



On advertisement call of the poison frog *Ameerega berohoka* (Dendrobatidae, Anura) from the Brazilian Cerrado

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The poison frog genus *Ameerega* (Dendrobatidae) currently contains 32 species. They are distributed from central Brazil into western Amazonia to the lower Andean versant. In addition, three trans-Andean species have been allocated to *Ameerega* (Andrade *et al.* 2013; Frost 2014). *Ameerega berohoka* (Vaz-Silva & Maciel 2011) was described based on specimens from central Brazil (type-locality: Arenópolis, GO) and it is assumed to occur in parts of western and southwestern state of Goiás (Frost 2014). More recently, Andrade *et al.* (2013) extended its distribution to the state of Mato Grosso. Here we re-describe the advertisement call of *A. berohoka*, providing additional information regarding its temporal structure and spectral traits. Our observations also consist of a new distribution record for this species to the state of Mato Grosso.

Field work was conducted on 16–17 November 2012 at the Parque Estadual Serra Azul (PESA), municipality of Barra do Garças (15.850767 S, 52.270808 W, approximately 533 m a.s.l.), state of Mato Grosso, Brazil. Recordings were made between 07:27–08:05 hrs. The most representative habitats in PESA are *cerrado rupestre* (hill savanna on rocky soil), *cerrado sensu stricto*, gallery forest and semi-deciduous forest (Sanchez & Pedroni 2011). The average annual rainfall in PESA is 1528 mm and the average temperature 25.5 °C (Pirani *et al.* 2009). One recorded male is deposited at Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia, municipality of Uberlândia, state of Minas Gerais, Brazil (AAG-UFU 1310).

TABLE 1. Advertisement call variables of *Ameerega berohoka* from the Parque Estadual Serra Azul (PESA), municipality of Barra do Garças, State of Mato Grosso, Brazil. Mean±SD (minimum-maximum). N = number recorded males; fifty analyzed calls/male.

Variables	<i>Ameerega berohoka</i> N=5 (250)
Call duration (s)	0.119±0.012 (0.090–0.173)
Intercall interval (s)	0.319±0.060 (0.140–0.695)
Calls/minute	144.02±21.55 (124.38–179.00)
Calls/second	2.60±0.55 (2.0–3.0)
Peak of dominant frequency (Hz)*	4118.2±63.7 (3919.0–4478.9)
Min. dominant frequency (Hz)*	2763.3±93.3 (2496.1–3092.9)
Max. dominant frequency (Hz)*	5056.5±158.7 (4618.3–5586.2)
Peak of fundamental frequency (Hz)	2165.4±135.3 (1921.9–2484.4)
Peak of 3 rd harmonic frequency (Hz)	6165.3±114.4 (5625.0–6468.8)
Air temperature (°C)	25–26

* = 2nd harmonic

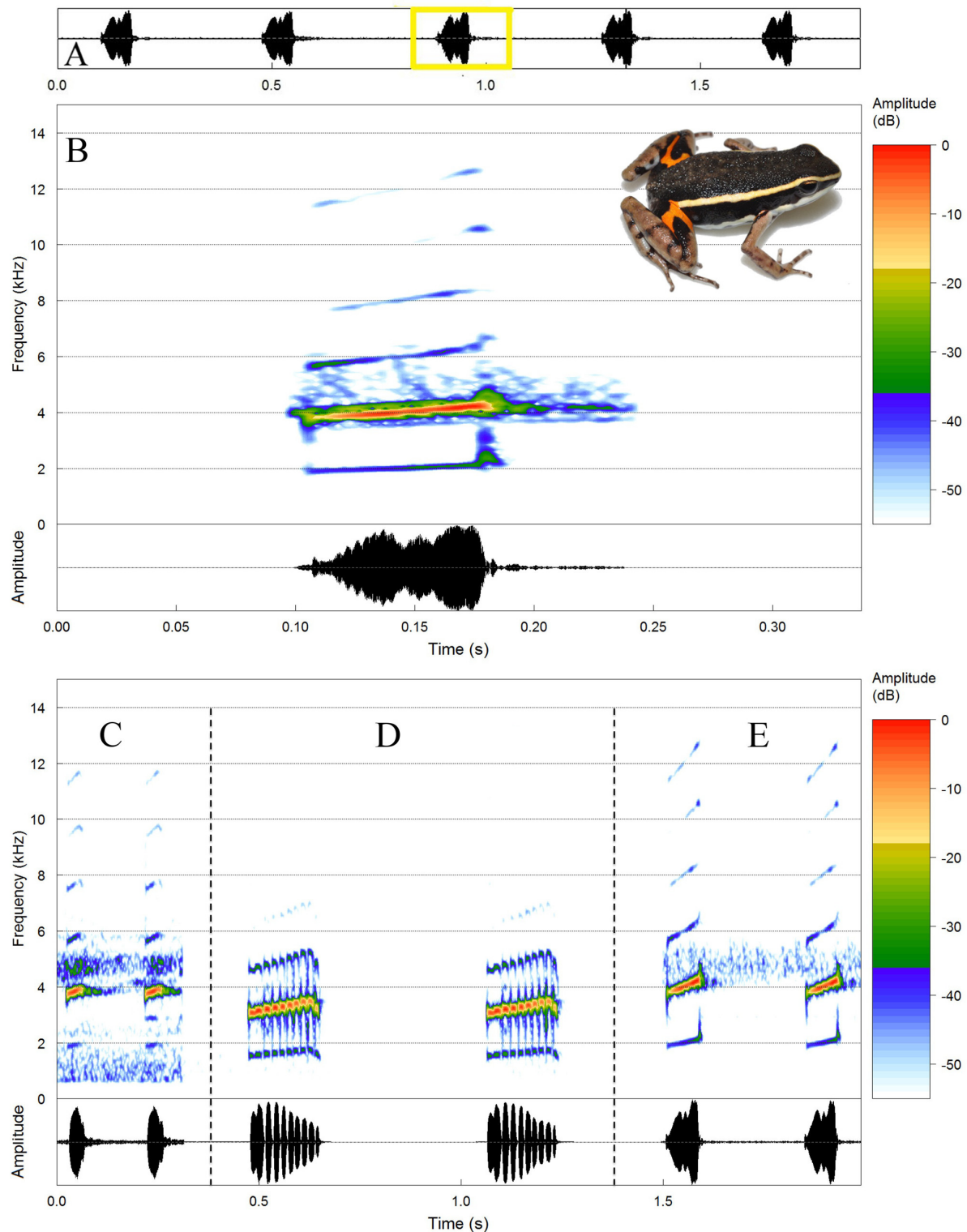


FIGURE 1. **A.** Oscillogram with five calls of *Ameerega berohoka* from the Parque Estadual Serra Azul, municipality of Barra do Garças, Mato Grosso, Brazil. **B.** Spectrogram (above) and corresponding oscillogram (below) detailing the call outlined in yellow. Sound file: AB_BG5. Recorded on 16 Nov 2012; 07:27h. Air temperature 26.0 °C. Spectrograms (above) and corresponding oscillograms (below) of calls of three *Ameerega* species from the Brazilian Cerrado: **C.** *A. braccata* from Santo Antonio de Leverger. Sound file: AB_MT. Recorded on 21 Nov 2012; 17:53h. Air 25 °C. Vouchered recording (AAG-UFU 1434); **D.** *A. flavopicta* from Santana do Riacho. Sound file: AF_MG3. Recorded on 04 Dec 2005; 7:05h. Air temperature 18.2 °C; **E.** *A. berohoka* from Barra do Garças. Sound file: AB_BG4. Recorded on 16 Nov 2012; 7:28h. Air temperature 26 °C. Two calls of each species are depicted. Within a species, the inter-call interval as recorded. Inset: an adult male of *A. berohoka* (AAG-UFU 1310) from Barra do Garças.

Call terminology followed Duellman and Trueb (1994) and McLister *et al.* (1995). Two hundred and fifty advertisement calls of five males were analyzed (50 calls/male). Pooled means and standard deviations were calculated considering mean values of individual males. Calls were recorded with a Marantz PMD 671, a Boss 864 (both coupled to Sennheiser ME67/K6 microphones) and a M-audio Microtrack II (Sennheiser ME66/K6) digital recorders. Air temperature was obtained for each recorded male. The recorders were set at 44.1 or 48.0 kHz and 16-bit resolution. Calls were analyzed using Raven Pro 1.5, 64-bit version (Bioacoustics Research Program 2012) with the following settings: window type = Hanning, window size = 256 samples, 3 dB filter bandwidth = 248 or 270 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), DFT size = 1024 samples (locked), and a grid spacing (spectral resolution) = 43.1 or 46.9 Hz. Temporal variables were analyzed in oscillograms and spectral variables in spectrograms. Dominant frequency and other frequencies were assessed through the 'Peak Frequency' function. Energy peaks are referred here as: 1) fundamental, 2) dominant (2nd harmonic) and 3) 3rd harmonic. Figures were generated using Seewave v.1.6 package (Sueur *et al.* 2008) on the R platform (version 3.0.2; R Development Core Team 2013). Seewave settings were as follows: Hanning window, 85% overlap, and 512 points resolution (FFT). Original analyzed calls (in wav format) are available at AmphibiaWeb (<http://amphibiaweb.org/>).

Values for quantitative variables of the advertisement call are detailed in Table 1 and spectrograms and oscillograms are depicted in Figure 1 (A–B). The advertisement call consists of an amplitude modulated (max. 50%) non-pulsed note with a discrete ascending frequency modulation throughout its duration. To the human ear, the call resembles a pure short-whistle and in the last 1/8th of each call, the sound intensity abruptly decreased. The calls of this species last 90–173 ms, are separated by intervals of 140–695 ms, and released at a rate of 125–179 calls/minute. Each note possess up to six harmonics. Dominant frequency varies around 3919–4479 Hz and coincides with the second harmonic. The fundamental frequency range is around 1922–2484 Hz and the air temperature varied from 25 to 26°C.

TABLE 2. Spectral variables of the advertisement calls of three species of the *Ameerega picta* group from the Brazilian Cerrado. Mean \pm SD (minimum-maximum). N = number of recorded males (number of analyzed calls).

Spectral Variables	<i>A. berohoka</i> N=5 (250)	<i>A. braccata</i> N=1(10)	<i>A. flavopicta</i> N=3(30)
Min. dominant frequency (Hz)*	2763.3 \pm 93.3 (2496.1–3092.9)	3129.5 \pm 69.2 (3017.2–3232.1)	2373.6 \pm 74.1 (2187.8–2579.6)
Max. dominant frequency (Hz)*	5056.5 \pm 158.7 (4618.3–5586.2)	4384.4 \pm 31.0 (4347.9–4438.5)	4187.5 \pm 53.4 (3979.3–4346.4)
Peak of dominant frequency (Hz)*	4118.2 \pm 63.7 (3919.0–4478.9)	3843.8 \pm 0.0 (3843.8–3843.8)	3288.1 \pm 50.3 (3186.9–3359.2)
Peak of fundamental frequency (Hz)	2165.4 \pm 135.3 (1921.9–2484.4)	1893.8 \pm 24.2 (1875.0–1921.9)	1660.2 \pm 50.2 (1550.4–1765.7)
Peak of 3 rd harmonic frequency (Hz)	6165.3 \pm 114.4 (5625.0–6468.8)	5695.3 \pm 74.1 (5625.0–5812.5)	4980.6 \pm 141.6 (4651.2–5254.1)

* = 2nd harmonic

For comparative purposes, we also present data on the advertisement calls of two other *Ameerega* species known to occur in the Brazilian Cerrado (Table 2 and Figure 1C–E; recordings also available on AmphibiaWeb). The location of advertisement calls and species are as follows: 1) *A. braccata* from Santo Antonio de Leverger (15.652611 S, 56.053442 W; see Forti *et al.* 2010 for a full description of the call); and 2) *A. flavopicta* from Santana do Riacho (19.300914 S, 43.601081 W; see details in Magrini *et al.* 2010).

Some characters of the advertisement call of *A. berohoka* were described in the initial description of this species, but were based on a poor quality (low amplitude) recording of a single male. Our analyses of several individuals provide additional information on the existence of harmonic structure and frequency modulation. Vaz-Silva and Maciel (2011) reported that the call consists of a single 10-pulsed note. However, we found that amplitude modulation is weak and not cyclic (Watkins 1968; Elemans *et al.* 2008). These results suggests that the call of *A. berohoka* is better defined as non-pulsed (see Fig. 1 B). *Ameerega berohoka* call is distinguished from that of *A. flavopicta* by being non-pulsed (Magrini *et al.* 2010) and that of *A. braccata* by being longer and by

having a slightly higher dominant frequency (see Table 2 and Figure 1 C and E). Our data on number of note types, note duration and frequency range of the dominant frequency are in accordance with Vaz-Silva and Maciel (2011).

Regarding the other species of the *A. picta* species group, *A. berohoka* is distinguished from *A. boehmei* by having a higher fundamental frequency (1.16–1.63 kHz in *A. boehmei*) and a higher call rate (1.28–1.97 calls/s in *A. boehmei*) (Lötters *et al.* 2009). The length of call of *Ameerega berohoka* (90–173 ms) is much longer than *A. picta* (50 ms), *A. altamazonica* (60–80 ms) and *A. hahneli* (11–18 ms) (Schlüter 1980; Morales 1992; Haddad & Martins 1994; De La Riva *et al.* 1996; Köhler & Lötters 1999; Twomey & Brown 2008). This species also differs from *A. hahneli* by having a lower pulse rate (5–9 calls/s in *A. hahneli*) (Schlüter 1980; Morales 1992; Haddad and Martins 1994; De La Riva *et al.* 1996; Köhler and Lötters 1999).

The occurrence of high frequency and harmonically-related acoustic bands have been neglected in many *Ameerega* call descriptions (Haddad & Martins 1994; Toledo *et al.* 2004; Costa *et al.* 2006; Magrini *et al.* 2010; Forti *et al.* 2010; Martins & Giaretta 2012). In our comparative analyses (Table 2), the fundamental frequency is always discernible, but weaker than the dominant (2nd harmonic). The presence of different harmonic bands in frog calls may be related to features such as sexual selection (Gridi-Papp *et al.* 2006) and to environs with different background noises (Lima & Eterovick 2013).

Recently, Andrade *et al.* (2013) expanded the known distribution of *A. berohoka* for the state of Mato Grosso (Itiquira), this record refers to the westernmost known locality for the species and the only one outside the Araguaia River basin. Our new record represents the northernmost (about 90 km northwest to its type locality) locality for the species.

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Appendix 1

Sound files (wav) of analyzed calls. All archives deposited in AAG's acoustic collection and are also available at AmphibiaWeb (<http://amphibiaweb.org/>).

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 AB_BG1:Ameerega_berohokaBarraGarcasMT1aIAH_AAGmt.wav
 AB_BG2:Ameerega_berohokaBarraGarcasMT2aFSA_AAGb.wav
 AB_BG3:Ameerega_berohokaBarraGarcasMT3aFSA_AAGb.wav
 AB_BG4:Ameerega_berohokaBarraGarcasMT4aAAGm671.wav
 AB_BG4:Ameerega_berohokaBarraGarcasMT4bAAGm671.wav
 AB_BG5:Ameerega_berohokaBarraGarcasMT5aAAGm671.wav
 AB_MT:Ameerega_braccataSVicenteMT1aTRC_AAGmt.wav
 AF_MG1: Ameerega_flavopCipoMG1bAAGb.wav
 AF_MG2: Ameerega_flavopCipoMG2aAAGb.wav
 AF_MG3: Ameerega_flavopCipoMG3aAAGb.wav