



Advertisement call of *Rhinella major* (Anura: Bufonidae) from the lower Amazonas River basin with comments on intraspecific variation

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The *Rhinella granulosa* group currently comprises 13 species that are distributed throughout South America, and Panama (Sanabria *et al.* 2010; Pereyra *et al.* 2015). *Rhinella major* (Müller & Hellmich) is a widely distributed species that occurs in the Chaco of Argentina e Paraguay, Brazilian and Bolivian Cerrado, as well as open environments along the Madeira, Tapajós, and Xingú rivers, reaching the mouth of the Amazon River (Narvaes & Rodrigues 2009).

The aim of this work is to describe the advertisement call of *Rhinella major* from a population occurring in the lower Amazon River, comparing it to the previously available acoustic data from Bolivia and Argentina, highlighting the reliable applicability of the acoustic dataset to assess the specific identity of this widespread toad species of the *R. granulosa* group.

Field work was conducted in April/2014, in the District of Monte Dourado, Municipality of Almeirim, Pará State, located in the lower Amazonas River basin, northern Brazil. Morphological and morphometric characters follow Narvaes & Rodrigues (2009): snout-vent length (SVL) and supratympanic crest length (STCR) were measured from two adult male specimens with calipers to the nearest 0.05 mm. Calls of four males were recorded using a Marantz digital recorder set at a sampling rate of 48 kHz and a 16-bit resolution, coupled to a Sennheiser ME67/K6 directional microphone. Calls were analyzed by C.S.B. with the software Raven Pro 32-bit version 1.5 (Bioacoustics Research Program 2012). Temporal traits were measured from oscillograms; spectral trait of dominant frequency was obtained through Peak Frequency measurement function (dominant frequency was obtained from notes). 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 = 46.9 Hz. Sound figures were generated with Seewave package version 1.7.3 (Sueur *et al.* 2008) of R version 3.1.0 (R Core Team 2014) with the following settings: window name = Hanning; window length = 256 samples (FFT); overlap = 85%. Acoustic definitions and terminology follow Duellman & Trueb (1994). Call voucher males were deposited 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 3571–3572.

Both adult male specimens (SVL 43.8–49.6 mm; Figs. 1A–B) have cephalic crests predominantly continuous; infraorbital crest short (Fig. 1C); parietal crest present (Fig. 1D); interorbital portion of the supraorbital crest slightly curved; supratympanic crest short (STCL 3.9–4.2% SVL); snout squared in dorsal view and straight in lateral view (Figs. 1C–D); loreal region, maxillary crest, and labial light stripe visible in dorsal view; interorbital area and area between tympanum and parotoid gland usually smooth, without granules; belly not pigmented (Fig. 1B); longitudinal dorsal stripe absent (Fig. 1A).

Males were observed calling in temporary pools and rainwater drainages in anthropogenic areas within the urban perimeter. Advertisement call of *Rhinella major* (Table 1; Figure 2) consists of long series of a single type of pulsed notes emitted at irregular intervals. Calls have an increase in the amplitude in their first third until they reach maximum amplitude, sustained throughout their duration. Call duration varies from 1.42 to 6.73 s (mean 4.17, SD = 0.96; N = 21). Calls have from 28 to 137 notes (mean 84.0, SD = 17.5; N = 21), emitted at rates varying from 19.2 to 21.8 notes per second (mean 20.3, SD = 0.6; N = 21). Note duration varies from 32 to 47 ms (mean 40.4, SD = 0.7; N = 250), and note interval from 4 to 18 ms (mean 9.5, SD = 1.9; N = 250). Notes have from 5 to 6 pulses (mean 5.8, SD = 0.2; N = 250), emitted at rates varying from 116.3 to 187.5 pulses per second (mean 145.1, SD = 4.6; N = 250). Dominant frequency varies from 2.44 to 3.09 kHz (mean 2.67, SD = 0.14; N = 250).

TABLE 1. Advertisement call traits of *Rhinella major* from the lower Amazonas River (Monte Dourado, Pará State), northern Brazil, and comparative acoustic data from Argentina and Bolivia. N = number of recorded males (analyzed calls). Mean±SD (min–max).

	<i>Rhinella major</i>		
	Köhler <i>et al.</i> (1997) Bolivia N = 1 (5)	Guerra <i>et al.</i> (2011) Argentina N = 4 (21)	Present study Lower Amazonas River, Brazil N = 4 (21)
Call duration (s)	5.28±1.41 (4.10–7.53)	5.10±1.60 (3.6–7.2)	4.17±0.96 (1.42–6.73)
Notes per call	96.4±23.5 (75–133)	84.3±18.5 (61–106)	84.0±17.5 (28–137)
Note duration (ms)	42.7±1.5 (40–48)	45.0±4.7 (42–52)	40.4±0.7 (32–47)
Note interval (ms)	-----	15.0±2.2 (13–18)	9.5±1.9 (4–18)
Note rate/s	18.3±0.4 (17.7–18.7)	16.8±1.8 (14.3–18.6)	20.3±0.6 (19.2–21.8)
Pulses per note	6	6.6±0.6 (6–8)	5.8±0.2 (5–6)
Pulse rate/s	139.5±4.8 (134.5–145.8)	-----	145.1±4.6 (116.3–187.5)
Dominant frequency (kHz)	2.96±0.06 (2.91–3.04)	2.73±0.18 (2.50–2.94)	2.67±0.14 (2.44–3.09)
Air temperature (°C)	24.5	20.0	27.9

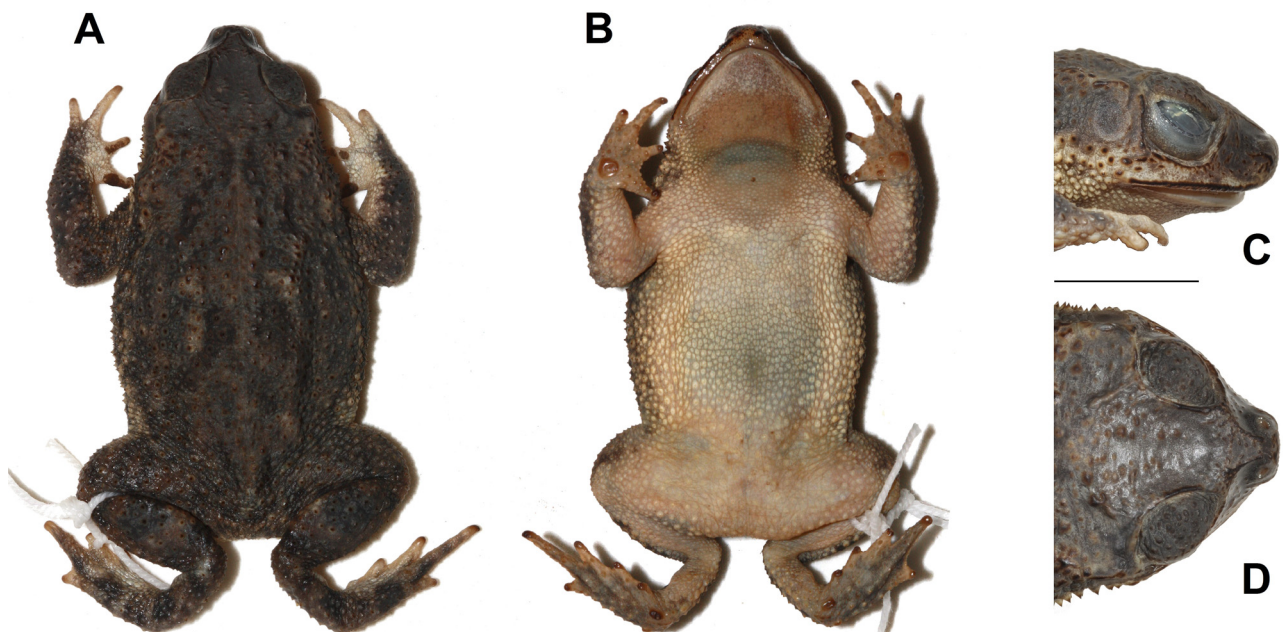


FIGURE 1. Dorsal (A) and ventral (B) views of the voucher male AAG-UFU 3572 (SVL = 49.6 mm), and lateral (C) and dorsal (D) views of its head. Scale = 8.5 mm.

Two morphological character states: parietal crest absent (present or barely discernible in our specimens) and cranial crests predominantly granulate (predominantly continuous in our specimens) were not in accordance to *R. major* diagnosis proposed by Narvaes & Rodrigues (2009). Even so, all the other morphological and coloration diagnostic features perfectly fitted its diagnosis, besides quite similar advertisement call traits compared to previously available data for Bolivian (Köhler *et al.* 1997) and Argentine (Guerra *et al.* 2011) populations. Thus, we believe that the divergent cranial crest character states might be attributed to intraspecific variation as there is no evidence to either assign these specimens to other species of the *R. granulosa* group or an undescribed species with the current available data.

The new acoustic data presented in this study came from a population located in the lower Amazon River basin, northern Brazil, approximately 2000 km northeastward to *R. major*'s type locality (São José de Chiquitos, Santa Cruz, Bolivia; Narvaes & Rodrigues 2009). Advertisement call descriptions for *R. major* from Bolivia (referred as *Bufo granulosa mini* Gallardo; Köhler *et al.* 1997) and Argentina (Guerra *et al.* 2011), despite the wide geographic distance, share a similar pattern both in temporal and spectral traits, except for notes per call and associated call duration, a highly

variable trait among *Rhinella* species (Table 1). Pulses per note seems to represent a reliable call trait to be employed for diagnosis of *R. major* in comparison with congeners of the *R. granulosa* group with available call descriptions: *R. major* has five to eight-pulse notes (six and seven-pulse notes are more commonly observed), whereas *R. bergi* has two-pulse notes (Guerra *et al.* 2011), *R. azarai*, *R. dorbignyi*, *R. fernandezae*, and *R. pygmaea* have three-pulse notes (Guerra *et al.* 2011; Carvalho *et al.* 2013); *R. centralis*, *R. granulosa*, *R. humboldti*, *R. merianae*, and *R. mirandaribeiroi* have four-pulse notes (Guerra *et al.* 2011; São-Pedro *et al.* 2011; Morais *et al.* 2012; Torres-Suárez & Vargas-Salinas 2013).

Therefore, we assume that bioacoustics might represent a useful dataset besides morphological features (*e.g.* cranial crests, snout shape; Narvaes & Rodrigues 2008) to assess the specific identity of *R. major* along its geographic distribution, given that call traits were similar (especially pulses per note) among three populations separated for over than 1000 km from each other, and might potentially include all the *R. granulosa* group as such trait has repeatedly been employed in acoustic diagnoses within this species group (Guerra *et al.* 2011, Carvalho *et al.* 2013).

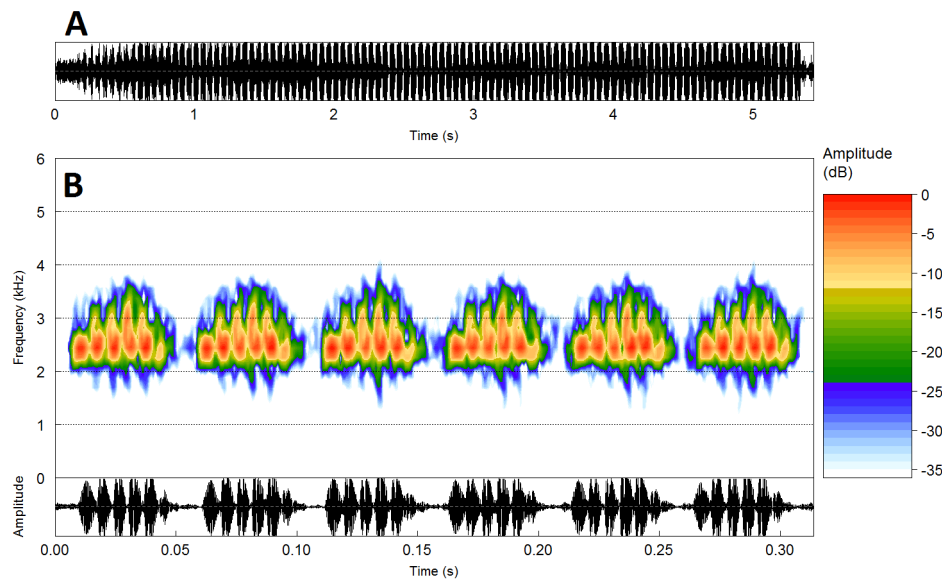


FIGURE 2. Waveform (A) of one advertisement call of *Rhinella major*; (B) spectrogram and respective oscillogram of six median six-pulse notes from A. Sound file: *Rhinella_major*MonteDouradoPA4bCSB_AAAGmt; recorded at 21:56, on 18 April 2014, in Monte Dourado (Almeirim), Pará, northern Brazil; air 27.9 °C, water 30.4 °C; unvouchered recording.

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