

Advertisement call and diel pattern of *Pseudis platensis* (Anura, Hylidae, Pseudinae) in the Brazilian Pantanal and a bioacoustical comparison with *Pseudis paradoxa*

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Abstract

Anuran advertisement calls are life history traits associated with sexual selection and reproduction, and they play an important role in premating isolation mechanisms between species. Comparative bioacoustical analyses of these calls can be useful for taxonomic purposes. Phylogenetic relationships within the genera *Pseudis* are controversial, especially those related to *P. platensis*, which is often considered a full species or a subspecies within *P. paradoxa*. The advertisement call of *P. platensis* has not been described in detail; therefore, call comparisons of these two conspecifics have not been possible. In this study, we describe the advertisement call and diel activity pattern of *P. platensis* in the Brazilian Pantanal and compare the calls of *P. platensis* and *P. paradoxa* to elucidate whether acoustical parameters may help resolve the present uncertain taxonomic treatment. The advertisement call of *P. platensis* lasted 0.21 ± 0.01 s and consisted of a short series of 8.05 ± 0.69 distinct pulses (9.34 ± 2.94 ms of pulse duration). The vocal activity of the species was largely restricted to a few hours after dusk (7–11 p.m.), which is inconsistent with the diurnal and nocturnal calling behavior described for other *Pseudis* congeners. The advertisement calls of *P. platensis* and *P. paradoxa* were similar in note duration, number of pulses per note and dominant frequency. The geographic variation of calls within species was similar to that found among species. This bioacoustical comparison does not provide new insights into whether *P. platensis* should be considered a distinct species.

Keywords: Activity pattern, acoustic monitoring, calling activity, frog, Pseudinae

Introduction

Anurans base their communication mainly on acoustical signals because of their cryptic and mostly nocturnal behavior (Wells 1977). Among anuran vocalizations, the advertisement call is the sound signal most frequently uttered by males and is by far the most studied (Guerra *et al.* 2018). Advertisement calls are primarily emitted during the breeding season in a reproductive context to attract females but can also serve to segregate calling males (Toledo *et al.* 2015). Moreover, such calls may act as a prezygotic isolation mechanism between species (Hoskin *et al.* 2005); thus, these calls are the most adequate acoustical signature for taxonomic purposes (Köhler *et al.* 2017). Indeed, comparative bioacoustical analyses have been employed to support species delimitation in anurans (Glaw & Vences 1991; Köhler *et al.* 2005).

Despite the exponential increase in the number of studies describing the calls of anurans, clear pitfalls are still observed (Guerra *et al.* 2018). For example, a recent review focusing on Brazilian anurans (the country with the largest number of anurans in the world) highlighted that 31% of the species had no published descriptions of their advertisement calls (Guerra *et al.* 2018). Among the Brazilian anurans with no call description in this review was *Pseudis platensis* Gallardo, 1961 (but see Barrio 1970 and Santana *et al.* 2016). It is a member of Hylidae, the family with the largest number of advertisement calls described (Guerra *et al.* 2018). This finding is surprising when

considering that *P. platensis* is a well-studied species (Fabrezi & Goldberg 2009; Fabrezi *et al.* 2009; Campião *et al.* 2010; Ceron *et al.* 2017) and has a large distribution range (Frost 2019). Nonetheless, Barrio (1970) provided information about the advertisement call of *P. platensis* from Santa Fe Province (Argentina), but he did not provide any information about some call parameters, such as pulse duration, bandwidth, lower and higher frequency, and number of calls or individuals measured. Additionally, Santana *et al.* (2016) described the calls of many populations of *Pseudis paradoxa* attributable to *P. platensis*, but the species was not considered a full species (see below). The diel activity pattern of the species remains unknown, but is essential for conducting field surveys when the detection probability is highest to maximize the cost effectiveness of monitoring programs (Dorcas *et al.* 2009).

According to Frost (2019), the genus *Pseudis* contains seven aquatic species (*P. bolbodactyla*, *P. cardosoi*, *P. fusca*, *P. minuta*, *P. paradoxa*, *P. platensis*, and *P. tocantins*), which are widespread in South American lowlands (Garda *et al.* 2010). However, the monophyly of the genus and the species status of *P. platensis* are controversial (Aguiar Jr. *et al.* 2007, Garda and Cannatella 2007). Some authors consider *P. platensis* a full species according to genetic analyses and morphological differences of adults and larvae with other *Pseudis* (Barg *et al.* 2003; Aguilar Jr *et al.* 2007). However, some authors have shown that *P. platensis* cannot be distinguished from *P. paradoxa* Linnaeus, 1758, based on molecular phylogenies (Garda and Cannatella 2007, Wiens *et al.* 2010), morphological differences among adults (Garda *et al.* 2010), and tadpole external morphology (Santana *et al.* 2016). Santana *et al.* (2016) described and compared tadpoles and published a bioacoustical comparison of the calls of *Pseudis* to identify acoustic characters that may help identify species within this group. These authors followed the nomenclature proposed by Garda *et al.* (2010); therefore, they did not consider *P. platensis* a full species and did not compare calls of *P. platensis* statistically against populations of *P. paradoxa*. Both species present an allopatric distribution, with *P. platensis* inhabiting northeastern Argentina, Paraguay, and the southern parts of Bolivia and Brazil (south of the Amazon Valley), while *P. paradoxa* is found in Trinidad and Tobago, Guianas (from Venezuela to French Guiana), Colombia, Peru, and the northern sections of Bolivia and Brazil (north of the Amazon Valley) (Frost 2019).

In this paper, we performed a detailed description of the advertisement call and diel activity pattern of *P. platensis* in the Brazilian Pantanal. We hope our results will increase knowledge about the calling behavior of the species and be useful for future studies about when and how to detect *P. platensis* in the field. We also compared, for the first time, the advertisement calls of *P. platensis* and *P. paradoxa*, hoping to provide insights into the taxonomic issues regarding the recognition of the species. We did not include other *Pseudis* species in the comparison because a recent review on this topic can be found in Santana *et al.* (2016).

Methods

Study area

The study was carried out in the northeastern part of the Pantanal Matogrossense National Park close to Pouso Alegre Lodge (Poconé locality, Mato Grosso, Brazil; 16°30'S, 56°45'W, Supplemental Figure 1). The Pantanal is the largest wetlands in the world, and up to 43 anuran species can be found on the Brazilian side (Strüssmann *et al.* 2007). The study area was located on the floodplain of the Cuiabá River, which is one of the main tributaries of the Paraguay River within the Pantanal. The Pantanal is seasonally inundated by the increase in the flood pulse of the Paraguay River (Junk *et al.* 2006); therefore, there is a marked seasonality with a pronounced dry season from May to September and a rainy season from October to April (Junk *et al.* 2006). The study area represented a mosaic of different forest formations and savannas. The climate in the region is tropical humid, with an average annual rainfall between 1,000 and 1,500 mm and a mean annual temperature of approximately 24°C (Junk *et al.* 2006).

Acoustic monitoring and analyses

We performed daily monitoring on 13 consecutive days from 20 January (starting hour: 10 p.m.) to 2 February 2013 (ending time: 9:15 p.m.) (rainy season) by using one Song Meter SM2 recorder (Wildlife Acoustics, USA, www.wildlifeacoustics.com), which was equipped with two omnidirectional microphones. The recorder was programmed to record (in stereo and .wav format) the first 15 minutes of each hour in 24/7 mode following the winter local time (GMT -4) and was configured with a sampling rate of 48 kHz and a resolution of 16 bits per sample. The SM2 recorder included an integrated data-logger that continuously logged air temperature every five minutes.

The left channel recordings were automatically scanned by using Kaleidoscope Pro 5.1.8. (Wildlife Acoustics, USA, www.wildlifeacoustics.com). This software is able to identify a target signal by using the signal parameters of the sounds that the user aims to detect. The signal parameters required to be introduced in Kaleidoscope are as follows: minimum and maximum frequency range (Hz), minimum and maximum length of detection (s) and maximum intersyllable gap (s), defined as the maximum allowable gap between syllables. We used some advertisement calls recorded during the study period and classified as *P. platensis* before starting a previous study (Schuchmann *et al.* 2014) to introduce accurate signal parameters into Kaleidoscope. In the following link we provide a compilation of sixteen snippets of *Pseudis platensis* and a 14-min recording length with several calls of the species made during the study period (http://cobra.ic.ufmt.br/?page_id=480). The signal parameters introduced were as follows: minimum and maximum frequency range (1,250–2,750 Hz), minimum and maximum length of detection (0.15–0.4 s) and maximum intersyllable gap (0.4 s). These signal parameters were used to maximize the possibility of detecting weaker signals of the species. We fitted the “distance from cluster center to include outputs in cluster.csv” as 2. This distance ranges from 0 to 2 and has an impact on the number of detected signals. The larger the value is, the larger the number of possible calls detected (and therefore of false positives). We used the largest possible value to detect the largest number of *P. platensis* calls.

The Kaleidoscope output reported a total of 179,716 events that matched the signal parameters. All events were visually and/or acoustically checked by the same observer (CPG) to separate false and true positives (correct detections made by Kaleidoscope). A total of 22,071 events were classified as *P. platensis* advertisement calls and used in posterior analyses.

Advertisement call measurement

While checking events in Kaleidoscope, recordings were labeled as containing high-quality advertisement calls (no background noise, no rain, etc.) according to visual and aural inspection. Among them, we selected eight recordings made on six different days (a maximum of two recordings on the same day) during dusk choruses, the maximum activity period of the species. For each recording, we measured a mean number of 7.6 ± 3.0 (SD) advertisement calls (range 3–11), for a total of 61 calls (489 pulses) measured. We were unable to precise the number of different males that emitted the measured calls.

The bioacoustic parameters were measured using Raven Pro 1.5 (Bioacoustic Research Program 2014) with the following configuration: Hamming window function; 71% brightness; 79% contrast; 256 point DFT size; and 50% time grid overlap. Spectral traits were measured from spectrograms: the dominant frequency was quantified using the Peak Frequency measurement function; lower and higher frequencies using the Frequency 5% and Frequency 95% functions and bandwidth using the Bandwidth 90% function. Temporal traits were measured from oscillograms. We measured call duration (s), pulse duration and interpulse intervals (ms). We followed the terminology recommended by Köhler *et al.* (2017) for call descriptions of anurans. However, because we cannot be sure whether calls recorded were uttered by one or more individuals, we did not include descriptions of the duration of internote intervals and note repetition rate (Köhler *et al.* 2017). Although calls measured within each recording could have been emitted by different individuals, bioacoustic parameters were averaged per recording to represent the smallest statistical unit of the analyses. Temporal and spectral measurements are presented as the mean $\pm$ standard deviation and the range (minimum and maximum values for all calls/pulses).

Date, time, mean air temperature (°C), and mean air humidity (%) for each recording analyzed can be found in Supplemental Table 1.

Advertisement call comparison

We compiled descriptions of the calls of *P. paradoxa* (Bosch *et al.* 1996; Tárrano 2010; Santana *et al.* 2016) and *P. platensis* (Barrio 1970) from the literature. Some of the *P. paradoxa* recordings of Santana *et al.* (2016) were classified as *P. platensis* for comparison according to the distribution range of the species (Frost 2019) (see Supplemental Table 2). For taxonomic purposes, we only considered acoustical parameters that could be homologically compared between species (note duration, dominant frequency and number of pulses per note) (Santana *et al.* 2016; Köhler *et al.* 2017). Differences in acoustic parameters between species were tested by one-way analysis of variance using

the recording location as an independent unit. Statistical analyses were performed using R 3.4.1 (R Development Core Team 2014).

Results

Advertisement call of Pseudis platensis

The advertisement call of *P. platensis* consisted of a short series of distinct pulses separated by silent intervals (Fig. 1). We referred to this train of pulses as a note. Notes were composed of 8.05 ± 0.69 distinct pulses (7.33–9.57, $n=61$). The note duration lasted 0.211 ± 0.019 s (0.185–0.265, $n=61$), while the pulse duration was 9.34 ± 2.94 ms (6.85–13.95, $n=489$). The interpulse intervals were 9.03 ± 3.21 ms (4.39–23.34, $n=423$). The average call dominant frequency was $1,991 \pm 208$ Hz (1,344–2,422, $n=61$), the lower frequency was 963 ± 163 Hz (594–1,250, $n=61$) and the higher frequency was $2,324 \pm 159$ Hz (1,562–2,891, $n=61$). The bandwidth was $1,360 \pm 173$ Hz (969–1,828, $n=61$). Notes had hardly noticeable harmonics in the 5,000–5,500 Hz frequency band.

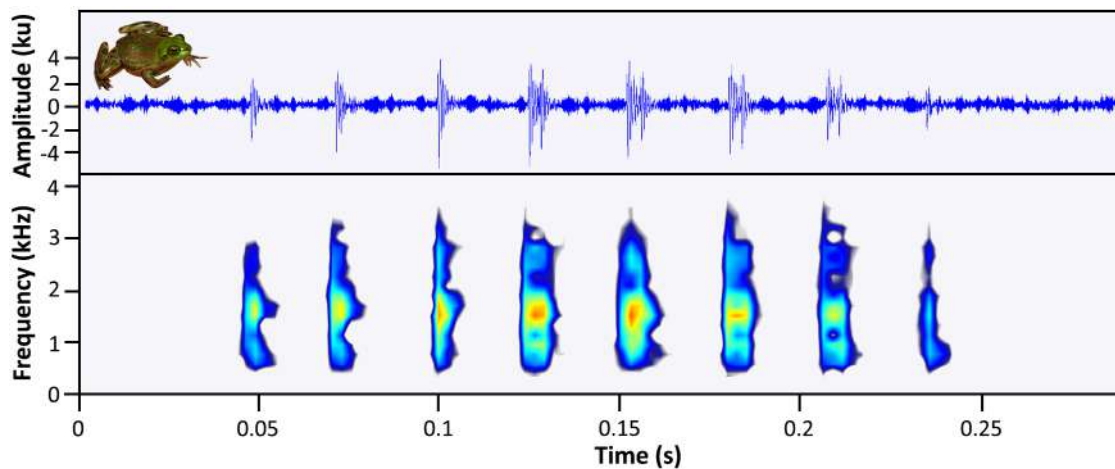


FIGURE 1. Oscillogram (Amplitude, ku) and audiospectrogram (Frequency range, kHz) of the advertisement call of *Pseudis platensis* recorded on 27 January 2013 in the Pantanal Matogrossense National Park, municipality of Poconé, state of Mato Grosso, Brazil. The call was recorded using a Song Meter SM2 recorder at 7:15 p.m., and the mean air temperature was 20°C. The figure was created using Raven Pro 1.5 with the following configuration: Hamming window function; 64% brightness; 74% contrast; 256 point DFT size; 50% time grid overlap; and Jet Black color scheme.

Diel activity pattern

The species was detected on all monitored days. The diel activity pattern of *P. platensis* in the northeastern part of the Pantanal Matogrossense National Park was concentrated in a few hours after dusk, with 93.8% of the total calls detected between 7 and 11 p.m. (Fig. 2). The hour with the highest calling activity of the species was 9 p.m., with 27.7% of the total calls detected during this hour-long period. Mean temperature during the study period at 9 p.m. was 28.2 ± 1.6 °C (Mean $\pm$ SD). The species was almost vocally inactive during the rest of the day, with only 3.1% of the calls detected between midnight and 5 p.m. (Fig. 2), when the mean temperature was 29.6 ± 3.7 °C.

Advertisement call comparison

The acoustic parameters were similar for both species. The mean note duration was identical (0.235 and 0.236 s for *P. platensis* and *P. paradoxa*, respectively), with no differences between them (ANOVA, $F_{1,11} = 0.004$, $P = 0.95$). Although far from significant, the notes of *P. platensis* had, on average, lower dominant frequencies (2,212 vs 2,379; ANOVA, $F_{1,11} = 1.28$, $P = 0.28$) and number of pulses (11.32 vs 12.37; ANOVA, $F_{1,11} = 0.42$, $P = 0.53$) than the notes of *P. paradoxa*. The mean values of the acoustic parameters for each recording location were plotted on a 3-D graph, and the results did not show a clear separation between the advertisement calls of both species (Fig. 3). We found

that the variation of acoustic parameters was more similar among subpopulations of the same species than between populations of *P. platensis* and *P. paradoxa* (Fig. 3).

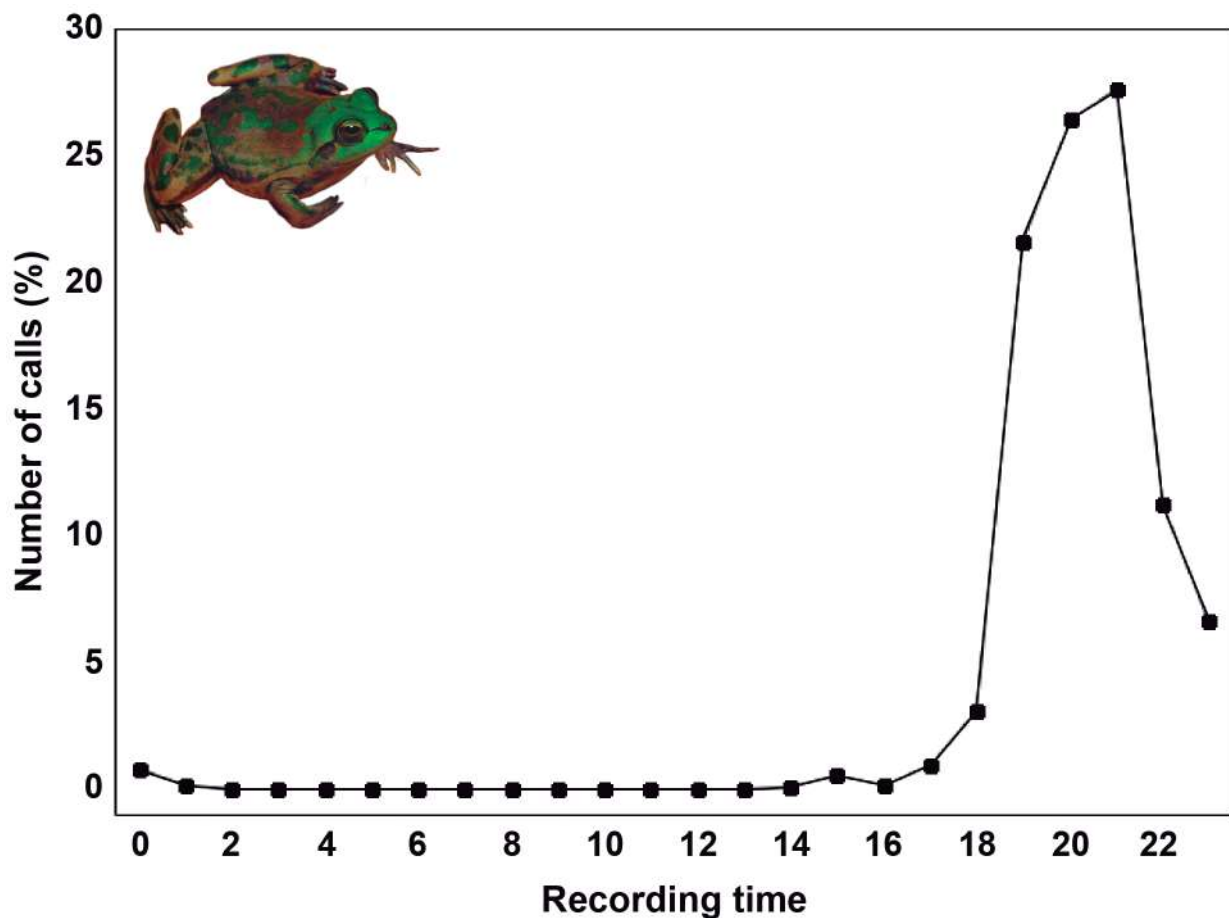


FIGURE 2. Diel pattern of calling activity of *Pseudis platensis* in the Pantanal Matogrossense National Park (Poconé municipality, Mato Grosso, Brazil). Calling activity was measured by acoustic monitoring from 20 January to 2 February 2014 at one site. The diel pattern is expressed as a mean percentage of calls detected at each recording time. Hours are expressed in winter local time (GMT -4). Photo: Mauro Teixeira Jr. © (Cáceres, Mato Grosso, Brazil).

Discussion

In this study, we provide the first detailed description of the advertisement call and diel calling pattern of *P. platensis* during 13 consecutive days. A prior description of the advertisement call of the species can be found in the literature, although little information about the call parameters was provided (Barrio 1970). The average note duration and number of pulses per note of *P. platensis* in the Brazilian Pantanal (0.21 and 8.1, respectively) were lower than the values reported by Barrio (1970) from Argentina (0.3 and 9.5, respectively). No other parameters were available for comparison. When considering the populations of *P. platensis* recorded by Santana *et al.* (2016), we found large differences between subpopulations of the species (Supplemental Table 2). It is well known that the calls of frogs may vary greatly between populations and even between individuals according to a multitude of factors, such as temperature and body size (Fine 1978, Bee *et al.* 2001). This phenomenon has also been found within the genus *Pseudis*. Kwet (2000) found that calls of *P. cardosoi* were more prolonged at lower temperatures. Intraspecific variation due to external factors usually refers to subtle differences in quantitative variables, although significant variations can also be found between those acoustical parameters considered more stable (Köhler *et al.* 2017). We cannot conclude whether the differences found between *P. platensis* subpopulations were related to uncontrolled factors between studies. However, the large differences found among sites suggest the existence of geographical variations between subpopulations of the species, as has been found for a large number of anurans, such as the Oyster Toadfish (*Opsanus tau*, Fine 1978) and the Green Toad (*Bufo viridis*, Castellano *et al.* 2000).

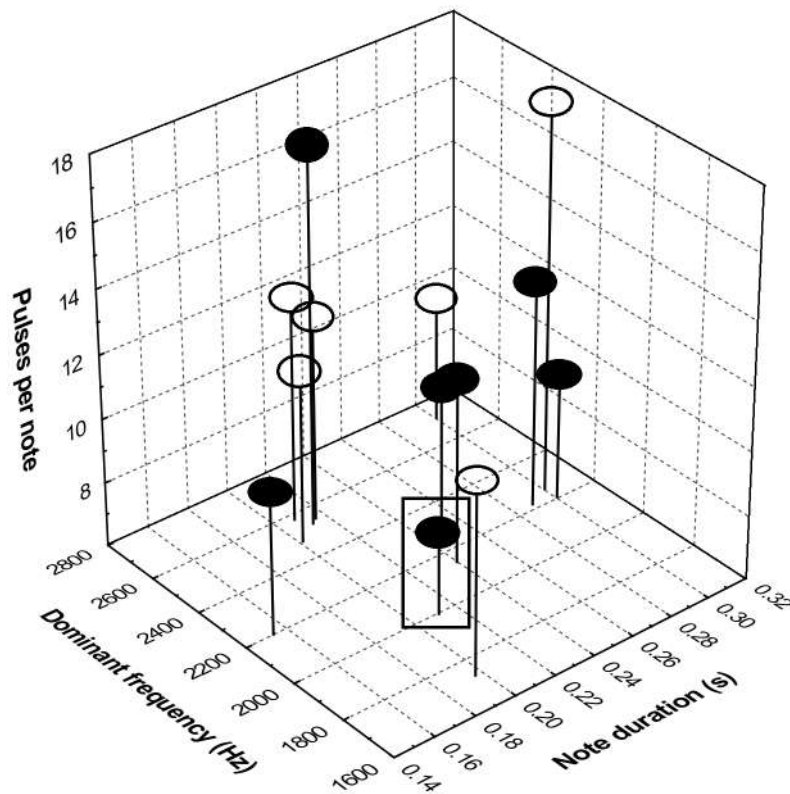


FIGURE 3. 3-D graph showing the distribution of *Pseudis platensis* (black circles) and *Pseudis paradoxa* (empty circles) advertisement calls according to three acoustical parameters. The rectangle highlights the position of the advertisement call described in the present work.

The structure of the advertisement call of *P. platensis* is pulsioned, which is similar to those emitted by other *Pseudis* species (Santana *et al.* 2016) and those of the related genus *Lysapsus* (Santana *et al.* 2013). This result is not surprising since closely related species often have advertisement calls with similar general structures due to joint evolutionary history (Goicoechea *et al.* 2010). Accordingly, our comparison of the advertisement calls of several populations of *P. platensis* and *P. paradoxa* showed that there are no differences between species for any of the three call parameters considered. We found that geographical variation between subpopulations of each species was as large as differences among species. Santana *et al.* (2016) indicated that the advertisement calls of *P. bolbodactyla*, *P. fusca*, and *P. paradoxa* were acoustically indistinguishable, although these species differ morphologically and do not form a monophyletic group within the genus. Therefore, the similar call structures observed for *P. platensis* and *P. paradoxa*, which represent closely related species, were to be expected.

This study is the first to analyze the diel calling activity pattern of *P. platensis*. The vocal activity of the species was almost restricted to the five hours after dusk, which is consistent with the typical diel vocalization cycles described for most anuran species (e.g., Bridges and Dorcas 2000, Akmentins *et al.* 2015). Higher vocal output at night has been suggested to be an anuran adaptation related to avoiding visual predators and skin desiccation (Pechmann and Semlitsch 1986, Santos and Grant 2011). However, it is inconsistent with the common vocal behavior of the genus *Pseudis*. Brandão *et al.* (2003) recorded diurnal and nocturnal calling activity for *P. bolbodactyla* (see also Silva *et al.* 2007) and indicated that this behavior is common for the genus based on the similar diel calling pattern found for *P. cardosoi* (Kwet 2000), *P. tocantins* (Brandão and Pères Jr. 2001), and *P. paradoxa* (Dixon *et al.* 1995). The presence of diurnal vocalizations in our dataset was anecdotal. This different calling behavior seems to be the only vocal parameter of *P. platensis* that differs from its congeners. Nonetheless, we are aware that our study was restricted to a short period (13 days) of time during the rainy season, and therefore, the diel activity pattern shown here might not be representative of the calling behavior of the species throughout the year. However, previous studies did not provide any detailed vocalization cycle (e.g., calls uttered per hour), and therefore, no direct comparison among species can be made. Further research using the same methodology in different periods of the year is needed to corroborate whether the diel calling activity pattern of *P. platensis* differs from that of other *Pseudis*.

Our description of the advertisement call of *P. platensis* was made using automated recorders and an omnidirectional microphone; therefore, we have no ability to determine individual identity or distance to recorder. Future studies should be performed using directional microphones and should record individual frogs and measure calling parameters that we did not include in this study, such as internote intervals and note repetition rate. However, we believe that our call description and bioacoustical comparison are not biased for the technique applied since only high-quality recordings were used. High-quality recordings obtained using the SM2 recorder have proven to be useful even for identifying bird individuals according to their acoustic signatures (Ehnes and Foote 2015). Our acoustical comparison did not provide new insights into whether *P. platensis* should be considered a full species. The study and comparison of advertisement calls between closely related species should be considered an independent line of evidence (together with the study of the external morphology, tadpoles or genetic analyses) within the integrative taxonomic approach, which may help to elucidate taxonomic issues regarding the recognition of anuran species (Padial and De la Riva 2009). The diel calling pattern of *P. platensis* in the Pantanal was different from what has been published for other *Pseudis* and was the only vocal parameter that differed from those described for *P. paradoxa*. Further research should be performed to precisely monitor both species in identical periods across the year and, if possible, compare the environmental conditions to unravel whether the calling behavior of *P. platensis* differs from that of *P. paradoxa*.

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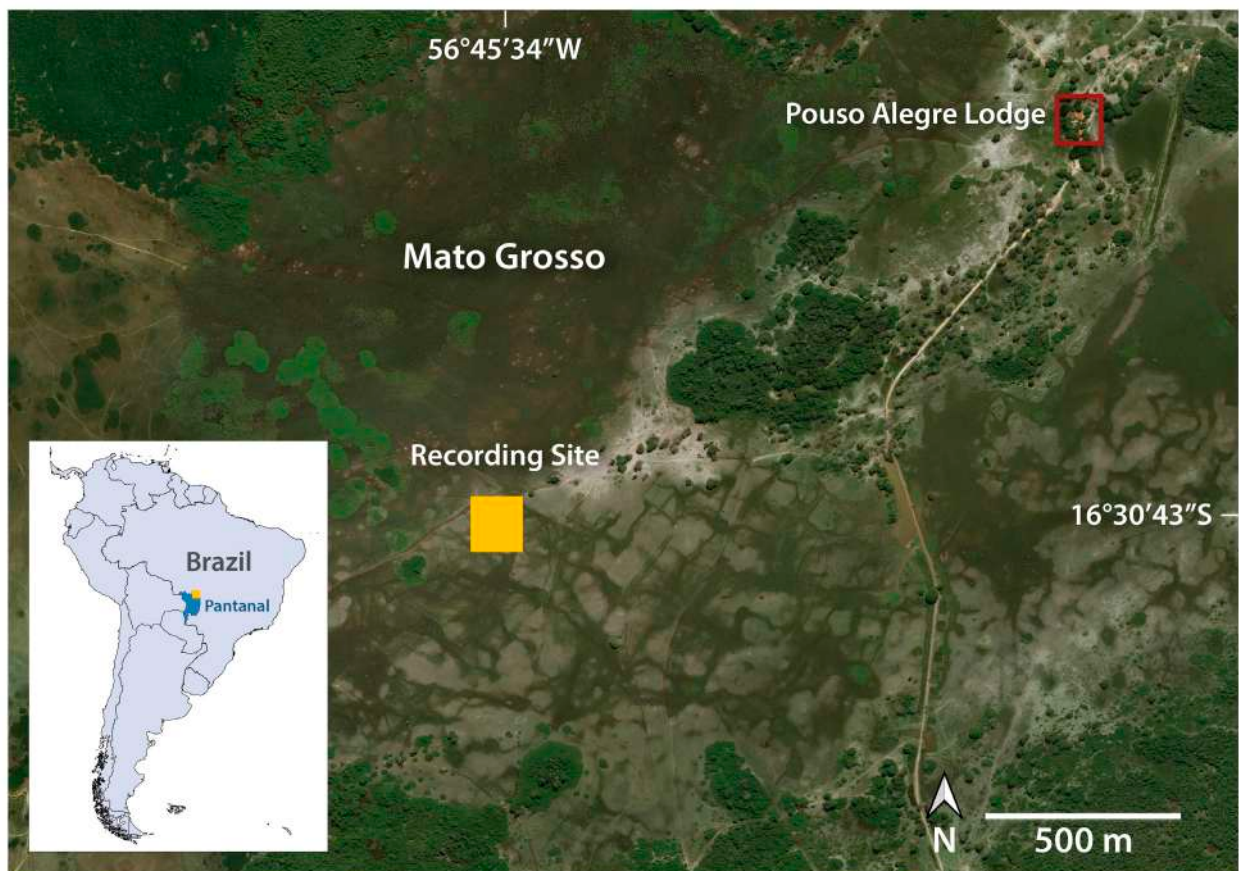
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SUPPLEMENTAL FIGURE 1: Location of the study area (16°30'S, 56°45'W) in the Pantanal Matogrossense National Park, Poconé municipality, Mato Grosso, Brazil. Pouso Alegre Lodge location is also shown. The inset shows the location of the study area (yellow square) in Brazil. Picture was extracted from Bing Satellite. Scale bar: 500 m.

SUPPLEMENTAL TABLE 1: Recording date, starting recording time, and number of calls measured for each recording considered in the study. Air temperature (°C) was measured using an integrated data-logger of the Song Meter SM2 recorder. Air humidity (%) was extracted from a weather station in the study area. Air humidity of the 29 of January was not recorded due to a failure of the weather station.

Recording	Calls	Date	Time	Air temperature (°C)	Air humidity (%)
1	11	21/01/2013	19 p.m.	26.9	89.8
2	11	21/01/2013	20 p.m.	26.4	90.4
3	6	22/01/2013	23 p.m.	27.7	84.3
4	3	24/01/2013	23 p.m.	29.0	91
5	7	25/01/2013	21 p.m.	27.2	90.4
6	11	27/01/2013	19 p.m.	30.8	79.0
7	6	27/01/2013	20 p.m.	30.2	87.5
8	6	29/01/2013	19 p.m.	31.3	-

SUPPLEMENTAL TABLE 2. Mean acoustical parameters for advertisement calls of *Pseudis paradoxa* and *Pseudis platensis* used in the study. Asterisks highlight cases in which the species was classified as *Pseudis paradoxa* but we considered it *Pseudis platensis* for comparison purposes, with the findings based on the distribution range of the species (Frost 2019).

Species	Locality	Dominant Frequency (Hz)	Duration (s)	Number of pulses/note	Reference
<i>P. paradoxa</i>	Santa Cruz/Bolivia	2711	0.3	9.5	Bosch et al. (1996)
<i>P. paradoxa</i>	Guárico State /Venezuela	2540	0.215	12	Tárano (2010)
<i>P. paradoxa</i>	Porto Velho /RO/Brazil	2476	0.201	10.9	Santana et al. (2016)
<i>P. paradoxa</i>	Pinheiro/MA/Brazil	1728	0.196	11.6	Santana et al. (2016)
<i>P. paradoxa</i>	Sucre/Venezuela	2250	0.3	17.6	Santana et al. (2016)
<i>P. paradoxa</i>	Apure/Mantecal /Venezuela	2571	0.208	12.6	Santana et al. (2016)
<i>P. platensis</i>	Santa Fé/Argentina	2200	0.3	9.5	Barrio (1970)
<i>P. platensis</i> *	Bela Vista/MS/Brazil	2524	0.212	17.2	Santana et al. (2016)
<i>P. platensis</i> *	Jaguaríuna/SP/Brazil	2196	0.153	10	Santana et al. (2016)
<i>P. platensis</i> *	Luis Antônio/SP/Brazil	2220	0.241	10.6	Santana et al. (2016)
<i>P. platensis</i> *	Poconé/MT/Brazil	2217	0.289	12.6	Santana et al. (2016)
<i>P. platensis</i> *	Corumbá/MT/Brazil	2141	0.239	11.3	Santana et al. (2016)
<i>P. platensis</i>	Poconé/MT/Brazil	1991	0.211	8.1	Present work