

Acoustic communication in a Neotropical frog (*Dendropsophus minutus*): vocal repertoire, variability and individual discrimination

Alessandro R. Morais^{1,2}, Vinícius G. Batista^{2,3}, Priscilla G. Gambale^{2,3}, Luciana Signorelli^{1,2}
& Rogério P. Bastos²

¹Programa de Pós-Graduação em Ecologia & Evolução, Universidade Federal de Goiás, Brazil

²Laboratório de Herpetologia & Comportamento Animal, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Brazil

³Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais, Universidade Estadual de Maringá, Brazil

Acoustic signals are the most important communication mechanism for many anuran species. However, acoustic parameters can be influenced by several factors (e.g., body size, air temperature and social context). In the present study, we investigated the influence of the air temperature, morphological characteristics and distance between calling males on acoustic parameters of *Dendropsophus minutus*. In addition, we studied the entropy values of calls, acoustic variability (both within and among males) and the potential for individual discrimination. The observations were carried out between January 2009 and April 2011, in Piracanjuba, Goiás, central Brazil. Males of *D. minutus* emitted single and compound calls, composed by notes A, B and C. We observed that vocalizations may be influenced by snout-vent length, body mass, air temperature and distance between calling males ($P < 0.05$ in all cases). Dominant frequency was the only variable classified as a static property. There was higher variability among males than within males in all parameters measured. Individual males of *D. minutus* may be discriminated by their vocalizations, with dominant frequency being the main acoustic variable used in individual discrimination. We conclude that the high variation in *D. minutus* calls might be important during mate choice process.

Key words: Brazilian Savanna, dynamic property, entropy, individual recognition, static property, vocalizations

INTRODUCTION

Individuals of several anuran species can be commonly found aggregated during the reproductive season (Aichinger, 1987). In such situations, efficient communication among individuals is fundamental, with interactions in several contexts (Ryan, 2001). The most important way of communication in anurans is by means of calling (Wells, 1977; Haddad, 1995). Vocalizations are produced in several social contexts, such as mate attraction (Ryan, 1991), competition for calling site (Bastos & Haddad, 1995, 2002), or maintenance of intermale distance (Bastos & Haddad, 2002).

Acoustic signals are influenced by morphological traits and the environment (Duellman & Trueb, 1994; Howard & Young, 1998; Lingnau & Bastos, 2007). A negative relationship between the dominant frequency and snout-vent length or body mass of males (Ryan, 1988; Toledo & Haddad, 2005; Bastos et al., 2011), as well as a positive relationship between temporal call features (e.g., call duration, pulse number or repetition rate) of acoustic signals and air temperature is often found (Ryan, 1988; Bastos et al., 2003; Pröhl, 2003; Lingnau & Bastos, 2007). A male may also alter his acoustic behaviour in response to nearby conspecifics by increasing call intensity (Bastos et al., 2011), shifting from advertisement calls to aggressive calls (Wells, 1977), changing temporal call parameters such as repetition rate (Wells, 1988), decreasing pulse repetition rate (Wells & Schwartz, 1984) or changing call complexity (Wells, 1988).

In spite of these variations in call properties, basic components of vocalizations allow the recognition of conspecific individuals (Wells, 1988). Gerhardt (1991) classified calls properties into static and dynamic, with some parameters more stereotyped (low variability) than others. Some authors have investigated this variation in acoustic parameters (e.g., dominant frequency, call duration) and found higher variability among males than within males (Bee et al., 2001; Pröhl, 2003; Briggs, 2010), but few studies so far investigated these aspects in Neotropical anurans with complex vocal repertoires (Wells, 1989; Tárano, 2002; Giasson & Haddad, 2006).

Dendropsophus minutus (Peters, 1872) is a widely distributed hylid in South America (Frost, 2011), and typical of open areas. Bokermann (1967) studied the vocalizations emitted by an individual of *D. minutus* in Campos de Jordão, São Paulo state. Later, Cardoso & Haddad (1984) investigated the acoustic variability in populations of this species and found a complex vocal repertoire composed by three different notes (e.g., A, B and C). These notes have different functions used in specific social contexts. Consequently, males may emit these notes separately (e.g., single call) or combined (e.g., compound calls). Herein, we analyzed the vocal repertoire of *D. minutus* to answer the following questions: i) Do individual characteristics, air temperature, and distance between males influence acoustic parameters? ii) Is there more variation in acoustic traits among than within males? and iii) Do acoustic parameters have the potential to discriminate between individuals?

Correspondence: Alessandro R. Morais, Programa de Pós-Graduação em Ecologia & Evolução, Universidade Federal de Goiás, Caixa Postal 131, Goiânia, Goiás, Brazil; *E-mail:* alessandrogn@hotmail.com

MATERIALS AND METHODS

We studied individuals of *D. minutus* in a temporary pond (16° 58' 22" S, 48° 57' 10" W; 1000 m², c. 840 m a.s.l.) in Piracanjuba, Goiás, central Brazil. The main vegetation formation in the region is the Cerrado, a highly seasonal Neotropical savanna with two seasons: rainy (between October and March) and dry (between April and September). Observations were carried out between January 2009 and April 2011, during three breeding seasons, totaling 12 visits and 60 hours of observations. Fieldwork was conducted from 1900h until 0000h, the peak of activity of the species in site.

Calls of 56 males were recorded with a MARANTZ PMD 222 or PMD 660 recorder and a Sennheiser ME66 microphone at about 50 cm from the calling frog. We measured the sound level of five to seven calls of 26 males with a Minipa digital decibelimeter (Type II; Time weighting=Fast; A-weighted) at 50 cm distance. After each recording session, we measured snout-vent length (SVL) of males to the nearest 0.05 mm with a caliper and their mass to the nearest 0.01 g with digital scales. Air temperature was recorded with a digital thermohygrometer to the nearest 0.1°C and distance between calling male and nearby male was measured with a tape.

Vocalizations were digitized and edited at sampling frequencies of 22 kHz and 16 bit resolution (.wav files) with a Pentium PC computer. We analyzed the temporal and spectral parameters of calls with Avisoft-Sonography light[®] and Cool Edit 96[®] programs, respectively. Frequency information was obtained through Fast Fourier Transformation (FFT) of 1024 points width. For each individual, we analyzed a mean of five single calls and five compound calls. The following acoustic parameters were measured: call duration (s), number of notes (notes/calls), note duration (s), pulse duration (s), dominant and fundamental frequency (Hz), repetition rate (calls/min) and types of call. Bioacoustics terminology followed Gerhardt (1998), Gerhardt & Huber (2002) and Wells (2007). Voucher individuals are housed at the Coleção Zoológica of the Universidade Federal de Goiás (ZUFG 4706–13; ZUFG 6064–65; ZUFG 6068–73; ZUFG 6078–84).

We used information theory (Shannon & Weaver, 1949) to measure the entropy values (E) per recording and, consequently, to obtain the complexity of vocal behaviour of *D. minutus*. Similar to Da Silva & Vielliard (2006), we obtained the repertoire of each male and, posteriorly, considered the probability (p_i) of each note (e.g., A, B and C) as its relative frequency of occurrence in the recording. We followed Shannon & Weaver (1949) to calculate the individual information ($I_i = \log_2(1/p_i)$) of each note and considering all notes emitted, we calculated the entropy values (as below) for each individual male.

$$(E = \sum_{i=1}^N p_i \cdot I_i)$$

We tested the assumptions of all tests prior to analysis. We used an analysis of variance (ANOVA) to compare body mass and SVL of individuals among breeding seasons (Zar, 1996). We used simple regressions to test

the influence of air temperature, distance to the nearest conspecific male, SVL and body mass on acoustic parameters of calls (simple and composite calls) and entropy values (E).

We only considered the note A to assess the variability of calls, since it is the most common. We calculated the inter-individual mean and standard deviation for each call parameter using the mean of each male. Therefore, we calculated the variability of call parameters by determining the coefficient of variation within and among males. The within-male coefficients of variation were calculated based on means and standard deviations of a recording session for each male, while the among-males coefficients of variation were based on the grand mean and standard deviation. Acoustic parameters with within-male coefficients of variation below 5% were considered static and those above 12% were considered dynamic (Gerhardt, 1991). We calculated the ratio of among-males and within-male coefficients of variation (CV_a/CV_w) to determine the variability among-males (Márquez & Eekhout, 2006). Thus, an acoustic property was considered as having more variability among-males than within-males when $CV_a/CV_w > 1$. Complementary, we used a Kruskal-Wallis non-parametric test to test if acoustic properties varied more among than within males (Zar, 1996).

To investigate if acoustic parameters of *D. minutus* have the potential to discriminate individuals in a breeding site we used a discriminant function analysis (Manly, 2008). We considered acoustic traits (e.g., call duration, pulse number, pulse duration and dominant frequency) of single calls (only note A). We calculated the discriminant function analysis with residuals of a multiple regression to control for the effect of the air temperature and SVL on acoustic parameters (Martin, 1972; Duellman & Trueb, 1994). Individuals were separated across canonical discriminant functions representing linear combinations of the original variables. Therefore, the contributions of original variables to discriminant functions were described with canonical coefficients using the cross-validation method. We used a forward stepwise selection in which all variables were tested to determine which one contributed most to the discrimination between individuals. These statistical analyses follow Gerhardt (1991), Zar (1996) and Manly (2008), with significance level ≤ 0.05 .

RESULTS

Dendropsophus minutus used shrubs, herbs and grass as calling sites, with perch heights ranging between 0.2 and 0.5 m (0.285±0.09 m; $n=24$ males). The average distance between calling males was 1.55±0.789 m (0.025–3 m; $n=24$ males). SVL and body mass of males were 23.14±1.36 mm (20.64–26.2 mm; $n=56$ males) and 0.757±0.118 g (0.5–1.0 g; $n=56$ males), respectively. Both SVL ($F_{(2,53)}=1.14$; $P=0.327$) and body mass ($F_{(2,53)}=1.37$; $P=0.262$) did not vary among breeding seasons. The average air temperature was 20.71±1.53 °C (range=18.4–24.1 °C).

We recorded 1183 calls (single and compound calls) emitted by 56 males. SPL of 246 calls varied between 67.0

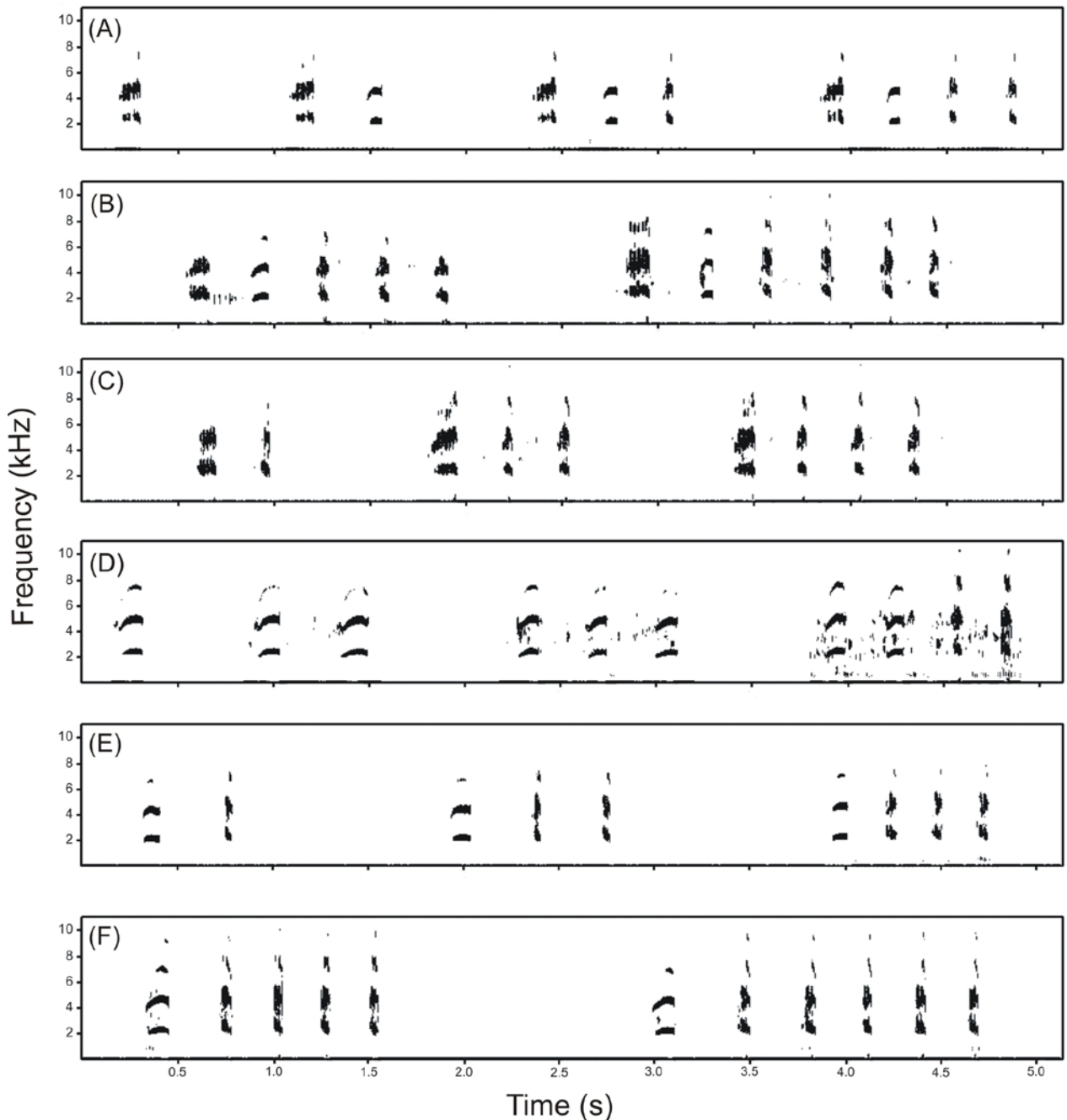


Fig. 1. Vocal repertoire of *Dendropsophus minutus*, Piracanjuba, central Brazil. A) Sonograms of A (19.7 °C), AB (19.7 °C), ABC (24.1 °C) and ABCC (24.1 °C) calls. B) Sonograms of ABCCC (19.7 °C) and ABCCCC (19.7 °C) calls. C) Sonograms of AC (22.73 °C), ACC (19.7 °C) and ACCC (19.7 °C) calls. D) Sonograms of B (23.4 °C), BB (23.4 °C), BBB (22.9 °C) and BBCC (20.9 °C) calls. E) Sonograms of BC (18.8 °C), BCC (18.8 °C), BCCC (20 °C) calls. F) Sonograms of BCCCC (20 °C) and BCCCCC (20 °C) calls.

and 95.4 dB ($X=80.48\pm 7.04$ dB). Vocalizations emitted were composed of three notes (A, B and C; isolated or combined). Each male emitted calls comprising of one to six notes (2.89 ± 1.49 notes/call; $n=56$ males). Sixteen distinct combinations of notes were emitted (Figs. 1 and 2).

Single calls

Only two out of 56 males were not recorded emitting single calls. Moreover, we did not record males emitting only note C, so we only obtained isolated notes A and B. Note A was the most common single call emitted,

representing about 77% ($n=911$ calls) of all recorded calls. This call has a pulsed structure and its mean duration was 0.143 ± 0.018 s (0.098–0.209 s; $n=261$ notes). The mean pulse number per note was 26.39 ± 3.65 (15.4–38 pulse per note; $n=261$ notes), with a duration ranging from 0.006 to 0.011 s (0.0079 ± 0.0013 s; $n=783$ pulses). The repetition rate of note A was 7.84 ± 4.6 notes per minute (0.5–26 notes/minutes; $n=54$ males), and the mean dominant frequency was 5001 ± 249.7 Hz (4425–5512 Hz; $n=261$ notes). The isolated note B has a harmonic structure and was emitted by only one male *D. minutus*. The highest amount of energy in this note is concentrated in the

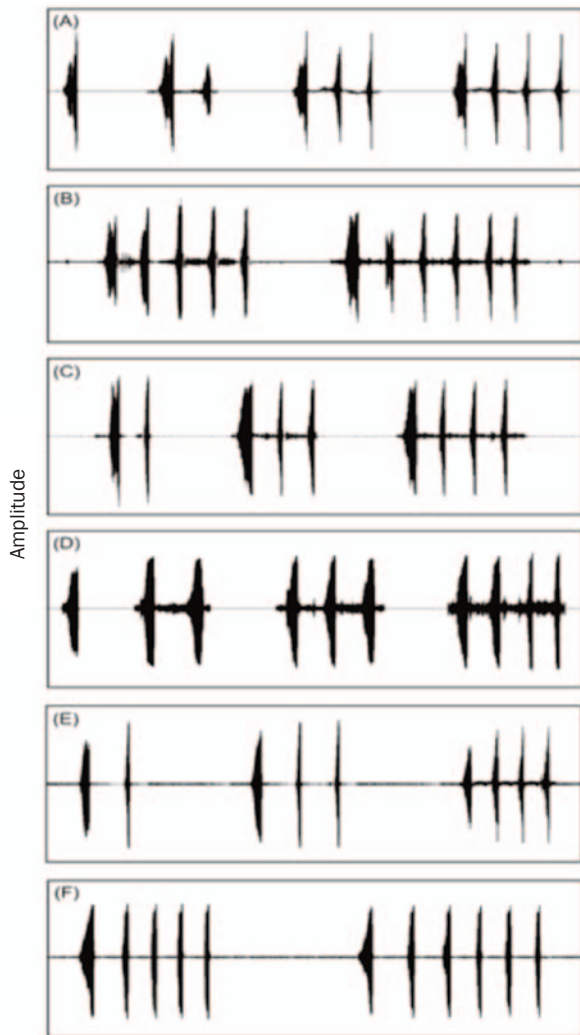


Fig. 2. Vocal repertoire of *Dendropsophus minutus*, Piracanjuba, central Brazil. A) Oscillogram of A (19.7 °C), AB (19.7 °C), ABC (24.1 °C) and ABCC (24.1 °C) calls. B) Oscillogram of ABCCC (19.7 °C) and ABCCCC (19.7 °C) calls. C) Oscillogram of AC (22.73 °C), ACC (19.7 °C) and ACCC (19.7 °C) calls. D) Oscillogram of B (23.4 °C), BB (23.4 °C), BBB (22.9 °C) and BBCC (20.9 °C) calls. E) Oscillogram of BC (18.8 °C), BCC (18.8 °C), BCCC (20 °C) calls. F) Oscillogram of BCCCC (20 °C) and BCCCCC (20 °C) calls. Oscillogram duration=5.1 s.

second harmonic. The mean dominant and fundamental frequencies were 4985.3 ± 40.7 Hz (4941–5021 Hz; $n=5$ notes) and 2496.7 ± 26.5 Hz (2470–2523 Hz; $n=5$ notes), respectively. The duration of this note varied from 0.121 to 0.157 s (0.137 ± 0.018 s; $n=5$ notes), while the repetition rate was 2.5 per minute ($n=1$ male).

Compound calls

Compound calls were composed by a combination of notes A, B and C (Table 1). Mean call duration was 0.871 ± 0.217 s (0.395–1.36 s; $n=185$ calls), with note numbers ranging between 2 and 6 per call (3.7 ± 1.35 note per call; $n=185$ calls). The dominant and fundamental frequencies were 4917.3 ± 213.9 Hz (4467.6–5260 Hz; $n=185$ calls) and 2436.6 ± 109.3 Hz (2217–2663 Hz; $n=185$ calls), respectively (Table 1). The repetition rate

of compound calls varied between one and six calls per minute. The average duration of note A (0.139 ± 0.017 s; range=0.123–0.197 s; $n=161$ notes) was higher than notes B (0.088 ± 0.0155 s; range=0.062–0.13 s; $n=189$ notes) and C (0.060 ± 0.0119 s; range=0.04–0.096 s; $n=268$ notes). Mean pulse number (26.55 ± 3.38 pulse/note; range=19–39 pulse/note; $n=141$ notes) and mean pulse duration (0.008 ± 0.001 s; range=0.005–0.014 s; $n=141$ notes) of note A were higher than pulse number (10.33 ± 2.44 pulse/note; range=5–15 pulse/note; $n=140$ notes) and pulse duration (0.007 ± 0.001 s; range=0.004–0.011 s; $n=140$ notes) of note C.

Dominant frequency was negatively related to body mass ($r^2=0.323$; $P<0.001$) and SVL ($r^2=0.299$; $P<0.001$; Fig. 3) in single calls (isolated note A). On the other hand, only SVL was positively influenced by repetition rate ($r^2=0.09$; $P<0.05$). Call duration was negatively influenced by air temperature ($r^2=0.09$; $P<0.05$). Distance between calling males and nearby males influenced negatively the repetition rate ($r^2=0.335$; $P<0.05$) of single calls. We found that both SVL ($r^2=0.175$; $P<0.01$) and body mass ($r^2=0.267$; $P<0.001$) were negatively correlated with the dominant frequency of compound calls (Fig. 3). We did not observe any relationship between other acoustic parameters and air temperature, morphological characteristics and distance between males ($P>0.05$ for all cases).

Mean entropy values per recording varied from 0.322 to 1.67 (0.946 ± 0.576 ; $n=56$ males), while mean total note number per recording varied between 13 and 64 notes (35.93 ± 12.16 ; $n=56$ males). We did not observe any relationship between entropy values and total note number per recording, air temperature, body mass, SVL and distance between calling males and nearby males ($P>0.05$ for all cases).

Only dominant frequency was a static property, since the mean within-male coefficient of variation was less than 5% (Table 2). The mean within-male coefficient of variation of call duration, pulse number and pulse duration were classified as intermediary properties (CV 5–12%; Table 2). The dominant frequency had a low among-male coefficient of variation ($CV=4.99\%$). Call duration and pulse number had also an intermediate among-male coefficient of variation (12.98% and 13.97%, respectively). Only pulse duration was a dynamic property, since the among-male coefficient of variation was higher than 30%. In all cases, the ratio CV_d/CV_w was higher than 1.0. We observed more variation between males than within males (Table 2; $P<0.05$) for all variables.

We conducted the discriminant analysis with only four acoustic variables (call duration, pulse number, pulse duration and dominant frequency) that generated three functions (Table 3). All functions had eigenvalues above 1.0, explaining 100% of the total variation among males. DF 1 (Wilks' Lambda=0.01; $F_{(32,132)}=37.32$; $P<0.001$) explained 55.45% of the variation and was largely represented by dominant frequency. DF 2 (Wilks' Lambda=0.013; $F_{(64,262)}=31.62$; $P<0.001$) explained 32.48% of the variation and was negatively related with pulse number per call. DF 3 (Wilks' Lambda=0.003; $F_{(96,390)}=23.69$; $P<0.001$) explained only 12.07% of

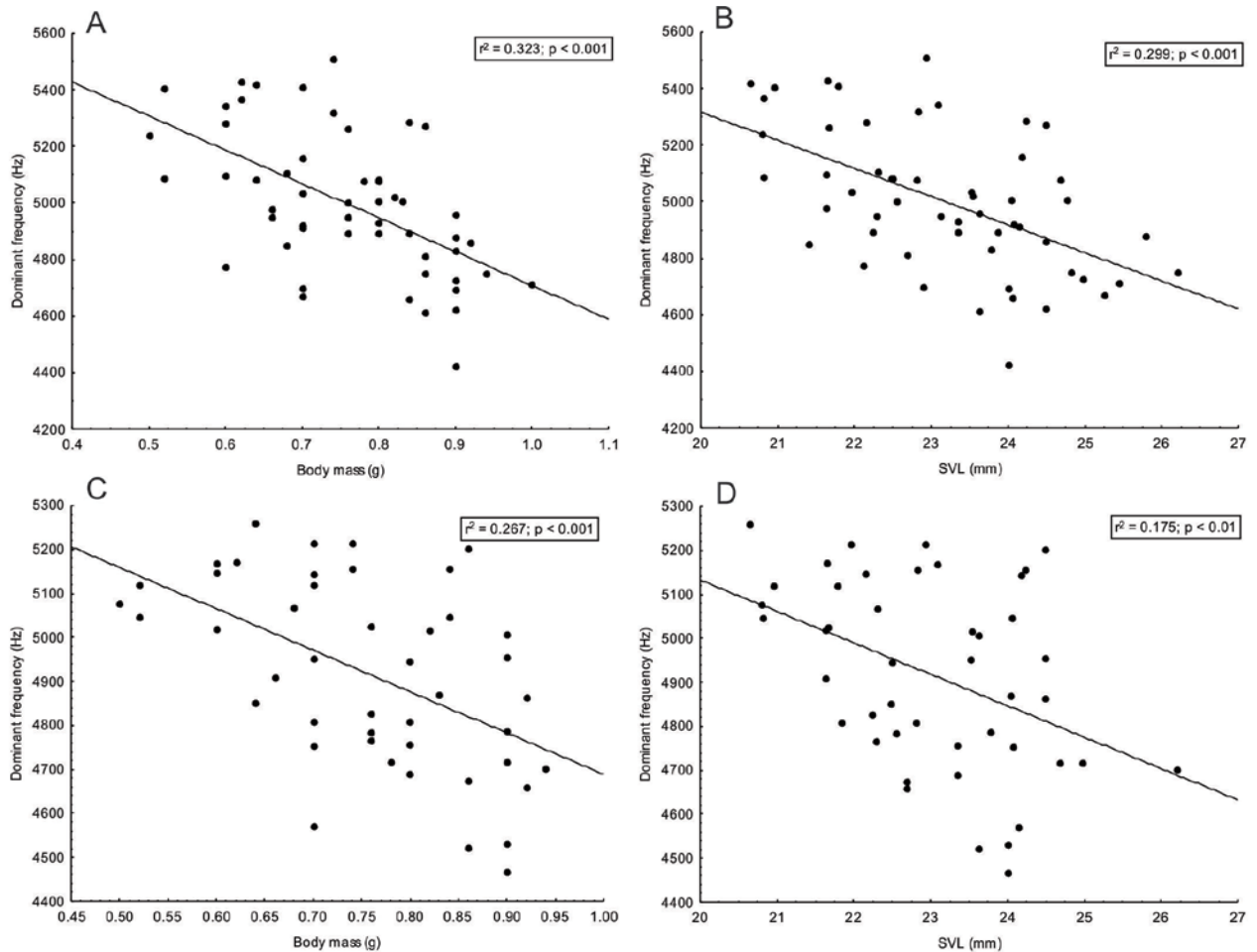


Fig. 3. Regression analysis between the dominant frequency of single (A and B) and compound (C and D) calls of *Dendropsophus minutus* and morphological characteristics.

variation among individuals and was negatively related to call duration.

DISCUSSION

The vocal repertoire of male *D. minutus* includes three notes (A, B and C) which may be emitted isolated or combined, leading to 16 different note combinations emitted by single individuals and showing that *D. minutus* is a species with a high acoustic variability. Although the mean values of acoustic parameters of notes differ from those described in other studies (e.g., Cardoso & Haddad, 1984; Heyer et al., 1990; Silva et al., 2008; Pombal, 2011), they are within the known range for *D. minutus*.

The most common vocalization was the isolated note A, following the patterns described for other populations from Nova Friburgo (Rio de Janeiro state) and Lages (Santa Catarina state) in southern Brazil (Cardoso & Haddad, 1984). On the other hand, the ACC call was the note combination most frequently emitted by males of *D. minutus* of populations from São Paulo state (Cardoso & Haddad, 1984). Despite considerable acoustic variability among populations of *D. minutus*, the same call characteristics can be recognized in different populations (Cardoso & Haddad, 1984).

We observed that some call parameters of *D. minutus* were influenced by air temperature, morphological characteristics and distance among calling males. The

dominant frequency was inversely correlated with SVL and body mass, similar to other anuran species (Howard & Young, 1998; Bastos et al., 2003; Toledo & Haddad, 2005; Giasson & Haddad, 2006; Briggs, 2010; Bastos et al., 2011). Other males can use the dominant frequency as an honest signal to evaluate the body size of the opponent to avoid physical combats between individuals (Briggs, 2010; Bastos et al., 2011).

Usually, temporal parameters of calls are not related to morphological characteristics (Bastos et al., 2003; Toledo & Haddad, 2005; Giasson & Haddad, 2006). However, in *D. minutus* repetition rate was positively influenced by SVL. Larger males may have a better physical condition than smaller males (Poole & Murphy, 2007), emitting more calls per minute or increasing call duration. Other factors such as air temperature and distance between males also influenced temporal parameters of calls. Air temperature is known to influence more temporal parameters than spectral parameters (Bastos & Haddad, 1995; Bastos et al., 2003; Guimarães & Bastos, 2003).

The vocal behaviour of *D. minutus* was influenced by the calling activity of nearby males. When the distance between nearby males was small, the focal male increased repetition rates of single calls (isolated note A), conforming with other studies on the influence of nearby males on focal males (Wells, 1988; Wagner, 1989; Bastos & Haddad, 1995, 2002; Bastos et al., 2011). Males of *D. ebraccatus*, *D. microcephalus* and *D. phlebodes* increase

Table 1. Acoustic parameters of the compound calls recorded for *Dendropsophus minutus* in Piracanjuba, Goiás, Brazil.

Calls	Call duration (s) mean±SD (range) (n)	Number of notes	Note duration (s) mean±SD (range) (n)	Dominant frequency (Hz) mean±SD (range) (n)	Analyzed/ Recorded calls
AB	0.469±0.05 (0.391–0.597) (18)	2	0.104±0.031 (0.038–0.159) (37)	4936.5±211.1 (4584–5192) (18)	18/25
ABC	0.809±0.072 (0.674–1.042) (74)	3	0.098±0.038 (0.038–0.22) (219)	4928.1±241.1 (4442–5326) (74)	74/102
ABCC	1.09±0.065 (0.983–1.229) (51)	4	0.085±0.034 (0.038–0.177) (208)	4970±227 (4425–5353) (51)	51/81
ABCCC	1.435±0.079 (1.33–1.542) (8)	5	0.086±0.03 (0.045–0.155) (40)	4843.9±242.7 (4478–5153) (8)	8/13
ABCCCC	1.655 (1) (1)	6	0.069±0.03 (0.054–0.131) (6)	4994 (1) (1)	1/1
AC	0.395 (1) (1)	2	0.084±0.045 (0.052–0.117) (2)	5008 (1) (1)	1/1
ACC	0.743±0.002 (0.742–0.745) (2)	3	0.087±0.046 (0.048–0.152) (6)	5059.5±57.3 (5019–5100) (2)	2/5
ACCC	1.008±0.048 (0.974–1.043) (2)	4	0.076±0.03 (0.045–0.124) (8)	5114.5±54.4 (5076–5153) (2)	2/4
BB	0.46±0.122 (0.375–0.601) (3)	2	0.109±0.04 (0.074–0.109) (3)	4896.6±76.8 (4808–4941) (3)	3/3
BBB	0.836 (1) (1)	3	0.118±0.01 (0.108–0.128) (3)	4888 (1) (1)	1/1
BBCC	1.032±0.025 (1.007–1.058) (3)	4	0.075±0.024 (0.048–0.119) (12)	4896.6±55.1 (4835–4941) (3)	3/3
BBCCC	1.069 (1) (1)	5	0.084±0.039 (0.048–0.141) (5)	4690 (1) (1)	1/1
BC	0.482 (1) (1)	2	0.0805±0.037 (0.054–0.107) (2)	4452 (1) (1)	1/1
BCC	0.697±0.08 (0.64–0.84) (7)	3	0.068±0.025 (0.039–0.141) (21)	5197±953 (4505–5192) (7)	7/8
BCCC	1.417 (5) (5)	4	0.07±0.04 0.047–0.131 (20)	4690 (5) (5)	5/8
BCCCC	1.624±0.204 1394–1784 (3)	5	0.0719±0.029 0.043–0.131 (15)	4694±98.1 (4637–4808) (3)	3/4

Table 2. Mean, standard deviation, and range of acoustic parameters of within and among male, coefficients of variation of call (isolated note A) in *Dendropsophus minutus*, in Piracanjuba, Goiás, Brazil. Numbers in *italics* are significant.

Acoustic parameter	mean±SD	Range	Within-male Mean <i>CV</i> %	Within-male range <i>CV</i> %	Between-male mean <i>CV</i> %	Ratio CV_a/CV_w	Kruskal-Wallis	
							H	<i>P</i>
Call duration (s)	0.139±0.018	0.098–0.209	7.08	1.322–29.5	12.98	1.83	166.63	<0.001
Pulse number/call	26.39±3.68	15.4–38	7.32	1.86–28.13	13.97	1.91	159.27	<0.001
Pulse duration (s)	0.008±0.002	0.006–0.0114	9.77	2.43–137.19	32.2	3.29	202.26	<0.001
Dominant frequency (Hz)	5001±249.7	4425–5512	1.65	0.245–4.935	4.99	3.01	223.54	<0.001

call complexity in response to nearby males (Wells, 1988). Males of *Acris crepitans* increase the rate of aggressive calls (Wagner, 1989).

To our knowledge this is the first study which calculates entropy values for an anuran amphibian. Da Silva & Vielliard (2006) have measured the structural complexity of the acoustic repertoire of the bird *Colibri serrirostris* through the use of entropy values. Similar to our results, the acoustic repertoire of *C. serrirostris* is not a predictor of entropy values, although a positive relationship between entropy value and proportion of used notes was found. Entropy values in *D. minutus* can likewise be used as a measure of acoustic variability.

Acoustic parameters of anuran calls may be classified into static or dynamic (Gerhardt, 1991). We found only the dominant frequency to be static, similar to other anuran species (Gerhardt et al., 1996; Bee et al., 2001; Bee & Gerhardt, 2001; Briggs, 2010; Rodriguez et al., 2010). Pulse duration was the most variable trait, as also observed for example in *Agalychnis moreletii* (Briggs, 2010).

Table 3. Standardized coefficients of canonical variables generated by discriminant function analysis.

	Discriminant Function Analysis		
	Root 1	Root 2	Root 3
Call duration (ms)	-0.788	-0.156	-1.424
Pulse number/call	0.367	-0.813	1.372
Dominant frequency (Hz)	0.851	-0.278	-0.449
Eigenvalue	11.17	6.54	2.43
Proportion of variance explained (%)	55.45	32.48	12.07
Total of variance explained (%)	55.45	87.93	100

Spectral parameters of calls (e.g., dominant frequency) are usually highly correlated with morphological structures (Bee & Gerhardt, 2001), therefore showing low variability. High variability of temporal parameters, on the other hand, allows males to quickly change their vocal behaviour during acoustic interactions (Castellano et al., 2002).

Our results suggest higher acoustic variation among than within male *D. minutus*, similar to other anurans (Howard & Young, 1998; Bee et al., 2001; Bee & Gerhardt, 2001; Pröhl, 2003; Briggs, 2010). Variation among males can be due to genetic variance, environmental induced phenotypic morpho-physiological variance, different adopted tactics and directional sexual selection (Giacoma & Castellano, 2001). The high among-individual variability indicates that individuals can be distinguished by their calls (Briggs, 2010). This form of individual recognition is known as neighbour-stranger discrimination (NSD) and has been described in *Lithobates clamitans* (Bee et al., 2001), *L. catesbeianus* (Bee & Gerhardt, 2001), *Oophaga pumilio* (Pröhl, 2003) and *Allobates femoralis* (Gasser et al., 2009), with acoustic properties that are more variable among males having an important role in this process. We found that the dominant frequency is the main acoustic property in individual discrimination, due to high CV_a/CV_w ratio. Previous studies (e.g., Bee et al., 2001; Bee & Gerhardt, 2001) also reported that spectral parameters are important in individual discrimination in frogs. However, the auditory system of anurans is limited to perceive relatively small among-male differences, and then individual discrimination in a chorus is based on a subset of signal properties that varies significantly between males (Bee & Gerhardt, 2001; Gasser et al., 2009). We also found that other acoustic parameters (e.g., call duration and pulse number) can also be useful in individual recognition of *D. minutus*.

ACKNOWLEDGEMENTS

We thank C. P. A. Prado, F. Nomura, M. Bilates, R. Márquez, and R. Lingnau for critical reading and valuable comments on the manuscript. RPB acknowledges CNPq

(Conselho Nacional de Desenvolvimento Científico e Tecnológico), ARM, PGG, and LS acknowledge CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq for fellowships. Diogo B. Provete reviewed the English language. Financial support was provided by CNPq (proc. 305836/2007-2) and FUNAPE (Fundação de Apoio à Pesquisa/UFG). Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/RAN) provided collecting permits.

REFERENCES

- Aichinger, M. (1987). Annual activity patterns of anurans in a seasonal neotropical environment. *Oecologia* 71, 583–592.
- Bastos, R.P. & Haddad, C.F.B. (1995). Vocalizações e interações acústicas de *Hyla elegans* (Anura, Hylidae), durante a atividade reprodutiva. *Naturalia* 20, 165–176.
- Bastos, R.P. & Haddad, C.F.B. (2002). Acoustic and aggressive interactions in *Scinax rizibilis* (Anura, Hylidae) during the reproductive activity in southeastern Brazil. *Amphibia-Reptilia* 23, 97–104.
- Bastos, R.P., Bueno, M.A.F., Dutra, S.L. & Lima, L.P. (2003). Padrão de vocalização de anúncio em espécies de Hylidae (anura) do Brasil Central. *Comunicações do Museu Ciências e Tecnologia* 16, 39–51.
- Bastos, R.P., Alcántara, M.B., Morais, A.R., Lingnau, R. & Signorelli, L. (2011). Vocal behavior and conspecific call response in *Scinax centralis* (Anura: Hylidae). *Herpetological Journal* 21, 43–50.
- Bee, M.A. & Gerhardt, H.C. (2001). Individual discrimination by territorial male bullfrogs (*Rana catesbeiana*): I. Individual variation in advertisement calls. *Animal Behaviour* 62, 1129–1140.
- Bee, M.A., Kozich, C.E., Blackwell, K.J. & Gerhardt, H.C. (2001). Individual variation in advertisement calls of territorial male green frog, *Rana clamitans*: Implications for individual discrimination. *Ethology* 107, 65–84.
- Bokermann, W.C.A. (1967). Notas sobre cantos nupciais de anfíbios brasileiros (Anura). III*. An. *Academia Brasileira de Ciências* 39, 491–493.
- Briggs, V.S. (2010). Call trait variation in Moretelli's tree frog, *Agalychnis moreletii* of Belize. *Herpetologica* 66, 241–249.
- Cardoso, A.J. & Haddad, C.F.B. (1984). Variabilidade acústica em diferentes populações e interações agressivas de *Hyla minuta* (Amphibia: Anura). *Ciência & Cultura* 36, 1393–1399.
- Castellano, S.B.R., Rinella, A.R. & Giacoma, C. (2002). The advertisement call of the European treefrogs (*Hyla arborea*): a multilevel study of variation. *Ethology* 108, 78–79.
- Da Silva, M.L. & Viellard, J. (2006). Entropy calculations for measuring bird song diversity: the case of the white-vented violet-ear (*Colibri serrirostris*) (Aves, Trochilidae). *Journal Razprave XLVII*, 37–49.
- Duellman, W.E. & Trueb, L. (1994). *Biology of Amphibians*. Baltimore: The John Hopkins University Press.
- Frost D.R. (2011). Amphibian species of the world online reference. American Museum of Natural History. <http://research.amnh.org/herpetology/amphibia/index.php>. Accessed 15 April 2012.
- Gasser, H., Amézquita, A. & Hödl, W. (2009). Who is calling? Intraspecific call variation in the Aromobatid frog *Allobates femoralis*. *Ethology* 115, 596–607.
- Gerhardt, H. C. & Huber, F. (2002). *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solution*. Chicago and London: University of Chicago Press.
- Gerhardt, H.C. (1991). Female mate choice in treefrogs: static and dynamic acoustic criteria. *Animal Behaviour* 42, 615–635.
- Gerhardt, H.C. (1998). Acoustic signals of animals: recording, field measurements, analysis and description. In *Animal Acoustic Communication*, 1–25. Hopp, S.L., Owren, M.J. & Evans, C.S. (eds). Berlin: Springer Verlag.
- Gerhardt, H.C., Dysin, M.L. & Tanner, S.D. (1996). Dynamic properties of the advertisement calls of gray treefrogs: patterns of variability and female choice. *Behavioral Ecology* 7, 7–18.
- Giacoma, C. & Castellano, S. (2001). Advertisement call variation and speciation in the *Bufo viridis* complex. In: *Anuran Communication*, 205–219. Ryan M.I. (ed). Washington and London: Smithsonian Institution Press.
- Giasson, L.O.M. & Haddad, C.F.B. (2006). Social interactions in *Hypsiboas albomarginatus* (Anura: Hylidae) and the significance of acoustic and visual signals. *Journal of Herpetology* 40, 171–180.
- Guimarães, L.D. & Bastos, R.P. (2003). Vocalizações e interações acústicas em *Hyla raniceps* (ANURA, HYLIDAE) durante a atividade reprodutiva. *Iheringia, Ser. Zool.* 93, 149–158.
- Haddad, C.F.B. (1995). Comunicação em Anuros (Amphibia). *Anais de Etologia* 1, 116–132.
- Heyer, W.R., Rand, A.S., Cruz, C.A.G., Peixoto, O.L. & Nelson, C.E. (1990). Frogs of Boracéia. *Arquivos de Zoologia* 31, 231–410.
- Howard, R.D. & Young, J.R. (1998). Individual variation in male vocal traits and female mating preferences in *Bufo americanus*. *Animal Behaviour* 55, 1165–1179.
- Lingnau, R. & Bastos, R.P. (2007). Vocalizations of the Brazilian torrent frog *Hylodes heyeri* (Anura: Hylodidae): repertoire and influence of air temperature on advertisement call variation. *Journal of Natural History* 41, 1227–1235.
- Manly, B.F.J. (2008). *Métodos estatísticos multivariados: uma introdução*. Porto Alegre: Bookman.
- Márquez, R. & Eekhout, X.R. (2006). Advertisement calls of six species of anurans from Bali, Republic of Indonesia. *Journal of Natural History* 40, 571–588.
- Martin, W.F. (1972). Evolution of vocalization in the Genus *Bufo*. In *Evolution in the Genus Bufo*, 279–309. Blair, W.F. (ed). Austin: University of Texas Press.
- Pombal, J.P. (2011). O espaço acústico em uma taxocenose de anuros (Amphibia) do sudoeste do Brasil. *Arquivos do Museu Nacional* 68, 135–144.
- Poole, K.G. & Murphy, C.G. (2007). Preferences of females barking treefrogs, *Hyla gratiosa*, for larger males: univariate and composite tests. *Animal Behaviour* 73, 513–524.
- Pröhl, H. (2003). Variation in male calling behavior and relation to male mating success in the strawberry poison frog (*Dendrobates pumilio*). *Ethology* 109, 273–290.
- Rodríguez, A., De la Nuez, D. & Alonso, R. (2010). Intraspecific variation in the advertisement call of the Cloud-Forest Frog *Eleutherodactylus glamyrus* (Anura: Eleutherodactylidae). *Journal of Herpetology* 44, 457–466.
- Ryan, M.J. (1988). Constraints and patterns in the evolution of anuran acoustic communication. In *The Evolution of the*

- Amphibian Auditory System*, 637–677. Frittsch, B., Ryan, M., Wilczynski, W., Walkowiak, W. & Hetherington, T. (ed). New York: John Wiley and Sons Inc.
- Ryan, M.J. (1991). Sexual selection and communication in frogs: some recent advances. *Trends in Ecology & Evolution* 6, 351–354.
- Ryan, M.J. (2001). *Anuran Communication*. Washington: Smithsonian Institution Press.
- Shannon, C. E. & Weaver, W. (1949). *The Mathematical Theory of Communication*. Chicago, University of Illinois Press.
- Silva, R.A., Martins, I.A. & Rossa-Feres, D.C. (2008). Bioacústica e sítio de vocalização em taxocenoses de anuros de áreas abertas no noroeste paulista. *Biota Neotropica* 8, 123–134.
- Tárano, Z. (2002). Vocal response to conspecific call variation in the Neotropical frog *Physalaemus enesefae*. *Journal of Herpetology* 36, 615–620.
- Toledo, L.F. & Haddad, C.F.B. (2005). Acoustic repertoire and calling site of *Scinax fuscomarginatus* (Anura, Hylidae). *Journal of Herpetology* 39, 455–464.
- Wagner, W.E. (1989). Graded aggressive signals in Blanchard's cricket frog: vocal response to opponent proximity and size. *Animal Behaviour* 38, 1025–1038.
- Wells, K.D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour* 25, 666–693.
- Wells, K.D. (1988). The effect of social interactions on anuran vocal behavior. In *The Evolution of the Amphibian Auditory System*, 433–454. Frittsch, B., Ryan, M., Wilczynski, W., Walkowiak, W. & Hetherington, T. (ed). New York: John Wiley and Sons Inc.
- Wells, K.D. (1989). Vocal communication in a neotropical treefrog, *Hyla ebraccata*: response of males to graded aggressive calls. *Copeia* 1989, 461–466.
- Wells, K.D. (2007). The ecology and behaviour of amphibian. Chicago: The University of Chicago Press.
- Wells, K.D. & Schwartz, J.J. (1984). Vocal communication in a neotropical treefrog, *Hyla ebraccata*: advertisement calls. *Animal Behavior* 32, 405–420.
- Zar, J.H. (1996). *Biostatistical Analysis*. Englewood Cliffs: Prentice-Hall.

Accepted: 2 July 2012