable Development Reserve, and in the municipality of Ferreira Gomes at Cachoeira Caldeirão hydroelectric power plant (Fig. 1). For both of these records, specimens were captured only for photographing (Fig. 4) and then released. The record from Ferreira Gomes represents the easternmost occurrence for this species. Considering its current distribution, we highly expect that *S. ruberoculatus* also occurs in the state of Pará, Brazil, and a confirmation of its occurrence in the northwestern portion of this state would fill a gap in its distribution. In documenting newly found populations of *S. ruberoculatus*, the present study contributes to the ecological and biogeographical knowledge of this species and will be useful in determining its conservation status.

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Authors' Contributions

Data curation: JCS, JPS, JDL, JRFL. Formal analysis: AGL. Resources: CECC. Supervision: CECC. Visualization: JCS, JPS. Writing – original draft: VAMBF, RTP, AGL. Writing – review & editing: FPS, PRS.

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