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# Description and comparison of advertisement calls of *Euparkerella brasiliensis* (Parker, 1926) and *E. cochranae* Izecksohn, 1988 (Amphibia: Anura: Strabomantidae)

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**Abstract.** The advertisement calls of two morphologically similar species of the endemic and poorly known genus *Euparkerella* are described and compared. The calls of *Euparkerella brasiliensis* and *E. cochranae* were recorded in the Parque Nacional da Tijuca, Rio de Janeiro and in the Campo Escoteiro Geraldo Hugo Nunes, Guapimirim respectively, both situated in the state of Rio de Janeiro, southeastern Brazil. These two species emit calls composed by a single note with a harmonic and pulsed structure, with short pulses repeated at regular intervals resulting in a quasi-periodic pulse train. The calls revealed notable differences between each other in the acoustic parameters measured mainly in the structural and temporal characters. The number of pulses and the repetition rate of pulses of *E. cochranae* was approximately twice as much as those of *E. brasiliensis*, which means that the call of *E. cochranae* was markedly more pulsed than that of *E. brasiliensis*. The present study shows that advertisement calls indeed provide us with useful characters to identity determination of *E. brasiliensis* and *E. cochranae.*

**Keywords.** Bioacoustics, taxonomy, acoustic signals, pulsed calls, pulse repetition rate, *Euparkerella*.

## Introduction

Advertisement calls are used in mate attraction and intermale spacing in anurans (Duellman and Trueb 1994; Gerhardt 1994). Since these calls are species-specific, acoustic communication is an important pre-zygotic isolation mechanism, maintaining species as discrete evolution units (Etges 1987; Cardoso and Vielliard 1990). The number of studies that use alternative or complementary approaches in species identification such as the analysis of acoustic data is growing (e.g. Heyer *et al*. 1990; Pombal *et al*. 1995; Napoli and Cruz 2005; Weber *et al*. 2005; Silva-Filho and Juncá 2006; Angulo and Reichle 2008).

*Euparkerella* Griffths, 1959 is a genus endemic to the Atlantic rainforest of southeastern Brazil (Frost 2010). This taxonomic group contains four species, *E. brasiliensis* (Parker, 1926), *E. cochranae* Izecksohn, 1988, *E. robusta* Izecksohn, 1988 and *E. tridactyla* Izecksohn, 1988 (Frost 2010; Izecksohn 1988). There is very limited information available about the members of the genus. The lack of published data is likely caused

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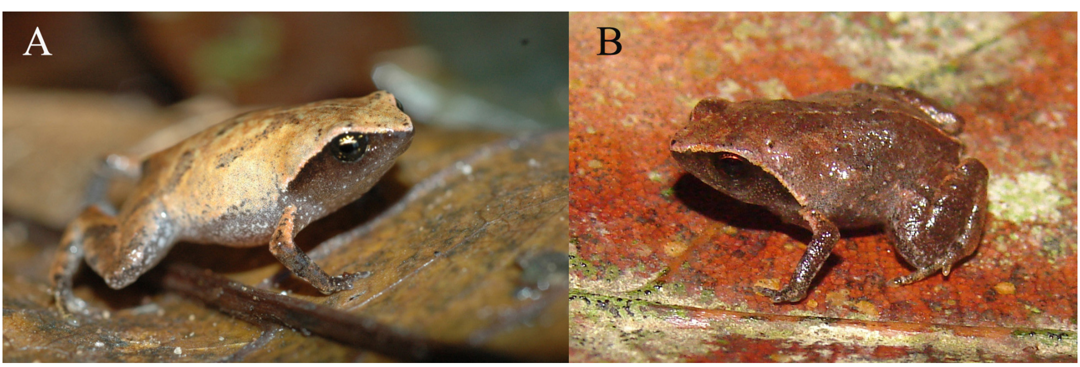


**Figure 1.** *Euparkerella brasiliensis* from Parque Nacional da Tijuca, Rio de Janeiro, Rio de Janeiro State (A); *Euparkerella cochranae* from Campo Escoteiro Geraldo Hugo Nunes, Guapimirim, Rio de Janeiro State (B). Note the morphological similarity between species (color is not an informative character). Photos: Sergio P. de Carvalho-e-Silva and Thiago Silva Soares.

**Table 1.** Voucher specimens of *E. brasiliensis* and *E. cochranae* with respective recordings.

| Species | Specimens | Recordings |
|---|---|---|
| *E. brasiliensis* | ZUFRJ 9579 | ASEC14833 and 14834 |
| | ZUFRJ 9586 | ASEC 14841 and 14842 |
| *E. cochranae* | ZUFRJ 11093 | ASEC 14850 and 14851 |
| | ZUFRJ 11095 | ASEC 14849 and 14852 |

by the limited geographical range, small body size and cryptic habit of the species.

Two species occur in Rio de Janeiro state, Brazil, i.e. *E. brasiliensis* and *E. cochranae* (Fig. 1). The first species is known from Serra dos Orgãos and coastal mountains in southeastern Rio de Janeiro state. The latter is known from the type locality in the Serra dos Orgãos as well as Morro São João (Frost 2010). Izecksohn (1988) stated that the overall morphological similarity among *E. brasiliensis* and *E. cochranae* might suggest a distinction only at subspecies level. This great similarity can be exemplified by Cochran (1955) who redescribed *E. brasiliensis* possibly based on a specimen of *E. cochranae* (see Izecksohn, 1988). Izecksohn (1988) commented superficially on the advertisement calls of both species and pointed out that their calls are distinctive characters allowing differentiation among the species, but without providing detailed acoustic measurements. Therefore, an accurate study of the acoustic characteristics of advertisement call will clarify the taxonomy among these closely related species.

The aims of the present study are to describe and compare the advertisement calls of *E. brasiliensis* and *E. cochranae*, and to comment on their taxonomic status.

## Materials and Methods

The calls of *E. brasiliensis* were recorded in the Parque Nacional da Tijuca (PNT) (between the coordinates 22º55' and 23º00' S, 43º11' and 43º19'W), in the Municipality of Rio de Janeiro, Rio de Janeiro State, and those of *E. cochranae* in the Campo Escoteiro Geraldo Hugo Nunes (between 22°34'33" and 22°35'05" S, 43°01'44" and 43°02'25" W), in the Municipality of Guapimirim, Rio de Janeiro State, from September 2006 to October 2008. Vocalizations were recorded with a Marantz PMD670 digital recorder, at sample rate of 44.1 Hz and sample size of 16 bits, a Sony WM-D6C tape recorder with cassette tapes TDK chrome 90 minutes and microphones Sennheiser ME-66, ME-67 and MKH-80. The calls recorded in cassette tape were digitalized at same sample rate and size of the digital recordings with Sound Forge 7.0. We analyzed advertisement calls with the software Raven Pro 1.3 from Cornell Laboratory of Ornithology (Bioacoustics Research Program). The parameters measured were: call duration; number of pulses per call (all pulses are considered); pulse rate; first and last pulse periods [measured from the beginning of one pulse to the beginning of the following one, thus encompassing the pulse duration and the interpulse interval (Weber *et al*. 2005)]; dominant frequency; and fundamental frequency. Numerical call parameters are given as range followed by mean $\pm$ SD in parenthesis. The temporal parameters were measured directly from the waveform and frequency parameters were measured directly from the audiospectogram. We also counted of the number of harmonically related frequencies observed in the audiospectogram and power spectrum. Even though these may vary with recording distance and quality, it is important to note their presence when possible (Angulo and Reichle 2008). Technical terms and definitions adopted follow Littlejohn (2001).

Ten calls of each species were analyzed in detail. The calls were individualized in the recordings that were deposited in the voice collection Arquivo Sonoro Professor Elias Pacheco Coelho (ASEC) of the Laboratório de Bioacústica, Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Brazil. Recordings were obtained in captivity with specimens captured from the same sites of field recording in order to obtain the voucher specimens. Two voucher specimens were obtained for each species, they were fixed in 10% formalin, preserved in 70% ethyl alcohol, and deposited at the Coleção de Anfíbios do Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro (ZUFRJ), Brazil (Table 1).

In order to compare each call parameter between species we performed the student *t* test. In addition, we performed multivariate variance analysis (MANOVA) to verify the acoustic difference between species. Assumptions of normality and homoscedasticity were verified with Kolmogorov-Smirnov and Levene's tests, respectively. We adopted the significance level of 0.05 (Zar 1984).

## Results

The behavior and calling sites of both species were similar. The males are shy and called sporadically in small choirs from scattered and concealed locations beneath leaf litter. They rarely started to call again after being disturbed by our proximity.

*Euparkerella brasiliensis* and *E. cochranae* presented advertisement calls characterized by a harmonic and pulsed structure, with short pulses repeated at regular intervals resulting in a regular trill or a quasi-periodic pulse train (see Littlejohn 2001). The pulse intervals of both species were long enough to enable distinction of these pulses by a human ear. The calls of both species did not show frequency modulation. The pulse periods increased throughout the call in both species.

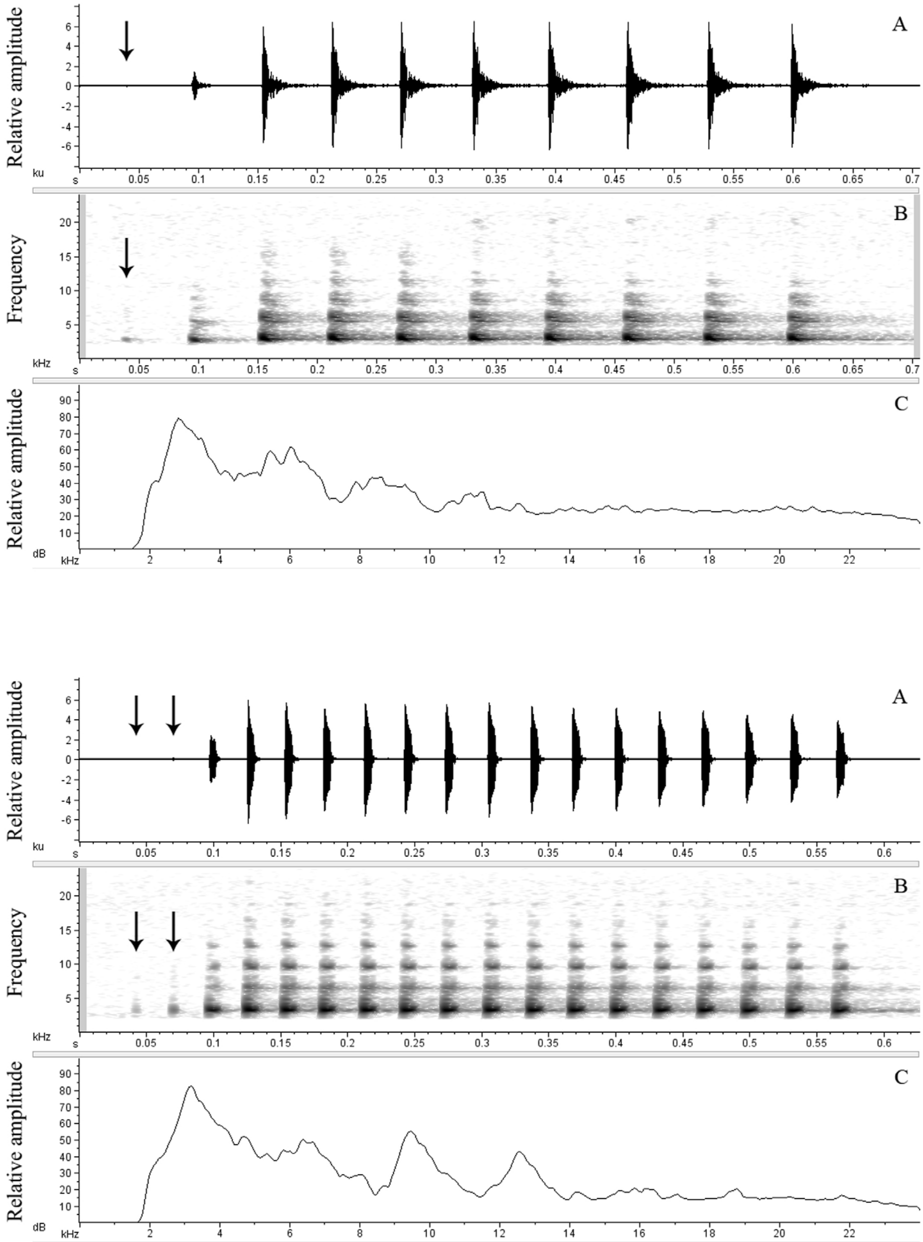


**Figure 2.** Advertisement call of *Euparkerella brasiliensis* above and *E. cochranae* below, recordings ASEC 14836 and ASEC 14847 respectively. Waveforms are flagged with "A", audiospectograms with "B" (window function Hann, amplitude logarithmic, filter bandwidth 2 KHz, overlap 99%, FFT size 512 points) and power spectrums with "C". The cryptic pulses are pointed by arrows.

In many calls the first pulses of the call train had low intensity, being sometimes imperceptible at first view and only distinguishable with a careful inspection of the corresponding oscilograms. However this was easier in the audiospectograms. Herein these pulses were named as cryptic pulses and are included in the number of pulses of the calls (Fig. 2).

**Table 2.** Measurement of advertisement call parameters for *Euparkerella brasiliensis*. In the first column, the number sequences of the recordings deposited in the voice collection. The asterisk marks the calls with voucher specimens.

| *Euparkerella brasiliensis* | Dominant frequency (Hz) | Call duration (s) | Number of pulses | First period (s) | Last period (s) | Repetition rate of pulses (s) | Number of visible harmonics |
|---|---|---|---|---|---|---|---|
| ASEC 14833* | 2812.5 | 0.443 | 9 | 0.06 | 0.069 | 20.32 | 6 |
| ASEC 14834* | 3000 | 0.46 | 8 | 0.061 | 0.069 | 17.39 | 6 |
| ASEC 14835 | 2812.5 | 0.51 | 9 | 0.06 | 0.068 | 17.65 | 6 |
| ASEC 14836 | 2812.5 | 0.566 | 10 | 0.056 | 0.07 | 17.67 | 7 |
| ASEC 14837 | 2812.5 | 0.546 | 9 | 0.063 | 0.076 | 16.48 | 5 |
| ASEC 14838 | 2625 | 0.542 | 9 | 0.054 | 0.077 | 16.60 | 6 |
| ASEC 14839 | 2812.5 | 0.506 | 9 | 0.059 | 0.067 | 17.79 | 6 |
| ASEC 14840 | 2812.5 | 0.553 | 9 | 0.061 | 0.072 | 16.27 | 6 |
| ASEC 14841* | 2812.5 | 0.552 | 9 | 0.06 | 0.076 | 16.30 | 6 |
| ASEC 14842* | 2625 | 0.63 | 10 | 0.059 | 0.084 | 15.87 | 5 |
| **Mean** | **2793.7** | **0.531** | **9.1** | **0.059** | **0.073** | **17.23** | **5.9** |

*Euparkerella brasiliensis* (Fig. 1A)

The advertisement call consisted of a single note composed of 8 to 10 pulses ($\bar{x}$ = 9.1; sd = 0.6; n = 10), all with attack shorter than decay. The intensity of the call rose gradually, or sometimes more suddenly, at the beginning (often, from the first to the third pulse of the call or from the second to third) and remained constant until the end of call. Call duration ranged from 0.443s to 0.630s ($\bar{x}$ = 0.531; sd= 0.054; n= 10). The values of dominant frequency were 2625, 2812.5 and 3000 Hz ($\bar{x}$ = 2793.7; sd = 106.4; n = 10), corresponding to the fundamental frequency band. The pulse periods increased throughout the call, and ranged from 0.054s to 0.063s ($\bar{x}$ = 0.059; sd = 0.002; n = 10) for the first period and 0.067s to 0.084s ($\bar{x}$ = 0.073; sd = 0.005; n = 10) for the last period. The call had harmonic structure, with the visible harmonics ranging from 5 to 7 ($\bar{x}$ = 5.9; sd = 0.6; n = 10). The repetition rate of pulses ranged from 15.87 to 20.32 ($\bar{x}$ = 17.23; sd = 1.28; n = 10) pulses per second. Frequently, the first pulse, and

**Table 3.** Measurement of advertisement call parameters for *Euparkerella cochranae.* In the first column, the number sequences of the recordings deposited in the voice collection. The asterisk marks the calls with voucher specimens.

| *Euparkerella cochranae* | Dominant frequency (Hz) | Call duration (s) | Number of pulses | First period (s) | Last period (s) | Repetition rate of pulses (s) | Number of visible harmonics |
|---|---|---|---|---|---|---|---|
| ASEC 14843 | 3375 | 0.375 | 14 | 0.028 | 0.03 | 37.33 | --- |
| ASEC 14844 | --- | 0.621 | 20 | 0.028 | 0.037 | 32.21 | --- |
| ASEC 14845 | --- | 0.633 | 20 | 0.03 | 0.038 | 31.59 | --- |
| ASEC 14846 | --- | 0.596 | 18 | 0.027 | 0.038 | 30.20 | 7 |
| ASEC 14847 | 3187.5 | 0.532 | 18 | 0.028 | 0.034 | 33.83 | 6 |
| ASEC 14848 | 3375 | 0.493 | 17 | 0.029 | 0.034 | 34.48 | 6 |
| ASEC 14849* | 3187.5 | 0.497 | 18 | 0.025 | 0.032 | 36.22 | 7 |
| ASEC 14850* | 3187.5 | 0.438 | 16 | 0.028 | 0.032 | 36.53 | 7 |
| ASEC 14851* | 3187.5 | 0.531 | 17 | 0.032 | 0.041 | 32.01 | 4 |
| ASEC 14852* | 3375.5 | 0.377 | 17 | 0.022 | 0.027 | 45.09 | 7 |
| **Mean** | **3267.9** | **0.509** | **17.5** | **0.028** | **0.034** | **34.95** | **6.3** |

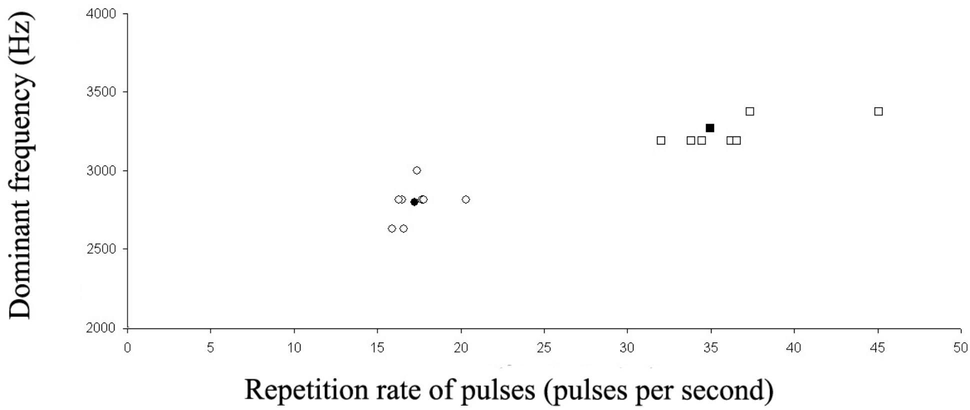


**Figure 3.** The circles represent the calls of *Euparkerella brasiliensis* and the squares the calls of E. cochranae. The black symbols represent the means. Note the complete separation between species.

sometimes the second, was cryptic and barely sighted due to its low intensity (Fig. 2; Table 2).

*Euparkerella cochranae* (Fig. 1B)

The advertisement call consisted of a single note composed of 14 to 20 pulses ($\bar{x}$ = 17.5; sd = 1.8; n = 10), all with attack shorter than decay. The intensity of the call rose gradually, or sometimes more suddenly, at the beginning (often, from the first to the third pulse of the call or from the second to third) and gradually fell at the end of call. Call duration ranged from 0.375 s to 0.633 s ($\bar{x}$ = 0.509; sd = 0.093; n = 10). The values of dominant frequency were 3187.5, 3375.0 and 3375.5 Hz ($\bar{x}$ = 3267.9; sd = 100.3; n = 7), corresponding to the fundamental frequency band. The pulse period increased throughout the call, and ranged from 0.022s to 0.032s ($\bar{x}$ = 0.028; sd = 0.003; n = 10) for the first period and 0.027s to 0.041s ($\bar{x}$ = 0.034 sd = 0.004; n = 10) for the last period. The call had harmonic structure, with the visible harmonics ranging from 4 to 7 ($\bar{x}$ = 6.3; sd = 1.1; n = 7). The repetition rate of pulses ranged from 30.20 to 45.09 ($\bar{x}$ = 35.95; sd = 4.26; n = 10) pulses for second. Frequently, the first and second pulses were cryptic and barely sighted due to their low intensities (Fig. 2; Table 3).

*Call Comparison*

Five parameters were significantly different between the two species based on the results of the student *t* test: the dominant frequency of *E. cochranae* was slightly higher than that of *E. brasiliensis* by approximately 400 Hz ($t = -9.25$; $p < 0.00$; n = 17); the first and last periods were longer in *E. brasiliensis* than in *E. cochranae* ($t = 26.68$; $p < 0.01$; n = 20; $t = 17.83$; $p < 0.01$; n = 20, respectively); the number of pulses of *E. cochranae* was approximately twice as much as that of *E. brasiliensis*, which means that the call of *E. cochranae* was markedly more pulsed than that of *E. brasiliensis* ($t = -14.22$; $p < 0.01$; n = 20) and the repetition rate of pulses of *E. cochranae* was much higher than that of *E. brasiliensis* ($t = -12.57$; $p < 0.01$; n = 20). Two parameters were not significantly different: the call durations ($t = 0.63$; $p = 0.53$; n = 20) and the number of harmonics ($t = -0.94$; $p = 0.36$; n = 17).

The multivariate variance analysis (MANOVA) revealed that the species are different considering the acoustic characteristics measured ($F = 229.02$; $p < 0.01$; n = 16).

## Discussion

Izecksohn (1988) described the calls of *E. brasiliensis* and *E. cochranae* as harsh and faint trills. The author also stated that the call of *E. cochranae* is clearly shorter and faster than call of *E. brasiliensis*. His miosperception of the duration of the calls was possibly influenced by the difference in repetition rates of pulses. Although we only measured the first and the last periods, the advertisement call of *E. cochranae* had pulse intervals visibly shorter than that of *E. brasiliensis*, resulting in a false perception of different call durations.

The marked divergence of repetition rate of pulses is due to similar call durations and different numbers of pulses. The quantification of the number of pulses is easy and does not depend neither on high recording quality nor accuracy of the software used in the call analysis. Moreover there is no overlap in the number of pulses between the species, enabling quick and easy distinction between them (Fig. 3). Information about the advertisement calls of *E. robusta* and *E. tridactyla* may help in clarifying the role of the repetition rate of pulses in the taxonomy of the genus.

The negative relationship between body size and dominant frequency has been discussed by several authors (e.g. Zweifel 1968). The body size difference between *Euparkerella* species can be responsible for the difference in dominant frequency. Indeed *E. cochranae* is smaller than *E. brasiliensis* by approximately 2.5 mm (Izecksohn 1988) and has a higher dominant frequency as expected.

Visible harmonics were noted to observe any possible difference between the patterns of energy distribution in the harmonics of *E. brasiliensis* and *E. cochranae*. The amount of energy of harmonics is a function of recording distance since their detection is associated with call intensity. Fewer distinguished harmonics can indicate a lower intensity of the call. However there was no significant difference between the number of visible harmonics of the species, even though a lower intensity of *E. cochranae* is expected due to its smaller body size resulting in less body muscles used to call. In order to test this hypothesis, a direct measurement of sound intensity of the calls of these species should be conducted.

The advertisement calls of *E. brasiliensis* and *E. cochranae* revealed notable differences in acoustic parameters, mainly in temporal characters such as number of pulses and repetition rate of pulses which did not overlap.

*Taxonomic Remarks*

The great morphological similarity between *E. brasiliensis* and *E. cochranae* raised doubts about distinction at species level, even though the few morphological features which distinguish the species can be constant (Izecksohn 1988). However, the present study shows that acoustic signals of advertisement call of the species indeed provide us with useful characters for species determination (cf. Barrio 1964), corroborating their full species status (e.g. Angulo *et al*. 2003; Kwet 2006; Kwet 2007), though more studies are needed to survey new morphological characters to this end.

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