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A reappraisal of the geographic distribution of *Bokermannohyla sazimai* (Anura: Hylidae) through morphological and bioacoustic approaches

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Abstract

A reappraisal of the geographic distribution of ***Bokermannohyla sazimai*** (Anura: Hylidae) through morphological and bioacoustic approaches. The type locality of *Bokermannohyla sazimai* is in the Municipality of São Roque de Minas, State of Minas Gerais, Brazil. In this paper, we reassess the geographic distribution of *B. sazimai* and provide additional information on variation of several other non-topotypic populations in comparison with topotypic populations (São Roque de Minas and Vargem Bonita), on the basis of three lines of evidence (color pattern, morphometry, and vocalizations). Differences obtained among all populations with respect to color pattern, morphometry, and advertisement calls were attributed to interpopulational variation, so that this variation was not enough to recognize any population as a distinctive lineage in comparison with the topotypic information available on *B. sazimai*.

Keywords: Amphibia, *Bokermannohyla circumdata* species group, Cerrado, Serra da Canastra, Southeastern Brazil, vocalizations.

Resumo

Reavaliação da distribuição geográfica de ***Bokermannohyla sazimai*** (Anura: Hylidae) sob abordagens morfológica e bioacústica. *Bokermannohyla sazimai* tem como localidade-tipo o município de São Roque de Minas, Minas Gerais, Brasil. Nesse trabalho, nós reavaliamos a distribuição geográfica de *B. sazimai* e fornecemos informações adicionais de variação de várias populações não-topotípicas em comparação com populações topotípicas (São Roque de Minas e Vargem Bonita), por meio de três linhas de evidência (padrão de coloração, morfometria e vocalizações). As diferenças encontradas entre todas as populações estudadas em relação ao padrão de coloração, morfometria e canto de anúncio foram atribuídas a variações interpopulacionais, de modo que essa variação não foi suficiente para o reconhecimento de qualquer população como uma linhagem independente frente às informações disponíveis de topótipos de *B. sazimai*.

Palavras-chave: Cerrado, grupo de *Bokermannohyla circumdata*, Serra da Canastra, sudeste do Brasil, vocalizações.

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Introduction

The *Bokermannohyla circumdata* group comprises 19 species (Carvalho *et al.* 2012) with its occurrence generally restricted to altitudinal riparian environments within the Atlantic Forest and Cerrado domains from northeastern to southern Brazil (Napoli and Pimenta 2009, Carvalho *et al.* 2012). *Bokermannohyla sazimai* (Cardoso and Andrade, 1982) was described from the Municipality of São Roque de Minas (State of Minas Gerais, Brazil), which lies on the easternmost section of the Parque Nacional da Serra da Canastra, and its currently known geographic range also includes a few additional localities north and northwestward to its type locality (Caramaschi *et al.* 2001, Giasson and Ribeiro 2006, Moura and Feio 2010). The original authors also provided a brief description of its advertisement call, which was re-described in Carvalho *et al.* (2012) based on topotypes.

In this paper, we reassess the geographic distribution of *Bokermannohyla sazimai* and provide additional information on variation in color pattern, morphometry, and vocalizations of several other non-topotypic populations in comparison with topotypic populations.

Materials and Methods

Specimens and vocalizations of *Bokermannohyla sazimai* were obtained from five localities in the State of Minas Gerais: 1) Sacramento (19º50'80" S, 47º18'64" W, ca 1120 m a.s.l.); 2) São Gotardo (19º19'27" S, 45º59'19" W, ca 1130 m a.s.l.); 3) at two sites in Perdizes: at the EPDA-Galheiro (19º13'89" S, 47º07'00" W, ca 930 m a.s.l.), and at the Sítio Bom Sucesso (19º30'10" S, 47º07'04" W, ca 975 m a.s.l.); 4) Serra do Salitre (19º06'19" S, 46º42'20" W, ca 1160 m a.s.l.); and 5) Uberaba (19º39'60" S, 47º42'56" W, ca 955 m a.s.l.). Additional K7 tape recordings were kindly made available by T.L. Pezzuti from Ibiá (19º29'11" S, 46º33'92" W, ca 960 m a.s.l.), and by L.G. Leone from Paracatu (17°18'79" S, 47º06'10" W, ca 660 m a.s.l.), State of Minas Gerais, for comparative purposes. Calls described in Carvalho *et al.* (2012) were also included in the present study for comparative purposes (see Table 2, Figures 3A, 4A).

Examined specimens (Appendix I) are housed in the following Brazilian zoological collections: Museu de Zoologia da Universidade Estadual de Campinas (ZUEC), Campinas, State of São Paulo; Célio F. B. Haddad collection (CFBH), Rio Claro, State of São Paulo; Collection of frogs of the Museu de Biodiversidade do Cerrado at the Universidade Federal de Uberlândia (AAG-UFU), Uberlândia, State of Minas Gerais; and Collection of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, State of Minas Gerais.

Twelve morphometric characters were measured with calipers to the nearest 0.1 mm. Seven measurements follow Duellman (1970): snout-vent length (SVL), head length (HL), head width (HW), eye diameter (ED), tympanum diameter (TD), shank length (SL), and foot length (FL); and five measurements follow Heyer *et al.* (1990): thigh length (TL), hand length (HAL), eye-nostril distance (END), fourth toe disk diameter (TDD), and third finger disk diameter (FDD).

We performed an Analyses of Variance (ANOVA) on snout-vent length for all populations we had sample sizes of seven or more male specimens, and a Principal Component Analysis was performed on a correlation matrix taking all (12) morphometric characters as an exploratory test to identify potential groups that may be related to previous assumptions without a priori classification of the samples into predetermined discrete units. Specimens from Paracatu, Serra do Salitre, and Uberlândia were not included in the PCA since we do not have either available specimens (Paracatu and Uberlândia) or measured specimens (Serra do Salitre). Considering that a Tukey a posteriori test set the population from Perdizes apart from all others (see Results section), we performed other PCA only including this population in comparison with topotype specimens. Both the ANOVA and PCA were performed on the R platform, v. 2.15.1 (R Development Core Team 2012). Detailed results of the PCA are summarized in Appendices II–III.

Advertisement calls were recorded with digital recorders (Boss BR 864 or M-audio Microtrack II) set at 44.1 or 48.0 kHz sample rate and resolution of 16 bits, coupled to directional microphones [Sennheiser K6/ME67 (Boss) or K6/ME66 (Microtrack)]. Bioacoustic variables were analyzed with SoundRuler software version 0.9.6.0. (Gridi-Papp 2007); sound graphs were obtained with Seewave (version 1.6.4) (Sueur *et al.* 2008), R (version 2.15.1) package (R Development Core Team 2012). Settings were Hanning window, 85% overlap, and 256 points resolution (FFT). Bioacoustic terminology and the assignment of letters to the note types (A and B) of *Bokermannohyla sazimai* advertisement call are according to Carvalho *et al.* (2012), referring to as the two distinctive notes of its advertisement call. Figured sound files are listed in Appendix IV.

Voucher specimens for call recordings are: Topotypes: AAG-UFU 4842–4846 (Vargem Bonita). Additional populations: AAG-UFU 1034–1035 (Sacramento); AAG-UFU 0610–0611, UFMG 3330–3332, ZUEC 12064 (Perdizes); ten specimens out of the series AAG-UFU 0350–0368 (São Gotardo); AAG-UFU 0553–0554, and AAG-UFU 1041–1044 (Uberaba).

Results

Color Pattern

Live and preserved specimens from all populations presented some degree of intra-populational variation. Dorsal coloration both in lived and preserved specimens varied from a pale cream/yellow to a medium brown among the populations. The diagnostic color pattern of reticulations on dorsum was present in all examined specimens. Still, there was intensity variation in the reticulated dorsal pattern, so that it varied from well-marked reticulated dorsa (Figures 1A, E) to an almost reticulation lacking pattern (Figures 1B-D), possessing reticulations only on flanks. The presence and extent of dark and/or lichenous light-colored dots, spots and blotches on dorsal surfaces of body and limbs, as well as the presence (well-defined or fragmented) or even the absence of transverse bars on the posterior surface of thighs were also observed among the populations (Figure 1). However, transverse bars on superior surface of thighs were present in all examined specimens.

Morphometry

Morphometric information is summarized in Table 1. Regarding size, the topotypic population (Serra da Canastra) practically embraced all the variation of other populations, except the population from Perdizes (Figure 2), which was significantly (F = 11.84; df = 4; $p < 0.001$) set apart from the topotypic population, as well as from all non-topotypic populations by its larger body size. PCA taking morphometric variables of all specimens into consideration recovered no relevant groupings (Appendix III). Likewise, PCA taking only topotypes and specimens from Perdizes showed no relevant differences (Appendix IV) in spite of the previously significant differences obtained in body size.

Vocalizations

Sacramento. Two males recorded (N = 20 notes A; 16 notes B). Bioacoustic variables are summarized in Table 2. Note A (Figure 3B) is composed of 17–21 pulses (mean 18.7, SD = 0.7), emitted at a rate of 51–59 notes/minute (mean 55.5, SD = 3.5). Note duration was 535–586 ms (mean 561.4, SD = 7.0), internote interval was 430–656 ms (mean 485.1, SD = 64.7). Dominant frequency was 2.34–2.63 kHz (mean 2.62, SD = 0.02). Note B (Figure 4B) is released in series of 5–6 notes/series (mean 5.3,

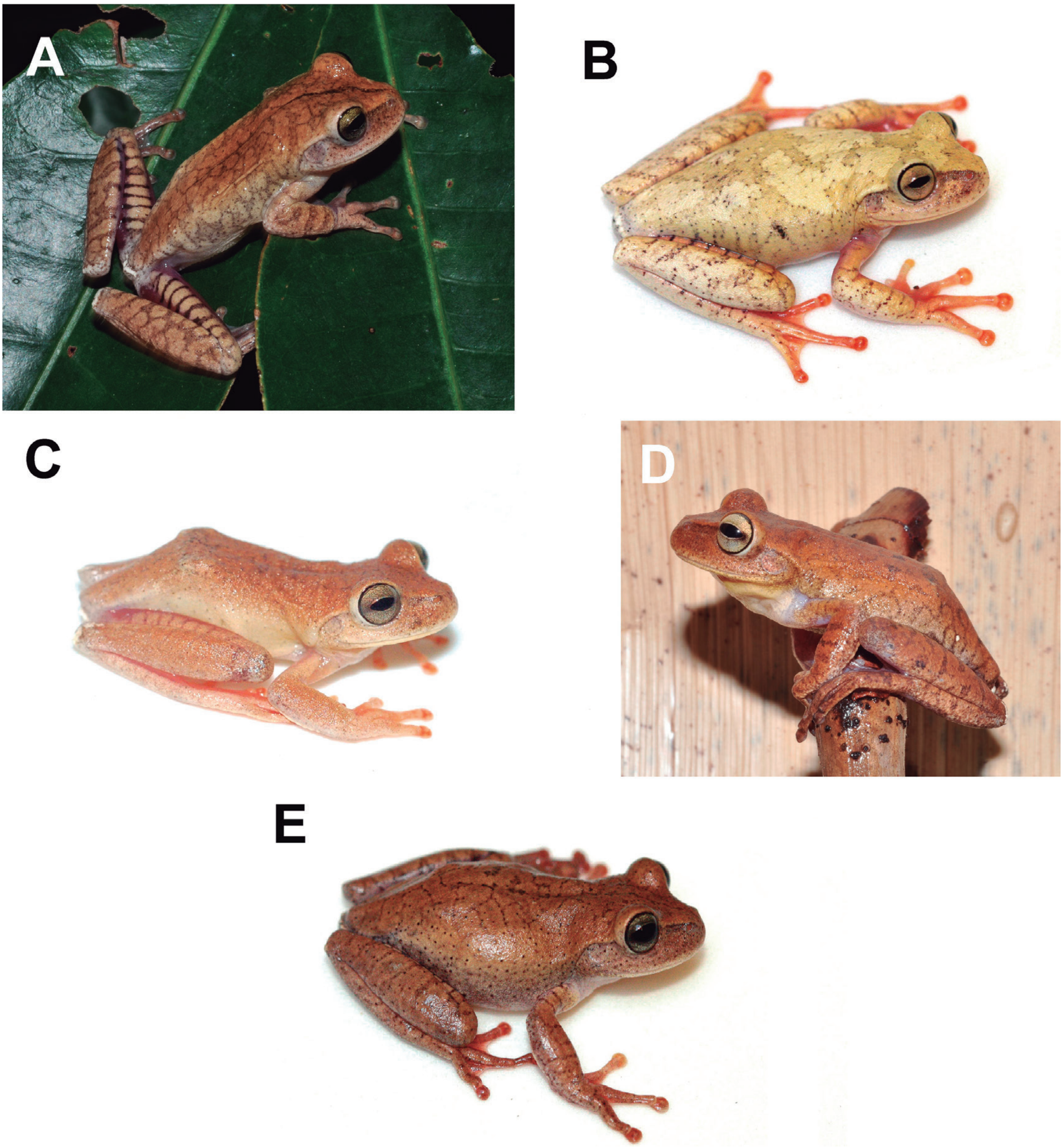

Figure 1. Adult male specimens of *Bokermannohyla sazimai* from several localities in the State of Minas Gerais, southeastern Brazil: A) Vargem Bonita; B) Sacramento; C) São Gotardo; D) Perdizes; E) Uberaba.

Table 1. Morphometric characters (in mm) of adult male topotypes of *Bokermannohyla sazimai* from the Serra da Canastra mountain range: municipalities of São Roque de Minas (seven paratypes) and Vargem Bonita (five topotypes); and adult male specimens of *B. sazimai* from the municipalities of Sacramento, Uberaba, São Gotardo and Perdizes, State of Minas Gerais, southeastern Brazil. Mean ± SD (range).

	Topotypic populations São Roque/Vargem Bonita (Serra da Canastra) *N* = 12	Sacramento (Serra da Canastra) *N* = 2	Uberaba *N* = 7	São Gotardo *N* = 18	Perdizes *N* = 13
SVL	32.4 ± 2.9 (28.3–36.4)	31.2 ± 1.5 (30.1–32.2)	32.8 ± 1.8 (30.3–36.2)	32.1 ± 1.4 (29.5–34.4)	35.6 ± 1.7 (32.4–38.2)
HL	11.9 ± 1.0 (10.1–13.3)	11.4 ± 0.6 (11.0–11.8)	12.1 ± 0.7 (11.5–13.1)	11.2 ± 0.5 (10.5–12.0)	12.6 ± 0.6 (11.4–13.5)
HW	11.3 ± 1.0 (9.5–12.2)	11.6 ± 0.1 (11.5–11.6)	11.6 ± 0.7 (10.7–12.7)	11.3 ± 0.4 (10.5–12.0)	12.5 ± 0.7 (11.4–14.2)
ED	3.2 ± 0.3 (2.8–3.6)	3.0 ± 0.0	3.0 ± 0.4 (2.7–3.6)	3.1 ± 0.1 (2.9–3.3)	3.4 ± 0.2 (3.0–3.8)
TD	2.0 ± 0.3 (1.6–2.3)	2.1 ± 0.1 (2.0–2.1)	1.9 ± 0.2 (1.6–2.2)	2.0 ± 0.2 (1.5–2.4)	2.3 ± 0.2 (2.1–2.8)
END	2.9 ± 0.3 (2.3–3.3)	2.5 ± 0.0	2.8 ± 0.3 (2.4–3.1)	2.7 ± 0.2 (2.3–3.3)	3.2 ± 0.2 (2.8–3.6)
TL	16.8 ± 1.4 (14.8–18.6)	16.9 ± 0.9 (16.2–17.5)	17.2 ± 1.1 (16.0–19.0)	16.5 ± 0.8 (15.2–18.0)	18.3 ± 1.0 (16.4–19.6)
SL	16.8 ± 1.4 (14.9–18.4)	16.5 ± 0.7 (16.0–17.0)	17.0 ± 0.9 (15.5–18.4)	16.5 ± 1.0 (14.7–18.0)	18.2 ± 0.8 (17.0–19.1)
FL	14.3 ± 1.5 (12.3–16.3)	15.1 ± 0.8 (14.5–15.7)	15.1 ± 0.8 (14.1–16.3)	14.2 ± 0.8 (12.7–15.5)	15.6 ± 1.2 (14.0–18.0)
HAL	10.3 ± 0.9 (9.0–11.4)	11.5 ± 0.5 (11.1–11.8)	10.7 ± 0.6 (10.0–11.9)	10.4 ± 0.5 (9.4–11.3)	11.4 ± 0.7 (10.6–12.7)
FDD	1.6 ± 0.2 (1.3–1.9)	1.5 ± 0.1 (1.4–1.6)	1.5 ± 0.4 (1.3–2.2)	1.6 ± 0.1 (1.4–1.8)	1.8 ± 0.2 (1.6–2.2)
TDD	1.6 ± 0.2 (1.2–1.8)	1.3 ± 0.1 (1.2–1.3)	1.4 ± 0.3 (1.1–1.8)	1.4 ± 0.1 (1.1–1.6)	1.7 ± 0.2 (1.5–2.2)

SD = 0.5) at a rate of 5–10 notes/minute (mean 7.0, SD = 2.6), and at a rate of 1–2 series/minute (mean 1.3, SD = 0.6). Note duration was 84–173 ms (mean 113.4, SD = 29.7), internote interval was 214–368 ms (mean 247.3, SD = 37.8). Note series duration was 1.55–1.83 s (mean 1.64, SD = 0.13). Dominant frequency was 2.44–2.63 kHz (mean 2.49, SD = 0.10).

São Gotardo. Ten males recorded (*N* = 178 notes A; 26 notes B). Bioacoustic variables are summarized in Table 2. Note A (Figure 3C) is composed of 13–37 pulses (mean 21.8, SD = 5.6), emitted at a rate of 48–80 notes/minute (mean 66.4, SD = 9.9). Note duration was 380–849 ms (mean 549.4, SD = 82.2), internote interval was 239–1614 ms (mean 568.0, SD = 348.3). Dominant frequency was 2.06–2.81 kHz (mean 2.55, SD = 0.21). Note type B (recorded from three males) (Figure 4C) consists of a multipulsed structure with initial isolated pulses (the presence and number of single pulses at the beginning of each note are variable within each

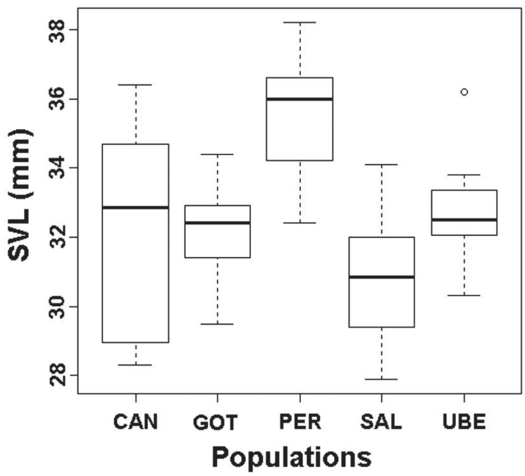

Figure 2. A boxplot depicting the SVL amplitude as function of *Bokermannohyla sazimai* populations from: type locality (CAN), São Gotardo (GOT), Perdizes (PER), Serra do Salitre (SAL), and Uberaba (UBE).

call) followed by several juxtaposed pulses. Note type B is released in series of 5–16 notes/series (mean 9.0, SD = 3.8) at a rate of 6–66 notes/minute (mean 38.7, SD = 26.5), and at a rate of 2–6 series/minute (mean 4.3, SD = 2.1). Note duration was 55–172 ms (mean 88.1, SD = 19.8), internote interval was 160–236 ms (mean 199.1, SD = 23.8). Note series duration was 1.55–4.32 s (mean 2.41, SD = 1.05). Dominant frequency was 2.24–2.81 kHz (mean 2.66, SD = 0.15).

Perdizes. Eight males recorded (*N* = 89 notes A). Bioacoustic variables are summarized in Table 2. Only note type A was recorded. Note A (Figure 3D) is composed of 12–24 pulses (mean 19.0, SD = 2.8), emitted at a rate of 51–69 notes/minute (mean 62.6, SD = 5.7). Note duration was 471–695 ms (mean 597.2, SD = 30.6), internote interval was 269–806 ms (mean 364.2, SD = 91.0). Dominant frequency was 2.24–2.76 kHz (mean 2.40, SD = 0.13).

Serra do Salitre. Two males recorded (*N* = 148 notes A). Bioacoustic variables are summarized in Table 2. Only note type A was recorded. Note A (Figure 3E) is composed of 7–21 pulses (mean 14.4, SD = 3.7), emitted at a rate of 77–85 notes/minute (mean 82.0, SD = 1.3). Note duration was 258–492 ms (mean 415.8, SD = 14.9), internote interval was 261–695 ms (mean 372.7, SD = 137.4). Dominant frequency was 2.24–2.58 kHz (mean 2.42, SD = 0.11).

Uberaba. Four males recorded (*N* = 39 notes A; 27 notes B). Bioacoustic variables are summarized in Table 2. Note A (Figure 3F) is composed of 12–27 pulses (mean 18.0, SD = 2.8), emitted at a rate of 39–64 notes/minute (mean 49.5, SD = 12.6). Note duration was 467–942 ms (mean 636.4, SD = 83.4), internote interval was 318–634 ms (mean 440.8, SD = 47.3). Dominant frequency was 2.44–2.62 kHz (mean 2.49, SD = 0.09). Note B (recorded from three males) (Figure 4D) consists of a multipulsed structure with initial isolated pulses (the presence and number of single pulses at the beginning of each note are variable within each call) followed by several juxtaposed pulses. Note type B is released in series of 4–7 notes/series (mean 5.6, SD = 0.9) at a rate of 9–57 notes/minute (mean 29.8, SD = 24.6), and at a rate of 2–9 series/minute (mean 5.0, SD = 3.6). Note duration was 58–136 ms (mean 86.2, SD = 9.4), internote interval was 151–232 ms (mean 190.1, SD = 21.6). Note series duration was 0.88–1.77 s (mean 1.31, SD = 0.27). Dominant frequency was 2.44–2.62 kHz (mean 2.50, SD = 0.10).

Distribution

Our surveys on *Bokermannohyla sazimai* recorded five additional distribution localities (Uberaba, Serra do Salitre, Perdizes, Ibiá, and Paracatu), and extended its north limit of distribution (Paracatu and Serra do Salitre) (Figure 5).

Discussion

The currently recognized distribution of *Bokermannohyla sazimai* has its southern limit

Table 2. Advertisement call variables of topotypes of *Bokermannohyla sazimai*: Municipalities of Vargem Bonita, Serra da Canastra mountain range, State of Minas Gerais (after Carvalho *et al.* 2012); and five additional populations of *B. sazimai* from the Municipalities of Sacramento, São Gotardo, Perdizes, Serra do Salitre, and Uberaba, State of Minas Gerais, southeastern Brazil. Mean ±_SD (range). *N* = number of specimens recorded (number of notes A / number of notes B).

Bioacoustic variables	**Topotypic population Vargem Bonita *N*=5 (74/221)**	**Sacramento *N*=2 (20/16)**	**São Gotardo *N*=10 (178/26)**	**Perdizes *N*=8 (89)**	**Serra do Salitre *N*=2 (148)**	**Uberaba *N*=4 (39/27)**
Note A duration (ms)	656.7 ± 97.8 (476–892)	561.4 ± 7.0 (535–586)	549.4 ± 82.2 (380–849)	597.2 ± 30.6 (471–695)	415.8 ± 14.9 (258–492)	636.4 ± 83.4 (467–942)
Internote interval (ms)	633.7 ± 320.7 (303–2109)	485.1 ± 64.7 (430–656)	568.0 ± 348.3 (239–1614)	364.2 ± 91.0 (269–806)	372.7 ± 137.4 (261–695)	440.8 ± 47.3 (318–634)
Pulses/note	26.7 ± 4.6 (13–36)	18.7 ± 0.7 (17–21)	21.8 ± 5.6 (13–37)	19.0 ± 2.8 (12–24)	14.4 ± 3.7 (7–21)	18.0 ± 2.8 (12–27)
Notes/minute	35.6 ± 16.0 (17–68)	55.5 ± 3.5 (51–59)	66.4 ± 9.9 (48–80)	62.6 ± 5.7 (51–69)	82.0 ± 1.3 (77–85)	49.5 ± 12.6 (39–64)
Dominant frequency (kHz)	2.47 ± 0.09 (2.24–2.58)	2.62 ± 0.02 (2.34–2.63)	2.55 ± 0.21 (2.06–2.81)	2.40 ± 0.13 (2.24–2.76)	2.42 ± 0.11 (2.24–2.58)	2.49 ± 0.09 (2.44–2.62)
Note B duration (ms)	100.9 ± 28.6 (80–165)	113.4 ± 29.7 (84–173)	88.1 ± 19.8 (55–172)	–	–	86.2 ± 9.4 (58–136)
Internote interval (ms)	175.2 ± 20.0 (124–199)	247.3 ± 37.8 (214–368)	199.1 ± 23.8 (160–236)	–	–	190.1 ± 21.6 (151–232)
Notes/series	10.1 ± 2.1 (6–15)	5.3 ± 0.5 (5–6)	9.0 ± 3.8 (5–16)	–	–	5.6 ± 0.9 (4–7)
Note B series duration (s)	2.65 ± 0.55 (1.57–3.93)	1.64 ± 0.13 (1.55–1.83)	2.41 ± 1.05 (1.55–4.32)	–	–	1.31 ± 0.27 (0.88–1.77)
Note series/minute	3.1 ± 1.0 (1–5)	1.3 ± 0.6 (1–2)	4.3 ± 2.1 (2–6)	–	–	5.0 ± 3.6 (2–9)
Notes/minute	31.9 ± 15.7 (6–54)	7.0 ± 2.6 (5–10)	38.7 ± 26.5 (6–66)	–	–	29.8 ± 24.6 (9–57)
Dominant frequency (kHz)	2.45 ± 0.12 (2.24–2.58)	2.49 ± 0.10 (2.44–2.63)	2.62 ± 0.15 (2.44–2.81)	–	–	2.50 ± 0.10 (2.44–2.62)

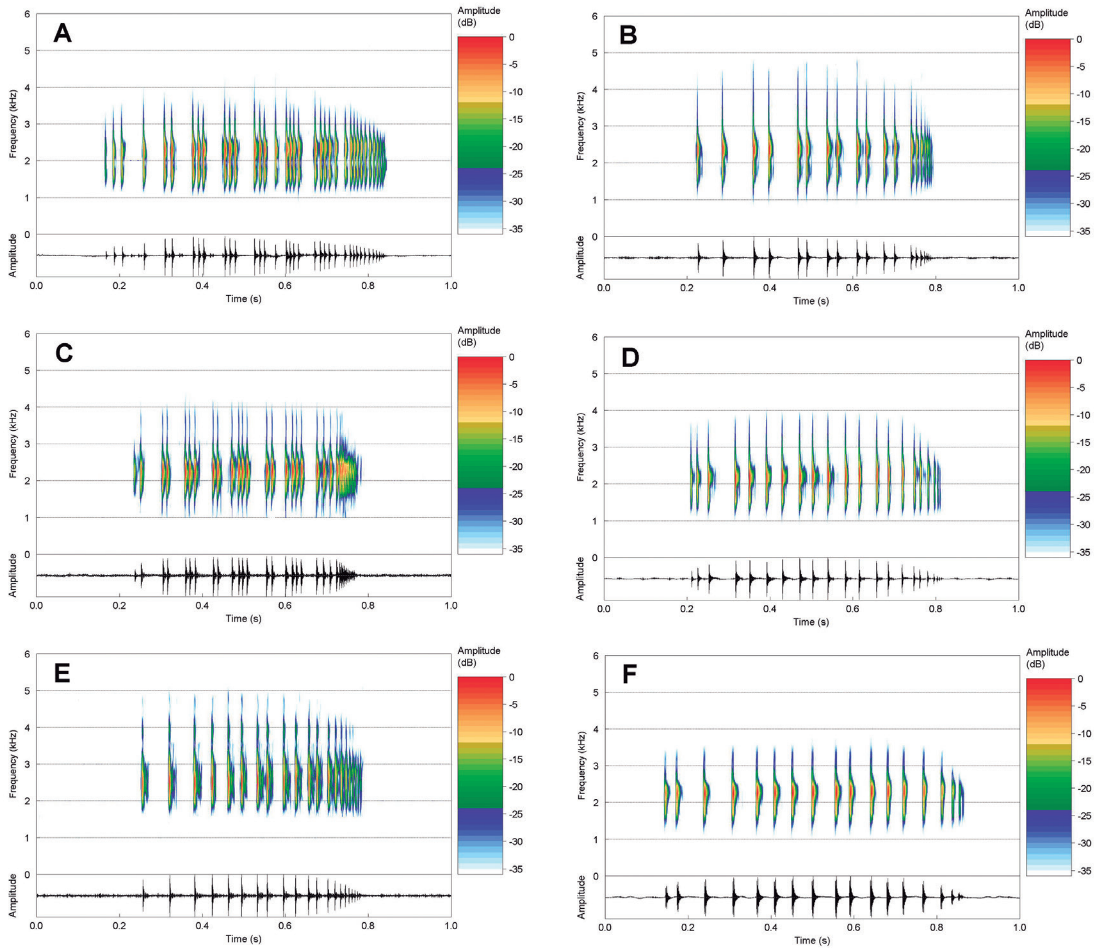

Figure 3. Audiospectrogram (above) and corresponding oscillogram (below) of the note type A of *Bokermannohyla sazimai* advertisement call from: A) Vargem Bonita; B) Sacramento; C) São Gotardo; D) Perdizes; E) Serra do Salitre; F) Uberaba. All localities in the State of Minas Gerais, southeastern Brazil.

represented by the Municipality of Vargem Bonita (Carvalho *et al.* 2012), northern limit in the Municipality of Nova Ponte (Caramaschi *et al.* 2001), eastern limit in the Municipality of São Gotardo (Caramaschi *et al.* 2001, present study), and western limit in the Municipality of Uberlândia (Moura and Feio 2010). Our newly gathered data reveal that *B. sazimai* occurs in disjunctive distribution associated to riparian environments in the State of Minas Gerais, southeastern Brazil (Figure 5). The record for Paracatu represents the northernmost and farthest (approximately 330 km northward in a straight line to its type locality) distribution for the species, unreported so far. Our report for Uberaba is the first record in the Grande River basin.

The species presented no relevant interpopulational discrimination with respect to

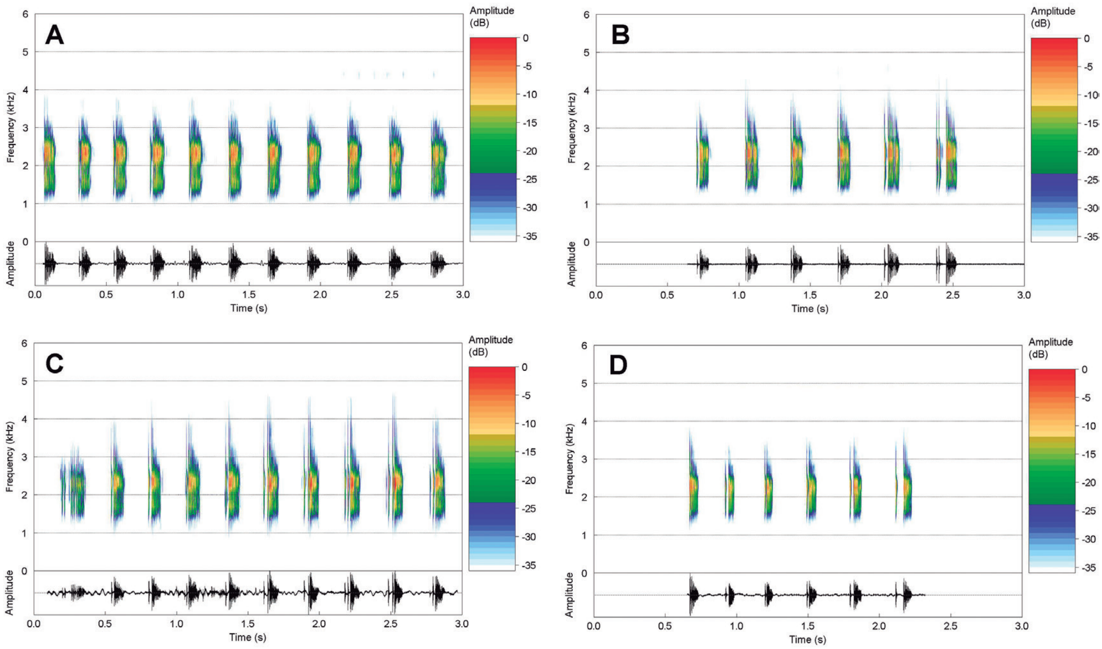

Figure 4. Audiospectrograms (above) and corresponding oscillograms (below) of a sequence (note series) of the note type B of *Bokermannohyla sazimai* from: A) Vargem Bonita; B) Sacramento; C) São Gotardo; D) Uberaba. All localities in the State of Minas Gerais.

the analyzed morphometric characters. It is noteworthy to state that variation with respect to dorsal color pattern and presence/absence of transverse bars on posterior thigh surfaces among examined specimens of *Bokermannohyla sazimai* were originally included as part of the set of diagnostic characters in the *Bokermannohyla circumdata* group. Despite some variation, the reticulated pattern on dorsum is still a valid character for the diagnosis of *B. sazimai*, whereas the presence of dark transverse bars on posterior thigh surfaces was quite variable (well-defined, fragmented, or immaculate). Regardless of such great variation in this character (at least three recognized character states), dark transverse stripes on superior thigh surfaces were present in all examined specimens of *B. sazimai*, still being a reliable character for its diagnosis.

Salient bioacoustic variation was observed among populations of *Bokermannohyla sazimai* with special reference to the pulse structure of note A, and the facultative emission of note B. Populations from Vargem Bonita (Figure 3A) and São Gotardo (Figure 3C) had pulses arranged in well-defined groups in note A, whereas the other populations had ill-defined or no groups instead (Figure 3). We expect that this variation should possibly be explained by individual variability, so that on the increase of recorded specimens from all populations, both well-defined and ill-defined or no pulse group structure pattern might be observed within each population. Males recorded from Perdizes and Serra do Salitre did not emit note type B, a usual feature in other populations (Figure 4). This note type was heard twice (not recorded) from a

Figure 5. Geographic distribution of *Bokermannohyla sazimai* in the State of Minas Gerais, southeastern Brazil: Square - São Roque de Minas (type locality); 1) Vargem Bonita (Carvalho *et al.* 2012); 2) Sacramento; 3) São Gotardo; 4) Serra do Salitre; 5) Ibiá; 6) Araxá (from Giasson and Ribeiro 2006); 7–8) Two localities in Perdizes (Sítio Bom Sucesso and EPDA-Galheiro, respectively); 9) Uberaba; 10) Nova Ponte (from Caramaschi *et al.* 2001); 11) Uberlândia (from Moura and Feio 2010); 12) Paracatu.

specimen from Perdizes (TRC, pers. obs.). The rare emission of note B in Perdizes leads us to believe that the population of Serra do Salitre could rarely emit this note type as well. We might assume that the facultative emission of note type B should possibly be ruled by some social context, and should be more thoroughly evaluated under an ethological approach so as to determine its role, thus classifying it as part or not of the advertisement call *B. sazimai*.

We were not able to assess bioacoustic information on the populations from Paracatu and Uberlândia. Both localities have been extensively explored, particularly the Municipality of Uberlândia and its outskirts, a study area for over ten years with no record of *Bokermannohyla sazimai* in the region, which was once reported in Moura and Feio (2010). Additional efforts to obtain bioacoustic information on all distributional records might improve the knowledge of the variability in morphological and bioacoustic characteristics of *B. sazimai* along its geographic distribution.

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Appendix I. Examined specimens of *Bokermannohyla sazimai*.

BRAZIL: MINAS GERAIS: Araxá: CFBH 11575; Ibiá: CFBH 18417–18419; UFMG 3424–3429; Perdizes: AAG-UFU 0610–0611, 2417, 2427–2429, 2615; UFMG 3330–3332; ZUEC 12063–12065; Sacramento: AAG-UFU 1034–1035; São Gotardo: AAG-UFU 0350–0368; São Roque de Minas: ZUEC 4194–4196, 4199–4200, 4212–4213; Serra do Salitre: UFMG 844–848, 851–859; Uberaba: AAG-UFU 0553–0554, 1041–1045; Vargem Bonita: AAG-UFU 4842–4846.

Appendix II. PCA eigenvectors, eigenvalues, and total variation explained based on twelve morphometric variables of adult male specimens of all analyzed populations of *Bokermannohyla sazimai*.

Variables	**PC1**	**PC2**	**PC3**
SVL	0.3038	-0.1266	0.3277
HL	0.2926	-0.0449	0.2043
HW	0.3119	-0.0790	0.0565
ED	0.2723	0.4013	-0.0144
TD	0.2711	0.2475	-0.0983
END	0.2606	0.2841	0.6686
TL	0.3077	-0.2986	-0.0413
SL	0.3020	-0.3049	0.0364
FL	0.2963	-0.3347	-0.0618
HAL	0.2813	-0.3238	-0.3781
FDD	0.2874	0.2749	-0.3519
TDD	0.2717	0.4450	-0.3424
Eigenvalues	3.0286	0.8817	0.7255
Percentage of variation	76.43%	6.48%	4.39%

Appendix III. PCA eigenvectors, eigenvalues, and total variation explained based on twelve morphometric variables of adult male specimens of *Bokermannohyla sazimai* based only on the topotypic and Perdizes populations.

Variables	PC1	PC2	PC3
SVL	0.2979	-0.3437	0.0944
HL	0.2860	0.1826	-0.0581
HW	0.3124	0.0472	-0.0123
ED	0.2733	0.0505	0.3919
TD	0.2891	-0.0050	0.3666
END	0.2598	0.1337	0.6149
TL	0.3007	-0.3167	-0.2258
SL	0.2966	-0.3588	-0.2350
FL	0.3034	-0.1345	-0.0894
HAL	0.2965	-0.1534	-0.2295
FDD	0.2826	0.3950	-0.33394
TDD	0.2607	0.6318	-0.1987
Eigenvalues	3.0897	0.8530	0.5388
Percentage of variation	76.55%	6.65%	4.32%

Appendix IV. Figured sound files.

Figure 3. A) Bokerm_sazimaiVargemBonitaMG3TRC_AAGmt; 23:05, 17 December 2009, air 19°C, water 21°C; B) Bokerm_sazimaiSacramentoMG1cLBM_AAGmt; 23:00, 13 February 2012, air 20°C, water 21°C; C) Bokerm_sazimaiSãoGotardoMG11TRC_AAGmt; 19:40, 26 March 2011, air 22°C, water 22°C; D) Bokerm_sazimaiPerdizesMG6bTRC_AAGmt; 19:40, 14 November 2011, air 18°C, water unmeasured; E) Bokermannohyla cf sazimai 2 trecho 2_2_57min; 19:10, 25 February 2009, air and water unmeasured; F) Bokerm_sazimaiUberabaMG6bTRC_AAGmt; 00:02, 2 February 2012, air 20°C, water 22°C.
Figure 4. A) Bokerm_sazimaiVargemBonitaMG2bTRC_AAGmt; 23:13, 17 December 2009, air 19°C, water 21°C; B) Bokerm_sazimaiSacramentoMG2bLBM_AAGmt; 23:05, 13 February 2012, air 20°C, water 21°C; C) Bokerm_sazimaiSãoGotardoMG2aTRC_AAGmt; 19:57, 24 March 2011, air 20°C, water 21°C; D) Bokerm_sazimaiUberabaMG6bTRC_AAGmt; 00:02, 2 February 2012, air 20°C, water 22°C.

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