# Intraspecific call variation in a Neotropical gladiator frog with a complex advertisement call

Rubens A.F. Turin[1], Renato C. Nali[2,*], Cynthia P.A. Prado[1]

**Abstract.** Vocalisation is one of the most conspicuous behavioural traits in different animals, but its emission can be variable within species, depending on environmental, morphological, and/or social factors. Understanding how acoustic parameters vary can provide information about sexual selection mechanisms that might have shaped the species' evolutionary history. We quantified and compared the variation of five call parameters within and among the males of *Bokermannohyla ibitiguara*, a Neotropical treefrog with complex reproduction and advertisement calls. The parameters were classified as static (dominant frequency), intermediate (note durations) or dynamic (pulse rate of the long note and call duration). Despite these differences, all of them varied more among individuals than within individuals, and most were associated with individual discrimination, showing potential acoustic recognition by males and females. A multiple regression analysis showed that all temporal parameters were affected by abiotic factors, except pulse rate of the long note; body size affected dominant frequency, duration of the long notes and call duration. This high variability in parameters, and the strong support for individual discrimination, indicate the existence of sexual selection mechanisms operating on calls; however, the individual recognition system is very complex and not limited to a single characteristic of the call. This is supported by the reproductive behavior of this species, including choosy females, elaborate courtships, and male-male vocal contests and physical combats. Fine-scale playback experiments with males and females will help us further understand sexual selection mechanisms in this and other acoustically oriented animals.

*Keywords*: amphibia, behavioural ecology, bioacoustics, cerrado, individual discrimination, sexual selection.

## Introduction

Vocalisation is an important behavioural characteristic of different animal groups including birds, mammals, insects, fishes and amphibians (Bradbury and Vehrencamp, 2011). In anuran amphibians, vocalisations are emitted only by males in most species, and are the major signal responsible for female attraction and territory maintenance (Gerhardt, 1994; Schwartz and Wells, 2007; Wells, 2007). Each anuran species has a vocal repertoire, with males emitting different types of calls depending on the social context (Wells, 1977; Wagner, 1989; Toledo et al., 2015). Several types of calls are known, such as the advertisement, territorial, fighting,

courtship, release, distress and amplectant call (Ryan, 2009; Toledo et al., 2015). Among these, the advertisement call is the most commonly emitted and, therefore, the most studied one (Gerhardt, 1994; Toledo et al., 2015). The study of variation in the acoustic properties of advertisement calls aids in understanding the evolutionary processes of sexual selection in anuran species (Howard and Young, 1998; Bee, Suyesh and Biju, 2013) and has been studied at two levels: within and among males (Gerhardt, 1991; Howard and Young, 1998). Based on within-male variation, call parameters that are more constant are called static, whereas dynamic parameters are more variable; in addition, intermediate parameters exist in between (Gerhardt, 1991). Studies show that quantitative data on acoustic parameters allow for discrimination and recognition among males and/or females from the same species (Bee et al., 2001; Morais et al., 2012; Pettit, Bourne and Bee, 2013; Gambale, Signorelli and Bastos, 2014).

1 - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Departamento de Morfologia e Fisiologia Animal, 14884-900 Jaboticabal, São Paulo, Brasil
2 - Universidade de São Paulo, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, 14040-901 Ribeirão Preto, São Paulo, Brasil
\*Corresponding author; e-mail: r_nali@yahoo.com.br

Vocalisations may represent an opportunity for indirect selection over other male characteristics because the acoustic parameters can be influenced by the individual's morphology (Gerhardt, 1994; McClelland, Wilczynski and Ryan, 1996). For example, the dominant frequency of the call is usually negatively correlated with male body size (Howard and Young, 1998; Morais et al., 2012, Nali and Prado, 2014a). Therefore, males can announce their size through call parameters to females and other males as part of sexual selection mechanisms (Howard and Young, 1998; Wells, 2007). Larger males can exhibit defence skills and maintain oviposition sites, becoming attractive to females and repelling intruders (Wells, 2007; Nali and Prado, 2012). However, call parameters must vary enough among different individuals at a breeding site to allow selection. Therefore, the relationship between the within and among-male variation of each parameter can uncover possible selection mechanisms for a given species (Gambale, Signorelli and Bastos, 2014).

Our focal species is a hylid from the genus *Bokermannohyla*, which includes 32 species distributed in forests or open areas throughout Brazil, except for the north region (Faivovich et al., 2005; Frost, 2017). *Bokermannohyla ibitiguara* is an endemic species of the Cerrado's phytogeographical formation, found in the Serra da Canastra, southeastern Brazil (Cardoso, 1983; Nali and Prado, 2012). This species is currently listed in the IUCN Red List of Threatened Species as Data Deficient (Caramaschi and Eterovick, 2004). Males vocalise during several months along streams associated with gallery forests and bushy vegetation (Nali and Prado, 2012). Advertisement calls are complex, consisting of short notes (territorial component) and long notes (advertisement component; Cardoso, 1983; Carvalho, Giaretta and Magrini, 2012; Nali and Prado, 2014a). Fight calls are emitted during physical combats (Nali and Prado, 2014b), and males can alter some acoustic parameters during aggressive

interactions (aggressive call; Nali and Prado, 2014a). Although acoustic parameters are apparently variable among males (Nali and Prado, 2014a), variations have neither been systematically quantified nor analysed in terms of their potential to contribute to individual identity. Thus, here we analysed the within and among-male variation of the acoustic parameters of the advertisement call in *B. ibitiguara* males and investigated the influence of male body size and abiotic factors on the call parameters. Given that acoustic parameters evolve under natural and sexual selection forces (e.g. environment characteristics, male-male interactions, and female choice of mates), we tested the following hypotheses: (i) that acoustic parameters vary within and among males, (ii) that the among-male variation in the acoustic parameters is higher than the within-male variation, and (iii) that some acoustic parameters could potentially be used to discriminate individuals in the breeding chorus.

## Materials and methods

The study was conducted at the Serra da Canastra National Park (SCNP), municipality of São Roque de Minas, southeastern Brazil. Climate of the region is seasonal, characterized by a rainy season during summer and a dry season during winter (Junior et al., 2010). Field work was conducted in five streams (coordinates: 20.2434°S, 46.4466°W; 20.2540°S, 46.4203°W; 20.2507°S, 46.4357°W; 20.2404°S, 46.5868°W; 20.2273°S, 46.6164°W); they are 1.3-20 km apart from each other in a straight line. Field trips occurred in January and February of 2013, totalizing 11 nightly surveys conducted approximately between 18:00 h and 0:00 h. Males of *B. ibitiguara* were found by active search at breeding sites.

Advertisement calls of each individual were recorded for at least 1 min and at a distance of approximately 1 m from males, using a digital recorder (Marantz Professional PMD-660) and a unidirectional microphone (Sennheiser ME66) at 16-bits resolution and 44 100 Hz. After each recording, air temperature was measured with an analogical thermometer (to the nearest 1°C) and the relative air humidity (%) was measured with a mini meteorological station (ADC Pro). We then measured the snout-vent length (SVL) and body mass of recorded males, using a Mitutoyo analogical calliper to the nearest 0.05 mm and Pesola dynamometers of 10 g or 20 g, to the nearest 0.1 or 0.2 g, respectively. Seven males were released back into their habitat after being marked by toe clipping (Waichman, 1992). Others

($n = 32$) were collected and euthanized with a spray of 10% lidocaine in the ventral region, fixed in 10% formalin and preserved in 70% alcohol (McDiarmid, 1994), as part of another ongoing research that required the collection of specimens (Nali, 2016). The collected specimens were deposited at the Coleção de Anfíbios Célio F. B. Haddad (CFBH), Universidade Estadual Paulista, Rio Claro, São Paulo state, Brazil (online supplementary table S1).

Acoustic analyses of 191 calls of the 39 males (1-10 calls for each individual) were performed using the Raven Pro 1.4 software (Cornell Lab of Ornithology, Ithaca, NY; FFT = 1024 resolution points, contrast = 80, and brightness = 75). The following call parameters were measured (e.g. Nali and Prado, 2014a): (1) call dominant frequency (Hz), (2) duration of the long note (s), (3) duration of the short notes' sequence (s), (4) pulse rate of the long note ($n$ pulses/0.5 s) and (5) call duration (s). The parameters chosen above are frequently under sexual selection pressure (Gerhardt, 1994) and potentially determine the reproductive success of males (Howard and Young, 1998). The dominant frequency was determined for the call as a whole, selecting the sequence of long and short notes, because there is no statistical difference between the dominant frequency of short and long notes in the species' advertisement call (Nali and Prado, 2014a). The duration of the short notes' sequence was measured from the start of the first short note to the end of the total sequence of short notes, and the duration of the call was calculated based on the total measurement of each advertisement call (sequence of long notes + interval between notes + sequence of short notes). The pulse rate was counted within 0.5 s starting from the onset of each long note, given that the latter pulses of the long notes are very short and difficult to measure, which could cause significant errors, as previously observed for this species (Nali and Prado, 2014a). The bioacoustics terminology followed Martins and Jim (2003) and Toledo et al. (2015).

To quantify the variability in the calls, the coefficient of variation (CV) of acoustic parameters was calculated as the ratio of the standard deviation over the mean, expressed in percentage (CV = SD/mean × 100). To obtain the within-male CV (CVw), the mean and standard deviation of each acoustic parameter were used for each male. The mean CVw was used as a representative measure of the population. The among-male CV (CVa) was calculated based on a general mean and standard deviation of the values of the individual parameters, obtaining a CVa value for each parameter (Pettit, Bourne and Bee, 2013). With respect to CVw, parameters with values up to 5% were classified as static, because of low variation, parameters with values between 5% and 12% were classified as intermediate and parameters with values above 12% were classified as dynamic (Gerhardt, 1991). Magnitude of the variations was calculated using the CVa/CVw ratio for each parameter and non-parametric Kruskal-Wallis was used to evaluate if the among-male variation was higher than the within-male variation (Morais et al., 2012; Gambale, Signarelli and Bastos, 2014).

For the following parametric analyses, three call parameters were transformed to achieve normality (dominant frequency: squared; duration of the long notes: multiplied by 10 and log-transformed; call duration: log-transformed), according to previous Shapiro-Wilk results and skewness and kurtosis values that evidenced non-normal distributions (Ghasemi and Zahediasl, 2012). A multiple linear regression analysis was used with the transformed variables to evaluate the influence of the abiotic variables (air temperature and relative air humidity) and body size (product of body mass and SVL) on the acoustic parameters of the advertisement calls of *B. ibitiguara* (Zar, 2010).

We used the regression coefficients from this analysis to remove the effects of air temperature and relative air humidity on the transformed parameters (e.g. Tessarolo et al., 2006; Kaefer and Lima, 2012; Guerra et al., 2017), but without correcting for the effects of SVL (e.g. Bee et al., 2010). Then, two multivariate analyses were performed to determine whether the acoustic parameters could be potentially used to discriminate among individuals (e.g. Gambale, Signarelli and Bastos, 2014). First, we used the mean values of the five acoustic parameters as variables for a principal component analysis (PCA), using 107 calls of 22 individuals (five calls for 21 individuals and two calls for one individual). We then included the scores generated to each factor as independent variables for a discriminant function analysis (DFA), removing possible autocorrelations among the variables. The classification success of the DFA was measured using the cross-validation 'leave-one-out' method, in which each advertisement call is attributed to a particular individual based on the discriminant function generated by the set of data containing $n-1$ calls (Pettit, Bourne and Bee, 2013; Gambale, Signarelli and Bastos, 2014); additionally, we calculated the Cohen's Kappa statistics (Cohen, 1960; McHugh, 2012).

The analyses were performed using the Statistica v. 10 software (StatSoft, 2011) and R platform version 3.3.3 (R Development Core Team, 2017), with signifcant values at $P \leqslant 0.05$.

## Results

We found adult males of *B. ibitiguara* mainly in the gallery forests along streams, vocalising on shrubs and trees (mean air temperature = 18.45°C, SD = 1.38, range = 16-22°C, $n = 34$; mean relative air humidity = 76.95%, SD = 5.93, range = 67.4-87.9%, $n = 37$). Mean SVL of males was 39.4 mm (SD = 4.24; range = 33.3-49.2 mm; $n = 39$) and mean body mass was 5.1 g (SD = 1.74; range = 2.7-11.5 g; $n = 39$). From 191 advertisement calls of 39 males registered at different sites, we analysed a total of 404 long notes and 187 short notes' sequences (fig. 1). The calls had a mean of four long notes (range = 1-14 notes), followed by a sequence of short notes with a mean duration of 1.9 s (range = 1.27-2.84 s). The average number
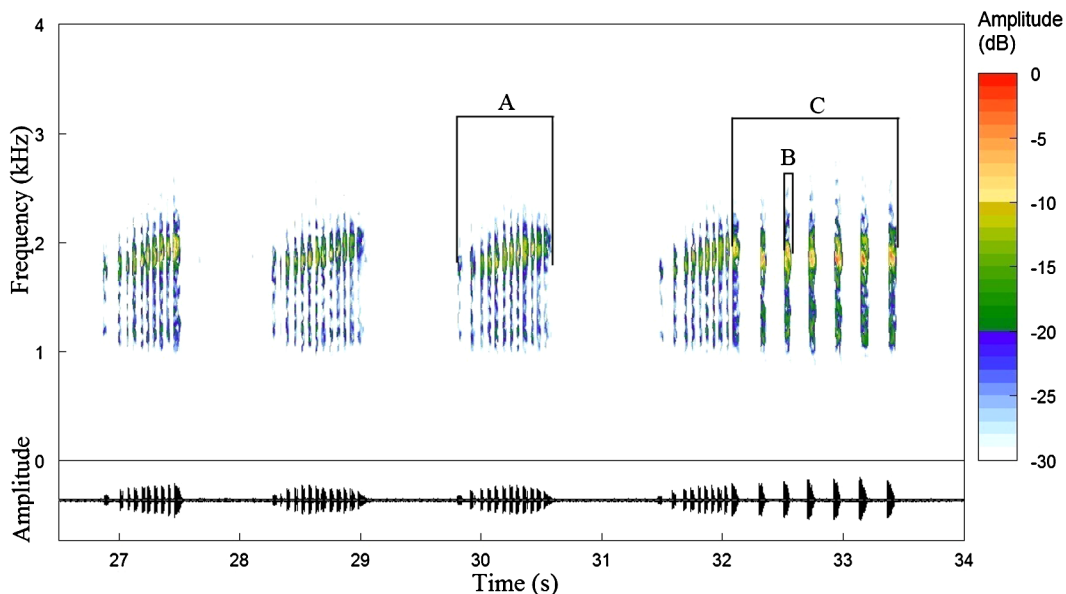
**Figure 1.** Spectrogram (above) and waveform (below) of the complex advertisement call of *Bokermannohyla ibitiguara* in the Serra da Canastra National Park, state of Minas Gerais, southeastern Brazil, composed by four long notes followed by a sequence of seven short notes (A = long note; B = short note; C = short notes' sequence). Recorded on 24 January 2013 at 21:00h, air temperature = 20°C, water temperature = 21°C, relative air humidity = 74.2%.

**Table 1.** Descriptive values of advertisement call parameters of *Bokermannohyla ibitiguara* males, coefficients of variation within (CVw) and among individuals (CVa), CVa/CVw ratio and statistical comparison between CVw and CVa (Kruskal-Wallis *H*). We also include a classification of each parameter according to the CVw (see text for details).

| Call parameters | Mean ± SD (range) | CVw (%) (range) | Classification | CVa (%) | CVa/CVw | *H* | *P* | *N* |
|---|---|---|---|---|---|---|---|---|
| Dominant frequency (Hz) | 1740.9 ± 233.5 (918-2246.65) | 3.65 (0-34.4) | static | 14.07 | 3.85 | 163.727 | <0.001 | 191 |
| Duration of the long note (s) | 0.964 ± 0.19 (0.595-1.328) | 10.55 (4.3-25.15) | intermediate | 21.57 | 2.04 | 240.357 | <0.001 | 404 |
| Duration of the short notes' sequence (s) | 1.90 ± 0.38 (1.27-2.84) | 11.11 (0-31.8) | intermediate | 23.33 | 2.10 | 169.231 | <0.001 | 187 |
| Pulse rate of the long note (*n* pulses/0.5 s) | 25 ± 5.05 (17-37) | 15.37 (4.72-43.27) | dynamic | 24.75 | 1.61 | 207.84 | <0.001 | 318 |
| Call duration (s) | 14.3 ± 8.3 (4-45) | 56.67 (14-132) | dynamic | 82.78 | 1.46 | 86.304 | <0.001 | 191 |

of short notes per sequence in this species is 5.5 (range = 4-8 notes; Nali and Prado, 2014a).

Means and coefficients of variation (CV) of each parameter are presented in table 1. The dominant frequency of the calls showed the lowest CVa (among individuals) and CVw (within individual), while call duration presented the highest coefficients. Among-male variability was higher than within-male variability for all parameters (CVa/CVw > 1), with call dominant

frequency presenting the highest value and call duration presenting the lowest value (table 1). This difference was statistically significant for all parameters (table 1).

The PCA analysis generated five factors extracted from the 107 calls of the five acoustic parameters (table 2). Resulting scores from the first three factors of the PCA analysis were used as entry values for the DFA, which demonstrated statistical significance, i.e., calls can be

**Table 2.** Results of the Principal Component Analysis (PCA) on the advertisement call parameters of *Bokermannohyla ibitiguara*, state of Minas Gerais, Brazil. Absolute values higher than 0.7 are shown in bold (see Cangelosi and Goriely, 2007).

| Call parameters | PCA Factors | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Dominant frequency | 0.308 | **0.898** | −0.308 | 0.048 | 0.008 |
| Duration of the long note | **−0.741** | 0.233 | 0.208 | −0.592 | −0.000 |
| Duration of the short notes' sequence | **−0.925** | −0.079 | −0.332 | 0.132 | 0.095 |
| Pulse rate of the long note | −0.578 | 0.301 | 0.673 | 0.347 | 0.000 |
| Call duration | **−0.916** | 0.003 | −0.362 | 0.143 | −0.093 |
| Eigenvalue | 2.676 | 0.959 | 0.833 | 0.512 | 0.018 |
| Variance (%) | 53.5 | 19.1 | 16.6 | 10.2 | 0.36 |
| Cumulative variance (%) | 53.5 | 72.7 | 89.3 | 99.6 | 100 |

**Table 3.** Standardized coefficients of the Discriminant Function Analysis (DFA) showing the relative importance of each PCA factor score in the composition of five roots of the discriminant functions in the advertisement calls of *Bokermannohyla ibitiguara*, state of Minas Gerais, Brazil. Absolute values higher than 0.7 are shown in bold (see Cangelosi and Goriely, 2007).

| PCA factor | Roots of the discriminant function | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Factor 1 | **1.167** | 0.454 | 0.113 |
| Factor 2 | 0.638 | **−1.113** | 0.316 |
| Factor 3 | **1.265** | −0.625 | −0.699 |
| Eigenvalue | 50.93 | 22.31 | 3.87 |
| Cumulative proportion (%) | 66 | 94 | 100 |

discriminated (Wilks' $\lambda < 0.001$; $F_{63,248} = 68.4$; $P < 0.001$). The DFA results were in agreement with the cross-validation 'leave-one-out' method, which demonstrated that 87.8% of the advertisement calls were correctly classified as belonging to the males that emitted them ($n = 22$ males), with Cohen's Kappa = 0.872, i.e., almost perfect agreement (1/22 or 4.5%; 95% confidence interval = 0.729-1.015). The first and second roots of the DFA explained together 94% of cumulative variance and correlated with the PCA factors 1, 2 and 3 (table 3). Of these, only the first and second factors presented values equal or higher than 0.7 and together explained 72.7% of the cumulative variance. This indicates that duration of the long notes, duration of the short notes' sequence, call duration and dominant frequency have potential to discriminate individuals, in order of importance.

The multiple regression analysis showed that the spectral parameter dominant frequency correlated negatively with body size of males, while temporal parameters duration of long notes and call duration correlated positively (table 4). This analysis also showed that abiotic variables affected temporal call parameters: duration of the long notes, duration of the short notes' sequence and call duration correlated negatively with air temperature; call duration correlated negatively with relative air humidity, and duration of the short notes' sequence showed the same tendency, but marginally non-significant (table 4).

## Discussion

Our results support the hypothesis that call parameters of *Bokermannohyla ibitiguara* vary within and among males, being classified as static, intermediate and dynamic (table 1). The among-male variation was higher than within-male variation for all parameters, indicating a possible source of selection for females in all of them and supporting our second hypothesis (Murphy and Gerhardt, 2002). Furthermore, most parameters analysed here were important for the individual discrimination of males, supporting our third hypothesis.

Previous studies on the variation of frog calls found higher among-male variation of acoustic parameters (Howard and Young, 1998; Bee et al., 2010; Morais et al., 2012; Pettit, Bourne and Bee, 2013; Gambale, Signorelli and Bastos, 2014; Guerra et al., 2017). The dominant frequency was the only one classified as static (lower within-male variation), as commonly

**Table 4.** Results of the multiple regression analysis between acoustic parameters of *Bokermannohyla ibitiguara* vs. abiotic factors and body size (mass × SVL), state of Minas Gerais, Brazil. Adjusted coefficient of determination ($R_a^2$), analysis of variance (ANOVA), observations (*n*), and partial regression coefficient (*b*) are indicated. Significant values are in bold ($P \leqslant 0.05$).

| Acoustic Parameters | n | Partial regression coefficient (*b*) | | | $R_a^2$ | ANOVA | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | Body size | Air temperature | Relative air humidity | | F | p |
| Dominant frequency | 34 | **−0.698810** | 0.043285 | 0.110592 | 0.4523 | 10.086 | <0.001 |
| Duration of the long note | 32 | **0.431736** | **−0.424485** | 0.071980 | 0.3033 | 5.499 | <0.05 |
| Duration of the short notes' sequence | 31 | 0.246806 | **−0.406661** | −0.362460 | 0.1578 | 2.874 | =0.054 |
| Pulse rate of the long note | 33 | 0.044247 | −0.184126 | −0.283095 | −0.0139 | 0.853 | =0.476 |
| Call duration | 32 | **0.456093** | **−0.389349** | **−0.349837** | 0.3156 | 5.766 | <0.05 |

observed in anurans (Gerhardt, 1991, 1994; Morais et al., 2012). This parameter varies across species, being either under stabilizing selection by females (Gerhardt, 1991; Pettit, Bourne and Bee, 2013), or under directional selection (Wollerman, 1998; Yasumiba, Alford, and Schwarzkopf, 2015). Although it represents a good parameter for individual recognition, due to its high repeatability (varies greatly among and little within individuals; Howard and Young, 1998), its classification as static depends on the species, and it may vary between breeding seasons of the same species (Gambale, Signorelli and Bastos, 2014). Usually, this parameter is negatively correlated with male body size (Ryan, 1988b; Howard and Young, 1998; Gingras et al., 2013; but see Pettit, Bourne and Bee, 2013), including that of *B. ibitiguara* (Nali and Prado, 2014a; this study), justifying the classification as a static parameter because each individual has a fixed morphology.

When considering among-male variations, the dominant frequency presented the highest CVa/CVw ratio among the parameters analysed. This parameter's large ratio suggests a potential for discrimination among calling males at the breeding site (e.g. Bee et al., 2001; Bee and Gerhardt, 2001; Morais et al., 2012), presenting enough variation for selection to occur. The possibility of individual discrimination based on the dominant frequency, which is correlated with male body size, also suggests that *B. ibitiguara* females could potentially choose larger

males among those in the population (Wollerman, 1998; Nali and Prado, 2014a; this study). Larger males could have an advantage in territorial interactions and oviposition site defence, as shown previously for the species (Nali and Prado, 2012; Nali and Prado, 2014a). However, the fact that other parameters have the potential for individual discrimination suggests that each call parameter contributes to individual identity (Bee and Gerhardt, 2001) inside a communication system in which notes, composed by distinct acoustic variables, pass through a complex selection filter.

The duration of the long notes and of the short notes' sequences were intermediate, suggesting a constant selection involving the characteristics of the call as a whole, which consists of long and short notes (Rand and Ryan, 1981; Nali and Prado, 2014a). Such conditions are particularly interesting in species like *B. ibitiguara*, which presents a mixed call with different messages; a previous study with direct observations and playback experiments in the field has shown that short notes of *B. ibitiguara* are the territorial/aggressive component of the call (directed to males during reproduction), while long notes are most likely the advertisement component (directed to females; Nali and Prado, 2014a). Therefore, the greater the complexity of the species' calls, the greater the amount of information provided in inter- and intrasexual contexts (see Ryan, 1988a). The difference between the static and dynamic categories

is extreme (Gerhardt, 1991), such that the intermediate parameters of the call can also suffer selection if they approach a dynamic classification (Pettit, Bourne and Bee, 2013). Within-male coefficients of these parameters were indeed closer to the intermediate/dynamic threshold of 12% (table 1). At least for the duration of the long note (advertisement component), it can serve as an indicator of the male body size and be under selection by females, given the correlation between these variables (Nali and Prado, 2014a; table 4).

Call duration and pulse rate were parameters classified as dynamic. As a whole, the compound call of the species is extremely variable, in terms of number of long notes per call, number of short notes per sequence, their duration and intervals (Nali and Prado, 2014; this study). Temporal parameters in species with more pulsed calls are generally more variable (Gerhardt, 1991), which could explain the large within and among-male variations in pulse rate and call duration for *B. ibitiguara*. In our case, pulse rate of the long note was not affected by abiotic factors or morphology, and was not important for call discrimination, which confirms its high variability. Additionally, we found a positive correlation between call duration and male body size for *B. ibitiguara*. As the energetic cost also depends on the body size of the animal (Gerhardt, 1994), this result indicates that larger males may invest more energy in emitting longer calls. This could increase male reproductive success, because females can select males that vocalise with a higher intensity and complexity and at a higher emission rate (Ryan, 1988a). This happens mostly in territorial species and species with a prolonged breeding pattern, such as *B. ibitiguara* (Nali and Prado, 2014a). It is also notable that call duration was the most variable parameter measured here, showing the highest CVw (57%) and CVa (83%). The high variability for call duration likely occurs because this is a combined phenotype of the duration of short notes, long notes, and the intervals between them, each with its own variability.

Although acoustic communication is crucial in most frog mating systems, its attractiveness depends on more than one acoustic property and their interactions, and its relative importance compared to other communication systems varies among species (Hirschman and Hödl, 2006; Narins et al., 2006; Schaefer and Ruxton, 2015; de Sá, Zina and Haddad, 2016). With respect to *B. ibitiguara*, we showed that call variation was larger among-males than within-males, and such variability probably allows for call-mediated sexual selection in this species, as commonly found in other anurans (Ryan, 1980; Gerhardt, 1991; Murphy and Gerhardt, 2002). However, since four out of five parameters measured are potentially important in male discrimination, individual recognition in this species is likely very complex, consistent with an elaborated breeding behaviour mediated by strong sexual selection mechanisms (Nali and Prado, 2012, 2014a, b; Nali, 2016). Moreover, the recognition system might be conditioned to abiotic factors, in a complex interplay with morphological characteristics such as body size (table 4; Ziegler, Arim and Bozinovic, 2016). Beyond recognition of potentially discriminating parameters, playback experiments could clarify the role of each parameter in the individual recognition and the reproductive behaviour of the species; these could analyse vocal interactions of resident males vs. neighbours and outsiders (i.e. from distinct populations), as well as among males and females (e.g. Howard and Young, 1998; Gerhardt and Brooks, 2009), preferentially taking into account abiotic factors such as temperature (e.g. Martinez-Rivera and Gerhardt, 2008). In any case, using a focal species with complex calls and other social interactions, such as *B. ibitiguara*, allows inferences on sexual selection and mechanisms of call evolution that could apply to other animal groups.

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