

# The advertisement and release calls of *Melanophryniscus pachyrhynus* (Miranda-Ribeiro, 1920) from the central region of Rio Grande do Sul, southern Brazil

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**Abstract.** The redbelly toad *Melanophryniscus pachyrhynus* (Miranda-Ribeiro, 1920) is a poorly known toad belonging to the *M. tumifrons* group, which inhabits rocky open areas on top of hilly landscapes of the Uruguayan Savanna ecoregion in Uruguay and southern Brazil. In this study we describe the advertisement and release calls from a redbelly toad population from the central Rio Grande do Sul, southern Brazil, based on recordings obtained during the winter season of 2011. The advertisement call consists of a complex call with two segments, the first segment comprising a series of single, unpulsed notes, and the second comprising a multi-pulsed note (a trill) highly variable in duration. The mean dominant frequency of the call is  $2668 \pm 154$  Hz (2261–2932 Hz) and mean call duration is  $37.07 \pm 23.97$  s (6.64–75.20 s). This feature, however, is highly variable depending on the duration of the second segment, which may be notably longer compared to calls of other species of the genus *Melanophryniscus*. The release call was recorded during amplexus of up to four individuals. It consists of a short trill with a dominant frequency of approximately 2245 Hz, emitted as a series of five to six pulsed notes, each comprising five or six pulses. The advertisement call of *Melanophryniscus pachyrhynus* is compared with those of four other *Melanophryniscus* species, and the similarities in habitat requirements and in the temporal pattern of reproduction for species of the *M. tumifrons* group are discussed.

**Keywords.** Call description, reproduction, redbelly toad, Bufonidae, grasslands, Uruguayan Savanna ecoregion.

## INTRODUCTION

The bufonid genus *Melanophryniscus*, currently composed of 26 species of small Neotropical toads, occurs from the interandean valleys of southern Bolivia to Paraguay, northern Argentina, southern and southeastern Brazil, and Uruguay (Frost, 2013). According to morphological features, these toads are arranged into three phenetic species groups: the *M. moreirae*, *M. stelzneri* and *M. tumifrons* groups (Cruz and Caramaschi, 2003). The *M. tumifrons* group currently includes eight species that share features such as highly developed warts with an

apical corneous spine on the dorsal surfaces and flanks, a dorsal color pattern without contrasting dots, and the presence of a frontal skin gland between the eyes (Cruz and Caramaschi, 2003; Naya et al., 2004).

*Melanophryniscus pachyrhynus* (Miranda-Ribeiro, 1920) is a poorly known toad belonging to the *M. tumifrons* group, but which is relatively abundant in southern Brazil (Vaz-Silva et al., 2008). Recently, based on a comparison of external morphology, osteological and molecular characters, Baldo et al. (2012) placed *M. orejasmirandai* as a junior synonym of *M. pachyrhynus* and showed that the distribution of *M. pachyrhynus* currently com-

prises localities from central and eastern Rio Grande do Sul, in Brazil, and from southern and northeast Uruguay. Its distribution seems to be associated with upland environments in the Uruguayan Savanna ecoregion, where it inhabits rocky open areas on top of hilly landscapes (Vaz-Silva et al., 2008; Baldo et al., 2012). This toad calls from herbaceous vegetation on the ground and breeds in small temporary streams (Santos et al., 2011).

Anuran advertisement calls are a useful taxonomic character because they are species-specific and serve as a mechanism of reproductive isolation (Wells, 1977). In spite of this, no information on the vocalizations of *M. pachyrhynus* is available, nor for the populations previously referred to as *M. orejasmirandai*. A recent bioacoustic analysis of the genus *Melanophryniscus* considered the calls of 13 species from the three phenetic groups, but *M. pachyrhynus* calls were not included (Baldo, 2011). In fact, there is a lack of call descriptions for species from the *M. tumifrons* and *M. moreirae* groups, in contrast with the call descriptions available for the *M. stelzneri* group (e.g., Baldo and Basso, 2004; Kwet et al., 2005; Ferrari and Vaira, 2008; Kurth et al., 2013).

In this study we describe the advertisement and release calls of *M. pachyrhynus* for a population from São Sepé, a municipality located in the central region of Rio Grande do Sul state, southern Brazil, comparing its advertisement call with those of four other species of *Melanophryniscus*. Additionally, based on data available in the literature, we discuss the similarities in habitat requirements and in the temporal pattern of reproduction for species of the *M. tumifrons* group.

## MATERIAL AND METHODS

Calls were recorded in the municipality of São Sepé (30°14'56.2"S, 53°35'22.4"W, 162 m a.s.l.), state of Rio Grande do Sul, Brazil, on 18<sup>th</sup> and 19<sup>th</sup> June 2011. This area belongs to the Planalto Sul-Rio-Grandense (or Serra do Sudeste), a pampaean region characterized by rock crystalline shield outcrops covered by a natural mosaic of grassland (Campos) and seasonal forests (IBGE, 2004). Historically, land use in the region was based on cattle raised on natural vegetation, but this economic activity is now being replaced by soybean (summer season) and wheat (winter season) cultivation. The local climate is classified as subtropical wet (Cfa in the Köppen classification), with rainfall evenly distributed throughout the year (1200–1600 mm), i.e., with no dry season (Overbeck et al., 2007). Summer temperatures are high (maximum 40 °C), while winter temperatures are low, with median values less than 15 °C during the three months period when frosts are common. Thus, climatic seasonality is mostly determined by variation in temperature and photoperiod (Both et al., 2008).

Calls were recorded with an Olympus VN-6200PC digital recorder with an internal microphone, at a distance of approx-

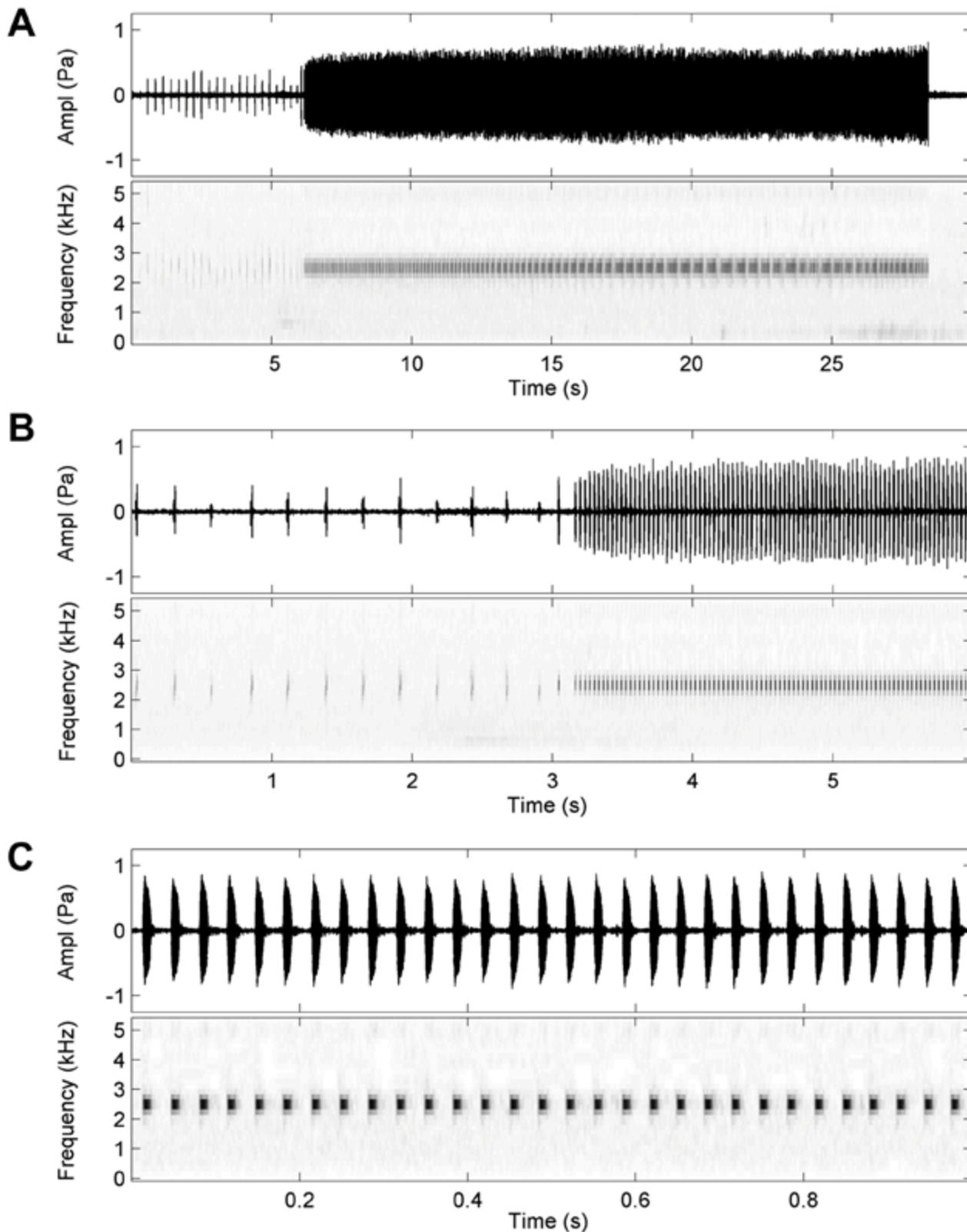
imately 50 cm from individuals, between 16:00 h and 21:00 h during two rainy days in the winter season. Air temperature at the calling sites ranged from 16 °C to 17 °C, and water temperature ranged from 16 °C to 18 °C. The advertisement call of *M. pachyrhynus* was described based on a total of 12 calls recorded from six individuals, and was compared to the advertisement calls described for four congeners, whose descriptions included similar call parameters: *M. krauczuki* (Baldo and Basso, 2004), *M. atroluteus* (Baldo and Basso, 2004), *M. montevidensis* (Kwet et al., 2005) and *M. dorsalis* (Kwet et al., 2005). The release call of *M. pachyrhynus* was described based on five calls recorded from one individual. We could not analyze the release calls of more individuals because the emission of these calls was sporadic and most calls were masked by the advertisement calls of other males. Three specimens were collected and deposited in the Herpetological Collection of the Universidade Federal de Santa Maria (Voucher specimens: ZUFMS 5193, 5195, 5243).

Calls were analyzed with SoundRuler software v. 0.9.6.0 (<http://soundruler.sourceforge.net>; Bee, 2004; Gridi-Papp, 2004) at a sampling frequency of 44100 Hz and a resolution of 16 bits. Oscillograms and audiospectrograms were also produced in SoundRuler software, with the following parameters: Fast Fourier Transformation (FFT) with 256 points, 100% frame, Hanning window type and 90% overlap. The following call parameters were analyzed: dominant frequency (Hz), call duration (s), 1<sup>st</sup> segment duration (s), number of notes of 1<sup>st</sup> segment, duration of notes of 1<sup>st</sup> segment (s), internote intervals of 1<sup>st</sup> segment (s), notes per s of 1<sup>st</sup> segment, 2<sup>nd</sup> segment duration (s), number of pulses of 2<sup>nd</sup> segment, duration of pulses of 2<sup>nd</sup> segment (s), interpulse intervals of 2<sup>nd</sup> segment (s), and pulses per s of 2<sup>nd</sup> segment. Values of call parameters are presented in the text as mean ± SD and range.

Terminology used for call parameters follows Heyer et al. (1990) and Duellman and Trueb (1994), and the definition of the term “note” follows the criteria of McLister et al. (1995), in which a note refers to a unit of sound consisting of one or more pulses, produced during a single airflow cycle. While recording the vocalizations of *M. pachyrhynus*, it was evident that each individual note of the first segment of the call corresponded to an airflow movement of the vocal sac, whereas pulses of the second segment (the trill) were all the result of a single airflow movement of the vocal sac, i.e., in the trills the vocal sac remained inflated all the time, with no airflow movements of the vocal sac as observed for the production of notes of the first segment. It should be noted that the definition of McLister et al. (1995) has been adopted in recent studies because it establishes a physiological basis for note emission (Orrico et al., 2009). Moreover, such definition could facilitate interpretations of the parameters of compound calls, such as those known for *Melanophryniscus* species (e.g., Kwet et al., 2005).

## RESULTS

The advertisement call of *Melanophryniscus pachyrhynus* consists of a compound call with two segments (Fig. 1 and Suppl. A1). The first segment comprises a series of



**Fig. 1.** Advertisement call of *Melanophryniscus pachyrhynchus* recorded in the municipality of São Sepé, Rio Grande do Sul, Brazil, on 18 June 2011, ~20:00 h, air temperature 16.5 °C, water temperature 16 °C. A) oscillogram (top) and audiospectrogram (bottom) of the entire call, B) oscillogram (top) and audiospectrogram (bottom) of a six second section of the call (1<sup>st</sup> and 2<sup>nd</sup> segments), C) oscillogram (top) and audiospectrogram (bottom) of one second section of the 2<sup>nd</sup> segment (the trill) of the call.

single, unpulsed notes ( $17 \pm 7$  notes; range 8–28 notes) with a mean duration of  $0.017 \pm 0.004$  s (0.010–0.026) and a mean time interval of  $0.234 \pm 0.034$  s (0.082–0.322 s) between them, emitted at a mean rate of  $3.9 \pm 0.5$  notes per second (2.9–4.3 notes/s). The first segment of the call has a mean duration of  $4.44 \pm 1.88$  s (1.85–7.67 s). The second segment of the call comprises a multipulsed note (a trill), highly variable in duration ( $25.27 \pm 15.80$  s; 4.79–45.75 s), with a mean of  $818 \pm 490$  pulses (164–1382 pulses). The mean pulse duration is  $0.012 \pm 0.002$  s (0.007–0.023 s) and the mean time interval between pulses is  $0.014 \pm 0.003$  s (0.007–0.030 s). Pulses are emitted at a mean rate of  $32.8 \pm 1.5$  pulses per second (30.2–34.3 pulses/s). The mean dominant frequency of the call is  $2668 \pm 154$  Hz (2261–2932 Hz), and the mean call duration is  $37.07 \pm 23.97$  s (6.64–75.20 s), but it is highly variable due to the duration of the second segment, which may be notably longer in comparison to calls of other species of the genus *Melanophryniscus* (Table 1).

The advertisement call of *M. pachyrhynchus* may be emitted in two different ways, i.e., the second segment

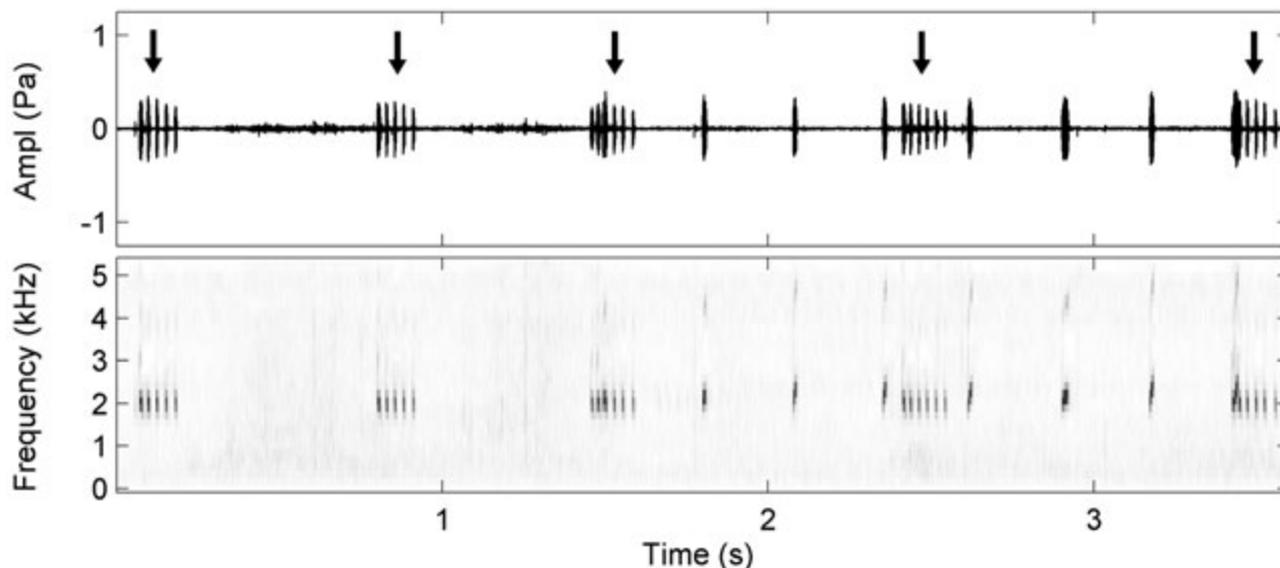
(the trill) alone or both segments in sequence. No males were observed emitting only the first segment, and we did not observe any apparent behavioral differences related to call emission.

Males of *M. pachyrhynchus* called along a temporary stream (50 cm wide) flowing through cultivated land (wheat), in shallow water among herbaceous vegetation based on the ground. The highest male abundance of *M. pachyrhynchus* in calling activity occurred at night (~21:00 h), totaling about 23 individuals. During this period we observed that males alternated calling activity with short dislocations (up to ~40 cm) down and/or upstream searching by females. At least three times we observed moving males being clasped by other males, resulting in an amplexus of up to four individuals. In these cases, clasped males tried to escape and displace the other male by moving along the substrate or by emitting a release call. The release call consists of a short trill, emitted as a series of five to six pulsed notes composed of five or six pulses each, with a dominant frequency of approximately 2245 Hz (Fig. 2 and Suppl. A2). The mean duration of the

**Table 1.** Comparison of temporal and spectral parameters of the advertisement call of *Melanophryniscus pachyrhynchus* (12 calls from six males; air temperature: 16–17 °C; water temperature: 16 °C) and four other *Melanophryniscus* species. Values are presented as mean, followed by range in parenthesis.

| Call parameters                                     | <i>M. pachyrhynchus</i> <sup>1</sup> | <i>M. krauczuki</i> <sup>2</sup> | <i>M. atroluteus</i> <sup>2</sup> | <i>M. montevidensis</i> <sup>3</sup> | <i>M. dorsalis</i> <sup>4</sup> |
|-----------------------------------------------------|--------------------------------------|----------------------------------|-----------------------------------|--------------------------------------|---------------------------------|
| Dominant frequency (Hz)                             | 2668<br>(2261–2932)                  | 3300<br>–                        | 3000<br>–                         | –<br>(2100–2800)                     | –<br>(2300–3200)                |
| Call duration (s)                                   | 37.07<br>(6.64–75.20)                | 32.699<br>(25.013–36.646)        | 7.523<br>(5.09–10.35)             | –                                    | –                               |
| 1 <sup>st</sup> segment duration (s)                | 4.44<br>(1.85–7.67)                  | 2.031<br>(1.128–3.160)           | 4.143<br>(2.575–5.36)             | 1.98<br>(1.0–4.5)                    | 1.89<br>(1.0–2.3)               |
| Number of notes of 1 <sup>st</sup> segment          | 17<br>(8–28)                         | 8.6<br>(6–12)                    | 20.6<br>(15–25)                   | 17<br>(7–28)                         | 13<br>(6–18)                    |
| Duration of notes of 1 <sup>st</sup> segment (s)    | 0.017<br>(0.010–0.026)               | 0.009<br>(0.005–0.023)           | 0.102<br>(0.006–0.174)            | 0.0313<br>(0.021–0.039)              | 0.0424<br>(0.02–0.05)           |
| Internote intervals of 1 <sup>st</sup> segment (s)  | 0.234<br>(0.082–0.322)               | 0.217<br>(0.147–0.837)           | 0.091<br>(0.006–0.229)            | 0.1035<br>(0.078–0.13)               | 0.1402<br>(0.08–0.17)           |
| Notes per s of 1 <sup>st</sup> segment              | 3.9<br>(2.9–4.3)                     | –                                | –                                 | –<br>(8–10)                          | –<br>(4–7)                      |
| 2 <sup>nd</sup> segment duration (s)                | 25.27<br>(4.79–45.75)                | 30.4548<br>(23.784–33.408)       | 3.012<br>(1.832–4.303)            | 1.58<br>(1.2–2.0)                    | 1.50<br>(0.6–2.2)               |
| Number of pulses of 2 <sup>nd</sup> segment         | 818<br>(164–1382)                    | 1298.5<br>(1018–1502)            | 222.38<br>(139–321)               | 147<br>(100–192)                     | 112<br>(54–162)                 |
| Duration of pulses of 2 <sup>nd</sup> segment (s)   | 0.012<br>(0.007–0.023)               | –                                | –                                 | 0.0051<br>(0.0045–0.006)             | 0.0071<br>(0.007–0.009)         |
| Interpulse intervals of 2 <sup>nd</sup> segment (s) | 0.014<br>(0.007–0.030)               | –                                | –                                 | 0.0052<br>(0.005–0.006)              | 0.0069<br>(0.0065–0.0075)       |
| Pulses per s of 2 <sup>nd</sup> segment             | 32.8<br>(30.2–34.3)                  | 43.67<br>(42.35–44.95)           | 75.44<br>(74.31–76.8)             | –<br>(85–95)                         | –<br>(74–78)                    |

Populations from: <sup>1</sup> São Sepé, central Rio Grande do Sul, Brazil (present study); <sup>2</sup> Misiones, Argentina (Baldo and Basso, 2004); <sup>3</sup> La Paloma, Uruguay (Kwet et al., 2005); <sup>4</sup> Torres, Rio Grande do Sul, Brazil (Kwet et al., 2005).



**Fig. 2.** Release call of a male of *Melanophryniscus pachyrhynus* emitted while being clasped by another male. Details with the arrows indicate the pulses of the release call, and the single note that appears around the third pulse onwards represents the beginning of an advertisement call of another nearby male. Call recorded in the municipality of São Sepé, Rio Grande do Sul, Brazil, on 18 June 2011, ~20:30 h, air temperature 16.5 °C, water temperature 16 °C. From top to bottom: oscillogram and audiospectrogram.

trills is  $0.131 \pm 0.009$  s (0.119–0.141) and the mean interval between them is  $0.699 \pm 0.157$  s (0.533–0.854).

Additionally, we observed two male–female pairs in axillary amplexus (Suppl. Fig. S1) during the night period between 20:00–20:40 h, but we did not observe the egg deposition. We returned to the breeding site after one week and observed *M. pachyrhynus* tadpoles in the pools remaining in the dry stream bed.

## DISCUSSION

The advertisement call of *M. pachyrhynus* differs from those of *M. atroluteus* (Kwet and Miranda, 2001; Baldo and Basso, 2004), *M. dorsalis* and *M. montevidensis* (Kwet et al., 2005) in having shorter notes in the first segment and because it has a noticeably longer second segment, consequently presenting a greater number of pulses. On the other hand, the advertisement call of *M. pachyrhynus* is similar to the call of *M. krauczuki* (Baldo and Basso, 2004), at least in the variable duration of the call and the greater number of pulses in the second segment, although the call of *M. pachyrhynus* can be longer. The mean dominant frequency of the advertisement call of *M. pachyrhynus* has an intermediate value in comparison with the calls of these species. No other formal call descriptions from the *M. tumifrons* group are available, but Maneyro and Kwet (2008) report that the advertisement call of *M. devincenzii* (*M. tumifrons* group) from

northern Uruguay is similar to that of other species in the *M. stelzneri* group, although also presenting a longer second segment.

Advertisement calls composed of long trills are known for species of *Rhinella* (Guerra et al., 2011; São-Pedro et al., 2011), *Peltophryne* (Hernández et al., 2010), *Frostius* (Juncá et al., 2012), and also for species of *Melanophryniscus* (Baldo and Basso, 2004), among other bufonids. Although some species with trilled calls are known to increase their call duration in response to other males (Wells, 2007), the highly variable duration of the advertisement call of *M. pachyrhynus* is remarkable within the genus *Melanophryniscus*, given that the shortest call recorded has 6.64 s and the longest has 75.20 s. Evidence gathered for anurans that call in dense choruses and at high rates revealed that this behavior implies a high energetic cost (Wells, 2001), although males can produce long calls at a slow rate in dense choruses, or produce short calls at a high rate in sparse choruses (Wells and Taigen, 1986).

As explosive breeders (*sensu* Wells, 1977), males of *M. pachyrhynus* probably experience a dense concentration of males over the course of a few days at the breeding sites. Thus, it is possible that the variation in call duration may be related to sexual selection dependent on the abundance of conspecific competitors at the breeding sites, since male-male competition for females is expected to be more significant than female choice in explosive breeders (Wells, 1977). Nevertheless, although the adver-

tisement call of *M. pachyrhynchus* constitutes a remarkable sexual signal, the functional significance of this exaggerated acoustic signal remains unclear and deserves further studies.

Release calls usually consist of a series of repeated broad-spectrum notes emitted by males when clasped by other males, indicating to a clasping male that he has grabbed an inappropriate mate (Wells, 2007). Thus, release calls constitute a signal used for sex recognition (Marco et al., 1998; Liao and Lu, 2009). Unlike advertisement calls, release calls are much less studied, although these calls are known for several bufonid species with explosive breeding (Garda et al., 2010; Guerra et al., 2011; Sanabria and Quiroga, 2012). Release calls of the genus *Melanophryniscus* were described for the first time recently for four species, consisting of a short trill which may or may not be frequency-modulated (Baldo, 2011). The release call of *M. pachyrhynchus* described here has this same structure and was recorded during dense aggregations of males, in which we observed amplexus of up to four individuals. Reproductive aggregations in some *Melanophryniscus* species are known to be male-biased, such as in the case of *Melanophryniscus rubriventris* and *Melanophryniscus* aff. *montevicensis* from Argentina, whose males outnumbered females at all breeding events studied (Vaira, 2005; Cairo et al., 2013). Moreover, males of *Melanophryniscus rubriventris* were found actively searching in order to obtain mates (Vaira, 2005). These results suggest that release calls may be common in species of *Melanophryniscus*.

Most species of the *M. tumifrons* group breed in temporary or permanent small streams, usually after heavy rainfall (Maneyro and Kwet, 2008; Santos et al., 2011). *Melanophryniscus pachyrhynchus* was found breeding in small temporary and permanent streams during spring and autumn in Uruguay (Prigioni and Langone, 1990; Lavilla and Langone, 2004; Maneyro and Carreira, 2012), and during early summer (Santos et al., 2011) and winter (present study) in ephemeral streams in southern Brazil. These results indicate that *M. pachyrhynchus* may breed at any season throughout the year, since there is enough rainfall to create ephemeral streams. Similar observations were made for *Melanophryniscus cambaraensis* in the Campos de Cima da Serra region, southern Brazil (Santos et al., 2010), and for *Melanophryniscus devincenzii* in northern Uruguay (Maneyro and Kwet, 2008). Based on these findings, we suggest that species of the *M. tumifrons* group probably share similar habitat requirements, as well as a similar temporal pattern of reproduction, i.e., without a marked climatic seasonality and dependent on rainfall patterns. Additional studies are still necessary in order to test this hypothesis for the *M. tumifrons* species

group, although at least for one species of the *M. stelzneri* group the breeding activity showed annual variation due to variation in rainfall patterns (Vaira, 2005).

The genus *Melanophryniscus* currently has 26 species of small Neotropical toads, but for many of them basic aspects of their natural history and ecology are still poorly known. This scenario is evident for the case of *M. pachyrhynchus*, which for many years was known only for its type locality, and is now considered relatively common in Rio Grande do Sul (Vaz-Silva et al., 2008). With regard to acoustic communication, call descriptions for species of the *M. tumifrons* and *M. moreirae* groups are necessary for a better comparative approach within the species groups, and attention should also be given to the occurrence of release calls within the genus. Furthermore, basic investigations including descriptions of breeding habitats, breeding behavior and calling activity, as well as reproductive events, could provide valuable information for this taxon.

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#### SUPPLEMENTARY MATERIAL

**Fig. S1.** Amplectant pair of *Melanophryniscus pachyrhynchus* observed in the municipality of São Sepé (30°14'56.2"S, 53°35'22.4"W, 162 m a.s.l.), Rio Grande do Sul, Brazil, on 18 June 2011, ~20:30 h, air temperature 16.5 °C, water temperature 16 °C.

**Audio file A1.** Advertisement call of *Melanophryniscus pachyrhynchus* recorded in the municipality of São Sepé (30°14'56.2"S, 53°35'22.4"W, 162 m a.s.l.), Rio Grande do Sul, Brazil, on 18 June 2011, ~20:30 h, air temperature 16.5 °C, water temperature 16 °C.

**Audio file A2.** Release call of a male of *Melanophryniscus pachyrhynchus* emitted while being clasped by another male, recorded in the municipality of São Sepé (30°14'56.2"S, 53°35'22.4"W, 162 m a.s.l.), Rio Grande do Sul, Brazil, on 18 June 2011, ~20:30 h, air temperature 16.5 °C, water temperature 16 °C. The single notes that appear around the third pulse onwards represent the beginning of an advertisement call of another nearby male.