Acoustic variation in the advertisement call of the Lime treefrog *Sphaenorhynchus caramaschii* (Anura: Hylidae)

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Abstract

Frog vocalizations are acoustic signals that contain specific recognition information, so detailed descriptions of calls are critical for improved taxonomic understanding and to establish boundaries between species. The study of the variation of acoustic properties, both within and among individuals, contributes to species diagnosis and can provide support for phylogenetic hypotheses. Herein we provide a more thorough description of the vocalizations of *Sphaenorhynchus caramaschii*, which includes analysis of the variation in call characteristics within and among individuals. We analyzed 305 calls of 14 males from three municipalities (Ribeirão Branco, Iporanga, and Ribeirão Grande) and compared the acoustic properties among these populations. Two types of notes were recognized in the advertisement call: a previously undescribed note with multiple pulses, called "A", and another note with single pulse, called "B". The temporal properties of the call were considered dynamic because of a high level of variation, and the spectral properties were considered static (with low variation) among calls from the same individual and those from different individuals. The advertisement call description presented in this paper is very distinct from the previous description, which was based on a single male recorded at Piraquara (140 km from the type-locality). Herein we discuss the implications of the variation in acoustic properties among populations. In addition, we present data on the effect of air temperature on dynamic acoustic properties and calling sites used by the species.

Key words

Vocalization, Anura, call variation, *Sphaenorhynchus*, acoustic properties.

Introduction

A critical component of reproductive behaviour for most anuran amphibians is the acoustic signal (Wells, 2007). The main sound produced by males during the reproductive season is the advertisement call, which is utilized for species recognition, sexual selection, and territory defense (Wells, 1977; Gerhardt & Davis, 1988; Toledo *et al.*, 2014). For these reasons, many of the acoustic properties of the advertisement call operate as pre-zygotic reproductive isolation mechanisms, and consequently are important for taxonomic inferences (Littlejohn, 1965; Duellman & Trueb, 1994; Goicoechea *et al.*, 2010).

Examining the variation in acoustic properties of advertisement calls at different organization levels (individuals, populations, species) contributes to efforts for taxonomic delineations among species and generates hypotheses about the biological meaning of acoustic traits (Giacoma & Castellano, 2001). Gerhardt (1991) classified the properties of frog calls as static or dynamic using a coefficient of variation, and defined static properties as those that vary 5% or less within individual males, and dynamic properties as those that vary 12% or more within individuals. Spectral properties of the call, includ-

ing the dominant frequency, are typically static (or stereotypical) and show little variation within and among conspecific males (Gerhardt & Huber, 2002). However, this property can vary significantly with body size of the calling male (Bee, 2002) or with geographic distance among populations (Smith *et al.*, 2003; Smith & Hunter, 2005). In contrast, temporal properties, such as call rate and call duration, are typically dynamic and usually vary according to environmental conditions and social context of the calling male (Castellano *et al.*, 2002; Lingnau & Bastos, 2007; Morais *et al.*, 2012).

Given the enormous anuran diversity and the high rate of new species description in South America, the vocal repertoires of most species remain unknown or poorly described (e.g. Cruz *et al.*, 2012; Lehr & Catenazzi, 2009; Targino *et al.*, 2009). The acoustic repertoire of *Sphaenorhynchus caramaschii* is poorly known, and the original call description was based in part on the advertisement call of a single male from municipality of Piraquara, in the southern Brazilian state of Paraná (Toledo *et al.*, 2007; 2014). This location is 140 km from the type-locality, where the species occurs in sympatry with its congener *S. surdus* (Toledo *et al.*, 2007). Therefore our study aimed (1) to re-describe the advertisement call of *S. caramaschii* based on more robust sampling and using recordings taken within and near the type-locality; (2) to present an analysis of the variation in call properties within and among individual males; (3) to evaluate the effect of air temperature on the dynamic properties of the advertisement call; and (4) to describe calling sites used by males.

Material and Methods

Study area. We obtained all recordings from permanent ponds in three municipalities of the southern portion of the state of São Paulo, southeastern Brazil: Ribeirão Branco (the type locality of *S. caramaschii*) (24°13′20″ S, 48°45′53″ W), Ribeirão Grande (24°16′30″ S, 48°24′54″ W) and Iporanga (24°32′52″ S, 48°41′05″ W). The recording of the male from Ribeirão Branco by Adão Cardoso on 11 November 1985 was acquired from the bioacoustic collection of Fonoteca Neotropical Jacques Vielliard (FNJV) (audio file access code: FNJV 11199). We recorded 13 additional males from Ribeirão Grande from 31 October to 1 November 2010 and 8−11 February 2011, and males from Iporanga on 28 November 2010.

The three municipalities are about 30 km apart and are characterized by perennial vegetation and high annual rainfall (CEPAGRI, 2013). The climate of Ribeirão Branco and Ribeirão Grande is humid subtropical (Köppen class Cwa), with mean annual temperature of 18.6 and 20.1°C, respectively, and with both sites located at an altitude varying between 600 and 850 meters above sea level. Iporanga is located 80 m a.s.l. with a tropical rainforest climate (Köppen class Af), and average annual temperature is 23.8°C (CEPAGRI, 2013).

Data collection. Our samples included calls from 14 males of *Sphaenorhynchus caramaschii* (one from Ribeirão Branco, nine from Ribeirão Grande, and four from Iporanga). For the 13 males that our team recorded in 2010-2011, we first recorded the vocalization, then recorded calling site data including perch type (emergent plants, water surface, or plants adjacent to water), height relative to water surface, and horizontal distance from water. Our digital recordings were made with a Marantz PMD660 recorder at a sampling rate of 48 kHz and 16 bit of sample size, and a Yoga EM 9600 microphone positioned about 1 m from each calling male. One male was recorded with an Uher 4000 analogical recorder and M538 microphone. For each recorded call sequence, the air temperature was measured with a thermometer (0.1°C accuracy). Recorded individuals were immediately collected to be retained as voucher specimens, fixed in 10% formalin, and later preserved in 70% alcohol and measured for snout-vent length (SVL) with digital callipers (0.1 mm accuracy). We deposited voucher specimens in the vertebrate collection of Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Brazil (ICMBio license number 23799-1), with the access codes VESALQ 678, 689, 716, 782, 800, 813, 881, 905, 926, and 935. We deposited calling records of four males in the Fonoteca Neotropical Jacques Vielliard, which are accessible by the numbers FNJV 31731-31734.

Acoustic analysis. We analyzed acoustic properties of 305 advertisement calls from 14 males with the software Raven pro 64 1.4 for Windows (Cornell Lab of Ornithology – Bioacoustics Research Program 2011), using FFT (Fast Fourier Transformation) = 1024 for spectral measurements and 256 for temporal visualization, and Overlap = 50% for both purposes. To clean the spectral window in every analysis, we used a frequency band-pass filter with 1200 Hz as the lower limit and 4400 Hz as the upper limit after an initial visual analysis to check the frequency positions. We analyzed the following quantitative call properties: number of total notes, number of notes "A", number of notes "B", call repetition rate, call duration, frequency range of note "A", frequency range of note "B", minimum frequency of note "A", minimum frequency of note "B", maximum frequency of note "A", maximum frequency of note "B", dominant frequency of note "A", dominant frequency of note "B", duration of note "A", duration of note "B", and pulse rate of note "A". The spectral measurements were obtained by selecting four variables in the "choose measurements" option in Raven 1.4: (1) Bandwidth 90% (Hz) – measures the band frequency included in 90% of the energy distribution (frequency range); (2) Peak Frequency (Hz) is the frequency with maximum energy (dominant frequency); (3) Frequency 5% (Hz) and (4) Frequency 95% (Hz) – which include the minimum and maximum frequency, ignoring the 5% above and below the frequency band formed by the energy distribution.

Call variation analysis. We assessed the variation in these acoustic properties with the coefficient of variation (CV = standard deviation/mean × 100) for each acoustic property, within (intraindividual variation) and among males (interindividual variation). Here we used the classification for variation by GERHARDT (1991), in which properties of the vocalization are considered static if they vary less than 5%, and dynamic if they vary 12% or more. The ratio of coefficients of variation (CVs ratio = among male CV/ mean of within male CV) was used to assess the magnitude of variability for each call property among males (e.g., BEE *et al.*, 2001; MÁRQUEZ & EEKHOUT, 2006).

Data analysis. To test the effect of air temperature on call rate, total number of notes per call, and call duration, we used a linear regression analysis. Our data met the assumptions of parametric tests. To perform the regressions, the acoustic characteristics were considered dependent variables and air temperature was considered the independent variable. Two multivariate discriminant analyses with the acoustic properties of notes "A" and "B" as discriminating factors were performed to compare advertisement calls among populations. Statistical tests were performed in SYSTAT 12 (WILKINSON, 2010) with a significance level of α = 0.05.

Results

Call description. The advertisement call of *Sphaenorhynchus caramaschii* is composed of 1 to 43 notes. Calls can contain two distinct notes: a long note with evident pulses called "A" and a short note with one pulse called "B" (Fig. 1). Both notes decrease in intensity through the duration of the note. Males produce calls that can be composed of sequences of note "A" with a single or several notes "B", or only sequences of notes "B".

The mean call repetition rate was 16 ± 8 calls per minute (ranged from 4 to 33; n = 14). Calls were composed of 0 to 26 notes "A" (mean = 1.3 ± 1.3; n = 305), and of 1 to 27 notes "B" (mean = 3.1 ± 3.6; n = 305). Values of the quantitative acoustic properties of note "A" and "B" are displayed in Table 1.

Within and among male variations. When considering within male variation, the temporal properties were considered dynamic because all individuals exhibited a mean coefficient of variation higher than 12%. The mean coefficient of variation of call duration, number of note "A", number of note "B" and total number of notes exceed 50%. All the spectral properties except range frequency (for both notes) exhibit low coefficients of variation, approximately 3% or less (Table 2).

Among males, temporal properties and range frequency of note "A" and note "B" exhibited coefficients of variation higher than 12%. Spectral properties, except range frequency, displayed low coefficients of variation,
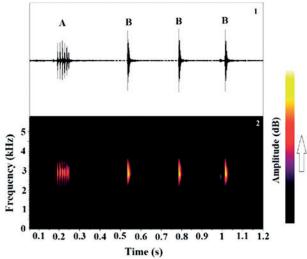

Fig. 1. Waveform (1) and spectrogram (2) of the advertisement call composed of notes "A" and "B" of *Sphaenorhynchus caramaschii* from municipality of Ribeirão Grande, state of São Paulo, southeastern Brazil. Air temperature 18.8 °C, relative humidity 85%.

below 6% (Table 2). All CV ratios were higher than 1, except for the number of notes "B" per call. In other words, the within male variation was greater than among male variation in almost all properties (Table 2).

Geographic distribution of acoustic properties. The general acoustic structures and the note "B" properties of the advertisement calls from Ribeirão Branco (type-locality), Iporanga and Ribeirão Grande were very similar (Fig. 2b). In contrast, these three populations were distinguishable by the note "A" properties in the discriminant analysis (Fig. 2a). Detailed comparisons of acoustic properties among populations can be found in Table 3, and the results of discriminant functions in Table 4.

Effects of air temperature on dynamic acoustic properties. There was no significant effect of air temperature on call repetition rate ($r^2 = 0.42$; F = 2.58; *P* > 0.05; n = 13), total number of notes ($r^2 = 0.51$; F = 4.31; *P* > 0.05; n = 13), or call duration ($r^2 = 0.02$; F = 0.22; *P* > 0.05; n = 13).

Calling site. Males of *S. caramaschii* were observed in reproductive activity in permanent pools during the rainy season. Calling males (n = 13) were found perched on emergent plants (76.9 %), on the water surface (15.4 %), and rarely on adjacent plants (7.7%; these calling males were observed up to 160 cm away from the water body). Mean perch height was 54.4 ± 66.4 cm (0 – 200 cm; n = 13).

Discussion

Call description. We provide a more thorough analysis of the advertisement call of *Sphaenorhynchus caramaschii*, specifically adding a multipulsed note (the note "A") not

Table 1. Acoustic properties (mean ± SD, range, sample size) of the advertisement call of 12 *Sphaenorhynchus* species.

Acoustic properties	S. caramaschii* (Males =14)	S. canga (Males = 6)	S. carneus (Males = 5)	S. dorisae (Males = 3)	S. lacteus (Males = 6)	S. mirim (Males = 2)
Call duration (s)	0.99 ± 1.27 (0.02 − 11.52) n = 305	0.20 ± 0.25 (0.008 − 1.23) n = 116	0.88 ± 1.36 (0.01 − 4.11) n = 16	0.32 ± 0.08 (0.24 − 0.42) n = 6	0.18 ± 0.10 (0.04 − 0.32) n = 14	0.044 ± 0.011 (0.034 − 0.101) n = 86
Frequency range of note "A" (Hz)	700 ± 129 (422 − 1172) n = 163	Missing	Missing	Missing	Missing	Missing
Duration of note "A" (s)	0.056 ± 0.017 (0.025 − 0.143) n = 163	0.010 ± 0.003 (0.005 − 0.020) n = 237	Missing	Missing	Missing	Missing
Minimum frequency of note "A" (Hz)	2498 ± 127 (2203 − 2766) n = 163	Missing	Missing	Missing	Missing	Missing
Maximum frequency of note "A" (Hz)	3198 ± 127 (2906 − 3422) n = 163	Missing	Missing	Missing	Missing	Missing
Dominant frequency of note "A" (Hz)	2856 ± 94 (2627 − 3141) n = 163	2841 ± 538 (2067 − 4550) n = 237	Missing	Missing	Missing	3262 ± 56 (3085 − 3398) n = 86
Number of pulses of note "A"	5.7 ± 1.3 (3 − 11) n = 163	2.5 ± 0.8 (1 − 5) n = 190	Missing	Missing	Missing	11.85 ± 2.94 (9 − 25) n = 48
Pulses rate of note "A" (pulses/s)	111 ± 40 (35 − 231) n = 163	247 ± 74 (59 − 444) n = 190	Missing	Missing	Missing	281 ± 24 (240 − 338) n = 48
Frequency range of note "B" (Hz)	587 ± 140 (281 − 984) n = 231	Missing	3302 ± 664 (2149 − 4517) n = 30	2280 ± 296 (1811 − 3096) n = 21	2254 ± 349 (1785 − 2891) n = 24	Missing
Duration of note "B" (s)	0.03 ± 0.01 (0.012 − 0.043) n = 231	0.008 ± 0.001 (0.005 − 0.009) n = 10	0.05 ± 0.009 (0.03 − 0.06) n = 30	0.06 ± 0.03 (0.02 − 0.10) n = 21	0.05 ± 0.02 (0.02 − 0.11) n = 24	Missing
Minimum frequency of note "B" (Hz)	2611 ± 140 (2156 − 2906) n = 231	Missing	938 ± 443 (109 − 1566) n = 30	1599 ± 176 (1198 − 1953) n = 21	970 ± 151 (723 − 1263) n = 24	Missing
Maximum frequency of note "B" (Hz)	3198 ± 161 (2859 − 3703) n = 231	Missing	4241 ± 390 (3679 − 5428) n = 30	3878 ± 221 (3594 − 4477) n = 21	3224 ± 249 (2741 − 3614) n = 24	Missing
Dominant frequency of note "B" (Hz)	2851 ± 114 (2438 − 3234) n = 231	2446 ± 264 (1722 − 2584) n = 10	2615 ± 92 (2497 − 2756) n = 30	2654 ± 238 (2437 − 3187) n = 21	1989 ± 93 (1875 − 2067) n = 24	Missing

Acoustic properties	S. orophilus (Males = 1)	S. palustris (Males = 2)	S. pauloalvini (Males = 2)	S. planicola (Males = 1)	S. prasinus (Males = 1)	S. surdus (Males = 3)
Call duration (s)	1.8 ± 0.34 (1.48 − 2.33) n = 4	0.165 ± 0.127 (0.014 − 0.508) n = 28	0.38 ± 0.12 (0.18 − 0.62) n = 9	0.33 ± 0.23 (0.16 − 0.49) n = 2	0.24 ± 0.04 (0.20 − 0.31) n = 3	0.89 ± 0.66 (0.36 − 1.91) n = 11
Frequency range of note "A" (Hz)	Missing	Missing	Missing	Missing	Missing	Missing
Duration of note "A" (s)	Missing	0.046 ± 0.015 (0.015 − 0.057) n = 8	Missing	Missing	Missing	Missing
Minimum frequency of note "A" (Hz)	Missing	Missing	Missing	Missing	Missing	Missing
Maximum frequency of note "A" (Hz)	Missing	Missing	Missing	Missing	Missing	Missing
Dominant frequency of note "A" (Hz)	(1500 − 2700)	3096 ± 246 (2760 − 3618) n = 54	Missing	Missing	Missing	Missing
Number of pulses of note "A"	Missing	5.5 ± 2.0 (2 − 8) n = 8	Missing	Missing	Missing	Missing
Pulses rate of note "A" (pulses/s)	150	120 ± 20 (80 − 140) n = 8	Missing	Missing	Missing	Missing
Frequency range of note "B" (Hz)	1709 ± 253 (1349 − 1907) n = 4	Missing	1231 ± 158 (1000 − 1809) n = 4	2187 ± 130 (2036 − 2262) n = 3	1983 ± 538 (1152 − 2974) n = 6	1934 ± 307 (1482 − 2247) n = 71
Duration of note "B" (s)	0.02 ± 0.003 (0.01 − 0.02) n = 4	0.014 ± 0.002; (0.010 − 0.018) n = 12	0.02 ± 0.006 (0.01 − 0.04) n = 4	0.13 ± 0.05 (0.08 − 0.17) n = 3	0.01 ± 0.004 (0.004 − 0.02) n = 6	0.02 ± 0.003 (0.02 − 0.02) n = 71
Minimum frequency of note "B" (Hz)	1314 ± 241 (1163 − 1674) n = 4	Missing	1645±82 (1404 − 1766) n = 4	1847 ± 65 (1810 − 1923) n = 3	1464 ± 147 (1252 − 1784) n = 6	1503 ± 323 (1268 − 2137) n = 71
Maximum frequency of note "B" (Hz)	3023 ± 100 (2884 − 3116) n = 4	Missing	2876 ± 111 (2681 − 3213) n = 4	4035 ± 65 (3960 − 4073) n = 3	3448 ± 432 (2849 − 4227) n = 6	3438 ± 161 (3181 − 3620) n = 71
Dominant frequency of note "B" (Hz)	2411 ± 0; n = 4	3091 ± 225 (2761 − 3446) n = 12	2306 ± 122 (2023 − 2583) n = 4	3000 ± 0; n = 3	2378 ± 112 (2250 − 2625) n = 6	2502 ± 284 (2290 − 2941) n = 71

mentioned in the previous descriptions (Toledo *et al.*, 2007; 2014). The vocalizations of the congeneric species *S. canga*, *S. palustris*, *S. mirim* and *S. orophilus* can also be composed of two distinct notes, distinguished through temporal differences (mainly in pulse number) (Heyer *et al.*, 1990; Nunes *et al.*, 2007; Lacerda *et al.*, 2011; Lacerda & Moura, 2013; Araujo-Vieira *et al.*, 2015).

However, for seven other *Sphaenorhynchus* species, the multipulsed note has not yet been observed (Toledo *et al.*, 2014; Table 1). We speculate that these two different notes probably have distinct social functions, as reported for other anurans (Narins & Capranica, 1978; Morais *et al.*, 2012). In order to describe the acoustic repertoire of diverse species of *Sphaenorhynchus*, many authors

Table 2. Within-male and among-males coefficients of variation (CV %) of acoustic properties of the advertisement call of *Sphaenorhynchus caramaschii* from southeastern Brazil.

	Within-male (mean %)	Within-male (range %)	Among male (%)	Classification	Type	CVs Ratio
Call duration	98.0	14.3 – 187.1	128.0	Temporal	Dynamic	1.31
Number of notes "A" per call	75.8	75.8 – 237.8	176.4	Temporal	Dynamic	2.33
Number of notes "B" per call	122.5	28.1 – 134.7	116.5	Temporal	Dynamic	0.95
Total number of notes per call	107.8	47.9 – 165.2	121.5	Temporal	Dynamic	1.13
Range frequency of note "A"	12.8	3.8 – 28.3	18.4	Spectral	Dynamic	1.44
Range frequency of note "B"	18.4	5.2 – 27.4	23.8	Spectral	Dynamic	1.29
Minimum frequency of note "A"	3.1	1.4 – 5.9	5.1	Spectral	Static	1.64
Minimum frequency of note "B"	3.1	1.0 – 6.1	5.4	Spectral	Static	1.73
Maximum frequency of note "A"	2.0	0 – 3.5	3.7	Spectral	Static	1.86
Maximum frequency of note "B"	2.7	0.9 – 6.4	5	Spectral	Static	1.84
Dominant frequency of note "A"	2.4	0.5 – 4.3	3.3	Spectral	Static	1.39
Dominant frequency of note "B"	2.7	0 – 5.5	4	Spectral	Static	1.5
Duration of note "A"	18.9	8.5 – 40.4	30.4	Temporal	Dynamic	1.61
Duration of note "B"	13.4	8.1 – 23.4	24.7	Temporal	Dynamic	1.84
Pulses rate of note "A"	16.9	1.7 – 28.5	36.3	Temporal	Dynamic	2.15

Table 3. Acoustic properties (mean ± SD, range, n) of four populations of *Sphaenorhynchus caramaschii* from southern and southeastern Brazil. *Type locality. **Data from Toledo *et al.*, 2014.

Acoustic properties (Means)	Localities			
	Ribeirão Branco* (n = 1)	Iporanga (n = 4)	Ribeirão Grande (n = 9)	Piraquara ** (n = 1)
Call duration (s)	1.181 ± 1.157 (0.180 – 6.365) (n = 31)	0.808 ± 1.125 (0.016 – 8.206) (n = 78)	1.032 ± 1.334 (0.024 – 11.521) (n = 196)	9.12 ± 2.63 (5.23 – 11.0) (n = 4)
Number of notes "A" per call	1.09 ± 0.83 (0 – 4) (n = 31)	0.88 ± 0.87 (0 – 4) (n = 78)	1.43 ± 2.63 (0 – 26) (n = 196)	Missing
Number of notes "B" per call	3.77 ± 4.62 (1 – 27) (n = 31)	2.62 ± 1.47 (0 – 27) (n = 78)	3 ± 3.2 (0 – 23) (n = 196)	Missing
Total number of notes per call	4.87 ± 5.25 (1 – 31) (n = 31)	3.51 ± 2.33 (1 – 43) (n = 78)	4.46 ± 5.3 (1 – 42) (n = 196)	28
Frequency range of note "A" (Hz)	671 ± 37 (603 – 732) (n = 14)	794 ± 108 (609 – 1172) (n = 33)	676 ± 130 (422 – 1031) (n = 116)	Missing
Duration of note "A" (s)	0.064 ± 0.010 (0.053 – 0.085) (n = 14)	0.039 ± 0.011 (0.025 – 0.072) (n = 33)	0.059 ± 0.016 (0.027 – 0.143) (n = 116)	Missing
Minimum frequency of note "A" (Hz)	2335 ± 54 (2240 – 2412) (n = 14)	2476 ± 86 (2250 – 2625) (n = 33)	2524 ± 128 (2203 – 2766) (n = 116)	Missing
Maximum frequency of note "A" (Hz)	3005 ± 66 (2929 – 3101) (n = 14)	3270 ± 50 (3188 – 3422) (n = 33)	3201 ± 112 (2906 – 3422) (n = 116)	Missing
Dominant frequency of note "A" (Hz)	2805 ± 112 (2627 – 3015) (n = 14)	2902 ± 125 (2719 – 3141) (n = 33)	2843 ± 75 (2672 – 3000) (n = 116)	Missing
Number of pulses of note "A"	5.8 ± 1 (5 – 8) (n = 14)	6.3 ± 1.4 (4 – 9) (n = 33)	5.5 ± 1.3 (4 – 11) (n = 116)	Missing
Pulses rate of note "A" (pulses/minute)	93 ± 20 (59 – 132) (n = 14)	166 ± 28 (83 – 231) (n = 33)	98 ± 31 (35 – 200) (n = 116)	Missing
Frequency range of note "B" (Hz)	545 ± 55 (474 – 603) (n = 20)	582 ± 139 (281 – 938) (n = 61)	594 ± 147 (281 – 984) (n = 150)	3302 ± 664 (2149 – 4517) (n = 30)
Duration of note "B" (s)	0.020 ± 0.003 (0.012 – 0.026) (n = 20)	0.023 ± 0.005 (0.016 – 0.037) (n = 61)	0.027 ± 0.007 (0.017 – 0.043) (n = 150)	0.05 ± 0.009 (0.03 – 0.06) (n = 30)
Minimum frequency of note "B" (Hz)	2466 ± 28 (2412 – 2498) (n = 20)	2625 ± 124 (2156 – 2906) (n = 61)	2625 ± 145 (2203 – 2906) (n = 150)	938 ± 443 (109 – 1566) (n = 30)
Maximum frequency of note "B" (Hz)	3010 ± 70 (2929 – 3101) (n = 20)	3207 ± 100 (3000 – 3516) (n = 61)	3219 ± 173 (2859 – 3703) (n = 150)	4241 ± 390 (3679 – 5428) (n = 30)
Dominant frequency of note "B" (Hz)	2771 ± 72 (2670 – 2885) (n = 20)	2889 ± 115 (2672 – 3141) (n = 61)	2846 ± 112 (2438 – 3234) (n = 150)	2615 ± 92 (2497 – 2756) (n = 30)
Call rate (calls/minute)	21	18.9 ± 13 (4 – 33) (n = 4)	14.4 ± 4.8 (8.3 – 25.3) (n = 9)	Uninformed

Table 4. Summary statistics of discriminant functions of the acoustic properties of the notes "A" and "B" per population.

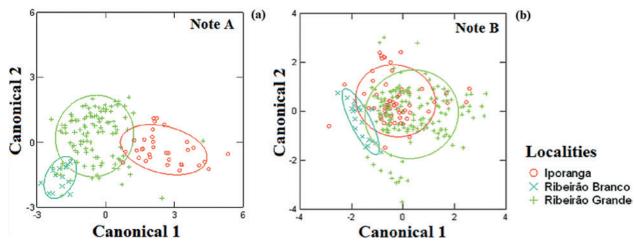| Note type		Discriminant Functions	
	Statistics	Canonical 1	Canonical 2
Note "A" (Wilks's Lambda = 0.267; F-ratio = 24.120; p <0.01)	Eigen value	1.846	0.314
	Proportion	0.855	1
	Canonical correlation	0.805	0.489
Note "B" (Wilks's Lambda = 0.697; F-ratio = 11.109; p <0.01)	Eigen value	0.334	0.075
	Proportion	0.817	1
	Canonical correlation	0.5	0.264

Fig. 2. Canonical scores plot from discriminant functions performed on acoustic properties of notes "A" (a) and "B" (b) of the advertisement call of *Sphaenorhynchus caramaschii*. Ellipses represent 95 % of confidence regions for each group.

have recognized two different notes, in which the simplest is the sexual signal and other more complex note has an aggressive function (Heyer *et al.*, 1990; Lacerda *et al.*, 2011; Lacerda & Moura, 2013; Araujo-Vieira *et al.*, 2015). We posit that differences associated with social context are not so easily recognized in the vocalizations of *S. caramaschii*. For any of these species, an experimental approach with playback tests should be performed to elucidate the functions of specific notes before assuming the social roles of divergent call notes.

Notes with intensity modulation may be universal for *Sphaenorhynchus* calls, since this has been noted for several species, including *S. caramaschii, S. canga, S. mirim, S. palustris* and *S. orophilus* (Heyer *et al.*, 1990; Lacerda *et al.*, 2011; 2013; Araujo-Vieira *et al.*, 2015; and the present study). We provide a more detailed comparison among call properties of different species in the genus *Sphaenorhynchus* in Table 1. To date, only three species of *Sphaenorhynchus* have unknown vocalizations: (1) *S. botocudo*; (2) *S. bromelicola*, and (3) *S. platycephalus*.

Although Toledo *et al.* (2007) and Toledo *et al.* (2014) analyzed only three calls from one male of *S. caramaschii*, the considerable difference in spectral properties of note "B" relative to our data (Table 3) may indicate the potential for the individual from Piraquara to belong to a different but closely related species. Some call properties, such as dominant frequency, have a highly specific recognition role, and females are generally well adapted to conspecific male calls (Ryan & Rand, 1993). However, differences in general temporal properties, such as the longer call reported by Toledo *et al.* (2007; 2014), should be interpreted with caution. That particular male recorded was in a very dense chorus (R. Lingnau, pers. obs.). Anuran males vocalizing in dense choruses can change their acoustic properties, particularly the temporal ones, to avoid auditory masking and to achieve a higher mating success (Green, 1990; Grafe, 1996). Furthermore, the temporal variables of the advertisement call of *S. caramaschii* exhibit high levels of among-male variation (see Table 2). These can potentially be influenced by extrinsic factors of social and environmental conditions, including air temperature and proximity to neighbors or partners for reproduction (Wells & Taigen, 1986; Gerhardt & Huber, 2002; Wong *et al.*, 2004; Lingnau & Bastos, 2007).

Within-male and among-male variations. For species with long advertisement calls, great variation in temporal properties, such as duration and number of notes per call, is usually expected between calls from an individual male. These longer calls are more susceptible to extrinsic influences of air temperature and social context of the calling male (Gerhardt & Huber, 2002). Spectral properties generally exhibit low variation within individuals, particularly dominant frequency, which is nearly identical from one call to another (Gerhardt & Huber, 2002). We confirmed this pattern of high and low variation in temporal and spectral properties respectively in the vocalizations analyzed for *S. caramaschii* in this study.

In some cases, the variation in acoustic properties among males should follow the same pattern found for the within-male variation for both dynamic and static variables, as it has been found for other species (Bee *et al.*, 2001; Forti *et al.*, 2015). However, the variation is generally greater, particularly for spectral properties that can differ in relation to variation in genes, body size, and morphological and physiological conditions of calling males (Bee, 2002; Gerhardt & Huber, 2002; Morais *et al.*, 2012; see Table 2). Our pattern of CV ratios (higher acoustic variation among than within male) was similarly found in other anuran species (Bee *et al.*, 2001; Bee & Gerhardt, 2001; Briggs, 2010; Morais *et al.*, 2012; Reichert, 2013). The combination of the high variability among males and the different acoustic properties of *S. caramaschii* indicates a possibility for individual recognition (*e.g.*, neighbor-stranger discrimination; see Bee *et al.*, 2001; Bee & Gerhardt, 2001; Gasser *et al.*, 2009; Morais *et al.*, 2012).

Acoustic properties on geographical point of view. The variation in acoustic properties of advertisement calls may be associated with gradients of geographic distance between populations (Smith *et al.*, 2003; Smith & Hunter, 2005; Forti *et al.*, 2012; Tsuji-Nishikido *et al.*, 2012). Such acoustic divergence between populations may be attributed to female preference, or simply an adaptive outcome from selection under different environmental conditions of distinct populations (Ryan *et al.*, 1990; Boul *et al.*, 2007). In this study it was not possible to explore this effect because two populations (Ribeirão Branco and Piraquara) were represented by only one individual, and any comparison would be limited by these low sample sizes. Furthermore, playback experiments should be used to support this hypothesis. However, if these males are representative of populations of these localities, the population of Piraquara would be the most divergent among the four. Piraquara is the most distant locality from the other three populations studied, so differences may correspond to geographic distance. To test this hypothesis, a larger sample of recordings must be taken from each population. However, this pronounced difference in relation to the other three populations suggests that the population from Piraquara may be considered a distinct species related to *S. caramaschii*. Nevertheless, additional samples and DNA analyses at this locality are required to test this supposition.

The discriminant functions have indicated differences in call properties among calls of the three populations sampled in relation to note "A", but not considering note "B" (Fig. 2). Similar differences of acoustic traits among populations using a fine scale to obtain spectral data (bioacoustic sampling techniques with a high FFT) were reported for other anurans (Wycherley *et al.*, 2002). This result suggests note "A" may be under pressure for divergence at the population level, which has not happened with note "B". Many factors, including distinct environmental conditions, female preference, and interspecific acoustic interactions are possible factors affecting acoustic traits among populations (Littlejohn, 1976; Höbel & Gerhardt, 2003; Faria *et al.*, 2013; Narins *et al.*, 2014). However, the question remains: why might different notes have different levels of evolution? One possibility is that the notes serve different functions in communication. Playback experiments with artificial and natural acoustic stimuli exposing sympatric and allopatric adults of both sexes may help to shed light on this subject.

The effects of air temperature on dynamic acoustic properties. We did not detect effects of air temperature on call repetition rate, total number of notes and call duration in the calls of *S. caramaschii*, as already noted for other species (Sullivan & Malmos 1994; Giacoma *et al.*, 1997; Navas & Bevier, 2001; Guimarães & Bastos, 2003; Wong *et al.*, 2004; Lingnau & Bastos, 2007). Even though relationships were not statistically significant, a high proportion of the variation in call rate and total number of notes, may still be explained by air temperature, as reflected by a large regression coefficient. Therefore,

temperature is likely an important factor influencing these variables. Air temperature strongly influences the metabolic rate of frogs and reflects the energy expended in the production of acoustic signals by males (Wells, 2007). However, this principle remains to be tested, but could be evaluated under controlled conditions by exposing males to a temperature gradient and obtaining measures of the acoustic properties to each male. Moreover, the analyzed properties (call rate, total number of notes per call, and call duration) are also influenced by the social context of the calling male (Gerhardt & Huber, 2002; Wong *et al.*, 2004; Lemes *et al.*, 2012).

Calling sites. The calling sites used by *S. caramaschii* males in the present study (permanent ponds with emergent vegetation) resemble those described for this species (Bertoluci & Rodrigues, 2002) and other congeneric species, including *S. surdus* (Conte & Machado, 2005), *S. palustris* (Nunes *et al.*, 2007), *S. bromelicolous* (Bokermann, 1966), *S. pauloalvini*, *S. prasinus* (Bokermann, 1973) and *S. mirim* (Lacerda *et al.*, 2011). Members of this genus usually share breeding sites with other frog species. Although they generally have been observed to utilize perches closer to the water, male *S. caramaschii* will also call from about 1 m in the vertical gradient (Bokermann, 1973) which includes perches used by other small tree frogs, including *Dendropsophus elegans*, *D. microps*, *D. minutus*, and *D. werneri*.

Final remarks

Our research contributes to the characters of bioacoustics of *Sphaenorhynchus caramaschii* and provides an improved view of species delimitation using acoustic evidences and different levels of variation. We have also raise some new hypotheses, and our work could be a first step for future experimental studies that target the functions of different vocal signals.

Acknowledgements

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