

# Release call of *Scinax eurydice* (Bokermann, 1968) (Anura, Hylidae) and advertisement call of northern populations

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**Abstract.** Vocalization is the most common type of communication among anurans. Within vocal repertoire anurans produce the advertisement and release calls, and both are useful for taxonomic approaches. *Scinax eurydice* is a hylid tree frog, endemic from Atlantic Forest. Its advertisement call is known only from a population in its southern distribution, State of Espírito Santo, Brazil. Besides, release calls within the genus *Scinax* are unknown. Herein we describe the release call of *S. eurydice* and compared the advertisement call between northern and southern populations of the species. The advertisement call from northern populations is composed of two distinct multipulsed notes and is notably different from southeastern populations, which presents three notes. Studies on differences in the advertisement calls provide valuable information to distinguish cryptic species, and help to understand patterns and functions of these behaviours in anuran assemblages.

**Key-words:** Atlantic Forest, bioacoustics, vocal repertoire, Hylid.

## Introduction

Vocalization is the most common type of communication among anurans, used in intra and inter-specific behavioural repertory (Duellman and Trueb, 1986). The advertisement, territory, encounter, and amplexus calls are known in the vocal repertoire within *Scinax* (Pombal Jr. et al., 1995; Bastos and Haddad, 2002; Magrini and Giaretta, 2010; Magrini et al., 2011), although the release call is not described for the genus. Release call is a signal emitted by males and females when tentatively amplexed or just touched in the dorsum, flanks, or axillary region, by another male

(Toledo et al. 2014). This type of call is commonly emitted during mating season, when males mistakenly clasp other males or unreceptive females for amplexus purposes (Gerhardt, 1994; di Tada et al., 2001).

*Scinax eurydice* (Bokermann, 1968) was described from Maracás, State of Bahia, Brazil, and it is widely distributed in eastern Brazil through the Atlantic Forest domain, from the State of São Paulo to the State of Paraíba (Hartmann, 2002; Arzabe and Carvalho-e-Silva, 2010). The advertisement call of *S. eurydice* was described by Pombal Jr. et al. (1995) from the region of Itaúnas, Municipality of Conceição da Barra, State of Espírito Santo, and by Magrini et al. (2011) from Municipality of Atibaia, State of São Paulo. Recently, species tree and population assignment tests supported the existence of two major clades corresponding to northern and southern populations Atlantic Forest in Brazil, each one composed of two other clades (Menezes et al., 2016).

These results have showed a possible hidden cryptic diversity named under *Scinax eurydice*, and given the need of integrative taxonomy approaches (Padial et al., 2010), based on multiples evidences such morphology, DNA base and acoustics, herein we describe, for the first time for the genus *Scinax*, the release call of *S. eurydice*, also, we compare the advertisement call between northern (Paraíba) and southern (Espírito Santo

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**Figure 1.** *Scinax eurydice* (CHUFPB7957, SVL 51.4 mm, male) collected at Reserva Biológica Guaribas, municipality of Mamanguape, State of Paraíba, Brazil.

and São Paulo) populations and discuss on the species taxonomy.

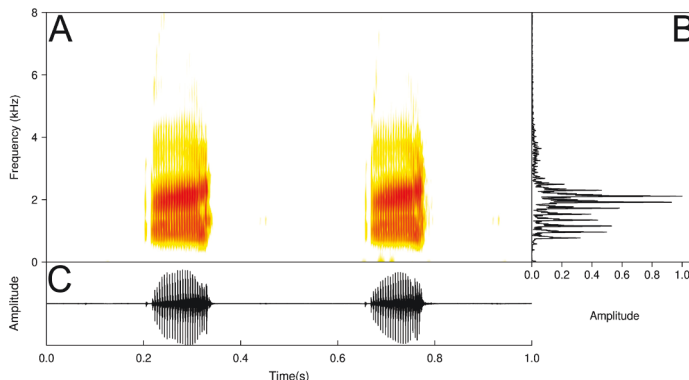
### Material and Methods

On 03 June 2011, males of *Scinax eurydice* (Figure 1) were found calling at the margin of a water bodies at Reserva Biológica Guaribas (6°44'32"S, 35°08'31"W), municipality of Mamanguape, State of Paraíba, Brazil. They began call at approximately 19:00hrs and the air temperature was 24.2°C. We collected one couple during amplexus. When separated from the female, the male (CHUFPB 7957, SVL 51.4 mm) emitted 23 release

calls (ZUFMS-DA009) while being handled. The advertisement call from this population was recorded two hours later (approximately 21h, air temperature 24.2°C) of another male of *S. eurydice* (CHUFPB 7956, SVL 52.2 mm) (ZUFMS-DA007 and ZUFMS-DA008). Calls were recorded *in situ* with an Olympus LS-10 linear PCM digital recorder coupled with an ATR55 Telemike TM directional microphone. Digital recordings were sampled at 44 kHz and 16 bit resolution and saved in uncompressed WAVE format. Voucher specimens are housed at herpetological collection of the Universidade Federal da Paraíba (CHUFPB), Brazil, and calls are deposited at Acervo de Mídia Digital da Universidade Federal de Mato Grosso do Sul – Anfíbios (ZUFMS-DA).

Males of *Scinax eurydice* were observed calling at Usina Japungu (6°59'8.15"S, 35°2'34.67"W), Municipality of Santa Rita, State of Paraíba. One male (CHUFPB4437, SVL 48.7 mm) (ASFURN594) was recorded on 29 April 2013, at 20:00hrs, air temperature 24.6°C, and another male (CHUFPB4470, SVL 52.2 mm) (ASFURN595, ASFURN596) was recorded on 17 May 2013, at 19:00hrs, air temperature 25.7°C. These calls were recorded with a PCM-M10 digital recorder coupled with a Rodes NTG2 directional microphone. Digital recordings were sampled at 48 kHz and 24 bit resolution and saved in uncompressed WAVE format.

The advertisement call of *S. eurydice* described by Pombal Jr. *et al.* (1995) was recorded at an air temperature of 27.5°C. This call was extracted from the Compact Disc - Sound guide of the Atlantic Rain Forest



**Figure 2.** Two release calls (ZUFMS-DA009) of *Scinax eurydice* (CHUFPB 7957, SVL 51.4 mm) from Reserva Biológica Guaribas, municipality of Mamanguape, State of Paraíba, Brazil. A) Spectrogram; B) Power spectrum of the second call; C) Oscillogram.

anurans (Haddad et al., 2005) (CD-ROM; track 34), with Windows Media Player version 12 for sonogram construction. The advertisement call parameters from Atibaia were based on Magrini et al. (2011).

**Table 1.** Acoustic parameters of the release call of *Scinax eurydice* from Reserva Biológica Guaribas, municipality of Mamanguape, State of Paraíba, Brazil. Values are presented as mean  $\pm$  standard deviation (range).

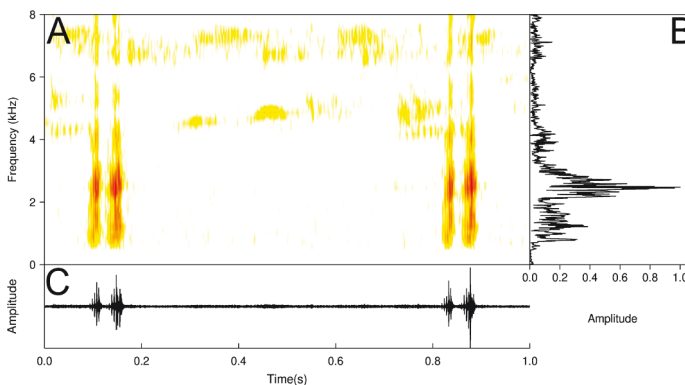
Acoustic parameters/Rate	Release call
<i>n</i> (calls)	23
Duration	0.116 $\pm$ 0.01 (0.102 – 0.149)
Pulses/call	22 $\pm$ 1.7 (20 – 26)
Pulses/second	184.9 $\pm$ 7.3 (172.4 – 200)
Concatenated pulse duration (s)	0.102 $\pm$ 0.01 (0.094 – 0.134)
Concatenated pulse/call	21 $\pm$ 1.7 (19 – 25)
Pulse/second (Concatenated part)	194.7 $\pm$ 7.0 (186.3 – 210)
Single pulse duration (s)	0.004 $\pm$ 0.001 (0.003 – 0.006)
Interval between components of pulses (s)	0.007 $\pm$ 0.002 (0.004 – 0.013)
Dominant Frequency (Hz)	1894.9 $\pm$ 397.1 (861.3 – 2067.2)

We analyzed the calls with Raven Pro 1.5 for Windows (Bioacoustic Research Program) and constructed audio spectrograms in R software using the package “seewave” (Sueur et al., 2008; R Development Core Team) with the following parameters: FFT window width = 256, Frame = 100, Overlap = 75, and flat top filter. We analyzed the following acoustic parameters: number of pulses per call, number of pulses per second, duration of concatenated pulses, number of concatenated pulse per call, pulse rate (pulse per seconds), single pulse duration, interval between components of pulses, and dominant frequency. Terminology in call descriptions follows Duellman and Trueb (1986), and the terminology for concatenated pulses follow Santana et al. (2013). Populations were labelled as “northern” (individuals recorded in Mamanguape and Santa Rita), and “southern” (calls from Conceição da Barra, Pombal Jr. et al., 1995; and from Atibaia, Magrini et al., 2011).

## Results

The release call of *S. eurydice* is a not harmonic multipulsed note and presents one first isolated pulse, followed by a group of concatenated pulses (Table 1, Figure 2).

The advertisement call of *Scinax eurydice* from Mamanguape is composed of two distinct not harmonic multipulsed notes (Figure 3). The first note is shorter than the second and presents fewer pulses. The advertisement call from populations of Santa Rita also consists of two distinct not harmonic multipulsed notes (Figure 4). The



**Figure 3.** Two advertisement calls (ZUFMS-DA007) of *Scinax eurydice* (CHUFPB7956, SLV 52.2 mm) from Reserva Biológica Guaribas, municipality of Mamanguape, State of Paraíba, Brazil. A) Spectrogram; B) Power spectrum of the second call; C) Oscillogram.

dominant frequency from Mamanguape ranges from 2411.7 – 2756.2 Hz, and from Santa Rita is 2625.0 Hz. Notes of both populations show the same duration and the first note presents one pulse at less (5 pulses). Other acoustic parameters of release and advertisement calls are presented in Tables 1 and 2.

## Discussion

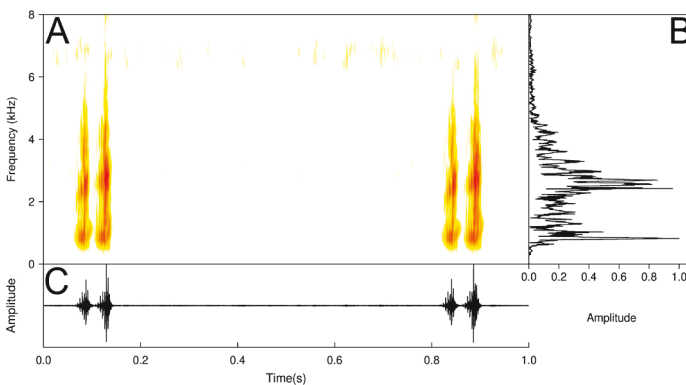
This is the first description of release calls for the genus *Scinax*. According to Pough *et al.* (2001), release calls may differ from advertisement calls in duration, number of pulses, and dominant frequency. Our results support this statement; because all analysed acoustic parameters of release calls (0.102 – 0.149 s, 172.4 – 200 pulse/call, 861.3 – 2067.2 Hz) differ from advertisement calls described here (0.067 – 0.072 s, 5 – 7 pulse/call, 2411.7 – 2756.2 Hz).

Release calls have potential to facilitate interspecific communication in heterospecific amplexus (Leary, 2001). However, some studies showed release calls as valuable source of taxonomic information (di Tada *et al.*, 2001 for *Rhinella spinulosa* group; Grenat and Martino, 2013 for *Odontophrynus americanus* and *O. cordobae*). This type of call differs specifically among several anuran species and might be phylogenetically informative to distinguish closely related taxa (Brown and Littlejohn, 1972; Sullivan and Wagner-Jr., 1988; di Tada *et al.*, 2001; Grenat and Martino, 2013; Vieira *et al.*, 2014).

The advertisement calls of *S. eurydice* from southeastern populations (Pombal Jr. *et al.*, 1995;

Magrini *et al.* 2011) (Figure 5) differ in all acoustic parameters from northern populations calls (Table 2), overlapping in the number of pulses of the note 1, number of pulses of the note 2, duration of the note 1, and the dominant frequency. The main difference is in the number of notes emitted in each call, being two notes in the call described in the present study, and three notes in calls described by Pombal Jr. *et al.* (1995) and Magrini *et al.* (2011). Magrini *et al.* (2011) described the call from Atibaia as presenting only a note, but here we consider that call as composed by three notes, as clearly presented in the sonogram (Figure 5A in Magrini *et al.* 2011). Furthermore, dominant frequency from northern populations (2411.7 – 2752.6 Hz) is lower than Conceição da Barra (2800 – 3800 Hz) and overlap with Atibaia (1180 – 2620 Hz).

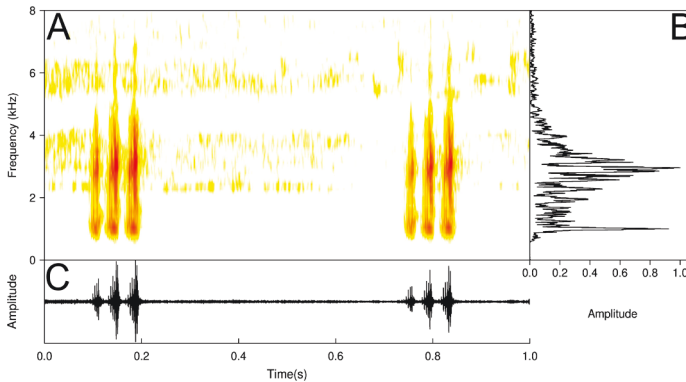
Anuran advertisement calls are species-specific (Gerhardt and Davis, 1988), and are considered as an efficient mechanism in reproductive isolation (Littlejohn, 1977); consequently, useful in taxonomic distinction of species (Wells, 1977; Duellman and Trueb, 1986). Our results should be treated with caution once we only compared the ends of a widely and continuous distribution (ca. 1500 Km airline between sampled populations). Furthermore, differences in number of notes can be influenced by environmental conditions, and/or individual behavioral characteristics of the studied specimens (Gerhardt and Huber, 2002; Hartmann *et al.*, 2006). However, our findings highlight the possibility of cryptic diversity under the name *S. eurydice*. Also, some studies considered species complex, that share the same distribution pattern of *S.*



**Figure 4.** Two advertisement calls (ASUFRN594) of *Scinax eurydice* (FSCHUFPB4437, SLV 48.7 mm) from Usina Japungu, municipality of Santa Rita, State of Paraíba, Brazil. A) Spectrogram; B) Power spectrum of the second call; C) Oscillogram.

**Table 2.** Acoustic parameters of the advertisement call of *Scinax eurydice* from Reserva Biológica Guaribas, Mamanguape, and Usina Japungu, Santa Rita, State of Paraíba, and from Conceição de Barra, State of Espírito Santo (Pombal Jr. et al., 1995; Magrini et al., 2011). Values are presented as mean  $\pm$  standard deviation (range).

Acoustic parameters	Mamanguape, Paraíba (present study – CHUFPPB7956)	Santa Rita, Paraíba (present study – FSCHUFPPB4437)	Santa Rita, Paraíba (present study – FSCHUFPPB4470)	Conceição da Barra, Espírito Santo Pombal Jr. et al. (1995)	Atibaia, São Paulo Magrini et al. (2011)
<i>n</i> (calls)	20	23	20	-	-
Duration (s)	0.070 $\pm$ 0.001 (0.067 – 0.072)	0.082 $\pm$ 0.002 (0.077 – 0.084)	0.073 $\pm$ 0.002 (0.069 – 0.077)	0.100 $\pm$ 0.005 (0.090 – 0.110)	0.11 $\pm$ 0.006 (0.102 – 0.116)
Notes/call	2	2	2	3	3
Pulse/Note 1	5	5	5	4.67 $\pm$ 0.50 (4 – 5)	-
Pulse/Note2	6 $\pm$ 0.366 (6 – 7)	7 $\pm$ 0.449 (6 – 7)	6	5.83 $\pm$ 0.86 (5 – 7)	-
Pulse/Note 3	-	-	-	5.83 $\pm$ 0.86 (5 – 7)	-
Duration Note 1 (s)	0.024 $\pm$ 0.001 (0.023 – 0.027)	0.034 $\pm$ 0.002 (0.030 – 0.036)	0.028 $\pm$ 0.002 (0.026 – 0.032)	0.019 $\pm$ 0.003 (0.017 – 0.030)	-
Duration Note 2 (s)	0.030 $\pm$ 0.001 (0.027 – 0.031)	0.034 $\pm$ 0.001 (0.031 – 0.038)	0.030 $\pm$ 0.002 (0.027 – 0.033)	0.024 $\pm$ 0.001 (0.020 – 0.026)	-
Duration Note 3 (s)	-	-	-	0.024 $\pm$ 0.002 (0.020 – 0.026)	-
Interval between Notes (s)	0.015 $\pm$ 0.001 (0.013 – 0.017)	0.015 $\pm$ 0.002 (0.009 – 0.017)	0.014 $\pm$ 0.002 (0.011 – 0.017)	-	-
Pulse/call	11 $\pm$ 0.4 (11 – 12)	12 $\pm$ 0.449 (11 – 12)	11	16 $\pm$ 0.81 (15 – 18)	-
Pulse/second	157.1 $\pm$ 6.3 (152.8 – 173.9)	144.6 $\pm$ 5.6 (130.9 – 153.8)	150.7 $\pm$ 4.9 (142.9 – 159.4)	842.1 $\pm$ 84.6 (600 – 944.4)	43.4 $\pm$ 5.9
Dominant Frequency (Hz)	2411.7 $\pm$ 104.2 (2411.7 – 2756.2)	2625	2625	2800 – 3800	(1180 – 2620)

**Figure 5.** Two advertisement calls of *Scinax eurydice* (Soundtrack 34, SVL 47 mm) from Conceição da Barra, State of Espírito Santo, Brazil, described by Pombal-Jr. et al. (1995). A) Spectrogram; B) Power spectrum of the second call; C) Oscillogram.

*eurydice* (e.g. *Proceratophrys boiei*, *Rhinella crucifer*, *S. alter*, *S. auratus* species complexes), composed by several species (Baldissera et al. 2004; Prado and Pombal Jr, 2008; Nunes et al., 2012), or pointed as a potentially species complex (e.g. *Hypsiboas semilineatus*, *H. faber*)

(Carnaval et al., 2009). Since past events influence the current distribution of many Atlantic Forest frogs lineages (Carnaval and Moritz, 2008; Fitzpatrick et al., 2009; Thomé et al., 2010; Thomé et al., 2012; Tonini et al., 2013; Gehara et al., 2013), the results presented

here confirm the proposal of Menezes *et al.* (2016), which state two major clades for *S. eurydice*, and may harboring undescribed taxa consistent with Pleistocene refugia (Carnaval *et al.* 2009).

*Scinax* is the most speciose genus within the subfamily Hylinae (Nunes and Pombal Jr., 2011), and has been taxonomically problematic owing its diversity (Pombal *et al.*, 1995). Furthermore, similar studies with different species of the genus, combined with the advertisement call, may contribute to its taxonomy.

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