



The advertisement call of *Bokermannohyla flavopicta* Leite, Pezzuti & Garcia, 2012 (Anura: Hylidae) from the mountains of Chapada Diamantina, Bahia, Brazil

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Bokermannohyla Faivovich, Haddad, Garcia, Frost, Campbell & Wheeler (2005) is a Brazilian treefrog genus currently composed of 32 species (Brandão *et al.* 2012; Leite *et al.* 2012; Frost 2015). The genus comprehends four, putatively monophyletic, species groups: *B. circumdata*, *B. claresignata*, *B. martinsi*, and *B. pseudopseudis* (*sensu* Faivovich *et al.* 2005). The *B. pseudopseudis* group includes nine species: *B. alvarengai* (Bokermann 1956), *B. flavopicta* Leite *et al.* 2012, *B. ibitiguara* (Cardoso 1983), *B. itapoty* Lugli & Haddad 2006a, *B. oxente* Lugli & Haddad 2006b, *B. pseudopseudis* (Miranda-Ribeiro 1937), *B. sagarana* Leite *et al.* 2011, *B. sapiranga* Brandão *et al.* 2012, and *B. saxicola* (Bokermann 1964).

Species of the *B. pseudopseudis* group are usually associated to *campos rupestres* (rupestrian grasslands) vegetation (Leite *et al.* 2011, 2012), a mosaic of habitat types including exposed grasslands, rock surfaces, cerrado, gallery forests, and small islands of arborescent vegetation, a typical phytophysognomy of the Espinhaço Range (Silveira *et al.* 2015). Therefore, Espinhaço Range is the richest area in species of this group (see Leite *et al.* 2011, 2012), with six of the nine currently known species; three in Minas Gerais (*B. alvarengai*, *B. saxicola*, and *B. sagarana*) and three in Bahia (*B. itapoty*, *B. oxente*, and *B. flavopicta*). An additional species (*B. ibitiguara*) occurs nearby on Serra da Canastra in the state of Minas Gerais. The two remaining species of the group are found at Planalto Central formation; *B. pseudopseudis* occurs in Chapada dos Veadeiros and Serra da Mesa, in the state of Goiás, while *B. sapiranga* is distributed from Distrito Federal to southern Goiás and northwestern Minas Gerais (Leite *et al.* 2012; Carvalho *et al.* 2013).

Bokermannohyla flavopicta is a narrowly distributed species only known for altitudes above 1500 m a.s.l. in the southwestern portion of Chapada Diamantina, a regional designation of the Espinhaço Range, in the state of Bahia (Leite *et al.* 2012). The present study aims to describe the advertisement call of *B. flavopicta* and compare it with calls described for the species of the *B. pseudopseudis* group.

The analyzed calls are from four male paratypes recorded by F.S.F. Leite on 12 December 2009, at Serra do Itobira (13°22'13.1"S, 41°52'40.8"W, 1616 m a.s.l.), and also on 9–13 January 2010 at Serra das Almas (13°31'13.9"S, 41°57'31.2"W, 1581 m a.s.l.), municipality of Rio de Contas, state of Bahia, Brazil. Voucher specimens are housed in the Coleção Herpetológica da Universidade Federal de Minas Gerais—UFMG—and call recordings in Coleção Bioacústica UFMG—CBUFMG—under collection records UFMG 4506, 4984–4985 and CBUFMG 372–389.

We recorded calls with a Marantz PMD660 digital recorder with sampling rate of 44.1 kHz and resolution of 16 bits coupled with a Sennheiser ME66/K6 unidirectional microphone. Spectral and temporal parameters were measured using Raven Pro 1.5 (Cornell Lab of Ornithology Research Program Bioacoustics Workstation) directly from the spectrogram and oscillogram respectively. Spectrogram was generated with window type Hann; window size = 512 samples; overlap = 70%; hop size = 3.49 ms; DFT size = 1024 samples and; grid spacing = 43.1 Hz. Sound graphics were obtained using Seewave (Sueur *et al.* 2008) package of R platform (R Core Team 2014), using Hanning window, FFT = 512 and 70% overlap.

Note terminology follows the physiological proposal from McLister *et al.* (1995), aiming to recover homology within produced sounds (see Robillard *et al.* 2006 for discussion). We considered the dominant frequency (DF) as the frequency range in which the most energetic part of the call is comprised (given in range from the lowest to the higher

values acquired through the spectrogram). The peak frequency (PF) was given by the Raven Pro 1.5 and is always within the DF. Temporal parameters were measured in agreement with Cocroft & Ryan (1995).

Relative note position (RNP) was calculated for each note as $RNP = a/b$, with a = note rank number and b = number of notes in the call. RNP range from 0 to 1 and represent the beginning and the end of the call, respectively. Interval between notes failed the Shapiro-Wilk normality test, therefore we conducted Kruskal-Wallis rank sum test for data comparison. Statistical analyses were conducted with software R 3.1.1 (R Core Team 2014). Abbreviations used furthermore: call duration (CD), interval between calls (CI), number of notes per call (NN), note duration (ND), interval between notes (NI), note rate (NR). Data are presented as mean \pm SD, range; n .

Obtained call parameters were compared to the published data available for the group. For comparison purpose, we only considered the note type A of *Bokermannohyla ibitiguara* (*sensu* Carvalho *et al.* 2012) as this is the more commonly emitted note type by this species. For *B. oxente* we considered only the short note (*sensu* Lugli & Haddad 2006b) due to its resemblance to the call of *B. flavopicta*.

The advertisement call of *B. flavopicta* is characterized by a series of short unpulsed notes emitted in long sequences (NN = 220.3 ± 57.5 , 182–336 notes/call; $n = 8$; Fig. 1). The amplitude rises through the first third of the call, is sustained halfway into the call and decreases in the final half towards the end (Fig. 1A and 1B). Call duration was 67.7 ± 19.4 , 56.4–111.5 s; $n = 8$. Longest call analyzed had more than 450 notes and was not fully recorded. Interval between calls was 47.2 ± 12.3 , 27.4–58.45 s; $n = 8$. Note duration was 34 ± 8 , 14–63 ms, $n = 2475$; amplitude of the note peaks around 13.9 ± 4.6 %, 5.0–26.9 % of notes' duration (call rise time = 10 ± 2.9 , 3–28 ms, $n = 1955$), and decreases until the end of it without frequency modulation; notes are emitted at a regular rate of 3.24 ± 0.13 , 3.01–3.39 notes/s; $n = 2475$. Interval between notes was 284 ± 89 , 188–1190 ms; $n = 867$ and differed through the call ($H = 552.8145$, $df = 4$, $p < 0.001$; Fig. 1C). The first fifth of the call had the longest intervals (1st fifth: 393 ± 146 , 241–935 ms; 1st vs. 2nd: $H = 231.51$, $df = 1$, $p < 0.001$; 1st vs. 3rd: $H = 334.94$, $df = 1$, $p < 0.001$; 1st vs. 4th: $H = 350.19$, $df = 1$, $p < 0.001$; 1st vs. 5th: 215.34 , $df = 1$, $p < 0.001$), followed by the second fifth, which is similar to the last one (2nd fifth: 266 ± 35 , 213–380 ms; 5th fifth: 268 ± 43 , 188–447; 2nd vs. 3rd: $H = 58.49$, $df = 1$, $p < 0.001$; 2nd vs. 4th: $H = 67.41$, $df = 1$, $p < 0.001$; 2nd vs. 5th: $H = 1.17$, $df = 1$, $p = 0.27$). The third and fourth fifths intervals of the call did not differ (3rd fifth: 245 ± 28 , 199–343; 4th fifth: 242 ± 27 , 188–349; 3rd vs. 4th: $H = 0.33$, $df = 1$, $p = 0.561$; 3rd vs. 5th: $H = 63.00$, $df = 1$, $p < 0.001$; 4th vs. 5th: $H = 75.68$, $df = 1$, $p < 0.001$). Dominant frequency ranged between 1.2 and 1.7 kHz; peak frequency was 1.3–1.6 kHz; 1.5 ± 0.08 ; $n = 2730$.

The call of *B. flavopicta* differs from all known advertisement calls of *B. pseudopseudis* group species due to its higher number of notes per call (186–336 notes per call in *B. flavopicta*; combined number of notes per call in the other species 5–79; see Table 1 for complete comparative results). From the calls of *B. saxicola*, *B. sapiranga*, *B. pseudopseudis*, and *B. oxente*, the call of *B. flavopicta* can be distinguished by its longer interval between notes (188–1190 ms in *B. flavopicta*; combined interval between notes of 50–124.5 ms in the other species; Eterovick & Brandão 2001; Lugli & Haddad 2006b; Brandão *et al.* 2012; Carvalho *et al.* 2013).

The higher peak frequency (1.3–1.6 kHz) differs the call of *B. flavopicta* from the calls of *B. pseudopseudis* and *B. sapiranga* (combined dominant frequency peaks 0.46–0.79 kHz; Carvalho *et al.* 2013). The higher number of notes per call differs it from *B. itapoty* (Lugli & Haddad 2006a). The non-harmonic structure distinguishes the call of *B. flavopicta* from *B. saxicola*, *B. pseudopseudis*, and *B. sapiranga*, which have harmonic structured calls. From the call of *B. ibitiguara* (pulsed notes), it differs by the non-pulsed structure and the shorter notes (Carvalho *et al.* 2012).

Taucce *et al.* (2015) recently described the advertisement call of *Bokermannohyla juiju* Faivovich, Lugli, Lourenço, and Haddad (2009) as a single unpulsed note emitted several times in a row. *B. juiju* was described based on a single male specimen from Chapada Diamantina, and is currently assigned to the *B. martinsi* group based on the presence of a hook-like humeral crest (Faivovich *et al.* 2009). However, if we consider the call of *B. juiju* as a sequence of unpulsed notes, his call is very similar to *B. flavopicta*. Although the call of *B. flavopicta* can easily be distinguished from *B. juiju* by the absence of harmonic structures, both species share the same pattern of short notes (ND = 47–66 ms in *B. juiju*; ND = 14–63 ms in *B. flavopicta*) emitted in extremely long sequences (over 378 notes in a row in *B. juiju*; over 450 in *B. flavopicta*).

Faivovich *et al.* (2005) included in their molecular analysis two out of the four species signed to the *Bokermannohyla pseudopseudis* group by the time. Although the number of recognized species has increased since then, no formal morphological synapomorphies have been proposed (Lugli & Haddad 2006a; Faivovich *et al.* 2009; Leite *et al.* 2011, 2012; Brandão *et al.* 2012). Species of the *B. pseudopseudis* group described since then were tentatively assigned based on external morphology, behaviour, and natural history. Eight of the nine currently described species already had their vocalizations described (Table 1). Unfortunately, the call description of *B. alvarengai* is simple (Sazima & Bokermann 1977), hampering detailed comparisons with other species. On the other hand, most advertisement calls described for the group are characterized by sequences of short unpulsed notes (combined ND = 14–146 ms; Table 1).

The exception is currently *B. ibitiguara*, whose call is characterized by long pulsed notes (ND = 435–1208 ms) emitted at irregular intervals, which resemble the calls described for the *B. circumdata* group (Carvalho *et al.* 2012 and references within).

TABLE 1. Call parameters of species of the *Bokermannohyla pseudopseudis* group. 1: Present work; 2: Carvalho *et al.* 2012; 3: Lugli & Haddad 2006a; 4: Lugli & Haddad 2006b; 5: Carvalho *et al.* 2013; 6: Brandão *et al.* 2012; 7: Eterovick & Brandão 2001. *Note type A; **Short note.

Species	Number of notes per call (range)	Note duration (range, ms)	Interval between notes (range, ms)	Dominant frequency (range, kHz)	Presence/number of harmonics	Note structure
<i>B. flavopicta</i> ¹	186–336	14–63	188–1,190	1.2–1.7	not present	unpulsed
* <i>B. ibitiguara</i> ²	1	435–1208	91–3575	1.50–2.20	not present	pulsed
<i>B. itapoty</i> ³	16–79	15–53	160–1,580	1.68–3.3	not present	unpulsed
** <i>B. oxente</i> ⁴	10–60	17.4–36.8	71.5–124.5	1.0–1.9	not present	unpulsed
<i>B. pseudopseudis</i> ^{5,7}	12–18	80–122	70–132	0.4–2.4	up to twelve	unpulsed
<i>B. sapiranga</i> ^{5,6}	3–8	55–146	92–132	0.5–0.7	up to twelve	unpulsed
<i>B. saxicola</i> ⁷	25–25	22–44	602	0.9–1.4	at least three	unpulsed

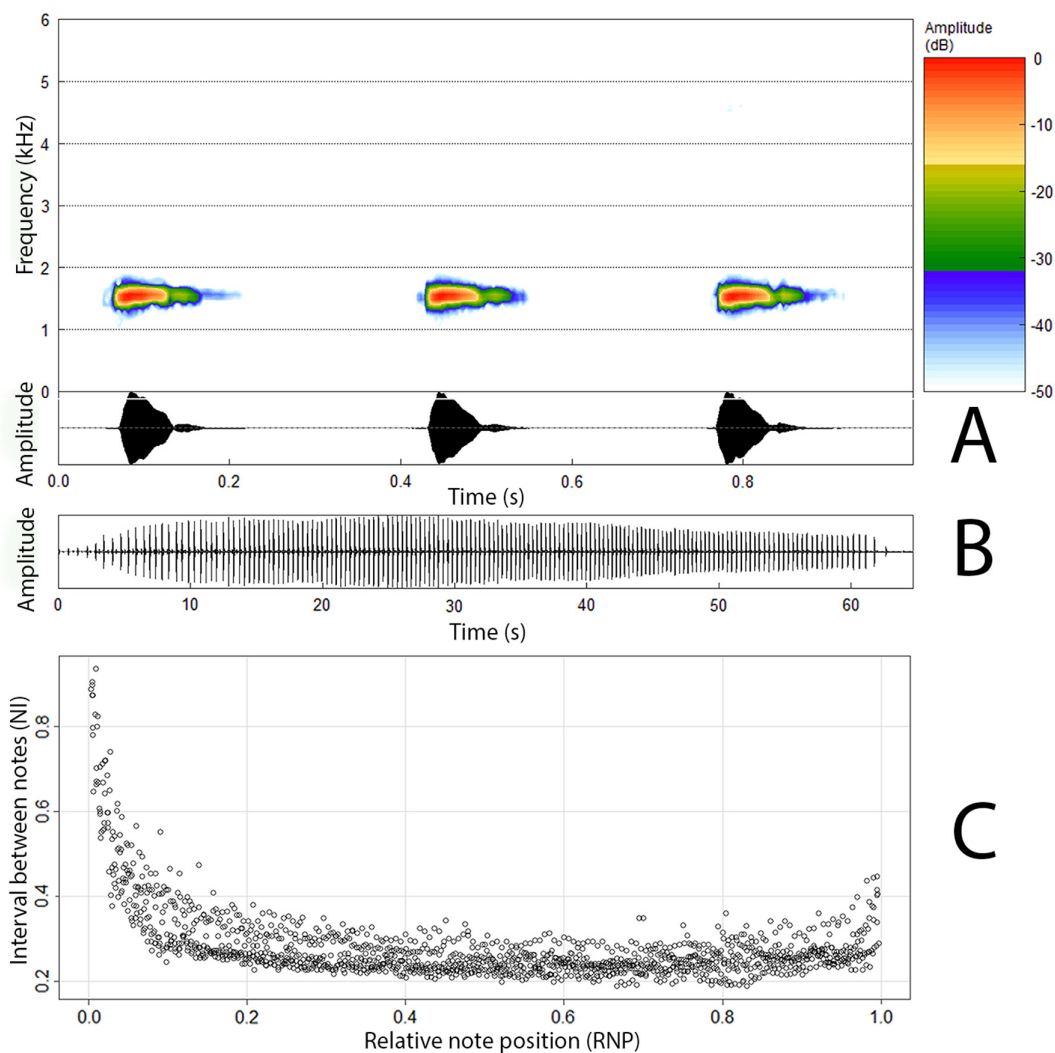


FIGURE 1. Spectrogram (A) and oscillogram (B) views of the advertisement call (CBUFMG 388) of *Bokermannohyla flavopicta* (UFMG 4985, paratype; recorded on 13 January 2010, 22h15, 20°C). C) Relationship between the note interval (NI) and the relative note position in the advertisement call of four males of *Bokermannohyla flavopicta* (n = 2475 notes; 8 calls) (RNP: from 0 to 1, representing the beginning and the end of the call, respectively).

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