

# Natural history, coloration pattern and conservation status of the threatened South Brazilian red bellied toad, *Melanophryniscus macrogranulosus* Braun, 1973 (Anura, Bufonidae)

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**Abstract.** Efforts to find and gather data on natural history, including geographic records, of species considered threatened are an important tool to assess and update its conservation status. Little is known about the threatened South American red belly toad, *Melanophryniscus macrogranulosus*, endemic from northeastern Rio Grande do Sul, southern Brazil. The main goal of this article is to provide information on natural history, geographic distribution, morphology and conservation of this toad, including new geographic records, and new data on color pattern, habitat use, and reproductive and defensive behavior. We conducted 30 field expeditions from 2005 to 2013 to the type locality and surroundings and examined the three major herpetological collections from Rio Grande do Sul. We described the live color pattern of juveniles and adults. The data on reproductive and defensive behavior was obtained in the field. We rediscovered the species on its type locality, after 45 years from previous records, and revealed the presence of *M. macrogranulosus* in five new localities. The color pattern varies ontogenetically from metamorphosed juveniles to adults. Newly metamorphosed individuals have dark gray dorsum and pale, partially translucent ventral surface. Adults have dorsum from light to dark green and ventral surface exhibiting a green or grayish blue coloration pattern with white spots and red patches. Associated with this suspected aposematic coloration we observed individuals employing *unken* reflex when disturbed. Reproductive activity was recorded after heavy rains in all four seasons. Males call during day and night, in small, shallow pools along temporary streams with clear water. Amplexus and struggles between males also involving a female were registered inside the water in the reproductive site. All records of *M. macrogranulosus* are inside the limits of the Atlantic forest, considered one of the most endangered Biome of Brazil. However, none is inside the limits of a protected area. Some possible threats observed include fragmentation and habitat destruction, pollution (improper discard of human waste) and the recent duplication of a paved road near the reproductive site. These impacts associated with the small extent of occurrence, justify the species category as Endangered (EN).

**Key words.** Amphibian, Atlantic forest, geographic distribution, color pattern, habitat use, reproductive behavior, defensive behavior.

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## Introduction

Efforts to locate new geographic records and gather data on the natural history of threatened species are important to assess and update the conservation status of these species (IUCN, 2012; IUCN Standards and Petitions Subcommittee 2013) and are one of the most important recommendations often found in red books of threatened species or other related documents (e.g. Fontana *et al.*, 2003; Machado *et al.*, 2008; IUCN, 2012). These data provide the framework to develop conservation strategies and are especially important when studying species with imprecise information about ecology, natural history, and geographical distribution (IUCN, 2012).

The Brazilian red belly toad, *Melanophryniscus macrogranulosus* Braun, 1973, is a threatened species endemic to northeastern Rio Grande do Sul, in southern Brazil. The species description was based on nine specimens collected in 1960 at locality reported only as “Torres, RS, Brasil” (municipality of Torres). From 1960–2003, no additional studies or records reported.

The precise location of the type locality was revealed by the first time after personal communication from the collector of the type series, Thales de Lema, a Brazilian herpetologist (Garcia and Vinciprova, 2003; Escobar *et al.*, 2004). Only Escobar *et al.* (2004) provided a geographic coordinate and, unfortunately, it was misled, ending in the middle of the Atlantic Ocean. Despite the obviously misplaced coordinates, the new information allows to assume that the referred locality is a single cave located in Dom Pedro de Alcântara, along the Federal Highway BR 101.

Considering the microendemism and the habitat destruction nearby the type locality, the species was considered regionally threatened with Vulnerable status in the State of Rio Grande do Sul (Marques *et al.*, 2002; Fontana *et al.*, 2003). Given the absence of recent records of the species, the regional assessors raised the possibility that *Melanophryniscus macrogranulosus* could be extinct (Garcia and Vinciprova, 2003). Using the same available information, in 2003 *M. macrogranulosus* was categorized as Critically Endangered at national level in Brazil (Fundação Biodiversitas, 2003; Ministério do Meio Ambiente, 2003).

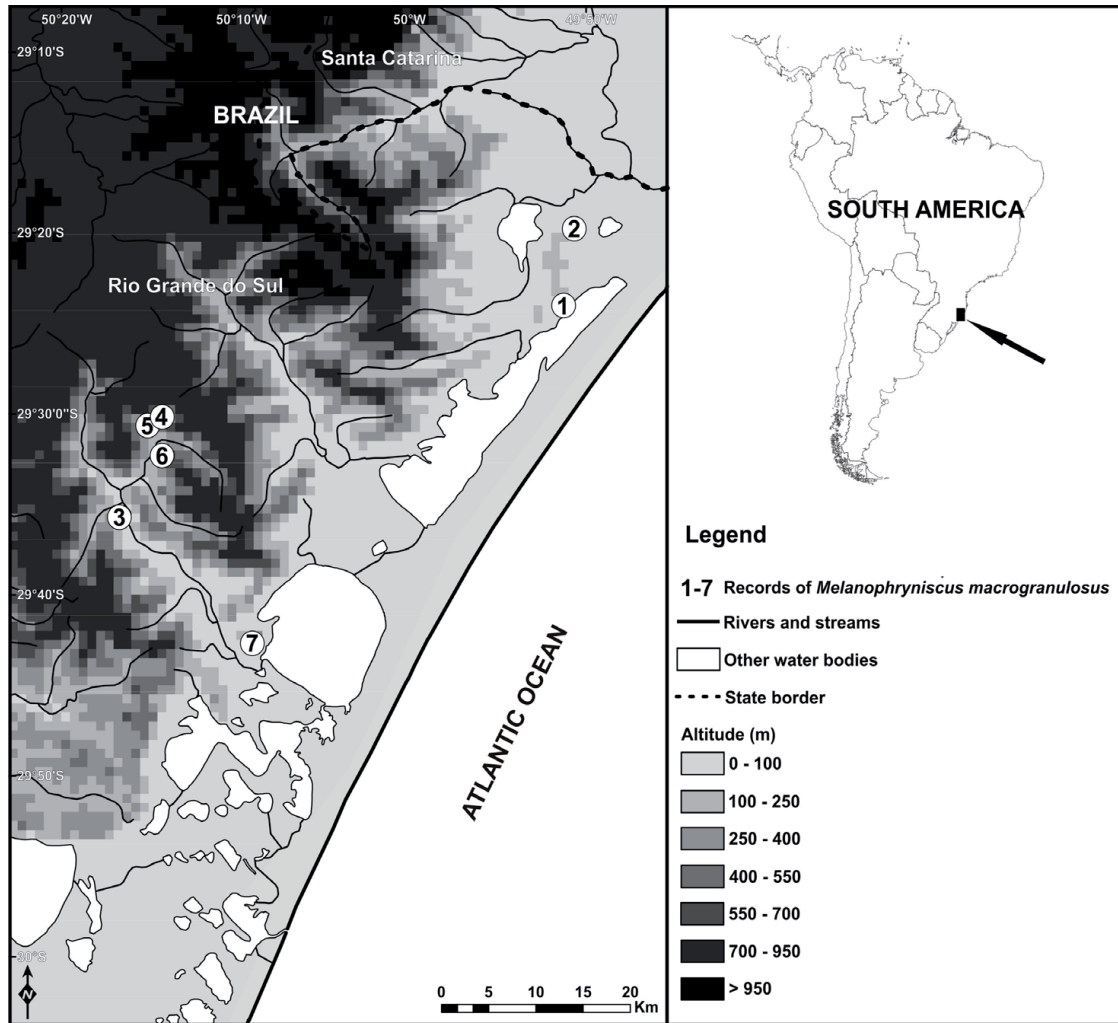
For 44 years the species was known only from its type locality until a second locality was recorded at Barra do Ouro district, municipality of Maquiné, Rio Grande do Sul, located ca. 40 km toward south of the type locality (Escobar *et al.*, 2004) (Fig. 1). The new register was only reported as “Barra do Ouro”, without

precise geographic coordinates. Besides a photograph of a live specimen and description of pattern coloration of the single individual collected (a juvenile), no other information about this toad was provided.

In 2008, the Red Book of Brazilian Threatened Fauna was published (Machado *et al.*, 2008) following the official red list published earlier (Fundação Biodiversitas 2003; Ministério do Meio Ambiente, 2003). In the Red Book the record of Escobar *et al.*, (2004) was considered, but no new information about *Melanophryniscus macrogranulosus* was added (Garcia, 2008). Compiling all these work mentioned above, the IUCN (International Union for Conservation of Nature and Natural Resources) red list categorized the species as Vulnerable (Silvano and Garcia, 2010). Recently, Poli *et al.* (2012) reported to have encountered the species again in Barra do Ouro, on September 2010. No voucher specimen was collected and geographic coordinates were not provided. However, Poli *et al.* (2012) reported information about habitat, climate, color in life and abundance of *M. macrogranulosus* at this locality. The most recent and in-depth assessment of the conservation status of all Brazilian species of amphibians - conducted under the auspices of Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) by the 49 Brazilian amphibian specialists from 32 institutions in four workshops from 2010–2012 - classified *M. macrogranulosus* as Endangered. The results of this workshop are not yet published. The latest regional assessment of conservation status was published recently in Rio Grande do Sul. The species was again categorized as Endangered (Decreto Estadual Nº 51.797 de 8 de Setembro de 2014).

More recently, Baldo *et al.* (2014) in a comprehensive review of larval morphology of *Melanophryniscus* described the tadpole external morphology, the buccal cavity and musculoskeletal anatomy of *M. macrogranulosus*, based on tadpoles from the type locality. A study focusing in climate changes and distribution of genus *Melanophryniscus* brought new information about the species and suggested some conservation actions (Zank *et al.*, 2014). No other information about the species is known.

The new assessment of the conservation status of *Melanophryniscus macrogranulosus* was based mostly on unpublished data. Therefore, the main goal of this article is to report these new data, including geographic records, coloration, habitat, reproductive and defensive behavior, as well as the main threats to the species.



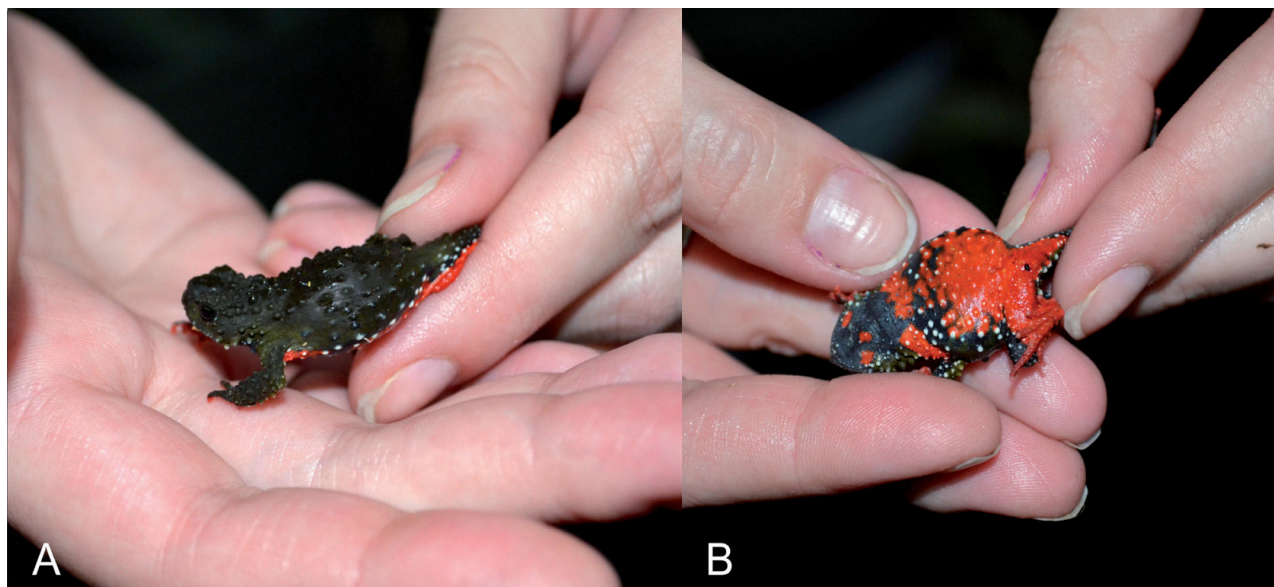
**Figure 1.** Collection localities of *Melanophryniscus macrogranulosus* in the State of Rio Grande do Sul, Brazil. All records are numbered 1–7: (1) Morro da Gruta, (type locality), (2) Mato dos Macacos, (next to Morro do Forno lagoon), municipality of Dom Pedro de Alcântara, (3) Barra do Ouro (Escobar, Maneyro and Di-Bernardo, 2004), (4) acima cascata do Garapiá (upper Garapiá waterfall, Barra do Ouro), (5) abaixo cascata do Garapiá, (lower Garapiá waterfall, Barra do Ouro), (6) Vale do Arroio Carvão (Barra do Ouro), (7) Morro do Cantagalo, municipailty of Maquiné. The arrow in inset map (right) indicates the frame on left on South America. All data, including vouchers numbers, geographic coordinates, collection date and additional data about each individual are detailed in Table 1 and referenced in the text.

**Materials and Methods**

To obtain additional information on the geographic distribution and natural history of *Melanophryniscus macrogranulosus*, from 2005–2013, we conducted 30 field expeditions, 17 to the type locality and 13 to new localities in the surrounding area *M. macrogranulosus* was likely to occur. Most observations and searches were made immediately following heavy rains, which is when species of *Melanophryniscus* tend to converge on temporary streams to breed (Garcia and Vinciprova, 2003; Achaval and Olmos, 2007), but we also searched for individuals away from water, under rocks, logs and other materials in the forest. Additionally, we examined

three of the most representative herpetological collections from southern Brazil to search for historical records of *M. macrogranulosus* as follows, *Coleção Herpetológica do Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul* (MCP), *Coleção Herpetológica do Departamento de Zoologia da Universidade Federal do Rio Grande do Sul* (UFRGS) and *Coleção de Anfíbios do Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul* (MCN).

For observed specimens, we recorded the coloration of juveniles and adults in life. We classified individuals in two categories based on snout–vent length (SVL) and



**Figure 2.** *Melanophryniscus macrogranulosus* from Morro do Cantagalo, municipality of Maquiné. Where: A, dorsal and B, ventral view (no voucher specimen, photographs: Brack, I.V.).

secondary sex characters. We define those groups based on SVL using the limit of minimum size of the less reproductive individual found during a breeding event, those with equal or bigger size were consider adult. Individuals < 27.7 mm SVL and lacking nuptial pads, vocal sac or eggs = juveniles; individuals > 27.7 mm SVL and/or presenting nuptial pads, vocal sac or eggs = adults. The data on reproductive activity, defensive behavior and vocalization site was obtained observing individuals from the type locality (locality 1) and Garapiá (locality 4 and 5; Fig. 1).

Several evidences were compiled, in the past 10 years, of habitat loss and degradation were this toad is found. We identified expansion of cities and road networks, conversion of forest into crops and pollution of the reproductive sites by human wastes and trash. To assess the conservation status of this toad we followed the IUCN criteria (IUCN, 2013). Extent of occurrence was calculated using Geocat® software. The collected material obtained during field expeditions was deposited on two herpetological collections (UFRGS and MCP).

## Results

### *Environmental data*

Our field expeditions and revision of herpetological collections rediscovered the species on its type locality after 45 years and revealed the presence of *Melanophryniscus macrogranulosus* in five new

localities. All localities were compiled, updated and georeferenced (Table 1 and Fig. 1). We found *M. macrogranulosus* in 2005 in the type locality, Morro da Gruta, (locality 1 in Fig. 1). The type locality is a small cave in a hill isolated by a paved Federal Highway (BR-101) surrounded by secondary forest, banana crop, small livestock areas and other disturbed open areas (Fig. 1 and 7) near the margins of the Itapeva lagoon. We discovered two reproductive new sites in Barra do Ouro (locality 4 and 5 in Fig. 1) with precise coordinates (Table 1). The new record Morro do Cantagalo (locality 7), Maquiné, is based on a photograph taken by Ismael Verrastro Brack, a biologist, on October 2012 (Fig. 2). This locality is on a hill isolated by a dirt road and the specimen was found walking on the ground in a place surrounded by secondary forest and banana crop (Brack, I.V., per. comm.).

### *Morphological variation*

We analyzed coloration of juveniles (n=6) and adult (n males=33; n female=9) individuals. Coloration varies ontogenetically. Newly metamorphosed individuals have a dark gray dorsum and pale, partially translucent ventral surface, lacking pigmentation; palmar and plantar surfaces pale (Fig. 3, A and B). Smaller juveniles had almost the same pattern of newly metamorphosed, except for palmar and plantar surfaces and the posterior region of venter that shows a pallid orange color (Fig. 3, C-F). Larger juveniles presented a more intense

**Table 1.** Records of *Melanophryniscus macrogranulosus* (all in Rio Grande do Sul, Brazil) numbered as in Fig. 1. Asterisks indicate new records. Vouchers are housed in Coleção de Anfíbios do Museu de Ciências Naturais da Fundação Zoobotânica do Rio Grande do Sul (MCN), Coleção Herpetológica do Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP) and Coleção Herpetológica, Departamento de Zoologia da Universidade Federal do Rio Grande do Sul (UFRGS).

Municipality	Locality	Voucher acronym/number	Coordinates/Altitude	Date	Complementary data
		MCN 01693			Female
		MCN 01694			Female
		MCN 01695			Male
		MCN 01696		30 Oct 1960	Male
		MCN 01697		<i>Braun, 1973</i>	Male
		MCN 01698		<i>(Type series)</i>	Male
		MCN 01699			Male
		MCN 01701			Male
		MCN 01702			Male
		UFRGS 2502		11 Aug 2005	Male in a temporary stream
		UFRGS 2978		20 Apr 2008	Juvenile
		MCP 11141		11 Aug 2009	Female
		MCP 11139		Set 2009	Male
		MCP 10319		13 Sep 2009	Male
Dom Pedro de Alcântara	1 - Morro da Gruta (Type locality)	MCP 10320	29°24'21.16" S, 49°51'1.48" W/		Male
		MCP 11140	31m	23 Sep 2009	Male
		UFRGS 4702		30 Oct 2010	Male walking in a trail inside the forest
		MCP 11576		14 Jan 2011	Female
		MCP 11577			Male
		MCP 11578			Male
		MCP 11936		9 Aug 2011	Male in calling activity
		MCP 11937			Male in calling activity
		MCP 11938		10 Aug 2011	Juvenile, hidden under a rock
		UFRGS 6448		21 Mar 2013	Male in a reproductive event
		UFRGS 6449			Male in a reproductive event
		UFRGS 6450			Male in a reproductive event
		UFRGS 6451			Male in a reproductive event
		UFRGS 6452			Male in a reproductive event
		UFRGS 6453			Male in a reproductive event
		UFRGS 6454			Male in a reproductive event
Dom Pedro de Alcântara	2 - Mato dos Macacos (next to Morro do Forno lagoon)*	MCP 10847	29°19' 37.6" S, 49°51'00" W/ 32m	30 Oct 2008	Juvenile
Maquiné	3 - Barra do Ouro	MCP 7943	Escobar et al., 2004	30 Apr 2004	Juvenile
		MCP 11935		03 Aug 2011	Male in calling activity
Maquiné	4 - Acima cascata do Garapiá (upper Garapiá waterfall, Barra do Ouro)*	UFRGS 6536	29°30'19.00"S, 50°14'29.03" W/ 230m	20 Jan 2013	Male crossing a dirt road
		UFRGS 6424		22 Feb 2013	Male walking in the reproductive stream
		UFRGS 6425			Female hidden under a rock
		UFRGS 2476		13 Nov 2004	Female
		MCP 8104			Male in amplexant pair with MCP 8105
Maquiné	5 - Abaixo cascata do Garapiá (lower Garapiá waterfall, Barra do Ouro)*	MCP 8105	29°30'33" S, 50°14'45" W/ 200m		Female in amplexant pair with MCP 8104
		UFRGS 2830		6 Jan 2008	Male, in a temporary stream
		UFRGS 2831		5 Jan 2008	Male in amplexant pair with UFRGS 2832
		UFRGS 2832			Female in amplexant pair with UFRGS 2831

Table 1. Continued.

Municipality	Locality	Voucher acronym/number	Coordinates/Altitude	Date	Complementary data
Maquiné	6 – Vale do arroio Carvão (Barra do Ouro)*	UFRGS 2080	29°32'29.95" S, 50°13'40.56" W/	14 Mar 2001	Male
		UFRGS 5762	370m	29 Dec 2001	Female
Maquiné	7 - Morro do Cantagalo*	Photograph record, (Figure 3)	29°42'44" S, 50°09'00" W/	13 Oct 2012	No sexed individual walking in a trail
Maquiné	No specified	MCP 9147		16 Oct 2003	Juvenile
	Barra do Ouro	UFRGS 6518		11 Jan 2012	Juveniles
	Barra do Ouro	UFRGS 6519			Male
	Barra do Ouro	UFRGS 6520			Male

coloration than smaller; dorsal surface of body and limbs dark green and ventral surface dark blue with white spots and some orange-red patches on the axils and belly (Fig. 3, G-H).

Adults have variable dorsal and ventral coloration pattern (Fig. 4). The dorsal color varies from light to dark green, some individuals are deep dark green, almost black. Ventral surface exhibits a green or grayish blue coloration pattern with red patches. A patch always present on abdominal region and axils and often in gular and pectoral region. Presence of small white spots covering all ventral surface, including limbs, throat, pectoral and abdominal regions. Palmar and plantar surfaces present red orange coloration.

Associated with this suspected aposematic coloration, individuals of *Melanophryniscus macrogranulosus* exhibited the *unken* reflex when disturbed (Fig. 5 A). Additionally, an important characteristic observed is that frontal swelling in non-adult individuals is absent or inconspicuous (Fig. 3, A, C, E and G), while is markedly present in adults (Fig. 3 D). Supplementary information of specimens collected and observed in the field are detailed in Table 1.

#### Reproductive activity

We observed reproductive activity of *Melanophryniscus macrogranulosus* after heavy rains in January and August 2011 and March 2013 at Morro da Gruta and upper Garapiá waterfall (localities 1 and 4), in June 2013 at upper Garapiá waterfall and lower Garapiá waterfall (localities 4 and 5), and an amplexant pair at lower Garapiá waterfall (locality 5) in November 2004. There were at least 20 individuals involved in the reproductive event in January of 2011 at the type locality. We observed males calling at Morro da Gruta,

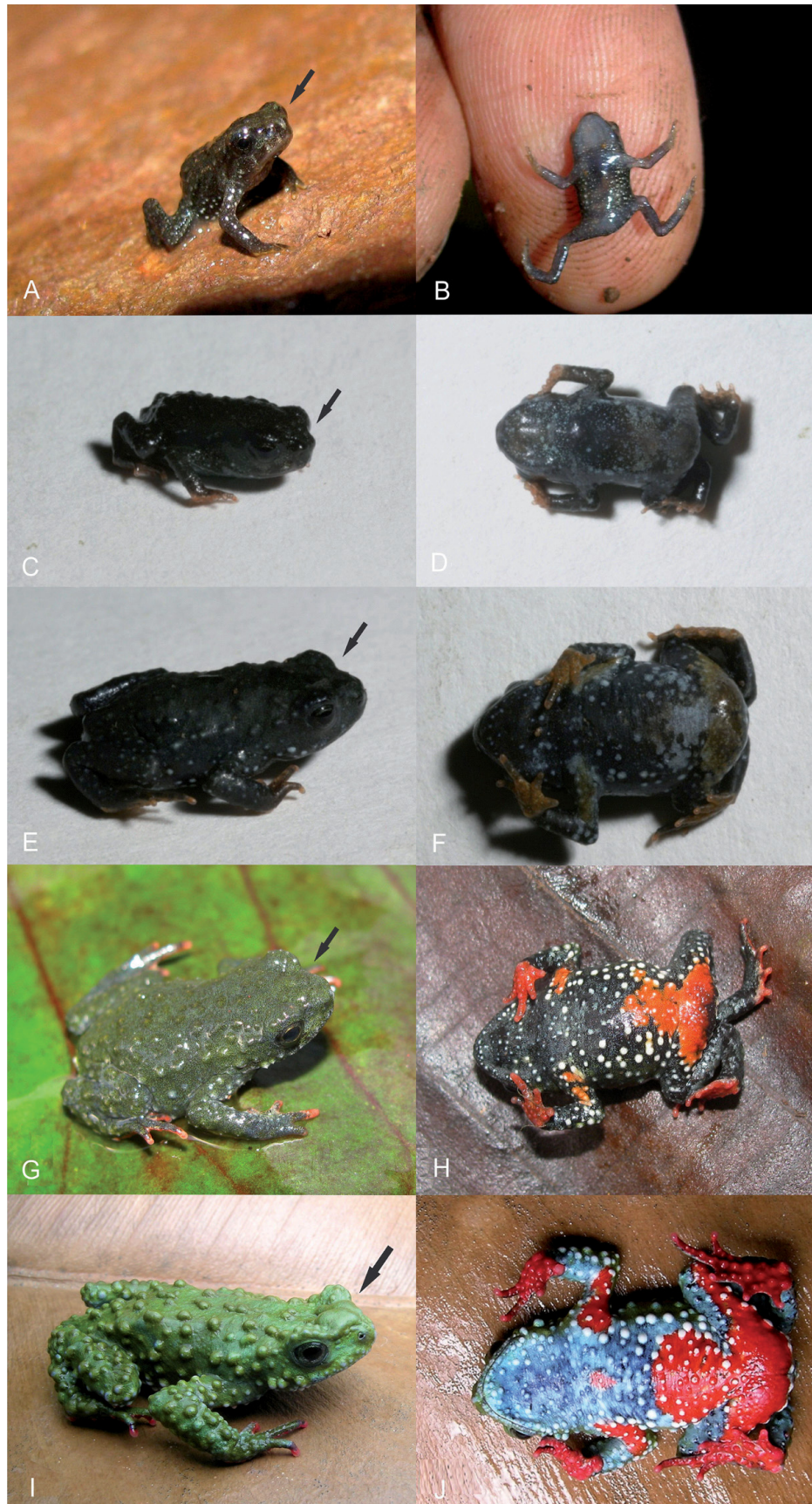
upper Garapiá waterfall and lower Garapiá waterfall (localities 1, 4 and 5) both diurnally and nocturnally in small, shallow pools along temporary, clear water streams (Fig. 5B), as well as some males calling hidden in small holes in the ground (formed by roots of trees) at type locality. We recorded amplexus (Fig. 5C) and struggles between males, sometimes including a female (Fig. 5D), inside the water at Morro da Gruta and lower Garapiá waterfall (localities 1 and 5). Spawnings were observed in shallow running water adhered to vegetation, rocks or on the ground.

#### Conservation

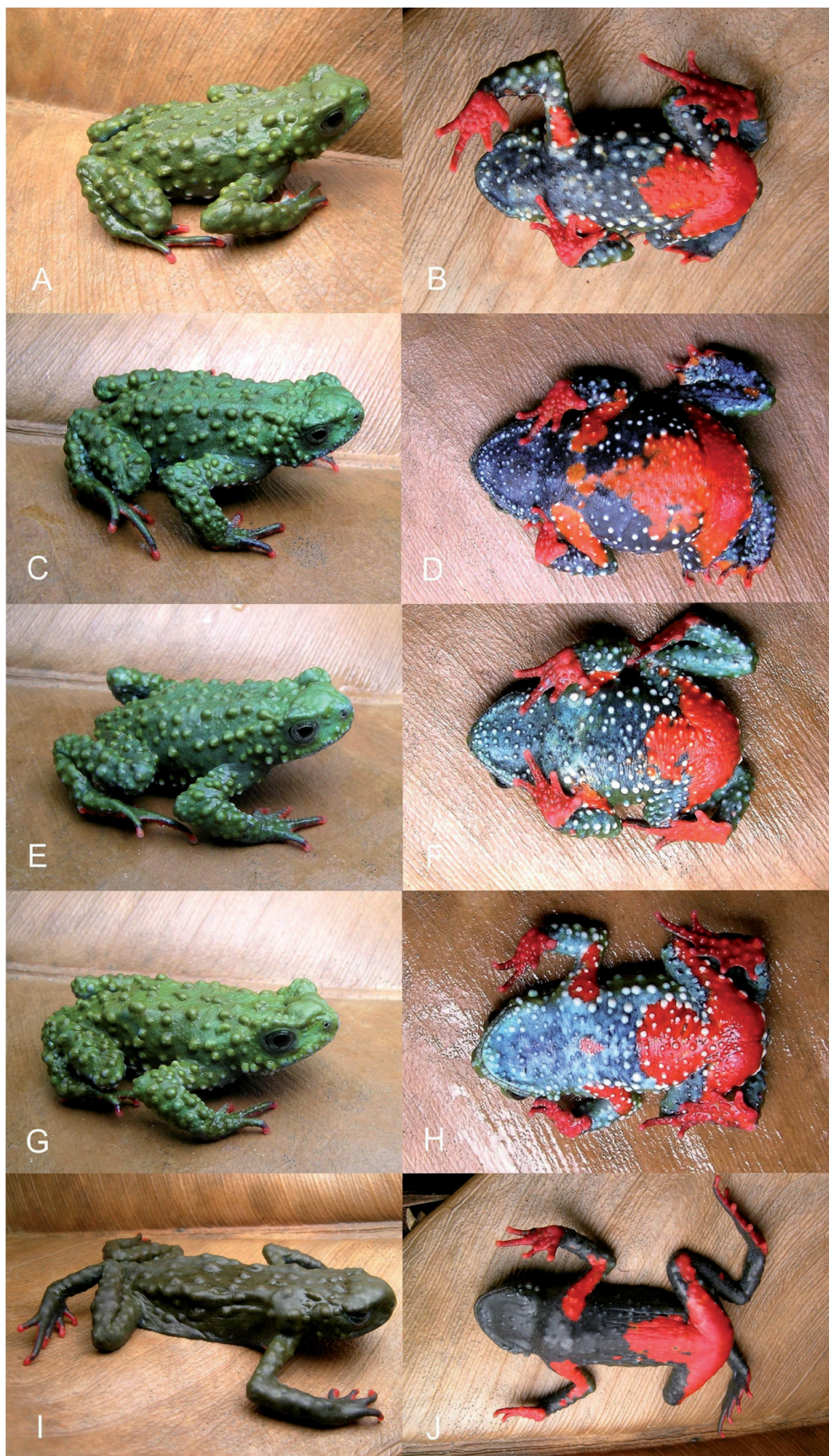
After several expeditions to the region, *Melanophryniscus macrogranulosus* has never been found in non-forested environments. The forested environments, particularly those where *M. macrogranulosus* occurs are reduced to few, small and discontinuous remnants mainly due the constant deforestation of areas for planting crops (e.g. banana, corn, bean) or for livestock (Fig. 6 and 7A). At least three localities (1, 4 and 5) are intensively visited by humans and, very often, we find trash and human waste at the site (Fig. 7B-E). Additionally, other possible impacts are found mainly at the type locality, as the recent duplication of the Federal Highway BR-101 that might have isolated the type population (Fig. 7) and increased noise pollution (produced by cars and trucks).

#### Discussion

Based on Escobar *et al.* (2004) we rediscovered *Melanophryniscus macrogranulosus* from the type locality in 2005 and discovered two reproductive sites in Barra do Ouro with precise coordinates. We believe that new records of *M. macrogranulosus* may be due



**Figure 3.** Dorsal and ventral view of five *Melanophryniscus macrogranulosus* specimens from the type locality, Morro da Gruta, Dom Pedro de Alcântara, RS, Brazil. Where: A-B newly metamorphosed toad (no voucher specimen); C-F juveniles (no voucher specimens), G-H juvenile (MCP 11938, 18,0mm SVL); I-J adult individual (MCP 11936, 31,2mm SVL). The black arrow indicates frontal swelling in different stages (Photographs C-F: Taran Grant; A, B, G, H: Valentina Caorsi; I-J: Patrick Colombo).



**Figure 4.** Dorsolateral (left) and ventral (right) pattern coloration of six adults of *Melanophryniscus macrogranulosus* from the type locality, Morro da Gruta, Dom Pedro de Alcântara, RS, Brazil. Where: A-B (UFRGS 4702), C-D (MCP 10319), E-F (MCP 10320), G-H (MCP11139), I-J (MCP 11141) – photographs of the specimen were taken immediately after the individual's death. (Photographs: Patrick Colombo).





**Figure 5.** Behavior and activity of *Melanophryniscus macrogranulosus* at reproductive site in the type locality, Morro da Gruta, Dom Pedro de Alcântara, RS, Brazil. Where: A, individual exhibiting presumable defensive behavior, *unken* reflex (UFRGS 2502); B, male in calling site between interval of calling activity; C, axillary amplexus; D, struggle between males including a female (no voucher specimens). (Photographs, A: Patrick Colombo; B-D: Valentina Caorsi).

the raise of efforts dispended in the past eight years by herpetologists working with the genus in Rio Grande do Sul. This is in part a result of the recent concern of Brazilian government about the conservation status of this endemic group of toads.

Additionally, *Melanophryniscus* species are often cited as cryptic species recorded mostly after heavy rains, mainly in the warm season (e.g. Achaval and Olmos, 1997; Langone, 1994). Some recent studies reported species reproducing after heavy rains but not seasonal (Santos et al., 2010). Most collection effort directed to found *M. macrogranulosus* were conducted after heavy rains, but, surprisingly we also found at least one individual in field trips during dry and non-reproductive periods. Previously assessments of the conservation status of this species considered only historical records and no effort to find the type population individuals or new information were done. Our results indicate the importance of systematic searching for species at any period even if they are considered rare.

As seen in two other species of the genus (Langone et al., 2008; Caorsi et al., 2012), newly metamorphosed *Melanophryniscus macrogranulosus* do not present very bright coloration. Pattern color in adults is variable enough to differentiate one individual from another, as seen in other species of the genus (Cairo and Di Tada, 2005; Caorsi, et al., 2012). The bright coloration and frontal swelling might be associated with the presence of toxic skin alkaloids sequestered from diet as chemical defenses against predation, pathogens, and parasites (Saporito et al., 2009; Saporito et al., 2012; Hantak et al., 2013). Such substances have been found in other species of the genus (e. g. Grant et al., 2012; Garraffo et al., 2013) including *M. macrogranulosus* (P. Colombo, unpub. data). The hypothesis is that younger individuals uptake these alkaloids gradually over lifetime (Saporito et al., 2009), developing concomitantly bright coloration and/or poison glands in Dendrobatidae (Saporito et al., 2010) and frontal gland in some *Melanophryniscus*.

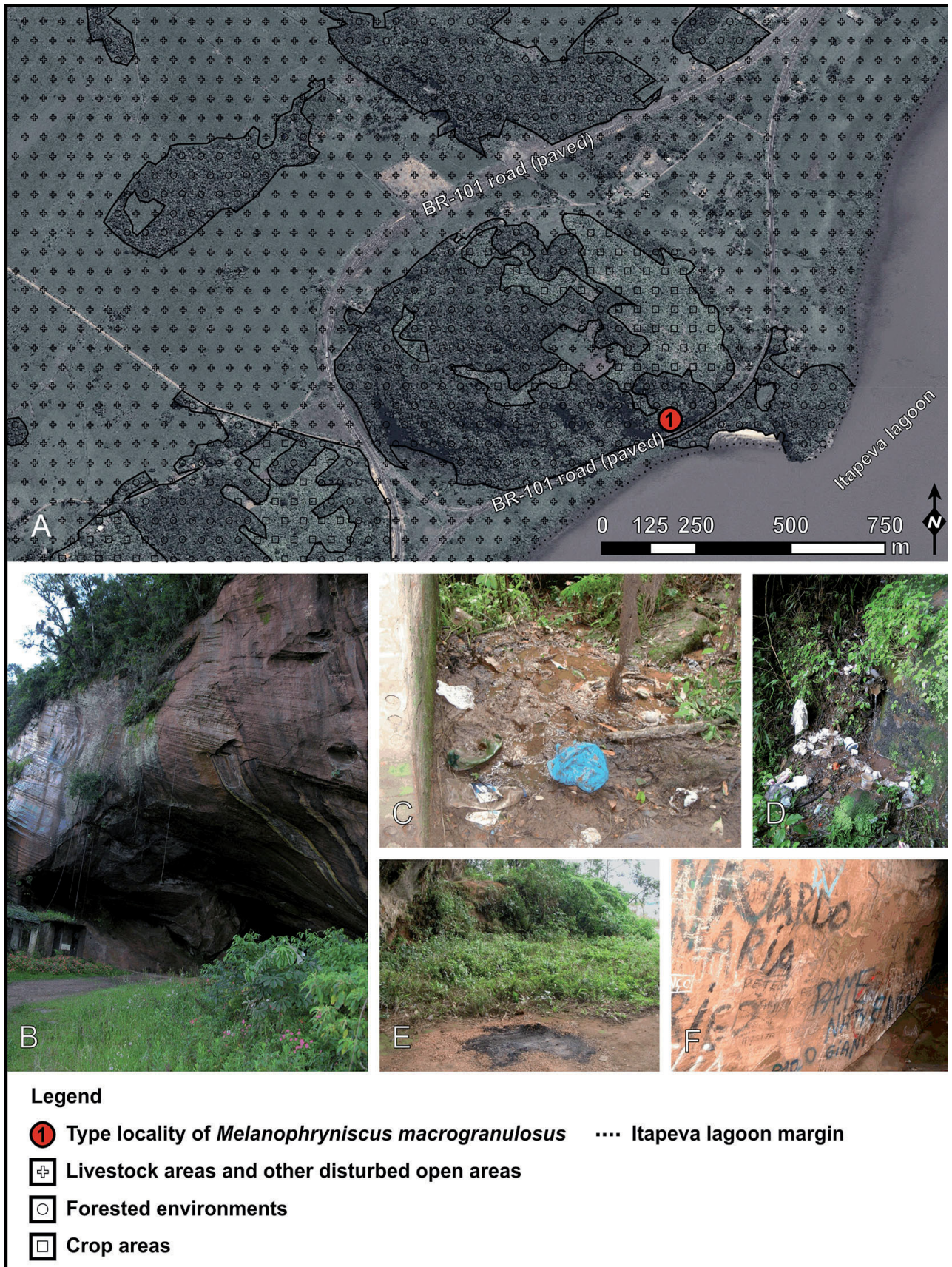
The presumed defensive behavior *unken* reflex



**Figure 6.** Disturbance observed along a decade in one reproductive site of *Melanophryniscus macrogranulosus* at Barra do Ouro, municipality of Maquiné, RS, Brazil: *Acima cascata do Garapiá* (upper Garapiá waterfall,). Where: A - B in 2001 and C - D in 2011. (Photographs: A-B: Cristiano Agra; C-D: Valentina Caorsi).

observed in *Melanophryniscus macrogranulosus* has also been reported to other species of the genus as *M. atroluteus* or *M. montevidensis* (Kwet and Miranda, 2001; Baldo and Basso 2004; Maneyro and Kwet, 2008), *M. cambaraensis* (Santos and Grant, 2011; Toledo et al., 2011), *M. cupreuscapularis* (Manzano et al., 2004), *M. devincenzii* (Maneyro and Carreira, 2012), *M. dorsalis* (Kwet et al., 2005), *M. krauczuki* (Baldo and Basso, 2004; Brusquetti et al., 2007), *M. montevidensis* (Langone, 1994; Kwet et al., 2005), *M. moreirae* (Almeida-Santos et al., 2010; Toledo et al., 2011), *M. pachyrhynchus* (Kolenc et al., 2003; Lema and Martins, 2011; Toledo et al., 2011; Maneyro & Carreira, 2012), *M. rubriventris* (Laurent, 1973), *M. simplex* (Grant et al., 2012) and *M. stelzneri* (Fernández, 1927; Herrman, 1988). This behavior associated with bright coloration also may be related to presence of toxic alkaloids as mentioned above.

The species seems to be an explosive breeder, as seen in other *Melanophryniscus* species (e.g. Baldo and Basso 2004; Achaval and Olmos, 2007; Santos et al., 2010; Cairo et al., 2013). Several evidences led to this conclusion, as reproductive events after heavy rains (i.e. in march 2013) with most individuals reproducing in temporary streams. Poli et al. (2012) registered 10 individuals in calling activity, probably in a reproductive event. We observed explosive-breeding events in different seasons and localities and in all we register fewer individuals than observed or reported in other species of the genus, *M. dorsalis* and *M. simplex* (Colombo, P. pers. obs.), *M. rubriventris* (Vaira, 2005). Another evidence of explosive breeding is the diurnal and nocturnal calling activity register in three different localities. Santos and Grant (2011) hypothesized that the diurnal and nocturnal breeding activity in *M. cambaraensis* might be due to the extremely short



**Figure 7.** Type locality of *Melanophryniscus macrogranulosus* and its main impacts with major categories of land use at vicinities. Where: A, satellite image from type locality, Morro da Gruta, extracted from Google Earth Pro©; B, general view of the entrance of the cave; C-D, trash deposited in the reproductive site, located in the left side of the cave; E-F, recently anthropogenic activity in the cave. (Photographs, B: Diego Baldo; C: Patrick Colombo; D-F: Ivan Borel).

duration of explosive reproductive events. Like other species of *Melanophryniscus* (Baldo and Basso, 2004; Goldberg *et al.*, 2006, Colombo *et al.*, 2007; Santos and Grant, 2011; Santos *et al.*, 2011), breeding occurred in temporary streams and males called in shallow water along the streams. The strategies of scramble competition (Wells, 2007) observed in the field as struggles to possess females were also observed in *M. rubriventris* (Goldberg *et al.*, 2006), *M. cambaraensis* (Caorsi *et al.*, unpub. data) and other bufonids (Peters, 1973; Davies and Halliday, 1978; Wells, 1979).

All records of *Melanophryniscus macrogranulosus* are inside the limits of the Atlantic forest (IBGE, 2004), considered one of the most endangered and devastated environment of Brazil (Myers *et al.*, 2000). We do not know exactly the association and dependence of this toad to forest, but all record sites of *M. macrogranulosus* are close to a forest remnant. This pattern was described in other species of the genus, including *M. admirabilis*, *M. cambaraensis*, and *M. simplex* (e.g. Di-Bernardo *et al.*, 2006; Colombo *et al.*, 2007; Santos and Grant, 2010). Some amphibian species may use the forest environments as shelter and food resource and leave the forest in to breed (Becker *et al.*, 2007).

The forested environments, particularly those of northeastern Rio Grande do Sul, where *M. macrogranulosus* occurs, suffer from several threats. Those forests are reduced to few, small and discontinuous remnants and some are intensively visited by humans and accumulate trash and human waste. The recently duplication of the highway next to type locality is also a concern. Increase of noise pollution produced by cars and trucks and road-kills were described as possible impacts for *Melanophryniscus* sp. that breeds close to the road (Cairo and Zalba, 2007). All these impacts associated with the small extent of occurrence known, 661.26 km<sup>2</sup>, justify the species category as endangered (Decreto Estadual N° 51.797 de 8 de Setembro de 2014).

Several conservation strategies can be employed to preserve *Melanophryniscus macrogranulosus*. Here we pointed priority actions based on the experience of the authors. Scientific research is always an important tool for conservation. This strategy was pointed by other authors (Garcia and Vinciprova, 2003; Garcia, 2008), and involves prospection of new populations, studies on genetic structure and population dynamics of this small toad. Currently, the first author is conducting a study on taxonomy, call structure, and prospection of new populations of this species.

We did not detect the presence of *Melanophryniscus macrogranulosus* in any protected area. In Maquiné municipality, locality 4, 5, and 6 occur in the buffer zone of Reserva Biológica da Serra Geral (Fig. 1), thus one conservation action could be the enlargement of that protected area to involve at least one of the populations found. Other priority region to conserve is the vicinities of the type locality in the area called “Complexo das Lagoas do Morro do Forno e Jacaré”. The same kind of habitat (forest, rocky outcrop and temporary streams) used by the toads to reproduce at the type locality was register in several other locations in this area. This region was also recognized as high relevant for biodiversity conservation by the project “Microcorredores Ecológicos de Itapeva” (Krob *et al.*, 2010). Locality 1 and 2 are covered by microcorridors suggested by the study, including the last one in a priority extreme deployment category. Additionally, several field works developed in this area revealed a great biodiversity in the region, including the new record of *M. macrogranulosus* in locality 2. Other conservation strategies are the capacitation and strengthening of environmental agencies and forest police as well as education initiatives and scientific outreach activities with local communities. These actions could help to diminish or stop the constant deforestation and degradation of the forests and associated habitats of this part of Rio Grande do Sul.

The data presented here show the most updated information on natural history of *Melanophryniscus macrogranulosus* and an actualized scenario of the conservation status of this small amphibian of southern Brazil. All data reported here have been used in the reevaluation of the species threat category in Rio Grande do Sul (Decreto Estadual N° 51.797 de 8 de Setembro de 2014) and Brazil (not yet published) and enforces the categorization of *Melanophryniscus macrogranulosus* as Endangered.

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