

# The advertisement call of *Melanophryniscus paraguayensis* (Anura: Bufonidae): a species endemic to Paraguay

El canto de advertencia de *Melanophryniscus paraguayensis* (Anura: Bufonidae): una especie endémica de Paraguay

Karina Núñez <sup>1</sup>, Griselda Zárate-Betzel <sup>2</sup>, Fátima Ortiz <sup>3</sup>, Marta Duré <sup>4\*</sup>

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## ABSTRACT

Reporting the characteristics of the advertisement call in anuran species is an essential aspect of their taxonomic characterization. The aim of the present study was to describe the advertisement call of *Melanophryniscus paraguayensis* in the Central department of Paraguay, an endemic and threatened species in the country. The advertisement call of *M. paraguayensis* is a complex call, featuring different note types, tonal and multi-pulsed, with a duration of 0.4–6.4 s, and a dominant frequency of 2239–2928 Hz. The data obtained were compared with available descriptions of advertisement calls from both the *Melanophryniscus stelzneri* group and other species within the genus *Melanophryniscus*.

**Keywords:** Amphibia, bioacoustics, *Melanophryniscus stelzneri* group

## RESUMEN

La descripción de las características del canto de advertencia de los anuros forma parte de la caracterización taxonómica de las especies. El objetivo del presente estudio fue describir el canto de advertencia de *Melanophryniscus paraguayensis*, una especie endémica y amenazada de Uruguay, en el departamento Central de este país. El canto de advertencia de *M. paraguayensis* es un canto complejo

- 1 Universidad Nacional de Asunción, Facultad de Ciencias Exactas y Naturales, Departamento de Biología, Núcleo de investigación de biodiversidad, San Lorenzo, Paraguay. [ranitapy@gmail.com](mailto:ranitapy@gmail.com)
  - 2 Universidad Nacional de Asunción, Facultad de Ciencias Exactas y Naturales, Departamento de Biología, Núcleo de investigación de biodiversidad, San Lorenzo, Paraguay. [grisel.zb@gmail.com](mailto:grisel.zb@gmail.com)
  - 3 Universidad Nacional de Asunción, Facultad de Ciencias Exactas y Naturales, Departamento de Biología, Núcleo de investigación de biodiversidad, San Lorenzo, Paraguay. [fatimanortiz@gmail.com](mailto:fatimanortiz@gmail.com)
  - 4 Centro de Ecología Aplicada del Litoral, CONICET-UNNE. Ruta 5, km 2.5, CP 3400, Corrientes, Argentina. [duremarta@gmail.com](mailto:duremarta@gmail.com)
- \* Corresponding author



con dos tipos de notas, tonales y multi-pulsadas, que tiene una duración de 0.4–6.4 s y una frecuencia dominante de 2239–2928 Hz. Los datos obtenidos se compararon con las descripciones disponibles de cantos de advertencia de especies del grupo *Melanophryniscus stelzneri* como de otras especies del género *Melanophryniscus*.

**Palabras clave:** Amphibia, bioacústica, grupo *Melanophryniscus stelzneri*

## INTRODUCTION

Advertisement calls of anurans emitted by males serve two main functions: attracting females for reproduction and transmitting territorial information to other males of the same species during the breeding season of anurans, being representative tool for their taxonomic identification (Köhler *et al.* 2017), once they are species specific (Duarte-Marin *et al.* 2022). Since call differences play a key role in distinguishing and identifying frog species, advancing methods and concepts in this area is crucial for biodiversity monitoring and effective conservation planning (Köhler *et al.* 2017).

Guerra *et al.* (2018) concluded that anuran families or genera with restricted distributions, many of which include recently described species, are the least known in terms of their calls or bioacoustic characteristics. This study specifically highlighted the genus *Melanophryniscus* Gallardo, 1961 (Bufonidae), as one of the least studied in relation to these aspects of its life history. *Melanophryniscus* comprises 31 species (Frost c2024) in three groups, *M. stelzneri*, *M. tumifrons* and *M. moreirae* groups (Cruz and Caramaschi 2003), but information on their acoustic repertoire is available for only half of them (Caorsi *et al.* 2020). Toads of this genus exhibit aposematic coloration and breed explosively in temporary water bodies within open environments such as grasslands, wetlands and savannas (Baldo *et al.* 2014). Their vocalizations are complex, consisting of two distinct types of notes (Forti *et al.* 2019, Mângia *et al.* 2019, Caorsi *et al.* 2020).

*Melanophryniscus paraguayensis* Céspedes and Motte, 2007, is one of six species found in Paraguay and is endemic to the natural grasslands of the central Oriental Region (Céspedes and Motte 2007, Weiler *et al.* 2013). This species is a member of the *M. stelzneri* group in

addition to the species *M. atroluteus* (Miranda-Ribeiro, 1920), *M. cupreuscapularis* Céspedes and Alvarez, 2000, *M. estebani* Céspedes, 2008, *M. formosus* (Gleich, 1925), *M. fulvoguttatus* (Mertens, 1937), *M. klappenbachi* Prigioni and Langone, 2000, *M. krauczuki* Baldo and Basso, 2004, *M. montevidensis* (Philippi, 1902), *M. rubriventris* (Vellard, 1947), and *M. stelzneri* (Weyenbergh, 1875) (Cruz and Caramaschi 2003, Baldo and Basso 2004, Céspedes and Motte 2007, Céspedes 2008, Frost c2024). *Melanophryniscus paraguayensis* is small (snout-vent length <30 mm) and has black dorsal coloration with yellow and red spots. Throughout its distribution, the species faces various anthropic pressures, leading to its categorization as “Vulnerable” (Motte *et al.* 2019, IUCN c2021). Despite being described over fifteen years ago, many key aspects of its life history remain unknown. Currently, only details of its diet are available (Núñez *et al.* 2021), and no data exist regarding its vocalizations. The main objective of this study is to describe the advertisement call of *M. paraguayensis* based on the analysis of vocalizations recorded at two sites within its distribution in Paraguay.

## MATERIALS AND METHODS

Recordings were obtained from two floodplains sites: an urban park in Asunción (25°15' South, 57°32' West, May 2018) and a private property in Nueva Italia (25°39' South, 57°31' West, May 2019), located 60 km apart. Calls were recorded in the field using a Yoga HT81 external directional microphone connected to a TASCAM DR40 digital recorder, positioned approximately 50 cm from the calling frogs. The recordings were made at a sampling frequency of 44.1 kHz with a resolution of 16 bits and saved as uncompressed WAV files. Air temperature and relative humidity at the microhabitat were measured at the time of recording using an AMPROVE digital thermohygro-

ter, and meteorological data were also requested from the Paraguay National Meteorological Center. Advertisement calls of four uncollected males were recorded both during the day and at night. Although the males recorded in this study were not collected, individuals from the same population had been sampled earlier and deposited as voucher specimens in the Zoological Collection of FACEN (CZCEN) (Núñez *et al.* 2021). The measurements taken, including snout-vent length and body mass of males, are reported here.

The sound recordings are archived in the bioacoustic repository of the same collection (Mp12052018PGG92, Mp13052018PGG95, Mp13052018PGG96, Mp04052019NIG168, Mp05052019NIG175, Mp05052019NIG176).

The analysis and description of the temporal and spectral parameters were performed using Raven Pro 1.6 software (Bioacoustics Research Program c2011), and the figure was generated with the Seewave (Sueur *et al.* 2008) and TuneR (Ligges *et al.* 2018) packages implemented in R (R Development Core Team c2022). Differences in call

frequencies were calculated using the Welch F Test in the software Past (Hammer *et al.* 2001). Temporal parameters were measured directly from the oscillograms, while spectral parameters were analyzed using spectrograms and the power spectrum.

To ensure comparability, the temporal and spectral parameters analyzed were based on previous studies of *Melanophryniscus* (Baldo and Basso 2004, Duré *et al.* 2015, Mângia *et al.* 2019, Caorsi *et al.* 2020), with adaptations from Köhler *et al.* (2017). The parameters measured included dominant frequency (Hz), call duration (s), inter-call interval (s), first segment duration (s), second segment duration (s), interval between segments (s), number of notes of first segment, first segment notes duration (s), inter-notes interval of first segment (s), pulse number of second segment, pulse rate of second segment. We compared the call parameters of *M. paraguayensis* with those of other species within the *M. stelzneri* group, as well as with other congeners for which advertisement calls have been described.



**Figure 1.** *Melanophryniscus paraguayensis*. (a) Amplexus recorded during the day, following the male's vocalization activity. (b) Amplexus recorded at night, without prior vocalization activity. (c) Coloration of the individual in its habitat. (d) Dorsal and (e) ventral view of a representative specimen (CZCEN 1432).

## RESULTS

*Melanophryniscus paraguayensis* vocalizes during the months of May and June, specifically in temporary water bodies that emerge in grasslands after rainfall (Fig. 1a). Although individuals are active in September, December, and February, no vocalization activity was detected during those months. Even though amplexus was observed in September, it was not preceded by any vocalization (Fig. 1b). We also observed physical fights and displacement among vocalizing males at both sites. The acoustic space of the species was shared with *Physalaemus cristinae* Cardozo, Tomatis, Duport-Bru, Kolenc, Borteiro, Pansonato, Confalonieri, Lourenço, Haddad, and

Baldo, 2023 during daytime recordings, and with *Elachistocleis bicolor* (Guérin-Méneville, 1838) and *Odontophrynus asper* (Philippi, 1902) during nighttime recordings.

Although many recordings were obtained, the calls with the least interference from wind and anthropogenic sounds were used for analysis. Thirty calls from four males were analyzed: eight calls from male 1 and fifteen calls from male 2 at the urban park, and three calls from male 3 and four calls from male 4 at the private property (Table 1). The morphometric data of males from the same populations correspond to values with a mean snout-vent length of  $22.4 \pm 1.9$  mm (19.8–25.7) and a mean weight of  $1.1 \pm 0.3$  g (0.8–1.6). (Fig. 1c, 1d, 1e) (Núñez unpublished data).

**Table 1.** Temporal and spectral parameters of advertisement calls of *Melanophryniscus paraguayensis* from two localities, Paraguay. Data are shown as mean (minimum and maximum values).

Characteristics	Male #1 n=8 calls	Male #2 n=15 calls	Male #3 n=3 calls	Male #4 n=4 calls
Study site	Asunción	Asunción	Nueva Italia	Nueva Italia
Date	12 may 2018	13 may 2018	04 may 2019	05 may 2019
Air temperature (°C)	18	19	23.1	not recorded
Humidity (%)	82	74	88	not recorded
Hour (hh:mm)	13:30	16:06	20:57	10:40
Dominant frequency (Hz)	2842.4	2733.3 (2670.1-2756.3)	2296.9 (2239.5-2325.6)	2885.5 (2842.4-2928.5)
Dominant frequency of first segment (Hz)	2782.7 (2670.1-2842.4)	2698.3 (2584-2756.2)	2265.3 (2239.5-2325.6)	2850.6 (2756.2-2928.5)
Dominant frequency of second segment (Hz)	2842.4	2750.5 (2670.1-2756.2)	2296.9 (2239.5-2325.6)	2863.9 (2842.4-2928.5)
Call duration (s)	0.5 (0.4-0.7)	2.6 (1.0-4.9)	2.0 (1.1-3.1)	2.7 (1.3-6.4)
Inter-call interval (s)	2.6 (1.4-5.6)	2.8 (1.0-10.0)	0.3 (0.3-0.3)	4.0 (3.5-4.4)
First segment duration (s)	0.1 (0.06-0.2)	1.4 (0.1-4.1)	0.5 (0.4-0.6)	1.5 (0.2-5.3)
Second segment duration (s)	0.3 (0.25-0.33)	0.8 (0.5-1.8)	1.4 (0.7-2.5)	1.0 (0.8-1.3)
Interval between segments (s)	0.1 (0.1-0.3)	0.3 (0.03-1.0)	0.1 (0.1-0.2)	0.2 (0.1-0.4)
Number of notes of first segment	1.6 (1-2)	3.9 (1-8)	3.3 (3-4)	5.3 (2-15)
First segment notes duration	0.04 (0.01-0.06)	0.05 (0.01-0.08)	0.04 (0.02-0.06)	0.03 (0.03-0.04)
Inter-notes interval of first segment (s)	0.12 (0.12-0.13)	0.4 (0.1-2.1)	0.1 (0.1-0.2)	0.3 (0.1-1.9)
Pulse number of second segment	12.6 (9-16)	38.9 (24-84)	64.3 (30-113)	58.3 (48-76)
Pulse rate of second segment	46.4 (32.9-49.9)	46.3 (43.2-47.7)	45.4 (45.1-45.8)	56.7 (48.9-59.9)

*Melanophryniscus. paraguayensis* emits an advertisement call of 0.4 to 6.4 seconds in length, characterized as a complex multi-note call with different note types: 1-15 tonal notes, which can be grouped or not, followed by a longer multi-pulsed notes (9-113 pulses) (Fig. 2, Table 1). The tonal notes may display upward frequency modulation (Fig. 2). The dominant frequency ranges from 2239 to 2928 Hz, with a lower frequency in nighttime calls (2239-2325 Hz) compared to daytime calls (2670-2928 Hz), a difference that is statistically significant ( $P = 0.0005$ ). However, there were no significant differences between the frequencies of the tonal and pulsed note segments ( $P = 0.1708$ ) (Table 1).

## DISCUSSION

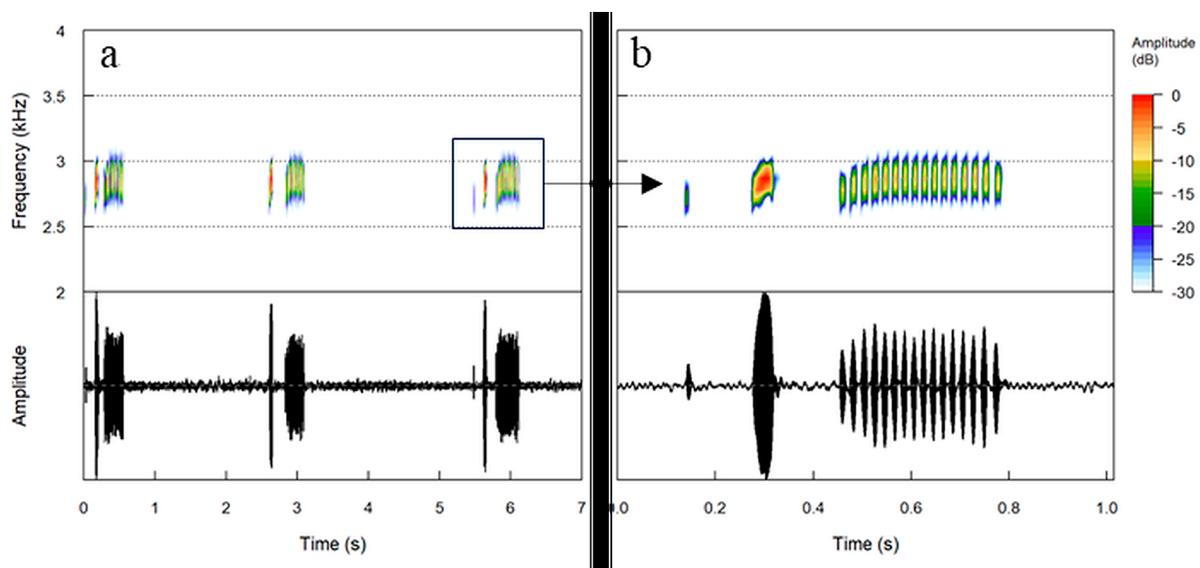
The reproduction of *M. paraguayensis* occurs explosively over short periods, similar to other species of the genus (Vaira 2005, Duré et al. 2015). The high density of males in temporary pools leads them to vocalize in close proximity, meaning their calls serve the dual purpose of attracting females and marking territories through fighting or displacing other males (Caldart et al. 2013, Duré et al. 2015, Caorsi et al. 2020).

Within the *M. stelzneri* group, advertisement calls have been documented for most species: *M. atroluteus* (Baldo

and Basso 2004), *M. cupreuscapularis* (Duré et al. 2015), *M. formosus* (Kwet et al. 2005), *M. klappenbachi* (Duré et al. 2024), *M. krauczuki* (Baldo and Basso 2004), *M. montevidensis* (Kwet et al. 2005), *M. rubriventris* (Ferrari and Vaira 2008) and *M. stelzneri* (Barrio 1964, Kwet and Miranda 2001). These species share a basic call structure consisting of short tonal notes followed by a trill of multi-pulsed notes, similar to *M. paraguayensis*.

In terms of call frequency, *M. paraguayensis* is similar to most species within the *M. stelzneri* group, except for *M. krauczuki*, which has a higher frequency (3300 Hz), and *M. rubriventris*, which has a lower frequency (<2000 Hz) (Table 2). However, the call duration of *M. paraguayensis* exhibits comparable values to those documented for *M. cupreuscapularis*, *M. klappenbachi* and *M. rubriventris* (Table 2). In contrast, other species in the group exhibit longer calls, with *M. krauczuki* having the longest call duration (Baldo and Basso 2004).

The number of notes in the first segment of the call of *M. paraguayensis* is comparable to that of *M. krauczuki* and *M. rubriventris*, while other species in the *M. stelzneri* group typically have more notes (Table 2). Regarding the number of pulses in the second segment, the call of *M. paraguayensis* is most similar to the call of *M. cupreuscapularis* and *M. klappenbachi*, as other species in the group have higher values (Table 2).



**Figure 2.** (a) Spectrogram (top) and oscillogram (bottom) of three advertisement calls of a male *Melanophryniscus paraguayensis* from Asunción, Paraguay (ambient temperature 18°C). (b) Spectrogram and oscillogram of the indicated call in higher temporal resolution.

**Table 2.** Comparison of the basic parameters of the advertisement calls of species in the *Melanophryniscus stelzneri* group and other congeners. Data are shown as mean (minimum and maximum values).

Species (authors describing the call), place of documentation	Calls (n), air temperature (°C)	Dominant frequency (Hz)	Call duration (s)	Number of notes of 1st segment	Pulse number of 2nd segment
<i>M. atroluteus</i> (Baldo and Basso 2004) Misiones, Argentina	12 calls, 1 male	3000	7.52 (5.09-10.35)	20.6 (15-25)	222.38 (139-321)
<i>M. cupreuscapularis</i> (Duré et al. 2015) Corrientes, Argentina	5 calls, 1 male, 22 °C	2270 (2176-2357)	3.02 (1.12-6.66)	10 (6-17) (grouped)	20.4 (16-24)
<i>M. formosus</i> (Kwet et al. 2005) Rio Grande do Sul, Brazil	7 calls, 2 males, 21 °C	(2300-3200)	unreported values	13 (6-18)	112 (54-162)
<i>M. klappenbachi</i> (Duré et al. 2024) Chaco, Argentina	49 calls, 5 males, 30 °C	(2239-2583)	1.85 (0.59-7.24)	6.97 (2-27)	42 (13-144)
<i>M. krauczuki</i> (Baldo and Basso 2004) Misiones, Argentina	5 calls, 1 male	3300	32.69 (25.01-36.64)	8.6 (6-12)	1298.5 (1018-1502)
<i>M. montevidensis</i> (Kwet et al. 2005) La Paloma, Uruguay	14 calls, 2 males, 24 °C	(2100-2800)	unreported values	17 (7-28)	147 (100-192)
<i>M. rubriventris</i> (Ferrari and Vaira 2008) Tiraxi, Jujuy, Argentina	12 calls, 4 males, 19 °C	1788 (1704-1846) 1st segment 1734 (1653-1789) 2nd segment	2.71 (1.40-3.25)	3.8 (2-7)	unreported values
<i>M. stelzneri</i> (Kwet and Miranda 2001) Córdoba, Argentina		(1600-2600)	7.3 (4.5-9.3)	26 (12-36)	127.8 (64-162)
<i>M. alipioi</i> (Forti et al. 2019) Ponta Grossa, Paraná, Brazil	18 calls, 4 males	unreported values	17.7 (6.7-25.8)	unreported values	unreported values
<i>M. cambaraensis</i> (Caorsi et al. 2020) Sao Francisco de Paula, RS, Brazil	13 calls, 8 males, 15.7-17 °C	unreported values	18.8 (12.6-33.4)	5.8 (3-11)	576.7 (314-948)
<i>M. macrogranulosus</i> (Caorsi et al. 2020) Maquiné e Morro da Gruta, RS, Brazil	19 calls, 11 males, 15.7-17 °C	unreported values	19.2 (11.9-29.1)	6.1 (2-14)	591 (461-811)
<i>M. moreirae</i> (Forti et al. 2019) Itamonte, Minas Gerais, Brazil	14 calls, 2 males	1856 (1766-1938)	1.83 (0.21-6.08)	unreported values	unreported values
<i>M. pachyrhynchus</i> (Caldart et al. 2013) São Sepé, Rio Grande do Sul, Brazil	12 calls, 6 males, 16-17 °C	2668 (2261-2932)	37.07 (6.64-75.20)	17 (8-28)	818 (164-1382)
<i>M. xanthostomus</i> (Mângia et al. 2019) Campo Alegre, Santa Catarina, Brazil	17 calls, 3 males, 18.8 °C	3395 (3101-3618)	18.6 (12.2-21)	13 (7-20)	685 (294-1033)
<i>M. paraguayensis</i> This study	15 calls, 1 male, 19 °C	2733.3 (2670.1-2756.3)	2.6 (1.0-4.9)	3.9 (1-8)	38.9 (24-84)

For species of the genus that do not belong to the *stelzneri* group, advertisement calls have been described for *M. alipioi* Langone, Segalla, Bornschein, and de Sá, 2008 (Forti *et al.* 2019), *M. cambaraensis* Braun and Braun, 1979 (Caorsi *et al.* 2020), *M. macrogranulosus* Braun, 1973 (Caorsi *et al.* 2020), *M. moreirae* (Miranda-Ribeiro, 1920) (Forti *et al.* 2019), *M. pachyrhynus* (Miranda-Ribeiro, 1920) (Caldart *et al.* 2013), *M. vilavelhensis* Steinbach-Padilha, 2008 (Forti *et al.* 2019), and *M. xanthostomus* Baldo, Bornschein, Pie, Ribeiro, Firkowski and Morato, 2015 (Mângia *et al.* 2019). We compared the calls of these species with that of *M. paraguayensis*, with the exception of *M. vilavelhensis*, as it was analyzed using different parameters that are not directly comparable (Table 2).

The advertisement calls of these species are generally of longer duration than those of *M. paraguayensis*, with the exception of *M. moreirae*, which exhibits a similar call duration. Regarding dominant frequency, *M. pachyrhynus* shows a comparable range, *M. moreirae* displays a lower frequency, and *M. xanthostomus* presents a higher frequency than *M. paraguayensis* (Table 2). For the remaining species, this parameter was not reported for the entire call. The number of notes in the first segment is similar between *M. paraguayensis* and the compared species, whereas the number of pulses in the second segment is substantially higher in all species for which this parameter was available (Table 2).

Although certain differences exist, the advertisement calls of *Melanophryniscus* species show considerable variability in both temporal and spectral parameters, with overlapping tonal and pulsed notes, as summarized by Mângia *et al.* (2019) and Caorsi *et al.* (2020). This variability may reflect the calls' dual function of attracting mates and marking territories, as suggested by Duré *et al.* (2015), and territorial components are sometimes incorporated into advertisement calls (Kohler *et al.* 2017). The general structure of the genus's advertisement calls consists of two types of notes organized into continuous segments, though the specific functions of these notes remain unknown (Mângia *et al.* 2019).

The advertisement call of *M. paraguayensis* shares greater similarity with *M. cupreuscapularis* and *M. klappenbachi*, whose populations are geographically close. In Paraguay, populations of *M. fulvoguttatus* (Mertens, 1937) and *M. devincenzii* Klappenbach, 1968 (*tumifrons* group) are also geographically close (Zank *et al.* 2014), but comparisons were not made as their calls have yet to be described.

Amphibians are the most threatened group of vertebrates worldwide due to disease, habitat loss and degradation, and the impacts of climate change (Luedtke *et al.* 2023). At the same time, many species remain poorly understood in terms of their natural history. In this context, bioacoustics emerge as a significant tool in conservation, enabling a more effective and less invasive evaluation of fauna, and aiding in the detection of environmental changes (Sugai *et al.* 2019). Thus, the identification of the calls of each species allows not only its taxonomic identification and the differentiation of other species in natural habitats but is also essential for acoustic monitoring (Penar *et al.* 2020). This technique complements other methods of diversity analysis and offers a comprehensive understanding of community composition (Boullhesen *et al.* 2021). Moreover, variations in the spectral and temporal parameters of calls within the same species, and knowledge of its acoustic repertoire, can indicate intra- and interspecific interactions as well as environmental changes (Kohler *et al.* 2017). This provides valuable information about ecosystem health and a better understanding of the biological patterns and processes that will guide future conservation and management strategies (Boullhesen *et al.* 2023).

In this report, we expand the knowledge of the acoustic repertoire of *M. paraguayensis*, which is endemic to Paraguay's grasslands and is categorized as vulnerable due to anthropogenic pressures in its range. There were no previous descriptions of its call, highlighting the importance of the data presented. Further data collection is necessary to better understand individual and population variations, as well as the effects of human interference on their calls.

## AUTHOR CONTRIBUTIONS

KN and MD: conceptualization and design. KN, GZ, FO: data collection. KN, GZ, FO and MD: data analysis and manuscript writing and review.

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