

12 **Abstract**

13 The genus *Mannophryne* includes 19 described species from Venezuela and two species from
14 Trinidad and Tobago. We described for the first time the advertisement call of *Mannophryne*
15 *lamarcai*, an endemic and Critically Endangered species from the Cerro Socopó at western
16 Venezuela. *M. lamarcai* was spatially restricted in appropriate habitats. Males were calling
17 actively on top of rocks and fallen trucks, along small streams. Calling activity was registered
18 between 13:00 - 19:00 h. The call of *M. lamarcai* comprises a single note arranged in call
19 groups of 37 to 202 notes ($130.35; \pm 45.62$) with five harmonics. The second harmonic is the
20 dominant with a frequency band of 3.7 kHz. The call lasts the duration of one note from 0.01
21 to 0.03 s (0.033 ± 0.006), while the call group lasts up to 16 s ($5.88 - 28.34; \pm 5.51$). Call bouts
22 were arranged in groups of eight trains ($6 - 13; \pm 1.73$), with an inter-note gap of 0.08 s (0.05
23 to $0.33; \pm 0.04$). The inter-note interval was longer during the first 4.5 s of the call group than
24 later.

25

26 *Keywords:* Advertisement call, endemic; collared frogs; Venezuela.

27 **Introduction**

28

29 The genus *Mannophryne* (La Marca 1992), or collared frogs (*sensu* Barrio-Amorós et al.
30 2010) includes 19 described species. High diversity and endemism are found in the forests of
31 the Cordillera de Mérida in the Andes, and the Coastal range of Venezuela, while only two
32 species (*M. trinitaris* and *M. olmonae*) are present in Trinidad and Tobago (La Marca 1994;
33 Barrio-Amorós et al. 2010). The systematic and taxonomy of this genus is still not fully
34 clarified, and there has been a high rate of description of new species in recent years (Barrio-
35 Amorós et al. 2010), along with taxonomic reassessment of described species (Manzanilla et
36 al. 2007).

37 Information about collared frogs' natural history, biology and ecology is extremely
38 scarce. Indeed, half of the species of collared frogs have been described taxonomically
39 without describing the advertisement calls. Quantitative description of advertisement call is
40 available only for nine *Mannophryne* species: *M. venezuelensis*, *M. trinitaris* (Manzanilla et
41 al. 2007), *M. olmonae* (Hardy 1983), *M. riveroi* (Edwards 1974, Barrio-Amorós et al., 2010),
42 *M. herminae* (Test 1956), *M. urticans*, *M. orellana*, *M. vulcano* and *M. collaris* (Barrio-
43 Amorós et al. 2010).

44 *Mannophryne lamarcai* (Mijares-Urrutia and Arends 1999) is an endemic and diurnal
45 frog from Cerro Socopó, Falcón at western Venezuela, *M. lamarcai* has been categorized as
46 Critically Endangered by habitat loss and restricted distribution (less than 100 km²; Mijares et
47 al., 2004). Although it has been observed sporadically in this area since its description (E.
48 Camargo, pers. comm.), several aspects of its ecology, including vocalization, remain
49 undocumented. The original qualitative description of their calls indicate that is characterized
50 by high frequency notes, uttered in trains or trills, the first notes repeated at slightly longer
51 periods than the subsequent ones (Mijares-Urrutia and Arends 1999). In this study, we make

52 the first quantitative description of *M. lamarcai*'s advertisement call.

53

54 **Materials and Methods**

55

56 *Study site:* Our field site was the type locality of this species: Cerro Socopó, located in the
57 north-western Venezuela between Falcón, Lara, and Zulia states (Mijares-Urrutia and Arends
58 1999). This is a small and isolated mountain (1,571 m of elevation) belonging to the Ziruma
59 mountain range. This area has suffered extensive land transformation and currently, only
60 small forest patches remain above 1,000 m. There are two rainy periods, from April to June
61 and August to October. The highest precipitation occurs in the months of April and August
62 and the minimum in January and February (Ministerio del Ambiente 2001).

63

64 *Sampling protocol:* We visited Cerro Socopo during rainy season in May and June 2014 as a
65 part of a preliminary survey to identify sampling locations. Two people systematically looked
66 for focal habitats (streams, rivers, ponds, etc.) in an area of approximately 2.5 km², between
67 1,036 and 1,405 m of elevation and identified five locations. We conducted acoustic and
68 visual encounter surveys on each location during at least one hour (Dood 2009), for two days
69 per month. We identified *M. lamarcai* in two of the five locations evaluated, “Socopo-3” and
70 “Socopo-4”.

71 “Socopo-3” and “Socopo-4” were separated by 1.5 km throughout a secondary road
72 located in the vicinity of humid habitats. Socopo-3 (10.47206 N; -70.81803 W; 1,093 m of
73 elevation) was in a narrow and shallow permanent stream (1.3 m wide x 20 cm deep), with
74 slow flow, clay soil, and sparse vegetation on its banks, surrounded by cattle-pastures and
75 scrubs. Socopo-4 (10.45906 N; -70.81393 W; 1,345 m) was located at a rocky stream (2 m
76 wide x 40 cm deep), with slow flow, clay soil, and dense vegetation on its banks (trees height

77 > 10 m, tree ferns, and plants from Cyclanthaceae, Araceae and Arecaceae families). The
78 stream in Socopo-4 was surrounded by a remnant of humid, premontane forest of proximately
79 0.9 km².

80

81 *Sound recording:* We performed acoustic recordings two hours before sunset, when males
82 called most actively (1600 to 1800 h). Specific recording conditions for Socopo-3 were
83 25.3°C and 72.9% of relative humidity, while for Socopo-4 were 31.4°C and 60.8% of
84 relative humidity. We used an H2n ZOOM digital handy recorder performing stereo
85 recordings at a variable sampling rate from 16bit/44.1 kHz up to 24bit/96kHz. Recorder was
86 mounted in a handy-held tripod and positioned approximately 1m from the focal frog. We
87 adjusted the gain setting of the recorder prior to the onset to assure the sounds were not
88 clipped and maintained the same gain setting throughout the recording (Bee et al. 2013). We
89 recorded from three to seven calling bouts per individual (n = 3 males). The sound dataset
90 included three files with a total of 9.3 minutes and 14 call groups.

91

92 *Acoustic analysis:* The calls were imported with Raven Pro software (v 1.5; Charif et al.
93 2010) to measure acoustic properties. Calls were recorded using 44.1 kHz and 16-bit
94 sampling size resolution and saved in .wav format. Raven Software was used to obtain
95 quantitative information and to generate spectrograms and oscillograms. Frequency
96 information was obtained from Fast Fourier Transform (FFT= 256; overlap 0.5) through a
97 Hahn Window. The terminology used for the description of the advertisement call follows
98 Heyer et al. (1990). We analyzed all call groups from all males (14 call groups, n = 3 males)
99 to measure dominant frequency bands (Hz), harmonics frequency bands (Hz), time interval
100 between call groups (sec), call groups per minute, call rate and note/internote duration.

101

102 **Results:**

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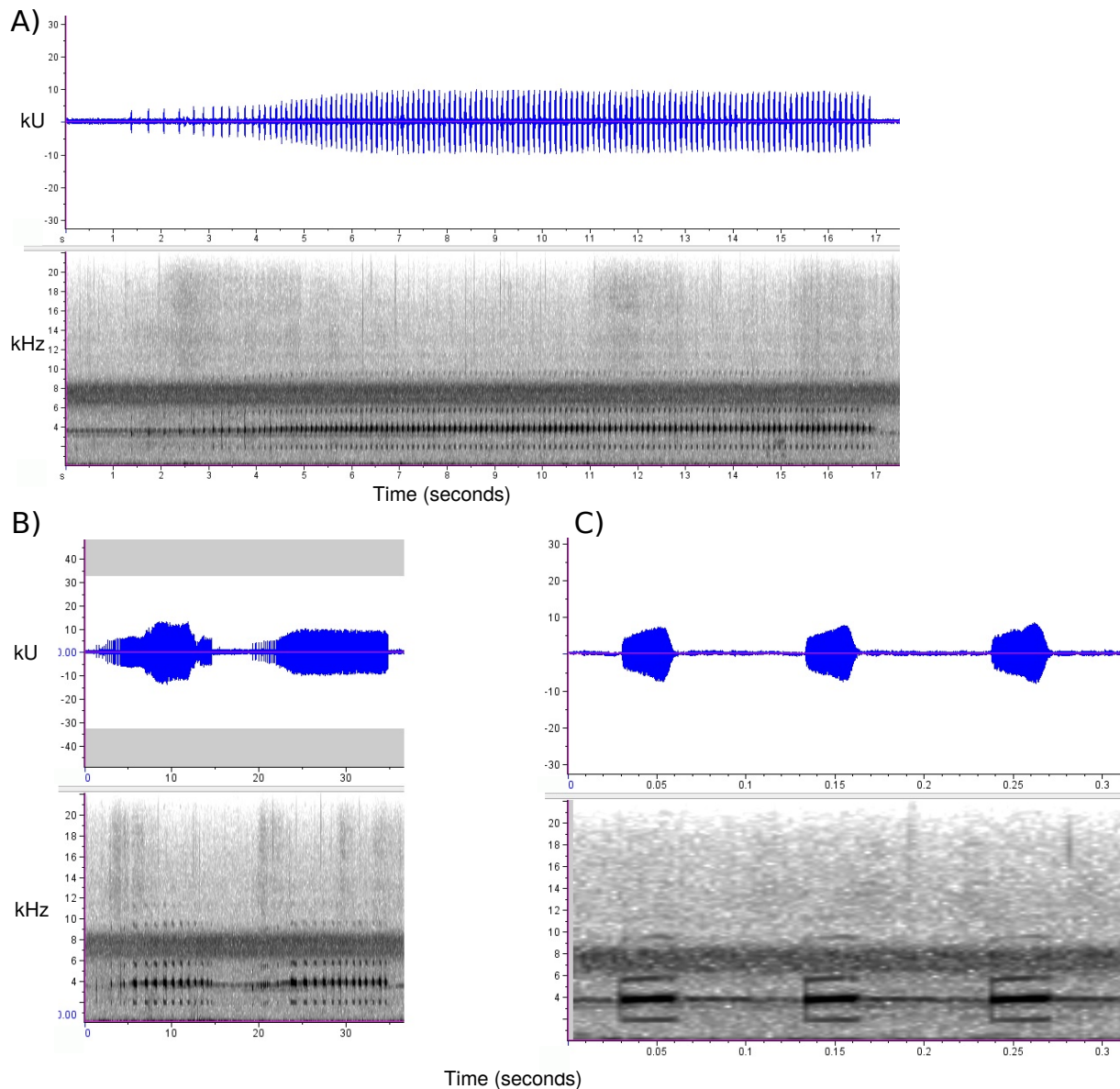
104 *Natural history:* *Mannophryne lamarcai* was spatially restricted to stream margins surrounded
105 by shrubby and forested habitats. We observed a total of four individuals in Socopo-3 (three
106 males, one female) and seven in Socopo-4 (five males, two females). Males were calling on
107 top of rocks and fallen logs, along small cascading, and streams. Males were aggregated (<2
108 m between individuals), calling simultaneously. Individuals uttered several call groups while
109 remaining in the same perch and displayed toe tip jumps in presence of females. Disturbed
110 frogs submerged in water and stayed on the bottom for at least one minute, and then resumed
111 calling in a nearby substrate (less than 3 m).

112

113 *Advertisement call:* Calling activity was registered between 13:00 h and about one hour after
114 dusk (19:00 h). During this period no other amphibian species vocalized, only the calls of
115 *Hypsiboas crepitans* were heard occasionally at dusk (18:30 h).

116 The call of *M. lamarcai* was characterized by a single note organized in call groups
117 of 37 to 202 notes (130.35; \pm 45.62). The call lasts the duration of one note from 0.01 to 0.03
118 s (0.033 ± 0.006), while the call group lasts up to 16 s (5.88 – 28.34; \pm 5.51; Figure 1A). Call
119 bouts were arranged in groups of eight trains (6 – 13; \pm 1.73; Figure 1B), with an inter-note
120 gap of 0.08 s (0.05 to 0.33; \pm 0.04). The inter-note interval was longer during the first 4.5 s of
121 the call group than later.

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123

124 **Fig. 1.** Advertisement call of *Mannophryne lamarcai*. A) Waveform and spectrogram of 17
 125 seconds of the call group showing 127 notes. B) Waveform and spectrogram of a 40 second
 126 sequence of two call groups showing groups of trains. C) Waveform and spectrogram of a 0.3
 127 second sequence of the call group showing three notes.

128

129 Vocalizations recorded at 40 cm showed five harmonics in the spectrum. The second
 130 harmonic was the dominant, with a frequency band from 3,445 to 3,962 Hz ($3,757.90 \pm$
 131 100.34) with low values at the beginning of the call (around 3,200 Hz). The fundamental
 132 harmonic frequency band ranges from 1,550.4 to 2,239.5 Hz ($1,949.97 \pm 123.20$). The third

133 harmonic frequency band ranges from 4,995.7 to 6,029.3 Hz ($5,605.82 \pm 198.27$). The fourth
134 harmonic frequency band ranges from 6,890.6 to 7,924.2 ($7,520.47 \pm 93.20$). The fifth
135 harmonic frequency band ranges from 9,130 to 9,991.4 ($9,573.51 \pm 87.59$). A sixth harmonic
136 was detected in some call groups with a frequency band from 10.9 to 11.54 kHz. The number
137 of notes (call) per second was ranged between 8 to 10 notes and up to 3 call groups per minute
138 (Figure 1C, Table 1).

139 **Table 1.** Published acoustic traits of advertisement call for *Mannophryne* species and values obtained in this study for *Mannophryne la-*
 140 *marcai*.

Species	Temporary parameters					Frequency parameters			Call description	Reference
	Call rate (notes/sec)	Call duration (sec)	Maximum duration (sec)	Number of notes per call*	Minimum duration (sec)	Dominant frequency (Hz)	Fundamental Frequency (Hz)	Frequency range		
<i>M. herminae</i>	10					4,500		2,500 – 6,750	A long series of clearly defined single notes	Test 1956
<i>M. olmonae</i>	3	13 – 128.3	0.13	36 – 347		4,800 – 5,700	2,100 – 4,200	2,100 – 5,700	Single notes repeated	Hardy 1983
<i>M. riveroi</i>	3 – 4					2,250 – 3,400	1,500	1,500 – 5,800	A series of thrilled notes	Edwards 1974
<i>M. trinitatis</i>	7		0.031		0.055					Edwards 1974
<i>M. venezuelensis</i>	4		0.048		0.018	3,731 – 4,647				Manzanilla et al 2007
<i>M. yustizi</i>									Unknown	
<i>M. neblina</i>									A series of short trills	Test 1956
<i>M. oblitterata</i>									Unknown	
<i>M. cordillerana</i>									Unknown	
<i>M. collaris</i>	8 – 9	16	0.04	132	0.03	3,547	3,181		A rapid series of chirps continuing for 10 – 20 sec	Barrio-Amorós et al 2010
<i>M. leonardo</i>									Unknown	Manzanilla et al 2005
<i>M. larandina</i>									Unknown	La Marca (1994)
<i>M. trujillensis</i>									Unknown	La Marca 2007
<i>M. speeri</i>									Unknown	La Marca 2009
<i>M. orellana</i>	10 – 13	6.8	0.04	73	0.03	3,423	3,036		Single notes repeated	Barrio-Amorós et al 2009

<i>M. urticans</i>	5 – 7	26.2	0.076	155	0.005	3,567	3,189	Single notes repeated	Barrio-Amorós et al 2010
<i>M. vulcano</i>	2 – 3	20	0.11	44	0.08	4,502 – 5,599	4,212 – 4,260	Single notes repeated	Barrio-Amorós et al 2010
<i>M. lamarcai</i>	8 – 10	0,08	0.06	130	0.01	3,445 – 3,962	1,550 – 2,239	Single note arranged in call groups, with a inter-note duration fairly stable throughout the call	This study

141 *Call group in *M. lamarcai*



143

144 **Fig. 2.** Views of specimens collected of *Mannophryne lamarcai*: A–B) Dorsal (left) and
 145 ventral (right) view of a adult female (nm0005, SVL =25.0 mm); C) Dorsal view of a male
 146 (nma0001, SVL = 21.3 mm). Photographs by Arlene Cardozo and Cecilia Lozano.

147

148 Discussion

149

150 As other *Mannophryne* species, the call of *M. lamarcai* is composed of a single note repeated
 151 in call groups. Compared with the published calls of other *Mannophryne* species, the mean
 152 dominant frequency of *M. lamarcai* (3,757.9 Hz) was higher than that of *M. urticans*, *M.*

153 *orellana*, and *M. collaris* (3,567, 3,424 and 3,547 Hz respectively; Barrio-Amorós et al.
154 2010), and *M. riveroi* (3,400 Hz; Edwards 1974); and lower than that of *M. olmonae* (5,700
155 Hz; Hardy 1983) and *M. venezuelensis* (4,647 Hz; Manzanilla et al. 2007). The dominant
156 frequency of *M. lamarcai* only overlap with the minimum values reported for *M.*
157 *venezuelensis* (3,730 Hz; Manzanilla et al. 2007), but the call of the former differs in the note
158 emission rate per second (between 8 - 10 in *M. lamarcai*. versus 4 in *M. venezuelensis*).

159 However, due the ample variability in definitions used by different authors to
160 measure acoustic traits, specific comparison between the call of *M. lamarcai* with those of
161 other species must be done with cautious. For example, Barrio-Amorós et al. (2010) reported
162 “the fundamental frequency” of the call for *M. urticans*, *M. orellana*, and *M. collaris*, but they
163 measured the lowest frequency of the second harmonic. Similarly, the dominant frequencies
164 reported by Manzanilla et al. (2007) for *M. venezuelensis* are the dominant frequency of the
165 initial portion of the call and the dominant frequency of the final portion of the call (called
166 minimum and maximum dominant frequency respectively).

167 While interspecific comparison of published values of frequencies bands between
168 *Mannophryne* species is unreliable, comparisons between temporal acoustic parameters could
169 be more truthful. In general, inter call (inter-note) duration is variable among *Mannophryne*
170 species, but for *M. lamarcai* was stable throughout the call group. Specific comparisons of
171 temporal acoustic parameters between geographically close species, *M. herminae* present at
172 the Coastal mountains range, *M. larandina* from Barbacoas (Lara state), and *M. caquetio*
173 present at Sierra de Churuguara (Falcon state) is limited by the lack of quantitative call
174 descriptions in these species. Only for *M. herminae* a qualitative reference of 10 notes per
175 minute is available (Test, 1956). In another hand, *M. collaris*, present at Cordillera de Mérida
176 (Barrio-Amorós et al 2010), has similar number of notes per call and note duration than those
177 observed for *M. lamarcai* (Table 2). However, we suspect that the impressive difference in

178 call duration between both species (16 s for *M. collaris* versus 0.08 s for *M. lamarcai*; Barrio-
179 Amorós et al 2010) is due a misleading definition of “call” of *M. collaris*, and probably
180 authors refer to a call group.

181

182 **Conclusion**

183 As other *Mannophryne* species, the advertisement call of *M. lamarcai* was composed of a
184 single note arranged in call groups, with a inter-note duration fairly stable throughout the call
185 group, and with a distinctive dominant frequency and length.

186

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194

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