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Advertisement call of species of the genus *Frostius* Cannatella 1986 (Anura: Bufonidae)

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Abstract. *Frostius pernambucensis* and *F. erythrophthalmus* are cryptic bufonid species recognized mainly by the iris color: yellow in *F. permambucensis* and red in *F. erythrophthalmus.* However, field studies showed that the iris color of *F. erythrophthalmus* could vary between yellow and red. To improve the recognition of these species we described the advertisement call of *Frostius pernambucensis* and *Frostius erythrophthalmus* and we tested if call characteristics are influenced by temperature, male size and perch height. We also report on a physical interaction between two males of *F. pernambucensis* and the associated vocalization, suggesting that *F. pernambucensis* has not a territorial call or encounter call. Comparing the advertisement calls, the call of *F. pernambucensis* was lower, shorter and with a smaller number of notes than the call of *F. erythrophthalmus*. Dominant frequency and fundamental frequency variation of the *F. pernambucensis* advertisement call were related to the male's size, while the call emission rate was related to air temperature. However, we could not find relationship among the acoustic characteristic of *F. erythrophthalmus* and male size or temperature.

Keywords. Vocalization, *Frostius pernambucensis*, *Frostius erythrophthalmus*, Atlantic forest, specific recognition.

INTRODUCTION

The genus *Frostius* Cannatella 1986 is endemic to the Atlantic Rainforest and is found in the Brazilian states of Paraíba (Pimenta and Caramaschi, 2007), Pernambu-

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co (Bokermann, 1962), Alagoas (Peixoto and Freire, 1998) and Bahia (Juncá and Freitas, 2001; Pimenta and Caramaschi, 2007). *Frostius* was suggested to be phylogenetically related to *Atelopus* Dumeril and Bibron, 1841, *Dendrophryniscus* Jiménez de la Espada, 1870, *Melanophryniscus* Gallardo, 1961, *Oreophrynella* Boulenger, 1895 and *Osornophryne* Ruiz-Carranza and Hernandez-Camacho, 1976 (Cannatella, 1986). The morphological characteristics and the cladistic analyses performed were indicative of a closer sister group relationship among the genus *Frostius* + (*Atelopus* + *Osornophryne*) or *Frostius* + *Atelopus* (Cannatella, 1986). The phylogenetical proximity with the genus *Atelopus* is expected considering the morphological similarities already pointed out by Bokermann (1962). Based on DNA characteristics, recent studies have indicated that *Osornophryne* (Frost et al., 2006), *Atelopus, Melanophryniscu*s and *Dendrophryniscus* (Frost et al., 2006, Pramuk et al., 2007) are basal bufonid genera, however, these authors did not include material from *Frostius* in their analyses.

Very little is known about the biology of *Frostius* species. Males vocalize perched on tree trunks and bushes of various heights (Juncá, pers. obs.). We know that *F. pernambuscensis* (Bokermman, 1962) reproduces in bromeliad phytothelms, where the endothrophic tadpoles complete their growth (Cruz and Peixoto, 1982; Juncá and Borges, 2002), whereas nothing is known about the reproductive biology of *F. erythrophthalmus* Pimenta and Caramaschi, 2007, which is suspected to be similar to that of *F. pernambuscensis* (Pimenta and Caramaschi, 2007).

The only two species known from this genus are very similar. They are distinguished by shape and color, the red iris of *Frostius erythrophthalmus* being the most conspicuous characteristic (Pimenta and Caramaschi, 2007). However, individuals of *F. erythrophthalmus* have been found presenting yellow iris color which is a feature assigned to F. pernambucensis (Solé, pers. obs.). The known distribution of *F. erythrophthalmus* is restricted to the south of Bahia state, while *F. pernambucensis* is known from the States of Paraíba, Pernambuco, Alagoas and northeastern Bahia. Apparently there is no overlap between the respective geographic distributions of these species.

The morphological similarity between the two species of the genus calls for efforts to find other diagnostic characteristics, for example, the recognition of their respective advertisement calls. Since the main function of this type of vocalization is to attract conspecific females (Wells, 2007), some degree of divergence between the advertisement calls of the two species is expected. Although the use of the advertisement call characteristics have been widely used in taxonomy (e.g., Castroviejo-Fisher et al., 2009, Vieites et al., 2009, Funk et al. 2012) , only a few are recognized as statics characteristics, with low individual variation (e.g., dominant frequency and pulse emission rate, Gerhardt, 1991). In many species these statics characteristics can be influenced by the male's size (e.g., Asquith et al., 1988), temperature (e.g., Castellano and Giacoma, 2000) geographic variation (Catellano et al., 2002, Smith et al., 2003) and habitat and micro-habitat (Ryan et al., 1990; Ryan and Wilczynsky, 1991; Preininger et al., 2007).

To contribute to the recognition of the two *Frostius* species, we compared the advertisement calls of *F. pernambucensis* and *F. erythrophthalmus* and tested if the size of males, air temperature and perch height influence the analyzed acoustic characteristics. We also describe a physical interaction between two males of *F. pernambucensis* and the associated vocalization.

MATERIAL AND METHODS

Males of the two *Frostius* species vocalized more during the night, but also during daytime, at different times. We recorded specimens of *Frostius* at night, from 1900 to 2300 h. We studied a *Frostius pernambucensis* population at Serra da Jibóia, municipality of Santa Terezinha, Bahia (12°51'S, 39°28'W). The hills extend for about 6 km with a maximum altitude of 850 m, with caatinga (typical xeric vegetation of Brazil) in the valleys, humid Atlantic rainforest on the slopes and rocky fields on the hilltops. They belong to a range of disconnected hills which extend from the coast of Bahia state towards the northwest and north of Baia de Todos os Santos region (Juncá, 2006).

We recorded the advertisement call of *Frostius erythrophthalmus* at three sites in the southern region of Bahia state: RPPN (Private Natural Heritage Reserve) Capitão, Itacaré municipality (five males), RPPN Boa União, Ilhéus municipality (two males) and Michelin Ecological Reserve, Igrapiuna municipality (one male). The Michelin Reserve has fragments of dense Atlantic Rainforest (13°50'S, 39°10'W, altitude 90 to 383 m) surrounded by banana, cocoa and rubber tree plantations. The annual precipitation reaches 2000 mm (Camurugi et al., 2010). The RPPN Boa União and Capitão are located in the south of the state of Bahia and are classified as tropical lowland (Oliveira-Filho and Fontes, 2000). According to Köppen (1936) the climate is classified as Af being hot and humid without a dry season; the average temperature is around 24 °C degrees and the annual rainfall can reach 2200 mm (Sá et al., 1982; Landau et al., 2003). The forest of this region consists of a mosaic in different successional stages, with a large concentration of epiphytes, bromeliads, great lianas, and a dense sub-canopy (Thomas et al., 1998; Jardim 2003).

In October 2008, we measured the advertisement call's sound pressure level (SPL) and snout-vent length (SVL) of 30 males of *Frostius pernambucensis* together with the air temperature. We used a decibel meter Minipa® MSL – 1351C to measure the SPL of three consecutive calls of each male at a distance of fifty centimeters from the individual. The arithmetic average of the three measurements was considered the sound intensity for each specimen. In April 2009, we recorded the advertisement call of twenty two males of *Frostius pernambucensis* using a Sony® WM-D6 Digital Audio Tape recorder, a directional microphone Yoga® HT-32OA, keeping the microphone 50 cm from the vocalizing male. During the two year study, we measured the height of the vocalization site and the air temperature with a digital Minipa® MV - 363 thermometer. After recording and measuring the call intensity, we measured the SVL of each male, putting light pressure on each individual's body using a wooden board, obtaining the measurement with a caliper (0.05 mm precision, 2008) and a plastic ruler (0.10 mm precision, 2009). Voucher specimens were deposited in The Zoology Museum of UEFS (MZUEFS 3708-3709).

In 2010, we recorded the advertisement call of *Frostius erythrophthalmus* with a Marantz® PMD 660 digital recorder and a Yoga® EM 9600 unidirectional microphone. After each recording, we collected the males and took the air temperature with a digital type K thermometer with an accuracy of 0.1 ºC (PCE Group-222). All the males recorded were photographed with a digital camera, whilst still alive to register the iris coloration. Males of *Frostius erythrophthalmus* were deposited at the Zoological Collection of The Santa Cruz State University, Zoological Museum of UEFS (MZUEFS 3687) and Zoology Museum 'Professor Adão José Cardoso', State University of Campinas (ZUEC 16631-33). The archives of the vocalization sound of *Frostius pernambucensis and Frostius erythrophthalmus* are deposited in the Sound library Sonoteca UEFS (*Frostius pernambuscensis* SUEFS 30.01-30.22; *F. erythrophthalmus* SUEFS100.09-100.12, 100.34, 100.35, 100.43 and 100.44)

Call analyses

We digitalized and analyzed the calls using the Canary 1.2.4 program. The following characteristics were measured: call duration (ms), interval between calls (s), call rate (call/s), pulse number, pulse rate (pulse/s), dominant frequency (kHz) and fundamental frequency (kHz). We calcu-

lated the mean and standard deviation for each characteristic analyzed of the males which had more than one call recorded. We verified if call characteristics are influenced by temperature, SVL and perch height (the last variable was only tested for *F. pernambucensis*) using multiple linear regression with BioEstat 5.0 software (Ayres et al., 2007).

We used Past 2.06 software (Hammer et al., 2001) to perform a principal component analysis (PCA) to verify if the acoustic characteristics of the advertisement call of these two species could separate the individuals into two groups, through the use of a correlations matrix, considering that the variables used were in different units. The characteristics used in this analysis were dominant frequency, fundamental frequency, number of pulses, pulse rate and call duration. The characteristics that were influenced by SVL or temperature had their values corrected by the partial coefficient regression, which is obtained in partial linear multiple regression. We used broken the stick method to identify the number of principal components to be retained (Hammer et al., 2001). To check the stability of the analysis we used Bootstrap with 1000 randomizations.

To determine if the call characteristics of *F. pernambucensis* are significantly different from those of *F. erythrophthalmus,* we used unequal variance t-test. The accepted probability was 0.05.

RESULTS

Frostius pernambucensis

In 2008, the males vocalized while perched on the vegetation with height variation from 0.20 to 200.00 cm (x = 114.62, SD = 52.5, n = 30). The vocalization SPL varied from 72.50 to 95.20 dB (x = 84.39 SD = 5.75, n = 30). The average SVL was 27.80 mm (SD = 0.22; n = 30) and the temperature varied from 17.60 to 22.10 ºC (x = 19.67, SD = 0.98, n = 30). The multiple linear regression was nearly significant for SPL ($F_{3,26}$ = 2.648, P = 0.07) and showed a significant relationship between SPL and temperature (partial coefficient regression b = 2.309, $t_{1,26}$ = 2.447, P = 0.02), while the relationships between SPL and SVL (partial coefficient regression b = -7.2438, $t_{1,26}$ = -1.7587, P = 0.09) or perch height (partial coefficient regression b = 0.0226, $t_{1,26}$ = -1.3892, P = 0.18) were not significant. That year, we observed an interaction between two males, which lasted about 5 minutes. Only the advertisement call was emitted and by only one of the males. The vocalization was alternated with visual displays. In these displays there was an extension of the hind limbs (Fig. 1). Physical interaction between these two males was common: one of the males passed beneath the other (Fig. 1A, 1B and 1E). During the observation the silent male tried to remove the resident male that was vocalizing and vice-versa. The sex of the silent individual was confirmed by a dilated gular region.

In 2009 we recorded three calls for each male of *Frostius pernambucensis* which vocalized perched in the vegetation with height variation from 0.30 to 2.53 m (x = 1.29, SD = 0.59, n = 21 males). The advertisement call of *F. pernambucensis* is long, lasting approximately 8 s, composed of a series of around 54 pulses and an emission rate of 7 pulses/s (Table 1, Fig. 2). The call emission rate was around 2 call/min. The dominant frequency was around 2.36 kHz (Table 1). A discreet frequency modulation is only observed in the first pulse and also in some calls in the second pulse (Fig. 2b). The SVL average for the males was 36.79 mm (SD= 0.21). The air temperature varied from 20 to 23 ºC (x = 20.88, SD = 0.83, n = 21).

The multiple linear regression was significant for dominant frequency ($F_{3,17}$ = 4.122, P = 0.02), fundamental frequency ($F_{3,17}$ = 3.166, P = 0.05) and call emission rate ($F_{3,17}$

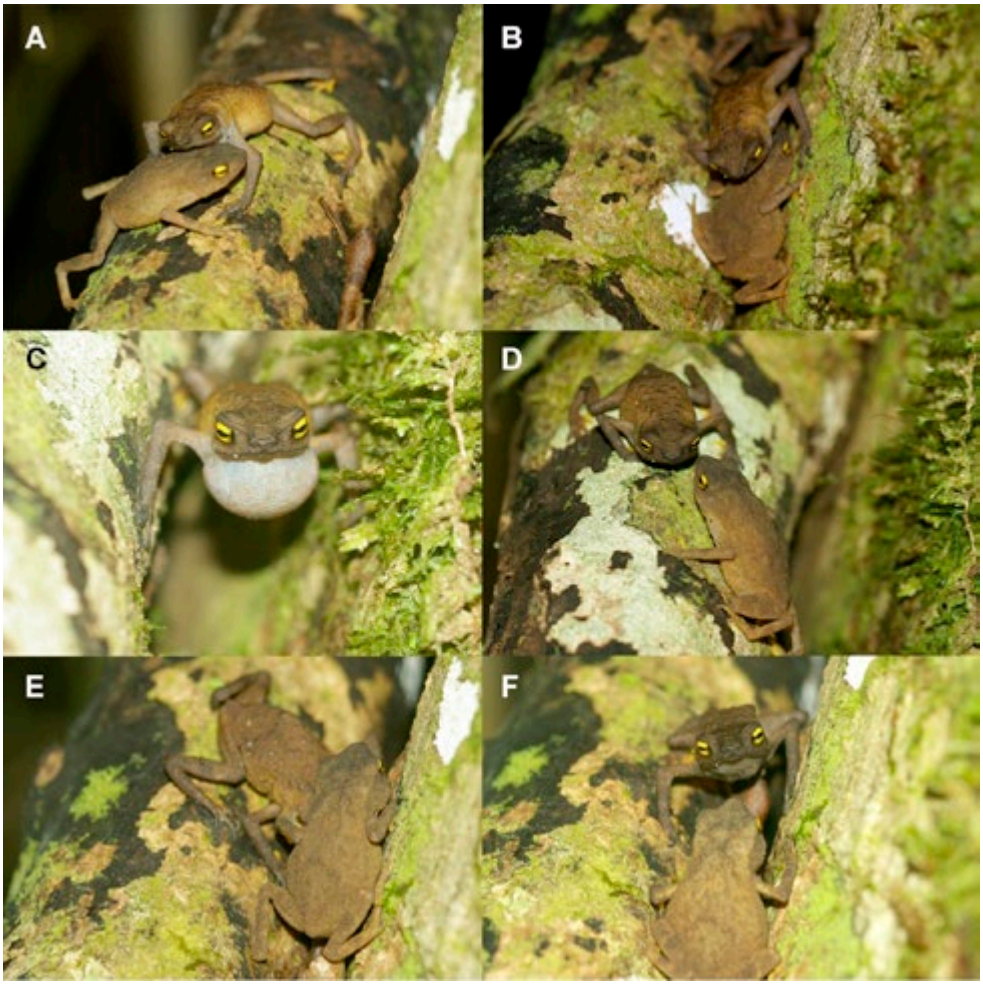

Fig. 1. Interactions between two males of *Frostius pernambucensis*, from municipality of Santa Terezinha, Bahia State, Brazil. The photos are in temporal sequence.

= 3.190, P = 0.05).The SVL was important for the dominant frequency variation (partial coefficient regression b = -0.2401, t = 2.917, P = 0.01) and fundamental frequency (partial coefficient regression b = -0.3435, t = 2.447, P = 0.02), whereas the temperature had a significant influence on the call emission rate (partial coefficient regression b = 0.2208, t = 2.650, P = 0.02). The perch height was not significantly related with any acoustic variable.

Frostius erythrophthalmus

The number of calls recorded for each male of *Frostius erythrophthalmus* varied from 1 to 3. The males vocalized at an approximate height of 1 m (x = 1.07, SD = 0.45, n = 8).

Table 1. Analyzed acoustic characteristics of the advertisement call of *Frostius* species.

	F. pernambuscensis (n = 96 calls)	*F. erythrophthalmus* (n = 21 calls)
	Serra da Jibóia	Ilhéus, Itacaré e Igrapiuna
Call duration (s)	7.54 ±1.53 (1.94-9.84)	10.59 ±3.10 (4.84-15.30)
Pulse number	52.64 ±9.93 (15-75)	69.23 ±22.48 (41-121)
Pulse rate (pulse/s)	7.02 ±0.44 (6.01-8.20)	6.77 ±1.30 (4.49-8.47)
Call rate (call/min)	1.68 ±0.38 (1.00-2.33)	1.94 ±0.70 (1.18-2.86)
Interval between calls (s)	28.49 ±9.56 (11.70-72.03)	29.42 ±11.73 (19.82-42.50)
Dominant frequency (kHz)	2.37 ±0.10 (2.11-2.54)	3.07 ±0.19 (2.76-3.47)
Fundamental frequency (kHz)	1.76 ±0.21 (0.75-2.04)	2.54 ± 0.32 (1.83-2.95)

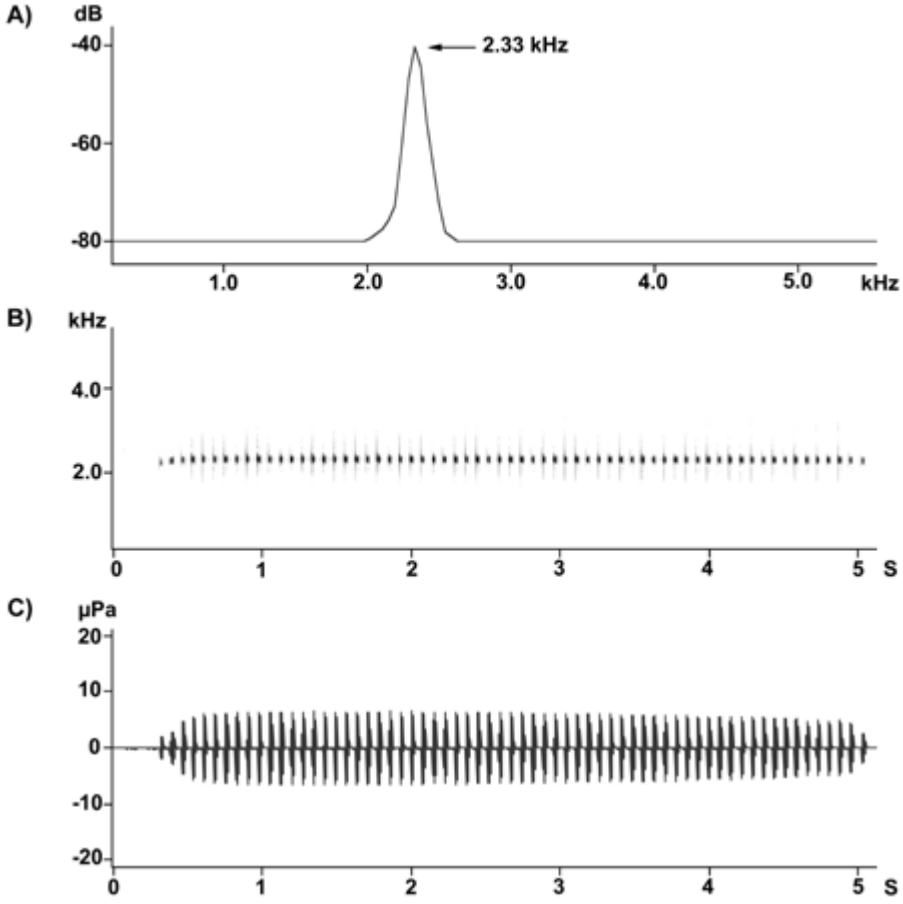

Fig. 2. Advertisement call of *Frostius pernambucensis,* from municipality of Santa Terezinha, Bahia State, Brazil. A) power spectrum, B) audiosectogram and C) oscillogram.

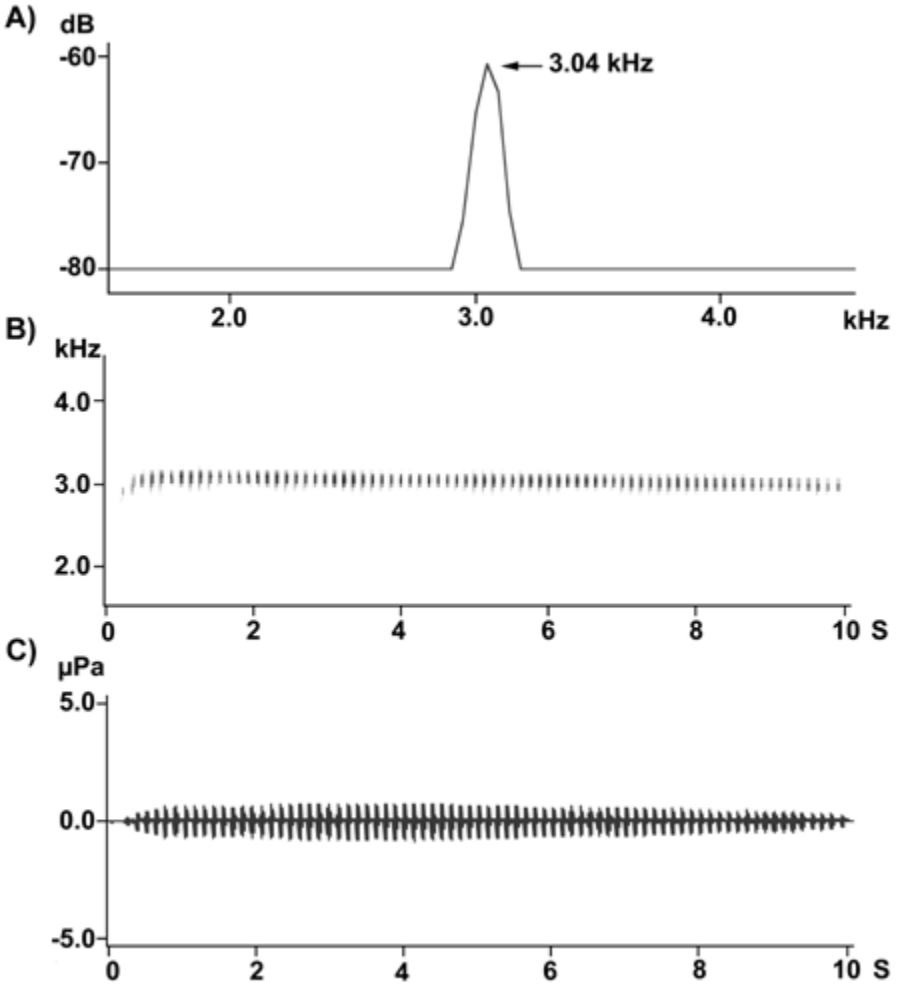

Fig. 3. Advertisement call of *Frostius erythrophthalmus*, from municipality of Itacaré, Bahia State, Brazil. A) power spectrum, B) audiosectogram and C) oscillogram.

The SVL varied from 20.00 to 23.24 mm (x = 21.30, SD = 1.03, n = 8) and the air temperature from 21.1 to 31.5 °C. The advertisement call of *F. erythrophthalmus* is similar to the call of *F. pernambucensis* in its general structure (Fig. 3) and they differed primarily in measured dominant frequency and fundamental frequencies, all of them higher in *F. erythrophthalmus* (Table 1). The multiple linear regression did not detect any relationship between the characteristics of the advertisement call of *F. erythrophthalmus*, SVL and temperature.

Using the Broken Stick method only the principal components 1 and 2 were retained and together they explained 84.9% of the variance (Table 2). The dispersion graph of the scores of the principal components 1 and 2 separated the two species (Fig. 4). All the vari-

Table 2. Variable loadings, eigenvalue and percentage of the variance for each principal component.

	PC1	PC2	PC3	PC4	PC5
Call duration	0.7647	-0.6279	0.1448	-0.00333	0.001066
Pulse number	0.8255	0.4905	-0.2257	-0.1646	6.35E-07
Pulse rate	0.8392	0.3821	-0.3542	0.1558	-1.05E-06
Dominant frequency	0.8497	-0.2423	0.4682	0.01039	-0.00093
Fundamental frequency	-0.04673	0.844	0.5337	0.02561	0.000526
Eigenvalue	0.7647	-0.6279	0.1448	-0.00333	0.001066
% Variance	53.89	31.036	14.03	1.0431	0.00005

ables showed loadings above 0.70 in PC1, except the note rate, that was the most important characteristic in the PC2 (Table 2).

The t-test showed a significant difference among population averages for the dominant frequency ($t = 2.590$, $P = 0.033$, $df = 7.71$) and fundamental frequency ($t = 4.644$, $P < 0.002$, $df = 7.77$), number of pulses ($t = -2.608$, $P = 0.032$, $df = 7.79$) and call duration ($t = -2.850$, $P = 0.022$, $df = 7.94$), but didn't indicate a difference in the pulse rate ($t = 0.180$, $P = 0.861$, $df = 7.67$).

During field work in the studied populations of *Frostius erythrophthalmus* we observed males with red and also yellow (Itacaré and Ilhéus) or only yellow irises (Igrapiuna).

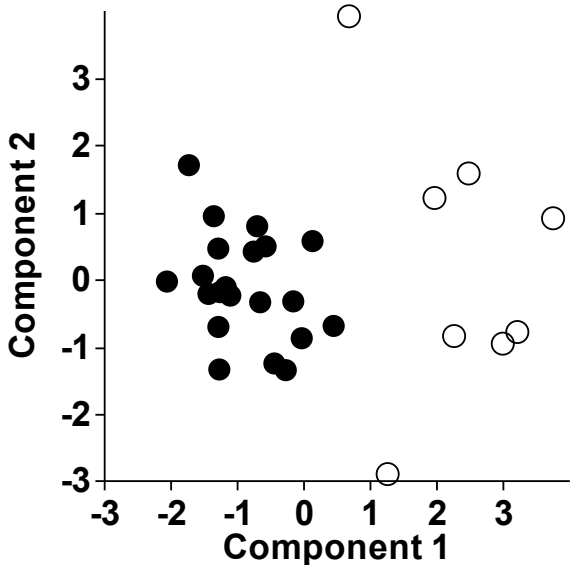

Fig. 4. Value dispersion of PC1 and PC2. Full circles *Frostius pernambucensis* and empty circles *Frostius erythrophthalmus*.

DISCUSSION

The two species of *Frostius* have similar advertisement calls, which are composed of a series of pulses emitted at regular intervals, a pattern of advertisement call found in many other bufonid species (Martin, 1972). Comparing the two species, the call of *F. pernambucensis* was lower, shorter and with a smaller number of notes than the call of *F. erythrophthalmus*. The differences of frequency, duration of the call and pulse emission rate are common among bufonid species which are phylogenetically close and show similar advertisement calls (Gergus et al., 1997; Garda et al., 2010). Although the pulse rate averages didn't show any significant differences between the two species of *Frostius*, it was an important variable which explained the variation in axis 2 of the PCA.

Various *Atelopus* species have the advertisement call registered (Cocroft et al., 1990) and at the very least only one call has ever been registered of *Osornophryne guacamaio* by a single male in the presence of a female (Gluesenkamp, 2001). These two genera are phylogenetically close to *Frostius* (Cannatella, 1986), and both genera have calls also formed by a series of pulses. Just as in the species of *Frostius*, *Atelopus* species also show advertisement calls with the same basic structure, but pulse rate, dominant frequency, and call length differ among species (Cocroft et al., 1990).

Many species of the genera *Atelopus* (Lötters et al., 1999) and at least *Osornophryne guacamayo* (Gluesenkamp, 2001) also have shiny coloration on the vent, including palmar and plantar surfaces and *Osornophryne* spawning has eggs with a lot of vitellus (McDiarmid and Gorzula, 1989; Glusenkamp, 2001), similar to *Frostius pernambucensis.* However, unlike *Frostius* species, the majority of species of both genera don't have tympanic membranes and the acoustic communication was considered less important than tactile or visual communication (Cocroft et al., 1990; Gluesenkamp, 2001). This way, interaction between two males of *Frostius pernambucensis* indicates that visual and tactile communication could also be important. The observed behavior during interaction indicates a dispute of vocalization site, suggesting the specie doesn't have a territorial or agonistic call, as reported for *Atelopus chiriquiensis* in aggressive encounters (Jaslow, 1979).

Usually, temperature has major influence on temporal properties of anuran acoustic signals (Navas and Bevier 2001, Lingnau and Bastos 2007), while the SVL has an influence on the spectral properties (e.g., Castellano et al., 2002, Giasson and Haddad, 2006). This relationship was also registered in *Frostius pernambucensis*, where the dominant frequency variation and the fundamental frequency of the advertisement call were related to the male's size, while the call emission rate was related to air temperature. The same analysis did not show any influence of the SVL or temperature on the characteristics of the advertisement call of *F. erythrophthalmus*. However, small sample size needs to be taken into account when interpreting this result. The sound pressure level measured to the advertisement call of *F. pernambucensis* is in the range of other anuran species, that vary from 72 to 116 dB (Wells, 2007). As *F. pernambucensis*, at least to some species the call intensity is not related to SVL (e.g., Passmore, 1981), although in other species large males tend to produce louder calls (Given, 1987; Arak, 1988). A possible influence of temperature on call SPL still remains an unexplored subject, although it was not found in at least one anuran species (Tárano, 2001). Although the perch height could be important to sound propagation (e.g., Kime et al., 2000), it did not influence any acoustic variable of *F. pernambucensis*.

Pimenta and Caramaschi (2007) did not report any variation in iris coloration in the populations they studied, however, the populations of *F. erythrophthalmus* in this study showed yellow (Igrapiuna) or yellow and red (Itacaré and Ilhéus) iris coloration. The identification of *F. erythrophthalmus* in these populations was made using other diagnostic characteristics reported by the authors of the specie (e.g., dorsal and ventral coloration, size and shape of tympanum, shape of fingers and toes). So, this study contributes to the diagnosis of two species in the field through the differences in the advertisement call, considering that the iris coloration can vary in *Frostius erythrophthalmus*.

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