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Advertisement calls of topotypes of *Elachistocleis matogrosso* (Anura: Microhylidae)

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The genus *Elachistocleis* PARKER, 1927 currently comprises 17 valid species, which are widely distributed across cis-Andean South America, from Panama and Colombia south to central Argentina, Uruguay, and southern Brazil (FROST 2017). Taxonomic arrangement within *Elachistocleis* has been a matter of discussion in recent years. The genus may still comprise many cryptic, undescribed species, as well as synonyms (NELSON 1973, LAVILLA et al. 2003, CARAMASCHI 2010, TOLEDO et al. 2010). Although representatives of *Elachistocleis* may exhibit different ventral colours and colour patterns, two general patterns were formally recognized: immaculate and spotted (e.g., PEREYRA et al. 2013). However, overall morphological similarities amongst, and variation within several species do not allow unequivocal specific identification without bioacoustical support, and differences in the advertisement calls have been mandatory for diagnosing new species (NUNES-DE-ALMEIDA & TOLEDO 2012, PEREYRA et al. 2013).

Described from the area of the central campus of the Federal University of Mato Grosso (UFMT), in the municipality of Cuiabá, state of Mato Grosso, mid-western Brazil, *Elachistocleis matogrosso* is a small-sized microhylid (snout–vent length ranging from 21.5–24.6 mm in males, 29.0–33.2 mm in females). Besides Mato Grosso, it also occurs in the Brazilian state of Mato Grosso do Sul, and probably in adjacent areas of eastern Bolivia and northern Paraguay (FROST 2017). Individuals of *Elachistocleis matogrosso* are described as having an immaculate belly (CARAMASCHI 2010), a character also reported for *E. bicolor* (GUÉRIN-MÉNEVILLE, 1838), *E. haroi* PEREYRA, AK-

MENTINS, LAUFER & VAIRA, 2013, *E. helianneae* CARAMASCHI, 2010, and *E. muiraquitana* NUNES-DE-ALMEIDA & TOLEDO, 2012. Diagnostic differences of *E. matogrosso* in comparison to the other four species with immaculate belly mainly rely in its smaller body size (CARAMASCHI 2010, NUNES-DE-ALMEIDA & TOLEDO 2012, PEREYRA et al. 2013). However, when describing *E. muiraquitana*, NUNES-DE-ALMEIDA & TOLEDO (2012) examined larger series of *E. matogrosso* and *E. helianneae* and showed that morphometric differences between these two species were not as evident as previously stated. The morphological diagnosis of *E. matogrosso* includes the presence of “a thin middle longitudinal light stripe, from the post-cephalic transverse skinfold to the vent, but absent on the head” (CARAMASCHI 2010). However, an interrupted or even absent stripe is also mentioned in the original description as an intraspecific variation (CARAMASCHI 2010), and topotypes may bear a complete light stripe, from the tip of snout to vent, similarly to *E. helianneae*. An integrative approach could shed some light on this subject, but bioacoustic data are available only for *E. helianneae*. We here provide information on the advertisement call of individuals of *E. matogrosso* obtained at the type locality.

Nocturnal fieldwork was carried at the campus of UFMT during the local rainy season, on 31 March 2011 and 1 December 2011. We recorded and analysed 69 advertisement calls from eight topotypes of *E. matogrosso*. All the specimens fit well the original description of *E. matogrosso* (CARAMASCHI 2010), except for the presence of a mid-dorsal stripe extending from the tip of snout to vent (Fig. 1) in

three of them. No other *Elachistocleis* species has been recorded from the university campus since *E. matogrosso* was described or even before that, based on material deposited at the “Coleção Zoológica de Vertebrados da Universidade Federal de Mato Grosso” (UFMT-A; Cuiabá, Mato Grosso, Brazil), which includes six voucher specimens of the present study.

Advertisement calls were obtained with a professional digital recorder (Marantz PMD 660) equipped with an external directional microphone (Yoga EM-9600). Digital recordings were sampled at a rate of 44.1 kHz, with 16 bit resolution. The files were subsequently saved as uncompressed wave files and archived in the Banco de Registros Bioacústicos of the Laboratório de Herpetologia do Instituto de Biociências da Universidade Federal de Mato Grosso (LH; Cuiabá, Mato Grosso, Brazil). Bioacoustic parameters were analysed using Raven Pro 1.3 (Bioacoustic Research Program 2012), with the following configuration: Hamming window function; brightness 71%; contrast 79%; DFT size 256 points, and time grid overlap 50%. The terminology in call descriptions follows that outlined by KÖHLER et al. (2017).

Calling males of *E. matogrosso* were found in a temporary pond, amidst dense vegetation, in highly motivated choruses. They call in an upright position, with the forelimbs supported on emergent vegetation and the posterior half of the body submerged. The advertisement call of *E. matogrosso* consists of a long train of pulses (Fig. 2) that here is referred to as a note. Each note is composed of 300–837 distinct pulses (mean 588 pulses per note; SD = 127), emitted at a rate of 158–282 pulses per second (mean 218.5 pulses per second; SD = 13.8). Duration of each pulse varies from 2–3 ms (mean 3 ms; SD = 0.3), and inter-pulse intervals vary from 1–2 ms (mean 2 ms; SD = 0.2). Note duration varies from 1.51–3.63 s (mean 2.68 s; SD = 0.5), and inter-note intervals vary from 4.3–51.5 s (mean 19.7 s; SD = 10.2). The notes are emitted at a rate of 2–4 notes per

minute (mean 2.7 notes per minute; SD = 1). The dominant frequency varies from 3937.5–4823.4 Hz (mean 4337.3 Hz; SD = 179) and the approximate prevalent bandwidth ranges from 2500 Hz to 5100 Hz.

Spectral and/or temporal parameters of the advertisement call of *E. matogrosso* (Table 1) differ from those of the other 12 congeners (values in parentheses) for which advertisement call descriptions are available. The note duration is shorter than in the calls of *Elachistocleis* cf. *bicolor* from Beni, Bolivia (5.3 s; REICHLÉ 1996), *E. erythrogaster* (3.9–4.2 s; KWET & DI BERNARDO 1998), *E. muiraquitana* (2.8–4.4 s; NUNES-DE-ALMEIDA & TOLEDO 2012) and *E. skotogaster* (3.3–4.8 s; LAVILLA et al. 2003), and is longer than in *Elachistocleis* cf. *bicolor* from Santa Cruz, Bolivia (1.5–1.9 s; DE LA RIVA et al. 1996), *E. cesarii* (1.5–2.6 s; TOLEDO et al. 2010), *Elachistocleis* cf. *ovalis* from Beni, Bolivia (1.4–2.6 s; DE LA RIVA et al. 1996) and *E. panamensis* (0.4–0.6 s; NELSON 1973). Pulse rate is higher than in *E. erythrogaster* (110–125 pulses/s; KWET & DI BERNARDO 1998), *E. haroi* (143–162 pulses/s; PEREYRA et al. 2013), *E. helianneae* (115.6–121.2 pulses/s; FONSECA et al. 2012), and *E. panamensis* (20–30 pulses/s; NELSON 1973).

The dominant frequency is lower in calls of *E. matogrosso* than in Bolivian samples of *Elachistocleis* cf. *bicolor* (5140–5755 Hz; REICHLÉ 1996, DE LA RIVA et al. 1996) and in *E. panamensis* (4800–5500 Hz; NELSON 1973), and higher than in *E. cesarii* (3450–3600 Hz; TOLEDO et al. 2010), *E. erythrogaster* (3100–4500 Hz; KWET & DI BERNARDO 1998), *E. muiraquitana* (3750–3930 Hz; NUNES-DE-ALMEIDA & TOLEDO 2012), *Elachistocleis* cf. *ovalis* (3715.5–3844.4 Hz; DE LA RIVA et al. 1996) and *E. skotogaster* (3458–3671 Hz; LAVILLA et al. 2003). In addition, pulse duration is shorter in *E. matogrosso* than in *E. cesarii* (3.82–4.6 ms; TOLEDO et al. 2010), *E. haroi* (3–4 ms; PEREYRA et al. 2013), *E. muiraquitana* (5–6 ms; NUNES-DE-ALMEIDA & TOLEDO 2012), and *E. panamensis* (3–4 ms; NELSON 1973).

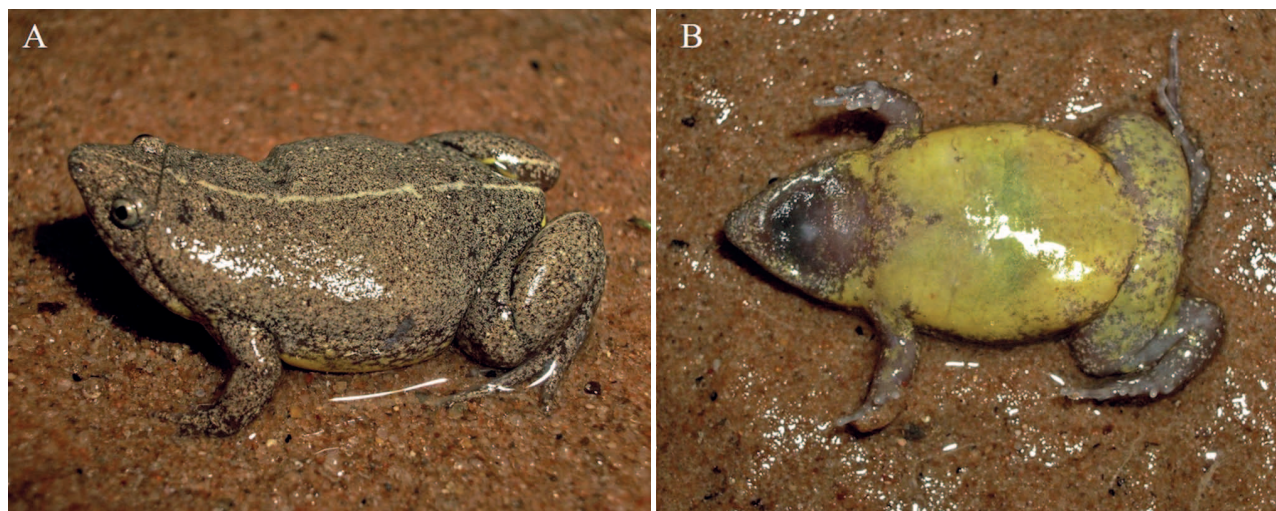


Figure 1. A topotypical male specimen of *Elachistocleis matogrosso* (UFMT 11700; from the campus of the Federal University of Mato Grosso, municipality of Cuiabá, state of Mato Grosso, mid-western Brazil), in life: (A) dorsal view, showing a light cream, thin vertebral stripe, from the tip of snout to vent, and (B) ventral view, showing the immaculate, yellowish belly.

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Table 1. Numerical parameters of topotypic calls of *Elachistocleis matogrosso* from municipality of Cuiabá, state of Mato Grosso, Brazil. Values are presented as mean \pm standard deviation (minimum–maximum).

Voucher record (number of notes analysed)	Voucher specimen (UFMT)	Air temp [°C]	Note duration [s]	Inter-note intervals [s]	Pulse dura- tion [ms]	Inter-pulse intervals [ms]	Pulse rate [pulses/s]	Dominant frequency [Hz]
LH 966 (n=5)	no voucher	24	1.93 \pm 0.07 (1.85–2.01)	28.1 \pm 8.9 (21.7–34.4)	3 \pm 0.06 (2.8–2.9)	2 \pm 0.06 (1.6–1.7)	233.2 \pm 2.6 (230–235)	4406.2 \pm 81 (4312.5–4453.1)
LH 967 (n=7)	UFMT-A 11701	24	1.82 \pm 0.1 (1.75–1.9)	22.1 \pm 15 (15.2–40.7)	3 \pm 0.03 (2.5–2.9)	2 \pm 0.04 (1.6–1.7)	183.1 \pm 25.2 (158–208)	3937.5
LH 968 (n=7)	UFMT-A 19186	25.3	1.75 \pm 0.3 (1.51–2.2)	22.4 \pm 11 (13.6–39.8)	3 \pm 0.02 (2.4–2.9)	2 \pm 0.02 (1.4–1.9)	218.5 \pm 5.7 (210.5–225.2)	4118.3 \pm 113 (3937.5–4312.5)
LH 969 (n=10)	no voucher	25.3	3.02 \pm 0.1 (2.8–3.2)	17.3 \pm 2.8 (14.5–22)	3 \pm 0.04 (2–3)	2 \pm 0.02 (1–2)	230.5 \pm 20.1 (203.5–282.1)	4409.9 \pm 89 (4306.6–4487.9)
LH 970 (n=9)	UFMT-A 19187	25.3	3.1 \pm 0.3 (2.55–3.63)	26.7 \pm 15 (12.5–48.4)	3 \pm 0.02 (2–3)	2 \pm 0.02 (1–2)	212.9 \pm 6.2 (206.7–228.0)	4316.2 \pm 64 (4263.6–4478.9)
LH 971 (n=11)	UFMT-A 19188	25.3	2.44 \pm 0.2 (2.15–2.7)	11.2 \pm 4.8 (4.3–20.9)	3 \pm 0.02 (2–3)	2 \pm 0.03 (1–2)	222.5 \pm 7.2 (214.7–232.9)	4404.5 \pm 263 (4005.2–4823.4)
LH 972 (n=11)	UFMT-A 19189	25.3	3.25 \pm 0.09 (3.15–3.44)	21.2 \pm 2.9 (18.2–28)	3 \pm 0.09 (2–3)	2 \pm 0.08 (1–2)	218.9 \pm 5.8 (212.4–232.1)	4353.6 \pm 89 (4220.5–4478.9)
LH 973 (n=9)	UFMT-A 11700	24	2.65 \pm 0.3 (2.31–3.4)	21.1 \pm 18.0 (6.1–51.5)	3 \pm 0.03 (2.6–3.4)	2 \pm 0.03 (1.4–1.9)	212.5 \pm 3.1 (204.9–215.5)	4411.9 \pm 147 (4306.6–4651.2)

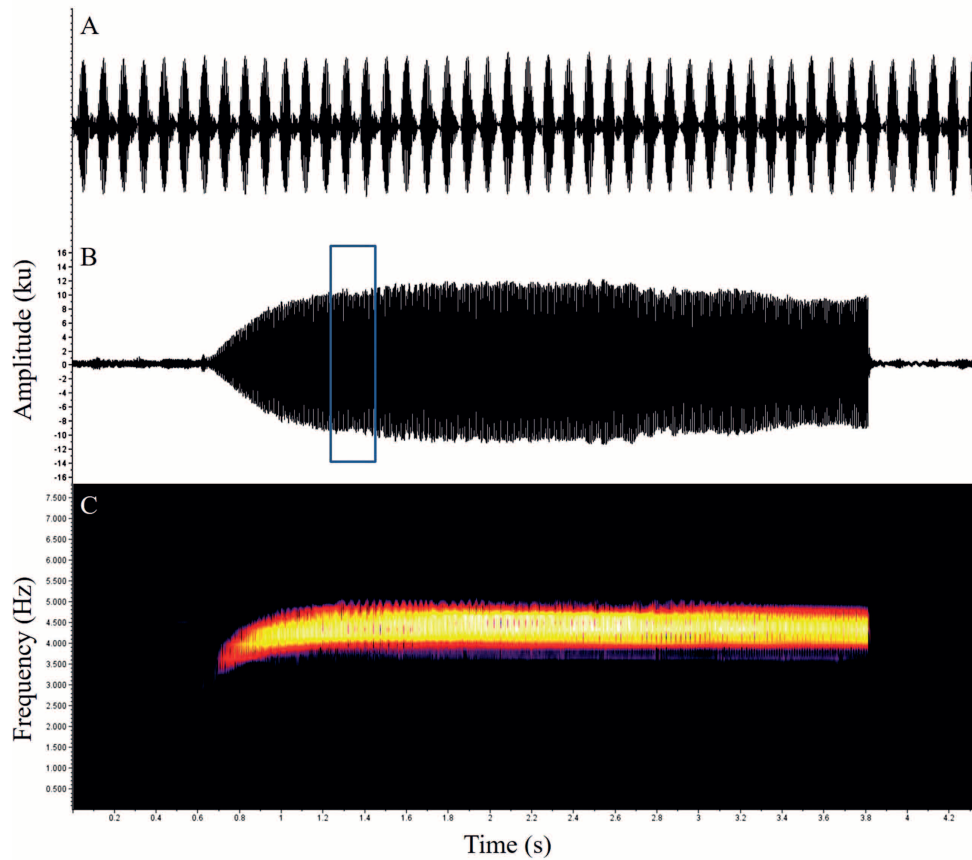


Figure 2. Oscillograms (A–B) and audiospectrogram (C) of the advertisement call of a topotypic specimen of *Elachistocleis matogrosso* (UFMT-A 19189), recorded on 1 December 2011 at the campus of the Federal University of Mato Grosso, municipality of Cuiabá, state of Mato Grosso, Brazil (LH 972, 21:00 h, air temperature 25.3 °C). Oscillogram (A) corresponds to 0.2 second section outlined by the blue rectangle in B, showing 45 pulses of the note.

Table 2. Numerical parameters of calls of *Elachistocleis* species with an immaculate belly. Values are presented as mean \pm standard deviation (minimum–maximum).

Species	Note duration [s]	Pulses per note	Pulse duration [ms]	Pulse rate [pulses/s]	Dominant frequency [Hz]	Locality	Source
<i>E. matogrosso</i>	2.68 \pm 0.5 (1.51–3.63)	588 \pm 127 (300–837)	2.76 \pm 0.003 (2.24–3.45)	218.5 \pm 13.8 (158–282)	4337.3 \pm 179 (3937.5–4823.4)	Cuiabá, Mato Grosso, Brazil	present work
<i>E. helianneae</i>	1.8 \pm 0.3 (1.3–2)	219 \pm 36.3 (156–245)	–	119.2 \pm 2.3 (115.6–121.2)	4410 \pm 231 (4134–4651)	Porto Velho, Rondônia, Brazil	FONSECA et al. (2012)
<i>E. cf. bicolor</i>	5.3 (4.37–6.35)	–	–	99.7	5140 (5080–5280)	Beni, Bolívia	REICHLE (1996)
<i>E. cf. bicolor</i>	1.77 \pm 0.2 (1.54–1.95)	–	–	–	5741.2 \pm 237 (5714–5755)	Santa Cruz, Bolívia	DE LA RIVA et al. (1996)
<i>E. haroi</i>	3.18 \pm 0.43 (2.35–3.74)	488.9 \pm 82.05 (343–605)	3.79 \pm 0.03 (3–4)	152.9 \pm 6.75 (143.45–161.86)	4560 \pm 0.08 (4330–4780)	El Algarrobal, Jujuy, Argentina	PEREYRA et al. (2013)
<i>E. muiiraquitan</i>	4.1 (2.86–4.42)	802.34	5.11 \pm 0.13 (5–6)	195.69	3780 \pm 0.03 (3750–3930)	Xapuri, Acre, Brazil	NUNES-DE-ALMEIDA & TOLEDO (2012)

From the four bioacoustic parameters mentioned above (note duration, pulse rate, dominant frequency and pulse duration), only pulse rate distinguishes *E. matogrosso* from *E. helianneae* (Tab. 2). Among the other three species with immaculate venter for which pulse rate is known, values fall within the range here reported for *E. matogrosso* (Tab. 2). Maximum variation in note duration in 12 species of *Elachistocleis* is 4.9 s (from 0.4 s in *E. panamensis* to 5.3 in *Elachistocleis cf. bicolor* from Beni, Bolivia), without any evident latitudinal or longitudinal trend. Variation in this parameter was 2.5 s (1.5–3.6 s) in our eight males of *E. matogrosso*. Note duration and thus number of pulses per note are highly variable between and within *Elachistocleis* species, depending on motivation and being considered weak characters for taxonomic distinctiveness between species in this genus (J. KÖHLER pers. comm.).

KÖHLER et al. (2017) recently pointed that the pulse rate within notes is potentially valuable for taxonomic purposes, as it is less influenced by external (e.g., temperature) or individual factors (e.g., motivation). Although the differences in this parameter presently support the distinctiveness of the morphologically highly similar *E. matogrosso* and *E. helianneae*, we call attention to the fact that bioacoustic information available for the latter species is very limited, based on five calls from a single recorded male (FONSECA et al. 2012). We here argue that additional data on pulse rate variation in calls of *E. helianneae*, as well as molecular genetics and additional information on e.g., ecology, behaviour, tadpole morphology, or osteology would help to clarify the taxonomic status of *E. matogrosso*.

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