



Geographic body size gradients in tropical regions: water deficit and anuran body size in the Brazilian Cerrado

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A recent interspecific study found Bergmann's size clines for Holarctic anurans and proposed an explanation based on heat balance to account for the pattern. However, this analysis was limited to cold temperate regions, and exploring the patterns in warmer tropical climates may reveal other factors that also influence anuran body size variation. We address this using a Cerrado anuran database. We examine the relationship between mean body size in a grid of 1° cells and environmental predictors and test the relative support for four hypotheses using an AIC-based model selection approach. Also, we considered three different amphibian phylogenies to partition the phylogenetic and specific components of the interspecific variation in body size using a method analogous to phylogenetic eigen vector regression (PVR). To consider the potential effects of spatial autocorrelation we use eigenvector-based spatial filters. We found the largest species inhabiting high water deficit areas in the northeast and the smallest in the wet southwest. Our results are consistent with the water availability hypothesis which, coupled with previous findings, suggests that the major determinant of interspecific body size variation in anurans switches from energy to water towards the equator. We propose that anuran body size gradients reflect effects of reduced surface to volume ratios in larger species to control both heat and water balance.

Body size gradients and thermoregulation have been conceptually linked since Bergmann (1847) proposed that cold macroclimates should harbor more large-bodied species than warmer areas, because the reduced surface area to volume ratios of larger animals would allow them to maintain body temperatures. Several interspecific studies in temperate to cold climates have supported Bergmann's rule and its associated heat conservation mechanism in endothermic organisms (mammals: Blackburn and Hawkins 2004, Rodríguez et al. 2006, Diniz-Filho et al. 2007; birds: Ramírez et al. 2008). However, the interspecific formulation of Bergmann's rule does not hold for endotherms in warm climates (Rodríguez et al. 2006, Medina et al. 2007, Greve et al. 2008, Rodríguez et al. 2008). On the other hand, although amphibians and reptiles remain largely underrepresented in the macroecological literature in comparison to mammals and birds, research on broad-scale body size gradients has not been exclusively limited to endotherms (Ashton 2002, Olalla-Tárraga et al. 2006, Olalla-Tárraga and Rodríguez 2007, Adams and Church 2008). Indeed, studies focused on vertebrates with limited capabilities for internal heat production are not only helpful to evaluate the generality of the patterns documented for mammals and birds but can generate insights into the energetic and physiological mechanisms that are likely

determining the spatial variation in body size across large scales.

In ectotherms, a relationship between thermoregulation and Bergmann's rule has also been supported to some extent in amphibians and reptiles since Lindsey (1966) first noted that some ectothermic vertebrate taxa show interspecific body size gradients in latitudinal space. For instance, anurans and lizards are believed to be thermoregulators (i.e. show behavioral control over their body temperatures through basking or selecting appropriate microhabitats) (Brattstrom 1963, Huey 1982, Hutchinson and Dupré 1992, but see Navas 2003) and Holarctic species in these groups increase in size towards colder macroclimates (Olalla-Tárraga et al. 2006, Olalla-Tárraga and Rodríguez 2007). In contrast, urodeles, which are believed to be thermoconformers (Brattstrom 1963, Hutchinson and Dupré 1992), are smaller in colder areas of the Holarctic region (Olalla-Tárraga and Rodríguez 2007), so thermoregulating abilities may also determine interspecific body size gradients in ectotherms. Nonetheless, ectotherms, including the best thermoregulators, may be less able to control body temperatures than endotherms. Therefore, it is not surprising that anurans and lizards have shallower size gradients and weaker relationships with ambient energy variables than endotherms (Olalla-Tárraga et al. 2006, Olalla-Tárraga and Rodríguez 2007), nor perhaps that

snakes, which also thermoregulate, have shown the converse to Bergmann's rule in the Holarctic (Olalla-Tárraga et al. 2006). In this latter case, the exception of snakes to the Bergmannian pattern displayed by anurans and lizards suggests that not only thermoregulation but the relative size of an organism determine the concordance with Bergmann's rule in thermoregulating ectotherms. That is, considering that snakes are generally larger than lizards and anurans, Bergmann's size clines might only be expected within groups of small sized species because longer heating times of larger animals limit the time available for other activities in colder high-latitude environments (see Olalla-Tárraga et al. 2006 for further discussion).

On the other hand, studies on amphibians and reptiles have been restricted to the Holarctic, which limits an extension of their conclusions to other regions. In this paper our primary goal is to document the body size gradients of anurans in a Neotropical region to compare with the patterns previously found for the Holarctic. Even though the Neotropical Realm has greater species richness of anurans than any other biogeographical region in the world (Duellman 1988), our knowledge of the distribution and ecology of the anuran faunas in the Neotropics is still rather limited (Ron 2000, Eterovick et al. 2005). In fact, this group is not an exception to the well-known Wallacean and Linnean shortfalls (i.e. a high number of species remain formally undescribed, and there is a paucity of distributional data for most species) (Bini et al. 2006). Furthermore, the lack of biological data is also apparent if we consider the difficulties in compiling body size data, a fundamental organismal trait, for many tropical anuran species (Cooper et al. 2008). These limitations prevent an analysis for the whole Neotropical region and lead us to focus on a single biome, the Cerrado, where enough biological information (extents of occurrence and body size) is ready available for all anuran species described so far (Diniz-Filho et al. 2004, 2005). After the Amazon rainforest, this is the second largest biome in Brazil (covers ca 204 million ha) and comprises enough environmental heterogeneity (Nimer 1989, Silva et al. 2006) that the potential consequences for anuran body size distributions should be apparent.

Here, we use an assemblage approach by examining the variation in average body size of all anuran species occurring in a $1^\circ \times 1^\circ$ grid of 181 cells in the Cerrado. Additionally, we use an information theoretic approach to investigate the relative support for a set of competing hypothesis that have been proposed to explain interspecific broad-scale body size gradients (Cushman 1993, Blackburn et al. 1999, Olalla-Tárraga and Rodríguez 2007). Specifically, we evaluate the following four potential explanations.

- 1) Heat balance: the heat conservation mechanism originally proposed to explain Bergmann's rule in endotherms was considered unlikely for ectotherms as their body temperatures rely both on heat loss and heat gain (Cushman et al. 1993). The heat balance hypothesis (Olalla-Tárraga and Rodríguez 2007) incorporates both processes and proposes interspecific Bergmann's rule patterns among those ectothermic taxa exhibiting thermoregulatory abilities when the larger species are below a certain body size threshold. Anurans are below this size
- 2) Water availability: amphibians are strongly dependent on water, which may determine their size patterns in the tropics. The rationale for this hypothesis is that the reduced surface-to-volume ratio of larger species makes them less prone to water loss, conferring a stronger desiccation tolerance in dryer areas (Nevo 1973, Ashton 2002). This hypothesis may be of special relevance in tropical regions with a pronounced dry season.
- 3) Primary productivity: in tropical climates, primary productivity is known to vary strongly depending on seasonality. Consequently, the productivity hypothesis predicts that large-bodied species will concentrate in highly productive environments because in less productive areas there may be insufficient food supply to maintain species with large body size (Rosenzweig 1968). This hypothesis was originally developed for mammals, which have much higher energetic requirements than anurans (Pough 1980). Hence, although a possible explanation that deserves testing, its relevance to anurans is unclear.
- 4) Habitat availability (topography \times macroclimate interaction): this hypothesis has been recently proposed to explain body size gradients of mammals in the Neotropical region. Mammals conform to Bergmann's rule in the northern parts of the Palearctic (Rodríguez et al. 2006) and the Nearctic (Blackburn and Hawkins 2004, Rodríguez et al. 2008), but not in the southern Nearctic and the Neotropics (Rodríguez et al. 2008). In the latter region, the geographic distributions of large-sized species are limited by the strong habitat zonation and reduced habitat areas in tropical/subtropical mountains and accumulate in the extensive habitat areas of the lowlands (Rodríguez et al. 2008). That is, mammal body size variation may be conditioned by topography and the effects that it exerts on habitat sizes. This hypothesis predicts larger bodied assemblages in the lowlands than in the mountains, although it should be noted that all anurans are small compared to most mammals.

Methods

Study area

The Cerrado is the most extensive woodland-savanna in South America (Silva and Bates 2002). Although it ranges in elevation from 300 to 2000 m. a.s.l., the topography is generally flat and only a few locations reach altitudes beyond 900 m (Clark 1992). The most important climatic feature of the Cerrado is its strong seasonality, with a dry period extending for up to six months, which has resulted in faunas and floras adapted to persist under prolonged dry conditions (Eiten 1972, Colli et al. 2002). In fact, because of the large number of endemic plant and vertebrate species (especially amphibians and reptiles, Silva and Bates 2002), the Cerrado has been considered a global biodiversity

hotspot, the only one dominated by tropical savanna and dry forests (Myers et al. 2000).

Anuran data

We used a distributional database for Cerrado anurans (Diniz-Filho et al. 2004, 2005). The database comprises 131 species belonging to eleven families (Aromobatidae, Brachycephalidae, Bufonidae, Cycloramphidae, Dendrobatiidae, Hylidae, Hylodidae, Leiuperidae, Leptodactylidae, Microhylidae and Ranidae) (Frost et al. 2006). We mapped the geographic distribution (extent of occurrence) of each species in a $1^\circ \times 1^\circ$ grid of 181 cells. For each species, we also compiled mean body size data (snout-to-vent length) (Colli et al. 2002, Diniz-Filho et al. 2004, 2005) and assigned a single body size measure (\log_{10} -transformed) to the distribution range of the species (Supplementary material Table S1). We then calculated the average \log_{10} body length (hereafter mean body size) across all species occurring in each grid cell (Blackburn and Hawkins 2004, Olalla-Tárraga et al. 2006, Olalla-Tárraga and Rodríguez 2007). It should be noted that our analyses do not incorporate intraspecific variation in body size since we assume that intraspecific spatial variation in body size is small relative to interspecific variation (Blackburn et al. 1999). Because we have insufficient data to validate fully this assumption, we did the following basic calculations to support our approach. We calculated the maximum to minimum body size ratio at the interspecific and intraspecific level for those species for which data exist. Using the complete Cerrado anurans database (131 species) we found a ratio of 13.63 for the interspecific level. We then estimated the intraspecific ratio using a subset of 16 species for which we have within-species variation in body size and we found the largest ratio for *Leptodactylus podicipinus* (2.25), although most ranged from 1 to 1.5. Thus, interspecific variation in Cerrado anurans appears to be much larger than intraspecific variation. Therefore, even if any intraspecific variation is geographically structured we not expect a strong influence in our analyses. This is a methodological assumption in most studies aimed to document body size patterns at an interspecific level and hence, albeit similar mechanisms may be operating at both taxonomic levels, our results must be interpreted in an explicitly interspecific context (Gaston 2008).

Hypotheses and environmental variables

We selected eight environmental predictors to evaluate the relative support for each hypothesis. They were obtained from GIS information layers in raster format and processed in ArcGIS 8.2 (ESRI 2002) to calculate average values for each grid cell. The hypotheses and their associated variables are: 1) heat balance hypothesis: we used minimum and mean annual temperatures as heat indicators and annual potential evapotranspiration (PET) as a combined measure of heat plus solar radiation inputs in the environment. Temperatures were obtained from a global 0.5° resolution database interpolated from weather station data for the period from 1961 to 1990 (New et al. 1999). The original PET dataset is based on a 50 yr time series from weather

stations (1950–1999) interpolated at a spatial resolution of 0.5° (Willmott and Kenji 2001). This variable was generated using a modified version of the Thornthwaite's formula (Willmott and Kenji 2001). 2) Water availability: annual precipitation and relative humidity were included as direct measures of water and moisture conditions in cells, and water deficit (WD) was used to measure the joint availability of biologically usable energy and water (Stephenson 1998, Rodríguez et al. 2005). Both the precipitation and relative humidity datasets were taken from New et al. (1999) and were originally generated from spatial interpolations of weather station data at 0.5° resolution for the period 1961–1990. We calculated WD as PET minus annual actual evapotranspiration (AET) (Stephenson 1998, Francis and Currie 2003). AET data were obtained from Willmott and Kenji (2001). 3) Primary productivity: we used the enhanced vegetation index (EVI), as a surrogate for net primary productivity. This and other vegetation indices have been widely used as indicators of plant productivity or standing crop in macroecological studies (Hurlbert and Haskell 2003, Hawkins 2004, Duro et al. 2007, Olalla-Tárraga and Rodríguez 2007). The EVI is generated from data collected by the Moderate Resolution Imaging Spectroradiometer (MODIS), and, similar to other vegetation indices, measures the greenness and density of the canopy, total standing biomass, green leaf area, and percent vegetation cover (Ferreira et al. 2003, Ratana et al. 2005). However, unlike other indices, EVI does not saturate in high biomass regions (Huete et al. 2002). EVI was calculated as monthly averaged composites for 2004–2005. 4) Habitat availability: elevation range is often used as an indirect measure of the effects of mesoscale climatic gradients on habitat variability (Hawkins and Diniz-Filho 2006, Rodríguez et al. 2008). Based on Janzen's hypothesis (1967), these authors argued that, in comparison to temperate montane areas, the greater climatic zonation associated with elevational changes in tropical regions generates sharp mesoscale habitat gradients. To incorporate the interaction between macroclimate and topographic relief and capture varying effects depending on the local inputs of environmental energy, together with elevation range we included its interaction with PET in multifactor models (Hawkins and Diniz-Filho 2006, Rodríguez et al. 2008). Elevation data were obtained from Clark (1992) and have an original resolution of 10 minutes.

Statistical analyses

Initially, we used Pearson correlations to explore the relationships between anuran mean body size and the environmental predictors. We then used ordinary least-squares multiple regression (OLS) to generate alternative models with different combinations of predictors and performed a model selection approach based on information theory to identify the best model using AIC (Burnham and Anderson 2002, Johnson and Omland 2004, Stephens et al. 2007). We also computed the ΔAIC_i of each model (i.e. $\Delta AIC_i = AIC_i - \min AIC$) to identify those models that are as good as the best model (i.e. with $\Delta AIC \leq 2$) (Burnham and Anderson 2002). Also, to evaluate the contribution of each variable to the models and assess the

Table 1. Pearson correlation coefficients of environmental variables and mean body size (snout-to-vent length, SVL).

Variable	Anuran SVL	Prec.	Rel. humid.	WD	Mean temp.	Min. temp.	PET	Range	EVI
Annual precipitation	<0.001	1							
Relative humidity	0.061	0.703	1						
Water deficit	0.555	-0.329	-0.406	1					
Mean annual temperature	0.170	-0.001	0.100	0.596	1				
Minimum temperature	0.263	0.071	0.109	0.667	0.960	1			
Potential evapotranspiration	0.300	-0.093	-0.030	0.745	0.821	0.815	1		
Elevation range	-0.260	-0.216	-0.189	-0.181	-0.417	-0.381	-0.361	1	
Enhanced vegetation index	0.063	0.333	0.375	-0.025	0.195	0.200	0.076	-0.124	1

relative support for each hypothesis, we used the standardized regression coefficients of the variables in the best models (Neter et al. 1996, Olalla-Tárraga et al. 2006). To minimize multicollinearity, we did not include highly correlated variables in multiple regression analyses. As a consequence, minimum and mean annual temperatures, highly correlated with PET, as well as precipitation, highly collinear with relative humidity (Table 1), were excluded. Additionally, in order to detect the presence of collinearity in our models, we calculated for each of them its condition number (CN) and the variance inflation factors (VIFs) of the predictors it includes (Belsey 1991, Neter et al. 1996, Lazaridis 2007). Variance inflation factors measure the degree to which collinearity inflates the estimated regression coefficients as compared to orthogonal predictors. The condition number is a commonly used index of the global instability of the regression coefficients calculated as the square root of the largest eigenvalue divided by the smallest eigenvalue of the correlation matrix. Typically, maximum VIF values lower than 10 and a CN lower than 5 indicate that collinearity is not a major problem (Belsey 1991, Neter et al. 1996, Lazaridis 2007).

Because cells in our spatial grid-based approach may not be fully independent, statistical problems associated with spatial autocorrelation are possible (Legendre 1993). To take this into account, we included eigenvector-based spatial filters truncated at the 1000 km distance as predictors in the regression models (the PCNM model – Borcard and Legendre 2002). These spatial filters have proven to be an effective tool to capture the spatial structure of the data at different spatial scales (Diniz-Filho and Bini 2005, Griffith and Peres-Neto 2006, Dormann et al. 2007). We used the maximization of the coefficients of determination in the regression between mean body size and the filters and the minimization in residual spatial autocorrelation as criteria to select non-redundant filters for the models and avoid “overcorrecting” for spatial autocorrelation (Griffith 2003, Diniz-Filho and Bini 2005). Because of strong collinearity between some filters and the environmental variables in the fitted model, we also excluded filters that were non-significant when added to the original environmental model. Additionally, we used spatial correlograms to evaluate if this approach removed the spatial autocorrelation of the residuals of our best multiple-regression models at all distance classes, which would indicate that the fitted models adequately describe the spatial variation in body size across all spatial scales (Diniz-Filho et al. 2003).

Some workers believe that it is necessary to adjust mean body sizes in grids by the number of species (Meiri and Thomas 2007). To accommodate this point of view we

used species richness values (SR) and $[1-\log_{10}(1/SR^2)]$ as weighting factors in a weighted least squares regression to recalculate the standardized coefficients of the variables included in our best models, as well as the coefficient of determination of each model (R^2).

Finally, we also analyzed body size variation using a numerical procedure based on the phylogenetic eigenvector regression (PVR) approach of Diniz-Filho et al. (2007), which allows partitioning total body size variation among species into a phylogenetic (P) and a specific component (S) (Diniz-Filho et al. 1998; see also Ramírez et al. 2008). The specific component S expresses the independent evolution in the trait after each species diverged from the others, so it can indicate adaptive processes related to climatic variation not confounded by deep history of lineages. However, there is no detailed phylogeny for the Cerrado species analyzed here, and using a phylogeny at higher hierarchical levels (i.e. family), as done by Ramírez et al. (2008), would be not too informative because there is actually a small number of families and most species would be concentrated in very few families (Hylidae, and Leiuperidae + Leptodactylidae = traditional Leptodactylidae) (Supplementary material Table S1).

Thus, instead of partitioning the total variation into P and S components as in PVR, we coded the taxonomic levels (species within genera and families) as dummy variables. We also used dummy coding for expressing higher-level relationships among families, based on phylogenies by Frost et al. (2006) Wiens (2007) and Roelants et al. (2007). For the last two phylogenies, families were clustered in the same way, using two dummy variables creating the Nobleobatrachia and (Natatanura + Microhylidae) clades. For Frost’s et al. (2006) phylogeny a different arrangement between families must be employed, mainly because these authors do not consider many of the “traditional” families as monophyletic. In this case, the genera were used to define the cluster of new families (Frost et al. 2006, p. 40). In general, using the better-known families as a reference only, Bufonidae and Dendrobatidae are closer to some Leptodactylidae, nested within a large clade that includes Ranidae, which is finally nested within a larger clade including Hylidae. In both cases, these taxonomic coding reflects phylogenetic clusters, and thus a nested ANOVA, using body size as response variable, can be used in comparative analyses in a way analogous to PVR (Harvey and Pagel 1991; see Diniz-Filho et al. 2005 for an application for anurans in Cerrado). In this case, the residuals of nested ANOVA estimate the S-component, and the R^2 of the model can be used to indicate the amount of phylogenetic effect in body size, as in PVR. Following

Diniz-Filho et al. (2007), the S-component of each species can then be averaged for each cell and regressed against the environmental predictors.

A recent species-level phylogenetic hypothesis for hylid frogs (Wiens et al. 2006) allowed us to conduct a complementary analysis within the Hylidae family. Considering the clusters of Hylidae genera in Wiens et al. (2006) phylogeny (i.e. Phyllomedusinae, Cophomantini, the clades Dendropsophus, Scinax and Lophiohylini) we used the abovementioned nested ANOVA analysis adding additional dummy variables. However, this increased slightly the proportion of variance from 61.9 to 68% (see Results) and S-components were in all cases highly correlated ($r > 0.89$), which suggest that including into the analyses such phylogenetic clusters within hylids does not affect qualitatively our main conclusions. Moreover, since there are no detailed species-level and generic-level relationships available for all families, we kept the analyses based on dummies for families, genus and higher order affinities as proposed by Frost et al. (2006), Wiens (2007) and Roelants et al. (2007).

All statistical analyses were performed with STATISTICA (StatSoft 2003) and SAM 3.0 (spatial analysis in macroecology; Rangel et al. 2006).

Results

We found a clear geographical gradient of anuran mean body size variation in the Cerrado, with the smallest mean sizes in the southwestern areas bordering the Pantanal, a wetland biome, and the largest sizes in the dryer areas of the northeast, near the limits of the Caatinga semi-arid region (Fig. 1). Consequently, mean body size was positively correlated with water deficit (Table 1), which described more variance than any other variable in the simple correlations ($R^2 = 0.31$). WD was also included in the two “best” multifactor models and had the highest standardized regression coefficients (Table 2). Both models had identical AIC values and described 45% of variance in mean body size. Potential evapotranspiration (PET) and relative humidity ranked second and third according to their regression coefficients (Table 2). The models also included topography, either directly or in interaction with PET, but the regression coefficients were low. Condition numbers and variance inflation factors were lower than 5 in all cases (Table 3), which indicates that collinearity among variables in these models is not a concern and we can use standardized regression coefficient values as estimates of the relative importance of each variable. Including non-redundant spatial filters in the models increased the percentage of described variance to 77% and removed residual autocorrelation in all cases – as indicated by spatial correlograms (not shown) – but this did not alter the variables or relationships described by the best models (Hawkins et al. 2007). Likewise, the best models remained qualitatively the same after accounting for anuran species richness with weighted least squares regression (Table 2), indicating that they were not affected by the uneven distribution of species richness throughout the Cerrado. Finally, the amount of phylogenetic signal in body size slightly differed when using the dummy variables derived from the three amphibian

phylogenies, being equal to 58.8% for nested ANOVAS based on Wiens (2007) and Roelants et al. (2007) phylogeny, and equal to 61.9% for nested ANOVA based on Frost et al. (2006). As expected, there was a high correlation ($r = 0.99$) between average S-components calculated for each ANOVA, since most body size variation can be “captured” by phylogenetic structures at genera and family levels and not by clusters of families which vary between the phylogenies used. Similar to the results for total body size, water deficit also had the highest regression coefficients in our multiple regression models for the average-S component of body size, which accounted for 34% of variance irrespective of the phylogenetic hypothesis used (Frost et al. 2006, Wiens 2007 or Roelants et al. 2007) (Table 4). We found a strong positive association between the geographical patterns in the specific component (S) of body size and water deficit (see also maps in Supplementary material Fig. S1), which suggests adaptive responses of species to climatic conditions independently of ancestry (Diniz-Filho et al. 2007).

Discussion

Our data identify a trend of increasing anuran body size along with decreasing available environmental water, as predicted by the water availability hypothesis. That is, the average body sizes were largest in the dryer northeast and smallest in the wetter southwest of the Cerrado biome. Water deficit, a measure of dryness levels in the environment or of climate drought (Stephenson 1998), was the best predictor of this gradient. The remaining hypotheses examined here did not receive substantial support, and only the secondary role of potential evapotranspiration in our regression models (associated to the heat balance hypothesis) merits attention (see below). Neither the habitat availability nor the primary productivity hypotheses played a significant role in determining anuran body size variation in the Cerrado region and, hence, we will not speculate on possible explanations for the lack of significance. However, it is possible that both hypotheses are important in tropical areas with a pronounced altitudinal gradient such as Andean slopes, a question that remains to be tested in future analyses.

Our assemblage approach, when combined with phylogenetic information, also found that the spatial pattern for the specific component (S) of body size is strongly related to water availability in the environment. Even in the presence of a strong phylogenetic inertia in body size variation (i.e. a high proportion of variance was explained by the phylogenetic structure in the data), we detected a clear signal of unique responses of each species to water deficit in the specific component (S). That is, species larger than expected by their phylogenetic relatedness are more frequent in drier environments, whereas species smaller than expected are mostly distributed in low water deficit areas. This finding suggests that there is a selective advantage for larger anurans under high water deficit conditions in tropical regions.

Most anurans need wet conditions to maintain their water balance, because they lose water from their skin at a rate similar to evaporation from a free water surface (Adolph 1933). Thus, they have little resistance to cutaneous

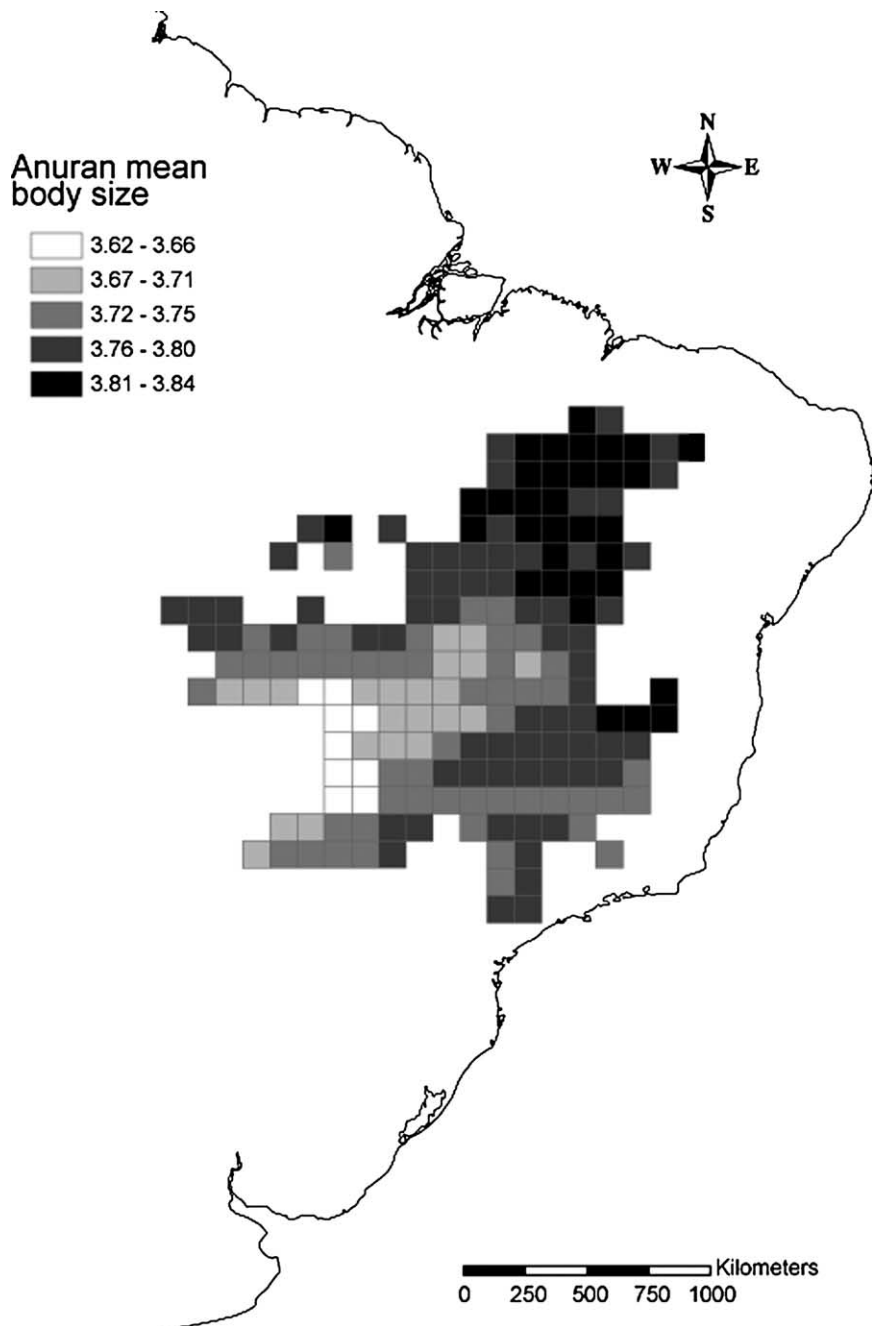


Figure 1. Mean anuran body size patterns in the Cerrado (131 species). Numbers in the legend are average \log_{10} -transformed snout-to-vent lengths (original units: millimeters).

evaporative water loss and are particularly sensitive to long droughts. The seasonal climate of the Cerrado is characterized by a six-month period of dry and hot conditions, which very likely imposes serious hydric constraints on its anuran fauna. Given that evaporative water loss is positively correlated with surface-to-volume ratios in anurans (Farrell and MacMahon 1969), a simple mechanism to reduce desiccation is decreasing the surface-to-volume ratio by increasing body size. The Cerrado has a southwest-to-northeast gradient of increasing dryness that may explain larger anuran sizes in the drier, northeastern areas.

Although the water availability hypothesis is most consistent with our data, only a third of the variance in

mean body size was described by water deficit alone, and our best multifactorial models explained only half of the variance (Table 2). This means that half of the spatial pattern of mean size is not associated with climatic gradients, suggesting that anuran tolerance to drought in the Cerrado is also influenced by size-independent traits. It has long been known that anurans have a wide array of adaptive mechanisms to withstand desiccation (Bentley 1966) and, observations in the neighboring, even drier (Silva 2004), Caatinga biome indicate that such mechanisms are common within the anuran communities in the dry Neotropics. According to Navas et al. (2004), many of the ~ 40 anuran species in the Caatinga have developed

Table 2. Multiple regression models for anuran mean body size in the Cerrado. Only the best models (i.e. $\Delta AIC \leq 2$) with their corresponding coefficients of determination (R^2) and the standardized regression coefficients of the predictors included in these models are shown. Env+Spa models include non-redundant spatial filters as predictors. Predictor variables are: Humidity = annual relative humidity; WD = water deficit; PET = annual potential evapotranspiration; Range = elevation range. The enhanced vegetation index (EVI) is not included among the predictors because it did not enter in any of the best models.

Model	Weighting variable	Predictors in model					Env		Env+Spa		
		Humidity	WD	PET	Range	PET \times Range	AIC	R^2	AIC	R^2	
1	None	0.274	0.658	-0.356	-0.098		1	-1206	0.455	-1343	0.765
2	None	0.269	0.650	-0.317		-0.087		-1206	0.454	-1342	0.764
1	$1 - \log_{10}(1/SR^2)$	0.458	0.747	-0.312	-0.154				0.446		0.758
2	$1 - \log_{10}(1/SR^2)$	0.449	0.735	-0.249		-0.133			0.446		0.758
1	SR	0.205	0.631	-0.379	-0.105				0.424		0.748
2	SR	0.200	0.624	-0.338		-0.096			0.425		0.748

physiological, behavioral and/or morphological adaptations to survive dry periods, including aestivation, phragmotic behavior and changes in skin morphology to improve water balance and thermal tolerance. Although the Cerrado is not as dry, it is likely that such size-independent mechanisms are common among their anuran species, which may account for the mean size variation not explained by climate.

Analyses in the Holarctic have also found relationships between climate and anuran mean body size gradients. Nonetheless, the Holarctic patterns depend more on the amounts of heat and light available in the environment (as indicated by a stronger negative relationship between body size and potential evapotranspiration) than on water availability (Olalla-Tárraga and Rodríguez 2007). Coupled with our present results (we found weaker relationships with PET), this suggests that anuran mean body size-climate relationships vary from temperate to tropical zones. In low energy regions (e.g. Holarctic), environmental energy availability is probably the primary driver of the body size-climate relationship, whereas water availability is the limiting factor in high energy macroclimates (e.g. Neotropics). This is not unexpected under the logic of the heat balance and water availability hypotheses. Both rely on a negative relationship between body mass and the surface-to-volume ratio, but differ in the proposed advantages of being large. That is, the heat balance hypothesis states that being large reduces heat loss, whereas the water availability hypothesis depends on a reduction in the amount of water that is evaporated from the skin. Therefore, a single explanation that incorporates both hypotheses can provide a general framework to understand body size gradients exhibited by this group across broad climatic gradients. This is similar to the water-energy conjecture of Hawkins et al. (2003) for broad-scale patterns of species richness, which

proposes that water availability is more critical in warm regions, whereas environmental energy is more critical in cold areas.

Because amphibians use environmental energy to warm their bodies, a very large body size may always improve water conservation, but it may simultaneously lead to insufficient heat gain under very low energy conditions, making it difficult to maintain heat balance (Olalla-Tárraga et al. 2006). Despite this, the fact that anurans as a group are larger towards the northern Holarctic suggests that, in general, the sizes attained by anuran species living at high latitudes are not constrained by heat gain (Olalla-Tárraga and Rodríguez 2007). Moreover, behavioural and/or physiological adjustments may help the larger, high latitude anuran species to reduce heating times, thus optimizing heat gain and converting their large bodies into an advantage for heat conservation. All in all, these arguments and results reinforce the idea that the heat balance and water availability mechanisms can be integrated into one hypothesis (the energy-water conservation hypothesis): a large body provides greater heat conservation in cold macroclimates and greater water conservation in dry tropical areas.

Exceptions to the above are likely to be found in extremely cold and dry regions, particularly because size-independent behavioral and physiological adaptations appear to be necessary for anurans inhabiting these areas to endure harsh environments (Storey and Storey 1996, Navas et al. 2004). In other words, a relatively large body size might not be enough to maintain optimum heat and water balances in extremely cold and dry habitats. Thus, anurans may be similar to other vertebrates, including mammals, which have size-independent adaptations to cope with cold (the burrowing capabilities of many rodents). These special adaptations have also been proposed to explain a lack of

Table 3. Condition numbers (CNs) and variance inflation factors (VIFs) of each predictor in our best multiple regression models. Values for environmental models (Env) and environmental models including non-redundant spatial filters as predictors (Env+Spa) are provided.

Model		Predictors in model					Condition number
		Humidity	WD	PET	Range	PET \times Range	
1	Env	1.536	3.390	3.029	1.206		3.495
2	Env	1.559	3.390	2.838		1.055	3.409
1	Env + Spa	2.658	4.623	3.626	1.273		4.334
2	Env + Spa	2.663	4.644	3.753		1.437	4.400

Table 4. Multiple regression models for the specific component of body size (S) obtained from the comparative phylogenetic analysis (see Methods). Results are presented for the best models in Table 2 and for two different amphibian phylogenies (Frost et al. 2006, Roelants et al. 2007). Analyses using Wiens (2007) phylogeny provided the same results than Roelants et al. (2007) and are not reported to avoid redundancy. Note that, contrarily to Table 2, AIC values are slightly lower for model 2 ($\Delta AIC \leq 1$). Presentation as in Table 2.

Model	Phylogeny	Predictors in model					Env	
		Humidity	WD	PET	Range	PET \times Range	AIC	R ²
1	Frost et al. (2006)	0.065	0.873	-0.451	-0.033		-1250	0.339
2	Frost et al. (2006)	0.061	0.861	-0.437		-0.043	-1251	0.331
1	Roelants et al. (2007)	0.054	0.867	-0.442	-0.046		-1242	0.343
2	Roelants et al. (2007)	0.049	0.862	-0.422		-0.056	-1243	0.345

conformity with Bergmann's rule in some endothermic groups (Rodríguez et al. 2006, Medina et al. 2007).

In conclusion, the geographical trend in anuran body size to increase towards high water deficit areas in the Cerrado suggests that the reduced surface to volume ratios associated with larger sizes reduces evaporative water loss. This generates greater resistance to desiccation, which may be critical for surviving in dry environments. In a parallel argument for colder regions, where energy availability – instead of water – is the main limiting factor, larger anuran species seem also to take advantage of a low surface to volume ratio to optimize their heat balance (Olalla-Tárraga and Rodríguez 2007). Therefore, in a context of latitudinal variation in the relative importance of energy and water from temperate to tropical regions, both hypotheses could be merged into a single explanation for anuran broad-scale body size gradients. If this proves to be general, it implies that qualitatively and quantitatively different environmental models may describe body size gradients in anurans depending on the extent and location of the study area across a latitudinal gradient.

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