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Revisiting the bioacoustics of *Sphaenorhynchus prasinus* (Anura: Hylidae): new data on vocalization of a lime treefrog from northeastern Brazil, including topotypes

LUIZ F. CARMO¹, IURI R. DIAS², GIOVANNI TANAJURA², OMAR ROJAS-PADILLA^{2,3},
MIRCO SOLÉ² & CAIO V. MIRA-MENDES⁴

¹Departamento de Vertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, 20940-040, Quinta da Boa Vista, Rio de Janeiro, Brazil

²Programa de Pós-Graduação em Zoologia, Universidade Estadual de Santa Cruz, Rodovia Jorge Amado, km 16, 45662-900, Ilhéus, Bahia, Brazil

³Dirección de Investigación en Diversidad Biológica Terrestre Amazónica, Instituto de Investigaciones de la Amazonía Peruana, 10170, carretera Fernando Belaúnde Terry, km 1, Tingo María, Huánuco, Peru

⁴Programa de Pós-Graduação em Ecologia e Conservação da Biodiversidade, Universidade Estadual do Maranhão, Av. Lourenço Vieira da Silva, nº 1000, 65055-310, São Luís, Maranhão, Brazil

Corresponding author: LUIZ F. CARMO, ORCID 0000-0002-9873-3186,
e-mail: luiz.carmo@ufrj.br

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Bioacoustic studies play an essential role in resolving taxonomic issues in anuran species, as advertisement calls act as a pre-mating barrier and can carry important phylogenetic information (KÖHLER et al. 2017, CARMO et al. 2024, SABBAG et al. 2024). In anurans, males typically produce calls during the breeding season to attract females, as well as other types of calls associated with different social roles (WELLS 2007, TOLEDO et al. 2015b). These different vocal patterns offer deeper insights into their behavioral biology and can be instrumental in studies on the evolution of communication (GERHARDT & HUBER 2002). In this context, documenting the bioacoustics of poorly known species is a critical step toward a more comprehensive understanding of their systematics, behavior, and conservation needs.

The genus *Sphaenorhynchus* TSCHUDI, 1838 currently comprises 14 recognized species of Neotropical treefrogs (sensu ARAUJO-VIEIRA et al. 2019, 2025). Three species are distributed in the Amazon basin (*S. dorisae*, *S. carneus* and *S. lacteus*) and the remaining ones in the Brazilian Atlantic Forest domain (PEREIRA et al. 2022, FROST 2025). These species are nocturnal and breed along temporary, permanent, or semi-permanent ponds in open areas and edges of forest fragments (BOKERMANN 1973, CRUZ 1973, CRUZ & PEIXOTO 1980, SILVA-NETO et al. 2022). One of these

species, *Sphaenorhynchus prasinus* BOKERMANN, 1973, is a small treefrog (snout–vent length of types 28–31 mm; BOKERMANN 1973) that is endemic to the Brazilian Atlantic Rainforest. Its range extends from the state of Paraíba to Minas Gerais and includes the coastal regions of the states of Pernambuco, Alagoas, Bahia, and Espírito Santo (ARAUJO-VIEIRA et al. 2016; VIEIRA et al. 2024). According to BOKERMANN'S (1973) original description, the species reproduces in temporary ponds in secondary forests, where males can be found calling when perched on floating vegetation or while they are partially submerged in water. This species was mainly collected in a temporary pond in the Cacao Research Center (type locality) in the municipality of Ilhéus, southern Bahia, Brazil. The advertisement call of the species was first described by BOKERMANN (1973), who mainly focused on qualitative characteristics. Later, TOLEDO et al. (2015a) described the advertisement call of *S. prasinus* based on six calls from a single male recorded in the municipality of Recife, state of Pernambuco, northeastern Brazil. Here, we present a redescription of the vocalization of *S. prasinus*, which includes calls from topotypic specimens. We also compare the results with descriptions of other *Sphaenorhynchus* species available in the literature.

Calling males were recorded from two localities in the state of Bahia in northeastern Brazil. Three individuals were

recorded in a temporary pond on the campus of Universidade Estadual de Santa Cruz (hereafter UESC; 14°47'45.73" S, 39°10'18.5" W, 34 m a.s.l.), in the municipality of Ilhéus, southern Bahia. This site is located 7 km from the Cacao Research Center (14°45'22.13" S, 39°13'52.94" W, 57 m a.s.l.), which constitutes the type locality referred to by BOKERMANN (1973) in the original description of *S. prasinus*. Additionally, a single male was recorded in the municipality of São Sebastião do Passé, Bahia, which is located 260 km north in a straight line from UESC. The climate of both localities is classified as 'Af' (tropical rainforest climate, without dry season), with annual precipitation ranging between 2200 and 2500 mm and average annual temperature between 24 and 26 °C (ALVARES et al. 2014).

The recordings at UESC were made on 27 November 2013, while the recordings in São Sebastião do Passé were made on 26 October 2014. Snout-vent lengths (SVLs) of all recorded males at UESC were measured with a precision caliper to the nearest 0.1 mm. For the recordings at UESC, each individual was recorded using a Marantz Professional PMD660 digital recorder (mono-configured, WAVE format, 24-bit resolution and 96000 Hz sample rate) coupled to a Sennheiser ME66/K6 unidirectional microphone, which was pointed in the direction of each calling male. In the municipality of São Sebastião do Passé, the calling male was recorded with a Tascam DR-1 digital recorder, configured in stereo audio (WAVE format, 16-bit resolution and sampling rate of 44100 Hz). This recording device was also coupled to a unidirectional Sennheiser ME66/K6 microphone and pointed in the direction of the calling male. The sound files were saved as WAV files and are publicly available at the Fonoteca Neotropical Jacques Vieliard (FNJV), Universidade Estadual de Campinas (UNICAMP), municipality of Campinas, Brazil (FNJV124979–84). Voucher specimens from UESC were euthanized with 2% lidocaine, fixed in 10% formaldehyde, and subsequently preserved in 70% ethanol (see MCDIARMID 1994). These specimens were deposited in the herpetological collection of the Museum of Zoology at UESC (MZUESC 24875–77). The recorded calling male from São Sebastião do Passé was not collected.

We performed acoustical editing of the calls using Adobe Audition CS6 (Adobe Systems Inc.). For the sound analysis, we used Raven Pro 1.6.5 software from the Cornell Laboratory of Ornithology (YANG 2024). Call illustrations were made in the R environment with the seewave and tuneR packages (SUEUR et al. 2008, LIGGES et al. 2016). We followed the homology-based naming procedure suggested by HEPP & POMBAL (2019) for describing acoustic signals. Specifically, we chose a note-centered approach because it has been commonly applied to members of this genus and it agrees with the definitions presented by KÖHLER et al. (2017). Both temporal and spectral parameters were measured with the window function Hann, by specifying a window size of 512 samples, a 3 dB filter bandwidth 124, and overlap of 99%. Calls were described based on the following Raven software functions to measure the acoustic properties: (1) call duration; (2) inter-call interval; (3) notes per call; (4) note duration; (5) note duration (last note);

(6) inter-note interval; (7) note repetition rate (per second); (8) average entropy; (9) bandwidth 90%; (10) dominant frequency; (11) center frequency; (12) frequency 5%; (13) first quartile frequency (frequency 25%); (14) third quartile frequency (frequency 75%); and (15) frequency 95%. Numerical parameters are given as ranges followed by their means and standard deviations in parentheses, statistical mode, when applicable, and by sample size [Min–Max ($x \pm SD$), mod; N]. Statistical mode is defined as the value that occurs most frequently in the dataset, used here as a measure of central tendency alongside the traditional use of the mean. Thus, values presented after the comma correspond to the mode, whereas values presented after the semicolon indicate the sample size. We compared the calls of *S. prasinus* with closely related species, though those call descriptions did not include all the call parameters that we analysed in this study.

The following description is based on 86 call emissions (412 notes) recorded from four males of *Sphaenorhynchus prasinus*. These calling males were recorded in UESC (24 calls of MZUESC 24875, SVL 30.6 mm; 30 calls of MZUESC 24876, SVL 31.3 mm; and 20 calls of MZUESC 24877, SVL 29.2 mm), while 12 other calls were from a male recorded in São Sebastião do Passé (not collected). Approximately 30 males vocalized on the floating vegetation present in the water body in São Sebastião do Passé, while only four calling males were recorded at UESC.

We detected a single call type, which we classified as an advertisement call based on observations during fieldwork (see TOLEDO et al. 2015b). All recorded calling males emitted this call, which totaled 86 emissions. These signals are composed of multipulsed notes, emitted at regular silent intervals, with an amplitude peak usually occurring at the end of the call duration. The call envelope shows an ascendant triangle shape (see oscillogram in Fig. 1A and B), with the following characteristics: a duration of 0.10–0.77 s (0.15 ± 0.07 , 0.15), inter-call intervals of 1.21–199.33 s (13.36 ± 14.36) composed of 4–21 (5 ± 1.88 , 5) notes with inter-note intervals of 0.014–0.041 s (0.029 ± 0.002 , 0.029; 34), and a note repetition rate of 27.29–42.83 (33.49 ± 2.54) notes/second. The number of pulses varied within each note, usually with a single pulse in the first (mean of 1.01 ± 0.169 ; $n = 138$ calls), and with two to four pulses in the last notes. The average entropy of the call varied between 2.49–3.59 (3.25 ± 0.24 , 3.37) bits, and the bandwidth 90% of 516.8–937.5 Hz (629.6 ± 112.3 , 562.5). The dominant frequency of the call ranged from 1875.0–2670.1 Hz (2187.8 ± 184.2 , 2062.5), with the 5% frequency varying between 1687.5–2584.0 Hz (2020.8 ± 213.6 , 1875.0), the 75% frequency varying from 2062.5–2756.3 (2220.8 ± 190.5 , 2062.5), the 95% frequency varying from 2250.0–2928.5 Hz (2452.9 ± 193.1 , 2437.5), and the center frequency varying between 2250.0–2928.5 (2452.9 ± 193.1 , 2437.5).

Following the classification proposed by TOLEDO et al. (2015b), the acoustic unit (i.e., advertisement call), was frequently emitted and could be heard from males that were apparently calling alone. The acoustic envelope of this call is similar to the call described in BOKERMANN's (1973) orig-

inal description and that of TOLEDO et al. (2015). BOKERMANN (1973) described the call of *S. prasinus* as being composed of about five short multipulsed notes, with a regular period of silence between them and having a short call duration. The quantitative results obtained in our study are consistent with those presented qualitatively in the original description of this species, thereby corroborating the preliminary conclusions of BOKERMANN (1973). These data reinforce the validity of field observations and confirm the

robustness of the data described by W. C. A. BOKERMANN during the early 1970s.

In light of phylogenetic relationships in which *S. prasinus* was recovered as the sister taxon to the *S. platycephalus* species group (ARAUJO-VIEIRA et al. 2019, 2025), previous studies on bioacoustics have shown that species within the clade typically produce less complex advertisement calls composed of multipulsed notes with a regular silence period between them (LACERDA & MOURA 2013, ARAU-

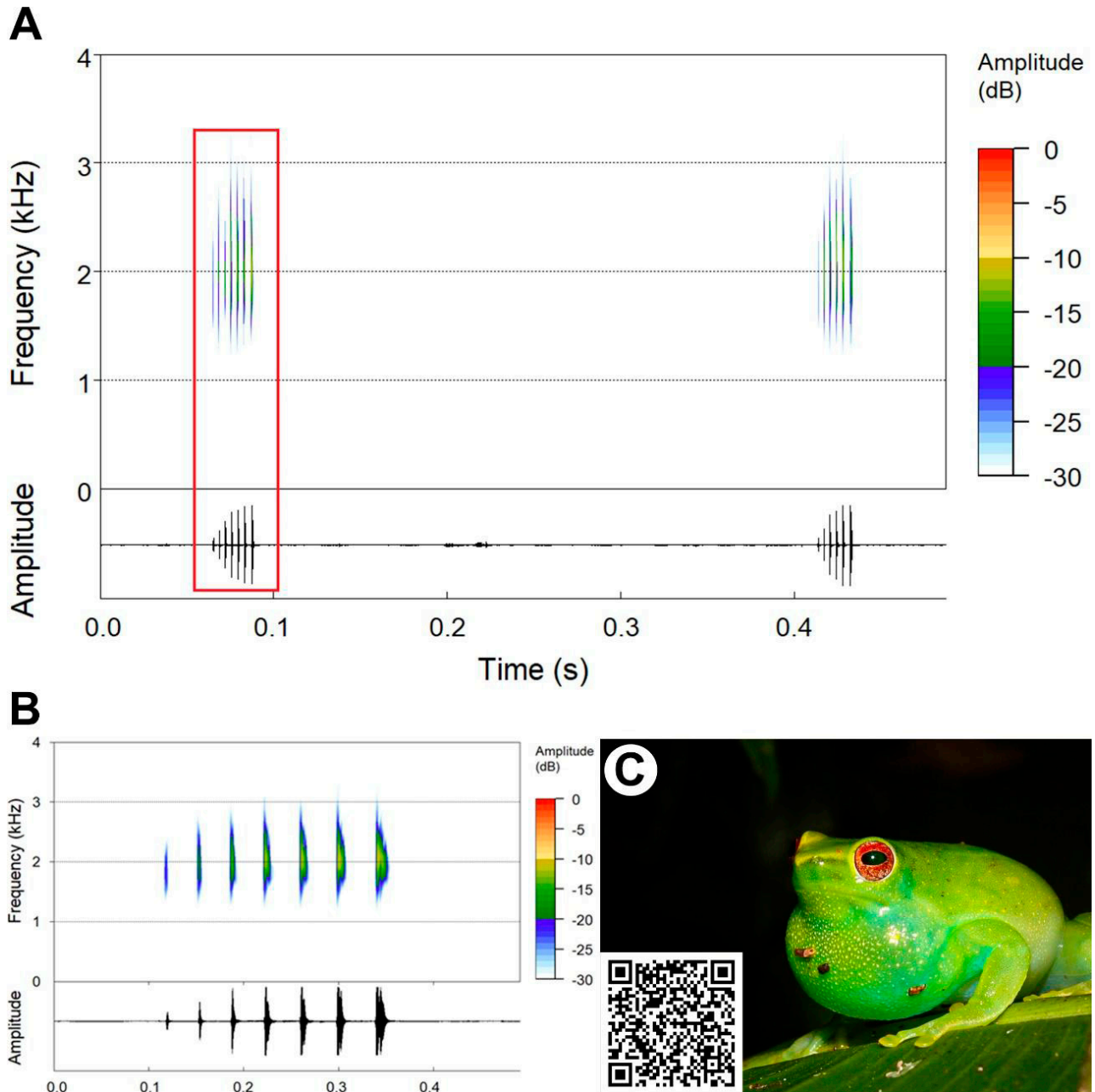


Figure 1. Advertisement call of *Sphaenorhynchus prasinus* recorded in the municipality of Ilhéus, Bahia: (A) Oscillogram and spectrogram showing general aspects of the structure of the advertisement call; (B) Oscillogram and spectrogram of one highlighted advertisement call, composed of seven notes; (C) Calling male recorded on 2019 in the municipality of Ilhéus (not collected), vocalizing on marginal vegetation (photographed by EDVALDO NETO). Note QR code links to the call recordings deposited at the Fonoteca Neotropical Jacques Vielliard (FNJV124979).

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Table 1. Acoustic parameters of advertisement calls of *Sphaenorhynchus prasinus* recorded at Universidade Estadual de Santa Cruz, municipality of Ilhéus and in the municipality of São Sebastião do Passé. Numerical parameters are each given as ranges followed by mean and standard deviation in parenthesis, mode, when applicable, and by sample size.

Localities	Ilhéus, Bahia			São Sebastião do Passé, Bahia	All individuals	Recife, Pernambuco (TOLEDO et al. 2015a)
Sound archives	FNJV124979–80	FNJV124981–82	FNJV124983	FNJV124984	N = 4	FNJV11200
Voucher specimens (SVL mm)	MZUESC 24875 (30.6)	MZUESC 24876 (31.3)	MZUESC 24877 (29.2)	Not collected	29.2–31.3 (29.8±1.70)	//
Nº. of calls analyzed	24	30	20	12	86	3
Call duration [s]	0.15–0.77 (0.19±0.13), 0.15	0.10–0.16 (0.13±0.02), 0.10	0.11–0.18 (0.14±0.02), 0.16	0.10–0.15 (0.14±0.01), 0.14	0.10–0.77 (0.15±0.07), 0.15	0.20–0.31 (0.24±0.04)
Inter-call interval [s]	1.21–22.45 (7.54±5.40)	5.26–20.90 (10.70±3.96)	8.71–199.33 (29.90±44.57)	2.62–14.94 (5.31±3.50)	1.21–199.33 (13.36±14.36)	//
Notes per call	5–21 (6.0±3.28), 5	4–5 (4.5±0.51), 5	4–6 (4.5±0.61), 4	4–5 (4.8±0.39), 5	4–21 (5.0±1.88), 5.0	//
Note duration [s]	0.003–0.017 (0.008±0.002), 0.009; 145	0.001–0.013 (0.006±0.003), 0.017; 136	0.003–0.017 (0.007±0.003), 0.010; 90	0.003–0.010 (0.005±0.001), 0.004; 59	0.001–0.017 (0.007±0.003), 0.009; 430	0.004–0.02 (0.01±0.004)
Note duration (last note) [s]	0.007–0.015 (0.010±0.002), 0.009	0.007–0.013 (0.009±0.002), 0.008; 30	0.008–0.017 (0.012±0.003); 20	0.005–0.010 (0.007±0.002); 12	0.005–0.017 (0.010±0.003), 0.009; 86	//
Inter-note interval [s]	0.022–0.038 (0.029±0.002), 0.029; 121	0.014–0.034 (0.030±0.003), 0.029; 106	0.026–0.032 (0.029±0.001), 0.030; 70	0.024–0.041 (0.029±0.004), 0.026; 47	0.014–0.041 (0.029±0.002), 0.029; 344	//
Note repetition rate [notes/s]	27.29–33.91 (31.83±1.72), 32.95	31.91–42.83 (34.31±2.79)	31.17–35.81 (33.37±1.60), 31.81	27.93–40.44 (34.95±2.90)	27.29–42.83 (33.49±2.54)	//
Average Entropy (bits)	2.96–3.55 (3.26±0.14)	2.02–3.59 (3.32±0.32)	3.09–3.53 (3.30±0.12)	2.49–3.30 (2.88±0.26)	2.49–3.59 (3.25±0.24), 3.37	//
Bandwidth 90% [Hz]	562.50–750.0 (578.1±52.9), 562.5	562.50–937.50 (759.4±128.67), 750.0	562.50–937.50 (759.4±128.67), 750.0	516.80–775.20 (610.1±15.30), 516.8	516.80–937.50 (629.57±112.26), 562.5	//
Dominant frequency [Hz]	1875.0–2062.5 (2054.7±38.3), 2062.5	1875.5–2250.0 (2225.0±95.14), 2250.0	2062.5–2062.5 (2062.5±0.00), 2062.5	2497.9–2670.1 (2569.7±71.88), 2497.9	1875.0–2670.1 (2187.8±184.18), 2062.5	2250.0–2625.0 (2378.0±112)
Center frequency [Hz]	1875.0–2062.5 (1984.4±94.4), 2062.5	1875.5–2250.0 (2187.5±113.7), 2250.0	2062.5–2062.5 (2062.5±0.00), 2062.5	2497.9–2670.1 (2585.0±73.43), 2497.9	1875.0–2670.1 (2154.9±210.01), 2062.5	//
Frequency 5% [Hz]	1687.5–1687.5 (1687.5±0.0), 1687.5	1687.5–1875.0 (1843.8±71.1), 1875.0	1687.5–1687.5 (1687.5±0.00), 1687.5	2153.3–2239.5 (2217.9±38.99), 2239.5	1687.5–2239.5 (1816.0±183.2), 1687.5	//
Frequency 25% [Hz]	1875.0–1875.0 (1875.0±0.0), 1875.0	1887.5–2062.5 (2043.8±75.50), 2062.5	1875.0–1875.0 (1875.0±0.00), 1875.0	2411.7–2584.0 (2497.9±51.94), 2497.9	1687.5–2584.0 (2020.8±213.6), 1875.0	//
Frequency 75% [Hz]	2062.5–2062.5 (2062.5±0.0), 2062.5	2062.5–2250.0 (2243.8±34.2), 2250.0	2062.5–2250.0 (2128.1±91.76), 2062.5	2583.9–2756.3 (2634.2±68.30), 2584.0	2062.5–2756.3 (2220.8±190.52), 2062.5	//
Frequency 95% [Hz]	2250.0–2437.5 (2265.6±52.9), 2250.0	2437.5–2812.5 (2465.9±76.7), 2437.5	2250.0–2625.0 (2446.9±128.69), 2437.5	2756.2–2928.5 (2828.01±71.93), 2756.2	2250.0–2928.5 (2452.9±193.1), 2437.5	//

JO-VIEIRA et al. 2015, ROBERTO et al. 2017, BOLOVON et al. 2020). In comparison to all species of the Sphaenorhynchini tribe, except *S. planicola* and *S. mirim*, the advertisement call of *S. prasinus* has the shortest inter-note interval, namely 0.022–0.038 s vs. the calls of *Gabohyla pauloalvini*, *S. botocudo*, *S. bromelicola*, *S. cammaeus*, *S. canga*, *S. caramaschii*, *S. carneus*, *S. dorisae*, *S. lacteus*, *S. palustris*,

S. platycephalus, and *S. surdus* which when combined exhibit 0.05–0.27 s inter-note intervals (TOLEDO et al. 2007, 2015a, ARAUJO-VIEIRA et al. 2015, LACERDA & MOURA 2013, FORTI et al. 2017, ROBERTO et al. 2017, BOLOVON et al. 2020, CARMO et al. 2021). Thus, compared to the described calls of the *S. platycephalus* species group, the advertisement call of *S. prasinus* differs considerably by the comparative-

ly shortest inter-note interval, reinforcing the species' distinct identity. This call pattern with multipulsed notes, with a regular silence period between them, was presented by BOLOVON et al. (2020) using the call-centered terminology proposed by KÖHLER et al. (2017).

A single call with a markedly higher number of notes (21 notes; MZUESC 24875) compared to the species' average (6.0 ± 3.28) was reported. Based on acoustic similarities, such as their acoustic envelope shape and relative positions, compared with calls classified here as advertisement calls, we did not consider this acoustic unit as a territorial call and suggest that it be defined as an outlier. Given that acoustic signals often serve more than one function or convey more than one type of message to their receiver, this single longer advertisement call might be issued with specific and differential contexts. Thus, future field studies are encouraged to investigate the influence of this call in interactions between individuals and also the presence of additional call types in *S. prasinus*, as well as determine their behavioural significance.

The dominant frequency of advertisement calls of *S. prasinus* was 1875.0–2670.1 Hz (2187.8 ± 184.2 , 2062.5). BOKERMANN (1973) reported a dominant frequency ranging between 1300–1500 Hz, while TOLEDO et al. (2015a) recorded a dominant frequency of *S. prasinus* from the municipality of Recife, state of Pernambuco, ranging between 2250–2625 Hz (2378 ± 112). These values can vary depending on the sample size (number of analysed calls), morphological conditions of calling males, such as the SVL, or even FFT used during the acoustics analysis. BOKERMANN (1973) did not report the FFT, while TOLEDO et al. (2015a) estimated FFT size of 250 (552 samples in the present study). TOLEDO et al. (2015a) examined only three calls of *S. prasinus*, which may represent an inadequate sampling effort to represent the intraspecific variation in acoustic traits of *S. prasinus*. Furthermore, male size is usually inversely correlated to dominant frequency of the advertisement call (see GERHARDT & HUBER 2002, TONINI et al. 2020), which may suggest that the calling male recorded in the state of Pernambuco possibly had a smaller SVL. Advertisement calls recorded from topotypic specimens at UESC are similar to the recorded calls in the municipality of São Sebastião do Passé (260 km distance in a straight line), showing minimal differences.

In comparison with other species of the Sphaenorhynchini tribe, *S. prasinus* has a lower dominant frequency than *Gabohyla pauloalvini* (CARMO et al. 2021), *S. carneus* (TOLEDO et al. 2015a), *S. mirim* (LACERDA et al. 2011), *S. palustris* (LACERDA & MOURA 2013) and *S. planicola* (TOLEDO et al. 2015a) (combined 2760–5812 Hz vs 1875–2670 Hz in *S. prasinus*). Nevertheless, the differences in dominant frequency to other species were minimal. Here, we consider, given the overlap of dominant frequency intervals, that there are no significant interspecific differences in this parameter between *S. prasinus* and *S. botocudo* (1720–2240 Hz; BOLOVON et al. 2020), *S. bromelicola* (1310–3000 Hz; BOLOVON et al. 2020), *S. cammaeus* (2250–3000 Hz; ROBERTO et al. 2017), *S. canga* (2070–4550 Hz;

ARAUJO-VIEIRA et al. 2015), *S. caramaschii* (2440–3230 Hz; TOLEDO et al. 2007), *S. dorisae* (2437–3187 Hz; TOLEDO et al. 2015a), *S. lacteus* (1875–2067 Hz; TOLEDO et al. 2015a), and *S. surdus* (2240–2370 Hz; TOLEDO et al. 2007).

Sphaenorhynchus prasinus occurs in the Atlantic Forest biome, one of the most threatened ecosystems in Brazil because it is currently undergoing intense habitat loss due to deforestation, fire, mining, pollution, and the effects of climate change (RIBEIRO et al. 2009, POTTIER et al. 2025). The known populations of *S. prasinus* are restricted to lowland areas, often associated with aquatic habitats that are increasingly degraded. Although the species is currently classified as “Least Concern” by the IUCN Red List of Threatened Species (IUCN 2023) and the national assessment of threatened species (BRASIL 2022), given its limited distribution within specific ecosystems of the Atlantic Forest, habitat loss represents a major risk to the species.

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