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Author(s): Claudio J. Bidau, Dardo A. Martí, and Diego Baldo

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## Inter- and Intraspecific Geographic Variation of Body Size in South American Redbelly Toads of the Genus *Melanophryniscus* Gallardo, 1961 (Anura: Bufonidae)

CLAUDIO J. BIDAU,<sup>1,2</sup> DARDO A. MARTÍ,<sup>3</sup> AND DIEGO BALDO<sup>3</sup>

<sup>1</sup>Instituto Oswaldo Cruz, FIOCRUZ, 21045-900 Rio de Janeiro, Rio de Janeiro, Brazil; E-mail: bidau50@gmail.com

<sup>3</sup>Departamento de Genética, Universidad Nacional de Misiones, 3300 Posadas, Misiones, CONICET, Argentina

**ABSTRACT.**—Amphibians show strong dependence on environmental variables (water balance, temperature). However, interactions affecting geographic distribution of body size are poorly known. We present an analysis of body size within and between species of an anuran genus using a climatic approach. We studied geographic body size distribution in 23 species of South American redbelly toads (*Melanophryniscus*) spanning 16° latitude, 22° longitude, and 2,400 m altitude. Body size was analyzed in relation to climatic parameters including temperature, precipitation, seasonality, evapotranspiration, and water balance at interspecific, interpopulational (all populations regardless of species), and intraspecific (populations within species) levels. LogSVL was regressed against climatic principal components scores using simultaneous autoregression. Interspecifically and interpopulationally, temperature and precipitation are the main factors responsible for the observed size clines, larger body sizes being associated with decreasing maximum ambient temperature and water availability. Intraspecific results for two species suggested comparable body-size trends. That temperature affects these size clines is reinforced by the strong positive correlation of logSVL with altitude. Because anurans strongly depend on water for survival and reproduction, it is reasonable that, besides temperature, larger body size is favored in drier environments, which is supported by the correlation between body size and coefficients of variation of annual rainfall: lower surface : volume ratios in larger species would help conserve water in unpredictable environments. Also, *Melanophryniscus* has reproductive peculiarities associated with ephemeral aquatic environments: explosive breeding synchronized with rainfall; eggs deposited in several clutches; and rapid tadpole development, which suggest a strong relationship between life history and water balance.

Ecogeographical rules that aim to quantify and understand the spatial distribution of biological traits such as body size at different levels in relation to positional or environmental variables, are of relevance in biogeographic and macroecological research (Ashton, 2001a; Gaston et al., 2008). The analysis of geographic patterns of body-size distribution of animals is central for several reasons. Body size is a fundamental property of animal taxa because it is intimately correlated with almost all life-history characteristics (Blueweiss et al., 1978; Roff, 1986; LaBarbera, 1989; Calder, 1996), and this has been well studied in amphibians (Morrison and Hero, 2003; Murray and Hose, 2005). Furthermore, body size is a highly variable trait affected by age, gender, phylogeny, and environment and, conversely, influences numerous ecological and evolutionary processes. Bergmann (1847) proposed that, in endothermic taxa, larger sizes are associated with higher latitudes and low temperatures, whereas smaller individuals tend to be found at lower latitudes and higher temperatures (James, 1970). Bergmann's rule assumes that body size is related to thermoregulation because of surface/volume relationships. However, several other explanations for body size clines have been suggested (Ashton et al., 2000; Meiri and Dayan, 2003; Medina et al., 2007; Rodríguez et al., 2008).

A further problem involves the intra- or interspecific application of Bergmann's rule, especially regarding causal mechanisms at both levels (Blackburn et al., 1999; Medina et al., 2007; Meiri and Thomas, 2007). Although the original definition was interspecific, Rensch (1938, 1959) and Mayr (1942, 1956) reformulated the rule for an intraspecific empirical pattern regardless of ecophysiological interpretations, and James (1970) stressed the importance of climatic factors in determining size clines at the intraspecific level. Mainly, the available literature deals with intraspecific body size clines and hypotheses other than the original thermoregulation explanation proposed to account for Bergmannian (and converse Bergmannian) body-size variation (Ashton, 2001a, 2004; Meiri and Thomas, 2007) in ectotherms (Ashton, 2001b, 2002; Ashton and Feldman, 2003; Blanckenhorn and Demont, 2004) and endotherms (Ashton et al., 2000; Meiri and Dayan 2003). However, practically all the studies cited have to do with

findings at the intraspecific, not the interspecific, level. In most taxonomic groups (including amphibians), there are few interspecific analyses of body-size trends, thus indicating the need for cross-species approaches. Indeed, a number of recent papers deal with the interspecific approach at broad geographic scales, including amphibians (Rodríguez et al., 2005; Olalla-Tárraga et al. 2006; Olalla-Tárraga and Rodríguez, 2007; Adams and Church, 2008).

Although Bergmann's rule was formulated for vertebrate endotherms, a number of studies in ectotherms, including insects (Ray, 1960; Mousseau, 1997; Blanckenhorn and Demont, 2004), fishes (Lindsey, 1966), amphibians, and reptiles (Ashton, 2001b; Lindeman, 2008), revealed Bergmannian and converse-Bergmannian trends, as well as the absence of such a trend (Miaud et al., 1999; Laugen et al., 2005; Morrison and Hero, 2003; Schäuble, 2004). Also, these studies are relevant to assess the possible effect of climate change on ectotherms (Tryjanowski et al., 2006; Teplitsky et al., 2008).

The genus *Melanophryniscus*, a putative monophyletic taxon (Graybeal and Cannatella, 1995), is the sister group of all other bufonid frogs (Frost et al., 2006) and currently represented by 25 described species and several species under description, distributed in southern Brazil, southern Bolivia, Paraguay, Uruguay, and central and northern Argentina (Baldo and Basso, 2004; Di-Bernardo et al., 2006; Langone et al., 2008). We studied the geographic body-size distribution in relation to environmental variables for 21 of the described species and some innominate species of *Melanophryniscus* to test Bergmann's rule across and within species and to explore possible environmental determinants.

### MATERIALS AND METHODS

We studied 1,151 adult specimens (788 males and 363 females) of 23 species (21 described and 2 under description) of South American redbelly toads of the genus *Melanophryniscus* from 129 localities that span approximately 16° latitude (S), 22° longitude (W), and 2,400 m altitude (Fig. 1; Table 1, Appendix 1). We used snout-vent length (SVL) as an estimator of body size. Mean SVL was calculated for each species and population separately for each sex because species of this genus exhibit female-biased sexual size dimorphism (SSD) (CJB

<sup>2</sup>Corresponding Author.

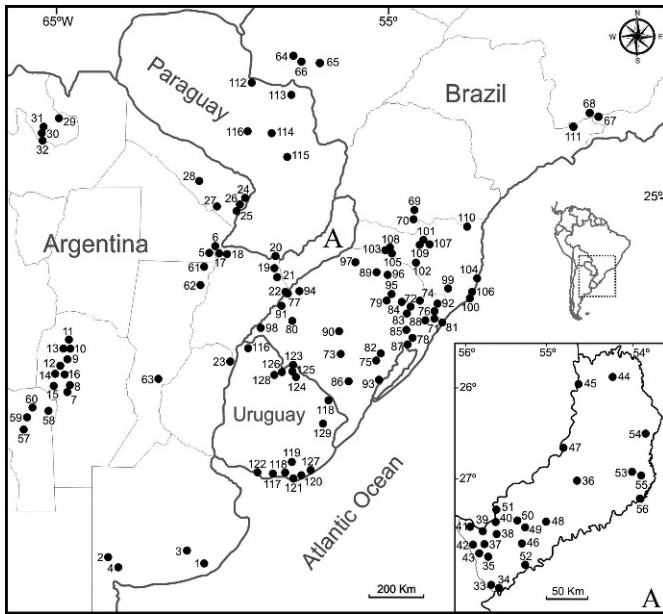


FIG. 1. Geographic distribution of the populations of *Melanophryniscus* species studied in this paper.

and DB, unpubl. data). All measurements were log transformed. We assessed normality of data within the whole sample, species, and localities with Kolmogorov-Smirnov tests.

Latitudinal, longitudinal, and altitudinal midpoints were calculated for each species distribution. However, we did not use geographic coordinates as independent variables because we generated direct estimates of climate characteristics (see below; Hawkins and Diniz-Filho, 2004). Instead, coordinates in decimal degrees were preset for each analysis using the SAM v.2 software (see below).

We processed a set of 13 climatic variables to use as predictors, including mean annual, maximum, and minimum temperatures (TMEA, TMAX, TMIN) and total annual,

TABLE 1. Species of *Melanophryniscus* studied in this paper and number of collected specimens.

Species	Number of individuals		
	Males	Females	Total
<i>M. atroluteus</i>	158	138	296
<i>M. cupreuscapularis</i>	4	1	5
<i>M. devincenzii</i>	108	26	134
<i>M. dorsalis</i>	31	24	55
<i>M. fulvoguttatus</i>	26	2	28
<i>M. klappenbachii</i>	26	11	37
<i>M. krauczuki</i>	90	27	117
<i>M. langonei</i>	2	1	3
<i>M. macrogranulosus</i>	8	3	11
<i>M. montevidensis</i>	6	3	9
<i>M. moreirae</i>	80	15	95
<i>M. orejasmirandai</i>	4	1	5
<i>M. pachyrhynchus</i>	24	30	54
<i>M. rubriventris</i>	30	14	44
<i>M. sanmartini</i>	10	6	16
<i>M. simplex</i>	17	7	24
<i>M. aff. devincenzii</i>	10	2	12
<i>M. spectabilis</i>	53	12	65
<i>M. stelzneri stelzneri</i>	29	13	42
<i>M. stelzneri cf. spegazzinii</i>	6	—	6
<i>M. tumifrons</i>	4	6	10
<i>M. sp. 1</i>	13	11	24
<i>M. sp. 2</i>	40	10	50
Total	788	363	1,151

maximum, and minimum, precipitation (PANN, PMAX, PMIN). Temperature and precipitation data were obtained from Cramer and Leemans (2001). We calculated seasonality for temperature and precipitation, calculated by their coefficients of variation (CVT, CVP) and by the difference between maximum and minimum monthly values (TMm, PMm). Thornthwaite's Actual Evapotranspiration (AET), Priestley-Taylor's Potential Evapotranspiration (PET), and Water Balance (WB) also were obtained for each midpoint and locality. We used vectors, data bases, and maps for AET, PET, and WB from Ahn and Tateishi (1994a,b). AET, PET, and WB data were obtained with the Geomatica FreeView V. 10.0 software (PCI Geomatics, Ontario, Canada, available from: www.pcigeomatics.com). All data are in mm/yr. Means, maximum, and range of these variables within geographic ranges of each species also were obtained, such that they can be paired with mean SVL for cross-species analysis. The complete set of climatic data for the studied area can be obtained from the corresponding author.

Because all these environmental variables show a high degree of colinearity, dimensionality of the predictors was reduced by means of Principal Component Analysis (PCA). The number of principal components retained and further used as predictors in correlation/regression analyses was established by the broken-stick criterion (Legendre and Legendre, 1998). To improve interpretation of the principal components, they were rotated to simple structure using VARIMAX criterion in SYSTAT v.8 (L. Wilkinson, SPSS, Inc., Chicago, 1998; Kline, 1994). After identification of the best PCA predictors, the best model (combination of variables with high loadings in that PCA) was identified using Akaike's Information Criterion (Burnham and Anderson, 2002).

Most biogeographic and macroecological data are spatially autocorrelated (Legendre, 1993; Diniz-Filho et al., 2003; Rangel et al., 2006), which requires using spatial statistic techniques. All spatial analyses were performed in SAM v.2 (Spatial Analysis in Macroecology [Rangel et al., 2006] available from: <http://www.ecoevol.ufg.br/sam>). Basically,  $\log_{10}SVL$  was regressed against climatic principal components scores with geographic coordinate variables (LON, LAT) using simultaneous autoregression (SAR). Also, altitude (ALT) and altitudinal range (ALTR) were included as body size predictors in SAR analyses. Analysis of variance (ANOVA) was performed with SYSTAT v.9.

Our analyses of geographic body size distribution involved three different levels. First, we performed SARs between species. Second, we repeated the regression analyses at the population level (within genus) regardless of species. Third, we performed a one-way ANOVA between populations within species, and the residuals were saved and regressed against the predictors of body size to quantify the within-species SVL variation. Finally, individual SARs were performed for those species with the highest number of populations. In all cases, error of Moran's I was examined for analyzing spatial structure.

No phylogeny of *Melanophryniscus* is available; thus, the phylogenetic component of across-species body size variation could not be evaluated.

## RESULTS

*Body-Size Variation in Melanophryniscus.*—At the species level, mean male SVL ranged between 19.72 mm in *Melanophryniscus krauczuki* and 37.35 mm in *Melanophryniscus rubriventris* (CV = 16.1), whereas mean female SVL ranged between 20.49 mm and 40.18 mm for *Melanophryniscus langonei* and *M. rubriventris*, respectively (CV = 16.8). Between populations regardless of species, the ranges for males and females were, respectively: 18.30 (*Melanophryniscus atroluteus*) and 41.18 (*M. rubriventris*)

TABLE 2. Principal Components Analysis of morphological data of species and populations of *Melanophryniscus*. Factors were extracted and rotated with the VARIMAX procedure with Kaiser Normalization for 13 environmental variables (for nomenclature of variables, see Materials and Methods). Values correspond to correlation coefficients between variables and factors. Relatively high loadings ( $|r| > 0.5$ ) are marked with an asterisk.

Predictor	Principal components					
	Interspecific			Interpopulational		
	PC1	PC2	PC3	PC1	PC2	PC3
AET	0.774*	0.398	0.327	0.721*	0.279	0.550*
PET	0.704*	-0.395	0.112	0.657*	-0.406	0.379
WB	0.276	0.866*	0.039	0.325	0.875*	0.114
PANN	0.660*	0.662*	0.166	0.339	0.141	0.919*
PMIN	0.238	0.914*	0.011	-0.823*	-0.320	-0.367
PMAX	0.875*	-0.074	0.177	0.554*	0.260	0.764*
CVP	0.110	-0.957*	-0.055	-0.064	0.104	0.987*
PMm	0.566*	-0.729*	0.142	-0.883*	-0.239	0.185
TMEA	0.477	0.024	0.873*	0.628*	0.655*	0.292
TMIN	0.685*	0.158	0.684*	-0.099	-0.926*	-0.279
TMAX	-0.056	-0.036	0.992*	0.326	0.890*	0.144
CVT	-0.885*	-0.225	-0.320	0.842*	0.068	0.249
TMm	-0.908*	-0.232	0.098	0.493	-0.767*	0.089
%Variance explained	48.4	27.5	12.8	53.5	22.4	13.5

(CV = 15.6), and 20.31 (*M. atroluteus*) and 40.18 (*M. rubriventris*) (CV = 13.9). All species showed female-biased SSD (CJB and DB, unpubl. data).

**Interspecific Analysis (Cross-Species).**—We performed a PCA of the climatic data for the 23 species of *Melanophryniscus*, using midpoints of each environmental variable calculated for the LAT and LON midpoints of each geographic distribution. Loadings of the predictors on the first three principal components are shown in Table 2. A SAR was performed using male or female mean logSVL as response variable and the three PCs as predictors. Results for males and females are shown in Table 3a. The best predictor of body size was PC3, a

TABLE 3. a, c. Standardized partial regression coefficients (b) and their respective *t*-values for the three principal components (PC1, PC2, PC3) derived from 13 environmental variables predicting patterns of body size distribution in males (M) and females (F) of 23 species of *Melanophryniscus*, from a simultaneous autoregressive (SAR) model. OLS, Ordinary Least Squares regression  $R^2$ ;  $R^2_{full}$  is the total coefficient of determination of the model (predictors + spatially structured error term);  $R^2_{pred}$  refers to the effects of the predictors independently of spatial structure;  $\rho$  is the autoregressive coefficient of the SAR model; Moran's *I* refers to the residual spatial autocorrelation; *b* are the partial slopes; *t* is Student's statistic. b, d. The same as above but including altitudinal range (ALTR) as a fourth body size predictor.

Predictor	<i>b</i>	<i>t</i>	OLS	$R^2_{full}$	$R^2_{pred}$	$\rho$	Moran's		
							I		
a. M	PC1	0.176	1.041	<i>ns</i>	0.345	0.335	0.998	0.258	0.019
	PC2	-0.316	-1.891	<i>ms</i>					
	PC3	-0.437	-2.588*						
b.	PC1	0.366	2.147*		0.467	0.448	0.999	0.258	0.036
	PC2	-0.325	-2.155*						
	PC3	-0.526	-3.360*						
	ALTR	-0.409	-2.407*						
c. F	PC1	0.169	-0.961	<i>ns</i>	0.289	0.283	0.994	0.252	0.013
	PC2	-0.206	-1.185	<i>ns</i>					
	PC3	-0.436	-2.478*						
d.	PC1	0.343	1.884	<i>ns</i>	0.388	0.376	0.999	0.252	-0.007
	PC2	-0.214	-1.321	<i>ns</i>					
	PC3	-0.517	-3.091**						
	ALTR	-0.374	-2.061*						

*ns*,  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; *ms*, marginally significant,  $0.05 < P < 0.07$ .

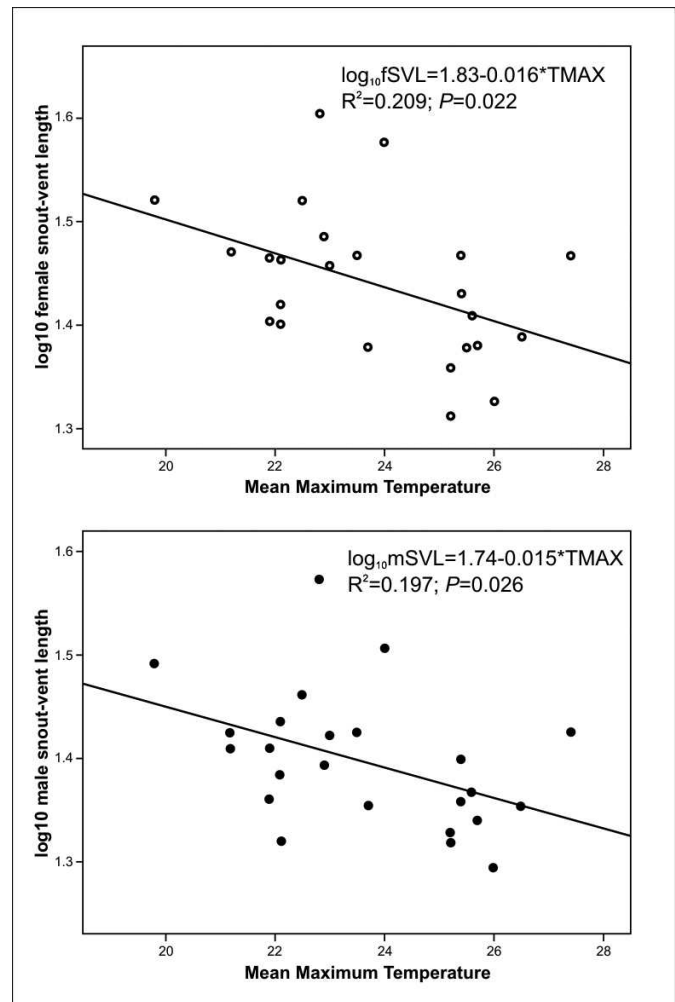


FIG. 2. Linear regressions between  $\log_{10}$  mean snout-vent length (SVL) and Mean Maximum Temperature (TMAX) for females (A) and males (B) of 23 species of *Melanophryniscus*. The regression equation, coefficient of determination ( $R^2$ ), and statistical significance ( $P$ ) are shown.

temperature component. To account for variations in altitude, we performed SARs for males and females separately including ALT and ALTR as a fourth explanatory variable. Although ALT was not a good predictor of body size, the altitudinal range of each species was (Table 3b, d). Furthermore, when ALTR was included the statistical significance of PC3 as a predictor of body size increased (Table 3).

We used the climatic variables with high loadings on PC3 (and PC2) to perform uni- and multivariate SARs. For univariate analyses, the best individual predictors of body size in both sexes were TMAX (males, OLS  $r^2 = 0.197$ , AIC = -129.004; females, OLS  $r^2 = 0.209$ , AIC = -125.467) (Fig. 2a,b) and CVP (males, OLS  $r^2 = 0.174$ , AIC = -128.307; females,  $r^2 = 0.096$ , AIC = -122.132). The best multivariate models found for both sexes included TMAX, CVP, and PMIN (males, OLS  $r^2 = 0.479$ , AIC = 132.776; females,  $r^2 = 0.394$ , AIC = 125.117).

**Population Analysis Independent of Species.**—A PCA of the 13 climatic variables for all populations was performed using LAT and LON of each population as geographic coordinates (Table 2). The results of the SARs for males and females are shown in Table 4. Again, PC3 and PC2 were the best predictors of body size (Table 4). When logSVL was regressed against the variables showing high loadings in PC2 and PC3, TMAX (males, OLS  $r^2 = 0.385$ ,  $P < 0.001$ , AIC = -672.322; females, OLS  $r^2 = 0.363$ ,  $P < 0.001$ , AIC = -492.282) and CVP (males,



TABLE 4. a, c. Standardized partial regression coefficients (*b*) and their respective *t*-values for the three principal components (PC1, PC2, PC3) derived from 13 environmental variables predicting patterns of body size distribution in males (M) and females (F) 129 natural populations of 23 species of *Melanophryniscus*, from a simultaneous autoregressive (SAR) model. OLS, Ordinary Least Squares regression  $R^2$ ;  $R^2_{full}$  is the total coefficient of determination of the model (predictors + spatially structured error term);  $R^2_{pred}$  refers to the effects of the predictors independently of spatial structure;  $\rho$  is the autoregressive coefficient of the SAR model; Moran's I refers to the residual spatial autocorrelation; *b* are the partial slopes; *t* is Student's statistic. b, d. The same as above but including altitude (ALT) as a fourth body size predictor.

	Predictor	<i>b</i>	<i>t</i>	OLS	$R^2_{full}$	$R^2_{pred}$	$\rho$	Moran's I
a. M	PC1	0.015	0.169 <i>ns</i>	0.404	0.372	0.776	0.710	0.210
	PC2	-0.282	-2.967**					
	PC3	-0.361	-4.027**					
b.	PC1	-0.004	-0.045 <i>ns</i>	0.437	0.428	0.811	0.710	0.192
	PC2	-1.155	-1.502 <i>ns</i>					
	PC3	-1.196	-1.851 <i>ms</i>					
	ALT	0.295	2.694**					
c. F	PC1	0.044	0.389 <i>ns</i>	0.324	0.283	0.796	0.604	0.342
	PC2	-0.137	-1.261 <i>ns</i>					
	PC3	-0.461	-4.449**					
d.	PC1	0.026	0.230 <i>ns</i>	0.328	0.299	0.783	0.604	0.349
	PC2	-0.058	-0.478 <i>ns</i>					
	PC3	-0.358	-2.795**					
	ALT	0.201	-1.354 <i>ns</i>					

*ns*,  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; *ms*, marginally significant,  $0.05 < P < 0.07$ .

$r^2 = 0.329$ ,  $P < 0.001$ , AIC = -669.398; females,  $r^2 = 0.097$ ,  $P = 0.05$ , AIC = -463.697) were the best univariate predictors of body size for both sexes (Fig. 3). The best multivariate model found for males included TMAX, CVP, PMIN, and WB (OLS  $r^2 = 0.505$ , AIC = -697.779). In females, the best model for prediction of body size at this level of analysis was the univariate TMAX. A female model, including the four variables explaining logSVL variation in males, yielded a lower AIC = -485.992.

**Within-Species SVL Variation.**—We performed one-way ANOVAs of logSVL between populations within species. For males and females, differences were highly significant (males,  $F_{22, 89} = 24.5$ ,  $P < 0.001$ ; females,  $F_{22, 59} = 12.6$ ,  $P < 0.001$ ). However, in both cases, when the standardized residuals were regressed against the principal components, no significant regressions were obtained (Table 5). Because the lack of significance was probably a result of the very dissimilar number of populations analyzed within each species, we performed single-species analyses and SAR analyses of those taxa where a relatively large number of populations was available, *M. atroluteus* (23) and *Melanophryniscus devincenzii* (18) (Table 6). In both species, no significant regressions were found for females. For males of *M. devincenzii*, SVL was highly correlated with all four independent variables and with PC1 and PC3 in *M. atroluteus* (Table 6).

#### DISCUSSION

Species with ample latitudinal or altitudinal geographic ranges are useful models for the analysis of body-size distribution at the intraspecific level. Wide distributions expose species to very different climatic conditions. Thus, not only factors such as temperature and rainfall, but also seasonality, evapotranspiration, primary productivity, predation, and competition pressure, may combine to produce different kinds of size clines (reviewed in Ashton et al., 2000; Meiri and Dayan, 2003; Bidau and Martí, 2007a,b).

In *Melanophryniscus*, the interspecific analysis of body-size variation strongly suggested that temperature is the main factor behind the observed size clines: Larger body sizes are associated with decreasing maximum ambient temperature (although not with mean annual temperature or an estimator of seasonality, TM-m). A negative correlation between temperature and body size is the expectation according to the original formulation of Bergmann's rule (i.e., decreasing surface to volume ratios with increasing body size).

Although Bergmannian patterns have been described in amphibians primarily at the intraspecific level (Ashton, 2002), a recent meta-analysis showed conflicting results with previously published studies (Adams and Church, 2008). These authors indicated that true Bergmannian patterns are not generally exhibited by anurans and salamanders and questioned the heat conservation hypothesis as a putative explanation for these patterns (Adams and Church, 2008). However, although it is clear that the analyses in Adams and Church (2008) do not support thermal body-size clines in *Plethodon*, the applicability of their results to the rest of amphibians is less doubtful. This is because their analyses were based on a very small fraction of about 6,000 species described to date and because the available data are often geographically and taxonomically biased (e.g., Gaston et al., 2008). Thus, caution is recommended when reaching conclusions about absence of patterns within a large group. At the interspecific level, a previous study of body size variation across species for amphibian faunas in the Holarctic (Olalla-Tárraga and Rodríguez, 2007) found that Bergmannian patterns do occur in anurans (whereas urodelans follow the converse to Bergmann's rule) related to amounts of energy availability. This is relevant because anurans appear to possess good thermoregulating abilities (Olalla-Tárraga and Rodríguez, 2007); thus, small increases in body mass could lead to a better performance in heat gain and conservation although this also may imply an upper limit to size increase (Olalla-Tárraga and Rodríguez, 2007).

The effect of temperature may reflect, in part, the well-known fact that ectothermic vertebrates (and also many invertebrates) tend to grow more slowly but reach larger sizes as rearing temperature decreases (Atkinson and Sibly, 1997; Atkinson et al., 2003; Morrison and Hero, 2003). Although this physiological response is not necessarily adaptive, it is likely that species occupying habitats with relatively wide variations of mean ambient temperature will be larger as a result of colder environments, which, in turn, may be adaptive with regard to heat conservation and, thus, be selected for. However, the prospective advantages of body size may not necessarily result from a thermoregulatory adaptation but perhaps depends on the different environments inhabited by a certain species, or group of species, of a secondary effect of body size (such as increasing tolerance to dry conditions, avoiding predators more efficiently, or having greater fasting endurance). Hence, direct physiological interactions with temperature, could result in intraspecific patterns if body size gradients have been coopted for functions in complex environments that show multiple environmental gradients. However, gradients in body size mimicking Bergmannian trends could appear independently of temperature gradients in anurans from a Neotropical region with high water deficit, as shown by Olalla-Tárraga et al. (2009). In this case, the authors attributed the body size clines to water availability related to surface : volume ratios: larger species would be more tolerant to desiccation than would smaller ones.

In *Melanophryniscus*, inhabiting areas with comparatively low water deficit, ambient temperature seems to better explain the observed Bergmannian clines. That temperature is the variable behind the size clines of *Melanophryniscus* is further reinforced by the observation that male and female logSVL are both strongly positively correlated with mean altitude. When

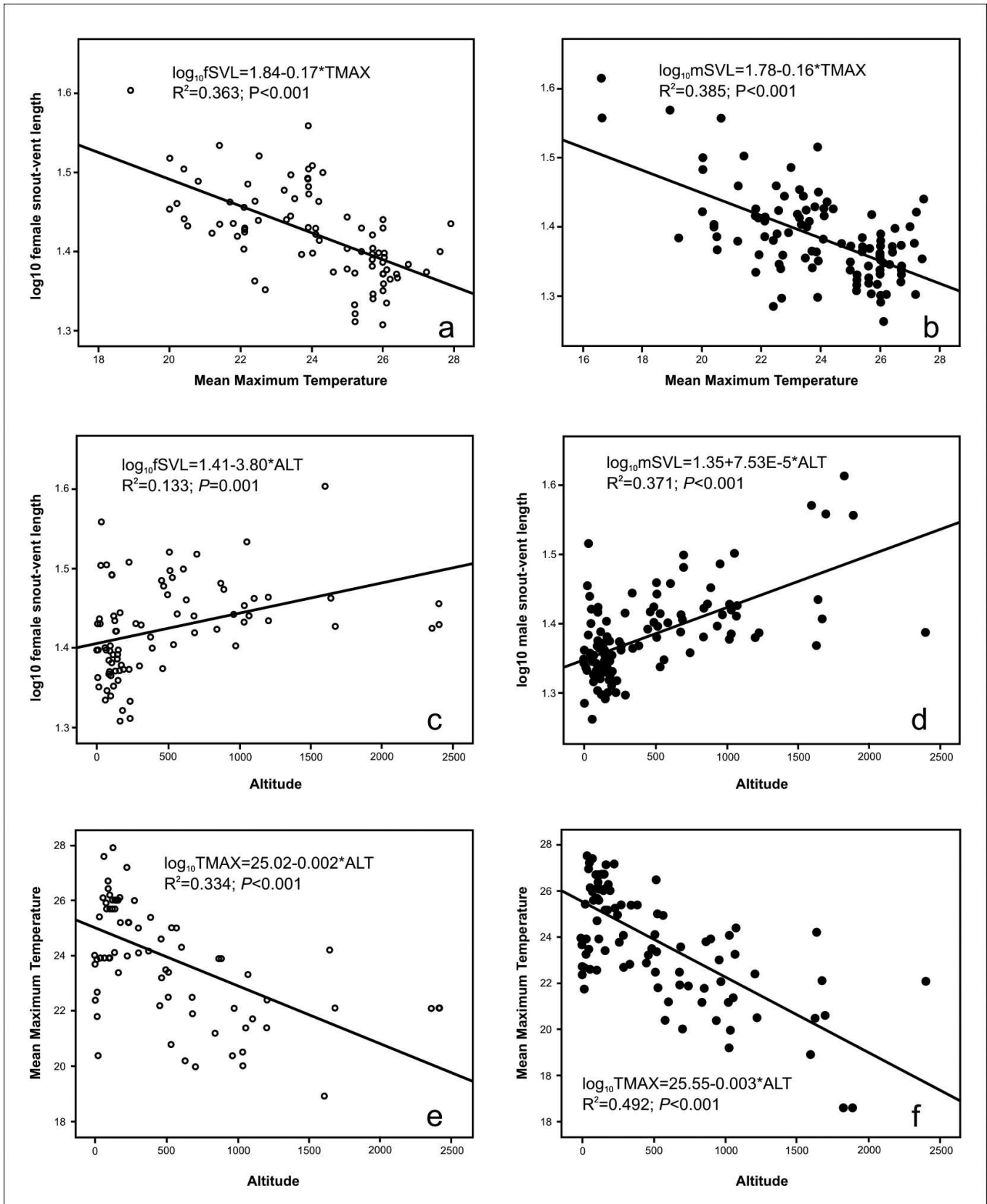


FIG. 3. Linear regressions between  $\log_{10}$  mean snout-vent length (SVL) for females (a, c, e) and males (b, d, f) from 82 and 114 populations of *Melanophryniscus* belonging to 23 species. a, c. SVL versus mean maximum temperature (TMAX); c, d. SVL versus altitude (ALT); e, f. The relationship between TMAX and ALT for the two groups of localities. The regression equation, coefficient of determination ( $R^2$ ), and statistical significance ( $P$ ) are shown.

*Melanophryniscus* body size was analyzed at the population level without consideration of species, TMAX (and also TMEAN) showed strong negative correlations for both sexes. However, some precipitation variables also were good predictors of body size at this level (see Results). This CVP signal appeared only when populations were considered as individual points regardless of species, probably because in this case a great deal of variation of CVP was included in the models. These results are by no means contradictory to the Bergmannian model: Olalla-Tárraga et al. (2009) found a positive correlation between anuran body size and water deficit in the Brazilian Cerrado. Because anurans are strongly dependent on water for survival and reproduction, it is reasonable, that besides temperature, a larger body size is favored in drier environments. CVP is a measurement of rainfall variation, and our results, agreeing with those of Olalla-Tárraga et al. (2009), suggest that larger sizes tend to occur in more seasonal environments with respect to precipitation: Lower surface : volume ratios in larger species would help conserve water in unpredictable environments. Also, the *Melanophryniscus* species have some reproductive peculiarities associated with ephemeral aquatic environments: explosive breeding synchronized with rainfall; eggs deposited in several clutches; and rapid tadpole development (Baldo and Basso, 2004; Vaira, 2005; Goldberg et al., 2006; Cairo et al., 2008).

In anurans, intraspecific Bergmannian patterns tend to occur in about 63% of the studied species when latitude or altitude is the independent variable (Ashton, 2002). However, the relationship with temperature is weaker: 50% of studied species in Ashton (2002). This suggests that other factors may be influencing Bergmannian-like clines. An intraspecific analysis of body-size variation was possible only in *M. atroluteus* and *M. devincenzii* where point data from a reasonable number of populations were available. Comparisons between inter- and intraspecific body size patterns within a group are of interest because there is not much published evidence about the concordance between both kind of patterns; and, if concordant, it is usually unknown whether mechanisms

TABLE 5. a, c. Standardized partial regression coefficients (*b*) and their respective *t*-values for the three principal components (PC1, PC2, PC3) derived from 13 environmental variables predicting patterns of body size distribution in males (M) and females (F) natural populations of 23 species of *Melanophryniscus*, from a simultaneous autoregressive (SAR) model using residuals of ANOVAs (see text) as dependent variable. OLS, Ordinary Least Squares regression  $R^2$ ;  $R^2_{full}$  is the total coefficient of determination of the model (predictors + spatially structured error term);  $R^2_{pred}$  refers to the effects of the predictors independently of spatial structure;  $\rho$  is the autoregressive coefficient of the SAR model; Moran's I refers to the residual spatial autocorrelation; *b* are the partial slopes; *t* is Student's statistic. b, d. The same as above but including altitude (ALT) as a fourth body size predictor.

	Predictor	<i>b</i>	<i>t</i>	OLS	$R^2_{full}$	$R^2_{pred}$	$\rho$	Moran's I
a. M	PC1	0.100	1.119 <i>ns</i>	0.013	0.012	0.038	-0.095	<0.001
	PC2	0.017	0.188 <i>ns</i>					
	PC3	-0.023	-0.259 <i>ns</i>					
b.	PC1	0.103	1.146 <i>ns</i>	0.013	0.011	0.041	-0.095	<0.001
	PC2	-0.004	-0.036 <i>ns</i>					
	PC3	-0.052	-0.393 <i>ns</i>					
	ALT	-0.045	-0.295 <i>ns</i>					
c. F	PC1	-0.006	-0.062 <i>ns</i>	0.004	0.006	0.016	-0.136	-0.006
	PC2	0.066	0.350 <i>ns</i>					
	PC3	-0.028	-0.269 <i>ns</i>					
d.	PC1	-0.041	-0.360 <i>ns</i>	0.010	0.011	0.044	-0.136	-0.002
	PC2	0.132	0.931 <i>ns</i>					
	PC3	0.044	0.298 <i>ns</i>					
	ALT	0.124	0.682 <i>ns</i>					

*ns*,  $P > 0.05$ .

TABLE 6. Standardized partial regression coefficients (*b*) and their respective *t*-values for the three principal components (PC1, PC2, PC3) derived from 13 environmental variables and altitude (ALT) predicting patterns of body size distribution in males (M) and females (F) of *Melanophryniscus atroluteus* and *Melanophryniscus devincenzii*, from a simultaneous autoregressive (SAR) model. OLS, Ordinary Least Squares regression  $R^2$ ;  $R^2_{full}$  is the total coefficient of determination of the model (predictors + spatially structured error term);  $R^2_{pred}$  refers to the effects of the predictors independently of spatial structure;  $\rho$  is the autoregressive coefficient of the SAR model; Moran's I refers to the residual spatial autocorrelation; *b* are the partial slopes; *t* is Student's statistic.

Species	Predictor	<i>b</i>	<i>t</i>	OLS	$R^2_{full}$	$R^2_{pred}$	$\rho$	Moran's I
<i>M. atroluteus</i>								
M	PC1	0.629	4.627**	0.599	0.606	1	0.074	0.001
	PC2	-0.071	-0.499 <i>ns</i>					
	PC3	0.472	3.522**					
F	ALT	0.293	2.049 <i>ns</i>					
	PC1	0.29	0.965 <i>ns</i>	0.302	0.304	0.999	-0.090	-0.076
	PC2	-0.682	-1.045 <i>ns</i>					
	PC3	0.169	0.814 <i>ns</i>					
	ALT	-0.239	-0.342 <i>ns</i>					
<i>M. devincenzii</i>								
M	PC1	-0.508	-3.341**	0.756	0.777	1	0.346	-0.122
	PC2	-1.120	-5.925**					
	PC3	0.524	4.124**					
	ALT	-0.956	-4.390**					
F	PC1	-0.143	-0.560 <i>ns</i>	0.242	0.344	1	-0.446	-0.442
	PC2	0.310	1.119 <i>ns</i>					
	ALT	0.198	0.844 <i>ns</i>					

*ns*,  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ .

operating at both levels are the same (Medina et al., 2007). However, the intraspecific results obtained lacked the consistency of the interspecific and interpopulation analyses, and females of both species did not show any significant correlation with principal components nor with altitude. Two explanations can be advanced. First, relatively small geographic distributions may explain a lack of strong environmental gradients that could affect body size significantly. For example, *M. atroluteus* occupies an area of 3.5° latitude and 4.5° longitude, whereas the altitudinal span is only 144 m (46–190). Mean annual temperature showed a 4°C difference between extreme latitudinal points (17.33°–21.35°C); but CV was very low (5.44), and most of the variability was a result of four of the southernmost samples, which, when eliminated from the analyses, produce a decrease of more than 2°C within the total sample (19.39°–21.36°) with a CV = 3.22. *Melanophryniscus devincenzii* occupies an area of 4.5° (latitude) and 3.5° (longitude) and a vertical span of almost 600 m (98–685). Here, TMEAN shows a much pronounced variation from 17.61°–25.12°C (CV = 8.17) as well as other environmental variables caused by the greater altitudinal span occupied by this species. However, these considerations do not explain the differences in response of males and females. In *M. atroluteus*, males are less variable in SVL than are females (CVs = 5.27 vs. 7.12), which could explain some growth constraint in males that could be more susceptible to environmental variation. In *M. devincenzii*, the situation is inverted, females being less variable (CVs = 3.62 vs. 5.53). This indicates that females reach more similar sizes than do males, thus suggesting that both species behave in opposite ways with respect to Rensch's rule (the hypothesis that sexual size dimorphism increases with body size when males are the larger sex and decreases when females are larger; Rensch, 1959). In turn, males and females have different growth patterns and different responses to the environment.

The results and conclusions presented in this paper require further testing. However, *Melanophryniscus* was shown to be a



suitable model for the analysis of different hypotheses regarding the existence of geographic body-size clines at a regional scale and within a probably monophyletic taxon, which displays special relationships between reproduction and environmental conditions and considerable number of relatively well-known species. Furthermore, there is growing evidence of frequent Bergmannian or Bergmannian-like clines at broad geographic scales and different taxonomic levels. Both thermo-regulation and avoidance of desiccation may be the primary factors determining body-size gradients in amphibians. This is of fundamental importance for establishing conservation measures for a globally endangered fauna in view of the risks imposed by impending climatic change and habitat loss.

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- chita (32°04'S, 64°33'W); (7) Estancia El Sauce, Villa General Belgrano (31°58'S, 64°34'W); (7) Estancia La Ponderosa, Los Reartes, Villa Berna (31°53'S, 64°35'W); (7) El Durazno, Calamuchita (32°04'S, 64°33'W); (7) Loma del Tigre, Calamuchita (32°04'S, 64°33'W); (7) Parador de la Montaña, Santa Rosa de Calamuchita (32°04'S, 64°33'W); (7) Calamuchita, Yacanto (32°03'S, 64°45'W); (8) Potrero Garay (31°47'S, 64°30'W); Departamento Colón: (9) Corral de Felipe, Cabana (31°13'S, 64°22'W); (10) Pozo Azul (30°57'S, 64°19'48"W); Departamento Ischilín: (11) Villa Albertina (30°41'S, 64°20'W); Departamento Punilla: (12) Flor Serrana, Tanti (31°23'S, 64°36'20"W); (12) Río Toro Muerto, Flor Serrana, Tanti (31°23'S, 64°36'20"W); (12) Sierras de Tanti (31°21'S, 64°36'W); (12) Tanti (31°21'S, 64°36'W); (13) La Cumbre, Cruz Chica (30°58'S, 64°29'W); (14) Pampa de Achala (31°35'S, 64°50'W); Puente Pérez, Pampa de Achala (31°48'S, 64°54'W); Departamento San Javier: (15) Hornillos (31°54'S, 64°59'W); Departamento Santa María: (16) Bosque Alegre (31°36'S, 64°33'W). CORRIENTES: Departamento Capital: (17) Barrio Lomas, Corrientes (27°28'S, 58°49'W); (17) Perichón (27°26'31'S, 58°45'08"W); (18) Laguna Brava (27°29'23"S, 58°42'47"W); Departamento Ituzaingo: (19) 15 km. NW from San Carlos (27°40'S, 56°01'W); Departamento Ituzaingo: (20) Rincón Santa María (27°28'S, 56°35'W); (21) Ruta Provincial N° 14, 22 km N from Gobernador Virasoro (27°53'27"S, 55°56'07"W); Departamento Santo Tomé: (22) Garruchos (28°10'S, 55°38'W). ENTRE RÍOS: Departamento Chajarí: (23) Chajarí, Arroyo Chajarí (30°44'01"S, 57°57'47"W). FORMOSA: Departamento Formosa: (24) Estancia Santa Catalina (25°51'S, 57°54'W); (25) Formosa (26°11'S, 58°11'W); (26) Reserva Guaycole (25°58'S, 58°10'W); Departamento Laishi: (27) Reserva Ecológica El Bagueal (26°10'S, 58°56'W); Departamento Patiño: (28) Reducción Bartolomé de las Casas (25°24'S, 59°34'W). JUJUY: Departamento Libertador General San Martín: (29) Abra Colorada, Parque Nacional Calilegua (23°40'S, 64°53'W); (29) Abra de Cañas, Parque Nacional Calilegua (23°40'S, 64°53'W); Departamento Manuel Belgrano: (30) Termas de Reyes (24°09'59"S, 65°29'22"W); (31) Tiraxi (24°00'45.36"S, 65°23'16.34"W); Departamento San Antonio: (32) Río Morado (= Huracalao) (24°19'40.6"S, 65°26'5"W). MISIONES: Departamento Apostoles: (33) Azara (28°4'S, 55°40'W); (34) Barra Concepción (28°05'59"S, 55°34'34"W); (35) Campo Gentilini, San José (27°44'S, 55°40'W); Departamento Concepción: (34) Campo San Lucas, Barra Concepción (28°05'59"S, 55°32'36"W); Departamento Canguas: (36) Dos de Mayo (27°01'S, 54°41'W); (36) El Paraisal, 2 de Mayo (27°02'S, 54°39'W); Departamento Candelaria: (37) 1 km W from Profundidad (27°34'S, 55°43'W); (37) Parque provincial Profundidad (27°34'S, 55°43'W); (38) Arroyo San Juan, Cerro corá (27°29'48"S, 55°36'54"W); (38) Ñu Pajú (27°29'25"S, 55°40'6"W); (38) Ruta Provincial N° 12 and Ruta Provincial N° 3 (27°27'46"S, 55°40'55"W); (39) Arroyo Garupá, Barrio UPCN (27°29'17"S, 55°44'21"W); (40) Reserva Privada Campo San Juan (27°22'55"S, 55°38'21"W); (40) Arroyo Santa Ana (27°23'42"S, 55°35'3"W); Departamento Capital: (41) Campus UNaM, Villa Lanús (27°26'11"S, 55°53'25"W); (42) Parada Leis (27°35'44"S, 55°50'7"W); (37) Parque Provincial Fachinal (27°38'S, 55°42'W); (43) Ruta Provincial N° 105, Manantiales (27°41'21"S, 55°48'12"W); Departamento Iguazú: (44) Arroyo Uruguai-Medio (25°51'S, 54°10'W); (45) Puerto Libertad (= Puerto Bemberg) (25°55'S, 54°36'W); Departamento Leandro N. Alem: (46) Leandro N. Alem (27°36'S, 55°19'W); Departamento Montecarlo: (47) Caraguoatá (26°34'S, 54°47'W); Departamento Oberá: (48) Campo Ramón (27°25'S, 55°2'W); (49) 11 de Noviembre, San Martín (27°28'S, 55°17'W); (50) Martires (27°25'S, 55°26'W); Departamento San Ignacio: (51) Reserva Privada Luis Jorge Velazquez (27°16'03"S, 55°34'8"W); Departamento San Javier: (52) Itacaruaré (27°52'S, 55°16'W); Departamento San Pedro: (53) Forestal Montreal, Arroyo Competidor (26°52'S, 54°02'W); (54) Parque Provincial Piñalito (26°26'S, 53°49'W); (55) Parque provincial Esmeralda (26°53'31"S, 53°52'45"W); (56) Parque provincial Moconá (27°10'S, 53°54'W). SAN LUIS: Departamento Capital: (57) Potrero de Funes (33°13'S, 66°14'W); (57) El Volcán (33°15'S, 66°12'W); Departamento Chacabuco: (58) Concarán (32°34'S, 65°15'W); Departamento Coronel Pringles; (59) La Carolina (32°48'S, 66°06'W); (60) Las Chacras (32°33'S, 65°47'W). SANTA FE: Departamento General Obligado: (61) Arroyo Sombrerito and Arroyo Los Amores (28°43'S, 59°27'W); (62) Florencia (28°02'S, 59°15'W); Departamento Las Colonias: (63) Esperanza (31°23'13.6"S, 60°55'3"W).

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#### APPENDIX 1.

Gazetteer of all localities of *Melanophryniscus* sampled in Argentina, Brazil, Paraguay, and Uruguay.

**ARGENTINA:** BUENOS AIRES: Partido Balcarce: (1) Sierra La Vigilancia (37°52'41"S, 58°02'W); Partido Saavedra: (2) Sierras de Puán, El Tropezón (37°37'S, 62°33'W); Partido Tandil: (3) Tandil (37°20'S, 59°07'W); Partido Tornquist: (4) Bosque Marengo, Parque provincial Tornquist (38°05'S, 62°11'W); (4) Tornquist, Parque provincial Tornquist (38°03'S, 62°01'W); (4) Sierra de la Ventana (38°03'S, 62°01'W); (4) Sierra de la Ventana, Abra Ventana (38°03'S, 62°01'W). CHACO: Departamento San Fernando: (5) 20 km from Resistencia (27°33'S, 59°08'W); (6) Resistencia (27°27'S, 58°59'W). CÓRDOBA: Departamento Calamuchita: (7) Athos Pampa, Rio Reartes (31°59'S, 64°42'W); (7) Calamuchita (32°04'S, 64°33'W); (7) Calmayo, Santa Rosa de Calamu-

chita (32°04'S, 64°33'W); (7) Estancia El Sauce, Villa General Belgrano (31°58'S, 64°34'W); (7) Estancia La Ponderosa, Los Reartes, Villa Berna (31°53'S, 64°35'W); (7) El Durazno, Calamuchita (32°04'S, 64°33'W); (7) Loma del Tigre, Calamuchita (32°04'S, 64°33'W); (7) Parador de la Montaña, Santa Rosa de Calamuchita (32°04'S, 64°33'W); (7) Calamuchita, Yacanto (32°03'S, 64°45'W); (8) Potrero Garay (31°47'S, 64°30'W); Departamento Colón: (9) Corral de Felipe, Cabana (31°13'S, 64°22'W); (10) Pozo Azul (30°57'S, 64°19'48"W); Departamento Ischilín: (11) Villa Albertina (30°41'S, 64°20'W); Departamento Punilla: (12) Flor Serrana, Tanti (31°23'S, 64°36'20"W); (12) Río Toro Muerto, Flor Serrana, Tanti (31°23'S, 64°36'20"W); (12) Sierras de Tanti (31°21'S, 64°36'W); (12) Tanti (31°21'S, 64°36'W); (13) La Cumbre, Cruz Chica (30°58'S, 64°29'W); (14) Pampa de Achala (31°35'S, 64°50'W); Puente Pérez, Pampa de Achala (31°48'S, 64°54'W); Departamento San Javier: (15) Hornillos (31°54'S, 64°59'W); Departamento Santa María: (16) Bosque Alegre (31°36'S, 64°33'W). CORRIENTES: Departamento Capital: (17) Barrio Lomas, Corrientes (27°28'S, 58°49'W); (17) Perichón (27°26'31'S, 58°45'08"W); (18) Laguna Brava (27°29'23"S, 58°42'47"W); Departamento Ituzaingo: (19) 15 km. NW from San Carlos (27°40'S, 56°01'W); Departamento Ituzaingo: (20) Rincón Santa María (27°28'S, 56°35'W); (21) Ruta Provincial N° 14, 22 km N from Gobernador Virasoro (27°53'27"S, 55°56'07"W); Departamento Santo Tomé: (22) Garruchos (28°10'S, 55°38'W). ENTRE RÍOS: Departamento Chajarí: (23) Chajarí, Arroyo Chajarí (30°44'01"S, 57°57'47"W). FORMOSA: Departamento Formosa: (24) Estancia Santa Catalina (25°51'S, 57°54'W); (25) Formosa (26°11'S, 58°11'W); (26) Reserva Guaycole (25°58'S, 58°10'W); Departamento Laishi: (27) Reserva Ecológica El Bagueal (26°10'S, 58°56'W); Departamento Patiño: (28) Reducción Bartolomé de las Casas (25°24'S, 59°34'W). JUJUY: Departamento Libertador General San Martín: (29) Abra Colorada, Parque Nacional Calilegua (23°40'S, 64°53'W); (29) Abra de Cañas, Parque Nacional Calilegua (23°40'S, 64°53'W); Departamento Manuel Belgrano: (30) Termas de Reyes (24°09'59"S, 65°29'22"W); (31) Tiraxi (24°00'45.36"S, 65°23'16.34"W); Departamento San Antonio: (32) Río Morado (= Huracalao) (24°19'40.6"S, 65°26'5"W). MISIONES: Departamento Apostoles: (33) Azara (28°4'S, 55°40'W); (34) Barra Concepción (28°05'59"S, 55°34'34"W); (35) Campo Gentilini, San José (27°44'S, 55°40'W); Departamento Concepción: (34) Campo San Lucas, Barra Concepción (28°05'59"S, 55°32'36"W); Departamento Canguas: (36) Dos de Mayo (27°01'S, 54°41'W); (36) El Paraisal, 2 de Mayo (27°02'S, 54°39'W); Departamento Candelaria: (37) 1 km W from Profundidad (27°34'S, 55°43'W); (37) Parque provincial Profundidad (27°34'S, 55°43'W); (38) Arroyo San Juan, Cerro corá (27°29'48"S, 55°36'54"W); (38) Ñu Pajú (27°29'25"S, 55°40'6"W); (38) Ruta Provincial N° 12 and Ruta Provincial N° 3 (27°27'46"S, 55°40'55"W); (39) Arroyo Garupá, Barrio UPCN (27°29'17"S, 55°44'21"W); (40) Reserva Privada Campo San Juan (27°22'55"S, 55°38'21"W); (40) Arroyo Santa Ana (27°23'42"S, 55°35'3"W); Departamento Capital: (41) Campus UNaM, Villa Lanús (27°26'11"S, 55°53'25"W); (42) Parada Leis (27°35'44"S, 55°50'7"W); (37) Parque Provincial Fachinal (27°38'S, 55°42'W); (43) Ruta Provincial N° 105, Manantiales (27°41'21"S, 55°48'12"W); Departamento Iguazú: (44) Arroyo Uruguai-Medio (25°51'S, 54°10'W); (45) Puerto Libertad (= Puerto Bemberg) (25°55'S, 54°36'W); Departamento Leandro N. Alem: (46) Leandro N. Alem (27°36'S, 55°19'W); Departamento Montecarlo: (47) Caraguoatá (26°34'S, 54°47'W); Departamento Oberá: (48) Campo Ramón (27°25'S, 55°2'W); (49) 11 de Noviembre, San Martín (27°28'S, 55°17'W); (50) Martires (27°25'S, 55°26'W); Departamento San Ignacio: (51) Reserva Privada Luis Jorge Velazquez (27°16'03"S, 55°34'8"W); Departamento San Javier: (52) Itacaruaré (27°52'S, 55°16'W); Departamento San Pedro: (53) Forestal Montreal, Arroyo Competidor (26°52'S, 54°02'W); (54) Parque Provincial Piñalito (26°26'S, 53°49'W); (55) Parque provincial Esmeralda (26°53'31"S, 53°52'45"W); (56) Parque provincial Moconá (27°10'S, 53°54'W). SAN LUIS: Departamento Capital: (57) Potrero de Funes (33°13'S, 66°14'W); (57) El Volcán (33°15'S, 66°12'W); Departamento Chacabuco: (58) Concarán (32°34'S, 65°15'W); Departamento Coronel Pringles; (59) La Carolina (32°48'S, 66°06'W); (60) Las Chacras (32°33'S, 65°47'W). SANTA FE: Departamento General Obligado: (61) Arroyo Sombrerito and Arroyo Los Amores (28°43'S, 59°27'W); (62) Florencia (28°02'S, 59°15'W); Departamento Las Colonias: (63) Esperanza (31°23'13.6"S, 60°55'3"W).

**BRAZIL:** MATO GROSSO DO SUL: (64) Bonito (21°8'S, 56°28'W); (65) Maracaju (21°38'S, 55°09'W); (65) Serra de Maracaju, Guia Lopes da Laguna (21°26'S, 56°07'W); (66) Jardim (21°28'S, 56°9'W); MATO GERAIS: (67) Abrigo Rebouças, Itamonte (22°23'S, 44°40'W); (67) Itatiaia, Itamonte, Brejo da Lapa (22°21'34.4"S, 44°44'13.4"W); RIO DE JANEIRO: (67) Parque Nacional Itatiaia (22°23'S, 44°38'W); (68) Itamonte (22°17'S, 44°53'W); PARANA: (69) Bituruna (26°10'S, 51°34'W); (70) Pinhao

(25°43'S, 51°37'W); **RIO GRANDE DO SUL:** (71) Aratinga, RS-486, São Francisco de Paula (29°19'12.6"S, 50°12'13.3"W); (72) Bento Gonçalves (29°10'S, 51°3'W); (73) Caçapava do Sul (30°30'S, 53°30'W); (74) Cambará do Sul, Itaimbezinho (28°39'S, 50°37'W); (75) Dom Feliciano (30°42'S, 52°7'W); (76) Fortaleza dos Aparados da Serra, P. N. Serra Geral Cambará do Sul (29°2'S, 50°9'W); (77) Garruchos (28°11'S, 55°38'W); (78) Gravataí, Fazenda Quatro Irmãos (29°56'S, 50°58'W); (79) Iliópolis (28°56'S, 52°7'W); (80) Manoel Viana (29°35'S, 55°29'W); (81) Morro da Gruta, Porto Fagundes, Porto Colônia, Dom Pedro de Alcântara (29°24'22.4"S, 49°51'05.1"W); (81) Torres (29°21'S, 49°44'W); (82) São Jerônimo, Fazenda Novosares (30°22'16"S, 51°54'07"W); (83) Nova Petrópolis, Linha Imperial (29° 22'S, 51°8'W); (84) Nova Roma (28°59'S, 51°24'W); (85) Novo Hamburgo, FINEP (29°41'S, 51°8'W); (86) Piratini (31°26'S, 53°06'W); (87) Porto Alegre (30°02'S, 51°12'W); (87) Porto Alegre, Morro da Agronomia (30°04'09"S, 51°08'10"W); (87) Porto Alegre, Morro de Santa Ana (30°35'S, 51°07'W); (87) Porto Alegre, Morro Teresópolis (30°02'S, 51°12'W); (87) Sítio do Mato, Belém Velho, Porto Alegre (30°07'S, 51°10'W); (87) Viamão, Parque Saint-Hilaire (30°05'55"S, 51°05'48"W); (88) Rincão dos Kroeff, Floresta Nacional São Francisco de Paula (29°25'S, 50°24'W); (89) Rondinha (27°49'S, 52°54'W); (90) Santa Maria (29°41'S, 53°43'W); (91) São Borja (28°38'S, 56°00'W); (92) São Jose dos Ausentes (28°50'S, 50°00'W); (93) São Lourenço do Sul (31°22'S, 51°58'W); (94) São Nicolau (28°10'S, 55°16'W); (95) Serafina Correa, UHE Boa Fé (28°43'S, 51°56'W); (96) Sertão (28°02'31"S, 52°13'28"W); (97) Tenente Portela (27°22'S, 53°45'W); (98) Uruguaiana, Km 170-BR (29°45'S, 57°04'W); (99) **SANTA CATARINA:** Boca da Serra, near of Bom Jardim do Sul; São Joaquim (28°20'S, 49°38'W); (100) Cabo de Santa Marta Grande (28°35'35"S,

48°49'40"W); (101) Caçador (26°46'S, 51°00'W); (102) Campos Novos (27°23'S, 51°12'W); (103) Gleba II, Floresta Nacional de Chapecó (27°10'54"S, 52°36'43"W); (104) Imbituba, Praia de Itapirubá (28°14'S, 48°40'W); (105) Itá, UHE (27°16'S, 52°19'W); (106) Laguna (28°29'S, 48°47'W); (107) Lebon Régis (26°50'58"S, 50°39'16"W); (108) Nova Teutonia (27°09'S, 52°25'W); (109) Rio das Antas (26°55'S, 51°04'W); (110) Rio Vermelho (= Curupá) (26°17'S, 49°20'W); **SÃO PAULO:** (111) Campos de Jordão (22°44'S, 45°35'W).

**PARAGUAY:** **ALTO PARAGUAY:** (112) Casado, Estancia Casilda (22°14'S, 57°55'W); **CONCEPCIÓN:** (113) Sapucaí (22°49'S, 56°24'W); **SAN PEDRO:** (114) Pavón (23°29'S, 56°59'W); (115) Primavera (24°30'S, 56°41'W).

**URUGUAY:** **ARTIGAS:** (116) Tomás Gomensoro (30°26'S, 57°26'W); **CANELONES:** (117) Carrasco (34°50'S, 56°01'W); **CERRO LARGO:** (117) Cuchilla del Mangrullo (32°14'S, 53°51'W); **LAVALLEJA:** (118) Cerro Cotto, 11.5 km E from Solís de Mataojo (34°36'S, 55°22'W); (119) Salto del Penitente (34°22'S, 55°03'W); **MALDONADO:** (118) Sierra de las Animas (34°42'S, 55°19'W); (120) Laguna Garzón (34°48'S, 54°34'W); (121) Maldonado (34°55'S, 54°58'W); **MONTEVIDEO:** (122) Barra de Santa Lucía (34°47'S, 56°20'W); **RIVERA:** (123) Campo Abasto, Rivera (30°54'S, 55°33'W); (123) Establecimiento Trinidad (30°53'S, 55°33'W); (124) Establecimiento Rocha, COFUSA (31°10'S, 55°30'W); (124) Establecimiento Vázquez (31°11'S, 55°21'W); (125) Estancia Trinidad, COFUSA. 20 km SE from Rivera (31°02'S, 55°33'W); (126) La Palma, Suarez (31°09'S, 55°55'W); **ROCHA:** (127) La Paloma (34°39'S, 54°10'W); **TACUAREMBO:** (128) Punta del Laureles (31°09'S, 56°09'W); (128) Rincón de Vasoura (31°12'S, 56°9'W); **TREINTA Y TRES:** (129) Bañado de Oliveras (33°07'10"S, 54°16'22"W).