

4-2008

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Recommended Citation

Langone, Jose A., Magno V. Segalla, Marcos Bornschein, and Rafael O. de Sá. "A New Reproductive Mode in the Genus *Melanzophryniscus* Gallardo, 1961 (Anura: Bufonidae) with Description of a New Species from the State of Paraná, Brazil." *South American Journal of Herpetology* 3, no. 1 (April 2008): 1-9. doi:10.2994/1808-9798(2008)3[1:anrmit]2.0.co;2.

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A NEW REPRODUCTIVE MODE IN THE GENUS *MELANOPHRYNISCUS* GALLARDO, 1961 (ANURA: BUFONIDAE) WITH DESCRIPTION OF A NEW SPECIES FROM THE STATE OF PARANÁ, BRAZIL

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ABSTRACT. A new species of bufonid toad of the genus *Melanophryniscus* is described from a mountaintop that is part of the Serra do Mar in the northeastern State of Paraná, Brazil. *Melanophryniscus alipioi* sp. nov. is distinguished from other known species by its uniformly dark brown dorsal color and a unique breeding site. The new species reproduces in bromeliads, a reproductive mode previously unknown for this genus. This species might be susceptible to current habitat loss.

KEYWORDS. Anura, Bufonidae, *Melanophryniscus alipioi*, new species, bromeligenous species.

INTRODUCTION

The genus *Melanophryniscus* Gallardo, 1961, is considered a basal, monophyletic, bufonid taxon (Graybeal and Cannatella, 1995; Frost *et al.* 2006) currently consisting of 20 described species distributed from southern Brazil, throughout Paraguay and southern Bolivia, to central and northern Argentina, and Uruguay (Frost, 2007; Di-Bernardo *et al.*, 2006). Some species have restricted distributions and/or are considered endangered taxa (IUCN *et al.*, 2004). The species in this genus have been clustered into three species groups: *moreirae* group, *tumifrons* group, and *stelzneri* group (Cruz and Caramaschi, 2003). These groups are loosely defined and lack diagnostic characteristics, except for the *M. tumifrons* group for which the presence of a frontal macrogland (*sensu* Naya *et al.*, 2004, based on a histological analysis of the skin) has been proposed as a synapomorphy for the group. This gland, referred as a frontal swelling, also was considered as a putative synapomorphy by Baldo and Basso (2004).

Herein we describe a new species of *Melanophryniscus* and report the occurrence of a new reproductive mode for the genus.

MATERIALS AND METHODS

Type specimens are deposited at Museu de História Natural Capão da Imbuia, Curitiba, Brazil (MHNCI);

Museu Nacional, Rio de Janeiro, Brazil (MNRJ); and Museu de Zoologia, Universidade de São Paulo, Brazil (MZUSP). All comparative material are listed and catalogue numbers are provided in Appendix I.

Measurements were taken with calipers to the nearest 0.02 mm; all measurements are provided in Table 1. Geographic coordinates were obtained using a Garmin Vista Global Positioning System (GPS).

Developing eggs and amplexic pairs were collected from bromeliads in the type locality on October 12, 2004. Amplexic pairs were taken to the laboratory where they laid additional eggs.

Melanophryniscus alipioi sp. nov. (Figs. 1 and 2)

Holotype – MHNCI 5186. Adult male, collected at Serra do Capivarí (25°07'49.0"S, 48°49'16.9"W; 1491 m altitude), part of the larger Serra do Mar, Campina Grande do Sul, State of Paraná, Brazil, on 12 October 2004.

Paratopotypes – MHNCI 5182-5185, 5187-5202, MNRJ 49769, MZUSP 132870-132872, collected with the holotype.

Diagnosis – Species of small size, without dorsal macrogland on snout; dorsal, lateral, and ventral body surfaces scattered with medium to large size glands, dorsal surfaces of limbs scattered with the same glands but these are slightly smaller and conical; dorsal color

TABLE 1. Measurements (mm) of paratypes of *Melanophryniscus alipioi* sp. nov. Snout-vent length (SVL), Head length (HL), Head width (HW), Eye diameter (ED), Interocular Distance (IOD), Internarial distance (IND), Eye-nostril distance (END), Upper eyelid length (UEL), Thigh length (TL), Shank length (SL), Foot length (FL), Hand Length (HL).

Males (n = 20)												
	SVL	HL	HW	ED	IOD	IND	END	UEL	TL	SL	FL	HL
x	21.06	6.19	6.81	2.04	2.92	1.72	1.88	2.87	8.20	7.58	8.02	5.29
S.D.	0.85	0.82	0.45	0.23	0.21	0.21	0.13	0.29	0.74	0.81	0.60	0.25
Max	22.66	7.00	7.50	2.52	3.30	2.11	2.00	3.50	9.00	8.50	8.83	5.70
Min	19.45	4.50	6.00	1.50	2.50	1.50	1.64	2.50	6.50	5.00	7.00	5.00
Females (n = 3)												
	SVL	HL	HW	ED	IOD	IND	END	UEL	TL	SL	FL	HL
x	25.26	6.35	7.22	2.41	3.24	2.16	1.96	3.19	9.37	25.26	6.35	7.22
S.D.	0.59	0.36	0.68	0.36	0.25	0.16	0.10	0.37	0.32	0.59	0.36	0.68
Max	25.64	6.60	8.00	2.68	3.49	2.32	2.03	3.62	9.59	25.64	6.60	8.00
Min	24.58	5.94	6.80	2.00	3.00	2.00	1.85	2.96	9.0	24.58	5.94	6.80

of head, body, and upper surfaces of the hind limbs uniformly dark brown; color of ventral surfaces dark brown, with large, irregular shaped, and uniformly bright red spots on each side of the pectoral area, extending slightly onto the ventral surface of the arms. The same uniformly bright red color is also found across the posterior abdominal area and on the ventral

surfaces of hands and feet, extending over the lateral surface of the digits and on the dorsal surface of the toe tips. On the ventral surface of body, a few scattered white dots are found over the red color. The pupil is surrounded by a golden iris, which is finely spotted or reticulated with dark brown markings. This species reproduces, i.e., calls and deposit eggs, in bromeliads.

Melanophryniscus alipioi is most similar to *M. moreirae* (Miranda-Ribeiro, 1920) in the macrohabitat that they occupy, i.e., open formation surrounded by forested areas in coastal mountains of southern and southeastern Brazil at high elevations (above 1200 m for *M. alipioi* and above 1800 m for *M. moreirae*). However, the two species can be distinguished by the following characteristics (characters of *M. moreirae* are in parenthesis): dorsum uniformly dark brown (dorsum dark grey to black or brownish with scattered light areas); ventral surface dark brown with bright red areas on pectoral, posterior abdominal, and femoral regions; bright red markings on the pectoral zone contained a few, very small, white spots (venter dark grey or black with bright red in the pectoral, abdominal and femoral areas; spots densely dotted with small white spots in throat, gular and pectoral area); calling and oviposition in bromelids (calling and oviposition in pools with running water). The nearest population reported of *M. moreirae* is found 490 km northeast of the type locality of *M. alipioi* at Queluz, State of São Paulo (22°25'44"S, 44°50'10"W) (Marques *et al.*, 2006).

The dorsal color of *M. alipioi* in life is similar to *M. krauczuki* Baldo and Basso, 2004; a species known only from a few localities in the Argentinean Province of Misiones and one locality in the Depart-

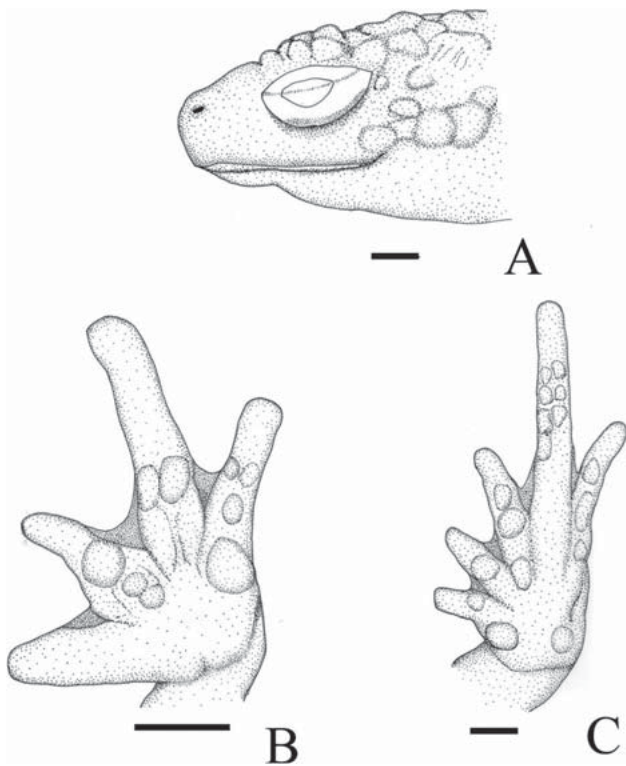


FIGURE 1. *Melanophryniscus alipioi* sp. nov., holotype, MHNCI 5186. (A) Lateral view of head. (B) Hand. (C) Foot. Bar = 1 mm.

ment of Itapua, Paraguay (Brusquetti *et al.*, 2007). *Melanophryniscus krauczuki* occurs in semi-natural environments communities and grasslands at low elevations (125-165 m, Baldo and Basso, 2004). Furthermore, the two species can be distinguished by the more extensive webbing in the foot of *M. krauczuki*; its ventral color consisting of a dark brown background with irregular orange spots and an orange-red femoral spot, and the presence of several white spots on the surface of the lower jaw.

The new species differs from species in the *tumifrons* group by the absence of a frontal macrogland. Furthermore, *Melanophryniscus alipioi* is easily distinguished from the other Brazilian members of the genus without frontal macrogland. From the recently

described *M. admirabilis* Di-Bernardo, Maneyro, and Grillo, 2006, from the State of Rio Grande do Sul, by the larger size of the later species (SVL 30-40 mm), its overall pale green dorsal color, and the presence of large, yellowish skin glands on dorsal, lateral, and ventral body surfaces. From *M. atroluteus* (Miranda-Ribeiro, 1920), distributed from Misiones Province, Argentina, throughout Uruguay reaching southern Paraguay and the northeastern and western regions of Rio Grande do Sul and presumably southern Santa Catarina, Brazil, because this species has a uniformly black dorsum (Kwet *et al.*, 2005). Furthermore, the texture of the dorsal skin of *M. atroluteus* has more and smaller skin glands than those found on the dorsal surface of *M. alipioi*. *Melanophryniscus dorsalis*



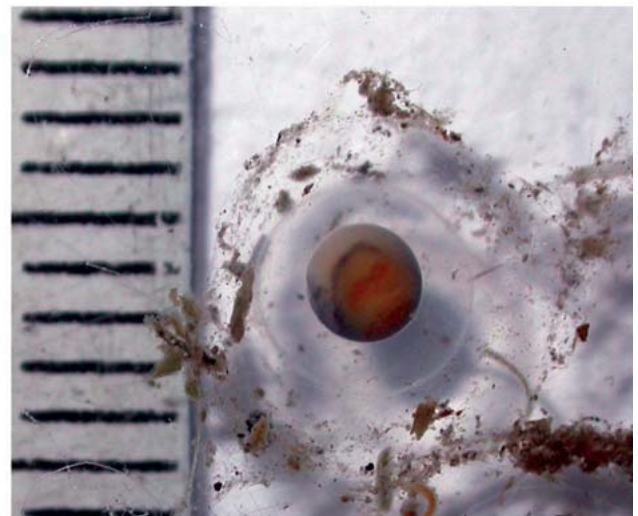
A



B



C



D

FIGURE 2. (A) *Melanophryniscus alipioi* sp. nov., holotype, MHNCI in its habitat. (B) *Melanophryniscus alipioi* sp. nov. in its habitat. (C) *Melanophryniscus alipioi* sp. nov., MZUSP 132968, specimen at the completion of metamorphosis. (D) Egg of *Melanophryniscus alipioi* sp. nov.

(Mertens, 1933), found in sandy habitats of the Atlantic coast of the states of Rio Grande do Sul and Santa Catarina, Brazil, exhibits a black dorsal color with a distinct red (brown in preservative) middorsal line, which is rarely absent but often interrupted or present only as a small trace behind the eyes and/or above anus (Kwet *et al.*, 2005); a middorsal line is absent in *M. alipioi*. *Melanophryniscus fulvoguttatus* (Mertens, 1937), from northern Argentina, Paraguay, and Mato Grosso do Sul, Brazil, can be easily differentiated from *M. alipioi* by its densely granular skin with black dorsal and ventral color dotted with numerous small and irregularly shaped yellow spots and yellow blotches on the suprascapular region (Kwet *et al.*, 2005). *Melanophryniscus montevidensis* (Philippi, 1902) is an exclusive sandy coastal inhabitant extending from the Rio de la Plata and Atlantic coast of Uruguay to the southernmost part of Rio Grande do Sul, Brazil. This species usually has yellow dorsolateral spots, but sometimes it is uniformly black (Kwet *et al.*, 2005). Also, the skin texture is uniformly less granular than that of *M. alipioi*.

In addition, all these species have allopatric distributions in relation to *M. alipioi* and they all breed in temporary ponds with moving or stationary water.

Description of holotype – Body stout; head slightly wider than long (HW/HL = 1.08), approximately one third of SVL; snout short, truncate in dorsal view, rounded in lateral profile, slightly protruding; nostrils directed laterally, oval, located at anterior tip of snout; eye large; pupil horizontally elliptical; eye diameter smaller than interorbital distance and larger than eye-nostril distance; tympanic membrane absent; parotoid gland absent; maxillae and premaxillae edentate; tongue narrow and elongate, posterior margin entire, posteriorly free for about two thirds of its length; vocal sac median, subgular; skin of dorsum rugose, scattered with medium to large, blunt, rounded glandular warts; warts without keratinized spines; these glandular warts extend over the ventral surface of body particularly in abdominal and gular areas; dorsal surface of limbs, hands, and feet scattered with the same warts but these are slightly more conical and smaller; lengths of fingers: I < II < IV < III; fingers blunt, short, slightly webbed at the base; subarticular tubercles developed; inner and outer metacarpal tubercles indistinguishable; palmar supernumerary tubercles conical, sparsely distributed; smooth, brown nuptial pads on thumb and second finger; lengths of toes: I < II < V < III < IV; toes slightly webbed at base; inner metatarsal tubercle rounded; outer meta-

tarsal tubercle oval, one and a half times smaller than inner one; subarticular tubercles conical; small, scattered supernumerary plantar tubercles

Measurements of holotype (mm) – Snout-vent length (SVL) 22.2, head length (HL) 5.8, head width (HW) 6.3, eye diameter (ED) 2.5, interorbital distance (IOD) 3.0, eye-nostril distance (END) 1.8, internarial distance (IND) 1.9, upper eyelid length (UEL) 3.0, thigh length (TL) 8.0, shank length (SL) 7.8, foot length (FL) 8.6, hand length 5.4.

Color in life – Dorsal color of head, body, and upper surfaces of the appendages uniformly dark brown; throat and gular surfaces dark brown; pectoral area with bright red spots on each side, these markings do not meet at the midline and extend over the ventral surface of the arm; belly, ventral surfaces of forearms and thighs, palmar surfaces of hands and fingers, ventral surfaces of feet and toes, lateral surfaces of digits, dorsal surfaces of fingers and toe tips of fore and hindlimbs bright red; bright red of the pectoral zone scattered with few and very little white spots; iris gold, finely spotted with dark brown.

Variation – The range of measurements of paratypes is given in Table 1. The variation observed mostly relates to differences in color pattern. Dorsally, one specimen (MHNCI 5182) had a light brown dorsum in life and the outer surface of its forearm was yellowish. Ventrally, the size and distribution of red color markings is variable, with some specimens being red on most of the belly and in part of the pectoral area (MHNCI 5195). A few specimens show very small red spots on gular region. Sexual dimorphism is evident in snout-vent length, with females being on average about 15% larger than males (SVL females 24.6-25.6 mm, $\bar{x} = 25.26$, SD = 0.59, $n = 3$; SVL males 19.4-22.7 mm, $\bar{x} = 21.06$, SD = 0.65, $n = 20$).

Adult breeding males have conical nuptial pads on thumb and usually on the second finger. A specimen at the completion of metamorphosis (MZUSP 132968) measured 6.8 mm in SVL. The overall coloration of this specimen is dark brown with a few small white dots disperse over the entire body including the appendages, but lacking the red markings that characterized the adult (Fig. 2C).

One of the paratypes (MHNCI 5187) has a clear abnormality on the right hand, where finger IV is missing (ectrodactyly); we cannot determine if this is the result of a developmental problem or the result of predation sometimes during its life.

Natural history – All specimens were collected during the day, between 12:00 and 17:00 hs. Except for two specimens, which were walking on the ground, all other specimens were collected inside bromeliads (*Vriesea platynema* Gaudichaud and *Aechmea ornata* var. *hoehneana* L.B. Smith) (Fig. 2B). Males of *M. alipioi* were observed calling inside the bromeliads (MHNCI 5187, 5188, 5189), two amplexic pairs were collected (i.e., MHNCI 5201 and MZUSP 132870, MZUSP 132871 and MZUSP 132872), and one egg cluster was collected in the axils of a bromeliad; the eggs were found just above the water collected inside.

Etymology – The new species is named in honor to Alipio de Miranda-Ribeiro (1874-1939), the first Brazilian zoologist that described new species of *Melanophryniscus* as *Atelopus* (*A. atroluteus*, *A. pachyrhynus*, and *A. moreirae*, Miranda-Ribeiro, 1920).

Distribution – *Melanophryniscus alipioi* is only known from the type locality in the northeastern State of Paraná, Brazil, at elevation above 1400 m. The area is included within the “campos de altitude” (sensu Safford, 1999), in the phytogeographic outline of Atlantic Forest. The species is likely to be endemic to the Serra do Capivari. The mountain area inhabited by this species undergoes periodical burnings due to unregulated fire use by the local residents (Andre Xavier Lima, pers. comm.)

DISCUSSION

Anurans have a wide diversity of reproductive modes, ranging from aquatic eggs with free-swimming larvae to truly viviparous species (Duellman and Trueb, 1986). Among these reproductive modes is phytotelm breeding, i.e., the use of water-holding plants as oviposition and larval development sites. Although phytotelm breeding has evolved in over 100 species of anurans across a wide phylogenetic spectrum (Lehtinen *et al.*, 2004), within the family Bufonidae it has been reported only in six genera. Among neotropical bufonids, only three genera have been reported to use phytotelmata for breeding; these are: *Dendrophryniscus brevipollicatus* (Carvalho, 1949) and related species (Izecksohn, 1994), *Frostius pernambucensis* (Cruz and Peixoto, 1982), and *Rhinella castaneotica* (Caldwell, 1993). The first two genera lay their eggs and tadpoles develop in bromeliads, whereas the later species uses the empty fruit cap-

sules of Brazil nuts for oviposition and development (Caldwell, 1993; Caldwell and de Araujo, 2004). All previously known species of *Melanophryniscus* deposit their eggs in freestanding water and they had been characterized as having exotrophic larvae that inhabit lentic and lotic environments (Altig and McDiarmind, 1999). The description of *M. alipioi* as a phytotelm breeding species represents a new reproductive mode for the genus and only the fourth neotropical bufonid species reported to have evolved this mode. *Melanophryniscus alipioi* was found associated with *Vriesea platynema* and *Aechmea ornata* bromeliads, but there is no available data on the physical and chemical characteristics of the microhabitat created in the water tanks of these species.

Several authors have provided morphotypes classifications of phytotelm anuran larvae and their reproductive modes. Lanoo *et al.* (1987) considered the following characters to classify “arboreal tadpoles” into six distinct groups: denticle pattern, body form, tail/body ratio, and dietary and habitat information. The tadpoles of *M. alipioi* would best fit the characteristics of Group 3; except that the tail/body ratio is 1.4-1.6, instead of the > 1.7 as defined for this group. Peixoto (1995) classified anuran larvae inhabiting bromeliads and, in the context of that classification, the tadpoles of *M. alipioi* correspond to the category of species breeding in bromeliads with free larval stage (“espécies bromeligenas com fase larvária livre” of Peixoto, 1995). Haddad and Prado (2005) provided a comprehensive revision of reproductive modes and added 10 additional reproductive modes to those previously summarized by Duellman and Trueb (1985) and Hödl (1990). *Melanophryniscus alipioi* should be added to Mode 6 of the later classification, corresponding to the first New World bufonid in this mode (although *Rhinella castaneotica* has phytotelm larvae, they are not found in tree holes or aerial plants, but in Brazilian nuts capsules on the ground).

According to these authors, bromeliad-inhabiting tadpoles could be endo or exotrophic. The larvae of *Dendrophryniscus*, *Frostius*, and *Fritziana* are endotrophic and characterized by a reduction or lack of keratinized mouthparts; e.g., *Dendrophryniscus*, *Frostius* (Izecksohn and Cruz, 1972; Cruz and Peixoto, 1982). The larvae of *M. alipioi* do have normal size marginal disc papillae, a keratinized beak, and normal developing rows of denticles. This suggests that the phytotelm larvae of this species are exotrophic. Furthermore, recently hatched larvae have the intestinal coils filled with light color material, likely vitellin; however, this is not the case of more advanced

developmental tadpoles. We have no evidence of an oophagous or macrophagous diet in this species (as reported for other bromeliad inhabiting tadpoles, e.g., *Osteopilus brunneus*, Lanoo *et al.*, 1987; *Anothea spinosa*, Taylor, 1954; Duellman, 1970; Jungfer, 1996; *Oophaga pumilio*, Starrett, 1960; Weygoldt, 1980; *Hoplophryne*, Noble, 1929; etc) and these larvae probably exhibit a detritivorous diet, feeding on plant and animal remains found in the bromeliads' water tank where they develop (similar to other bromeliad inhabiting tadpoles, e.g., *Bromeliohyla bromeliacia*, Stuart, 1948; Duellman, 1970; *Phyllodytes luteolus*, Bokermann, 1966; *Crossodactylodes*, Peixoto, 1981, 1983; *Allobates bromelicola*, Dixon and Rivero-Blanco, 1985, etc).

There is little comparative data for egg size (i.e., ovum without egg's capsules) and time of hatching in *Melanophryniscus*. Data on egg size has been reported for: *M. krauczuki* ranging from 1.7-2.0 mm (Baldo and Basso, 2004), *M. moreirae* between 2.0-2.5 mm (Bokermann, 1967; Starret, 1967), *M. stelzneri* between 1.4-2.0 mm (Echeverria, 1998; Bustos Singer and Gutiérrez, 1997). In addition, egg size of *M. montevidensis* is 2.0 mm (J. Langone, pers. obs.) and that of *M. atroluteus* and *M. aff. tumifrons* is approximately the same as *M. krauczuki* (D. Baldo, pers. com.). The egg diameter of *M. alipioi* ranges between 2.5-3.0 mm (diameter including egg's capsules is about 7-8 mm, Fig. 2D). Other relevant egg's size data for bufonids are those for *Dendrophryniscus brevipollicatus* with 2 mm (Carvalho, 1949; Izecksohn and Cruz, 1972) and *D. minutus* with 0.8 mm (Duellman and Lynch, 1969). The egg's diameter of *Frostius penambucensis* is 2 mm (Cruz and Peixoto, 1982), and we assumed this size refers to egg diameter without egg capsules. Although the egg sizes of exotrophic larvae ranges from 0.8 to 4.0 mm and that of endotrophic larvae from 1.8 to 10.0 mm, egg size in itself is a poor indicator of endotrophy (Thibaudeau and Altig, 1999). It is interesting to note that the eggs of *M. alipioi* are not significantly larger than those reported for other species of *Melanophryniscus* and although the species is a phytotelm breeder, it does not exhibit other traits associated with endotrophy. Most free-swimming larvae hatch from the egg capsules at about Gosner stage 22. Echeverria (1998) reported that larvae of *M. stelzneri* hatch about 50 hs post fertilization at Gosner stage 21. The larvae of *M. alipioi* hatch from the eggs' capsules at Gosner stage 29-30 (MZUSP 132961), that is, at comparatively advanced stages of development. This is in agreement with the concept that late hatching occurs in species in which

oviposition takes place out of standard lentic and lotic water environments (Alcala, 1962).

The lack of bright color in the specimens at the end of metamorphosis of *M. alipioi* is consistent with our observations on the color of recently metamorphosed individuals of *M. montevidensis* and *M. orejasmirandai*. The lack of aposematic color in this stage is interesting; it is possible that the uniform dark color may have a selective advantage, i.e., environmental camouflage for the recently metamorphosed individuals, whereas the characteristic aposematic colors and the associated "unken reflex" that characterize this genus would appear later, likely once the individuals have reach a minimal critical size. The SVL of recently metamorphosed *M. alipioi* is similar to that reported for other species, e.g. *M. moreirae* (SVL = 6.5-7.9 mm, Bokermann, 1967), *M. orejasmirandai* (SVL = 6.5-7.5 mm, Prigioni and Langone, 1990), and *M. stelzneri* (SVL = 6.0 mm, Echeverria, 1998).

We observed that the locality inhabited by *M. alipioi* lacks standing ponds or running water; furthermore, the terrain has a pronounced angle that would make it difficult for water to accumulate. In addition, we did not found water accumulating in other plants besides large bromeliads. Consequently, the water accumulated in bromeliads seems to be the only source of water for egg deposition and larval development available to *M. alipioi*. No other anuran species was observed in this habitat. The lack of a phylogenetic framework for this genus precludes us to determine the new species closest relatives; however, the unique mode of reproduction has probably evolved in this species as a result of its altitudinal isolation.

Based in Haffer (1979) and Simpson (1979), Guix *et al.* (1998) suggested that the current restricted distribution of *M. moreirae* to high elevations in southeastern Brazil could be the result of environmental changes during the Pleistocene (ca 1.8 mya). Pleistocene glacial periods resulted in a reduction of wet forested areas and an expansion of savanna-like formations; among the later are the high elevation grasslands ("campos de altitude") that presently occur in mountains of SE Brazil above 1800 m (Behling *et al.*, 2001). Pleistocene glacial and interglacial periods impacted the extent and distribution of open and semi-open habitats inhabited by the genus *Melanophryniscus* and potentially affected gene flow and genetic differentiation of these species and other grassland specialists anurans. Overall, the last glacial period in SE Brazil occurred between 30,000 and 18,000 years before present (BP) and, during that period, grassland

vegetation was extensive and dominated the landscape into the early and possible mid Holocene (Behling, 1997; Behling *et al.*, 2001). These extensive grasslands may have begun to retreat about 17,000 years BP when wet conditions, similar to present climate, allowed the expansion of *Araucaria*, cloud, and rainforests (Behling *et al.*, 2002). The extant high altitude grasslands, and its anuran inhabitants, may have become isolated since about 5,500 years BP and definitively in the last 1,000-1,500 years BP (Behling, 1995, Behling *et al.*, 2002).

Previous studies placed *Melanophryniscus* as a basal bufonid (Graybeal, 1997; Frost *et al.*, 2006). A recent molecular study supports the basal position of this genus and suggests that the origin and early diversification of Bufonidae occurred during the Upper Cretaceous (Pramuk *et al.*, 2008). Overall, *Melanophryniscus* are savanna-like specialists and assuming that the genus evolved in association with similar lowland and open habitats, then changes of sea level that resulted in marine transgressions and regressions in the southern portion of South America during the Cenozoic (Lundberg *et al.*, 1998; Ortiz-Jaureguizar and Cladera, 2006) are likely to have contributed to the genus diversification throughout the arid, grasslands, and rocky areas that it occupies. However, regarding species inhabiting SE Brazil, and specifically for the species currently inhabiting high altitude grasslands, we need to also consider Pleistocene climatic changes as previously suggested (Guix *et al.* 1998). It is likely that species of *Melanophryniscus* inhabiting SE Brazil would have had more extensive distributions during pleistocenic glacial periods as the grassland expanded during those dryer and cooler periods. These more extensive distributions among *Melanophryniscus* populations would have been interrupted, to a greater or lesser extent, during the interglacial periods and could have impacted on any population differentiation that may have started by isolation during the glacial periods. However, repeated glacial and interglacial periods throughout the Pleistocene could have resulted in “in-situ” speciation of isolated populations in open, savanna-like habitats and explain the restricted distribution and endemisms we found today among species of *Melanophryniscus* inhabiting the Atlantic coastal chain of mountains in southern Brazil. This is particularly the case for species like *M. moreirae* and *M. alipioi* that inhabit high elevation grasslands in SE Brazil. At one end of the geological time, *Melanophryniscus* species inhabiting “campos de altitude” could have begun to differentiate as long ago as 1.8 mya and through-

out the Pleistocene or, at the other end of geological time, could be the result of the most recent disruption of continuous populations occupying more extensive grasslands that took place after the last glacial period and in the last 17,000-5,000 years. It is more likely that speciation and current diversity of *Melanophryniscus* in SE Brazil is the result of a mixture of older and younger events. Under this scenario, the unique reproductive mode of *M. alipioi* could have evolved in the last 1.8 mya to 5,000 years ago. If the reproductive mode is an earlier evolutionary event, it may be ancestral to a clade of closely related species of *Melanophryniscus* that today are restricted to high elevation grasslands, “campos de altitude”, throughout the mountain ranges of SE Brazil. Alternatively, phytotelm breeding in *Melanophryniscus* could have evolved later and independently in *M. alipioi*, and maybe in other yet unknown species, as a result of their isolation in high elevation grasslands during the last 17,000 years. These scenarios suggest the need of further field work in high altitude grasslands to fully assess the extent of their unique diversity.

RESUMO

Uma nova espécie de bufonídeo do gênero *Melanophryniscus* é descrita de um morro que faz parte do Serra do Mar no nordeste do Estado do Paraná, Brasil. *Melanophryniscus alipioi* sp. nov. é distinto de todas as outras espécies conhecidas no gênero por sua coloração dorsal uniformemente marrom escuro e pelo local de procriação sem igual. A espécie nova se reproduz, vocaliza e deposita os ovos em bromélias, uma estratégia até então desconhecida para o gênero. Esta espécie poderia estar ameaçada por destruição do seu hábitat.

ACKNOWLEDGMENTS

We are grateful to Osmar dos Santos Ribas for identification of the bromeliads species. This study was partially supported through NSF Award #0342918 to RdS and WRH.

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Submitted 24 October 2007

Accepted 16 January 2008

APPENDIX I

Additional specimens examined deposited in Museo Nacional de Historia Natural y Antropología (MNHN), Montevideo, Uruguay.

Melanophryniscus atroluteus – Uruguay: *Artigas* (MNHN 4444). Arroyo Tres Cruces Artigas (MNHN 1484). Barra del Ayo Yacuí (MNHN 1483). *Salto*: El Espinillar. ANCAP (MNHN 399; 400; 609; 5011; 5012). Brasil: *Rio Grande do Sul*: Rio Santa Maria-São Gabriel (MNHN 1283).

Melanophryniscus dorsalis – Brazil: *Rio Grande do Sul*: Municipality of Torres (MNHN 2225; 5181). *Santa Catarina*: Praia do Sol. Municipality of Laguna (MNHN 5398-5402).

Melanophryniscus fulvoguttatus – Paraguay: *Concepción*: Estancia San Luis de la Sierra. Apa-Bergland (MNHN 6188). *Guairá*: Near Coronel Martínez (MNHN 4086. 4087).

Melanophryniscus montevidensis – Uruguay: *Montevideo*: Carrasco (MNHN 393; 403; 405; 406; 4892-4896). Pajas Blancas (MNHN 394). *Canelones*: Atlántida (MNHN 598). Near Aeropuerto Internacional de Carrasco (MNHN 407; 408; 1155-1157). *Maldonado*: Barra del Arroyo Maldonado (MNHN 613).

Melanophryniscus moreirae – Brazil: *Rio de Janeiro*: Itatiaia (MNHN 829; 794; 1211; 5196-5199).

