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Complex Call with Different Messages in *Bokermannohyla ibitiguara* (Anura, Hylidae), a Gladiator Frog of the Brazilian Cerrado

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Abstract.—The Gladiator Frog, *Bokermannohyla ibitiguara,* is a hylid endemic to the Brazilian Cerrado with a complex call composed of long and short notes. We describe the influence of male size and temperature on call properties and investigate the social contexts of the different notes of the call through direct observations and playback experiments conducted in the field. The dominant frequency correlated negatively with male body size, whereas the duration of the long note correlated positively; there was no correlation between pulse rate and male body size. Air temperature correlated negatively with the duration of the long note and positively with pulse rate. Playback experiments with males indicated that they did not change the proportion of long to short notes in the presence of an intruder (= playback), but rather, they changed the short notes, which became longer, with a lower dominant frequency and more pulses with variable intervals. We interpreted this call as an aggressive call. Our results suggest that females may choose males based on dominant frequency and duration of the long note, traits that might be under sexual selection. Moreover, short notes appear to be the territorial component of the mixed call, and the aggressive call is a variation of this component.

Resumo.—*Bokermannohyla ibitiguara* é um hilídeo endêmico do Cerrado brasileiro que possui um canto complexo, composto de notas longas e curtas. Neste estudo, descrevemos a influência do tamanho do macho e da temperatura do ar nas propriedades acústicas do canto, investigando também os contextos sociais das diferentes notas através de observações diretas e experimentos de *playback* conduzidos no campo. O tamanho do macho correlacionou-se negativamente com frequência dominante do canto e positivamente com duração da nota longa; não houve correlação com taxa de pulsos. A temperatura do ar correlacionou-se negativamente com duração da nota longa e positivamente com a taxa de pulsos. Os experimentos de *playback* mostraram que machos não alteram a proporção de emissão de notas curtas e longas na presença de um intruso (= *playback*); ao invés disso, machos modificam as notas curtas, tornando-as mais longas, com frequência dominante mais baixa e com mais pulsos com intervalos variáveis. Nós interpretamos tal canto como sendo o canto agressivo. Nossos resultados sugerem que, potencialmente, as fêmeas podem escolher os machos avaliando a frequência dominante do canto e a duração da nota longa, características que podem estar sob seleção sexual. Além disso, as notas curtas parecem ser o componente territorial do canto misto, sendo o canto agressivo uma variação de tal componente.

Sexual selection has been defined as a selective force that acts differently on each sex or sexual function because of the different strategies adopted, favoring sex-specific reproductive adaptations (Darwin, 1871; Carranza, 2009). Sexual selection drives the ability of individuals of one sex (generally males) to compete for mates (intrasexual selection) and also affects the reproductive traits evaluated during mate choice (intersexual selection) (Krebs and Davies, 1993). In anurans, some of the traits associated with the "best" quality males can be body size, reproductive site fidelity, territory quality, and call properties, each one indicating stronger or healthier individuals (Lips, 2005).

The study of male calling behavior is a good way to assess sexual selection and mating preferences in anurans (e.g., Gerhardt et al., 2000, 2007; Murphy and Gerhardt, 2002). In general, the most studied and most common sound emitted by males is the advertisement call, the main function of which is female attraction and maintenance of spacing (Wells, 1977a; Murphy and Gerhardt, 2002; Martins and Jim, 2003). Male success in obtaining mates can be related to large body size (Morris, 1989; Bastos and Haddad, 1996; Bernard, 2007), which often is correlated negatively with the dominant frequency of the advertisement call (e.g., Howard and Young, 1998; Toledo and Haddad, 2005). Therefore, females may be capable of choosing males based on their call properties (e.g., Howard and Young, 1998; Murphy and Gerhardt, 2002; Gerhardt et al., 2007).

Sexual selection mechanisms mediated by calling activity in frogs are usually much more complex, because most species emit more than one type of call in different social interactions (Duellman and Trueb, 1994). Apart from the advertisement call, males also emit an aggressive call, which is important in competing for females and is part of territorial behavior (e.g., chases and fights) (Martins et al., 1998; Nali and Prado, 2012). High energy expenditure related to calling activity generally drives the ability of male frogs to display modulated calls (Gerhardt and Huber, 2002), such that conspecific males can emit different calls in the presence of predators, females, or possible rival males (e.g., Ryan, 1985; Byrne, 2008). The occurrence of mixed or complex calls, those with two or more types of notes emitted in different proportions (Cardoso and Haddad, 1984; Lingnau et al., 2004), also indicates that calling behavior may be plastic, with males adjusting calls according to the social context (Wells, 1988; Bee et al., 2000). Thus, an accurate description of the different parts and structures of the call is necessary to avoid misinterpretations of crucial social interactions in anurans; effective methods for such studies include direct observations in the field and playback experiments (Meuche et al., 2012; Reichert and Gerhardt, 2013).

Bokermannohyla ibitiguara (Cardoso, 1983) is a stream-dwelling treefrog endemic to the Brazilian Cerrado. Males are territorial and engage in physical combat, attempting to puncture rivals with their well-developed prepollical spines (Nali and Prado, 2012). Despite being common throughout its distribution, its basic biology is poorly known (Nali and Prado, 2012). This frog is included in the Data Deficient category by the International Union for the Conservation of Nature (IUCN) (Caramaschi and Eterovick, 2004). The advertisement call is composed of long notes followed by a sequence of secondary short notes (Cardoso, 1983; Carvalho et al., 2012; this study). According to

Cardoso (1983), when two males call next to each other, commonly one emits only long notes, whereas the other one emits only short notes. The occurrence of mixed calls and the plasticity of call emission depending on the social context led us to hypothesize that long and short notes have different social functions. Thus, through direct observations and playback experiments in the field, we tested the hypothesis that long notes are related to female attraction, whereas short notes function as an aggressive call. Also, we examined whether male response to playback is associated with body size as well as the influences of body size and air temperature on call properties.

MATERIALS AND METHODS

Study Area and Data Collection.—Fieldwork was conducted on private farms in the municipality of Sacramento, State of Minas Gerais, southeastern Brazil (Fig. 1). We sampled frogs at two permanent streams (20.2727°S, 47.0735°W, 677 m in elevation; 20.2056°S, 47.1316°W, 839 m in elevation) and one temporary stream (20.2194°S, 47.1058°W, 880 m in elevation; datum WGS 84). The climate is markedly seasonal, with a hot and rainy summer (October to March) and a dry winter (April to September); the topography is mountainous, with flat or rounded hills reaching up to 1,500 m in elevation, with deep valleys and many streams (Dietz, 1984). The vegetation consists of cerrado, grassland fields at higher elevations, gallery forests in the valleys, and patches of semideciduous forest (Dietz, 1984).

Observations of male calling behavior occurred throughout the species' breeding season (October 2010 to June 2011). Individuals were located via auditory and visual searches at breeding sites and observed with headlamps, usually with red lights, reducing the interference with behavior (e.g., Pough et al., 1983).

Vocalization Analyses, Male Body Size, and Temperature.—Calls were recorded using a Marantz PMD-660 digital recorder (Mahwah, NJ) and Sennheiser ME66 unidirectional microphone (Marlow, Buckinghamshire, UK) at a 16-bit resolution and 44,100 Hz. Air temperature and time of recording were recorded. Calls were analyzed using the software Raven Pro 1.3 (Cornell Lab of Ornithology, Ithaca, NY) with FFT (Fast Fourier Transformation) = 512 points.

We use the following terms throughout the text (adapted from McLister et al., 1995; Martins and Jim, 2003): 1) call/ vocalization: a sequence of sounds produced and emitted in a temporal pattern; 2) note: the total amount of sound energy generated by the male during an air flow cycle, observed through trunk and vocal sac inflation and deflation; and 3) pulses: subdivision of the note in smaller temporal units.

To evaluate whether the parameters of males' advertisement calls were influenced by male body size or temperature, we performed linear regression analyses between these variables and the following acoustic parameters: 1) mean dominant frequency (Hz)—mean of approximately five long notes and five sequences of short notes for each male; 2) duration of the long note (s)—mean of 10 notes for each male; and 3) pulse rate (N pulses/0.5 s), counting from the onset of five long notes for each male. We used these parameters because they are known to be preferred by female anurans (Gerhardt, 1994). The dominant frequencies of the long and short notes were analyzed together because they were statistically similar ($t = 0.13$; $P = 0.89$). The snout–vent length (SVL) of males was measured in the field with an analogical caliper (0.05 mm resolution), and male body mass was measured with a dynamometer (0.1 g resolution). Males were identified by unique natural marks (spots, scars,

and unpigmented regions; Bradfield, 2004). At each study site, we took one or more pictures and described the natural marks of each recorded male, such that we had a visual guide for each individual in the field.

Social Functions of Notes: Direct Observations and Playback Experiments.—We observed male behavior and recorded focal male calls in natural conditions to evaluate the emission of different parts of the call depending on the number of males attending the chorus or the distance of the nearest calling neighbor (DNN). We expected an increased emission of short notes—hypothesized to have a territorial function—in larger choruses and at short DNNs. Thus, we used Student's t-test to compare the following: 1) the mean number of short notes emitted by males calling alone or with one other male; and 2) the mean number of short notes emitted by males in groups of three to five individuals. ANOVA was used to compare the number of short notes emitted for three different ranges of DNN: 101–500 cm; 501–1,000 cm; and above 1,000 cm. Distance categories were based on previous observations of distance between males (Nali and Prado, 2012).

For the playbacks, we used advertisement calls recorded at the study site from October to December 2010. We analyzed the following parameters: 1) mean dominant frequency (Hz); 2) mean duration of long notes (s); 3) mean duration of short notes' sequences (s); 4) mean time interval between notes (s); and 5) mean number of long notes emitted in sequence. For parameters 1, 2, and 3, we analyzed approximately five notes and five sequences of short notes for each individual ($N = 15$ males); for number 4, we analyzed 15 intervals for each individual ($N = 5$ males); and for number 5 we also used the latter individuals ($N = 32$ sequences). After determining the mean values for each parameter of this subset (1: 1,860 Hz; 2: 0.95 s; 3: 1.45 s; 4: 0.64 s; 5: 6 notes), we used, from our recordings, specific notes with values close to these averages. We then constructed playbacks of 1 min each, according to each treatment, using the free software Audacity 1.3 Beta (Audacity Developer Team, Pittsburgh, PA). Acoustic sampling continued until March 2011, and those measures were added later in the advertisement call description.

We calculated the mean sound pressure level (SPL) for seven males at a distance of 50 cm (mean 82 ± 7 dB) using a Brüel and Kjær type 2232 SPL meter (Nærum, Copenhagen, Denmark). For each test, we used a mini Philips AZ-302S system (Eindhoven, Netherlands) with the playback track in original WAV format, adjusting the volume to emit the same SPL at 50 cm, thus simulating the most natural condition of the male's call (Rosso et al., 2006). This equipment was adequate for evaluation although we note that the ideal would be the use of potent amplifiers and fixed speakers (e.g., Gerhardt et al., 2000; Murphy and Gerhardt, 2002). The use of such equipment is logistically difficult because this species resides in gallery forests and commonly calls from perches on vegetation (Nali and Prado, 2012).

For the playback experiment, we held the sound source 50 cm from the male, recording the male's call without playback (control period; 1 min). After that, we played the playback recording for 1 min and recorded the male's response during the playback (test period; Giasson and Haddad, 2006). This procedure was performed for three different treatments, each with its own playback track: T1) call with only long notes; T2) call with only sequences of short notes; and T3) mixed call (long and short notes). For both the control and test periods, we counted the numbers of short and long notes emitted by the male. Each individual was tested only once per treatment ($N =$
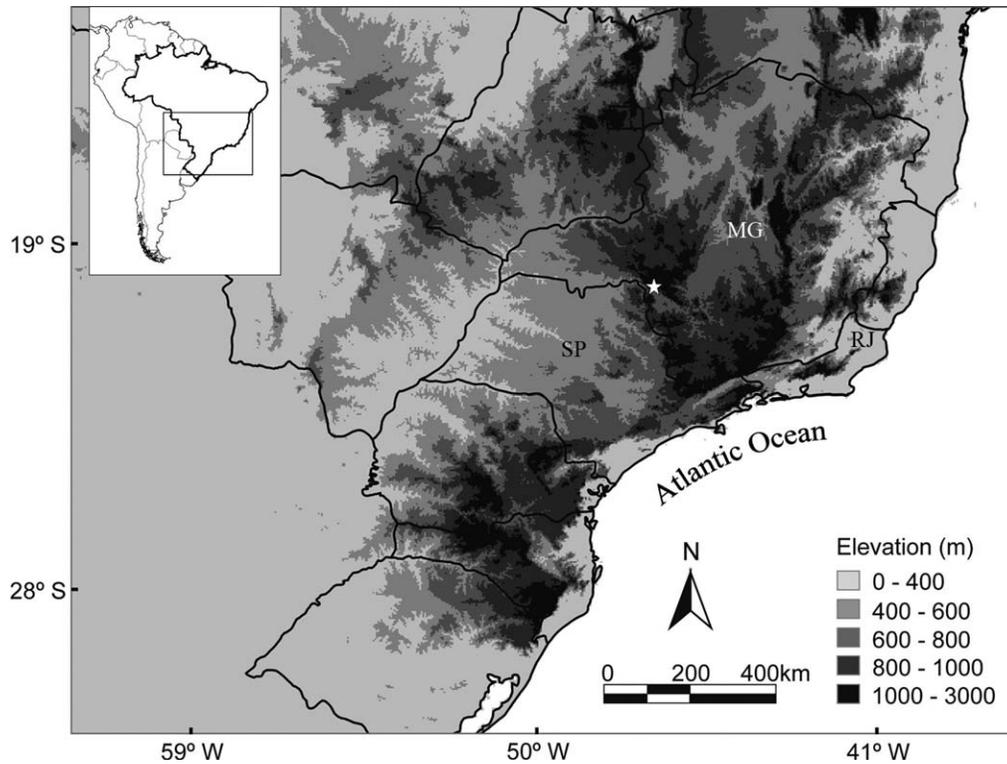
FIG. 1. Location of Brazil in South America; in detail, our study site (star) in the Cerrado of Minas Gerais state (MG), southeastern Brazil.

41 males); in a few cases, two tests using the same male occurred, but those were separated by at least one period of noncalling activity (Luna et al., 2010). We performed 16 replicates for T1 and T2 and 17 for T3. However, because many males practically stopped calling during the playback—which was assumed to be lack of response—we were able to consider only 7 replicates for T1, 13 for T2, and 11 for T3.

Differences in the mean numbers of short and long notes before and during the playback were tested using a t-test for dependent samples (Zar, 1999). We expected that, if short notes had the function of an aggressive call, the number of short notes should increase when an intruder was simulated (via playback); similarly, if long notes had the main function of female attraction, the number of long notes should decrease during such a simulation.

The replicates without male response to playback provided data to investigate whether males of the nonresponding group were smaller in size than were males that responded to the playbacks. The mean SVL and body mass of the responding and nonresponding males were compared through Student's t-test.

All data sets were checked for normality with a Shapiro-Wilk test, and statistical analyses (Zar, 1999) were performed in the program Statistica v.10 (Statsoft, Tulsa, OK).

RESULTS

Advertisement Call Properties, Body Size, and Temperature.— *Bokermannohyla ibitiguara* males emitted mostly the complex advertisement call but occasionally emitted only long notes for a long period of time (Table 1). During periods of low activity, males could emit sequences of short notes but less intensively and at a low repetition rate.

The dominant frequency of calls was correlated negatively with both male body mass (Fig. 2A) and male SVL (Fig. 2B). The duration of the long note correlated positively with male body mass (Fig. 2C) and male SVL (Fig. 2D). The duration of the long note also correlated negatively with air temperature (Fig. 3A), whereas pulse rate correlated positively (Fig. 3B). Pulse rate was

TABLE 1. Acoustic parameters of *Bokermannohyla ibitiguara* advertisement call based on both types of commonly emitted notes (short and long notes). Males ($N = 48$) recorded in the municipality of Sacramento, Minas Gerais state, southeastern Brazil, from October 2010 to March 2011. Dominant frequencies of short and long notes were analyzed together because they were statistically similar (see text for details).

| Parameter	Mean	SD	Range	N
Dominant frequency (Hz)	2,024	205	1,378–2,842	454
Duration of short note (s)	0.053	0.008	0.042–0.065	80
Duration of short notes' sequence (s)	1.45	0.35	0.72–2.28	70
Number of short notes per sequence	5.5	1.1	4.0–8.0	151
Duration of long note (s)	0.80	0.17	0.44–1.52	408
Time interval between long notes (s)	0.64	0.30	0.31–2.34	75
Number of long notes emitted in sequence	6	4	1–17	32
Pulse rate of long note (N pulses/0.5 s)	25	7	9–46	190
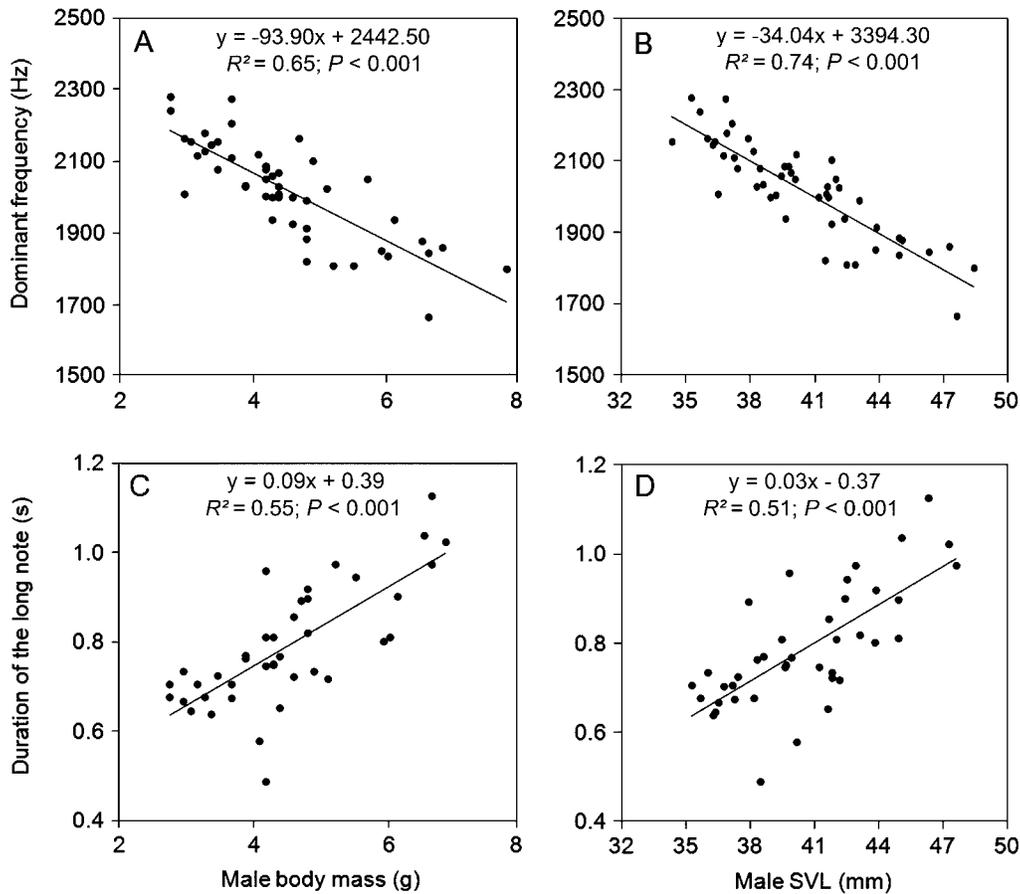
FIG. 2.   Regression analyses between acoustic parameters of the advertisement call and male body size in *Bokermannohyla ibitiguara*, municipality of Sacramento, Minas Gerais, Brazil. A and B: 49 males; C and D: 40 males.

not correlated with male body mass ($R^2 = 0.03$; $N = 38$; $P = 0.313$) or male SVL ($R^2 = 0.006$; $N = 38$; $P = 0.651$).

Direct Observations and Analyses of Natural Choruses.—On 21 November 2010, we observed two males approximately 60 cm from each other. A female nearby was noticed by the males. In this case, both males emitted mostly long notes, in antiphony. On 25 January 2011, we observed a calling male, a satellite male,

and a female nearby. In this case, the calling male also emitted mostly long notes.

On 20 November 2010, we observed two males with no females nearby, emitting mostly short notes and a few long notes. In two other separate events (18 January and 27 February 2011), we observed a calling male turning to a satellite male and emitting short notes. In the event on 18 January, this happened
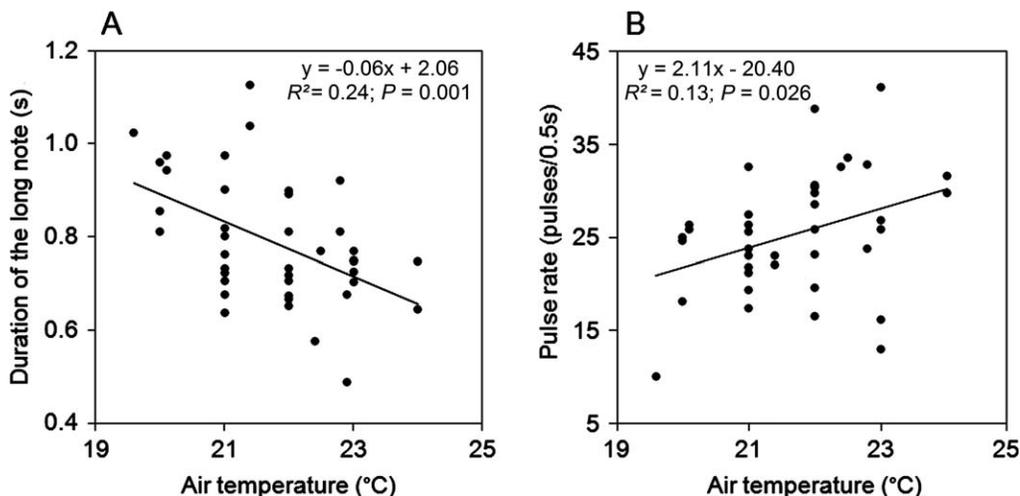

FIG. 3.   Regression analyses between temporal acoustic parameters of the advertisement call of *Bokermannohyla ibitiguara* and air temperature, municipality of Sacramento, Minas Gerais, Brazil. A: 40 males; B: 38 males.
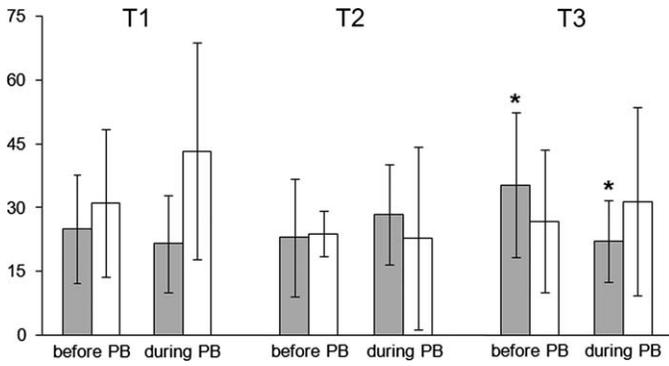
FIG. 4. Mean number of notes (vertical axis) emitted by males before and during each playback (PB) treatment (T1, T2, T3). The * indicates significant statistic differences ($P < 0.05$). Gray bars = long notes; white bars = short notes (see text for details).

twice: 1) in the middle of a courtship event; and 2) before the calling male engaged in a fight with the satellite male (details in Nali and Prado, 2012).

The number of short notes emitted by individuals alone or with another calling male was not different from those emitted by males in groups of 3 to 5 individuals ($t = -0.54$; $P = 0.59$). Additionally, there was no difference in the emission of short notes among any of the ranges of DNN ($F = 1.31$, $P = 0.29$).

Playback Experiments and Aggressive Call.—Considering each playback treatment, we found no difference between the numbers of short and long notes emitted before and during the playback (Fig. 4), except for T3 (mixed call with long and short notes), for which males significantly reduced the emission of long notes during the playback ($t = 3.75$, $P < 0.05$, Fig. 4). However, we observed that short notes emitted in response to the playbacks were slightly different from the short notes emitted before the playback, but the number of short notes per sequence was similar. The differences in these short notes are related to the dominant frequency, duration and pulse structure (Table 2; Fig. 5).

We found no difference in mean SVL between males that responded and males that did not respond to T1 ($t = 1.72$; $P = 0.12$), T2 ($t = -0.09$; $P = 0.93$) or T3 ($t = 0.02$; $P = 0.98$). Controlling for mean male body mass, we found no difference for T2 ($t = 0.28$; $P = 0.78$) or T3 ($t = 0.11$; $P = 0.91$), but for T1 (only long notes), responding males were heavier than non-responding ones ($t = 2.22$; $P < 0.05$). Considering all treatments together, we found no difference either in SVL ($t = 0.32$; $P = 0.75$) or in mass ($t = 0.61$; $P = 0.55$) between responding and nonresponding males.

DISCUSSION

In our study, the dominant frequency and duration of notes were similar to those described for a different population of *B.*

ibitiguara (Carvalho et al., 2012); however, the intervals between long notes were approximately half of the duration reported there. Additional parameters measured here serve as a complement to the previous redescription (Table 1). The dominant frequency of *B. ibitiguara* calls was negatively correlated with male body size (e.g., Cocroft and Ryan, 1995; Toledo and Haddad, 2005; Gingras et al., 2013), which is explained by the negative acoustic relationship between laryngeal size and its vibration, which is, in turn, influenced by body size (Martin, 1972; McClelland et al., 1996). Because dominant frequency in *B. ibitiguara* signals male size, females may select males through the evaluation of the call frequency, as reported for other species (Ryan et al., 1992; Márquez and Bosch, 1997). Females may have an advantage in choosing larger males because this species is territorial (Nali and Prado, 2012), and larger males may monopolize better-quality spawning sites. Females of *B. ibitiguara* may also use duration of the long note to evaluate males because this trait was positively correlated with male size. However, males emitted longer calls at lower temperatures, indicating that evaluation of this call trait by females might depend on air temperature. Pulse rate did not correlate with male body size but was also influenced by air temperature. The evolution of anuran call properties and female choice is very complex because calls are composed of a set of characters evolving at different rates and influenced by morphology (e.g. size; Klump and Gerhardt, 1987; Gerhardt, 1994; this study) and abiotic factors (Cocroft and Ryan, 1995; Howard and Young, 1998; Oseen and Wassersug, 2002). Further studies on female preferences are needed to better comprehend sexual selection mechanisms in this species.

Bokermannohyla ibitiguara males commonly emit a mixed call, and our results indicate that one part of the call may be directed to females and another part directed to males. The advantage of mixed calls is that they share the functions of female attraction and male aggressiveness, lowering energetic costs related to aggressive calls' emission (Wells, 1988, 2007). These calls may be less attractive to females (Wells, 1988, 2007), but the aggressive part is important, because male territoriality in frogs is often related to higher reproductive success (Wells, 1977b; Ursprung et al., 2011). In *B. ibitiguara*, long notes might constitute the advertisement part, as we observed, on two occasions, males emitting mostly these notes with a female nearby; however, playback experiments with females are needed to confirm this hypothesis. Short notes are most certainly directed toward males, and they can be grouped into two types: one emitted under natural conditions and the other during the intruder simulation (= playback), the latter of which was also sporadically heard under natural conditions. These calls are emitted in a similar pattern, because the number of short notes per sequence did not differ between them. However, they exhibited important differences, because the call emitted when an intruder was simulated had a lower dominant frequency, was longer and included groups of structured pulses with variable

TABLE 2. Mean ± SD of acoustic parameters of the short notes recorded before and during the playback experiments. The results for the *t*-test for dependent samples are shown ($N = 8$ males, 10 notes before and 10 notes during playbacks). Range in parentheses.

| Parameter	Before playback	During playback	*t*-test
Dominant frequency (Hz)	2,026.3 ± 127.9 (1,877.7–2,239.5)	1,953.1 ± 115.0 (1,826.0–2,187.8)	$t = 6.06$; $P < 0.001$
N notes per sequence	5.1 ± 0.9 (4.0–6.8)	4.5 ± 0.9 (3.2–6.1)	$t = 1.42$; $P = 0.20$
Note duration (s)	0.053 ± 0.008 (0.042–0.065)	0.077 ± 0.014 (0.059–1.000)	$t = -5.79$; $P < 0.001$
Pulse structure	One group of short pulses	Groups of pulses and short pulses	–
Call type	Territorial component of mixed call	Aggressive call	–
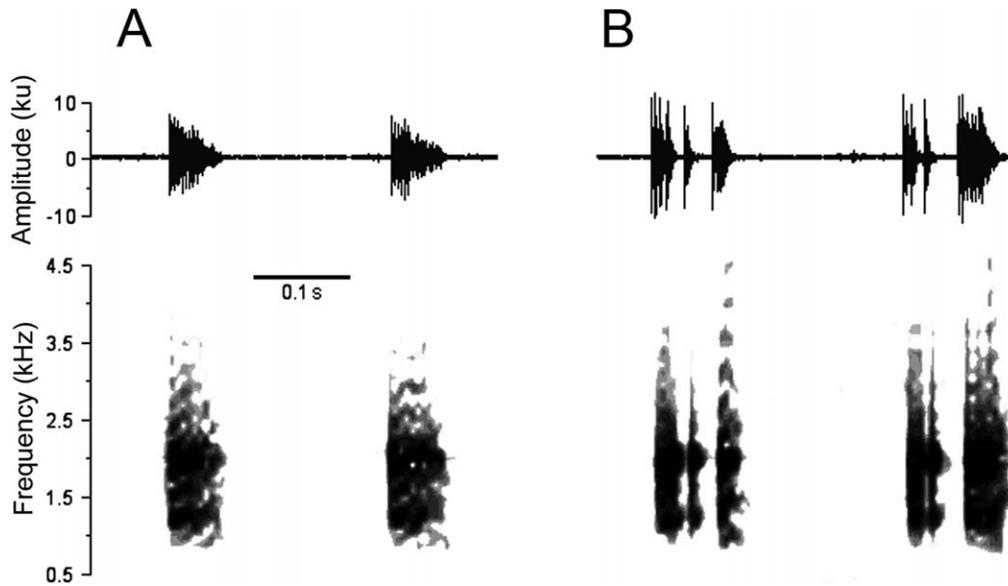
FIG. 5. (A) Two short notes commonly emitted by *Bokermannohyla ibitiguara* males under natural conditions and (B) two short aggressive notes emitted during playback. Oscillogram above and spectrogram below. In B, notes appear to be more intense and composed of separate groups of pulses with variable intervals, whereas in A, notes are composed of one group of short pulses.

intervals. Based on these results, we propose that the short notes of the mixed advertisement call have a territorial function, whereas the aggressive call itself, emitted when there is an intruder near the resident male, is a variation of the common short notes (Table 2).

If the short notes of the mixed advertisement call indeed have a territorial function, why did our playback experiments not confirm such an assumption? Males did not reduce the emission of long notes, except for the T3 treatment, and did not increase the emission of short aggressive notes during the playbacks. One possible explanation for this is that the aggressiveness of *B. ibitiguara* call is related not to the number of short notes emitted (e.g., Giasson and Haddad, 2006), but rather to the modulation of some parameters of the original short notes, as observed here in the short notes emitted in response to playbacks (aggressive call). During playbacks, short notes became longer with a lower dominant frequency and exhibited more pulses and short pulses with variable intervals, a change very similar to that observed in the African Reed Frog, *Hyperolius marmoratus* (Grafe, 1995). In *Pseudacris crucifer*, a North American hylid, males increased the duration of aggressive calls and the number of aggressive calls in response to increased synthetic call intensity (Schwartz, 1989). Although the number of short notes in *B. ibitiguara* remained the same, aggressive short notes were longer, showing an increased energy investment of the male. Furthermore, the dominant frequency of the short notes emitted during the playbacks was significantly lower than that of the normal short notes. It is known that males may lower the dominant frequency of calls when interacting vocally with nearby males (Howard and Young, 1998; Bee et al., 2000), and specifically, when emitting aggressive calls (Reichert and Gerhardt, 2013), most likely to increase their apparent body size and fighting ability, thereby avoiding fights (see below). Finally, the groups of pulses with variable intervals in the aggressive call were audibly more aggressive than the single group of short pulses from the original short notes of the mixed call. Thus, *B. ibitiguara* might show escalated aggressive calls (*sensu* Wells,

1988; Martins et al., 1998), in which males may vary the aggressive and advertisement elements as rival males gradually approach. In this case, males can vary the aggressive element in terms of the dominant frequency, duration, and pulse structure of the short notes when there is an intruder nearby.

Males of *B. ibitiguara* emitted the same number of short notes regardless of the distance of the nearest neighbor and the number of males attending the chorus (i.e., short notes are always present). These short notes were mostly the common short notes of the mixed call. Males of *B. ibitiguara* tend to maintain some space among themselves along streams, establishing territories (Nali and Prado, 2012). Because fights are costly (Robertson, 1986; Martins and Haddad, 1988), male frogs strongly avoid them, and disputes are generally solved by means of visual or acoustic signals (Robertson, 1986; Martins and Haddad, 1988; Giasson and Haddad, 2006), during which males evaluate each other's ability to fight (Parker, 1974). This appears to be true for *B. ibitiguara*, because we observed a male–male fight only once during all fieldwork (Nali and Prado, 2012) when the satellite male was especially persistent (Nali and Prado, 2012). Thus, the territorial part of the call (short notes) might be the common territorial signal for this species, functioning as a constant aggressive signal.

Because males of *B. ibitiguara* tend to avoid fights, their capacity to lower the dominant frequency of aggressive calls in the presence of an intruder can be explained by the "bluff of size" hypothesis (Wagner, 1992), where the signal sent to the opponent is deceptive, such that males appear to be larger than they actually are. The assessment of the opponent's traits related to fighting ability, such as body size, is an important part of the decision of whether to engage in a fight, which fits the concept of game theory (Myerson, 1991), a widespread concept used to explain conflicts between individuals. Additionally, the strategy of engaging in fights only as the last resort can also be seen as an evolutionarily stable strategy (ESS; Maynard-Smith and Price, 1973) because *B. ibitiguara* males tend to exhibit this sequence of

behaviors to prevent any unnecessary injuries, which might be adaptive. If all males are capable of behaving this way, it is probable that an alternative strategy will not evolve, as assumed by the ESS theory.

We expected that smaller males would be more inhibited by the playback than larger males, because 1) those are generally less likely to win male combats (Wells, 1978; Katsikaros and Shine, 1997) and 2) the winner of the fight observed for *B. ibitiguara* was indeed heavier and larger (Nali and Prado, 2012). However, we did not find a difference in size between responding and nonresponding males, except for body weight when analyzing T1 independently. Among the responding males, even the smaller ones lowered the dominant frequency of their short notes, considered here as aggressive calls, increasing their apparent fighting ability and attempting to defend their territories without actually fighting. The lack of response to playbacks by some males could be explained by male condition, degree of male activity, social context, or even by the presence of the observer and artificial reproduction of the calls during the playbacks. Moreover, a visual component is known to mediate important aggressive responses in male frogs (Giasson and Haddad, 2006; Luna et al., 2010), which was not considered here.

Our study indicated that *B. ibitiguara* vocal repertoire is much more complex than recognized previously and that further studies on calling behavior, call properties, and sexual selection are needed. Our results on the species' vocal behavior will inform future studies on male–male social interactions and the mechanisms of mate selection in this hylid frog endemic to the biodiversity hotspot (Myers, 2003) of the Brazilian Cerrado.

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