

RESEARCH ARTICLE

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

Sarah Mângia¹, Diego José Santana¹, Lucas Ribeiro Mariotto²

¹Universidade Federal de Mato Grosso do Sul, Instituto de Biociências, Laboratório de Zoologia, Av. Costa e Silva, s/n, Cidade Universitária, 79070-900, Campo Grande, MS, Brazil

²Universidade de São Paulo, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Departamento de Biologia, Avenida Bandeirantes, 3900, Monte Alegre, 14040-901, Ribeirão Preto, SP, Brazil
 Corresponding author: Lucas Ribeiro Mariotto (lucasmariotto@gmail.com)

<http://zoobank.org/1F5BD8E4-DB3D-4ACD-B993-6D318A60EB42>

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 Pomboal-Jr 1998, Carvalho and Giarretta 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 Boclaer et al. 2010, Peloso et al. 2012), and its monophyly is supported by morphological, biochemi-

cal, 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 Caetetal, 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).

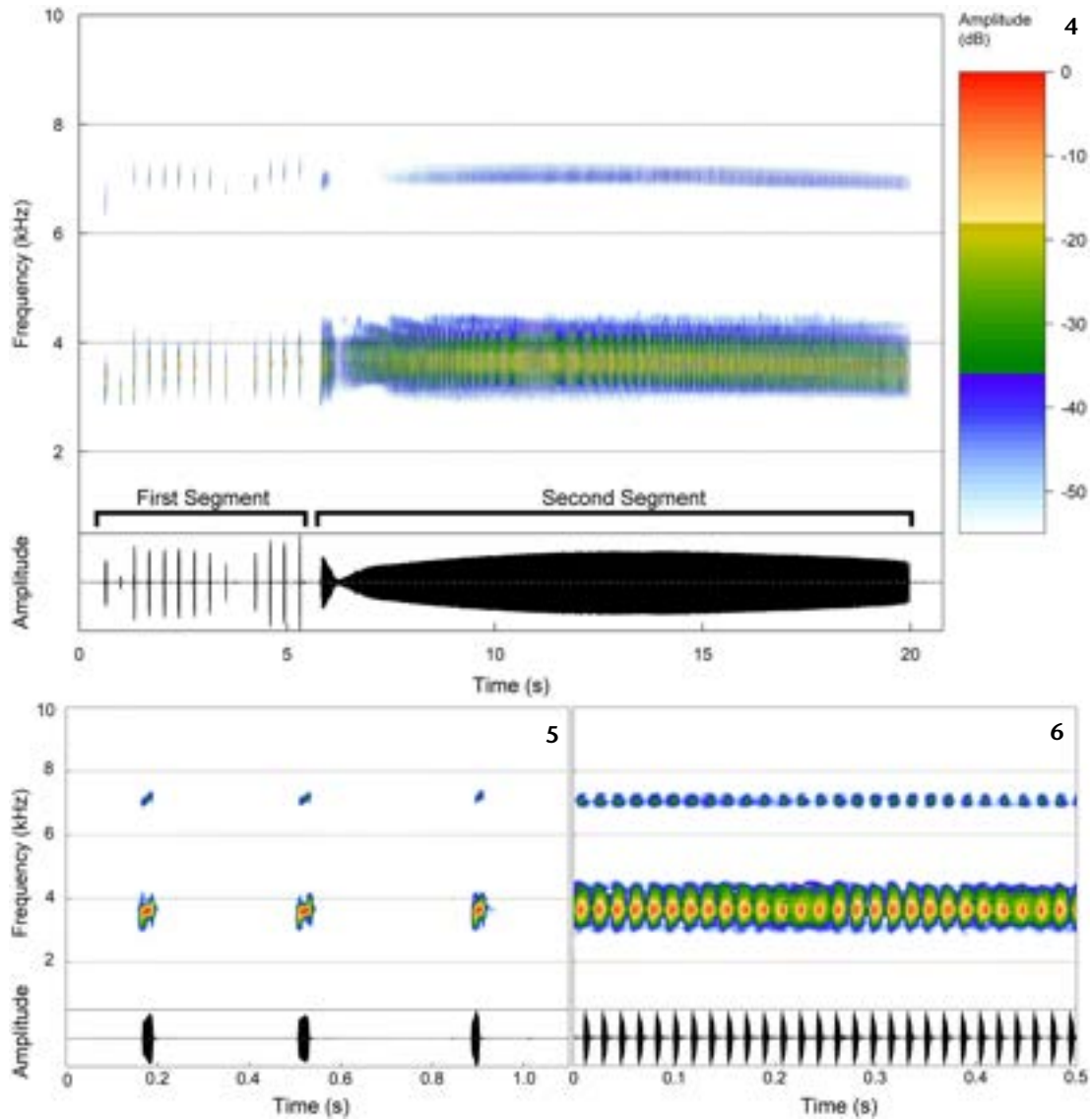
We compared the advertisement call of *M. xanthostomus* with nine congeners, although those descriptions did not include all the call parameters that we analyzed (see references in Table 1).

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 spectrogram of one call; (5) oscillogram and spectrogram of three notes from the first segment; (6) oscillogram and spectrogram of 28 pulses from the second segment.

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	<i>M. xanthostomus</i> ¹	<i>M. atroluteus</i> ²	<i>M. atroluteus</i> ³	<i>M. cupreuscapularis</i> ⁴	<i>M. dorsalis</i> ⁵	<i>M. dorsalis</i> ⁶	<i>M. klappenbachii</i> ⁷
Calls (n)	17 calls, 3 males 18.8 (a)	12 calls, 1 male 17.0 (w)	14 calls, 2 males 21.5 (a)	20 calls, 4 males 22.0 (a)	3 calls, 1 male 21.0 (a)	7 calls, 2 males 21.0 (a)	11 calls 22.0–24.0 (a)
Temperature (°C)	3395 (3101–3618)	3000	(1900–3100)	2270 (2176–2357)	(2600–3200)	(2300–3200)	1900
Dominant frequency (Hz)	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)
Call duration (s)	82,949 (9,258–221,673)	8,489 (2,922–7,954)	–	2,100 (1,350–2,780)	–	–	–
Inter-call interval (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)	–
1 st 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)	–
2 nd segment duration (s)	0,265 (0,054–0,609)	0,026 (0,003–0,079)	–	0,100 (0,040–0,160)	–	–	–
Interval between segments (s)	–	–	–	–	–	–	–
Number of notes of 1 st segment	13 (7–20)	20,6 (15–25)	17,6 (11–26)	10 (6–17)	19 (18–20)	13 (6–18)	3–4
1 st 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 1 st 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 1 st segment	0,003 (0,002–0,004)	–	(8–9)	–	–	(4–7)	–
Number of pulses 2 nd 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 2 nd 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	<i>M. krauczukii</i> ⁸	<i>M. montevideensis</i> ⁹	<i>M. pachyrynus</i> ¹⁰	<i>M. rubriventris</i> ¹¹	<i>M. rubriventris</i> ¹²	<i>M. rubriventris</i> ¹³	<i>M. stelzneri</i> ¹⁴
Calls (n)	5 calls, 1 male 17(w)	14 calls, 2 males 24(a)	12 calls, 6 males 16–17(a) 16–18(w)	12 calls, 4 males 19(a)	12 calls, 4 males 19(a)	12 calls, 4 males 19(a)	<i>M. stelzneri</i> ¹⁴
Temperature (°C)	3300	(2100–2800)	2668 (2261–2932)	1 st seg. 1788 (1704–1846) 2 nd seg. 1734 (1653–1789)	1 st seg. 1721 (1515–1845) 2 nd seg. 1687 (1535–1783)	1 st seg. 1841 (1754–1944) 2 nd seg. 1782 (1705–1869)	FH (2200–2600) 2H (4600–5200) 3H (7000–7600)
Dominant frequency (Hz)	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)
Call duration (s)	21,988 (18,196–25,737)	–	–	–	–	–	–
Inter-call interval (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)
1 st 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)
2 nd segment duration (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)	–
Interval between segments (s)	–	–	–	–	–	–	–
Number of notes of 1 st 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)
1 st 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 1 st 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)
Notes per s of 1 st segment	–	–	3,900 (2,900–4,300)	–	–	–	(5,000–6,000)
Number of pulses 2 nd segment	1298,500 (1018–1502)	147 (100–192)	818 (164–1382)	–	–	–	–
Pulses per s of 2 nd 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)

Specimens from: ¹Campos Alegre, Santa Catarina, Brazil (present study); ²Misiones, Argentina (Baldo and Basso 2004); ³São José dos Ausentes, Rio Grande do Sul, Brazil (Kvet and Miranda 2001); ⁴Corrientes, Argentina (Duré et al. 2015); ⁵Laguna, Santa Catarina, Brazil (Kvet et al. 2005); ⁶Jorres, Rio Grande do Sul, Brazil (Kvet et al. 2005); ⁷Paraguay (Kurth et al. 2013); ⁸Misiones, Argentina (Baldo and Basso 2004); ⁹La Paloma, Uruguay (Kvet et al. 2005); ¹⁰São Sepé, Rio Grande do Sul, Brazil (Caldart et al. 2013); ¹¹Tiraxi, Jujuy, Argentina (Ferrari and Vaira 2008); ¹²Abra Colorada, Jujuy, Argentina (Ferrari and Vaira 2008); ¹³Canto Del Monte, Argentina (Ferrari and Vaira 2008); ¹⁴Calamuchita, Córdoba, Argentina (Kvet and Miranda 2001); ¹⁵Santa 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 *Fritziana* 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. pachyrhynchus* (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* Céspedes & Alvarez, 2000, *M. dorsalis* (Mertens, 1933), *M. klappenbachi* Prigioni & Langone, 2000, *M. montevidensis* (Philippi, 1902), *M. stelzneri* (Weyenbergh, 1875), except from *M. krauczuki*, *M. pachyrhynchus*, 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 pachyrhynchus* 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. pachyrhynchus*, 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 evolutionary 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.

ACKNOWLEDGMENTS

We are thankful to all friends involved directly and indirectly to this work, specially to Carlos E. Conte, Renato C.

Nali and Daniel Son for suggestions and the help during the fieldworks. We are thankful to H.L. Doerrier and C.T. Ferriolli for the English editing. To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq processes 132559/2012-9, 167888/2014-5 and 311492/2017-7), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Boticário for the grants. To the SISBIO (#35005) and FUNDEMA (#013/12-GEMAP) for the research licenses.

LITERATURE CITED

- Baldo D, Basso NG (2004) A new species of *Melanophryniscus* Gallardo, 1961 (Anura: Bufonidae), with comments on the species of the genus reported for Misiones, Northeastern Argentina. *Journal of Herpetology* 38: 393–403.
- Baldo D, Candiotti FV, Haad B, Kolenc F, Borteiro C, Pereyra MO, Zank C, Colombo P, Bornschein MR, Sisa FN, Brusquetti F, Conte CE, Nogueira-Costa P, Almeida-Santos P, Pie MR (2014) Comparative morphology of pond, stream and phytotelm-dwelling tadpoles of the South American Redbelly Toads (Anura: Bufonidae: *Melanophryniscus*). *Biological Journal of the Linnean Society* 112: 417–441. <https://doi.org/10.1111/bij.12296>
- Barrio A (1964) Peculiaridades del canto nupcial de *Melanophryniscus stelzneri* (Weyenbergh) (Anura: Brachicephalidae). *Physis* 24: 435–437.
- Bevier CR, Gomes FR, Navas CA (2008). Variation in call structure and calling behavior in treefrogs of the genus *Scinax*. *South American Journal of Herpetology* 3(3): 196–206. <https://doi.org/10.1670/144-03A>
- Bornschein MR, Firkowski CR, Baldo D, Ribeiro LF, Belmonte-Lopes R, Corrêa L, Morato SAA, Pie MR (2015) Three New Species of Phytotelm-Breeding *Melanophryniscus* from the Atlantic Rainforest of Southern Brazil (Anura: Bufonidae). *PlosOne* 10(12): e0142791. <https://doi.org/10.1371/journal.pone.0142791>
- Caldart VM, Santos TG, Maneyro R (2013) The advertisement and release calls of *Melanophryniscus pachyrhynchus* (Miranda-Ribeiro, 1920) from the central region of Rio Grande do Sul, southern Brazil. *Acta Herpetologica* 8(2): 115–122.
- Caramaschi U, Cruz CAG (2002) Taxonomic status of *Atelopus pachyrhynchus* Miranda-Ribeiro, 1920, redescription of *Melanophryniscus tumifrons* (Boulenger, 1905), and descriptions of two new species of *Melanophryniscus* from the state of Santa Catarina, Brazil (Amphibia, Anura, Bufonidae). *Arquivos do Museu Nacional* 60(4): 303–314.
- Carvalho TR, Giaretta AA (2013) Bioacoustics reveals two new syntopic species of Adenomera Steindachner (Anura: Leptodactylidae: Leptodactylinae) in the Cerrado of central Brazil. *Zootaxa* 3731(3): 533–551.
- Daly JW, Wilham JM, Spande TF, Garraffo HM, Gil RR, Silva GL, Vaira M (2007) Alkaloids in bufonid toads (*Melanophryniscus*): temporal and geographic determinants for two Argentinian species. *Journal of Chemical Ecology* 33(4): 871–887. <https://doi.org/10.1007/s10886-007-9261-x>
- Duellman WE, Trueb L (1986) *Biology of Amphibians*. Baltimore, Johns Hopkins University Press, 670 pp.
- Durê MI, Schaefer EF, Kehr AI (2015) Acoustic Repertoire of *Melanophryniscus cupreuscapularis* (Céspedes and Álvarez 2000) (Anura: Bufonidae): Advertisement, Encounter, and Release Calls. *Journal of Herpetology* 49(1): 53–59.
- Erdtmann LK, Lima AP (2013) Environmental effects on anuran call design: what we know and what we need to know. *Ethology Ecology & Evolution* 25(1): 1–11. <https://doi.org/10.1080/03949370.2012.744356>
- Ferrari L, Vaira M (2008) Análisis comparativo de la estructura del canto de anuncio de tres poblaciones de *Melanophryniscus rubriventris* (Vellard, 1947) (Anura: Bufonidae). *Cuadernos de Herpetologia* 22: 25–34.
- Frost DR (2018) *Amphibian Species of the World: an Online Reference*. New York, American Museum of Natural History, v. 6.0. <http://research.amnh.org/herpetology/amphibia/index.html>
- Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sá RO, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto M, Moler P, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC (2006) The Amphibian Tree of Life. *Bulletin of the American Museum of Natural History* 297: 1–291.
- Gerhardt HC (1991). Female mate choice in treefrogs: static and dynamic acoustic criteria. *Animal Behaviour* 42(4): 615–635. [https://doi.org/10.1016/S0003-3472\(05\)80245-3](https://doi.org/10.1016/S0003-3472(05)80245-3)
- Gerhardt HC, Davis MS (1988) Variation in the coding of species identify in the advertisement calls of *Litoria verreauxi* (Anura: Hylidae). *Evolution* 42: 556–563.
- Haddad CF, Pombal-Jr JP (1998) Redescription of *Physalaemus spiniger* (Anura: Leptodactylidae) and description of two new reproductive modes. *Journal of Herpetology* 557–565.
- Heyer WR (1971) Mating calls of some frogs from Thailand. *Fieldiana Zoology* 58: 61–82.
- Köhler J, Jansen M, Rodríguez A, Kok PJR, Toledo LF, Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M (2017) The use of bioacoustics in anuran taxonomy: Theory, terminology, methods and recommendations for best practice. *Zootaxa* 4251: 1–124. <https://doi.org/10.11646/zootaxa.4251.1.1>
- Kurth M, Hörnes D, Esser S, Rödder D (2013) Notes on the acoustic repertoire of *Melanophryniscus klappenbachi* Prigioni & Langone, 2000. *Zootaxa* 3626: 597–600.
- Kwet A, Miranda T (2001) Zur Biologie und taxonomie der Schwarz-kro te *Melanophryniscus atroluteus* (Miranda-Ribeiro, 1920). *Herpetofauna* 23: 19–27.
- Kwet A, Maneyro R, Zillkens A, Mebs D (2005) Advertisement call of *Melanophryniscus dorsalis* (Mertens, 1933) and *M. montevidensis* (Phillipi, 1902), two parapatric species from southern Brazil and Uruguay, with comments on morphological variation in the *Melanophryniscus stelzneri* group (Anura: Bufonidae). *Salamandra* 41: 3–20.

- Kwet A, Lingnau R, Di-Bernardo M (2010) Pró-Mata: Anfíbios da Serra Gaúcha, sul do Brasil. Tübingen, Brasilien-Zentrum, University of Tübingen, 148 pp.
- Langone JA, Segalla MV, Bornschein M, de Sá RO (2008) A new reproductive mode in the genus *Melanophryniscus* Gallardo, 1961 (Anura: Bufonidae) with description of a new species from the state of Paraná, Brazil. *South American Journal of Herpetology* 3(1): 1–9.
- Larson PM, de Sá RO, Arrieta D (2003) Chondrocranial, hyobranchial and internal oral morphology in larvae of the basal bufonid genus *Melanophryniscus* (Amphibia: Anura). *Acta Zoologica* 84: 145–154.
- Maneyro R, Loebmann D, Tozetti A, Fonte LFM (2017) Anfíbios das planícies costeiras do extremo sul do Brasil e Uruguai. São Paulo, Anolis Books, 176 pp.
- Martin WF (1972) Evolution of vocalization in the genus *Bufo*. In: Blair WF (Ed.) *Evolution in the genus Bufo*. Austin, University of Texas Press, 279–309.
- McDiarmid RW (1971) Comparative morphology and evolution of frogs of the neotropical genera *Atelopus*, *Dendrophryniscus*, *Melanophryniscus* and *Oreophrynella*. *Bulletin of the Los Angeles County Museum of Natural History Science* 12: 1–66.
- Morton E S (1975) Ecological sources of selection on avian sounds. *The American Naturalist* 109(965): 17–34. <https://doi.org/10.1086/282971>
- Pansonato A, Mudrek JR, Simioni F, Alves Martins I, Strüßmann C (2014) Geographical variation in morphological and bioacoustic traits of *Pseudopaludicola mystacalis* (Cope, 1887) and a reassessment of the taxonomic status of *Pseudopaludicola serrana* Toledo, 2010 (Anura: Leptodactylidae: Leiuperinae). *Advances in Zoology* 2014: 1–13. <https://doi.org/10.1155/2014/563165>
- Peloso PLV, Faivovich J, Grant T, Gasparini JL, Haddad CFB (2012) An extraordinary new species of *Melanophryniscus* (Anura, Bufonidae) from Southeastern Brazil. *American Museum Novitates* 3762: 1–32.
- Sueur J, Aubin T, Simonis C (2008) Equipment review: seewave, a free modular tool for sound analysis and synthesis. *Bioacoustics* 18: 213–226.
- Van Bocxlaer I, Loader SP, Roelants K, Biju SD, Menegon M, Bossuyt F (2010) Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads. *Science* 327: 679–682.
- Wells KD (2007) *The ecology and behavior of amphibians*. University of Chicago Press, 1400 pp.
-
- Submitted: April 10, 2018
Accepted: May 11, 2018
Available online: July 31, 2019
Editorial responsibility: Mauricio O. Moura
-
- Author Contributions: LRM conducted the field works, recording the vocalizations and collecting the specimens, SM, DJS and LRM analyzed the data and wrote the paper.
Competing Interests: The authors have declared that no competing interests exist.
© 2019 Sociedade Brasileira de Zoologia. Published by Pensoft Publishers at <https://zoologia.pensoft.net>