The complex vocalization of *Scinax cardosoi* (Anura: Hylidae), with comments on advertisement calls in the *S. ruber* Clade

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Abstract

The complex vocalization of Scinax cardosoi (Anura: Hylidae), with comments on advertisement calls in the S. ruber Clade. The complex vocalization of Scinax cardosoi from a population of Minas Gerais in southeastern Brazil is described and compared with available acoustic data for the other species of the S. ruber Clade. Three distinct types of high-pitched, pulsed calls were identified, and are referred to as "short," "long," and "trilled" calls. Short calls (16–66 ms) resemble squeaks, and consist of a pulsed signal (8–28 pulses/call) with regular amplitude modulations throughout their duration; the amplitude peak occurs at about the midpoint of the call duration. Long calls (268–518 ms) resemble giggles, and have lower amplitude than short calls; typically, they consist of pulsed note series (1–6 notes/call). Trilled calls resemble insect chirps and have the lowest amplitude of the three call types; they consist of long (1.1-3.0 s) pulsed note series (9-25)notes/call). Acoustic data are taxonomically informative in the Scinax ruber Clade and provide phenotypic characters diagnosing S. cardosoi in addition to those features proposed in its original description. The vocalization repertoire of S. cardosoi resembles the complex vocal repertoires (i.e., multiple call types emitted in variable combinations) of members of the S. catharinae Clade more than some species of the S. ruber Clade, which tend to have simpler call structures (i.e., a single type of multipulsed note).

Keywords: acoustic repertoire, amphibians, call functions, Minas Gerais state, southeastern Brazil.

Received 2 April 2015. Accepted 13 October 2015. Distributed December 2015.

Resumo

A vocalização complexa de Scinax cardosoi (Anura: Hylidae), com comentários sobre cantos de anúncio no Clado de S. ruber. A vocalização complexa de Scinax cardosoi de uma população do estado de Minas Gerais no sudeste brasileiro é descrita e comparada com os dados acústicos disponíveis para outras espécies do Clado de S. ruber. Três tipos diferentes de cantos pulsados e agudos foram identificados, denominados cantos "curto," "longo" e "trinado". Cantos curtos (16-66 ms) lembram chios e consistem em sinais pulsados (8-28 pulsos/canto) com modulações de amplitude regulares ao longo de sua duração e pico de amplitude aproximadamente na metade de sua duração. Cantos longos (268-518 ms) lembram risadinhas e têm menor amplitude quando comparados a cantos curtos; tipicamente, consistem em séries de notas pulsadas (1-6 notas/canto). Cantos trinados lembram zunidos de insetos e apresentam a menor amplitude dentre os três tipos de cantos; consistem em séries muito longas (1,1-3,0 s) de notas pulsadas (9-25 notas/canto). Dados acústicos são taxonomicamente informativos no Clado de S. ruber, fornecendo evidências fenotípicas à diagnose de S. cardosoi além das características propostas em sua descrição original. O repertório vocal de S. cardosoi assemelha-se mais aos repertórios vocais complexos (vários tipos de cantos emitidos em combinações variadas) de espécies do Clado de S. catharinae do que ao de algumas espécies do Clado de S. ruber, os quais tendem a ter uma estrutura do canto mais simples (um tipo único de nota multipulsionada).

Palavras-chave: anfíbios, funções do canto, Minas Gerais, repertório acústico, sudeste do Brasil.

Introduction

Anuran vocalizations can contribute to the resolution of taxonomic issues—e.g., revealing cryptic diversity (Heyer *et al.* 1996, Carvalho and Giaretta 2013)—and the determination of identities of species with variable or poorly known phenotypic characters (Padial *et al.* 2008). They also are useful in detecting macroevolutionary patterns (Cocroft and Ryan 1995, Robillard *et al.* 2006), as well as in conducting biodiversity inventories or monitoring frogs in visually inaccessible habitats (Obrist *et al.* 2010).

Scinax currently comprises 113 species distributed from eastern and southern Mexico, Central America, and throughout South America to Argentina and Uruguay (Frost 2015). Faivovich (2002) proposed two major clades in the genus—the *S. catharinae* and the *S. ruber* clades— both of which were corroborated by Faivovich *et al.* (2005) with a larger taxon sampling. Two species groups are recognized in the *S. ruber* Clade—the *S. rostratus* and the *S.* *uruguayus* groups; several other species remain unassigned to either of the species groups (Faivovich *et al.* 2005). Vocalizations (particularly advertisement calls) have supplemented species-level diagnoses in *Scinax* (De la Riva 1993, Nunes *et al.* 2012).

Scinax cardosoi (Carvalho-e-Silva and Peixoto 1991) was described from Teresópolis (Rio de Janeiro state) and Domingos Martins (Espírito Santo state), southeastern Brazil, and is assigned to the *S. ruber* Clade (*sensu* Faivovich *et al.* 2005). The species recently has been reported from three municipalities of Minas Gerais state in southeastern Brazil (Pinto *et al.* 2009, Linares *et al.* 2011), but its vocalization has not been described to date.

Herein, the vocalization of *Scinax cardosoi* is described from a population near (~45 km) its type locality (Teresópolis, Rio de Janeiro) in Minas Gerais. The vocalization is compared to published reports of advertisement calls for the other species of the *S. ruber* Clade that are not assigned to a species group.

Materials and Methods

Fieldwork was conducted from 30 November–4 December 2011 in the Municipality of Chiador (21°55' S, 42°51' W), Minas Gerais state in southeastern Brazil; this locality is fewer than 50 km north of the type locality of *S. cardosoi* (Teresópolis, Rio de Janeiro state). Voucher specimens (ICMBio/SISBIO permit # 30059) are housed in the Collection of Amphibians of the Museu de Biodiversidade do Cerrado at the Universidade Federal de Uberlândia (AAG-UFU) under the following accession numbers: AAG-UFU 0681–0687; all specimens but the female AAG-UFU 0681 are call vouchers.

Comparisons of morphological, morphometric, and coloration characters are based on those of the original description of S. cardosoi (Carvalho-e-Silva and Peixoto 1991). Snoutvent length (SVL) was measured with calipers to the nearest 0.05 mm following the methodology of Duellman (1970). Calls of six males were recorded using a Sennheiser K6/ME66 directional microphone (positioned approximately 1 m from calling males) and a M-Audio Microtrack-II digital recorder set at a sampling rate of 48 kHz and a resolution of 16 bits. Air and water temperatures were measured with a digital thermometer after vocalizations were recorded for 1-2-min. Calls were analyzed with the software Raven Pro 32-bit 1.5 Beta version (Bioacoustics Research Program 2012). Temporal traits were measured from oscillograms; the dominant frequency was obtained through "Peak Frequency" measurement function. Raven Pro settings: window size = 256 samples; window type = Hann; 3dB filter bandwidth = 270 Hz; overlap = 85%; hop size = 0.79 ms; DFT size = 1024 samples; grid spacing = 46.9Hz. Sound figures were generated with Seewave package version 1.7.3 (Sueur et al. 2008), R platform version 3.1.0 (R Core Team 2014), with the following settings: window type = Hann; window length = 128 samples (FFT); overlap = 85%.

Acoustic terminology follows that of Carvalho et al. (2015). We realize that the functions of calls cannot be classified without being investigated in a behavioral/experimental framework. Our categorizations of calls are based on information that could be gathered in field while recording S. the cardosoi vocalizations; we consider these a practical way to facilitate interspecific comparisons. Our assignment of calls to vocalization categories follows the suggested nomenclature proposed by Toledo et al. (2015). The rarest call (hereinafter "trilled" call) was recorded from only one isolated male and it might be considered in a reproductive context; if so, the trilled call probably is either the advertisement call or at least the major advertisement signal. The other two call types (hereinafter "short" and "long" calls) usually were emitted either as single calls or intermingled with one another in phrases. These seem to have reproductive significance in signaling the position of one male to the nearest conspecific male (recognized function for advertisement calls; Gerhardt 1994). Additionally, the short and long calls may figure in aggressive behavior between conspecific males because neighboring calling males were observed to broadcast phrases of these two call types at high rates (aggressive call definition fide Wells 2007). Thus, different call types may convey different messages to males and females, respectively (Narins and Capranica 1978).

The species compositions of clades and species groups of *Scinax* are those of Faivovich (2002) and Faivovich et al. (2005). Bilate and Lack (2011) summarized advertisement call traits for several species of the S. ruber Clade. These authors proposed a straightforward classification of calls based on their duration-i.e., short (up to ~350 ms) and long (> 350 ms) calls, which we adopt to facilitate interspecific comparisons. Acoustic comparisons include only the unassigned species of the S. ruber Clade (i.e., all species except those in S. rostratus and S. uruguayus groups).

Results

Our specimens match the original diagnosis of *Scinax cardosoi* by Carvalho-e-Silva and Peixoto (1991), as follow. (1) Adult males small (SVL 18.7–19.9 mm, mean = 19.4; N = 6); female larger (SVL 24.4 mm; N = 1). (2) Upper shank surfaces with oblique bars (Figure 1A). (3) A broad, longitudinal stripe extending from eye to inguinal region; a thin, occasionally fragmented vertebral stripe (Figure 1A). (4) Ventral surfaces (throat, forearms, shanks, and flanks) with distinctive dark-colored mottled pattern (Figure 1B). (5) Lateral aspect of head acuminate.

Male *Scinax cardosoi* were observed to call from perches 0.5–1.0 m above the ground on grassy vegetation in temporary, rock-bottomed pools near forest borders and in clearings. *Bokermannohyla circumdata* and *S. hayii* were also in calling activity at the locality. Three distinct types of high-pitched, pulsed calls were identified (Table 1; Figures 2–4), herein referred to as "short," "long," and "trilled" calls. Short calls (Figure 2B) resemble squeaks and consist of a pulsed signal with regular amplitude modulations throughout their duration, and have amplitude peak about midway during the call duration. This call type was emitted by all six males (N = 73 calls), at highly variable rates, ranging from 3.1-57.1 calls/min (mean = 17.0, SD = 20.1). Short call duration varies from 16– 66 ms (mean = 40.0, SD = 8.1), with intervals from 0.5-11.9 s (mean = 5.8, SD = 4.6). Almost all short calls are emitted as single notes, but two males (N = 4 series) emitted two-note series (Figure 3A). In all two-note series, the second note has fewer pulses and a shorter duration. In the cases of two-note series, intervals between notes are shorter, varying from 5-87 ms (mean = 63.0, SD = 28.6). Short calls have 8-28 pulses (mean = 20.6, SD = 2.9) and are emitted at high rates varying from 340.0-678.6 pulses/s (mean = 525.1, SD = 90.8). The dominant frequency varies from 3281-4828 Hz (mean = 4429.1, SD = 91.9).



Figure 1. Dorsal (A) and ventral (B) views of the adult male AAG-UFU 0682 (SVL: 18.7 mm) of *Scinax cardosoi*, collected in Chiador, Minas Gerais state, southeastern Brazil.

Long calls (Figure 2C) were emitted by only three males (N = 14 calls) and have a lower amplitude than do the short calls; some portions of the long calls have ill-defined amplitude modulations, typically consisting of pulsed note series, resembling giggles. Long calls are highly variable in temporal traits (especially call interval and rate, pulse number, grouping, and spacing), and are emitted at highly variable rates, ranging from 2.0-37.2 calls/min (mean = 15.5, SD = 19.0). They vary from 268-518 ms (mean = 427.0, SD = 80.2) and have 31-68 pulses (mean

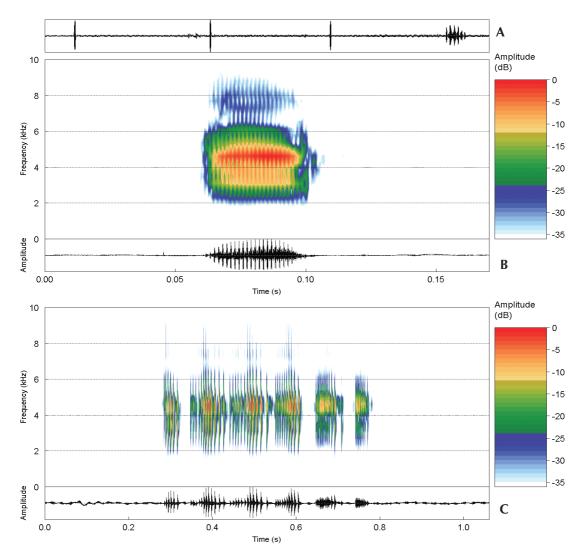


Figure 2. Short and long calls of *Scinax cardosoi*. Waveform (**A**) of a series (~11 s) of three short calls followed by one long call; (**B**) spectrogram and respective oscillogram of the first short call in A; (**C**) spectrogram and respective oscillogram of the long call in A. Sound file: Scinax_cardosoiChiadorMG2LBM_AAGmt; recorded at 00:20 h, on 3 December 2011; air 16°C, water 19°C. Recorded in Chiador, Minas Gerais state, southeastern Brazil.

= 52.7, SD = 12.6) emitted at rates ranging from 78.5–147.6 pulses/s (mean = 124.4, SD = 9.7). In contrast to short calls, which almost always are emitted as single notes, long calls typically occur in series (Figure 2C), but one long call was emitted as a single note (Figure 3B). Long

calls are formed by 1–6 notes (mean = 3.7, SD = 1.3). The dominant frequency varies from 4266–4500 Hz (mean = 4386.1, SD = 34.8).

Short and long calls were emitted in complex phrases at high rates in one recording, but in most cases, long calls were emitted at irregular

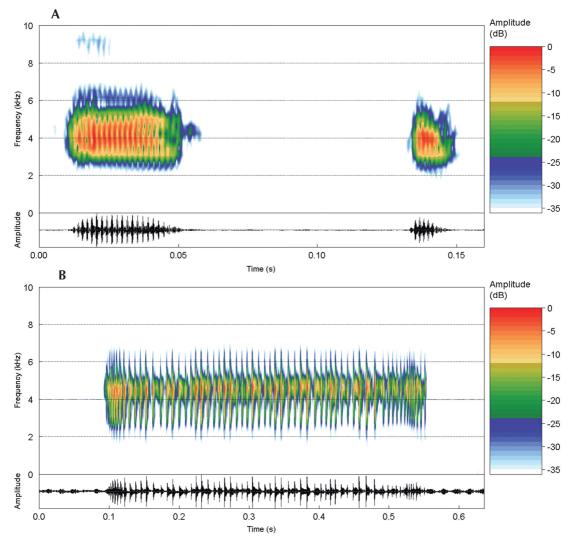


Figure 3. Variation in short and long call types (spectrograms and respective oscillograms). (A) Short calls emitted as a two-note series. Sound file: Scinax_cardosoiChiadorMG2LBM_AAGmt; recorded at 00:20 h, on 3 December 2011; air 16°C, water 19°C; (B) long call emitted as a single note. Sound file: Scinax_cardosoiChiadorMG5LBM_AAGmt; recorded at 01:30 h, on 3 December 2011; air 16°C, water 19°C. Recorded in Chiador, Minas Gerais state, southeastern Brazil.

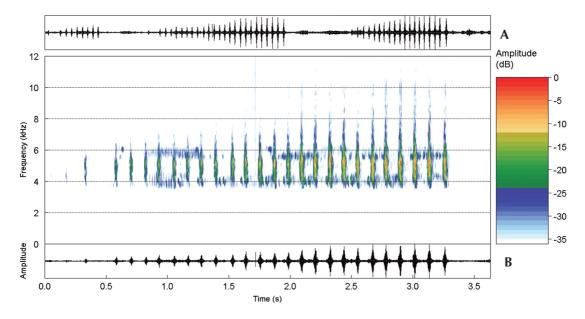


Figure 4. Waveform (A) of a series of three trilled calls (ca. 9 s); (B) spectrogram and respective oscillogram of the second call in A. Sound file: Scinax_cardosoiChiadorMG1LBM_AAGmt; recorded at 22:20 h, on 30 November 2011; air 20°C, water 21°C. Recorded in Chiador, Minas Gerais state, southeastern Brazil.

Table 1.	Acoustic traits of	Scinax cardosoi	<i>i</i> from Chiado	r, Minas G	ierais state,	southeastern	Brazil. N =	number of
	recorded males (analyzed calls). I	Mean ± SD (m	in–max).				

	Short call <i>N</i> = 6 (73)	Long call $N = 3$ (14)	Trilled call $N = 1$ (3)	
Call duration (ms)	40.0 ± 8.1 (16–66)	427.0 ± 80.2 (268–518)	2043.7 ± 929.3 (1100–2958)	
Call interval (s)	$5.8 \pm 4.6 \ (0.5-11.9)$	-	-	
Notes/call	$1.1 \pm 0.1 (1-2)$	3.7 ± 1.3 (1–6)	17.7 ± 8.1 (9–25)	
Note rate/s	-	-	$8.6 \pm 0.5 (8.2 - 9.2)$	
Pulses per call	20.6 ± 2.9 (8–28)	52.7 ± 12.6 (31–68)	-	
Pulse rate/s	525.1 ± 90.8 (340.0–678.6)	124.4 ± 9.7 (78.5–147.6)	-	
Call rate/min	17.0 ± 20.1 (3.1–57.1)	15.5 ± 19.0 (2.0–37.2)	-	
Dominant frequency (Hz)	4429.1 ± 91.9 (3281–4828)	4386.1 ± 34.1 (4266–4500)	5125.0 ± 71.6 (5063–5203)	
Air temperature (°C)		16.0–20.0		
Water temperature (°C)		19.0–21.0		

rates intermingled with short calls, which was the most frequent call type emitted among the recordings.

Trilled calls (Figure 4) were emitted by only one male (N = 3 calls) and have the lowest amplitude of all three call types; the call resembles an insect chirp, and consists of a long (call duration > 1 s) pulsed-note series. The duration of the trilled call varies from 1100– 2958 ms (mean = 2043.7, SD = 929.3); the call is composed of 9–25 notes (mean = 17.7, SD = 8.1), and is emitted at rates varying from 8.2–9.2 notes/s (mean = 8.6, SD = 0.5). The dominant frequency varies from 5063–5203 Hz (mean = 5125.0, SD = 71.6).

Discussion

The advertisement calls of members of the *Scinax ruber* Clade often are described as a single type of pulsed note. The vocalizations of *S. cardosoi* do not coincide closely with this pattern because they are composed of different call types that may be functionally significant in reproductive and aggressive activities. Such a distinctive vocal repertoire with variable emission patterns aids in the distinction of *S. cardosoi* from its congeners of the *S. ruber* Clade.

The trilled call of Scinax cardosoi (apparently the major advertisement signal) matches the long call (call duration > 350 ms) category of Bilate and Lack (2011); this call type differentiates S. cardosoi from its congeners that are unassigned to a species group in the S. ruber Clade because its duration (1100-2958 ms) is longer than those species having short calls (combined range values: 70-340 ms): S. auratus (Nunes et al. 2007), S. blairi (Fouquette and Pyburn 1972), S. caldarum (Magrini and Giaretta 2010), S. castroviejoi (De la Riva et al. 1994), S. chiquitanus (De la Riva et al. 1994), S. cretatus (Nunes and Pombal Jr. 2011), S. cuspidatus (Pombal Jr. et al. 1995a), S. danae (Duellman 1986), S. elaeochrous (Duellman and Pyles 1983), S. eurydice (Pombal Jr. et al. 1995a), S.

fuscovarius (Pombal Jr. et al. 1995a), S. granulatus (Conte et al. 2010), S. hayii (Pombal Jr. et al. 1995a), S. ictericus (Duellman and Wiens 1993), S. juncae (Nunes and Pombal Jr. 2010), S. lindsayi (Pyburn 1992), S. manriquei (Barrio-Amorós et al. 2004), S. maracaya (Cardoso and Sazima 1980), S. nasicus (De la Riva et al. 1994), S. oreites (Duellman and Wiens 1993), S. pachycrus (Carneiro et al. 2004), S. perereca (Pombal Jr. et al. 1995b), S. quinquefasciatus (Duellman 1971), S. ruber (Duellman and Pyles 1983), S. similis (Bilate and Lack 2011), and S. tigrinus (Nunes et al. 2010).

Scinax cardosoi has a longer call duration (1100-2958 ms) than other species with long calls (\geq 350 ms), as follow: S. crospedospilus (250-445 ms; Magrini et al. 2011), S. duartei (420-680 ms; Magrini et al. 2011), and S. wandae (442-710 ms; Pombal et al. 2011). It has a higher dominant frequency (5063-5203 Hz) than S. acuminatus (830-1560 Hz; Magrini et al. 2011), S. alter (1300-4600 Hz; Pombal Jr. et al. 1995a), S. boesemani (1289-1529 Hz; Duellman and Pyles 1983), S. cabralensis (3700-4220 Hz; Drummond et al. 2007), S. curicica (2440-3660 Hz; Pugliese et al. 2004), S. exiguus (4000–4800; Duellman 1986), S. fuscomarginatus (2960-4594 Hz; Brusquetti et al. 2014), S. imbegue (1200-5000 Hz; as S. altera in Pombal Jr. et al. 1995a), S. madeirae (3369-3879 Hz; Brusquetti et al. 2014), S. rogerioi (1380-3190 Hz; Pugliese et al. 2009), S. rupestris (2067–2239 Hz; Araujo-Vieira et al. 2015), S. squalirostris (4048-4392 Hz; Pombal et al. 2011), and S. tymbamirim (4000-4300 Hz; Nunes et al. 2012).

Acoustic data, therefore, are taxonomically informative in the *Scinax ruber* Clade and provide additional characters that supplement the diagnosis in the original description of *S. cardosoi* by Carvalho-e-Silva and Peixoto (1991). Moreover, the vocalizations of *S. cardosoi* (squeaks-short calls, giggles-long calls, and insect chirps-trilled calls) are more similar to the complex vocal repertoires (multiple call types emitted in quite variable combinations) of species of the *S. catharinae* Clade (e.g., *S. aromothyella*, *S. berthae*, and *S. rizibilis*; Bastos and Haddad 2002, Pereyra *et al.* 2012), than they are to some species of the *S. ruber* Clade. The vocal repertories of the latter tend to have simpler call structures (a single type of multipulsed note emitted regularly; Pombal Jr. *et al.* 1995a, Bilate and Lack 2011).

The behavioral functions of the various calls of *Scinax cardosoi* must be addressed by studies in an experimental framework to identify on the specific context of each call type described in this study. It is possible that this array of call types and variable emission patterns (phrases) operate in more than one behavioral context (i.e., reproductive and aggressive behaviors).

Acknowledgments

This study was supported by CNPq and FAPEMIG. A research grant was awarded to AAG by CNPq. Doctoral fellowships were awarded by FAPESP to TRC and by CNPq to LBM. We thank two anonymous reviewers, Ignacio De la Riva, Jaime Bertoluci, and Linda Trueb for the comments and suggestions made in earlier versions of this study.

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Editor: Ignacio De la Riva