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First record of the genus *Promops* (Chiroptera, Molossidae) in the state of Rio de Janeiro, Brazil

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ABSTRACT
Bioacoustic data have been expanding knowledge on bat species. The objectives of this work were to describe the first occurrence of *Promops* at the state of Rio de Janeiro, to provide data on the acoustic parameters found at Ilha Grande, and to provide schedules of the species' foraging feeding activity. We had a high capture effort with mist nets, but we did not have any capture of *P. centralis,* for which we obtained acoustics recordings. We increased the number of species recorded for the state of Rio de Janeiro up to 79. This study reassures the importance of using bioacoustic methods alongside mist nets on fieldwork.

Key words: Activity time, Atlantic Forest, bioacoustic parameters, echolocation calls, *Promops centralis.*

RESUMO — Primeiro registro do gênero *Promops* (Chiroptera, Molossidae) para o Estado do Rio de Janeiro, Brasil.
Dados bioacústicos têm expandido o conhecimento de espécies de morcegos. Os objetivos foram registrar a primeira ocorrência de *Promops* no estado do Rio de Janeiro, fornecer dados dos parâmetros acústicos encontrados na Ilha Grande assim como dos horários da atividade de alimentação. Tivemos um grande esforço de captura com redes de neblina, mas não tivemos nenhuma captura de *P. centralis*, no entanto, obtivemos registros acústicos. Aumentamos o número de espécies registradas para o estado do Rio de Janeiro para 79. Este estudo reafirma a importância do uso de métodos bioacústicas ao lado de redes de neblina no trabalho de campo.

Palavras-chave: Ecolocalização, horário de atividade, Mata Atlântica, parâmetros bioacústicos, *Promops centralis*.

Promops Gervais, 1856 is one of the bat genera from the subfamily Molossinae (Molossidae) present in the Southern Hemisphere which fly high, and are fast and strong flyers able to catch insects with their wings (Gardner 2008). Like all aerial insectivorous bats, *Promops* bats use echolocation both to navigate and during foraging (Jung et al. 2014; Hintze et al. 2020). Its echolocation calls present two main types, which can alternate irregularly: (1) upward modulated frequency calls, and

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(2) downward modulated frequency calls. They are also easily recognizable to the species level by the frequencies used (Jung et al. 2014; Hintze et al. 2020). *Promops centralis* is distinguished from *P. nasutus* by its maximum energy frequency and call duration (Jung et al. 2014; Arias-Aguilar et al. 2018): *P. centralis* typically emits low frequency calls of long duration, while *P. nasutus* emits higher frequency calls of short duration. Therefore, although *P. centralis* and *P. nasutus* are sympatric in many regions within their ranges, differences in their calls allow for an easy way to separate them (Hintze et al. 2020).

Two of the three species of the genus *Promops* have records in Brazil: *Promops centralis* Thomas, 1915 and *Promops nasutus* (Spix, 1823) (Garbino et al. 2020), while *Promops davisoni* Thomas, 1921 is restricted to Peru, Ecuador, and Chile (Gregorin & Chiquito 2010; Ossa et al. 2018). *Promops nasutus* has a wide distribution range in Brazil, with several capture records (Ramos et al. 2013). *Promops centralis* is restricted to few capture records at the states of Acre (Nogueira et al. 1999), Amazonas (Barnett et al. 2006), Mato Grosso do Sul (Cáceres et al. 2008; Fischer et al. 2015), and Pará (Gregorin & Taddei 2000). New occurrences of *P. centralis* were recorded in Brazil with confirmed bioacoustic data at the states of Espírito Santo, Goiás, Minas Gerais, Paraíba, Pernambuco, Rio Grande do Norte, Rio Grande do Sul, and Tocantins (Silva & Bernard 2017; Hintze et al. 2020). Both species are categorized as Least Concern by the International Union for the Conservation of Nature's (IUCN) Red List of Threatened Species (Barquez & Diaz 2015; Solari 2019), but are not listed on the official list of endangered fauna of Brazil because they are not endangered (ICMBio 2018).

Information regarding bat distribution and richness is important, as they are known to provide important ecosystem services (Kunz et al. 2011). Studies with bioacoustic data have been increasing the knowledge of several bat species' distribution, mostly insectivorous, which are harder to capture by mist nets than herbivorous species, adding information about many species (Sampaio et al. 2003; González-Terrazas et al. 2016; Silva & Bernard 2017; Hintze et al. 2020). Acoustic methods, along with mist nets, are considered essential to improve bat studies, mainly because of the subsampling of insectivore bat taxa (e.g., Murray et al. 1999; O'Farrell & Gannon 1999; Duffy et al. 2000; Sampaio et al. 2003; MacSwiney et al. 2008; Silva & Bernard 2017).

Brazilian Ilha Grande is one of the areas with the highest number of recorded bat species (n= 36) in the state of Rio de Janeiro (Costa et al. 2021). Until now, 10 species of insectivorous bats from the families Emballonuridae, Furipteridae, Molossidae, and Vespertilionidae were known to occur on the island, captured by mist nets or at their daytime shelters (Costa et al. 2021). Between 2014 and 2018, we sampled the island using mist nets and bioacoustic methods, to increase the possible detection of insectivorous species.

The objectives of this work were to describe the first occurrence of *P. centralis* in the state of Rio de Janeiro, to provide data on acoustic parameters for the species found in the state of Rio de Janeiro, and to provide its feeding activity times.

Ilha Grande is located in the municipality of Angra dos Reis, Rio de Janeiro State (Brazil), and is an island with an area of about 190 km^2 and rugged relief (Oliveira 2002). The climate is tropical and humid, with a mean temperature of 22 °C, and an annual rainfall over 2,200 mm, being January the rainiest month and July the driest (INEA 2011). The island consists of predominantly dense evergreen ombrophilous forest, and to a lesser extent sandbank vegetation, mangrove (Oliveira & Coelho-Netto 2000), and rocky outcrops. Ilha Grande is one of the most well-preserved spots of Atlantic Forest in Brazil, and is considered an ecologic sanctuary, included in the Atlantic Forest Biosphere Reserve by UNESCO since 1992 (Davis et al. 1997; Guedes-Bruni & Lima 1997), and a World Heritage Site together with Paraty (https://whc.unesco.org/en/list/1308/).

We adapted our survey to a long-term ecological research methodology (RAPELD) (Magnusson et al. 2005). In the first module we sampled ten plots located at the Ilha Grande State Park between two villages (Vila do Abraão and Vila Dois Rios), and in the second module we sampled four plots located at the Biological Reserve of Praia do Sul between two beaches (Praia Longa and Praia do Sul) (Fig. 1). The first module was inside an Ombrophilous Dense Submontane and Montane Forest (Veloso et al. 1991), whereas the second module was inside an area of Ombrophilous Submontane Forest, sandbank vegetation, and mangrove. The plots sampled were located at different altitudes, from sea level to 692 m a.s.l.

We conducted fieldwork between December 2014 and August 2018, with a total of 56 sampled nights, using the 14 plots as sampling units for recording bat echolocation calls. The calls, emitted by bat species during flight while foraging nearby, were recorded using a Wildlife Acoustics SM2BAT+ (www.wildlifeacoustics.com). We configured the SM2BAT+ to record automatically with a sampling rate of 384 kHz, to trigger to sounds above 12 dB, gain 0.0 dB and echo filter threshold 5 dB, beginning at sunset and stopping at sunrise; recorders were set to record 3 min with 12 min intervals. To improve the recording of bat calls, we also positioned microphones with an angle of 45° from the ground, and at 1.5 meters. In addition to the acoustic sampling, we set 13 mist nets (10 x 3 m, 19 mm mesh, by Ecotone® 719/10) every night in each plot, from sunset to sunrise. Research followed guidelines for collections, and was approved by the institutional animal care and use committee (Permanent IBAMA Capture License no. 12548 and 10361; SISBIO no. 45702-4; INEA no. 63/2015; CEUA: no. 008/2018).

For a preliminary acoustical analysis we used CallViewer18, a MATLAB based software (Skowronski & Fenton 2008). The parameters of the spectrogram were set to a Fourier quick transformation of size 1024, we used Hamming windows and the length of the windows was fixed at 1 ms, and the threshold to 10 dB. Later, we used the software Bat Sound v.3.31 (Pettersson Elektronik AB, Uppsala, Sweden) to manually obtain five variables for each detected call: the interval between pulses (in ms), the total duration of the call (ms), the minimum frequency observed (kHz), the maximum frequency observed (kHz), the maximum energy frequency (kHz). For this study, we adopted the same acoustic variables used by Hintze et al. (2020). We also used the software Raven Pro 1.6 (Cornell Lab of Ornithology 2014) to obtain the sonogram, and built the spectrograms using Hamming windows, 1024 DFT size, and 96% overlap.

We calculated the mean and standard deviation of the analyzed variables (mean ± standard deviation): inter-pulse interval, call duration, minimum frequency, frequency with maximum energy, maximum frequency and duty cycle. Only sequences with a minimum of three pulses were analyzed (Lloyd et al. 2006; Ratcliffe et al. 2011), feeding buzzes and the immediate pulses before and after were not analyzed. Species identification was performed according to the available literature for the region (e.g., Barataud et al. 2013; Jung et al. 2014; Arias-Aguilar et al. 2018; Hintze et al. 2020). The recordings described in this paper can be accessed at the sound library of the Brazilian Society of Chiroptera Studies (SBEQ) at the website https://www.sbeq.net/bioacustica in Atlantic Forest (Mata Atlântica), records 231 to 234. The recorder automatically registers the recording time, but since there is a significant variation of the sunset hour depending on the season, we converted the recording time into minutes after sunset (MAS). To precisely determine the sunset time, we used the software Moonphase 3.3 software (Tingstrom 2009).

After a mist-netting effort of 257,790 h*m^2, we registered no captures of *P. centralis* with this methodology. However, we obtained acoustic recordings of *P. centralis* at one of the plots (latitude -23.176666; longitude -44.298055) (Fig. 1) on the west side of the island, in sandbank vegetation. We recovered three acoustic sequences of *P. centralis* during sampling night number 41, on October 12th, 2016, and one sequence during sampling night number 52, on November 26th, 2017 (Table 1). The activity we registered varied from 45 minutes to 10 hours after sunset. We also recorded a feeding buzz at 18:45h, 45 minutes after sunset (Fig. 2b; Table 1).

The values of all variables (inter-pulse interval, call duration, minimum frequency, frequency with maximum energy, maximum frequency and duty cycle) are represented as mean and standard deviation (minimum and maximum) (Table 2). All calls recorded in this study presented almost the same call type, structure, and pattern (Fig. 2a, b, c).

This is the first record of *P. centralis* for Rio de Janeiro State, increasing the number of species recorded in the state to 79 (Peracchi & Nogueira 2010; Moratelli et al. 2011; Dias et al. 2013; Delciellos et al. 2018). This record is approximately 235 km from the nearest previous record in municipality Ijaci, Minas Gerais State (Fig. 1) (Hintze et al. 2020). The records of *P. centralis* in the state of Rio de Janeiro was expected, based on a species distribution modeling (SDM) study (Hintze et al. 2020). Using fieldwork may be essential to support those models because reliability and validity are of the uttermost importance for species conservation efforts (e.g., Siqueira et al. 2009; Teixeira et al. 2013; Teixeira et al. 2014). In this study, we used bioacoustics to validate a species occurrence prognosis made by an SDM study.

The bats of Rio de Janeiro State are considered well sampled, with studies verifying its high species richness (Bergallo et al. 2003; Peracchi & Nogueira 2010; Stevens 2013). However, only mist-net capture methods or active search at daytime shelters were used. Similar to the study by Silva & Bernard (2017) and despite our large capture effort (using mist nets), we did not capture *P. centralis*. However, and as in Silva & Bernard (2017), we recorded *P. centralis* only using bioacoustics methods.

In this study, we recorded calls of *P. centralis* on a sandbank vegetation area, in the state Biological Reserve of Praia do Sul, considered to be the best-preserved site on the island with no human traffic allowed in the area. However, this species has been recorded in both disturbed and undisturbed areas with a variety of habitats (Nogueira et al. 1999; Gregorin & Taddei 2000; Barnett et al. 2006; Cáceres et al. 2008; Fischer et al. 2015; Silva & Bernard 2017; Hintze et al. 2020), adding support to consider *P. centralis* a ubiquitous species.

Many insectivorous bat species do have activity records related to the time of sunset, usually restricted to periods close to dusk or dawn, with a bimodal pattern (e.g., Bateman & Vaughan 1974; Erket 1978; Marques 1986; Esbérard & Bergallo 2010). However, some species may also have their activity time unrelated to sunset (e.g., Sanderson et al. 2006; Costa et al. 2011), and other variables might influence their activity, such as moonlight (Appel et al. 2017). Since we only recorded four *P. centralis*' acoustic sequences, we cannot infer much about its activity and behavior. Still, we recorded the species at several moments during the night (Table 1), so we can probably assume that *P. centralis* is active all night long. Some studies state that *P. centralis* only starts its foraging activity later in the night (Nogueira et al. 1999), but we also found feeding activity occurring 135 minutes after sunset.

Previous to our study, several bat species inventories were made on this island (e.g., Esbérard et al. 2006; Costa et al. 2021). Ilha Grande is a very well sampled site with more than 1,000 Phyllostomidae bat captures, which is considered to be the minimum effort to describe most bat assemblages in the Atlantic Forest biome (Bergallo et al. 2003). Despite many previous studies and the high sampling effort we made using mist nets, we were not able to capture *P. centralis*. Bioacoustic methods are known for recording insectivorous species not commonly captured by mist nets (Sampaio et al. 2003; Jung et al. 2014), as with this study. With this new occurrence of the species in Ilha Grande, we add support to the importance of using different methods in fieldwork.

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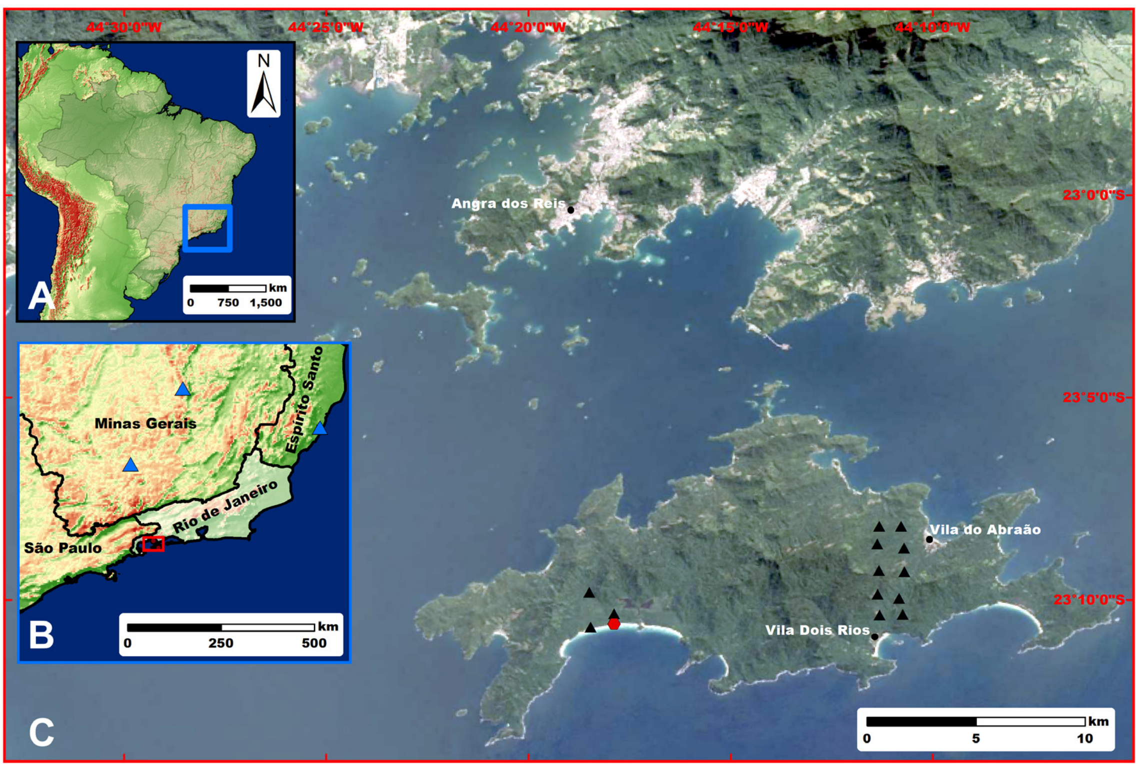

Figure 1. Partial map of South America highlighting southeastern Brazil (A), Rio de Janeiro State (B) and Ilha Grande (C) showing the 14 plots studied (black triangles). Blue triangles (B) show previous records of *Promops centralis* in the state of Minas Gerais and Espírito Santo. The red circle indicates the point where we recorded *Promops centralis*' calls (C).

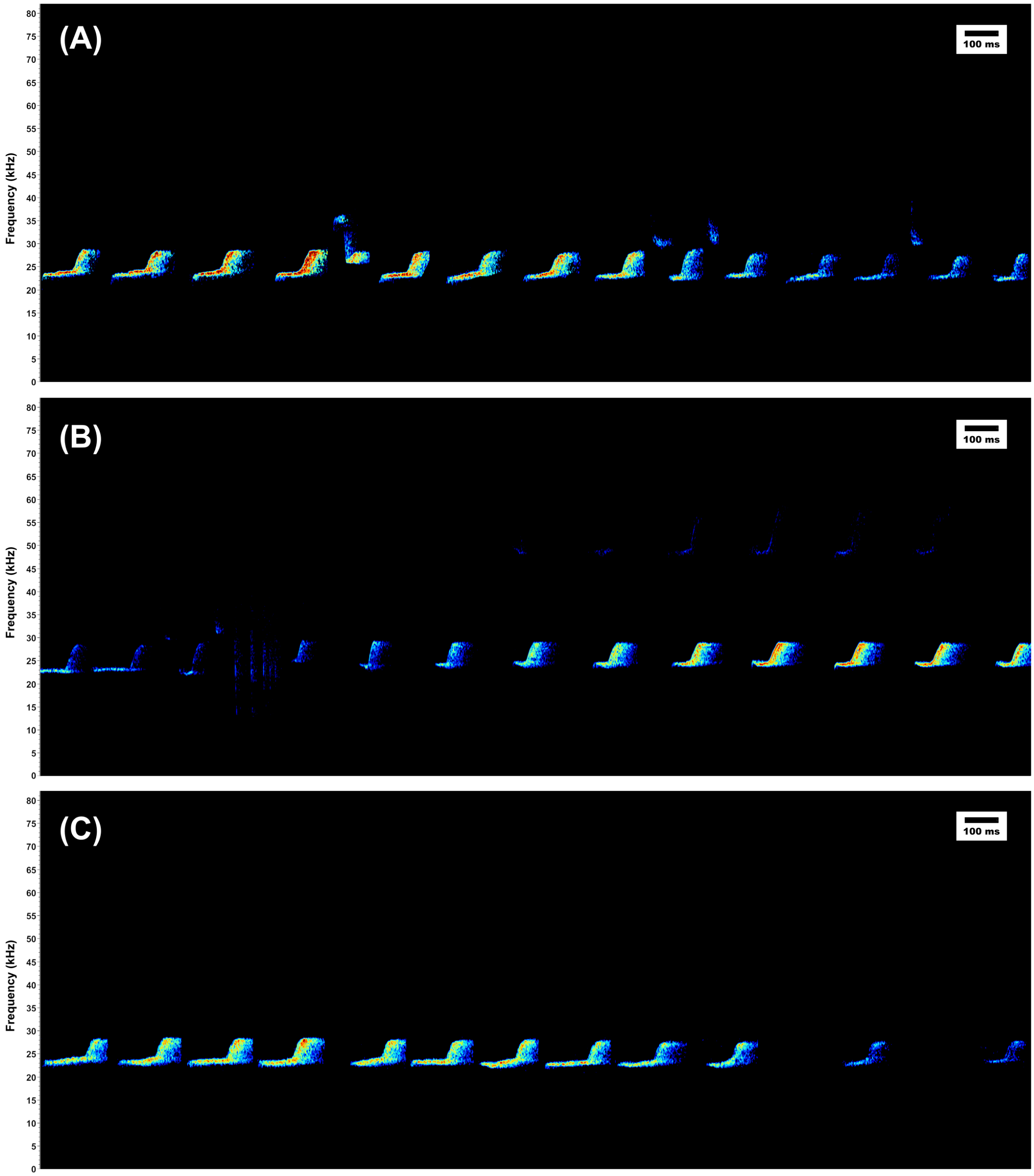

Figure 2. Echolocation calls of *Promops centralis* recorded in Ilha Grande, Rio de Janeiro State (Brazil), showing sequences with low calls alternating with higher calls (A), a feeding buzz (B), and showing only low calls (monotone sequence) (C).

Table 1. Call signals sequence of *Promops centralis* based on figure 2, day and time of the recordings and minutes after sunset (MAS), recorded between October 2016 and November 2017 in Ilha Grande, Rio de Janeiro State, Brazil. Date is represented as day/month/year.

Sequence	Date	Time	MAS (min)	Observations
A	12/10/2016	18:45:00	45	
B	12/10/2016	20:30:12	135	With feeding-buzz
C	13/10/2016	04:15:22	600	
D	27/11/2017	01:58:56	451	

Table 2. Compared call characteristics of Promops centralis recorded between October 2016 and November 2017 in Ilha Grande (Rio de Janeiro State, Brazil) and by Hintze et al. (2020). Call Type was based on Hintze et al. (2020). Call Structure: CF, constant frequency; FMu - upward modulated frequency; qCFu, upward quasi-constant frequency. Data is presented as mean ± standard deviation, and the minimum-maximum ranges of the parameters (in parentheses). IPI, inter-pulse interval; Duration, call duration; Fmin, minimum frequency; FME, frequency with maximum energy; Fmax, maximum frequency; DC, duty cycle; and NC, number of analyzed calls.

Call Type	Call structure	IPI (ms)	Duration (ms)	Fmin (kHz)	FME (kHz)	Fmax (kHz)	DC (%)	NC	Reference
Low Type II	CF-FMu-qCFu	229.8 ± 36.4 (154.6 – 357.1)	123.3 ± 39.5 (31.7 – 199.8)	21.9 ± 0.8 (20.2 - 24.0)	24.9 ± 2.1 (22.5 - 28.1)	28.9 ± 0.5 (27.7 - 30.2)	34.2 ± 9.5 (8.2 - 49.0)	41	This study
Low Type II	CF-FMu-qCFu	90.2 ± 88.3 (23.4–423.9)	105.1 ± 36.7 (22.0–178.2)	24.8 ± 0.7 (22.5–27.8)	28.9 ± 1.4 (24.8–31.5)	31.4 ± 0.9 (27.8–33.8)	53.3 ± 20.9 (17.5–87.2)	88	Hintze et al. (2020)

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