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RESEARCH ARTICLE

The advertisement call of the phytotelm-breeding *Melanophryniscus xanthostomus* (Anura: Bufonidae)

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ABSTRACT. Vocalizations are often useful for understanding taxonomic relationships among anuran species. Despite this usefulness, vocalizations are described in only nine of 29 in *Melanophryniscus* Gallardo, 1961. Here we describe the advertisement call of *Melanophryniscus xanthostomus* Baldo, Bornschein, Pie, Ribeiro, Firkowski & Morato, 2015 of a population from Serra Dona Francisca, municipality of Campo Alegre, state of Santa Catarina, Brazil. The advertisement call (of three males, total of 17 calls) comprises two segments (the first with short and single notes, followed by a multi-pulsed note), with a duration of 12.194–20.986 s, and dominant frequency of 3101–3618 Hz (first and second segments combined). The advertisement call of *M. xanthostomus* differs from its congeners mostly by the higher number of pulses in the second segment (294–1033; from 16 to 321 in the other *Melanophryniscus* species), except *Melanophryniscus krauczuki* Baldo & Basso, 2004 (1018–1502 pulses in the second segment). This is the first described call of a phytotelm breeding *Melanophryniscus*, but it presents the same prototype (a compound call formed by two segments, the first composed of short notes followed by a long trill) of its congeners not-phytotelm breedings. This might indicate the advertisement call of *Melanophryniscus* as a conserved trait and thus can be considered diagnostic for the genus.

KEY WORDS. Atlantic Forest, bioacoustic, call description, natural history, vocalization.

INTRODUCTION

Vocalizations are imperative to solve major taxonomic problems in many distinct anuran groups (Haddad and Pombal-Jr 1998, Carvalho and Giaretta 2013, Pansonato et al. 2014). The most common vocalization among anuran repertoire is the advertisement call (Wells 2007), which is species-specific and therefore useful for species identification (Gerhardt and Davis 1988). The advertisement calls of many species-groups have a prototype pattern, well diffused among species – e.g., Microhylidae, Bufonidae (Heyer 1971, Martin 1972) –, and can be the synapomorphic characteristic for lineages.

Melanophryniscus Gallardo, 1961 toads (29 valid species) are distributed in South America (Argentina, Bolivia, Brazil, Paraguay, and Uruguay) (Frost 2018). In phylogenies, the genus has been recovered as the sister taxon of all other bufonids (e.g., Frost et al. 2006, Van Bocxlaer et al. 2010, Peloso et al. 2012), and its monophyly is supported by morphological, biochemical, behavioral, and molecular evidence for adults (McDiarmid 1971, Daly et al. 2007, Peloso et al. 2012) and tadpoles, based in a comparative description of the larvae of 23 *Melanophryniscus* species (Larson et al. 2003, Baldo et al. 2014). The genus is traditionally grouped into three species groups (*M. tumifrons*, *M. stelzneri*, and *M. moreirae* species groups) based mainly on morphology and coloration patterns (Caramaschi and Cruz 2002). Some species of *Melanophryniscus* described after 2003 have not been assigned to any group.

The toad *Melanophryniscus xanthostomus* Baldo, Bornschein, Pie, Ribeiro, Firkowski & Morato, 2015 was recently described based on individuals from Serra do Quiriri, Campo Alegre, Condomínio Vale dos Lagos and Reserva Particular do Patrimônio Natural Caetezal, Joinville and Morro do Boi, Corupá, municipalities from the state of Santa Catarina, Brazil, and was not assigned to any species groups. Some information about the natural history of *M. xanthostomus* is provided in the original description of the species (Bornschein et al. 2015),

including data of vocalization sites, and period that males were found calling (from September to February). However, the advertisement call was not described.

Given the rapid increase of the species description in the genus, the search for useful characteristics, as vocalizations, is crucial important to help resolve their taxonomy and to test evolutionary hypothesis. However, from the 29 species of *Melanophryniscus*, only nine have the advertisement call described (see Caldart et al. 2013, Duré et al. 2015). Because anuran advertisement calls are species-specific (Gerhardt and Davis 1988) and therefore, are useful in the distinction of the species and an important character for taxonomy (Duellman and Trueb 1986), in the present study, we described the advertisement call of *M. xanthostomus* for a population from Serra Dona Francisca, Campo Alegre, Santa Catarina, Brazil. We also compared the calls of *M. xanthostomus* with the nine other species of *Melanophryniscus*.

MATERIAL AND METHODS

We recorded advertisement calls of three males (FURB 22851 – SVL 18.6 mm, FURB 22822 – SVL 19.7 mm, FURB 22713 – SVL 16.9 mm, total of 17 calls, Figs 1–3) at Serra Dona Francisca (-26°12'52.54"S, 49°13'04.92"W), Campo Alegre, Santa Catarina, on November 3rd, 2013. Calls were recorded with a Marantz PMD661 digital recorder coupled with a YOGA HT-81 directional microphone. Recordings were made around midnight (air temperature 18.8 °C, humidity 65%). We digitalized the recordings at 44.1 kHz, resolution of 16 bits. The three specimens were collected and deposited on the Zoology Collection of Universidade Regional de Blumenau (FURB), Santa Catarina, Brazil, under the numbers above.

We analyzed calls in RAVEN PRO 1.5 for Mac (Bioacoustics Research Program 2012) and constructed audio spectrograms in R using the package seewave (Sueur et al. 2008) with the following parameters: FFT window width = 256, Frame = 100, Overlap = 75, and flat top filter. We analyzed acoustic parameters normally used for species of *Melanophryniscus*: dominant frequency (Hz), call duration (sec), call interval (sec), first segment duration (sec), second segment duration (sec), interval between first and second segment (sec), number of short notes, duration of short notes (sec), interval between short notes (sec), note rate of the first segment (the ratio of the absolute number of notes and the absolute duration of the segment), pulse number of the second segment, and pulse rate of the second segment (the ratio of the absolute number of pulses and the absolute duration of the segment). Terminology of call descriptions follows Köhler et al. (2017).

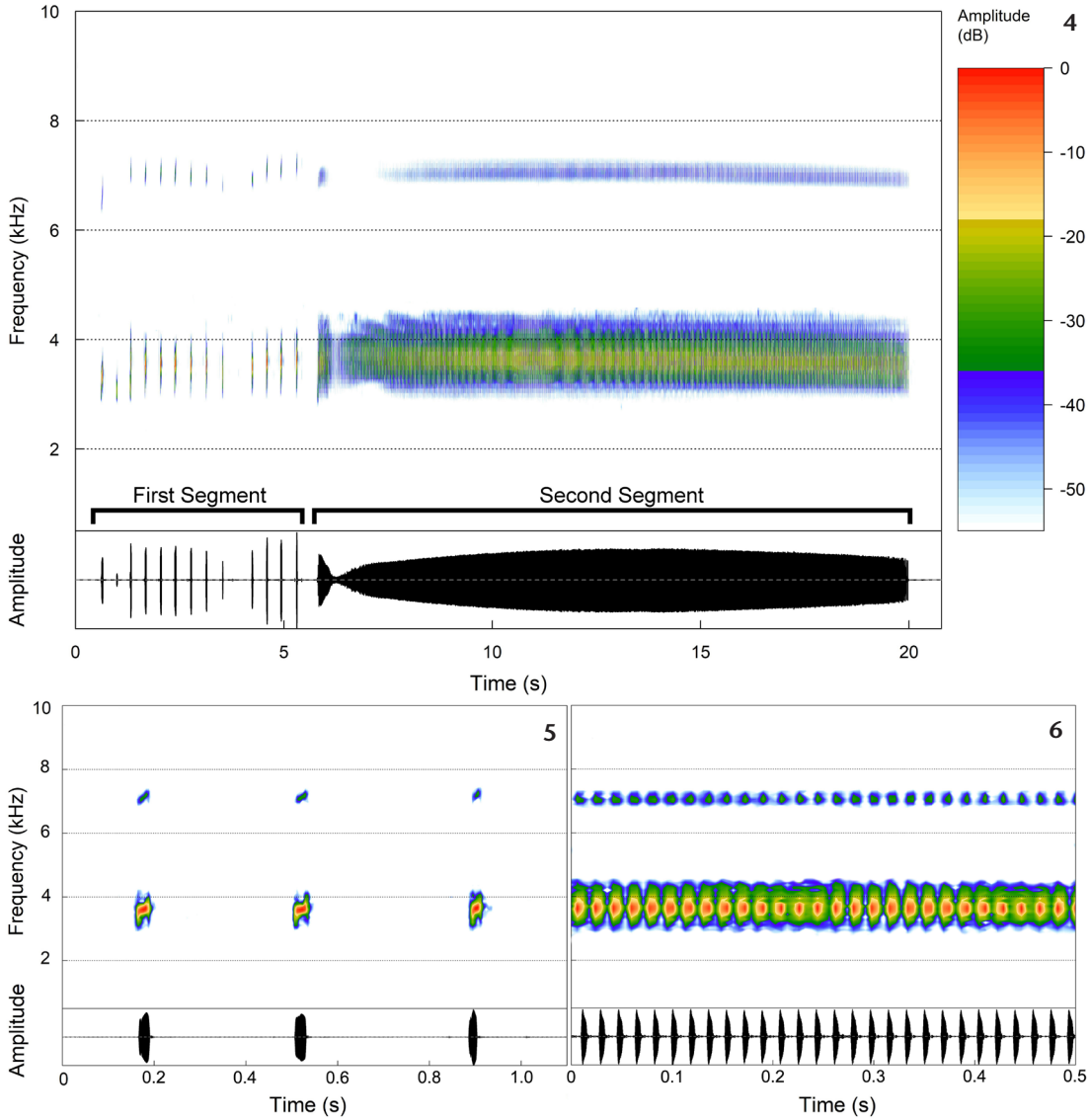
Figures 1–3. Specimens of *Melanophryniscus xanthostomus*: dorsal view (1) and ventral view (2) from FURB 22851 specimen and FURB 22713 specimen in life (3).

We compared the advertisement call of *M. xanthostomus* with nine congeners, although those descriptions did not include all the call parameters that we analyzed (see references in Table 1).

RESULTS

The advertisement call of *M. xanthostomus* is a compound call formed by two segments (Table 1, Fig. 4–6). The first segment is composed by short and single notes (13 ± 4; range 7–20 notes), with a duration of 0.011–0.057 s (0.027 ± 0.010 s), and interval duration of 0.242–0.774 s (0.325 ± 0.109 s) between the short notes, with a pulse rate of 0.002–0.004 s (0.003 ± 0.001 s). The duration of the first segment is 1.774–6.794 s (4.115 ± 1248.98 s). The second segment consists of a multi-pulsed note (a long trill) of 6.278–18.318 s of duration (14.189 ± 2979.77 s), with 294–1033 pulses (685 ± 179.59 pulses), and pulse rate of 0.04–0.06 pulses per second (0.05 ± 0.01 pulses/s). The advertisement call (first and second segments combined) presents a duration of 12.194–20.986 s (18.577 ± 2443.70 s), and dominant frequency of 3101–3618 Hz (3395 ± 180.32 Hz). The interval duration between the first and the second segments is 9.258–221.673 s (82.949 ± 78878.03 s).

The advertisement call of *M. xanthostomus* is emitted with both segments in sequence, not only the first or the second



Figures 4–6. Advertisement call of *Melanophryniscus xanthostomus* (LM380, SLV 18.6 mm): (4) oscillogram and spectogram of one call; (5) oscillogram and spectogram of three notes from the first segment; (6) oscillogram and spectogram of 28 pulses from the second segment.

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Table 1. Comparison of temporal and spectral parameters of the advertisement call of *Melanophryniscus xanthostomus* and nine other congeneric species. Values are means with the range in parentheses. (a) Air temperature; (w) water temperature.

Call parameters	*M. xanthostomus*[1]	*M. atroluteus*[2]	*M. atroluteus*[3]	*M. cupreuscapularis*[4]	*M. dorsalis*[5]	*M. dorsalis*[6]	*M. klappenbachi*[7]
Calls (n)	17 calls, 3 males	12 calls, 1 male	14 calls, 2 males	20 calls, 4 males	3 calls, 1 males	7 calls, 2 males	11 calls
Temperature (ºC)	18.8 (a)	17.0 (w)	21.5 (a)	22.0 (a)	21.0 (a)	21.0 (a)	22.0–24.0 (a)
Dominant frequency (Hz)	3395 (3101–3618)	3000	(1900–3100)	2270 (2176–2357)	(2600–3200)	(2300–3200)	1900
Call duration (s)	18.577 (12.194–20.986)	7.523 (5.090–10.350)	3.700 (3.400–4.200)	3.023 (1.120–6.660)	–	–	0.507 (0.292–0.652)
Inter-call interval (s)	82.949 (9.258–221.673)	8.489 (2.922–7.954)	–	2.100 (1.350–2.780)	–	–	–
1st segment duration (s)	4.115 (1.774–6.794)	4.143 (2.575–5.360)	2.500 (1.800–3.200)	2.688 (0.780–6.390)	3.700 (3.500–4.100)	1.890 (1.000–2.300)	–
2nd segment duration (s)	14.189 (6.278–18.318)	3.012 (1.832–4.303)	1.100 (0.500–1.400)	0.23 (0.19–0.27)	2.37 (2.0–2.6)	1.50 (0.6–2.2)	–
Interval between segments (s)	0.265 (0.054–0.609)	0.026 (0.003–0.079)	–	0.100 (0.040–0.160)	–	–	–
Number of notes of 1st segment	13 (7 – 20)	20.6 (15–25)	17.6 (11–26)	10 (6–17)	19 (18–20)	13 (6–18)	3–4
1st segment notes duration (s)	0.027 (0.011–0.057)	0.102 (0.006–0.174)	–	0.020 (0.010–0.040)	0.054 (0.030–0.065)	0.042 (0.020–0.050)	–
Inter-notes interval of 1st segment (s)	0.325 (0.242–0.774)	0.091 (0.006–0.229)	(0.085–0.100)	0.270 (0.090–3.150)	0.153 (0.130–0.190)	0.140 (0.080–0.170)	–
Notes per s of 1st segment	0.003 (0.002–0.004)	–	(8–9)	–	–	(4–7)	–
Number of pulses 2nd segment	685.000 (294.000–1033.000)	222.380 (139.000–321.000)	87.600 (45.000–116.000)	20.400 (16.000–24.000)	161.000 (152.000–173.000)	122.000 (54.000–162.000)	43.000 (26.000–53.000)
Pulses per s of 2nd segment	47.960 (40.550–56.700)	75.440 (74.310–76.800)	(85.000–95.000)	88.950 (96.770–84.030)	(62.000–74.000)	(74.000–78.000)	86.000 (80.340–88.910)

Continues

Table 1. Continued.

Call parameters	*M. krauczucki*[8]	*M. montevidensis*[9]	*M. pachyrhynus*[10]	*M. rubriventris*[11]	*M. rubriventris*[12]	*M. rubriventris*[13]	*M. stelzneri*[14]	*M. stelzneri*[15]
Calls (n)	5 calls, 1 male	14 calls, 2 males	12 calls, 6 males	12 calls, 4 males	12 calls, 4 males	12 calls, 4 males		
Temperature (ºC)	17(w)	24(a)	16–17(a) 16–18(w)	19(a)	19(a)	19(a)		20.5(a)
Dominant frequency (Hz)	3300	(2100–2800)	2668 (2261–2932)	1st seg. 1788 (1704–1846) 2nd seg. 1734 (1653–1789)	1st seg. 1721 (1515–1845) 2nd seg. 1687 (1535–1783)	1st seg. 1841 (1754–1944) 2nd seg. 1782 (1705–1869)	(1600–2600)	FH (2200–2600) 2H (4600–5200) 3H (7000–7600)
Call duration (s)	32.699 (25.013–36.646)	–	37.070 (6.640–75.200)	2.710 (1.400–3.250)	3.150 (2.030–4.170)	2.130 (1.320–3.790)	7.300 (4.500–9.300)	
Inter-call interval (s)	21.988 (18.196–25.737)	–	–	–	–	–	–	
1st segment duration (s)	2.031 (1.128–3.160)	1.980 (1.000–4.500)	4.440 (1.850–7.670)	0.912 (0.296–1.489)	1.438 (0.606–2.318)	0.617 (0.231–1.449)	4.200 (2.500–6.300)	
2nd segment duration (s)	30.455 (23.784–33.408)	1.580 (1.200–2.000)	25.270 (4.790–45.750)	1.365 (0.691–1.648)	1.413 (0.558–2.251)	1.323 (0.772–2.125)	2.100 (1.800–2.400)	0.500
Interval between segments (s)	0.258 (0.085–0.827)	–	–	0.435 (0.212–0.929)	0.299 (0.111–0.404)	0.187 (0.210–0.524)	–	
Number of notes of 1st segment	8.6 (6–12)	17 (7–28)	17 (8–28)	3.8 (2–7)	4.7 (2–8)	2.9 (1–6)	26 (12–36)	
1st segment notes duration (s)	0.009 (0.005–0.023)	0.0313 (0.021–0.039)	0.017 (0.010–0.026)	0.039 (0.042–0.048)	0.044 (0.033–0.043)	0.044 (0.035–0.050)		0.110
Inter-note interval of 1st segment (s)	0.217 (0.147–0.837)	0.103 (0.078–0.130)	0.234 (0.082–0.322)	0.285 (0.209–0.496)	0.316 (0.171–0.987)	0.268 (0.157–0.254)	(0.110–0.130)	0.090
Notes per s of 1st segment	–	–	3.900 (2.900–4.300)	–	–	–		(5.000–6.000)
Number of pulses 2nd segment	1298.500 (1018–1502)	147 (100–192)	818 (164–1382)	–	–	–		
Pulses per s of 2nd segment	43.670 (42.350–44.950)	(85.000–95.000)	32.800 (30.200–34.300)	54.000 (51.000–58.000)	48.000 (45.000–61.000)	57.000 (54.000–64.000)	(65.000–85.000)	70.000

Speciemens from: [1]Campo Alegre, Santa Catarina, Brazil (present study); [2]Misiones, Argentina (Baldo and Basso 2004); [3]São José dos Ausentes, Rio Grande do Sul, Brazil (Kwet and Miranda 2001); [4]Corrientes, Argentina (Duré et al. 2015); [5]Laguna, Santa Catarina, Brazil (Kwet et al. 2005); [6]Torres, Rio Grande do Sul, Brazil (Kwet et al. 2005); [7]Paraguay (Kurth et al. 2013); [8]Misiones, Argentina (Baldo and Basso 2004); [9]La Paloma, Uruguay (Kwet et al. 2005); [10]São Sepé, Rio Grande do Sul, Brazil (Caldart et al. 2013); [11]Tiraxi, Jujuy, Argentina (Ferrari and Vaira 2008); [12]Abra Colorada, Jujuy, Argentina (Ferrari and Vaira 2008); [13]Canto Del Monte, Argentina (Ferrari and Vaira 2008); [14]Calamuchita, Córdoba, Argentina (Kwet and Miranda 2001); [15]Santa Rosa de Calamuchita, Córdoba, Argentina (Barrio 1964).

segment separately. In November 2013, we observed six males calling on bromeliads at ground level, in two forest fragments at 1027 m altitude. Most toads can be found in bromeliads closer to the ground, which were usually under 1 m high, as well as inside those that fell in the leaf litter. Also we found one female near an egg clutch at the fence of pitfall trap. In January 2014, we registered only one male calling during the fieldworks. No individuals were capture in pitfall traps in the forest fragment. Other species calling along with *M. xanthostomus* in the forest were *Fritizana* sp., *Cycloramphus bolitoglossus* (Werner, 1897) and *Adenomera araucaria* Kwet & Angulo, 2002.

DISCUSSION

The natural history of the phytotelma-using *Melanophryniscus* species is very different from their congeners, because of the reproductive mode and tadpole morphology (Langone et al. 2008, Baldo et al. 2014), and the type of habitats they inhabit (other species occurs and lay the eggs in freestanding water) (Kwet et al. 2010, Maneyro et al. 2017). Concerning its call parameters, this species showed some temporal and spectral characteristics that suggest some acoustic adaptations for forest environments, such as described in the Acoustic Adaptation Hypothesis (AAH) (Morton 1975, Erdtmann and Lima 2013). According this hypothesis, calls in forest environments will: (1) be longer in length, (2) have a lower repetition rate, (3) have lower minimal, maximal and dominant frequencies and (4) have a smaller frequency bandwidth (Morton 1975).

The advertisement call of *M. xanthostomus* differs from its congeners by the higher number of pulses in the second segment (294–1033; from 16 to 321 in the other *Melanophryniscus* species), except from *Melanophryniscus krauczuki* Baldo & Basso, 2004 (1018–1502 pulses in the second segment) and *M. pachyrhynus* (Miranda-Ribeiro, 1920) (164–1382 pulses in the second segment). The lowest pulse rate of the second segment (40.55–56.70 pulses per second) differs *M. xanthostomus* from most of its congeners (62 to 95 pulses per second in *M. atroluteus* (Miranda-Ribeiro, 1920), *M. cupreuscapularis* Céspedez & Alvarez, 2000, *M. dorsalis* (Mertens, 1933), *M. klappenbachi* Prigioni & Langone, 2000, *M. montevidensis* (Philippi, 1902), *M. stelzneri* (Weyenbergh, 1875), except from *M. krauczuki*, *M. pachyrhynus*, and *M. rubriventris* (Vellard, 1947) (42.35–44.95, 3020–34.30, 45.00–64.00 pulses per second, respectively). The total call and the second segment durations of *M. xanthostomus* (12.19–20.99 s, 6.28–18.32 s, respectively) are only shorter than *M. krauczuki* (25.01–36.65 s, 23.78–33.41 s). *Melanophryniscus pachyrhynus* reaches higher values of call and second segment durations (6.64–75.20 s, 4.79–45.75 s) than *M. xanthostomus*, however, the values overlap. The higher number of notes in the first segment (7–20) of the advertisement call of *M. xanthostomus* differs from *M. klappenbachi* (3–4) and *M. rubriventris* (2–7). The dominant frequency is higher (3395 Hz) and the amplitude range is lower (± 500 Hz) than other species of *Melanophryniscus*, except from *M. krauczuki* (dominant frequency 3300 Hz), *M. cupreuscapularis* (amplitude ± 200 Hz) and *M. rubriventris* (amplitude between 100-300 Hz). The interval between calls is longer in *M. xanthostomus* (82.949 s) than other species. The other acoustic parameters, such as first segment duration and interval between segments, varies among species of the genus, but all the values overlap. Even with some acoustic traits that corroborate with the AAH, we observed that the advertisement calls are similar among *Melanophryniscus* species.

Species of *Melanophryniscus* have an advertisement call composed of short notes followed by a long trill (e.g., Kwet et al. 2005, Caldart et al. 2013, Duré et al. 2015), as we observed to *M. xanthostomus*. Most species, including *M. xanthostomus*, have the first segment composed by one group of short notes, except *M. cupreuscapularis* and *M. dorsalis* (from Laguna, Santa Catarina, Brazil), which have two or more groups of short notes, separated by a higher distance than that among notes (Kwet et al. 2005, Duré et al. 2015). *Melanophryniscus xanthostomus* emit the advertisement call with both segments in sequence as described for other species of the genus (see Duré et al. 2015), except *M. pachyrhynus*, that also emit calls with the second segment (the trill) alone (Caldart et al. 2013). The functional differences of the two segments of the advertisement call of *Melanophryniscus* species might be investigate in future bioacoustic and behavior studies of these species.

Thus, it is remarkable that the advertisement call of *M. xanthostomus* presents the same prototype (a compound call formed by two segments, the first composed of short notes followed by a long trill) of its congeners not-phytotelm breedings. Biological concerns, such as species recognition, sexual selection, physiological traits or body size (Gerhardt 1991, Bevier et al. 2008), may explain the similarities of advertisement calls among *Melanophryniscus* species, while some evidences showed contrary pattern expected for AAH. Some amphibians species showed larger body mass and lower call frequencies in open-land environments (Bevier et al. 2008), such as we detected for *M. xanthostomus* (higher dominant frequency associated to forest environment). But the open land and stream inhabitant *M. krauczuki* (Baldo and Basso 2004) also showed higher dominant frequency, suggesting other environment variables may be associated, such as stream noise. However, the lack of phylogenetic hypothesis for the genus and other described calls from phytotelm breeding *Melanophryniscus*, makes it hard to test this hypothesis at this moment. Thus, future phylogenetic comparative studies, testing the AAH, could bring new explanations for the evolutinary acoustic patterns of *Melanophryniscus*. Once this is the first described call of a phytotelm breeding *Melanophryniscus*, we highlight the need to know the call of the other species with the same reproductive mode in order to confirm this proposal.

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