

Advertisement calls, intraspecific variation and species diagnosis of five Brazilian species of
***Elachistocleis* (Anura: Microhylidae: Gastrophryninae)**

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Abstract

In this paper, we describe for the first time the advertisement call of three *Elachistocleis* species (*E. surumu*, *E. magna*, *E. matogrosso*); and we redescribe the calls of two species (*E. helianneae*, *E. muiiraquitan*). Additionally, we evaluate intraspecific variation in morphology and color patterns, and comment on the species diagnosis in the genus. The advertisement call of the five species of *Elachistocleis* have a stereotyped temporal and spectral structure, which consists of a long sustained multipulsed note with an upsweep in frequency at the onset, followed by a constant frequency along the note, and are emitted at irregular intervals. The newly collected series of *Elachistocleis* in northern and western Brazil showed intraspecific variation in morphology and coloration that had not been previously reported. Coloration was listed as a diagnostic feature in species original descriptions, but our series exhibited color patterns somewhat variable within each species, and sometimes did not allow for species differentiation. Our results indicated that the three species with greater degrees of morphological resemblance (*E. helianneae*, *E. matogrosso*, *E. muiiraquitan*) differed in microtemporal and spectral traits of their advertisement calls. Moreover, the microtemporal trait of pulse duration in *E. helianneae* was divergent, lasting approximately double the duration of pulse in the calls of the other four species studied. Therefore, pulse duration might be informative to the assignment of specimens/populations to *E. helianneae* based on this distinctive acoustic feature, a species widely distributed across Amazonian lowland forests and Amazonian savannas in northern South America. Our results bring novelties on species diagnosis and intraspecific variation in *Elachistocleis*, based on acoustic evidence and morphological/chromatic patterns, and accentuate the importance of comprehensive assessments for accurate species-level identification in this genus of Neotropical frogs.

Key words: Amazon Rainforest, Bioacoustics, Cerrado, Neotropics, Oval frogs, Taxonomy

Introduction

Microhylid frogs represent one of the four most species-rich anuran families, containing over 600 species (Frost 2017), and the monophyly of this taxon is supported by morphological (larvae and adults) and molecular evidence (Haas 2003; Van der Meijden *et al.* 2007; De Sá *et al.* 2012). *Elachistocleis* currently comprises 17 species that are widely distributed throughout the Neotropical region from Panama to central Argentina (Frost 2017); this microhylid genus is included in the South American vicariant clade Gastrophryinae (Van Bocxlaer *et al.* 2006; Van der Meijden *et al.* 2007).

In anurans, vocalizations in reproductive context, namely advertisement calls, are of importance in taxonomic delimitations, given that they may function as a premating isolating mechanism (Blair 1955; Littlejohn 1965) and may then provide a means for species differentiation. Twelve out of 17 *Elachistocleis* species have their advertisement calls described up to date.

Here we describe for the first time the advertisement call of three *Elachistocleis* species: *E. surumu* Caramaschi, 2010, *E. magna* Toledo, 2010, and *E. matogrosso* Caramaschi, 2010; and redescribe the calls of two species: *E. helianneae* Caramaschi, 2010 and *E. muiiraquitan* Nunes-de-Almeida & Toledo, 2012, based on newly gathered data. Also, we highlight intraspecific variation in morphology and color patterns, and comment on the species diagnoses.

Material and Methods

All species were recorded using digital recorders (Marantz PMD 660, 670, 671) and directional microphones (Sennheiser ME67/K6 and ME66/K6). Recordings were made at sampling rates of 44.1 or 48.0 kHz, and samples sizes of 16 or 24 bits. Information on each species is as follows (all field collections and sound recordings were made in Brazil; Fig. 1): *Elachistocleis surumu* was recorded in the municipality of Cantá (2°17'22.36"N, 60°38'33.85"W; 88 m above sea level), state of Roraima, in June 2016 and July 2016. *Elachistocleis magna* was recorded in three municipalities: (i) Costa Marques (12°26'50.53"S, 64°13'33.93"W; 135 m a. s. l.) in January 2016; (ii) Espigão

77 d'Oeste (11°17'41.27"S, 60°55'8.05"W, 201 m a. s. l.; type locality), and (iii) Vilhena
 78 (12°42'55.46"S, 60° 6'56.07"W, 583 m a. s. l.) in January 2017; all in the state of Rondônia.
 79 *Elachistocleis matogrosso* was recorded in the municipality of Cuiabá (15°26'13.58"S,
 80 56°03'44.54"W, 205 m a. s. l.; type locality), state of Mato Grosso, in January 2017. *Elachistocleis*
 81 *muiraquitan* was recorded in the municipality of Rio Branco (9°57'21.49"S, 67°52'0.78"W, 155 m
 82 a. s. l.), state of Acre, in February 2017. *Elachistocleis helianneae* was recorded in two
 83 municipalities: (i) Itapuã do Oeste (9°11'12.67"S, 63°11'20.18"W, 97 m a. s. l.), state of Rondônia,
 84 in January 2017; and (ii) Macapá (0°00'23.07"S, 51°05'08.52"W, 16 m a. s. l.), state of Amapá, in
 85 March 2017.

86 Specimens are housed in the Collection of amphibians (AAG-UFU) of the Museu de
 87 Biodiversidade do Cerrado at Universidade Federal de Uberlândia (Uberlândia, Minas Gerais,
 88 Brazil). Additional specimens were examined in the following Brazilian collections: Herpetological
 89 Collection of the Universidade Federal do Acre (UFAC-RB), Rio Branco, state of Acre; Museu de
 90 História Natural “Adão J. Cardoso” (ZUEC-AMP) at Universidade Estadual de Campinas
 91 (UNICAMP), state of São Paulo; Collection Célio F. B. Haddad (CFBH) at Universidade Estadual
 92 Paulista (UNESP), Rio Claro, state of São Paulo. See Appendices I–II for further information on
 93 specimens examined, call vouchers, and sound recordings.

94 The snout-vent length (SVL), head length (HL), and head width (HW) were measured with
 95 calipers to the nearest 0.05 mm according to Caramaschi (2010). The acoustic analysis was
 96 conducted in Raven Pro 1.4, 64-bit version (Bioacoustics Research Program 2012); sound figures
 97 were produced using Seewave package, version 1.7.3 (Sueur *et al.* 2008), in R, version 3.1.0 (R
 98 Core Team 2014). Raven Pro settings: window size = 512 samples; window type = Hann; 3dB filter
 99 bandwidth = 124 or 135 Hz; overlap = 85%; hop size = 1.60 or 1.75 ms; DFT size = 512 samples;
 100 grid spacing = 86.1 or 93.8 Hz. Seewave settings: window = Hann; window length = 256 samples
 101 (Figs. 2–6), FFT = 128 samples (Figs. 7–8); overlap = 90% (Figs. 2–6), overlap 99% (Figs. 7–8).

102 Spectrograms were produced using a relative amplitude scale of 36 dB indicated by colors: red is
 103 the maximum amplitude (= 0 dB). A 500-Hz high pass filter was applied to the calls in Figs. 2–8.

104 Call traits used in this description are as follows: call duration (duration of one vocal
 105 emission, which corresponds to one multipulsed note in *Elachistocleis*), call rate (duration that
 106 comprised all the calls from a recording, and then adjusted to one minute using cross-multiplication;
 107 given as calls/minute), intercall intervals (interval from one call to the next), call rise time (relative
 108 amplitude peak of the call), pulse duration (measured from the beginning of one pulse to the
 109 beginning of the next), pulse rate (obtained from the measurement of duration of ten pulses from
 110 mid-call, and then extrapolated to one second; given as pulses/s), dominant frequency (frequency
 111 containing the greatest energy in the call), minimum and maximum frequency (the lowest and
 112 highest frequency detected in the spectrogram). Temporal traits were analyzed in the oscillogram
 113 window, spectral traits in the spectrogram window. Pulse duration was quantified from the average
 114 of ten pulses at the midpoint of each call. The dominant frequency was quantified through Peak
 115 Frequency measurement function; minimum and maximum frequencies through Frequency 5% and
 116 Frequency 95% functions, respectively; and the call rise time through Peak Time function.

117 Calls of the five *Elachistocleis* species studied here are pulsed, and these are always
 118 incomplete, *i.e.*, there is no complete (100%) amplitude modulation between pulses (see Figs. 7–8).
 119 However, we recognized two distinctive portions composing the pulses in the call of *E. helianneae*:
 120 high amplitude portions, containing most energy and amplitude peak, which are connected to the
 121 next pulse by low amplitude portions (Figs. 7–8); in a few cases, we detected very short periods of
 122 silence between pulses (≤ 1 ms), based on temporal quantification at low FFT size (128), which we
 123 did not consider as well-defined intervals between pulses.

124 A Principal component analysis (PCA) was applied on a correlation matrix to the acoustic
 125 dataset (mean values for each recorded male). Seven out of nine acoustic traits measured were
 126 considered: call rate and intercall interval were excluded, because of smaller sample sizes. Values
 127 from this dataset were normalized. The first three principal components (PCs) obtained were then

128 applied to a model of Multivariate Analysis of Covariance (MANCOVA) as dependent variables.
 129 The temperatures of air and water, measured while recording the calls, were included (separately)
 130 as covariates to evaluate the temperature effects on the acoustic variance. In this step, *Elachistocleis*
 131 *muiraquitana*, for which temperatures were not measured, was excluded. A few calls from one male
 132 recorded in Costa Marques (Rondônia) were tentatively assigned to *E. helianneae* based on their
 133 particular microtemporal structure (see Discussion; Fig. 8). Still, we restricted our analysis for this
 134 population to a qualitative characterization, since we do not have an actual voucher from this
 135 locality. Calls from Macapá (Amapá) do not have voucher males either, but this species of
 136 *Elachistocleis* is already known and the only reported up to date in the Brazilian state of Amapá
 137 (Costa-Campos & Freire 2015).

138 Additionally, we conducted a Discriminant Analysis of Principal Components (DAPC) to
 139 assess the relationships between different clusters focused on between-group variability (= between
 140 species). The DAPC relies on data transformation using PCA as a prior step to DA, which ensures
 141 that variables submitted to DA are uncorrelated, and that their number is less than that of
 142 individuals analyzed. This multivariate method defines a model in which dataset variation is
 143 partitioned into a between-group and a within-group component, and yields synthetic variables that
 144 maximize the first while minimizing the second (Jombart *et al.* 2010). Principal component scores
 145 are provided in Appendix III; component loadings, eigenvalues, and variation explained in
 146 Appendix IV. Analytical procedures were implemented in R, using the packages *vegan* v. 2.3.1
 147 (Oksanen *et al.* 2013) and *ade4* v. 2.0.0 (Jombart 2008).

148

149 **Results**

150

151 ***Species Identification***

152 Regarding the diagnostic morphological/chromatic features from the original descriptions, the
 153 specimens collected by us showed some variation, especially in SVL, HW, HL, and patterns of

spots, blotches and stripes. The species identification and the variation previously unreported are listed as follows:

Elachistocleis surumu (Table 1; Fig. 2): our series (N = 8 adults: seven males and one female) mostly match the diagnosis by Caramaschi (2010): (1) small-sized species: male SVL 26.4–30.8 mm (mean: 28.2, SD: 1.5); (2) head wider than long (males): HL about 77% of HW (range: 63–84%; mean: 77, SD: 8); (3) small/discrete post-commissural gland; (4) dorsum smooth; gray with small and lighter gray spots irregularly distributed; (5) vertebral stripe absent; (6) venter gray with many irregular cream spots regularly distributed; (7) an undefined color transition between dorsal and ventral regions; (8) small, defined white spots on axilla and groin; (9) a broad and irregular longitudinal stripe on the posterior surface of thighs; and (10) a longitudinal stripe on knee and tarsus absent. However, we observed unreported variation in belly color patterns, female SVL, distribution of spots on axilla and groin, and thigh stripe. The male AAG-UFU 5571, for instance, had the belly covered with white spots laterally on a dark-colored background and intermingled with irregular yellow blotches. The SVL of the adult female AAG-UFU 5572 is 38.2 mm, far beyond the maximum female SVL reported in the original description (SVL 23.2–26.9 mm; Caramaschi 2010). The adult male AAG-UFU 5445 did not possess defined white spots on axilla or groin, even though white spots were observed on the anterior surface of thighs. Besides, the stripe on the posterior surface of thighs was absent in this specimen.

Elachistocleis magna (Table 1; Fig. 3): our series (N = 16 adults: 14 males and two females) perfectly fit the original species diagnosis (Toledo 2010): (1) ventral coloration in preserved individuals is white with gray spots or gray with white spots (depending on the prevalence of one of the colors); (2) grayish dorsum in preserved individuals; (3) presence of post-commissural gland; (4) lack of red blotches in the hidden surface of legs or red stripes on the legs; and (5) SVL larger than 31 mm in adult individuals: males (range: 28.5–33.5 mm; mean: 31.4, SD: 1.7) and females (range: 39.5–43.8 mm; mean: 41.7, SD: 3.0). The trait (4) was observed only in live specimens, since those blotches tend to fade and become pale orange in preserved specimens.

180 *Elachistocleis helianneae* (Table 1; Fig. 4): our series (N = 2 adults: one male and one female)
 181 essentially fit the original species diagnosis (Caramaschi 2010): (1) small-sized species (male SVL:
 182 28.4 mm; female SVL: 33.5 mm); (2) HL slightly smaller than HW (HL about 88% of HW in the
 183 male); (3) post-commissural gland poorly developed; (4) dorsum smooth, grayish brown with
 184 minute scattered light gray spots in preserved specimens; (5) light cream longitudinal stripe from
 185 the snout to vent; (6) immaculate venter; (8) well-delimited color limits between the dorsal and
 186 ventral regions; (9) no spots on the axilla or groin; (10) a broad and irregular cream line on the
 187 posterior surface of the thighs, and a large and light cream spot on the proximal/internal surface of
 188 tibiae; and (11) a narrow light cream stripe surrounding the knees and reaching the mid-length of
 189 the tarsus. The variation was only observed in the female AAG-UFU 5801, for which the HL is
 190 slightly greater than HW (HL about 121% of HW), and the trait (11) was absent.

191 *Elachistocleis matogrosso* (Table 1; Fig. 5): some divergences were found in our series (N =
 192 4 adults: three males and one female) in comparison with the original diagnosis (Caramaschi 2010):
 193 (1) small-sized species: male SVL 23.1–27.3 mm (mean: 25.9, SD: 2.4) and female SVL 36.1 mm.
 194 In the original description, the male SVL varies from 21.5–24.6 mm and female SVL from 29.0–
 195 33.2 mm, generally smaller than the specimens collected by us; (2) in the original species diagnosis,
 196 the mid-dorsal longitudinal light stripe was reported to extend from the post-cephalic transverse
 197 skinfold to the vent, absent on the head, which could be observed in the specimens AAG-UFU 5955
 198 and AAG-UFU 5953, but the mid-dorsal longitudinal stripe is either complete (from the tip of the
 199 snout to the vent; AAG-UFU 5954) or at least it extends beyond the post-cephalic dermal fold
 200 toward the tip of the snout (AAG-UFU 5720); (3) the venter was originally described as
 201 immaculate, but the individual AAG-UFU 5953 has dots scattered on ventral surfaces. The other
 202 traits agree with those in the species diagnosis: (4) HL shorter than HW, HL 87.5% of the HW in
 203 males, and HL 98% of the HW in the female; (5) post-commissural gland poorly developed; (6)
 204 dorsum smooth and grayish in preserved individuals; (7) presence of light spots on the axilla or
 205 groin, and a broad and irregular line on the posterior surface of the thighs; (8) a large, light spot on

the proximal/internal surface of tibiae; (9) a narrow and light stripe surrounding the knees and reaching the mid-length of the tarsus; and (10) the limits between dorsal and ventral regions poorly defined in all specimens analyzed.

Elachistocleis muiiraquitana (Table 1; Fig. 6): our series (N = 3 adult males) mostly agrees with the original diagnosis (Nunes-de-Almeida & Toledo 2012): (1) small-sized species: male SVL 28.6–29.7 mm (mean: 29.2, SD: 0.6); (2) HL shorter than HW, HL about 81% of HW; (3) post-commissural gland poorly developed; (4) dorsum smooth uniformly dark brown; (5) venter immaculate with limits between the dorsal and ventral regions well-defined; (6) a broad and irregular line on the posterior surface of the thighs; (7) a large, light spot on the proximal/internal surface of tibiae; and (8) a narrow, light stripe surrounding the knees and reaching the mid-length of the tarsus. The only divergence is related to the mid-dorsal longitudinal light stripe: in the specimen AAG-UFU 5842, the stripe extends from the vent to mid-dorsum; but in AAG-UFU 5843–5844, the stripe is complete, extending to the tip of the snout (stripe from the post-cephalic transverse skinfold to the vent, but never reaching the head; Nunes-de-Almeida & Toledo 2012).

Acoustic analyses

The advertisement call of the five species of *Elachistocleis* analyzed in the present study have a stereotyped temporal and spectral structure, which consists of a long sustained multipulsed note with an upsweep in frequency at the very onset, followed by a relatively constant frequency along the note, and is emitted at irregular intervals. There is no noticeable interval between pulses and calls have a progressive increase in amplitude and tend to have their amplitude peak in the final portion of the call. Acoustic descriptions (mean values and SDs for each species can be found in Table 2) for each species are as follows:

Males of *Elachistocleis surumu* were calling at night (19:00–00:00 h) in abandoned pisciculture ponds with shallow water (< 30 cm) and bordered by short grassy vegetation, inside natural depressions on the ground filled with rainwater at forest edge or in flooded areas covered

232 with herbaceous/grassy vegetation and cattle farming pasture (non-native grasses). The call of *E.*
 233 *surumu* (N = 12 males, 60 calls; Fig. 2) lasts 0.9–2.3 s, being emitted at rates of 2.4–6.9 calls per
 234 minute, and intercall interval from 16.4–34.7 s. The call rise time varies from 63–98% of the call.
 235 Pulses are emitted at 224–269 pulses/s, whose duration is always 4.0 ms (Table 2; Fig. 7). The call
 236 dominant frequency ranges from 3.5–4.1 kHz, the minimum frequency from 2.8–3.6 kHz, and the
 237 maximum frequency from 4.1–4.3 kHz.

238 Males of *Elachistocleis magna* were calling in flooded areas bordered by herbaceous/grassy
 239 vegetation and pasture (non-native grasses) at the type locality (Espigão d'Oeste) and rain-filled
 240 temporary pools in urban areas (Costa Marques and Vilhena). Calling activity was observed
 241 between 19:00– 22:00 h. The call of *E. magna* (N = 19 males, 50 calls; Fig. 3) lasts 1.2–2.5 s, being
 242 emitted at rates of 3.0–8.0 calls per minute, and intercall interval from 8.0–37.3 s. The call rise time
 243 varies from 39–97% of the call. Pulses are emitted at 223–278 pulses/s, whose duration is around
 244 4.0 ms (Table 2; Fig. 7). The dominance frequency ranges from 3.1–3.8 kHz, the minimum
 245 frequency from 2.7–3.4 kHz, and the maximum frequency from 3.4–4.0 kHz.

246 Males of *Elachistocleis helianneae* were calling at night (19:00–02:00 h) among rain-filled
 247 temporary pools in urban areas. The call of *E. helianneae* (N= 14 males, 66 calls; Fig. 4) lasts 1.3–
 248 3.0 s, being emitted at rates of 3.3–16.6 calls per minute, and intercall interval from 7.7–24.6 s. The
 249 call rise time varies from 43–95% of the call. Pulses are emitted at 118–137 pulses/s, whose
 250 duration varies from 7.0–8.3 s (Table 2; Fig. 7). The dominant frequency ranges from 4.2–4.7 kHz,
 251 the minimum frequency from 3.9–4.4 kHz, and the maximum frequency from 4.4–4.9 kHz.

252 Males of *Elachistocleis matogrosso* (N= 6 males, 24 calls; Fig. 5) were calling at night
 253 (21:30–00:00 h) among rain-filled temporary pools in rural areas (natural remnants of Cerrado and
 254 anthropogenic areas). The call lasts 1.3–1.8 s, being emitted at rates of 3.5–8.4 calls per minute, and
 255 intercall interval from 5.0–25.6 s. The call rise time varies from 61–98% of the call. Pulses are
 256 emitted at 223–236 pulses/s, whose duration is around 4.0 ms (Table 2; Fig. 7). The dominant

frequency ranges from 4.3–4.7 kHz, the minimum frequency from 3.6–4.3 kHz, and the maximum frequency from 4.5–4.8 kHz.

Males of *Elachistocleis muiraquitana* were calling at night (20:00–22:00 h) in rain-filled temporary pools in a green area covered with short grasses at the university campus (UFAC, Rio Branco). The call of *E. muiraquitana* (N = 2 males, 7 calls; Fig. 6) lasts 0.7–0.8 s, being emitted at a rate of 3.9 calls per minute, and a 28.8-second interval between the two calls recorded. The call rise time varies from 80–85% of the call. Pulses are emitted at 210.5–234.0 pulses/s, whose duration varies from 4.0–4.5 ms (Table 2; Fig. 7). The dominant frequency ranges from 3.5–3.9 kHz, the minimum frequency from 3.1–3.5 kHz, and maximum frequency from 4.0–4.1 kHz.

Revisiting between-species diagnosis

Elachistocleis surumu and *E. magna* differed in ventral coloration, according to Caramaschi (2010). Color patterns in the newly collected series of both species varied to the extent that they could not be differentiated from each other, neither could they by differences in size: male SVL 28.5–33.5 mm (mean 31.4, SD = 1.7; N = 14) in *E. magna*, male SVL 26.4–30.8 mm (mean 28.2, SD = 1.5; N = 7) in *E. surumu*.

Species with an immaculate venter are *E. matogrosso*, *E. helianneae*, and *E. muiraquitana*. Morphological traits did not accurately distinguish our specimens of *E. matogrosso* from those of *E. helianneae*. The original description of these species (Caramaschi 2010) listed the following characteristics to the differentiation between them: (1) HL/HW proportion (smaller in *E. matogrosso* than in *E. helianneae*); (2) mid-dorsal stripe from the post-cephalic dermal fold to the vent in *E. matogrosso* (mid-dorsal stripe from tip of snout to vent in *E. helianneae*); (3) absence of minute light spots on dorsum and dorsal surfaces of members in *E. matogrosso* (present in *E. helianneae*); (4) dorsal gray color of the snout invading the loreal region almost to the upper lip border in *E. matogrosso* (loreal region conspicuously white in *E. helianneae*); and (5) stripe on the posterior surface of the thighs broad and irregular in *E. matogrosso* (thin and well-defined in *E.*

helianneae). Among the specimens analyzed by us, the traits #2–5 were highly variable within each one of these species (see the species identification section earlier).

Elachistocleis muiiraquitan was reported to be the species with the longest head and the largest SVL among congeners with immaculate venter (compared to *E. helianneae*, *E. matogrosso*, and *E. bicolor*; Nunes-de-Almeida & Toledo 2012). Our results agree to this statement. By contrast, the following color patterns proposed as diagnostic characteristics (dorsal stripe, limits between dorsal and ventral regions, minute light spots on dorsum and dorsal surfaces of members) were not useful to distinguish *E. muiiraquitan* from *E. helianneae* or *E. matogrosso*.

Acoustic comparisons

The first three PCs accounted for ca. 91% of the total variance explained in the dataset of acoustic traits. The DAPC analysis showed that all three spectral traits and temporal traits of pulse duration and pulse rate accounted the most for species discrimination along PC1, whereas call duration and call rise time contributed the most along PC2 (Fig. 9). It is worth highlighting that only *Elachistocleis surumu* and *E. muiiraquitan* could not be discriminated based on the acoustic traits analyzed (Fig. 9), but they can be easily distinguished from each other by their distinctive coloration (see Results; Figs. 2, 6).

The multivariate analysis of covariance (MANCOVA) discriminated all the groups (five species) with a high significance despite the effects of the covariate temperature: the five species were recovered as distinct taking into account the potential effects of air temperature (Pillai trace = 1.75, $F_{9,135} = 21.13$, $df = 9$; $p < 0.001$); these species were also recovered as distinct groups taking into account the potential effects of water temperature (Pillai trace = 1.75, $F_{9,135} = 20.95$, $df = 9$; $p < 0.001$). This analysis also showed that the covariates (air and water temperatures) did not have significant effects on the acoustic variance found for the five species of *Elachistocleis*: air (Pillai trace = 0.10, $F_{3,43} = 1.57$, $df = 3$; $p = 0.21$); water (Pillai trace = 0.04, $F_{3,43} = 0.59$, $df = 3$; $p = 0.63$).

309 Discussion

310 The advertisement call of all *Elachistocleis* species analyzed in this study falls under the
 311 stereotyped calling pattern recognized for the genus, which consists of long-lasting and sustained,
 312 fast-pulsed and high-pitched calls, which resemble a buzzing sound (Figs. 2–6). This acoustic
 313 stereotypy with respect to a shared acoustic structure is most likely the result of the phylogenetic
 314 history of this microhylid genus, presumably sharing high degrees of phylogenetic signal among
 315 species' advertisement calls (Erdtmann & Amézquita 2009; Gingras *et al.* 2013).

316 The collection of new specimens of five *Elachistocleis* species in northern and western
 317 Brazil revealed intraspecific variation in morphology and coloration that had not been reported up
 318 to the present moment. Coloration patterns were listed as diagnostic features in species original
 319 descriptions, but they varied to different extents among the specimens collected for each species,
 320 and hence not reliable traits for species differentiation in some cases. Of particular significance is *E.*
 321 *magna* and *E. surumu*. Our results showed that one cannot distinguish these species unequivocally
 322 based on any of the features listed by Caramaschi (2010), and have overlapping SVL ranges (Table
 323 1; Figs. 2–3). Despite the high degree of phenotypic similarity between these species, our acoustic
 324 analysis revealed that their calls (especially spectral traits), on the other hand, might be relevant for
 325 their differentiation (Fig. 9).

326 Our results also indicate that the three species with greater degrees of morphological
 327 resemblance, sharing an immaculate venter (*E. helianneae*, *E. matogrosso* and *E. muiiraquitana*), had
 328 distinctive microtemporal (pulse duration; Figs. 7–8) and spectral (dominant frequency) features in
 329 their advertisement calls (Table 2), which could be of importance in the differential diagnosis of
 330 these morphologically similar species of *Elachistocleis*, including that *E. matogrosso* and *E.*
 331 *helianneae* could not be differentiated unambiguously by any evidence other than their calls (Fig.
 332 9). Pulse duration in *E. helianneae* was divergent, lasting approximately double the duration of
 333 pulses in the calls of the other four species in this study (Figs. 7–8).

Our call redescrptions for *E. helianneae* and *E. muiiraquitán* did not exhibit substantial differences in comparison with earlier descriptions, except for call duration in *E. muiiraquitán*, which was remarkably shorter in our recordings. In the first call description for *E. muiiraquitán* (Nunes-de-Almeida & Toledo 2012), these authors divided the call into an “initial and modulated portion of the call” with duration, varying from 0.12–0.22 s, and a “final and sustained portion of the call”, varying from 2.86–4.42 s. Our sample of calls (both portions included) had shorter duration, lasting 0.6–1.0 s. We suggest that this temporal difference is partly because the males recorded by us were not in high calling activity (DLB, field obs.). Besides, the distinctive call trait of *E. helianneae* (pulse duration) was not provided in the earlier call description for this species (Fonseca *et al.* 2012).

Therefore, this microtemporal trait (pulse duration) should be informative to the assignment of specimens/populations to *E. helianneae* and its discrimination from the apparently morphologically cryptic *E. matogrosso*. *Elachistocleis helianneae* is a species with occurrence in Bolivia and southern Amazon (Brazilian states of Rondônia e Amazonas), with a disjunct distribution in the Amazonian savannas of the Brazilian state of Amapá (Costa-Campos & Freire 2015; Frost 2017). It is noteworthy that the two latter regions are separated over 1500 km by forests across the great Amazon basin (Fig. 1). Furthermore, calls recorded (unvouchered males) from the border of Brazil and Bolivia (Costa Marques, Brazilian state of Rondônia) should probably be assigned to *E. helianneae*, based on the markedly long pulse duration (Fig. 8). Therefore, this locality is tentatively accepted as the westernmost range of this species within Brazil’s territory (Fig. 1).

Our results bring novelties on species diagnosis for five Brazilian species of *Elachistocleis* based on acoustic evidence and morphological/chromatic patterns, and highlight the importance of taking into account the vocal patterns in this Neotropical group of frogs for future studies in the taxonomic and systematic fields. Furthermore, future research to address the extent to which call variability in *Elachistocleis* is allied to physical factors (e.g., temperature), morphology (e.g., body

size and mass) and other sources of variability could tell us more about an appropriate set of acoustic traits for species differentiation in this taxonomically complex group of frogs. However, our preliminary results on the effects of temperature on call traits of *Elachistocleis* showed that this physical factor did not explain the variance found in the call traits analyzed here. Besides, studying in detail how these species produce their calls could give us insights into evolutionary novelties related to sound production, as demonstrated by Giaretta *et al.* (2015) for the South American microhylid *Dermatonotus muelleri*, and also underlying processes that might have shaped the evolution of the particular and stereotyped vocal communication system in New World microhylids.

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469

470 **Appendix I.** List of specimens examined.

471

472 *Elachistocleis bicolor*—BRAZIL: RIO GRANDE DO SUL: Bom Jesus (CFBH 18199);
 473 SANTA CATARINA: São Domingos (CFBH 36285).

474 *Elachistocleis cf. carvalhoi*—BRAZIL: TOCANTINS: Araguacema (CFBH 10301, 10304).

475 *Elachistocleis cesarii*—BRAZIL: GOIÁS: Alto Paraíso de Goiás (AAG-UFU 0794); MATO
 476 GROSSO: Pontal do Araguaia (AAG-UFU 1315–16; AAG-UFU 3479); MINAS GERAIS:
 477 Ituiutaba (AAG-UFU 0342); Perdizes (AAG-UFU 2693–95); Sacramento (AAG-UFU 0889);
 478 Uberlândia (AAG-UFU 0340; AAG-UFU 2111); SÃO PAULO: Campinas (AAG-UFU 2986).

479 *Elachistocleis helianneae*—BRAZIL: AMAZONAS: Humaitá (topotypes: CFBH 37253–
 480 56); RONDÔNIA: Itapuã do Oeste (AAG-UFU 5800–01).

481 *Elachistocleis magna*—BRAZIL: RONDÔNIA: Costa Marques (AAG-UFU 5288–99);
 482 Espigão d'Oeste (AAG-UFU 5776–77; UFAC-RB 3229, 3249–50, 3265, 3276, 3310; holotype:
 483 ZUEC-AMP 11384; paratypes: CFBH 5156–58; ZUEC-AMP 11382–83, 11385–86); Vilhena
 484 (AAG-UFU 5766–67).

485 *Elachistocleis matogrosso*—BRAZIL: MATO GROSSO: Cuiabá (topotypes: AAG-UFU
 486 2125, 5842–44).

487 *Elachistocleis muiiraquitan*—BRAZIL: ACRE: Rio Branco (AAG-UFU 5842–44).

488 *Elachistocleis surumu*—BRAZIL: RORAIMA: Cantá (AAG-UFU 5442–47, 5571–72);
 489 Mucajaí (AAG-UFU 5488).

490

491 **Appendix II.** Information on sound recordings for *Elachistocleis* species. Abbreviations for Brazilian states: AC (Acre), AP (Amapá), MT (Mato

492 Grosso), RO (Rondônia), RR (Roraima). Letters following the number in sound file names (e.g., 6a-c) indicate the number of recordings for that male

493 (three recordings for male #6).

Sound file	Species	Accession number (AAG-UFU)	Locality	Date (dd-mm-yyyy)	Air / water (°C)
Elachist_surumuCantaRR1aAAGm671	<i>E. surumu</i>	—	Cantá, RR	20-07-2016	28.0 / 30.0
Elachist_surumuCantaRR2aAAGm671	<i>E. surumu</i>	—	Cantá, RR	20-07-2016	28.0 / 30.0
Elachist_surumuCantaRR3aAAGm671	<i>E. surumu</i>	—	Cantá, RR	21-07-2016	26.0 / 29.0
Elachist_surumuCantaRR4aAAGm671	<i>E. surumu</i>	—	Cantá, RR	21-07-2016	26.0 / 29.0
Elachist_surumuCantaRR5aAAGm671	<i>E. surumu</i>	—	Cantá, RR	21-07-2016	26.0 / 29.0
Elachist_surumuCantaRR6a-cAAGm671	<i>E. surumu</i>	5571	Cantá, RR	23-07-2016	26.0 / 27.0
Elachist_surumuCantaRR7aAAGm671	<i>E. surumu</i>	—	Cantá, RR	24-07-2016	27.0 / 27.0
Elachist_surumuCantaRR8aAAGm671	<i>E. surumu</i>	—	Cantá, RR	24-07-2016	27.0 / 27.0
Elachist_surumuCantaRR9a-cAAGm671	<i>E. surumu</i>	—	Cantá, RR	23-07-2016	26.0 / 27.0
Elachist_surumuCantaRR10a-bAAGm671	<i>E. surumu</i>	—	Cantá, RR	23-07-2016	26.0 / 27.0
Elachistocleis_sumuruCantaRR1a-bTRC_AAGm671	<i>E. surumu</i>	—	Cantá, RR	20-06-2016	25.3 / 29.1
Elachistocleis_sumuruCantaRR2aTRC_AAGm671	<i>E. surumu</i>	—	Cantá, RR	20-06-2016	25.3 / 29.1
Elachist_magnaCostaMarquesRO1a-bAAGm671	<i>E. magna</i>	—	Costa Marques, RO	10-01-2016	24.0 / 27.0
Elachist_magnaCostaMarquesRO2a-cAAGm671	<i>E. magna</i>	—	Costa Marques, RO	10-01-2016	24.0 / 27.0
Elachist_magnaCostaMarquesRO3aAAGm671	<i>E. magna</i>	—	Costa Marques, RO	10-01-2016	24.0 / 27.0
Elachist_magnaCostaMarquesRO4aFSA_AAGm671	<i>E. magna</i>	—	Costa Marques, RO	08-01-2016	27.0 / 27.0
Elachist_magnaEspigaoDoeste1a-cBFVT_AAGm	<i>E. magna</i>	—	Espigão D'Oeste, RO	24-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoeste2aBFVT_AAGm	<i>E. magna</i>	—	Espigão D'Oeste, RO	24-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoeste3a-bBFVT_AAGm	<i>E. magna</i>	—	Espigão D'Oeste, RO	24-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoesteRO1a-bDLB_AAGm671	<i>E. magna</i>	—	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoesteRO2a-cDLB_AAGm671	<i>E. magna</i>	5776	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoesteRO3a-cDLB_AAGm671	<i>E. magna</i>	—	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoesteRO4aDLB_AAGm671	<i>E. magna</i>	—	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoesteRO5aDLB_AAGm671	<i>E. magna</i>	—	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoesteRO6aDLB_AAGm671	<i>E. magna</i>	—	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1

Elachist_magnaEspigaoDoesteRO7a-bDLB_AAGm671	<i>E. magna</i>	—	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1
Elachist_magnaEspigaoDoesteRO8a-bBTRC_AAGm671	<i>E. magna</i>	—	Espigão d'Oeste, RO	29-01-2017	25.4 / 28.1
Elachist_magnaVilhenaRO1a-cDLB_AAGm671	<i>E. magna</i>	—	Vilhena, RO	27-01-2017	22.7 / 27.0
Elachist_magnaVilhenaRO2a-dDLB_AAGm671	<i>E. magna</i>	—	Vilhena, RO	27-01-2017	22.7 / 27.0
Elachist_magnaVilhenaRO3a-dDLB_AAGm671	<i>E. magna</i>	5766	Vilhena, RO	27-01-2017	22.7 / 27.0
Elachist_helianneaelTapuaOesteRO1a-dDLB_AAGm671	<i>E. helianneae</i>	—	Itapuã do Oeste, RO	31-01-2017	26.0 / 26.0
Elachist_helianneaelTapuaOesteRO2aDLB_AAGm671	<i>E. helianneae</i>	—	Itapuã do Oeste, RO	31-01-2017	26.0 / 26.0
Elachist_helianneaelTapuaOesteRO3a-cDLB_AAGm671	<i>E. helianneae</i>	—	Itapuã do Oeste, RO	31-01-2017	26.0 / 26.0
Elachist_helianneaelTapuaOesteRO4a-bBFTV_AAGm	<i>E. helianneae</i>	—	Itapuã do Oeste, RO	31-01-2017	25.5 / 26.8
Elachist_helianneaelTapuaOesteRO5aBFTV_AAGm	<i>E. helianneae</i>	—	Itapuã do Oeste, RO	31-07-2017	25.5 / 26.8
Elachist_helianneaelTapuaOesteRO6aTRC_AAGm	<i>E. helianneae</i>	5800	Itapuã do Oeste, RO	30-01-2017	25.6 / 26.7
Elachist_helianneaelTapuaOesteRO7a-bTRC_AAGm671	<i>E. helianneae</i>	—	Itapuã do Oeste, RO	31-01-2017	25.1 / 26.9
Elachist_helianneaelTapuaOesteRO8aTRC_AAGm671	<i>E. helianneae</i>	—	Itapuã do Oeste, RO	31-01-2017	25.1 / 26.9
Elachist_helianneaeMacapaAP1aAAGm661MK2	<i>E. helianneae</i>	—	Macapá, AP	26-03-2017	26.0 / 28.0
Elachist_helianneaeMacapaAP3aAAGm661MK2	<i>E. helianneae</i>	—	Macapá, AP	26-03-2017	26.0 / 28.0
Elachist_helianneaeMacapaAP6aAAGm661MK2	<i>E. helianneae</i>	—	Macapá, AP	31-03-2017	28.0 / 28.0
Elachist_helianneaeMacapaAP7aAAGm661MK2	<i>E. helianneae</i>	—	Macapá, AP	31-03-2017	28.0 / 28.0
Elachist_helianneaeMacapaAP8aAAGm661MK2	<i>E. helianneae</i>	—	Macapá, AP	31-03-2017	28.0 / 28.0
Elachist_helianneaeMacapaAP9aAAGm661MK2	<i>E. helianneae</i>	—	Macapá, AP	31-03-2017	28.0 / 28.0
Elachist_helianneaeCostaMarques1a-cAAGm671	<i>E. helianneae</i>	—	Costa Marques, RO	11-01-2016	27.0 / 27.0
Elachist_matogrossoCuiabaMT1aDLB_AAGm671	<i>E. matogrosso</i>	—	Cuiabá, MT	25-01-2017	28.5 / 25.0
Elachist_matogrossoCuiabaMT2a-cDLB_AAGm671	<i>E. matogrosso</i>	—	Cuiabá, MT	15-02-2017	24.5 / 26.0
Elachist_matogrossoCuiabaMT3a-bDLB_AAGm671	<i>E. matogrosso</i>	5953	Cuiabá, MT	15-02-2017	24.5 / 26.0
Elachist_matogrossoCuiabaMT4a-bDLB_AAGm671	<i>E. matogrosso</i>	5954	Cuiabá, MT	15-02-2017	24.5 / 26.0
Elachist_matogrossoCuiabaMT5a-cDLB_AAGm671	<i>E. matogrosso</i>	—	Cuiabá, MT	15-02-2017	24.5 / 26.0
Elachist_matogrossoCuiabaMT6aDLB_AAGm671	<i>E. matogrosso</i>	—	Cuiabá, MT	15-02-2017	24.5 / 26.0
Elachist_matogrossoCuiabaMT7aDLB_AAGm671	<i>E. matogrosso</i>	—	Cuiabá, MT	15-02-2017	24.5 / 26.0
Elachist_muiraquitanoRioBrancoAC1a-dDLB_AAGm671	<i>E. muiraquitana</i>	5842	Rio Branco, AC	10-02-2017	No data
Elachist_muiraquitanoRioBrancoAC2a-bDLB_AAGm671	<i>E. muiraquitana</i>	—	Rio Branco, AC	10-02-2017	No data

Appendix III. Component scores from the first three PCs for each male recorded of *Elachistocleis surumu* (SUR), *E. magna* (MAG), *E. helianneae* (HEL), *E. matogrosso* (MAT), and *E. muiraquitana* (MUI).

Individual	PC1	PC2	PC3	Individual	PC1	PC2	PC3
SUR1	0.423	-0.790	-0.659	MAG1	1.739	-1.235	-1.049
SUR2	0.236	-0.274	0.322	MAG2	2.081	0.332	-1.022
SUR3	0.312	-1.514	0.008	MAG3	1.150	0.224	-1.496
SUR4	0.250	-2.142	0.060	MAG4	1.170	1.080	0.521
SUR5	0.087	-0.632	-0.300	MAG5	2.355	0.507	-0.468
SUR6	-0.105	-0.714	-0.293	MAG6	2.460	2.455	1.144
SUR7	-0.032	-0.277	-0.472	MAG7	2.891	-0.174	-0.338
SUR8	0.361	-0.877	-0.756	MAG8	2.764	0.555	-0.178
SUR9	1.304	-0.236	1.476	MAG9	2.919	0.122	-0.344
SUR10	0.764	-1.295	0.485	MAG10	2.569	0.870	0.650
SUR11	1.728	-1.304	0.429	MAG11	2.226	1.109	0.608
SUR12	0.968	-0.420	-0.443	MAG12	1.870	0.333	-1.354
HEL1	-3.370	0.745	-0.495	MAG13	2.718	0.170	-0.074
HEL2	-3.289	0.086	-1.151	MAG14	2.648	0.396	-0.668
HEL3	-2.517	0.058	0.412	MAG15	2.779	1.630	0.238
HEL4	-3.794	0.474	0.034	MAG16	2.495	0.902	-0.285
HEL5	-2.662	-0.635	-0.241	MAG17	1.351	1.820	0.187
HEL6	-2.525	1.207	0.203	MAG18	0.477	0.604	-0.917
HEL7	-2.302	-0.009	-0.885	MAT1	-0.785	-0.558	1.643
HEL8	-2.735	-1.016	-0.092	MAT2	-1.427	-1.220	1.800
HEL9	-3.025	2.166	-0.117	MAT3	-0.598	-0.172	2.037
HEL10	-2.998	-0.170	-0.875	MAT4	-0.256	-1.125	1.170
HEL11	-3.925	0.371	-1.211	MAT5	-1.539	-2.232	0.586
HEL12	-2.138	-0.263	-1.007	MAT6	-1.180	-1.027	0.924
HEL13	-3.097	2.870	1.365	MUI1	0.781	-1.609	0.574
HEL14	-2.897	1.737	0.117	MUI2	1.321	-0.902	0.194

498 **Appendix IV.** Component loadings of the call traits analyzed for *Elachistocleis* species, and eigenvalues and variance explained for the first three PCs.

Call trait	PC1	PC2	PC3
Call Duration	-0.233	0.578	-0.423
Pulse Duration	-0.399	0.271	-0.224
Pulse Rate	0.431	-0.170	0.188
Dominant Frequency	-0.438	-0.211	0.275
Maximum Frequency	-0.432	-0.223	0.308
Minimum Frequency	-0.453	-0.085	0.239
Call Rise Time	-0.131	-0.679	-0.713
Eigenvalue	2.116	1.112	0.828
Variance (%)	64.0	17.7	9.8

TABLE 1. Morphological traits (mm) for adult specimens of five *Elachistocleis* species collected in northern and western Brazil for this study (AAG-

UFU accession numbers in Appendix I). Values given as mean±SD (minimum–maximum). M = male, F = female. Sample sizes in brackets.

Trait/species	<i>E. surumu</i>		<i>E. magna</i>		<i>E. helianneae</i>		<i>E. matogrosso</i>		<i>E. muiraquitana</i>	
	M (7)	F (1)	M (14)	F (2)	M (1)	F (1)	M (3)	F (1)	M (3)	F (1)
Snout Vent Length	28.2±1.5 (26.4–30.8)	38.2	31.4±1.7 (28.6–33.5)	41.7±3.0 (39.5–43.8)	28.4	33.5	25.9±2.4 (23.1–27.3)	36.2	29.2±0.6 (28.6–29.7)	
Head Length (HL)	5.0±0.4 (4.3–5.5)	5.8	5.0±0.6 (3.9–5.9)	6.1	5.6	6.0	4.7±0.4 (4.3–5.0)	6.2	4.4±0.1 (4.2–4.5)	
Head Width (HW)	6.5±0.5 (5.8–7.3)	7.6	5.3±0.4 (4.2–5.7)	6.7±0.2 (6.6–6.9)	4.6	6.8	5.3±0.3 (4.9–5.6)	6.4	5.5±0.6 (5.0–6.1)	
HL / HW (%)	76.6±8.0 (63.0–84.0)	76.8	95.0±10.1 (73.0–112.0)	91.5±3.0 (89.6–93.3)	87.5	120.8	87.5±27.6 (84.4–89.7)	97.6	80.6±8.2 (73.3–89.5)	

TABLE 2. Acoustic traits for five *Elachistocleis* species recorded in northern and western Brazil (see Appendix II for further information). Values

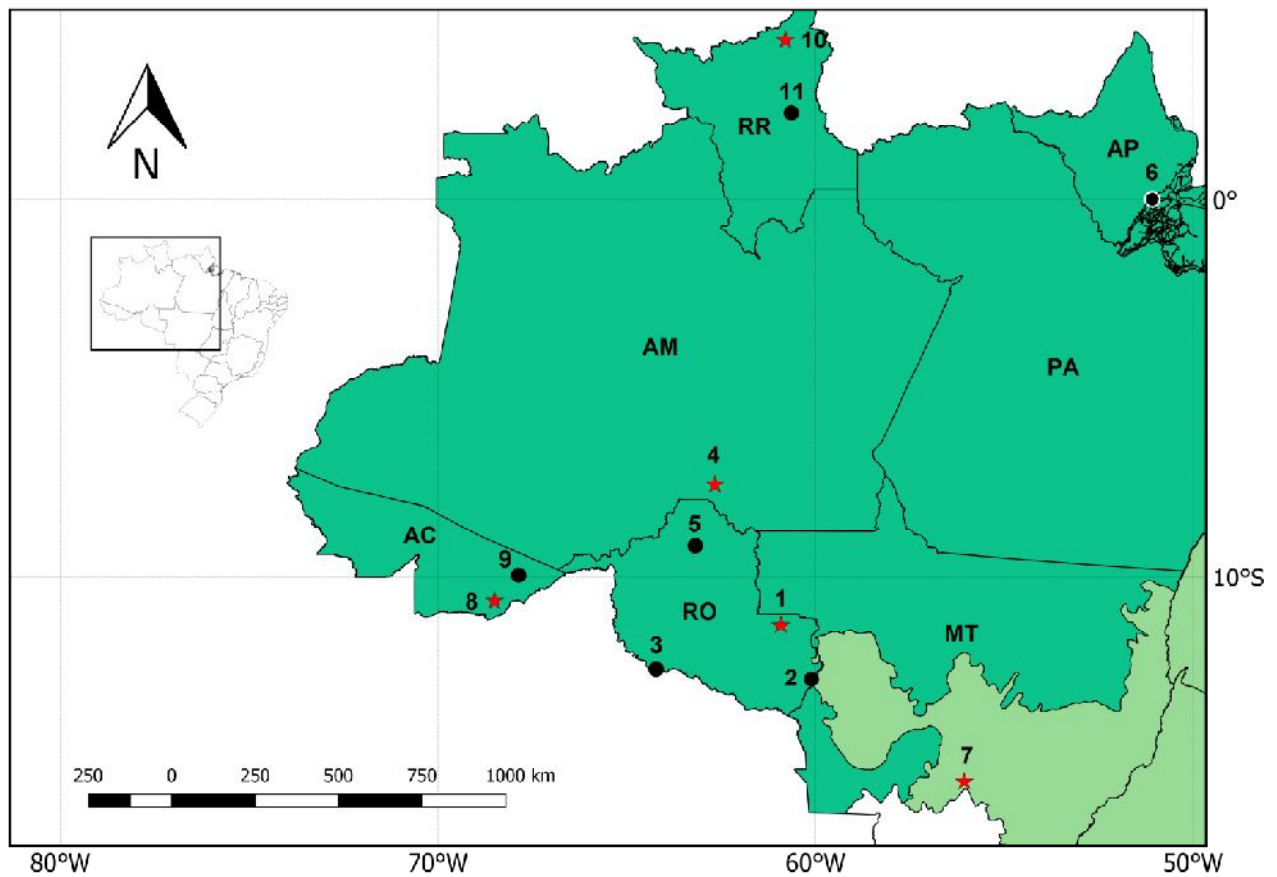
given as mean±SD (minimum–maximum). N = number of males recorded/number of calls analyzed.

	<i>E. surumu</i> N = 12/60	<i>E. magna</i> N = 19/50	<i>E. helianneae</i> N = 14/66	<i>E. matogrosso</i> N = 6/24	<i>E. muiraquitana</i> N = 2/7
Call duration (s)	1.57±0.44 (0.91–2.29)	1.79±0.43 (1.15–2.52)	2.24±0.52 (1.31–2.95)	1.51±0.16 (1.26–1.79)	0.78±0.07 (0.73–0.83)
Intercall interval (s)*	24.23±6.08 (16.35–34.68)	21.00±9.76 (7.92–37.30)	16.13±5.43 (7.70–24.60)	20.21±7.70 (5.02–25.58)	28.84
Call rate (calls/minute)**	3.60±1.29 (2.41–6.86)	4.38±1.62 (2.85–8.04)	5.75±3.44 (3.33–16.56)	5.04±1.76 (3.46–8.44)	3.90
Call rise time (%)	86.1±9.1 (62.8–98.0)	70.7±0.1 (38.7–97.0)	80.6±15.6 (43.0–95.0)	77.2±11.5 (60.9–98.0)	82.7±3.8 (80.0–85.0)
Pulse duration (ms)	4.0±0.0	4.0±0.1 (3.8–4.4)	7.9±0.4 (7.0–8.3)	4.1±0.2 (4.0–4.4)	4.3±0.4 (4.0–4.5)
Pulse rate (pulses/s)	243.0±17.3 (224.3–269.3)	258.2±15.6 (222.7–277.7)	125.3±5.6 (118.1–136.8)	229.2±4.4 (222.8–235.7)	222.5±16.9 (210.5–234.4)
Dominant frequency (kHz)	3.88±0.17 (3.48–4.11)	3.37±0.20 (3.14–3.75)	4.39±0.12 (4.18–4.65)	4.46±0.14 (4.28–4.69)	3.74±0.29 (3.53–3.94)
Maximum frequency (kHz)	4.20±0.78 (4.10–4.30)	3.61±0.16 (3.39–3.99)	4.58±0.12 (4.39–4.85)	4.66±0.12 (4.52–4.82)	4.05±0.12 (3.96–4.13)
Minimum frequency (kHz)	3.46±0.23 (2.81–3.62)	2.98±0.19 (2.74–3.38)	4.13±0.15 (3.92–4.05)	4.03±0.21 (3.61–4.25)	3.28±0.26 (3.10–3.46)

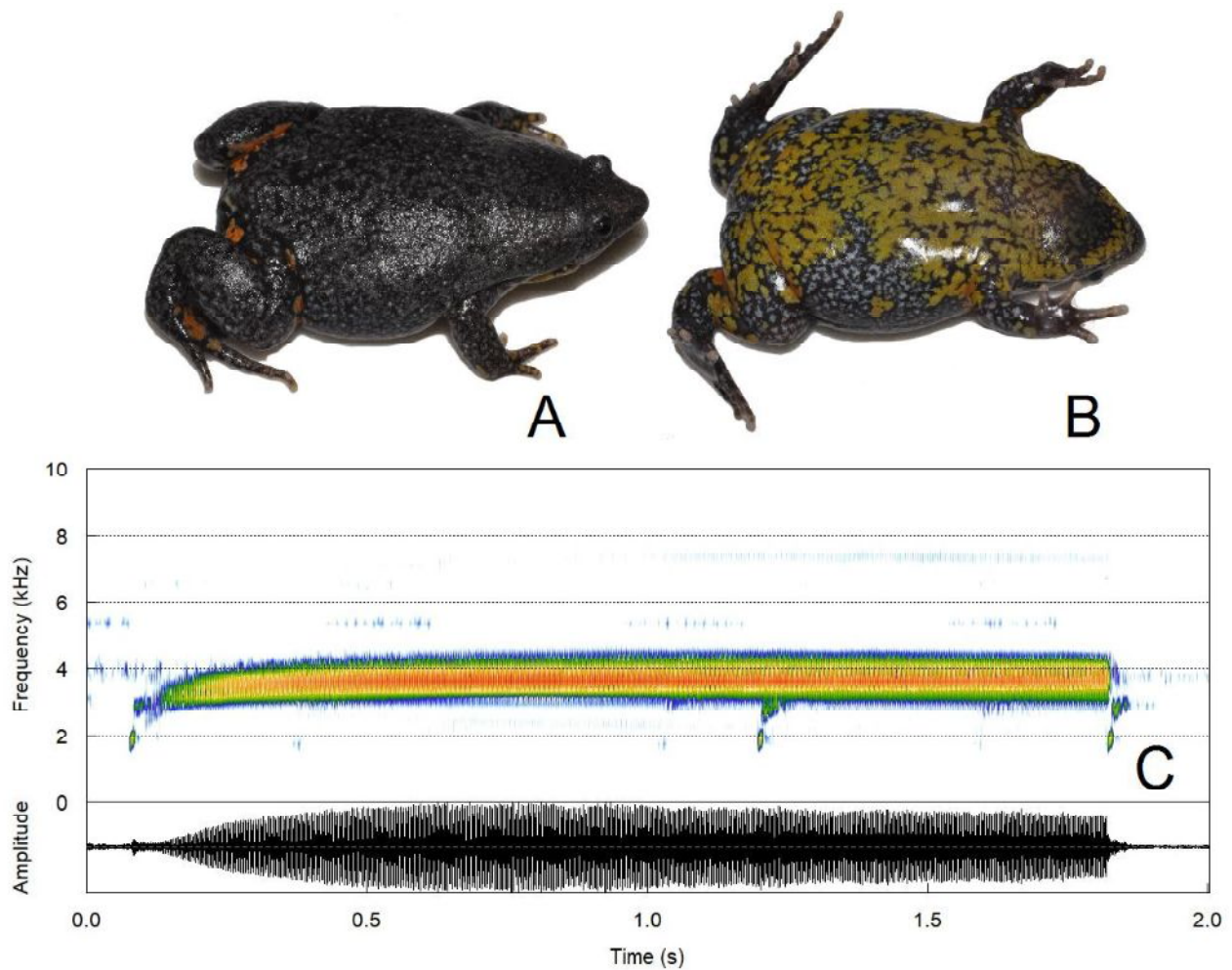
* Values for intercall interval were obtained from 12 (*E. surumu*), 15 (*E. magna*), 14 (*E. helianneae*), 5 (*E. matogrosso*), and 1 (*E. muiraquitana*)

individual, respectively.

** Values for call rate were obtained from 11 (*E. surumu*), 15 (*E. magna*), 14 (*E. helianneae*), 5 (*E. matogrosso*), and 1 (*E. muiraquitana*) individual, respectively.



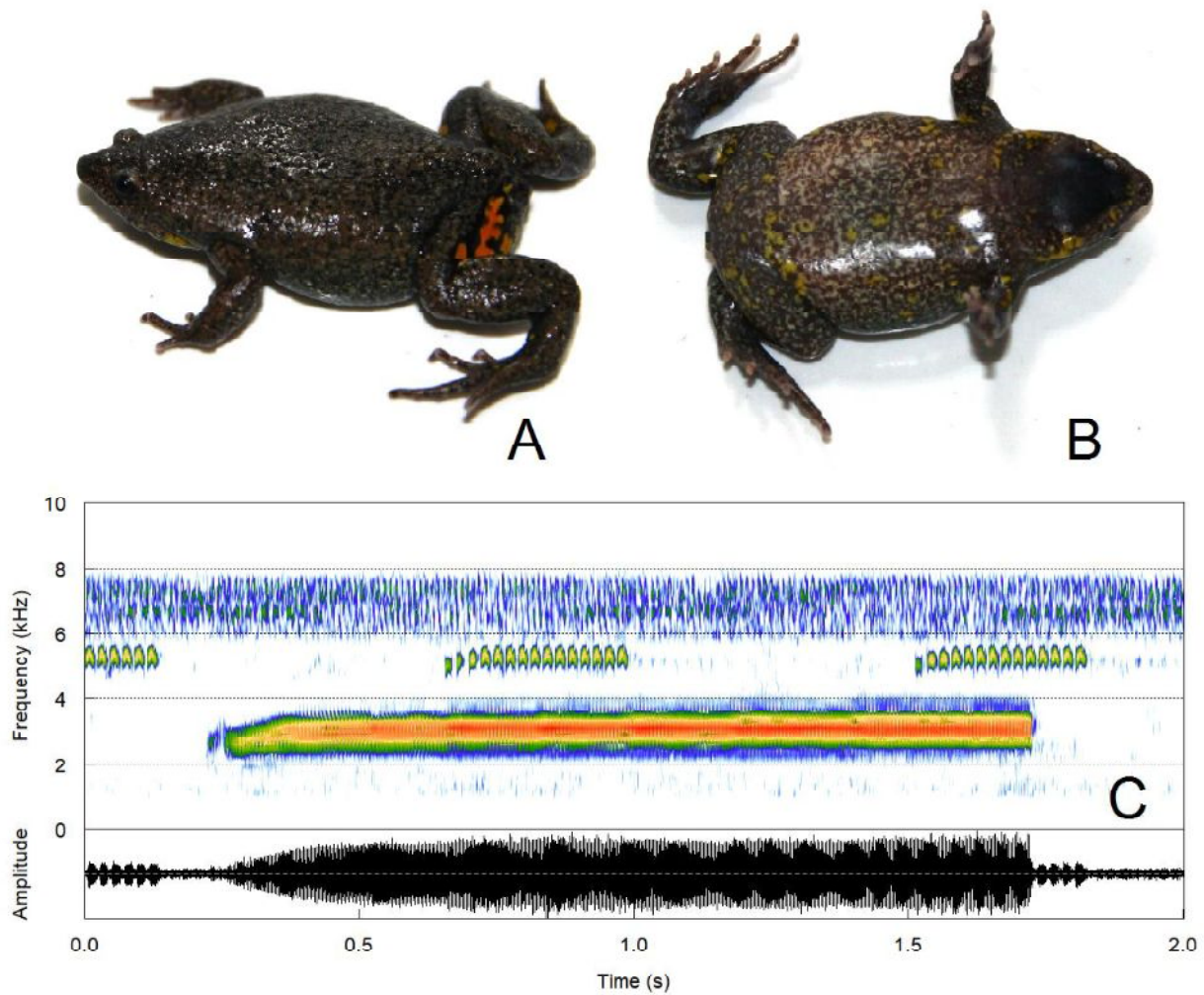
511
 512 **FIGURE 1.** Localities from where acoustic data for species of *Elachistocleis* were collected in
 513 northern and western Brazil for this study. Colors on the map correspond to the Brazilian Amazon
 514 Rainforest (dark green) and the Brazilian Cerrado (light green). Abbreviations for the Brazilian
 515 states: AC (Acre), AM (Amazonas), AP (Amapá), MT (Mato Grosso), RO (Rondônia), RR
 516 (Roraima). Type localities are identified by red-colored stars: *E. magna* (1–3; 1: Espigão d'Oeste, 2:
 517 Vilhena, 3: Costa Marques); *E. helianneae* (3–6; 3: Costa Marques, 4: Humaitá, 5: Itapuã do Oeste,
 518 6: Macapá); *E. matogrosso* (7: Cuiabá); *E. muiiraquitan* (8–9; 8: Xapuri, 9: Rio Branco); *E. surumu*
 519 (10–11; 10: Vila Surumu, 11: Cantá).



521

522 **FIGURE 2.** (A-B) Dorsolateral and ventral views of a call voucher of *Elachistocleis surumu*. Adult
 523 male specimen (AAG-UFU 5571, SVL: 29.4 mm) from Cantá, state of Roraima, northern Brazil;
 524 (C) Spectrogram (above) and oscillogram (below) of one advertisement call recorded from the male
 525 in A-B. Sound file: Elachist_surumuCantaRR6bAAGm671.

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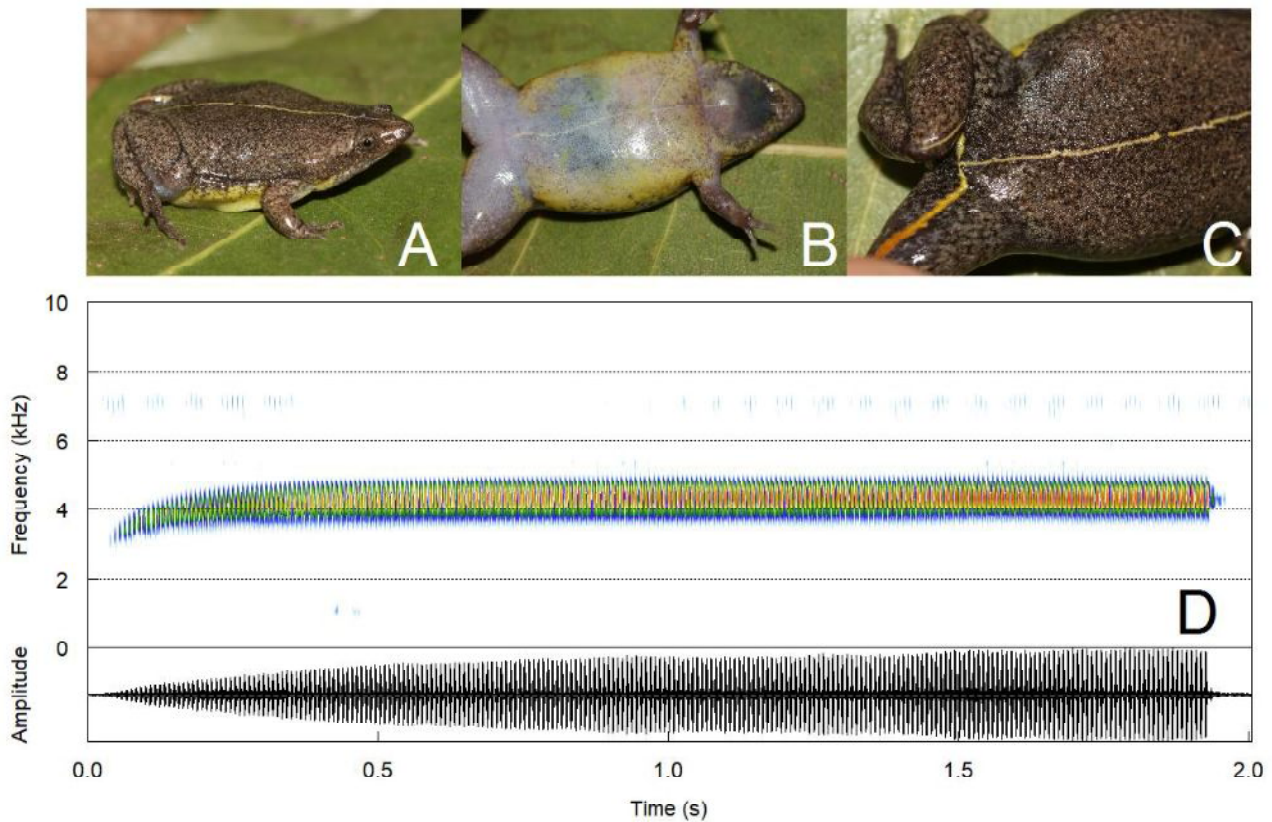


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528 **FIGURE 3.** (A-B) Dorsolateral and ventral views of a call voucher of *Elachistocleis magna*. Adult
 529 male specimen (AAG-UFU 5776, SVL: 33.3 mm) from Espigão d'Oeste (type locality), state of
 530 Rondônia, northern Brazil; (C) Spectrogram (above) and oscillogram (below) of one advertisement
 531 call recorded from the male in A-B. Sound file:

532 Elachist_magnaEspigaoDoesteRO2bDLB_AAGm671.

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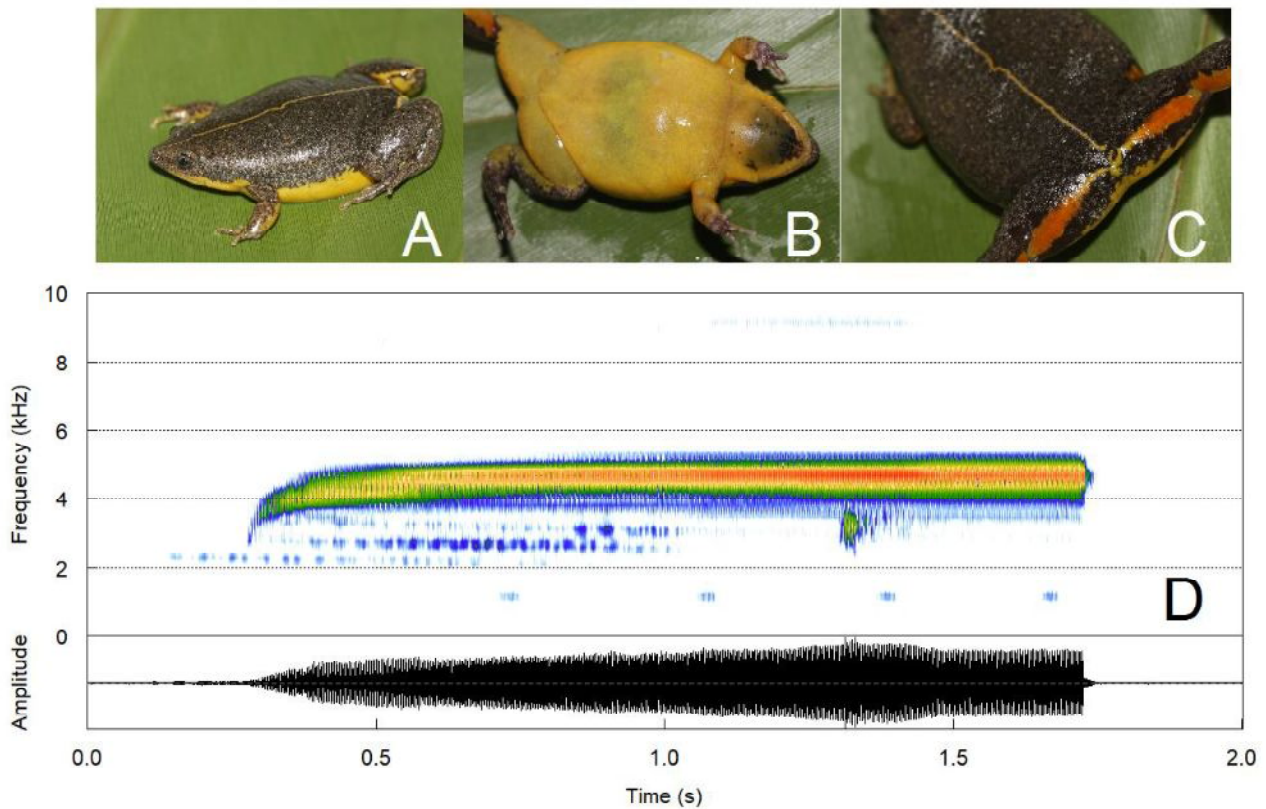


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535 **FIGURE 4.** (A-C) Dorsolateral and ventral views, and posterior surface of the thighs of a call
 536 voucher of *Elachistocleis helianneae*. Adult male specimen (AAG-UFU 5800, SVL: 28.4 mm) from
 537 Itapuã do Oeste, state of Rondônia, northern Brazil; (C) Spectrogram (above) and oscillogram
 538 (below) of one advertisement call recorded from the male in A-C.

539 Sound file: Elachist_helianneaeItapuaOesteRO6aTRC_AAGm671.

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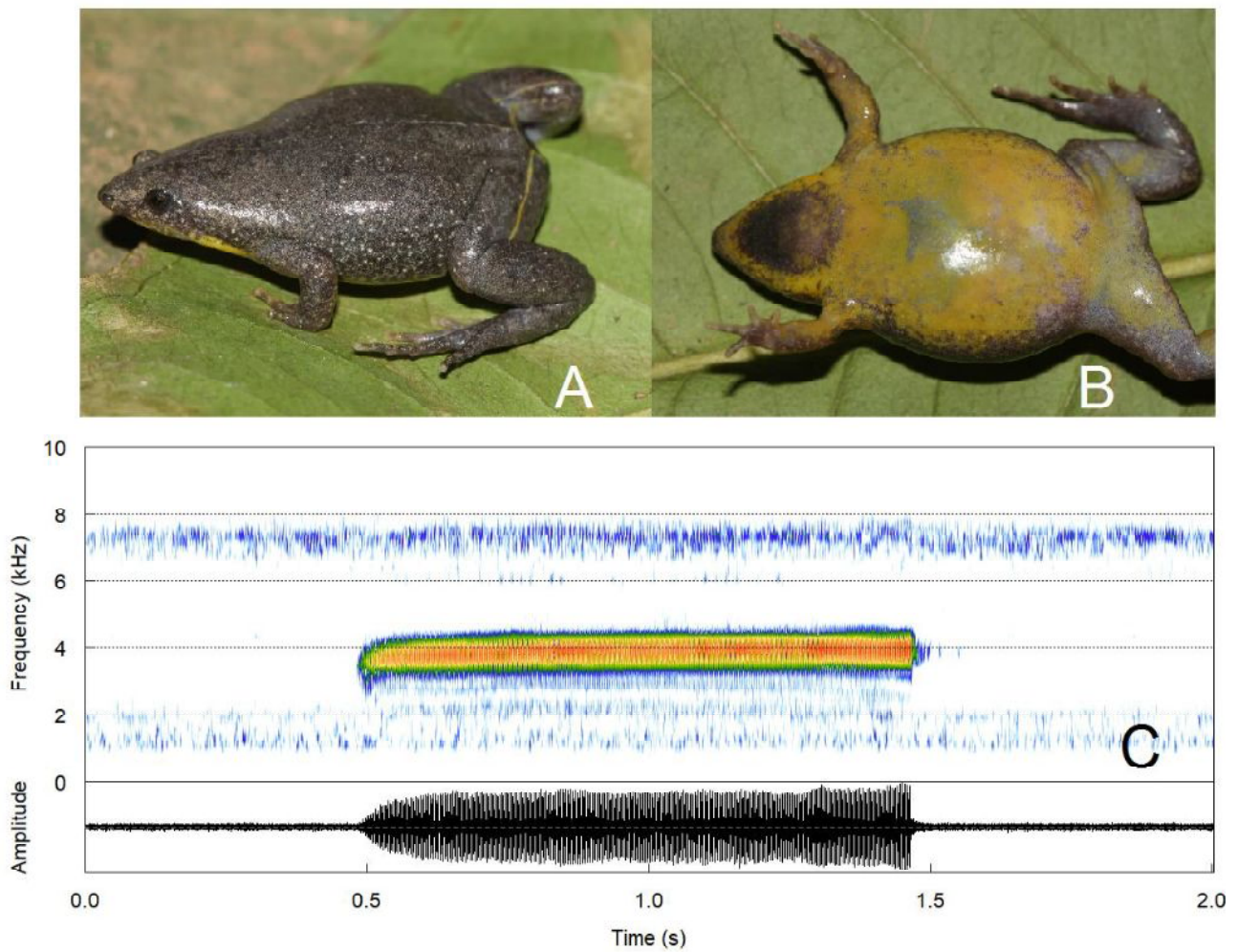
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FIGURE 5. (A-C) Dorsolateral and ventral views, and posterior surface of the thighs of a call voucher of *Elachistocleis matogrosso*. Adult male specimen (AAG-UFU 5954, SVL: 27.4 mm) from Cuiabá (type locality), state of Mato Grosso, western Brazil; (C) Spectrogram (above) and oscillogram (below) of one advertisement call recorded from the male in A-C. Sound file: Elachist_matogrossoCuiabaMT3aDLB_AAGm671.



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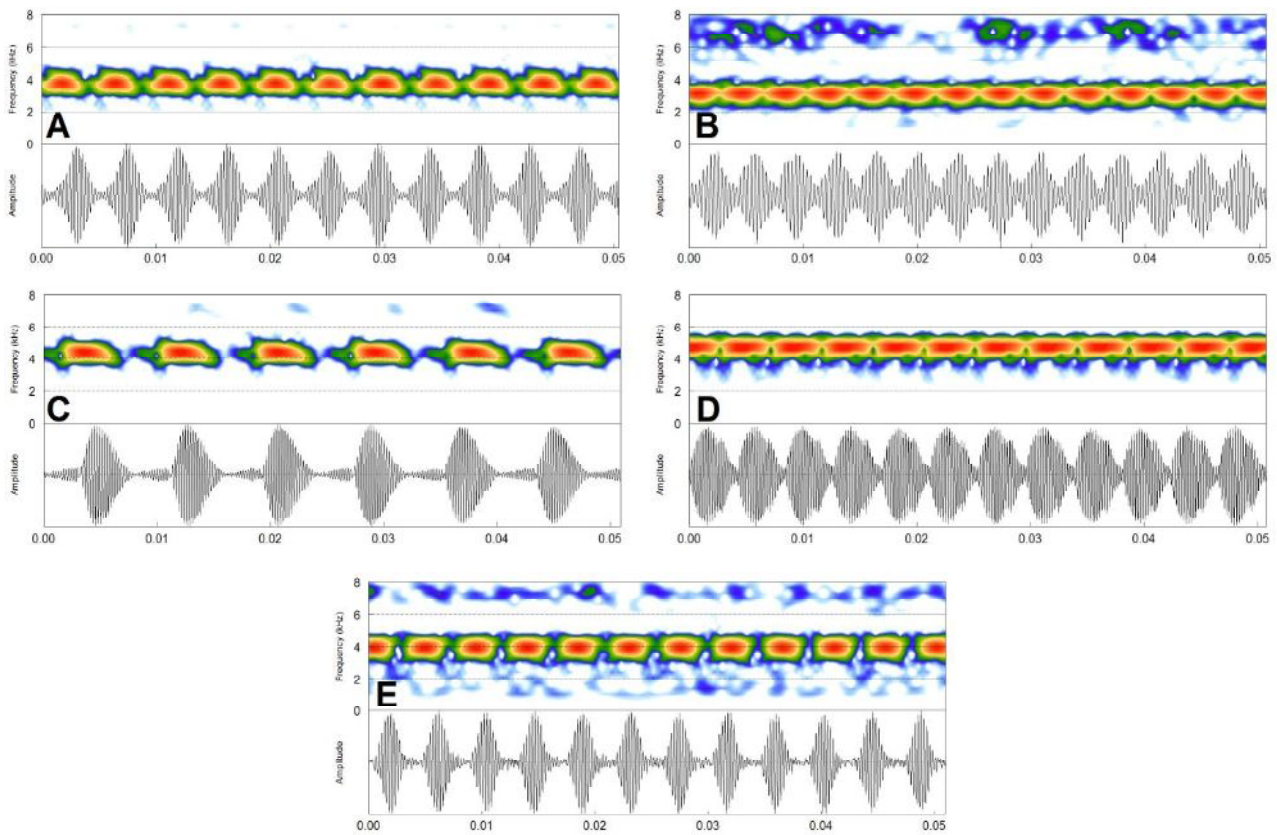
549 **FIGURE 6.** (A-B) Dorsolateral and ventral views of a call voucher of *Elachistocleis muiraquitana*.

550 Adult male specimen (AAG-UFU 5842, SVL: 28.6 mm) from Rio Branco, state of Acre, northern

551 Brazil; (C) Spectrogram (above) and oscillogram (below) of one advertisement call recorded from

552 the male in A-B. Sound file: Elachist_muiraquitanaRioBrancoAC1aDLB_AAGm671.

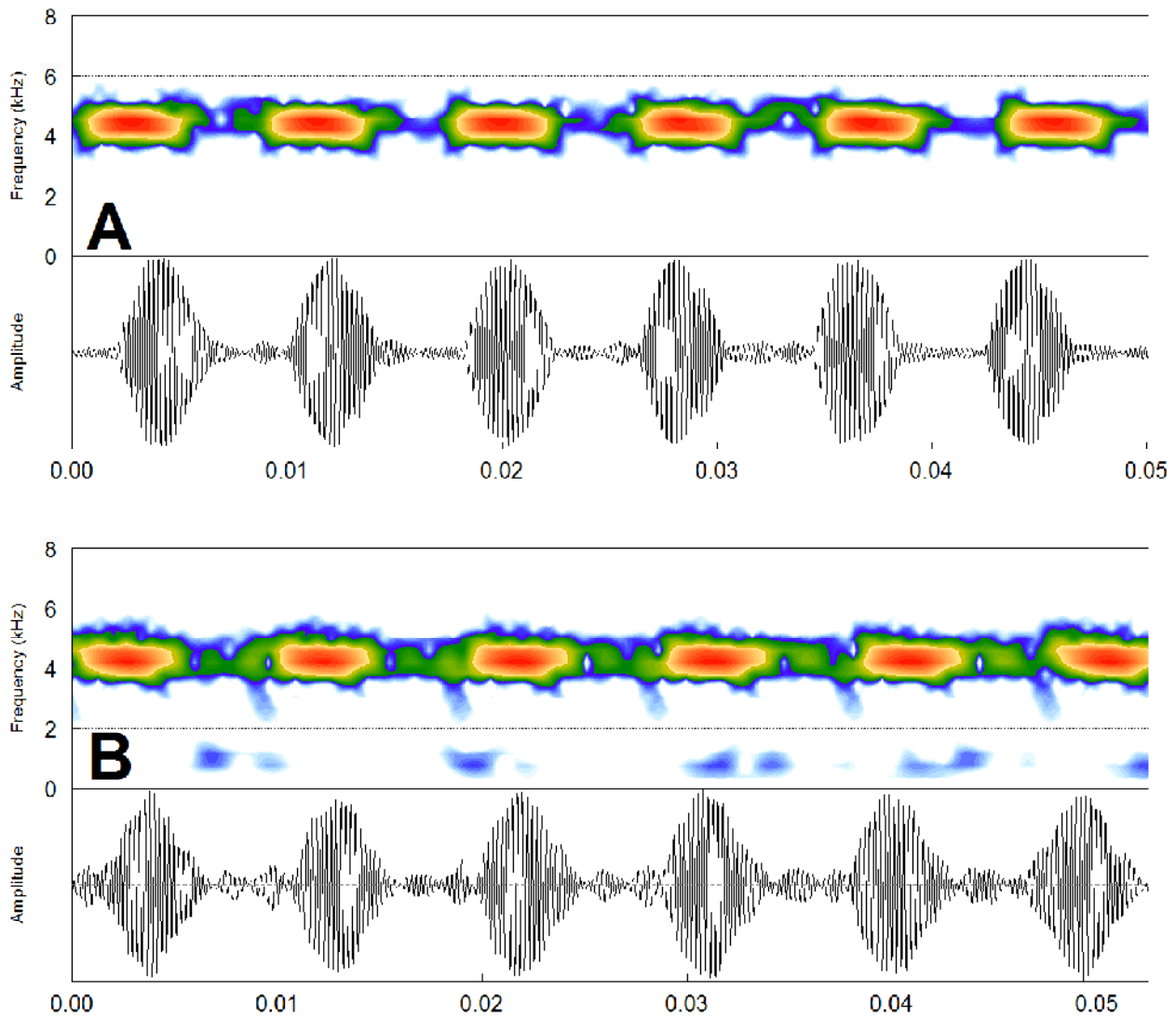
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555 **FIGURE 7.** Spectrograms and corresponding waveforms from sections (ca. 50 ms; x axis) at the
 556 approximate midpoint of calls in Figs. 2–6 detailing pulse shape and emission rate: (A) *E. surumu*;
 557 (B) *E. magna*; (C) *E. helianneae*; (D) *E. matogrosso*; (E) *E. muiiraquitana*.

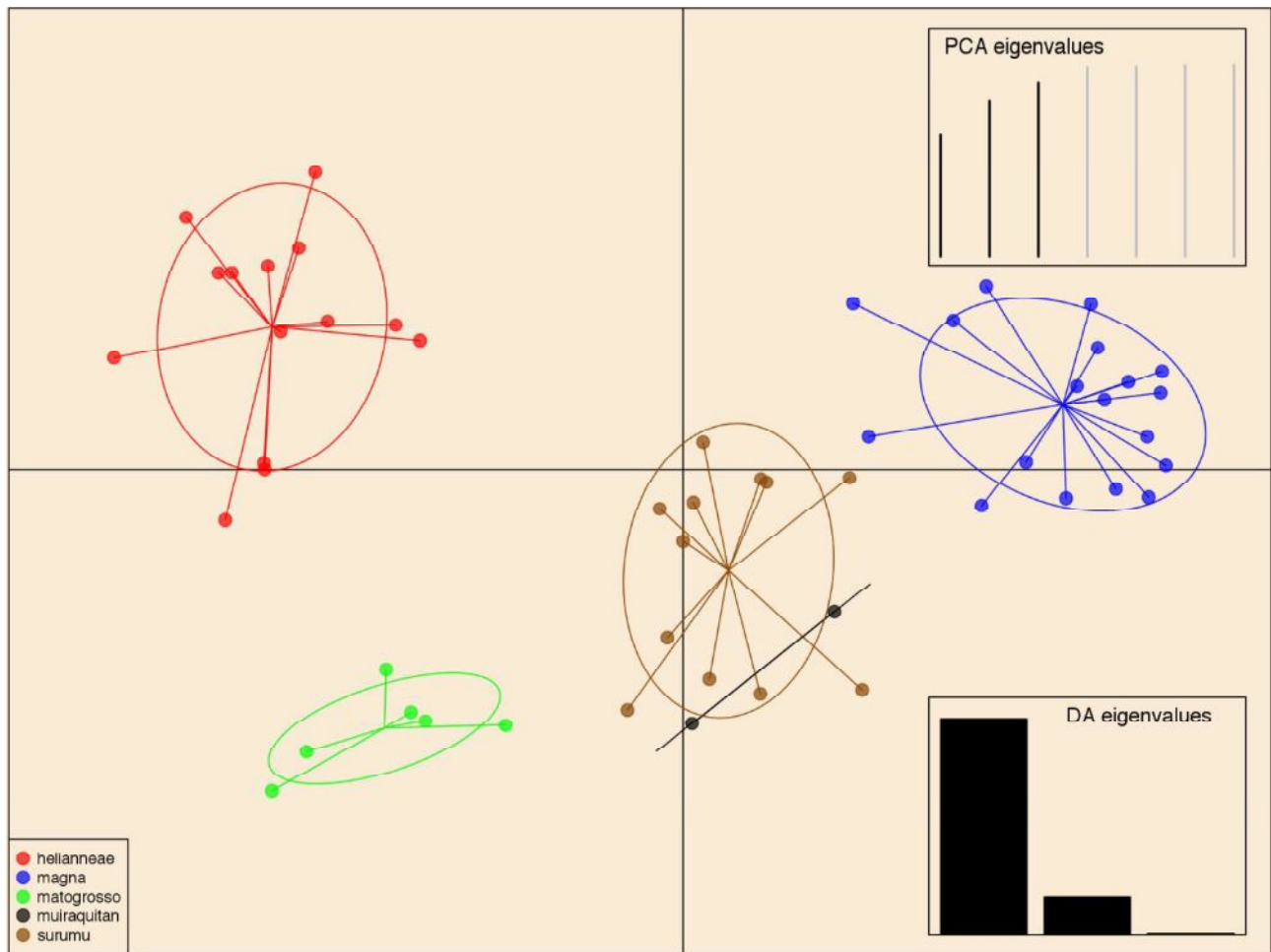
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560 **FIGURE 8.** Spectrograms and corresponding waveforms from sections (ca. 50 ms; x axis) at the
 561 approximate midpoint of calls detailing pulse shape and emission rate of *Elachistocleis helianneae*
 562 from (A) Macapá (Amapá), and (B) Costa Marques (Rondônia); both localities in northern Brazil.
 563 Sound files: (A) Elachist_helianneaeMacapaAP1aAAGm661MK2; (B)
 564 Elachist_helianneaeCostaMarquesRO1cAAGm671.

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566

567 **FIGURE 9.** DAPC scatterplot on the acoustic dataset of five *Elachistocleis* species. The first three
 568 first PCs and first three DAs were retained for this analysis (values in Appendices III–IV).