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Third time's a charm: reviewing the advertisement call of *Gabohyla pauloalvini* (Anura: Hylidae: Scinaxinae), an endemic species from Brazil' Atlantic Forest

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Abstract

The Neotropical tribe Sphaenorhynchini is composed of 15 recognized species commonly named as "lime treefrogs". *Gabohyla pauloalvini* occurs in Atlantic Forest lowlands through the states of Bahia and Espírito Santo, Brazil. Based on field observation, literature review, and evaluation of audio files, we conclude that previous bioacoustic descriptions were erroneously assigned to *G. pauloalvini*. Here, we describe for the first time the advertisement call of *G. pauloalvini* from two disjunct populations. In addition, we provide new distribution records and an updated map for this species. The advertisement call of *G. pauloalvini* is composed of 1–5 closely spaced notes, with duration of 0.016–1.976 s, inter-note intervals of 0.340–1.25 s, and an average dominant frequency (= fundamental frequency) of 4966.0 Hz. Harmonics are visible in the spectrogram. Three new populations of *G. pauloalvini* have been recorded and extended the distribution by 60 km south. Nevertheless, *G. pauloalvini* is still known only from few localities. We highlight the importance of depositing audio recordings and specimens in scientific collections to promote systematics and natural history studies.

Key words: Acoustic communication, bioacoustics, Sphaenorhynchini, vocalization

Introduction

Communication comprises information transfer via signals that evolve from the interaction between emitter and receiver (Bradbury & Vehrencamp 2000; Gerhardt & Huber 2002). Bioacoustics is one of the main mediators of social interactions of several taxonomic lineages (Gerhardt & Huber 2002; Chen & Wiens 2020). Despite different types of communication in anurans (e.g., seismic, visual, or tactile) (Narins 1990; Hödl & Amézquita 2001; Waldman & Bishop 2002; Hartmann *et al.* 2005; Furtado *et al.* 2019), acoustic signals represent the main-interaction tactic

for most species (Wells 1977). These acoustic signals, usually conspicuous and stereotyped, are used in intra and interspecific interactions (Gerhardt & Huber 2002; Wells & Schwartz 2007; Toledo *et al.* 2015a). Acoustic signals are an important source of characters that provide essential information for studies dealing with systematics and natural history (Wells & Schwartz 2007; Padial & De la Riva 2009; Goicoechea *et al.* 2010; Hepp & Pombal 2017). Although more than one type of acoustic signal has been documented for many species, the call emitted by males in reproduction is the most known and explored for calling behaviour research (Wells 1977; Toledo *et al*. 2015a; Köhler *et al.* 2017; Guerra *et al*. 2018).

The hylid tribe Sphaenorhynchini Faivovich *et al.*, 2018 currently comprises 15 species of lime treefrogs grouped into two genera: *Sphaenorhynchus* Tschudi, 1838 and *Gabohyla* Araujo-Vieira, Luna, Caramaschi, & Haddad, 2020 (Araujo-Vieira *et al.* 2020). *Gabohyla* is a monotypic genus recently described to allocate *G. pauloalvini* (Bokermann, 1973) that was removed from the genus *Sphaenorhynchus* and suggested as its sister taxon (Araujo-Vieira *et al.* 2019, 2020). *Gabohyla pauloalvini* (Fig. 1) is an endemic species to Brazil's Atlantic Forest from the states of Espírito Santo and Bahia (Bokermann 1973; Almeida *et al.* 2011; Frost 2021). The type locality of *G. pauloalvini* is the Cacao Research Center (Centro de Pesquisas do Cacau, 14°47'08"S, 39°13'22"W, 50m" by "14.785556° S, 39.222778° W), municipality of Ilhéus, state of Bahia, northeastern Brazil (Bokermann 1973). It is found along coastal plains associated to lentic ecosystems, and males vocalize from vegetation 0.5 to 1 m above the water (Bokermann 1973). This species is considered DD by IUCN Red list of threatened species, because little data is available about its geographic distribution, abundance of known populations and ecological and natural history notes (Peixoto & Pimenta 2004).

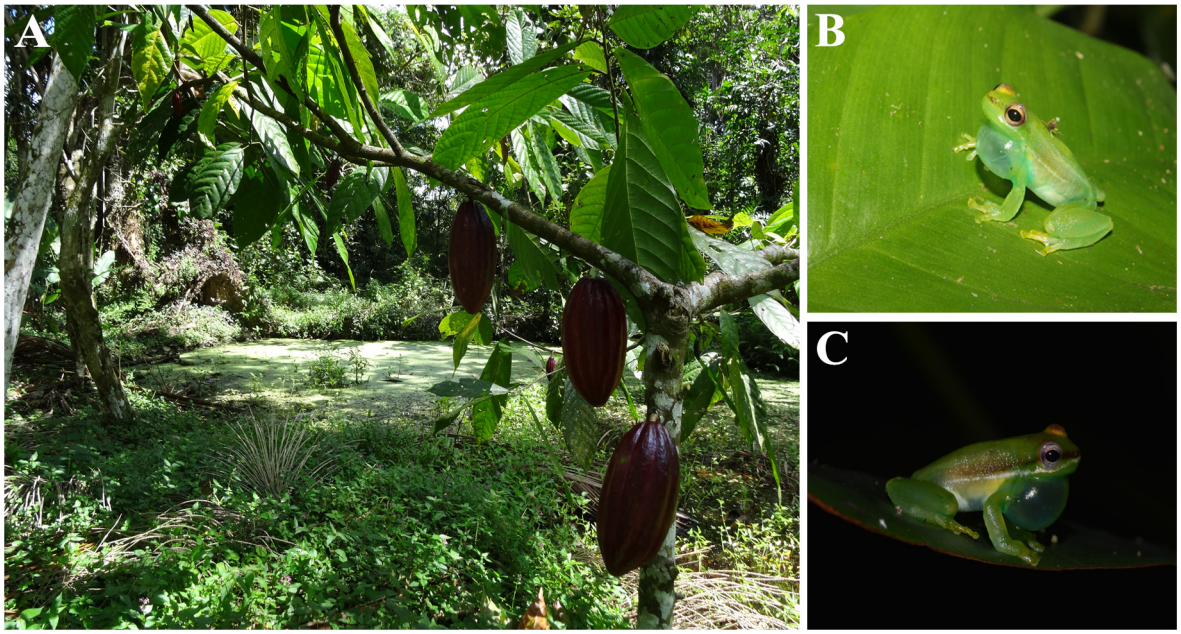

FIGURE 1. Breeding habitat of *Gabohyla pauloalvini* in the municipality of Ilhéus, state of Bahia (**A**). Calling males of *Gabohyla pauloalvini*: Universidade Estadual de Santa Cruz, municipality of Ilhéus, state of Bahia (**B**); and Estação Biologia Marinha Augusto Ruschi, municipality of Aracruz, state of Espírito Santo (**C**).

At least one type of call has been described for each of the 15 species of the Sphaenorhynchini tribe (Guerra *et al.* 2018; Forti *et al.* 2019; Bolovon *et al.* 2020). However, for many of them, the number of recorded calling males is remarkably insufficient to represent population variation. The advertisement call of *Gabohyla pauloalvini* was first described by Bokermann (1973) in the species description and latter redescribed by Toledo *et al.* (2015b). Based on field observation, literature review and evaluation of the audio files used by Bokermann (1973) and Toledo *et al.* (2015b), we conclude that both descriptions were probably erroneously assigned to *G. pauloalvini*. Thus, herein we describe for the first time the advertisement call of *G. pauloalvini* from two disjunct populations. In addition, we provide new distribution records and an updated map for this species.

Material and Methods

Study areas

We sampled three localities: i) a semi-permanent pond at: Universidade Estadual de Santa Cruz (hereafter UESC; 14.795833° S, 39.172222 ° W), municipality of Ilhéus, state of Bahia, northeastern Brazil, about 5.5 Km from the species type locality; ii) a semi-permanent pond at Ponta da Tulha (hereafter Tulha; 14.604654° S, 39.065667° W), also in the municipality of Ilhéus, about 26 Km from the species type locality; iii) and a semi-permanent swamp at Estação Biologia Marinha Augusto Ruschi (hereafter EBMAR; 19.9692° S, 40.1436° W), district of Santa Cruz, municipality of Aracruz, state of Espírito Santo, southeastern Brazil, about 585 km from the species type locality. The pond at UESC is surrounded by a "cabruca" (cocoa plantation growing in the shadow of old grown Atlantic Forest trees) while the pond at Tulha is surrounded by secondary Atlantic Coastal Forest. EBMAR is characterized as having a Dense Ombrophilous Forest type, constituted by coastal plain and hillside forest (Xavier 1998), also known as Tabuleiros Forest and Coastal Forest. The region's climate is classified as Aw, with annual precipitation ranging between 1,300 to 1,600 mm and average annual temperature between 22 and 24 ºC (Alvares *et al*. 2014).

Data collection

At UESC, recordings (n = four males) were obtained in November, 2014 and July, 2018, from 6:00 pm to 8:00 pm, and air temperature varying from 21 to 22 ºC (Minipa MT-241 +/- 1°C). At Tulha, recordings (n = two males) were obtained in July, 2010, from 7:30 pm to 8:30 pm, and air temperature varying from 20.5 to 21 ºC (Minipa MT-241 +/- 1°C). At EBMAR recordings (n = seven males) were performed in November 2019, from 6:30 pm to 11:00 pm, air temperature varying from 19 to 24 ºC (Kestrel 3000 +/- 1°C). Acoustic signals were recorded with a Sennheiser ME45 external microphone (Sennheiser, Wedemark-Wennebostel, Germany) coupled with a Marantz PMD 660 digital recorder (Marantz, Kanagawa, Japan) at a sampling rate of 48 kHz and 16 bits resolution; and a Tascam DR-40 (Tascam, California, EUA) digital recorder coupled with internal microphone, at a sampling rate of 48 kHz and 24 bits resolution. Calls of each male were recorded for at least one minute from a distance between 50-90 cm from the calling males.

To complement the data on the geographical distribution of *Gabohyla pauloalvini*, we reviewed specimens housed at the Museu de Zoologia da Universidade Estadual de Santa Cruz (MZUESC).

Data analysis

Bioacoustic analyses were performed using Raven Pro 1.5 software from the Cornell Laboratory of Ornithology (Bioacoustics Research Program 2014). Spectrograms were produced using a window function Hann, 512 point DFT size, 99% time grid overlap, 3 dB filter bandwidth of 135 and 124, brightness 55%, and contrast 80%. For description of the calls we used note-centered terminology (sensu Köhler *et al.* 2017). Quantitative parameters (summarized in Table 1 are shown as ranges [Minimum (Min) – Maximum (Max)] followed by Mean (x) ± Standard Deviation (SD), and sample size (n) [Min – Max (x ± SD, n)]. In order to ascertain the variation in the acoustic parameters, we calculated the with-in-individual coefficient of variation (CVw) for measured parameters of each male following Gerhardt (1991). Likewise, between-individual coefficient of variation (CVb) was calculated for each parameter. Acoustic parameters that showed the CVw below 5% were classified as static and parameters that showed the coefficient of variation above 12% were considered dynamic (Gerhardt 1991). Values between 5 and 12% were considered as intermediated (5% < CV < 12%; Gerhardt 1991). The acoustic variability patterns, both intra- and interindividual were determined for each population calculating the ratio between CVb and CVw, with values > 1.0 responding as variations in acoustic parameters that are greater between males than within males (Márquez & Eekhout 2006). Acoustic signals here described were classified according to the classification system proposed by Emmrich *et al.* (2020).

Voucher specimens from state of Bahia were anesthetised and killed with lidocaine 2%, fixed in formaldehyde 10%, and subsequently preserved in 70% ethanol [usual techniques described by McDiarmid (1994)]. These specimens were deposited in the MZUESC (10490 and 21234). Recorded calling males from the state of Espírito Santo were not collected. Sound files are deposited and available at the Fonoteca Neotropical Jacques Vielliard (FNJV) and are listed in the Appendix.

TABLE 1. Acoustic parameters of the advertisement call of two populations of *Gabohyla pauloalvini*. Abbreviations: CVw = within-male coefficients of variation (S = Static, I = Intermediate, D = Dynamic); CVb = between-male coefficients of variation; and CVb/CVw ratios.

Individuals (n)	*Gabohyla pauloalvini* seven males					*Gabohyla pauloalvini* six males				
Locality	Municipality of Aracruz, state of Espírito, Brazil					Municipality of Ilhéus, state of Bahia, Brazil				
Acoustic parameters	**Range (Mean ± SD); n**	**Category**	**Mean CVw**	**CVb**	**CVb/ CVw**	**Range (Mean ± SD); n**	**Category**	**Mean CVw**	**CVb**	**CVb/CVw**
Call duration (s)	0.016–1.976 (0.88 ± 0.424); 70	D	47.95%	78.7%	1.64	0.18–1.413 (0.379 ± 0.408); 78	D	72.3%	107.6%	1.5
Inter-call interval (s)	2.203–89.937 (28.54 ± 30.966); 52	D	108.5%	118.2%	1.09	1.792–108.7 (32.83 ± 41.53); 61	D	60.4%	126.5%	2.1
Notes per call	1–5 (2.63 ± 0.789); 70	D	29.98%	48.5%	1.62	1–3 (1.506 ± 0.553); 78	D	20.94%	36,7%	1.75
Notes duration (s)	0.013–0.037 (0.020 ± 0.002); 169	I	11.75%	17.64%	1.5	0.018–0.041 (0.025 ± 0.004); 117	I	9.81%	14.6%	1.49
Inter-note interval (s)	0.340–1.038 (0.50 ± 0.141) 102	D	28.12%	50.3%	1.79	0.448–1.25 (0.726 ± 0.175); 37	D	18.21%	24.1%	1.32
Pulses (repetition) rate	281.37–492.61 (396.75 ± 30.29); 138	I	7.63%	11.9%	1.56	297.06–470.81 (375.05 ± 41.01); 117	S	4.98%	10.9%	2.19
Pulses per note	5–13 (8.28 ± 1.05); 143	D	12.79%	24.0%	1.88	7–13 (9.24 ± 0.94); 117	I	8.54%	10.2%	1.19
Dominant frequency (= fund. freq.) (Hz)	4218.8–5062.5 (4745.5 ± 220.5); 169	S	1.71%	4.67%	2.7	4312.5–5718.8 (5186.6 ± 306.7); 117	S	2.67%	5.9%	2.21
Frequency 5%	3843.8–4593.8 (4385.4 ± 171.6); 169	S	2.72%	3.91%	1.47	4031.2–5250.0 (4806.5 ± 296.8); 117	S	2.24%	6.18%	2.85
Frequency 95%	4500–5812.5 (5064.8 ± 282.1); 169	S	2.98%	5.57%	1.96	4968.8–6468.8 (5705.7 ± 106.3); 117	S	1.86%	6.78%	3.64

Results

We analyzed 148 calls (286 notes) of *Gabohyla pauloalvini*, of which 78 calls (117 notes) from six males at UESC and Tulha (state of Bahia), and 70 calls (169 notes) from seven males at EBMAR (state of Espírito Santo). We recorded only one type of call emitted by males of *G. pauloalvini*. Based on field observations, this call was classified as advertisement call because it attracted females (n = 3).

The advertisement call of *G. pauloalvini* consists of 1–5 (1 = 27.2%, 2 = 31%, 3 = 18.8%, 4 = 16.2%, 5 = 6.8%; n = 148) short pulsed notes, duration of 0.016–1.976 s (0.63 ± 0.416; n = 148) and inter-call intervals of 1.997–99.318 s (30.685 ± 36.248; n = 113). In some calling males, we observed that some notes presented the last pulses fused in a pulsatile note (total of 26 pulsatile notes; Fig. 2B), which made it impossible to count pulse number and to measure pulse parameters properly. Each note was composed of 5–13 pulses [8.76 ± 0.995; n = 260 notes (only those with no pulsatile structure)], duration of 0.013–0.041 s (0.022 ± 0.003; n = 286). The inter-note intervals varied between 0.340–1.250 s (0.613 ± 0.158; n = 139). Harmonics and sidebands are visible in the spectrogram. Sidebands were caused by the pulse rate of the call (Fig. 2C). Pulse repetition rate ranged from 281.37–492.61 pulses/s (402.56 ± 37.88; n = 255). The dominant frequency was equal to the fundamental frequency, varying from 4500.0–5718.8 Hz (4966.0 ± 163.4; n = 286), with 5% and 95% frequencies (excluding the lower and higher portions of recording that concentrate 5% of energy each, respectively) between 3843.8–4593.8 Hz (4208.3 ± 234.2; n = 286) and 4500–5812.5 Hz (5385.2 ± 194.2; n = 286), respectively. Our analysis of intra- and interindividual variation showed that all measured parameters from both populations of *G. pauloalvini* were higher between males than within males (ratio between CVb and CVw with values > 1.0). Pulses per note, inter-note intervals and pulse repetition rate are the least variable parameters, especially intra-individually, among the temporal parameters of advertisement call of *G. pauloalvini.* Call duration and inter-call interval are highly variables and greater variation between males than within males (CVb/CVw ratio). Likewise, spectral parameters showed greater variation between males than within males, although in both populations all spectral parameters are static (see Table 1).

The population from EBMAR extends the known geographic distribution by approximately 60 km south from its nearest locality (straight line), municipality of Linhares, state of Espírito Santo, Brazil (Almeida *et al.* 2011) (Fig. 3). In addition, we found three new populations from the municipality of Almadina (14.700139° S, 39.630000° W - MZUESC 10202–10203; 14.693611° S, 39.631667° W - MZUESC 10226–10230) and Uruçuca (14.623722° S, 39.353944° W - MZUESC 8111), in the state of Bahia, Brazil (Fig. 3).

Discussion

The advertisement call described in the present study differed from the descriptions presented by Bokermann (1973) and by Toledo *et al.* (2015b). We conclude that both descriptions were probably erroneously assigned to *G. pauloalvini.* Two sympatric species can be heard in the recording used by Bokermann (1973) (FNVJ 32038): *Boana atlantica* in the foreground and a second species in the background with higher pitched call. Bokermann (1973) mentioned that the vocalization of *G. pauloalvini* resembles *Dendropsophus branneri* (Cochran, 1948) and *D. oliveirai* (Bokermann, 1963) (i.e. high pitched calls; see Nunes *et al.* 2007; Santana *et al.* 2011). However, Bokermann (1973) description clearly refers to *Boana atlantica* based on the structural and spectral parameters (see Napoli & Cruz 2005 and listen to FNJV 13053-54, 13057, 30920-21, 33768, and 34137) (Fig. 4B). After analyzing the vocalization used by Toledo *et al.* (2015b) (FNJV 11898), we argue that it probably refers to the sympatric *Sphaenorhynchus prasinus* (see Bokermann 1973; Toledo *et al.* 2015b; and listen to FNJV 0011200 and 0032027) based on both structural (high number of notes per call) and spectral parameters (low pitched vocalization) (Fig. 4A). Therefore, the present study is the first to report the advertisement call of *Gabohyla pauloalvini*. This comparison and conclusion were only possible due to the availability of voucher sound material. Thus, we highlight the importance of depositing audio recordings in scientific collections or online repositories allowing future replication studies (see Toledo *et al.* 2015c).

Compared to other species of the Sphaenorhynchini tribe, the advertisement call of *G. pauloalvini* can be distinguished from the calls of all species (except *S. carneus*) by its dominant frequency above 4500 Hz (Lacerda *et al.* 2011; Lacerda & Moura 2013; Araujo-Vieira *et al.* 2015; Toledo *et al.* 2015b; Forti *et al.* 2017; Roberto *et al.* 2017; Bovolon *et al.* 2020). *Gabohyla pauloalvini* is one of the smallest species of the Sphaenorhynchini tribe, jointly

with *S. carneus* (see Toledo *et al.* 2007). Usually, male size is inversely correlated to dominant frequency of the advertisement call (Gerhardt & Huber 2002; Tonini *et al.* 2020). The call duration of *G. pauloalvini* (0.01–1.976 s, n = 148) differs from *S. carneus* (0.01–4.11 s, n = 16; Toledo *et al.* 2015b). The advertisement call of *G. pauloalvini* resembles calls of *S. botocudo*, *S. bromelicola*, *S. cammaeus*, *S. canga*, *S. carneus*, *S. dorisae* and *S. lacteus* by having fewer notes (1–5 rhythmically sparse within the sequences separated by periods of silence [considered as a call series by Bovolon *et al.* (2020)].

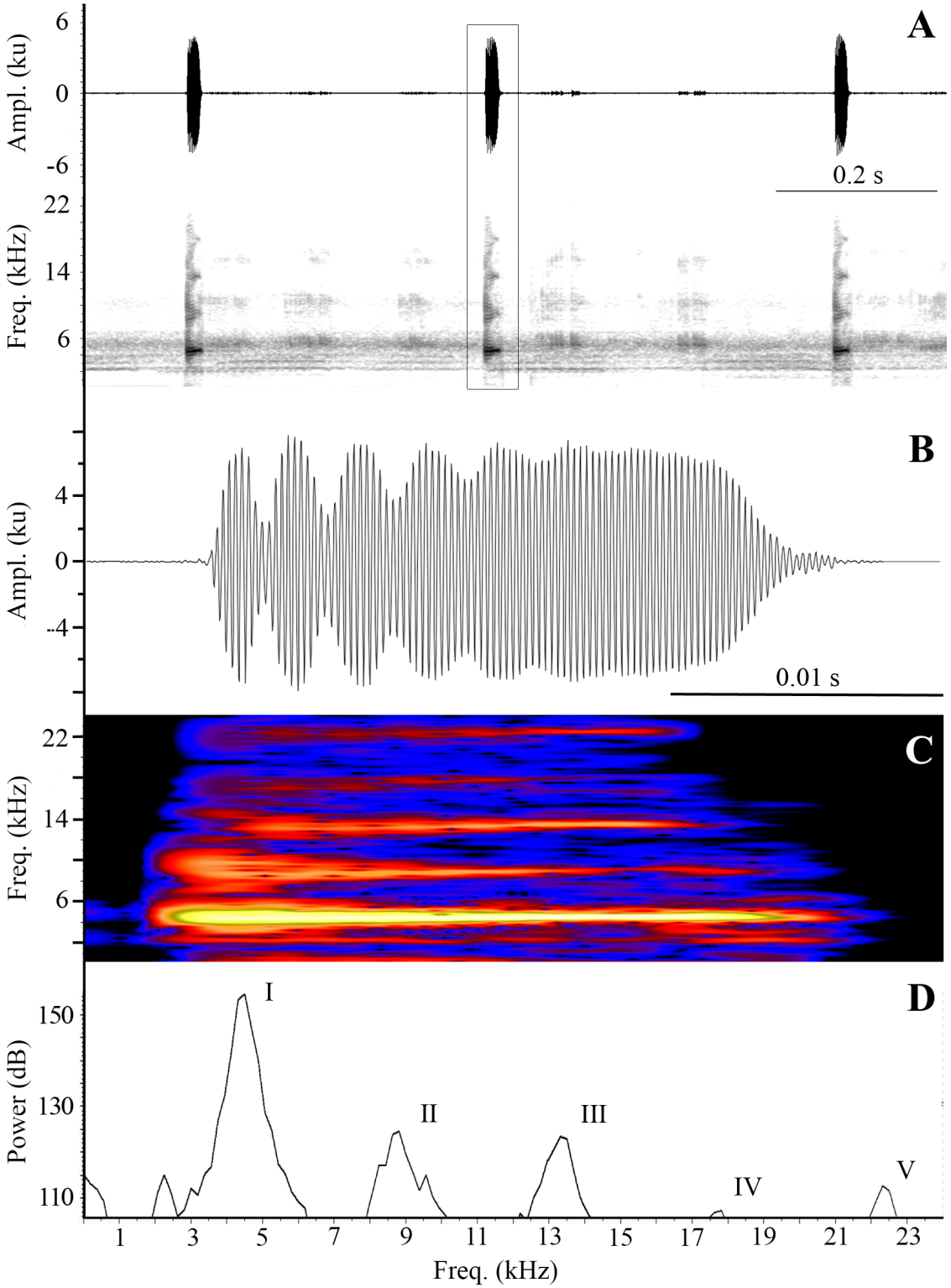

FIGURE 2. Advertisement call of *Gabohyla pauloalvini* from the Estação Biologia Marinha Augusto Ruschi, municipality of Aracruz, state of Espírito Santo, southeastern Brazil. Oscilogram and spectrogram showing three notes emitted in sequence (**A**). Oscilogram (**B**), spectrogram (**C**) and power spectrum (**D**) of a single note, indicating five visible harmonics numbered I to V.

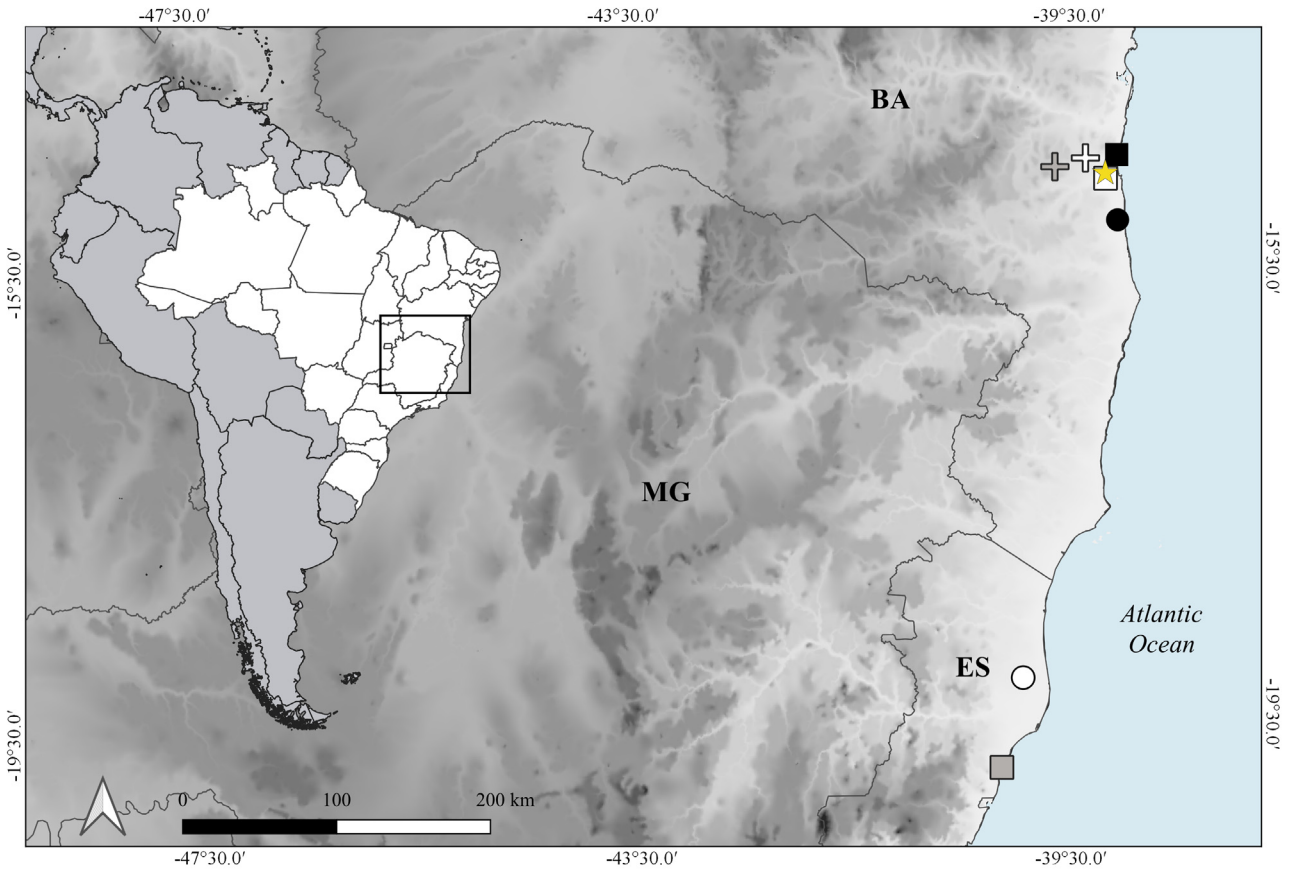

FIGURE 3. Geographic distribution of *Gabohyla pauloalvini*: type locality in the Centro de Pesquisas do Cacau (yellow star; Bokermann 1973), Universidade Estadual de Santa Cruz (white square; present study) and Ponta da Tulha (black square; present study), in the municipality of Ilhéus, state of Bahia; municipality of Una, state of Bahia (black circle; Freitas *et al.* 2009); Estação Biologia Marinha Augusto Ruschi, municipality of Aracruz (grey square; present study), municipality of Linhares (white circle; Almeida *et al.* 2011), state of Espírito Santo; municipality of Almadina (grey plus) and Uruçuca (white plus) in the state of Bahia (new occurrence records; present study). States of Minas Gerais (MG), Bahia (BA), and Espírito Santo (ES).

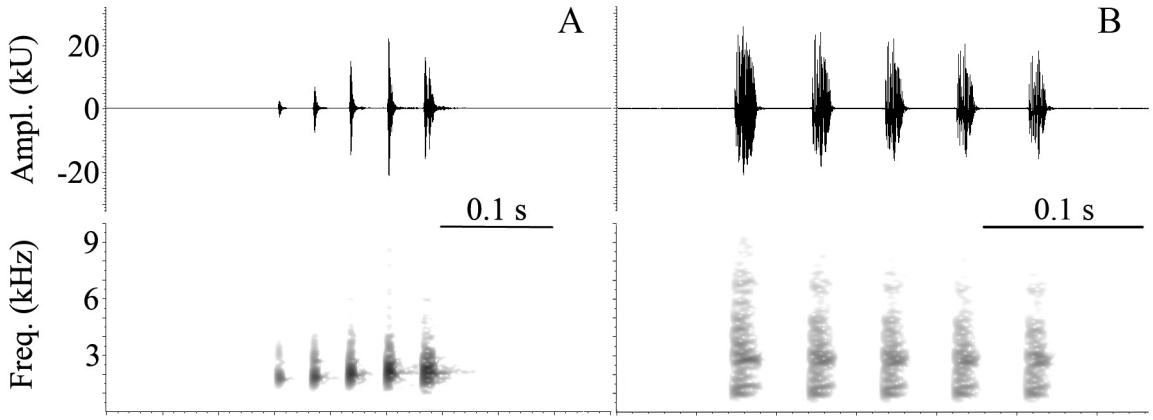

FIGURE 4. Advertisement calls of *Sphaenorhynchus prasinus* (FNJV0011200) and *Boana atlantica* (FNJV0030920): Oscilograms and spectrograms showing a single call of *S. prasinus* **(A)** and *B. atlantica* **(B)**. Filter bandwidth (Hz): 135 (**A** and **B**).

We recorded only advertisement calls of *G. pauloalvini* although some species of Sphaenorhynchini usually emit territorial calls prior to the advertisement calls [*S. canga* (Araujo-Vieira *et al.* 2015), *S. cammeus* (Roberto *et al.* 2017), *S. lacteus* (listen FNJV 11191), *S. botocudo* (Lacerda & Moura 2013), *S. platycephalus* (Heyer *et al.* 1990), *S. surdus* (listen FNJV 11198), *S. planicola* and *S. prasinus* (LFC, personal observations)]. Because *Gabohyla* is the sister taxon of all remaining species of Sphaenorhynchini (Araujo-Vieira *et al.* 2019), we suggest that the territo-

rial call may represent a derived character state of the communication in the evolutionary history of the clade. To test this hypothesis, we argue that more studies should be conducted on the acoustic communication of the species within this clade.

In most anurans, the patterns of variation in acoustic parameters are correlated with male mate selection by females, because each parameter contains biologically significant information (Gerhardt 1991). On one hand, the examined spectral parameters had little variation among the two populations of *G. pauloalvini*, whereas their CVw were smaller than 5%. On the other hand, temporal parameters had higher variation at different levels according to the classification of Gerhardt (1991). These results corroborate with the pattern reported for several other anuran species (Guerra *et al.* 2017; Luna-Dias & Carvalho-e-Silva 2019; Vélez & Guajardo 2021). In acoustic communication, the static properties of signals are significantly more important for intraspecific recognition (Gerhardt 1991; Wollerman 1998; Lin *et al.* 2020). As a result of stabilizing selection, females usually choose males with calls at or near the mean of the population (Gerhardt 1991). Contrastingly, the dynamic properties of acoustic signals with higher CVw are generally understood to be adaptive advantage, driven by directional selection, for mate choice, because females prefer extreme values of male calls (Gerhardt 1991, 1994; Wollerman 1998; Friedl 2006; Köhler *et al.* 2017). Therefore, we suggest that all spectral parameters contributed to species recognition and the temporal parameters might encode information on mate quality in response to their conspecific.

Most Sphaenorhynchini species call perched on marginal and floating vegetation of ponds (e.g., Lacerda *et al.* 2011; Forti *et al.* 2017; Roberto *et al.* 2017). Interestingly, *G. pauloalvini* calls on branches and leaves of shrub and arboreal vegetation, between 2-3 m high (EBMAR, our study). They can also use perches between 0.50 and 1 m high on aquatic vegetation (Bokermann 1973). A similar calling site was reported for *Dendropsophus branneri* by Bokermann 1973, which may have hampered the detection of this species. *Gabohyla pauloalvini* is still known from only few localities and presents a wide sampling gap between the extreme south of Bahia and the north of Espírito Santo (see Fig. 3). We are confident that the description of the advertisement call and the availability of sound archives on digital platforms will facilitate the recording of new populations. Therefore, we highlight the importance of depositing audio recordings and specimens in scientific collections to promote systematics and natural history studies.

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APPENDIX 1. Sound archives (voucher number) deposited in the Fonoteca Neotropical Jacques Vielliard.

Gabohyla pauloalvini, state of Bahia, municipality of Ilhéus, Universidade Estadual de Santa Cruz (UESC): FNJV0050419, 0050420, 0050421, 0050422, 0050423; Ponta da Tulha: FNJV0050424, 0050425, 0050426, 0050427, 0050428, 0050429, 0050430, 0050431, 0050432, 0050433, 0050434, 0050435; state of Espírito Santo, municipality of Aracruz, Estação Biologia Marinha Augusto Ruschi (EBMAR): FNJV50436, 50437, 50438, 50439, 50440, 50441, 50442.