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Advertisement calls of two species of the *Sphaenorhynchus platycephalus* group and the aggressive call of *S. bromelicola* (Anura: Hylidae: Scinaxinae)

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Anuran acoustic communication plays several roles in social interactions, transmitting information in reproductive, aggressive, and defensive contexts (Wells 2007, Toledo et al. 2015a, Köhler et al. 2017). Among the several call types recognized, the advertisement call is the most commonly emitted and studied one (Toledo et al. 2015a). Males emit advertisement calls to attract females, which in turn use call features to assess information as to the specific identity, body size, and physical condition of the calling male (Ryan 1980, Forester & Czarnowsky 1985). Therefore, the advertisement call is species-specific, and key to both sexual and natural selection processes (Ryan 1980, 1983, Forester & Czarnowsky 1985, Tobias et al. 2011). Hence, accurate advertisement call descriptions are fundamental for studies in different fields, such as taxonomy and systematics (Haddad & Pombal jr 1998, Canedo & Pombal 2007, Hepp et al. 2015, Andrade et al. 2016; Forti et al. 2017a), natural history (Muscat et al. 2019, Zornosa-Torres & Toledo 2019), evolution (Tobias et al. 2011, Toledo et al. 2015b, Forti et al. 2018), conservation (Forti et al. 2016), and biodiversity monitoring (Alvarez-Berríos et al. 2016, Sugai et al. 2019, Augusto-Alves et al. 2020).

The Neotropical frog genus *Sphaenorhynchus* Tschudi, 1838 (Hylidae: Scinaxinae) is distributed in the Amazon and Orinoco river basins in South America, and in the Brazilian Atlantic rainforest (Araujo-Vieira et al. 2019, Frost 2020). This genus is currently composed by 14 species (Araujo-Vieira et al. 2020, Frost 2020), and the advertisement calls of all its species have been described, except for *S. bromelicola* and *S. botocudo* (Forti et al. 2019), both of which form part of the *S. platycephalus* species group (Araujo-Vieira et al. 2019). Herein, we describe the advertisement calls of these two remaining species based on recordings from 1965 and 2009, deposited in the collection of the Fonoteca Neotropical Jacques Vielliard (FNJV; Universidade Estadual de Campinas, Unicamp, Brazil). These recordings were made at their respective type localities (Bokermann 1966, Caramaschi et al. 2009).

We analysed 41 advertisement calls from two males of *Sphaenorhynchus botocudo* (FNJV 44121–44122), and 50 advertisement calls and one aggressive call from two males of *S. bromelicola* (both males in the same recording, FNJV 31937). Recordings were digitized with a sampling rate of 44.1 kHz and a sample size of 16 bits for *S. botocudo*, and with 96 kHz and 16 bits for *S. bromelicola*. Prior to analyses, we normalized recordings to the maximum amplitude of -1.0 dB, after removing DC offset (i.e., vertically centring the waveforms on zero), using the software Audacity 2.3.1 (Audacity Team 2019). We measured spectral and temporal parameters from the recordings using Raven Pro 1.5 (Bioacoustic Research Program 2014). Spectral parameters were measured from spectrograms and temporal parameters were measured from oscillograms. Configurations adopted

for analyses were: 69% brightness; 59% contrast; and Fast Fourier Transform length (FFT) of 512.

Calls were characterized using the following parameters: call duration; inter-call interval, call series interval, note duration (used for the aggressive call), inter-note interval (used for the aggressive call), and dominant frequency (using the peak frequency function in Raven). Bandwidth is provided as minimum frequency (using the frequency 5% function in Raven, which excludes 5% of the energy below the selected call) and maximum frequency (using the frequency 95% function in Raven, which excludes 5% of the energy above the selected call). Numerical values are provided as mean ± standard deviation (range in parentheses). We follow the call-centred terminology suggested by Köhler et al. (2017) and the taxonomy presented by Araujo-Vieira et al. (2019).

We used the definition of 'call' as a unit of vocalization, which is separated from other calls by a silent period that is usually longer than the call duration (Köhler et al. 2017); and the definition of 'call series' as calls organized in groups, which are separated from other call series by a silent period that is longer than the inter-call interval (the pattern of these groups can vary or be predictable; Köhler et al. 2017).

The advertisement call of *S. botocudo* is simple, pulsatile, and modulated with a decreasing frequency, and has a dominant frequency of 1.9 ± 0.2 (1.7–2.2) kHz (Fig. 1A; Table 1). This call was emitted alone (54%; n = 15) or organized in series of two calls (46%; n = 13). Individuals of *S. botocudo* were observed calling partially submerged in a pond at the type locality, in the municipality of Mucurici, state of Espírito Santo. Calling males presented a pale orange dorsal coloration and a red lateral stripe (Fig. 2), which distinguishes them from the greenish pattern reported for this and all congeneric species.

The advertisement call of *S. bromelicola* is simple, pulsatile, and modulated with decreasing frequency. Calls were emitted in series of 2.9 ± 1.2 (2–5) calls (Fig. 1B; Table 1). The mean dominant frequency was 2.4 ± 0.5 (1.3–3.0) kHz. Additional spectral and temporal advertisement call parameters of both species are summarized in Table 1.

The only aggressive call of *S. bromelicola* recorded was emitted before an advertisement call (Fig. 1C). It was pulsatile, had a total duration of 241 ms, a mean note duration of 7 ± 1 (5–10; n = 18) ms, and an inter-note interval of 7 ± 11 (0–45; n = 17) ms. The dominant frequency was 2.4 kHz (range 1.1–2.6 kHz).

Previous studies described the advertisement calls of species in the *S. platycephalus* species group as being composed of several notes (Toledo et al. 2007a, 2015b, Lacerda & Moura 2013, Araujo-Vieira et al. 2015, Forti et al. 2017b, Roberto et al. 2017). Here, following the bioacoustical nomenclature proposed by Köhler et al. (2017), we classified a call as a unit separated from other calls by a silent period that was longer than the call duration. This means that what said previous studies referred to as a note, we considered a call, and what they referred to as a call, we considered a call series. In order to standardize the advertisement call descriptions in the *S. platycephalus* species group, we present the comparable (same classification) values in Table 1.

With the descriptions provided in the present study, all *Sphaenorhynchus* species have now their typical advertisement calls described (Forti et al. 2019). These descriptions could be useful not only for species identification, but also for studies of the evolution of acoustic traits in the genus.

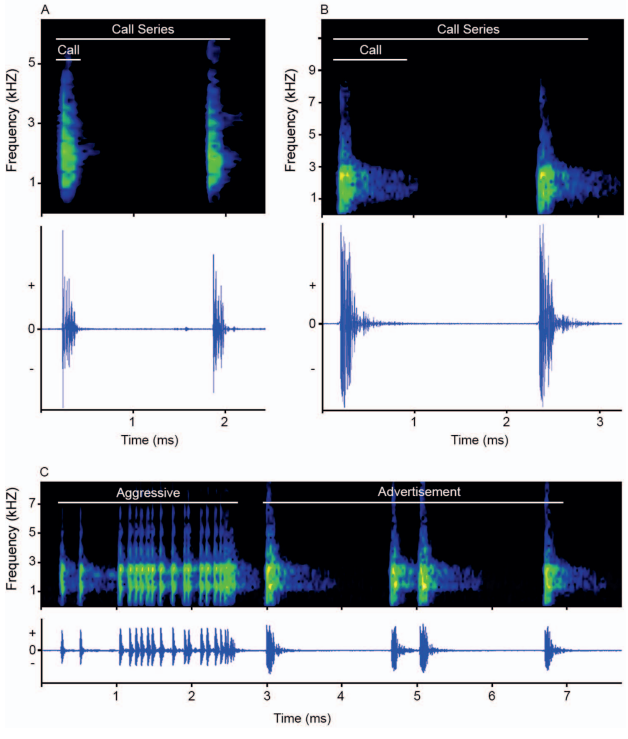

Figure 1. Spectrogram (above) and waveform (below) of the advertisement calls of A) *Sphaenorhynchus botocudo*, B) *S. bromelicola*, and C) the aggressive call preceding an advertisement call of *S. bromelicola*.

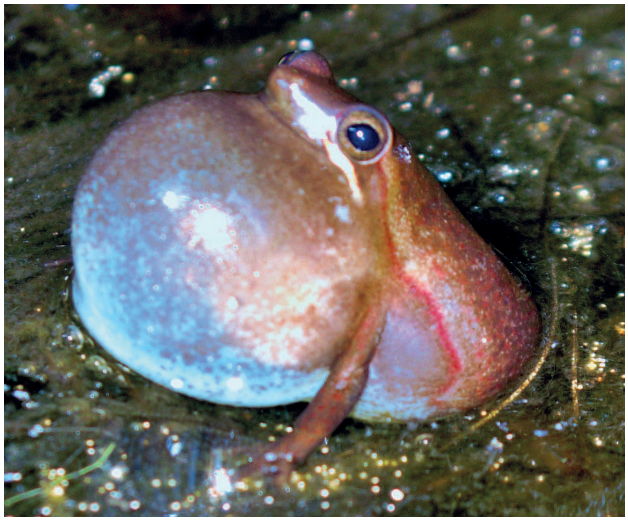

Figure 2. Adult male of *Sphaenorhynchus botocudo* photographed while calling. September 2009, municipality of Mucurici, state of Espírito Santo, Brazil (type locality).

Table 1. Spectral and temporal parameters of the advertisement calls of *Sphaenorhynchus* spp. of the *S. platycephalus* species group; values are presented as means ± standard deviations (minimum – maximum; number of calls analysed/number of males analysed).

	S. botocudo	*S. bromelicola*	*S. cammaeus*	*S. canga*	*S. caramaschii*	*S. palustris*	*S. platycephalus*	*S. surdus*
Calls per call series	1.46 ± 0.5 (1–2; 41/2)	2.88 ± 1.16 (2–5; 50/2)	2.5 ± 0.5 (2–3; 50/4)	2.05 ± 1.43 (1–9; 116/6)	–	1.9 ± 0.7 (1–4; 28/2)	8–12/1	18–22; 27/3
Call duration (ms)	18 ± 2 (16–21; 41/2)	30 ± 5 (19–42; 50/2)	10 ± 3 (8–30; 127/4)	10 ± 3 (5–20; 237/6)	30 ± 10 (12–43; 231/14)	17.0 ± 5.1 (10.7–35.8; 54/2)	20 ± 3 (10–20; 4/1)	21.70 ± 4.43 (13.0–32.0; 27/3)
Inter-call interval (ms)	145 ± 4 (138–152; 13/2)	145 ± 60 (7–212; 33/2)	200 ± 20 (170–240; 77/4)	160 ± 20 (50–230; 119/6)	–	148.6 ± 12.1 (126.7–169.5; 26/2)	190 ± 20 (160–270; 4/1)	68.29 ± 6.93 (52.0–86.0; 27/3)
Call series interval (s)	11.25 ± 4.47 (6.48–26.76; 26/2)	15.93 ± 14.94 (1.32–49.73; 15/2)	14.7 ± 80 (46.8–42.1; 45/4)	8.47 ± 3.79 (2.74–20.19; 107/6)	–	–	–	–
Pulse duration (ms)	–	–	–	4 ± 2 (1–17; 467/6)	–	–	–	–
Minimum frequency (kHz)	1.13 ± 0.1 (1.03–1.29; 37/2)	1.27 ± 0.18 (0.94–1.50; 50/2)	0.98 ± 0.17 (0.52–1.64; 127/4)	–	2.61 ± 0.14 (2.16–2.91; 231/14)	–	1.65 ± 0.06 (1.61–1.74; 4/1)	1.34 ± 0.13 (0.98–1.54; 27/3)
Maximum frequency (kHz)	2.71 ± 0.11 (2.50–2.93; 37/2)	2.81 ± 0.18 (2.62–3.19; 50/2)	3.83 ± 0.27 (3.21–4.84; 127/4)	–	3.20 ± 0.16 (2.86–3.70; 231/14)	–	3.03 ± 0.05 (2.97–3.10; 4/1)	3.41 ± 0.17 (3.17–3.85; 27/3)
Dominant frequency (kHz)	1.93 ± 0.14 (1.72–2.24; 37/2)	2.39 ± 0.45 (1.31–3.00; 50/2)	2.76 ± 0.19 (2.25–3.00; 127/4)	2.84 ± 0.54 (2.07–4.55; 237/6)	2.85 ± 0.11 (2.44–3.23; 231/14)	3.1 ± 0.25 (2.76–3.62; 54/2)	2.41 ± 0; 4/1	2.29 ± 0.03 (2.24–2.37; 27/3)
Reference	Present study	Present study	Roberto et al. (2017)	Araujo-Vieira et al. (2015)	Forti et al. (2017b)	Lacerda & Moura (2013)	Toledo et al. (2015b)	Toledo et al. (2007a)

Sphaenorhynchus botocudo and *S. palustris* are closely related and are suggested to be sister species by the phylogeny of Araujo-Vieira et al. (2019). However, we found a marked dissimilarity in their advertisement calls: *S. botocudo* presents a dominant frequency of 1.9 ± 0.2 kHz (the lowest dominant frequency in the *S. platycephalus* group) and *S. palustris* presents a dominant frequency of 3.1 ± 0.3 kHz (Lacerda & Moura 2013). The type localities of these species are close and sympatric populations may exist, which could have imposed major selective pressures favouring call divergence (see Toledo et al. 2015b).

Other studies have described a second call type for congeneric species (Lacerda & Moura 2013, Araujo-Vieira et al. 2015, Roberto et al. 2017), which was named Type II call. We identified a similar pattern and decided to classify the second call type of *S. bromelicola* as an aggressive call, based on the general pattern observed in Neotropical, aggressive, and generally territorial anurans whose aggressive calls are composed of series of notes that are emitted at faster rates compared to their advertisement calls (e.g., Wells & Schwartz 1984, Toledo et al. 2007b). The territorial call is a subcategory of the aggressive call (Toledo et al. 2015a), but we could not relate the recorded *S. bromelicola* call to a territorial dispute, so that the proper social context must be left for future studies to explore.

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