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Vocal repertoire of *Scinax v-signatus* (Lutz 1968) (Anura, Hylidae) and comments on bioacoustical synapomorphies for *Scinax perpusillus* species group

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Abstract. Herein we describe the vocal repertoire of *Scinax v-signatus*, compare it to the other species belonging to the *S. perpusillus* species group and discuss the bioacoustical synapomorphies proposed for the group. We recorded an advertisement call and an aggressive call of *S. v-signatus*. The advertisement call is similar to the one described for others species of the *Scinax perpusillus* group. Similarly to *Scinax cosenzai*, the first note in the advertisement call of *S. v-signatus* is the longest. The aggressive call was preceded by a series of advertisement calls. We suggest that bioacoustical parameters proposed as synapomorphies for the *Scinax perpusillus* group are not valid, as they are also observed in species belonging to other groups within the genus.

Keywords. Advertisement call, Bromeligenous species, Aggressive call, Atlantic Rain Forest, Taxonomy, Anuran bioacustic, Raven.

The Neotropical genus *Scinax* Wagler, 1830 include 113 recognized species (Frost, 2015) distributed from southern Mexico to the east coast of Argentina (Faivovich, 2002). It is composed of the *S. ruber* and *S. catharinae* clades with the later including the *S. perpusillus* and *S. catharinae* species groups (Faivovich, 2002). *Scinax perpusillus* species group is endemic to the Brazilian Atlantic Forest and was first suggested based on its bromeligenous habitat and absent or reduced membrane between toes I-II and II-III (Peixoto, 1987). The group currently includes 13 described species and its monophyly has never been formally tested (Alves-Silva and Silva, 2009). Furthermore, Pombal and Bastos (2003) suggested bioacustical characters as possible synapomorphies.

The specificity of anurans' calling repertoire is an important taxonomic and phylogenetic tool (Duellman and Trueb, 1994; Goicoechea et al., 2010; Taucce et al., 2012). Despite this importance, only eight species of the *Scinax perpusillus* group had their call described: *S. arduous* (Pombal and Bastos, 2003), *S. belloni* (Peres and Simon, 2011), *S. cosenzai* (Lacerda et al., 2012.), *S. littoreus* (Pontes et al., 2013.), *S. peixotoi* (Brasileiro et al., 2007) and *S. perpusillus* (Pombal and Bastos, 2003). Moreover, *S. arduous* (Pombal and Bastos, 2003), *S. littoreus* (Pontes et al., 2013) and *S. perpusillus* (Pombal and Bastos, 2003) had their aggressive/territorial calls described and *S. insperatus* (Silva and Alves-Silva, 2011) and *S. v-signatus* (Alves-Silva and Silva, 2009) had an antiphonic interaction described. Besides those studies,

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Heyer et al. (1990) described the advertisement call of an unnamed species belonging to the *Scinax perpusillus* group from Estação Biológica de Boracéia, municipality of Salesópolis, state of São Paulo.

Scinax v-signatus (Lutz, 1968) is endemic to the Serra dos Órgãos mountain range, Rio de Janeiro state, Southeastern Brazil (Silva and Alves-Silva, 2013). Alves-Silva and Silva (2009) described its vocalization based on males in antiphony and restricted their study to a behavioral/reproductive focus. Herein we describe the vocal repertoire of *S. v-signatus* and compare its advertisement call with others species belonging to the *S. perpusillus* group. Furthermore, we also compare it to the calls described for its sister group – *S. catharinae* group *sensu* Faivovich 2002 – and discuss the use of bioacoustical data as a synapomorphy for the *S. perpusillus* group.

We recorded 261 calls from six specimens of *Scinax v-signatus*. Recordings were made at different localities throughout the Serra dos Órgãos mountain range, Rio de Janeiro state, Southeastern Brazil. Voucher specimens are housed at the Coleção Herpetológica of Universidade Federal de Minas Gerais (UFMG-AMP) and Coleção de Anuros do Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia (AAG). Two individuals (only one of which was collected: UFMG 14106) were recorded on 18 January 2013, at São Pedro da Serra district, municipality of Nova Friburgo, (22°18'47.03"S, 42°20'13.92"W; 25 °C; Datum WGS84). In the same district, we recorded two other individuals on 19 January 2013. One individual was recorded on 23 January 2013 at Macaé de Cima district, also in the municipality of Nova Friburgo (22°28'40.08"S, 43°15'9.36"W; 25 °C; Datum WGS84). One individual (AAG-UFU 0742) was recorded on 14 December 2011, at Maria Mendonça district, municipality of Trajano de Moraes (22°11'32.60"S, 42°11'16.29"W; 20 °C; Datum WGS84). Whilst this individual was being kept in a plastic bag, it emitted a different type of call. Recordings were made using digital recorders Marantz PMD 660 coupled with a Sennheiser ME66 unidirectional microphone in all individuals, apart from those recorded on 19 January 2013, in which we used a Sony ICD P620 with internal microphone. The first featured sample rate of 44.1 kHz, whereas the second 11.025 kHz. Both recorders had a 16 bit resolution and each recording was made at a distance of 20 cm to 1 m between specimen and microphone.

Oscillograms and spectrograms were produced and analyzed using software Raven Pro 1.4 (FFT = 256, 89% Overlap and Hann window). Terminology follows Duellman and Trueb (1994) and Toledo et al. (2015). Parameters measured include call duration, interval between calls, note duration, interval between notes, number of notes per call, dominant frequency and peak frequency. Temporal parameters were measured directly from the waveform. Dominant frequency is given as range and is considered as the frequency band comprising 90% of energy within the call. It was acquired through the parameters "Frequency 5%" and "Frequency 95%" from Raven Pro 1.4 (Charif et al., 2010). The peak frequency represents the peak of energy within the call and was acquired using the parameter "Peak Frequency" from Raven Pro 1.4.

We compared our results to all data available in the literature. Whenever values in tables and textual description presented incoherencies, we used the textual description. Silva and Alves-Silva (2011) referred as notes and pulses what is commonly treated as calls and notes (see discussion in Lacerda et al., 2012). Therefore, we treated it as calls and notes. Alves-Silva and Silva (2009) did not stablish bioacoustics parameters for the call of *Scinax v-signatus*, we did not compare it to our results.

The advertisement call of *Scinax v-signatus* (Fig. 1A) consist of a series of 1-9 pulsed notes. It features duration

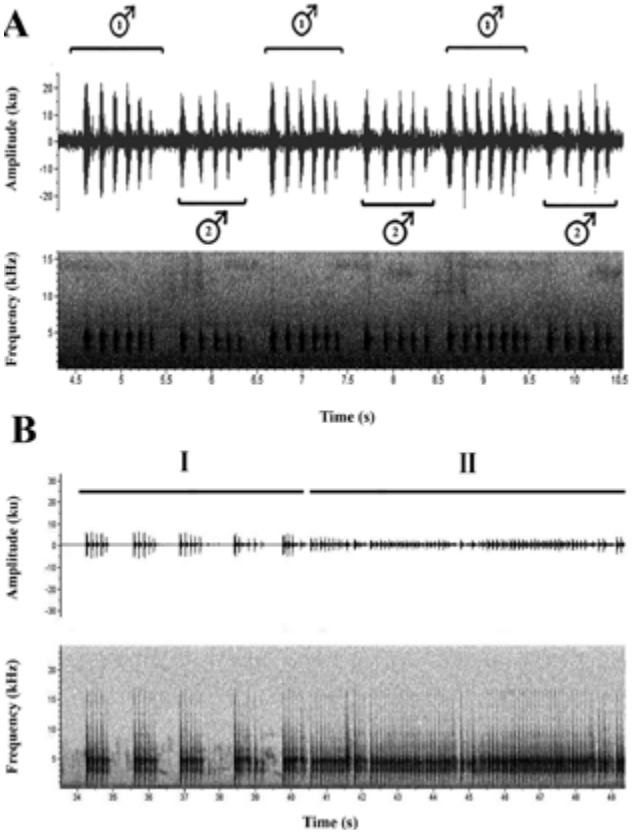

Fig. 1. Oscillogram and spectrogram of advertisement and aggressive calls of *Scinax v-signatus*: A) two distinct male in an interaction; B) mixed call of *Scinax v-signatus*, I refers to the initial advertisement call and II refers to the aggressive call.

between 77 and 1,076 ms (602 ± 184 ms; n = 240 calls) (Table 1) and the interval between calls ranges between 142-2702 ms (1141 ± 0.483 ms; n = 183 intervals), with a tendency to shrink at the end of a series of calls. The first note is the longest with duration of 45-127 ms (73.0 ± 13.8 ms; n = 260 notes). The remaining notes have duration of 14-101 ms (52.0 ± 13.8 ms; n = 946 notes). Interval between notes ranged from 26 to 242 ms (87.0 ± 25.0 ms; n = 784 intervals between notes). The notes had 1-23 pulses (16 ± 7 pulses). Dominant frequency ranged between 2,282-5,250 Hz. The peak frequency varied among populations, ranging between 2,497 and 4,500 Hz (3,937 ± 0.407).

Whilst being kept on a plastic bag, one male emitted an aggressive call preceded by a series of 20 advertisement calls (Fig. 1B). This call was emitted by one male, alone and inside a plastic bag. Thus, we suggest that this type of call has an aggressive context (Toledo et al., 2015). This call consisted of a series of 360 pulsed notes ranging from 20 to 350 ms (40.0 ± 39.0) at rate of 451.8 notes per minute. Dominant frequency ranged from 2,625 to 5,625 Hz and peak frequency of 2,812-4,875 Hz (4,312 ± 695 Hz).

Our results indicate that the advertisement calls of *Scinax v-signatus* resemble the ones from *S. arduous*, *S. belloni*, *S. cosenzai*, *S. littoreus*, *S. perpusillus*, and *S. peixotoi*. Those species share a similar pattern of call duration, interval between calls, number of notes, pulses per note, and dominant frequency. On the other hand, the call of *S. peixotoi* had higher interval between notes, and *S. belloni* has more pulses per note that *S. v-signatus*' advertisement call. This high similarity is also observed in the aggressive call, where only the call duration and number of notes had a higher value than others.

When analyzing the call of *Eleutherodactylus coqui*, Narins and Capranica (1978) showed that the first note has a territorial importance. In their study, neighboring males only responded when the first note was played and the presence of the second note had no influence on male's behavior. Calls that carry multiple information (e.g. advertisement and territorial calls) are likely to reduce energy expenditure (Wells, 1988). Heterogeneous character was found on call of *Scinax v-signatus*. The first notes of all calls are longer than others ones. These same characters are related to *S. cosenzai*, other species

Table 1. Advertisement and agressive (*) calls of *Scinax perpusillus* group species.

Species	Call duration (ms)	Call interval (ms)	Number of notes	Note duration (ms)	Note interval (ms)	Number of pulses/ note	Pulse duration (ms)	Dominant frequency (Hz)	Reference
S. arduous	198.0-328.0	234.0-283.0	4-6	14.0-45.0	23.0-51.0	5-13	2.0-4.0	3802-4682	Pombal and Bastos (2003)
S. belloni	590.0-690.0	1410-3960	2	17.0-21.0	28.0-36.0	28-35	-	3078	Peres and Simon (2011)
S. belloni	100.0-120.0	1410-3960	3	16.0-24.0	27.0-36.0	24-31	-	3078	Peres and Simon (2011)
S. cosenzai	177.0-2066	1900-4320	2-14	1.8-111.0	10.0-516.0	1-33	177.0-2066	3375.9-4571.2	Lacerda et al. (2012)
S. littoreus	174.0-287.0	980.0-14197	2-5	118.0-348.0	14.0-38.0	2-9	4.0-24.0	4306.6-4651.2	Pontes et al. (2013)
S. littoreus	204.0-282.0	1009-7027	2-4	118.0-291.0	24.0-38.0	2-8	-	4306.6-4478.9	Pontes et al. (2013)
S. littoreus	174.0-287.0	980.0-2781	2-9	255.0-348.0	26.0-29.0	3-9	-	4512.7-4651.2	Pontes et al. (2013)
S. littoreus	201.0-223.0	2122-14197	3-4	230.0-371.0	25.0-27.0	2-4	-	4306.6	Pontes et al. (2013)
S. perpusillus	92.0-174.0	776.0-1067	3-6	7.0-18.0	9.0-59.0	3-5	2.0-5.0	4554-4856	Pombal and Bastos (2003)
S. aff. *perpusillus*	250.0-400.0	-	4-5	30.0-70.0	-	4-10	-	3500-5900	Heyer et al. (1990)
S. peixotoi	146.0-232.0	438.0-2400	3-5	9.0-28.0	438.0-2400	4-9	1.0-3.0	3617-3963	Brasileiro et al. (2007)
S. v-signatus	**77.0-1076**	**142.0-2702**	**1-9**	**14.0-127.0**	**26.0-242.0**	**1-23**	**-**	**2282-5250**	**This study**
*S. arduous**	53.0-64.0	82.0-116.0	1	53.0-64.0	-	18-22	2.0-4.0	3760-4445	Pombal and Bastos (2003)
*S. insperatus**	-	-	25-50a	-	630.0-860.0	155-434b	-	4479-4665c	Silva and Alves-Silva (2011)
*S. insperatus**	-	-	23-47a	-	50.0-631.0	223-500b	-	4479-4665c	Silva and Alves-Silva (2011)
*S. littoreus**	267.0-322.0	981.0-1590	14-22	20.0-49.0	9.0-18.0d	2-4	1.0-4.0e	5304.6-5439	Pontes et al. (2013)
*S. littoreus**	274.0-349.0	689.0-915.0	16-31	16.0-34.0	12.0-27.0d	2-4	1.0-4.0e	5356.2-5439	Pontes et al. (2013)
*S. littoreus**	220.0-298.0	1228-1374	8-12	22.0-58.0	3.0-23.0d	2-4	1.0-4.0e	5292	Pontes et al. (2013)
*S. perpusillus**	314.0-400.0	748.0-792.0	6-12	8.0-42.0	8.0-64.0	1-6	4.0-9.0	4902-4918	Pombal and Bastos (2003)
*S. perpusillus**	183.0-508.0	-	1	183.0-508.0	-	-	-	4770-4743	Pombal and Bastos (2003)
S. v-signatus*	**47820.0**	**-**	**360**	**2.0-350.0**	**12.0-183.0**	**-**	**-**	**2625-5625**	**This study**

of *Scinax perpusillus* species group (JVL pers. obs.). In the same way, the aggressive call of *S. v-signatus* was preceded by an advertisement call, composing a mixed call series.

According to Pombal and Bastos (2003), the *Scinax perpusillus* species group is distinguishable from the *S. catharinae* group and the *S. ruber* clade by having advertisement calls composed of three to six pulsed notes spaced by 23-59 ms. However, those parameters overlap in some species belonging to the *S. catharinae* group (e.g. *S. angrensis* and *S. littoralis*, Garey et al., 2012). On other hand, some species signed to the *S. perpusillus* species group have advertisement calls with more than six notes per call and longer intervals between notes (i.e. *S. littoreus*, *S. peixotoi* and *S. v-signatus*). Based on our results, we suggest that bioacoustical parameters should no longer be considered synapomorphies for the S. *perpusillus* species group. However, new studies focusing on relationships within *Scinax* considering bioacoustical characters using modern phylogenetic approaches could elucidate this issue.

The range of bioacoustical terminology in the literature reinforces the difficulty of establishing comparisons between calls of different species. Within the genus *Scinax*, species recognition and identification is not simple (Pombal et al., 1995; Lourenço et al., 2014). The high complexity of their vocal repertoires does not make it easier. In view of this discussion, we emphasize the importance of the careful use of bioacoustics in a genus known for its high diversity and difficult taxonomy.

NOTE ADDED IN PROOF

The authors did not includes in this manuscript, the nomenclatural modifications, proposed to the genus Scinax by "Duellmann, W., Marion, A., Blair, H. (2016). Phylogenetics, classification, and biogeography of the treefrogs (Amphibia: Anura: Arboranae). Monograph Zootaxa 4104: 001-109" as either the results of the article "Carvalho, T.R., Martins, L.B., Giaretta, A.A. (2015). The complex vocalization of the *Scinax cardosoi* (Anura: Hylidae), with comments on adverrisement call in the *S. ruber* Clade. Phyllomedusa 14: 127-137)" because the manuscript was accepted before the publication of these articles.

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