Revisiting the advertisement call features of *Scinax montivagus* (Anura: Hylidae)

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**ABSTRACT**

In the present study, we revisit the advertisement call of *Scinax montivagus* based on new recordings from Rio de Contas, state of Bahia, Brazil. The species’ call consists of a single type of multipulsed note of relatively short duration (mean = 192 ms), having two non-harmonically related emphasized frequency bands. The dominant frequency corresponds either to the lower or the higher band, or it may shift between both along call emissions. Herein, we assess and discuss call features that diverge from what is in the literature and also provide further details on the species call. We reinforce that detailed and standardized advertisement call descriptions of different *Scinax* species would contribute to acoustic diagnosis between them.

**Introduction**

The advertisement call is the main type of acoustic signal emitted by anuran males during the breeding season and functions in the attraction of conspecific females [1, 2] and in among-males spacing [3]. Since the advertisement call may act as a prezygotic reproductive isolation mechanism, it is widely used in taxonomic studies to help in species delimitation [4], especially in cases of cryptic species [5–8]. Furthermore, advertisement calls can be useful to address questions on macroevolutionary patterns [9], conservation status [10], and acoustic landscape monitoring [11, 12].

Currently, *Scinax* Wagler, 1830 comprises 73 species distributed in South and Central Americas, and in some Caribbean islands [13]. The genus was formerly composed by two major clades, those of *S. ruber* and *S. catharinae* clades [14, 15], but a recent taxonomic change based on molecular analysis [16] reallocated species of the *Scinax catharinae* clade to *Ololygon* Fitchinger, 1843 and species of the *S. uruguayus* group (formerly group of *S. ruber* clade) to *Julianus* Duellman, Marion, and Hedges, 2016; therefore, *Scinax* is currently composed solely by species of the *S. ruber* clade, with the exception of species of the formerly *S. uruguayus* group. The genus and its component species have a long and sometimes problematic taxonomic history [14], due to the morphological conservatism and the large diversity within it [17, 18], being frequent the description of new species [19–22] and synonymizations [e.g. 15, 23, 24]. These facts point out to the need to use lines of evidence other than morphology, such as molecular and acoustics characterizations, to uncover cryptic diversity in *Scinax* [17]. In this sense, several studies have been done in an endeavor to properly document interspecific variation of vocal patterns in the genus [25–34]. The advertisement call of *Scinax* species is usually consisted of a single multipulsed note with variable duration [25, 31–35] and sometimes with more than one emphasized frequency band [25, 30, 36], what seems to be relevant in species-specific recognition [25, 37].

*Scinax montivagus* Juncá, Napoli, Nunes, Mercês, and Abreu, 2015 is a recently described species apparently endemic to the Serra do Espinhaço Range in state of Bahia (BA), where it is known from some few sites around the type locality [38]. Herein we revisit its advertisement call based on recordings from Rio de Contas (BA), aiming to (1) provide further details on the species’ advertisement call; (2) report on differences in a call trait regarding the literature; (3) reinterpret the species’ call frequency features.

**Methods**

**Data collection and species identification**

We recorded and collected specimens in Pico das Almas, municipality of Rio de Contas [13°29′53.32″S; 41°57′52.44″W (WGS84 datum), 1540 m above sea level], Espinhaço Range, state of Bahia, northeastern Brazil. We recorded six males with a Marantz PMD 671 digital
recorder (sampling rate of 48.0 kHz and 16 bits resolution) coupled to a Sennheiser ME67-K6 directional microphone, which was positioned at a distance of approximately 2 to 3 m from the calling males. We made the recordings on 25 November 2016, between 19:00 and 20:00 h, and air temperature at 25°C. Call voucher specimens and recordings (see Table A1) are housed in the Coleção de Anuros do Museu de Biodiversidade do Cerrado (AAG-UFU), Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.

Our specimens (n = 6 males; Figure 1) match the original species diagnosis [38] in the following features: (1) moderate adult size (25.9–26.8 mm SVL); (2) snout rounded in dorsal and lateral views; (3) straight and well-marked canthus rostralis; (4) dorsum background with a sparse and irregular dark-spotted pattern; two specimens (AAG-UFU 5625 and 5629) have fragmented brown blotches forming an inverted parenthesis in scapular region; (5) advertisement call duration of 142–239 ms, dominant frequencies ranging from 1.7 to 1.8 kHz (lower-frequency band) and 4.0–4.4 kHz (higher-frequency band), and 12–16 pulses per call.

**Acoustic analysis**

We analyzed 10 calls of each male (n = 60) using the Raven Pro 1.5 software [39] with the following settings: window type Hann, window size of 256 samples, 3 dB filter bandwidth of 248 Hz, 90% overlap (locked), hop size of 0.590 ms, DFT size of 1024 samples and grid spacing at 43.1 Hz. We applied a filter at 200 Hz prior analysis to reduce background noise (wind). We manually measured temporal traits in the oscillogram and obtained dominant frequency values using the “Peak Frequency” function of Raven [39]. We generated sound figures with the seewave package v. 1.7.6 [40] in the R platform v. 3.1.1 [41], using the following settings: window = Hanning, overlap = 90%, and FFT = 256 (for Figure 2) or 1024 (for Figure 3). Relative amplitude scale of 36 dB in spectrograms (Figures 2 and 3) is represented by colors (red being the maximum amplitude). We followed Bang and Giaretta [29] for acoustic terminology and definitions, except for pulse rate and call rate which were measured according to Cocroft and Ryan [9].

**Results**

The advertisement call (Figure 2; Table 1) consists of a single type of multipulsed note with duration of 142–239 ms (short call sensu Bilate and Lack [32]), emitted at intervals of 1.4–4.8 s. Calls have 12–16 pulses that increase in amplitude from the first to the third or fourth pulse, until it reaches a plateau towards the end of the call. In some cases, pulses can raise gradually in amplitude until final portion of the call. Pulses have regular internal amplitude modulations (typically from 2 to 3), except for the first one that can be weakly modulated (i.e. the pulse has ill-defined amplitude modulation). Pulses are released at a rate of 66–81/s. The first pulses last 6–9 ms, mid-call pulses 7–10 ms, and last pulses 7–12 ms. Calls are emitted at rates of 11–38/min, and have two emphasized (i.e. most energetic) and not harmonic-related frequency bands (Figures 2 and 3(a,b)). At FFT of 256, pulses have the shape of an “I” or an inverted “L” (Figure 2). The lower-frequency band (LFB) (Figure 2(a,b)) peaks at 1679–1851 Hz and the
higher-frequency band (HFB) (Figure 2(c,d)) at 3996–4431 Hz. Dominant frequency corresponded to the LFB in one individual and to the HFB in other four; in a male, it shifted between LFB and HFB along the call emission. At FFT of 1024, both LFB and HFB are embedded in several sidebands through the call’s bandwidth (Figure 3(a)).

Discussion

Juncá et al. [38] recognized the advertisement call of *Scinax montivagus* as being composed of two harmonic bands, but as demonstrated herein, this relationship does not seem to exist. A harmonic series is a set of component frequencies that are all integer multiples of a fundamental frequency ($f_0$, $2f_0$, $3f_0$, and so on; see Bradbury and Vehrencamp [42]). However, it is noticeable (FFT = 1024; Figure 3(a,b)) that these frequency bands are embedded in several peaks representing sidebands resulting from amplitude modulations [43]. For a harmonic series, the next peak following the LFB (fundamental frequency = 1679–1851 Hz) should be its double, but instead, it is at approximately 2300 Hz (Figure 3(b)). Furthermore, the first pulse, which is

Table 1. Advertisement call features of *Scinax montivagus* from Rio de Contas (present study), topotypes (Miguel Calmón [38]), and from Mucugê, all in the Espinhaço range, state of Bahia, northeastern Brazil. Data are presented as mean ± SD (minimum-maximum). HFB = Higher-frequency band; LFB = Lower-frequency band.

| Call traits          | Rio de Contas n = 60 calls (6 males) | Type locality n = 268 calls (4 males) | Mucugê n = 69 calls (3 males) |
|----------------------|--------------------------------------|---------------------------------------|-----------------------------|
| Call duration (ms)   | 192 ± 32 (142–239)                   | 200 ± 10 (140–240)                    | 220 ± 20 (190–230)          |
| Call rate (calls/min)| 26.3 ± 9.8 (11–38)                   | -                                     | -                           |
| Call interval (s)    | 2.5 ± 1.3 (1.4–4.8)                  | 2.2 ± 1.0 (0.7–6.9)                  | 2.8 ± 2.6 (0.5–19.3)        |
| Number of pulses     | 14.3 ± 1.7 (12–16)                   | 12.6 ± 0.8 (9–15)                    | 14.2 ± 1.7 (11–19)          |
| 1st pulse duration (ms) | 7.0 ± 1.0 (6.1–9.0)                 | -                                     | -                           |
| Mid-call pulse duration (ms) | 8.6 ± 0.9 (7.4–9.8)           | -                                     | -                           |
| Last pulse duration (ms) | 9.1 ± 1.5 (7.5–11.7)            | -                                     | -                           |
| 1st portion pulse interval (ms) | 6.8 ± 1.0 (5.7–8.3)            | -                                     | -                           |
| Mid-call pulse interval (ms) | 5.5 ± 0.9 (4.0–6.6)            | -                                     | -                           |
| Last portion pulse interval (ms) | 5.4 ± 0.7 (4.4–6.2)           | -                                     | -                           |
| Pulse rate (pulses/s) | 73.0 ± 5.6 (66.4–81.0)         | 60.6 ± 1.5 (53.4–64.1)               | 63.6 ± 4.3 (52.9–70.5)      |
| HFB (kHz)            | 4.1 ± 0.2 (4.0–4.4)                 | (3.0–5.6)                             | 4.1 ± 0.2 (3.8–4.4)        |
| LFB (kHz)            | 1.8 ± 0.1 (1.7–1.8)                 | (0.8–2.7)                             | 1.8 ± 0.0 (1.7–1.9)        |
| Temperature (°C)     | 25                                   | 18                                    | 19–20                       |

Figure 2. (a) Spectrogram (top) and its respective oscillogram (bottom), and (b) power spectrum of an advertisement call of *Scinax montivagus* depicting the lower frequency band as the dominant one (sound file = Scinax_montivagusRioContasBA4aAAgm671). (c) Spectrogram (top) and its respective oscillogram (bottom), and (d) power spectrum of a call depicting the higher frequency band as the dominant (sound file = Scinax_montivagusRioContasBA6aAAgm671). Recordings from Rio de Contas, state of Bahia, northeastern Brazil. Spectrograms with an FFT of 256. See further details on sound recordings in Table A1.

Figure 3. (a) Spectrogram of the same call in Figure 2(a) with FFT of 1024 samples (arrow points to the frequency range of the first pulse), and (b) its respective power spectrum showing several frequency peaks around the two main ones (LFB = 1.67 kHz, HFB = 3.96 kHz).
weakly amplitude modulated, does not have the HFB apparent in its frequency range (Figure 3(a)).

We found slight differences in pulse rate (66–81 pulses/s, n = 60 calls) when compared to the values reported by Juncá et al. [38] (53–70 pulses/s, n = 337 calls, n = 7 males), possibly due to random effects related to small sample or interpopulational variation. Also, call variations may be heavily influenced by extrinsic factors, such as temperature variations (our values were higher than those of Juncá et al. [38]; see Table 1). Therefore, only through a statistical analysis encompassing larger samples, standardized call descriptions from other populations, as well as considering environmental variables (e.g. temperature, humidity), that we could address a putative intraspecific variability in *Scinax montivagus* advertisement call.

We assert that deeper acoustic analyses and a standardized methodology (as intended by Köhler et al. [4]) could raise a more comparable acoustic dataset in which diagnostic call features might be revealed. Once this dataset is raised, it could serve as a framework to understand macroevolutionary patterns through comparative methods [44–46], as well as to aid in conservation efforts involving acoustic monitoring [12]. Furthermore, considering that several *Scinax* species have advertisement calls composed of two non-harmonically related main emphasized frequency bands [25, 28, 30], studies on biomechanical/physiological process could elucidate how these species produce such complex calls, as well as how they are able to shift the dominance between these frequency bands along call emissions.

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**Authors’ contribution**

AAG conceived and designed this study. AAG recorded and collected the specimens. AGL conducted the acoustic analysis. AGL and DLB interpreted the results and wrote the first drafts of this manuscript. The final version was reviewed by all authors.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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Table A1. List of analyzed sound files of *Scinax montivagus* from Rio de Contas, state of Bahia, northeastern Brazil.

| Sound file                                | Voucher (AAG-UFU) | Recording Time | Date (dd/mm/yyyy) |
|-------------------------------------------|-------------------|----------------|-------------------|
| Scinax_montivagusRioContasBA1aAAGm671     | 5624              | 18:58 h        | 25/11/2016        |
| Scinax_montivagusRioContasBA2aAAGm671     | 5625              | 19:09 h        | 25/11/2016        |
| Scinax_montivagusRioContasBA3aAAGm671     | 5626              | 19:18 h        | 25/11/2016        |
| Scinax_montivagusRioContasBA4aAAGm671     | 5627              | 19:25 h        | 25/11/2016        |
| Scinax_montivagusRioContasBA5aAAGm671     | 5628              | 19:27 h        | 25/11/2016        |
| Scinax_montivagusRioContasBA6aAAGm671     | 5629              | 19:30 h        | 25/11/2016        |