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ARTICLE

Redescription of the advertisement calls of *Scinax tigrinus* and *Scinax maracaya* (Anura: Hylidae) and an evaluation of their differential diagnosis

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ABSTRACT: (Redescription of the advertisement calls of *Scinax tigrinus* and *Scinax maracaya* (Anura: Hylidae) and an evaluation of their differential diagnosis). Tree-frogs of the genus *Scinax* comprise a conspicuous group within Hylidae, and their distribution ranges from southeast Mexico to northern Argentina. In this work, we redescribe and compare the advertisement calls of *S. maracaya* and *S. tigrinus* in order to evaluate their differential diagnosis. We also extend the known distribution of *S. tigrinus* approximately 200 km south from the type locality. Our description of the advertisement call of *S. tigrinus* is consistent with that presented in the literature, and therefore, allows for the extension of its distribution range. In relation to the calls of *S. maracaya* compared to their original description in literature, we report some differences regarding the frequency and pulse duration. When the advertisement calls of the two species were compared, we were unable to distinguish any diagnostic feature, however, significant differences were found for the duration and number of pulses per call. *S. tigrinus* and *S. maracaya* represent an example of two species that can be differentiated morphologically, mainly by their dorsal color pattern and texture, but are relatively acoustically conserved. The similarity between calls is expected as these species are allopatric, and therefore, there are no selective pressures leading to character displacement between calls.
Keywords: Advertisement call, Taxonomy, Cerrado biome, *Scinax*, *Scinax tigrinus* distribution.

RESUMO: (Redescrição dos cantos de anúncio de *Scinax tigrinus* e *S. maracaya* (Anura: Hylidae) com uma avaliação de suas diagnoses diferenciais). Pererecas do gênero *Scinax* compreendem um grupo conspícuo dentro de Hylidae e sua distribuição se extende do sudeste do México até o norte da Argentina. No presente trabalho, re-descrevemos os cantos de anúncios de *S. maracaya* e *S. tigrinus* e comparamos seus cantos a fim de avaliar suas diagnoses diferenciais. Também estendemos a distribuição conhecida de *S. tirinus* ca. de 200 km para o sul da localidade tipo. Nossa caracterização do canto de anúncio de *S. tigrinus* está de acordo com o apresentado na literatura. Em relação aos cantos de *S. maracaya* e a descrição original na literatura, algumas diferenças em relação à frequência e duração dos pulsos são observadas. Quando comparados os cantos de anúncios de ambas as espécies, nenhuma característica diagnóstica pôde ser observada entre elas, apesar de que diferenças significativas foram encontradas na duração e número de pulsos por canto. *S. tigrinus* e *S. maracaya* representam um exemplo onde espécies podem ser diferenciadas morfologicamente, principalmente pela coloração e textura dorsais, mas são relativamente conservadas acusticamente. A similaridade entre os cantos é esperada, visto que as espécies ocorrem em alopatria e, portanto, não há pressão seletiva causando o deslocamento de caráter entre os cantos.
Palavras chave: Canto de anúncio, taxonomia, Bioma Cerrado, *Scinax*, distribuição de *Scinax tigrinus*.

INTRODUCTION

Tree-frogs of the genus *Scinax* Wagler, 1830 comprise a conspicuous group within Hylidae. There are approximately 65 recognized species (Frost 2016) which are distributed through the Neotropics, from southeast Mexico to northern Argentina (Duellman *et al*. 2016, Frost 2016). Recent studies have supported *Scinax* monophyly on both morphological and molecular bases (Faivovich 2002, Faivovich *et al.* 2005, Duellman *et al*. 2016). *Scinax maracaya* (Cardoso & Sazima) and *Scinax tigrinus* Nunes, Carvalho & Pereira are two species that inhabit the Brazilian Cerrado biome. In the original description of *S. tigrinus* (Nunes *et al*. 2010), the authors recognized that *S. tigrinus* morphologically resembles *S. maracaya*, however, an acoustic comparison was not provided, probably due to a small sample size. The genus comprises a great number of species, and therefore, the taxonomy is complex and sometimes confusing. Therefore, in this study we aimed to redescribe the advertisement calls of *Scinax maracaya* and *S. tigrinus*, based on newly gathered data, in order to evaluate, mainly through acoustics, their differential diagnosis.

MATERIALS AND METHODS

Voucher specimens and call recordings are housed in the Coleção de Anuros do Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia (AAG-UFU; Uberlândia, Minas Gerais, Brazil). *Scinax tigrinus* specimens and recordings were collected from Presidente Olegário, Reserva Particular do Patrimônio Natural (RPPN), Fazenda Vereda Grande (18°11'25.98" S 46°30'58.80" W; approximately 1000 m above sea level

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a.s.l.; datum WGS84) in the state of Minas Gerais (MG) and from Cristalina (16°44'9.18" S 47°34'14.06" W; approximately 1200 m a.s.l.; datum WGS84; in the state of Goiás (GO). *S. maracaya* specimens and recordings were collected from São Roque de Minas, Parque Nacional da Serra da Canastra (MG) (20°14'35.36" S 46°26'46.66" W; approximately 850 m a.s.l.; datum WGS84). Measurements of the snout-vent length (SVL) were taken from collected adult males using a Mitutoyo caliper (to the nearest 0.1 mm). We also compared the morphology and color patterns of specimens with those described in the literature (Cardoso & Sazima 1980, Nunes *et al.* 2010) to confirm species identity. The specimens analyzed can be found under the following accession numbers: *Scinax tigrinus,* Presidente Olegário – Reserva Vereda Grande: AAG-UFU 1665–71, 4990; Cristalina: 1793–94; *Scinax maracaya*, São Roque de Minas – Parque Nacional da Serra da Canastra: AAG-UFU 606–7, 663.

Vocalizations were recorded in the field using Marantz PMD 671 and MicroTrack recorders (set at 44.1 kHz sampling rate and 16-bit resolution), which were coupled to Sennheiser K6/ME67 and K6/ME66 microphones, respectively. Calls were analyzed using Raven Pro 1.5 software (Bioacoustics Research Program, 2012) with the following settings: Hanning window, window size of 256 samples; 3dB filter bandwidth of 270 Hz, 90% overlap (locked), hop size of 0.542 ms, Discrete Fourier Transform (DFT) size of 1024 samples, and grid spacing at 46.9 Hz. The brightness and contrast were set to 60% and 70%, respectively, for *S. maracaya*, and both were set to 70% for *S. tigrinus*. All other settings were set to the default software settings. Calls were filtered up to 200 Hz prior to the analyses in order to reduce the background (wind) noise. Temporal features were measured from oscillograms, and the spectral features from spectrograms. The dominant frequency was measured using the "peak frequency" function of the software. The acoustic terminologies and definitions are listed in Table 1. The sound figures were made using the Seewave package (version 1.7.6; Sueur *et al.* 2008) in R software (version 3.1.2; R Core Team 2013), using the following settings: Hanning window, 85% overlap, and Fast Fourier Transform (FFT) with 256 points.

To evaluate the statistical significance of differences for features of the calls (call duration and number of pulses per call) between the two species, we conducted an Exact Wilcoxon Mann-Whitney Rank Sum Test ("wilcox_test" in the coin package; Hothorn *et al.* 2008) using R software. Values of $p \leq 0.05$ were considered to be statistically significant.

RESULTS

Morphology

Scinax tigrinus (Fig. 1A). SVL ranging from 26.7 to 28.6 mm (mean = 27.9 mm; SD = 0.6 mm; n = 10 males); dorsum characterized by a beige to dark-brown background with elongated dark blotches, transversely disposed, outlined by beige lines and with small light spots irregularly disposed all over the dorsum; in preservative, presence of dark and light (intercalated) transversely disposed stripes on thigh; tarsal region with a lack of dermal fold or tubercles; granulose dorsal skin; vocal sac with smooth texture; round inner metacarpal tubercle and cordiform outer metacarpal tubercle.

Scinax maracaya (Fig. 1B). SVL ranging from 27.5 to 29.0 mm (mean = 28.4 mm; SD = 0.7 mm; n = 3 males); dorsum characterized by a brown to dark-brown background with elongated dark blotches and some smaller blotches irregularly disposed, outlined by cream lines; in preservative, presence of dark and light (intercalated) transversely disposed stripes on thigh; tarsal region with a lack of dermal fold or tubercles; smooth or scattered granulated dorsal skin; vocal sac with smooth texture; round inner metacarpal tubercle and cordiform outer metacarpal tubercle.

Advertisement calls

Scinax tigrinus. Call measurements are summarized in Tab. 2. Four males from Presidente Olegário (MG) and two from Cristalina (GO) were recorded and, due to their similarities, all samples were pooled for the analyses. The advertisement call (Fig. 2A) consisted of a single multipulsed note with duration of 78–339 ms (n = 60 calls), with 4–14 pulses per call (n = 60). The call rate ranged from 38.5–92.6 calls/min (n = 18). Calls were emitted at

Table 1. Acoustic terminologies and definitions employed in the present study.

Acoustic traits	Definition
Call duration (ms)	Time from beginning to end of one call (calls corresponded to one single note in both *Scinax tigrinus* and *S. maracaya*). A call was defined as being the sound produced during a single exhalation cycle.
Number of pulses	Total number of pulses within a single call.
Call rate	Number of calls emitted in a series, expressed as calls/minute.
Interval between calls	Time between the end of one call and the beginning of another.
Pulse duration (ms)	Time between the beginning and end of a single pulse within a call. The first, middle and last pulses were measured.
Pulse interval (ms)	Time between the end of one pulse and the beginning of another. Measured during the beginning, middle and last portion of each call.
Pulse rate	Total number of pulses divided by the call duration, expressed as pulses/second.
Dominant frequency (kHz)	Frequency with the greatest energy, determined from an entire call.

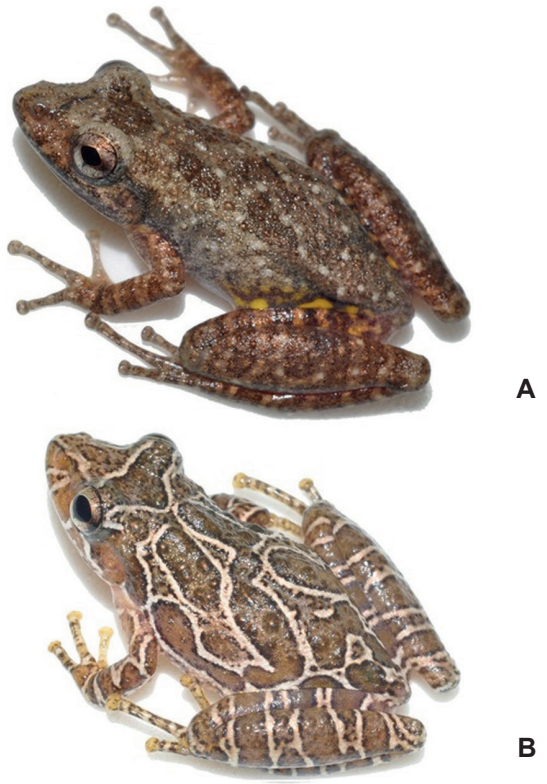

Figure 1. A. Adult male in life of *Scinax tigrinus* (voucher specimen AAG-UFU 1665) collected from Presidente Olegário (state of Minas Gerais). B. Adult male in life of *S. maracaya* (voucher specimen AAG-UFU 0052) collected from São Roque de Minas (state of Minas Gerais).

irregular intervals of 0.39–2.78 s (n = 43 intervals). The majority of calls had an ascendant amplitude modulation from the first to third pulse, until it reached a plateau, which was sustained throughout the call duration. Pulses were well defined with complete amplitude modulation between each pulse. The first pulse always had a lower intensity, with a duration of 9–19 ms (n = 40 pulses); mid-call pulses had a duration of 11–18 ms (n = 40), and the last pulses had a duration of 11–23 ms (n = 40). The interval between pulses during the first part of the call had a duration of 6–11 ms (n = 40 intervals), mid-call the duration was 7–11 ms (n = 40), and in the last part of call the duration was 6–14 ms (n = 40). The pulse rate ranged from 38.7–53.7 pulses/s (n = 60). The dominant frequency ranged from 2.76–3.46 kHz (n = 60).

Scinax maracaya. Call measurements are summarized in Tab. 2. Four males from São Roque de Minas (MG) were recorded. The advertisement call (Fig. 2B) consisted of a single multipulsed note with duration of 169–510 ms (n = 40 calls), with 7–20 pulses per call (n = 40 calls). The call rate ranged from 44.9–60.4 calls/min (n = 7). Calls were emitted at irregular intervals of 0.65–2.83 s (n = 36 intervals). The majority of calls had an ascendant amplitude modulation from the first to the third pulse, until it reached a plateau, which was sustained throughout the call duration. Pulses were well defined with complete amplitude modulation between each pulse. The first pulse always had a lower intensity, with a duration of 10–17 ms (n = 40 pulses); mid-call pulses had a duration of 11–19 ms (n = 40), and the last pulses had a duration of 11–17 ms (n = 40). The interval between pulses in the first part of call had a duration of 6–11 ms (n = 40 intervals), mid-call the duration was 7–10 ms (n = 40), and the last portion had a duration of 6–12 ms (n = 40). The pulse rate ranged from 39.2–49.1 pulses/s (n = 40). The dominant frequency ranged from 2.39–3.09 kHz (n = 40).

Table 2. Acoustic features of the advertisement calls of *Scinax tigrinus*, collected from Presidente Olegário (state of Minas Gerais) and Cristalina (state of Goiás), and *S. maracaya*, collected from São Roque de Minas (state of Minas Gerais), compared with those described in the literature (type locality of both species). Measurements are presented as range (mean ± standard deviation). N/A, data not available.

Call features	*Scinax tigrinus*		*Scinax maracaya*	
	Present study (60 calls, 6 males)	**Nunes *et al.* 2010 (40 calls, 2 males)**	**Present study (40 calls, 4 males)**	**Cardoso and Sazima 1980 (N/A)**
Call duration (ms)	78–339 (213 ± 41)	80–340 (210 ± 60)	169–510 (306 ± 42)	280
Call rate (calls/min)	38.5–92.6 (62.6 ± 13.8)	N/A	44.9–60.4 (49.5 ± 5.7)	42
Interval between calls (s)	0.39–2.78 (1.82 ± 1.84)	0.60–6.53 (1.07 ± 0.95)	0.65–2.83 (1.05 ± 0.15)	N/A
Number of pulses	4–14 (9 ± 1)	4–15	7–20 (12 ± 1)	11 or 12
First pulse duration (ms)	9–19 (13.5 ± 0.2)	N/A	10–17 (13.4 ± 1.5)	N/A
Mid-call pulse duration (ms)	11–18 (13 ± 1)	N/A	11–19 (15 ± 2)	10
Last pulse duration (ms)	11–23 (15.2 ± 1.7)	N/A	11–17 (13.5 ± 0.6)	N/A
First portion pulse interval (ms)	6–11 (8.1 ± 1.1)	N/A	6–11 (8.4 ± 0.8)	N/A
Mid portion pulse interval (ms)	7–11 (8 ± 1)	N/A	7–10 (8 ± 1)	N/A
Last portion pulse interval (ms)	6–14 (8.6 ± 1.6)	N/A	6–12 (8.6 ± 1.3)	N/A
Pulse rate (pulses/s)	38.7–53.7 (45.8 ± 5.9)	N/A	39.2–49.1 (42.3 ± 5.4)	N/A
Dominant frequency (kHz)	2.76–3.46 (3.13 ± 0.2)	2.93–3.27 (3.06 ± 0.03)	2.39–3.09 (2.99 ± 0.04)	1.00–3.50
Minimum frequency (kHz)	1.15–1.25 (1.19 ± 0.04)	N/A	0.96–1.30 (1.16 ± 0.09)	N/A
Maximum frequency (kHz)	3.61–4.74 (4.11 ± 0.40)	N/A	3.40–4.28 (4.01 ± 0.24)	N/A
Temperature (°C)	21	N/A	16–20 (18 ± 2)	25

The calls of both species overlapped in all measured features, however, we identified significant differences for the call duration (Z = 2.132, p = 0.0381) and the number of pulses per call (Z = 2.345, p = 0.0190), both of which were longer in the calls of *Scinax maracaya* (Fig. 3).

DISCUSSION

The specimens that we collected from Cristalina (GO) and Presidente Olegário (MG) match the species description for *S. tigrinus* regarding their morphological and coloration patterns. In the original description (Nunes *et al.* 2010), the call characterization was based on two males. Our acoustic data are consistent with the description by Nunes *et al*. (2010) for the dominant frequency, call duration and the number of pulses. Therefore, the new recordings from the species in Cristalina and Presidente Olegário extend their geographical distribution to approximately 200 km south from the type locality. However, even with this range extension, *S. tigrinus* remain an endemic species to the Cerrado biome of Central Brazil.

In relation to the advertisement call originally described by Cardoso & Sazima (1980) (probably only one male recorded) for *S. maracaya*, we describe similar results for the call duration, call rate, number of pulses and the dominant frequency. However, our measurements for the pulse duration were higher than that previously reported (15 vs. 10 ms in the original description). Cardoso & Sazima (1980) also considered a frequency band at around 500 Hz to be a call component (see Fig. 6 in Cardoso & Sazima 1980), which may actually represent background noise. The main differences between the

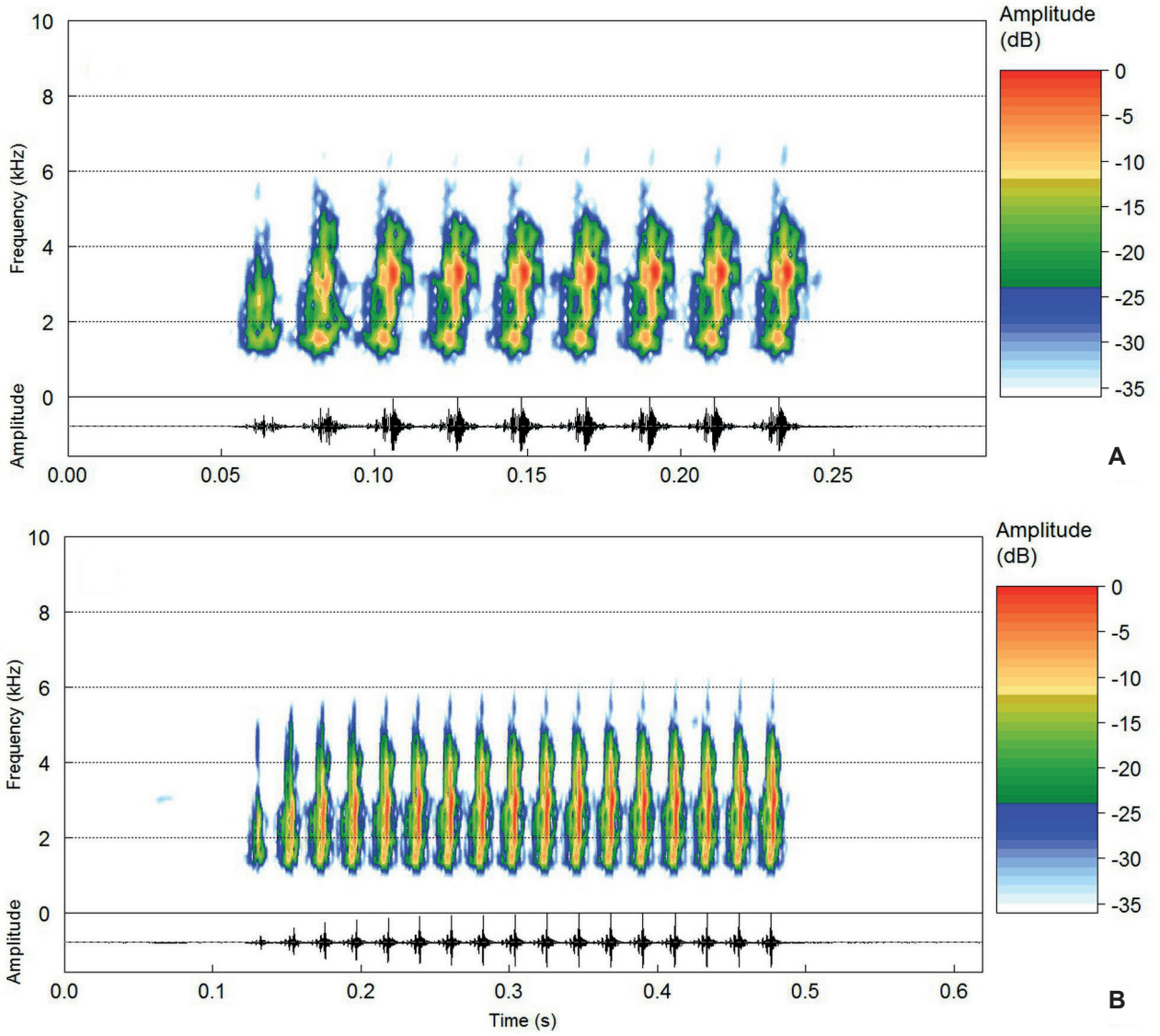

Figure 2. A. Spectrogram and waveform of the advertisement call of *Scinax tigrinus*. The specimen was collected at 21:00 during October 2010 from Presidente Olegário (state of Minas Gerais), with air and water temperatures of 21 ºC and 25 ºC, respectively (sound file Scinax_tigrinusPresOlegarMG2dAAGm671). B. Spectrogram and waveform of the advertisement call of *Scinax maracaya*. The specimen was collected at 21:20 during November 2011 from São Roque de Minas (state of Minas Gerais), with an air temperature of 18.1 ºC (sound file Scinax_maracayaMG12cTRCmt).

pulse duration measured for the call sample in the current study and that reported in the literature may be explained by imprecise measurements (e.g. older recording devices and analysis tools). In addition, as the morphology and coloration patterns of our samples were comparable to the original description (Cardoso & Sazima 1980), we are confident in attributing the species name to the specimens collected in São Roque de Minas.

The majority of morphological features of our specimens mostly matched the original descriptions of both *Scinax tigrinus* and *S. maracaya*. However, with the assessment of new non-topotypical populations, we were unable to make clear between-species discrimination based on some of the characteristics employed by Nunes *et al*. (2010). For instance, the SVL, tarsal region (with lack of dermal folding and tubercles) and the metacarpal tubercles were some characters that we observed for both species that were not useful between species discrimination. On the other hand, the dorsal coloration patterns and texture represented reliable characters so as to diagnose *S. tigrinus* in comparison with *S. maracaya*. We are aware that these indistinctive characteristics could be attributed to intraspecific differences, and therefore, these variations should be revisited with the reevaluation of type material or topotypical specimens in order to clearly establish the morphological diagnosis between the two species.

The general structure of the advertisement calls of *Scinax tigrinus* and *S. maracaya* were similar in regard to their spectral and temporal features. However, these features were found to be statistically different because the calls of *S. maracaya* were longer and had a higher number of pulses when compared to *S. tigrinus* (Fig. 3). Due to the substantial overlap for all measured features, none of the analyzed acoustic features should be considered diagnostic between the species. The quantitative parameters measured are known to be dynamics properties of calls (*sensu* Gerhardt (1994)) and are expected to vary considerably depending on the temperature. We did not evaluate the effect of temperature on call measurements in this study, however, we believe that the impact on the observed values was low (temperature presented in Tab. 2). Henceforth, given this approach of evaluating both the morphological features and advertisement signals, we report that the dorsal coloration pattern and texture represents the most straightforward characteristic that should be employed in both species discrimination.

In this study we have reported another instance where species (*S. tigrinus* and *S. maracaya*) can be differentiated based on their morphological features, despite their calls being relatively acoustically conserved, with a similar pattern to that previously reported in other groups of frogs (Garcia *et al.* 2001, Martins & Giaretta 2013, Teixeira *et al.* 2013). It is expected that species occurring in allopatry will show similar acoustic traits and structures (Duellman & Pyles 1983), since there is a lack of selective pressures (e.g. acoustic interference for mate recognition), which seems to be the case for *S. tigrinus* and *S. maracaya*. Therefore, there was no evident process of reproductive character displacement (Brown & Wilson 1856, Höbel & Gerhardt 2003) that would lead the acoustic divergence between the two species, as previously reported in populations in sympatry (Höbel & Gerhardt 2003), syntopy (Martins *et al*. 2016), and in cryptic species (Carvalho & Giaretta 2013). It is also noteworthy that the stereotyped multipulsed pattern reported for these two species is common among the *Scinax* genus (Pombal Jr. *et al.* 1995, Carneiro *et al.* 2004, Magrini *et al.* 2011, Faria *et al.* 2013), probably representing a phylogenetically conserved feature (Erdtmann & Amézquita 2009).

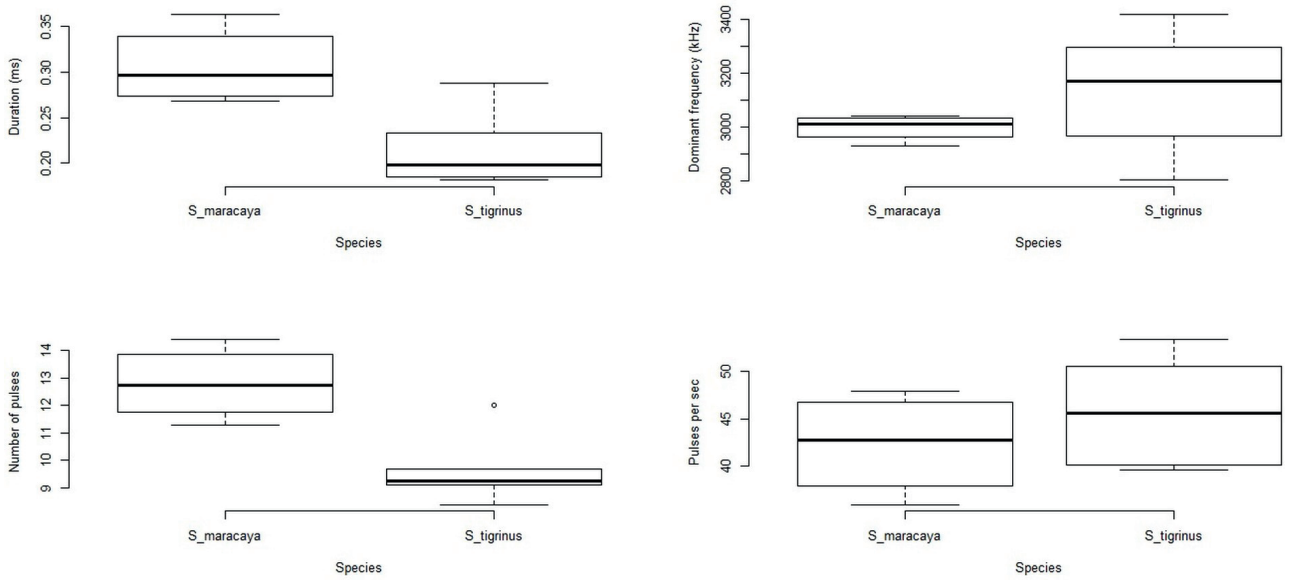

Figure 3. Box-and-whisker plots for four features of the advertisement calls of *Scinax tigrinus* and *Scinax maracaya*. *Scinax tigrinus* data was collected from Cristalina (state of Goiás) and Presidente Olegário (state of Minas Gerais), and *S. maracaya* data was collected from São Roque de Minas (state of Minas Gerais). The duration and number of pulses were significantly different between species ($p < 0.05$).

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