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Biophysical Modeling of Water Economy Can Explain Geographic Gradient of Body Size in Anurans

Sidney F. Gouveia,[1],* Rafael P. Bovo,[2] Juan G. Rubalcaba,[3] Fernando Rodrigues Da Silva,[4] Natan M. Maciel,[5] Denis V. Andrade,[6] and Pablo Ariel Martinez[7]

1. Department of Ecology, Federal University of Sergipe, São Cristóvão, Sergipe 49100000, Brazil; 2. Department of Physiology, Institute of Biosciences, University of São Paulo, São Paulo 05508090, Brazil; 3. Department of Biology and Geology, Physics and Inorganic Chemistry, Rey Juan Carlos University, Mostoles, Madrid 28933, Spain; 4. Department of Environmental Sciences, Federal University of São Carlos, Sorocaba, São Paulo 18052780, Brazil; 5. Department of Ecology, Federal University of Goiás, Goiânia, Goiás 74001970, Brazil; 6. Department of Zoology, São Paulo State University, Rio Claro, São Paulo 13506900, Brazil; 7. Department of Biology, Federal University of Sergipe, São Cristóvão, Sergipe 49100000, Brazil

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ABSTRACT: Geographical gradients of body size express climate-driven constraints on animals, but whether they exist and what causes them in ectotherms remains contentious. For amphibians, the water conservation hypothesis posits that larger bodies reduce evaporative water loss (EWL) along dehydrating gradients. To address this hypothesis mechanistically, we build on well-established biophysical equations of water exchange in anurans to propose a state-transition model that predicts an increase of either body size or resistance to EWL as alternative specialization along dehydrating gradients. The model predicts that species whose water economy is more sensitive to variation in body size than to variation in resistance to EWL should increase in size in response to increasing potential evapotranspiration (PET). To evaluate the model predictions, we combine physiological measurements of resistance to EWL with geographic data of body size for four different anuran species. Only one species, *Dendropsophus minutus*, was predicted to exhibit a positive body size–PET relationship. Results were as predicted for all cases, with one species—*Boana faber*—showing a negative relationship. Based on an empirically verified mathematical model, we show that clines of body size among anurans depend on the current values of those traits and emerge as an advantage for water conservation. Our model offers a mechanistic and compelling explanation for the cause and variation of gradients of body size in anurans.

Keywords: allometry, biophysical model, ectotherms, evaporative water loss, macrophysiology, water conservation hypothesis.

Introduction

Body size of animals modulates multiple biological and ecological processes, such as resource consumption, interactions, population dynamics, community assembly, adaptation, and evolution (Peters 1983; Smith and Lyons 2013). Therefore, the existence of climate-driven gradients of this trait implies that environmental settings will affect such processes in some relevant way (Gaston et al. 2008; Pacifici et al. 2017). In the mid-nineteenth century, the physiologist Karl Bergmann predicted a negative body size–temperature relationship at broad spatial scales as a product of the effects of the allometry of surface-area-to-volume ratio on the retention and dissipation of body heat in endotherms (Salewski and Watt 2017). Christened Bergmann's rule, this relationship has received increasing support for endotherms (e.g., Ashton 2001; Blackburn and Hawkins 2004; Clauss et al. 2013; for counterexamples, see Salewski and Watt 2017) and has proven to be helpful for understanding their response to human impact (Santini et al. 2017). However, the applicability of Bergmann's rule to ectotherms has remained inconclusive (e.g., Ray 1960; Ashton 2002; Adams and Church 2008; Pincheira-Donoso 2010; Olalla-Tárraga 2011), precluding a clear picture of their response to environmental changes.

In view of this elusiveness, alternative explanations for the patterns of body size for ectotherms have been provided. For example, the water conservation hypothesis (Nevo 1973; Ashton 2001) suggests that the larger the animal, the more water it can conserve due to the logarithmic scaling of the volume-to-surface ratio. This rationale is analogous to the heat conservation hypothesis but with water instead of heat.

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* Corresponding author; email: sgouveia@ufs.br.

ORCIDs: Gouveia, http://orcid.org/0000-0003-4941-7440; Bovo, http://orcid.org/0000-0003-4345-6430; Rubalcaba, http://orcid.org/0000-0003-4646-070X; Da Silva, http://orcid.org/0000-0002-0983-3207; Maciel, http://orcid.org/0000-0001-5654-0645; Andrade, http://orcid.org/0000-0002-7662-2848; Martinez, http://orcid.org/0000-0002-5583-3179.

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This hypothesis was recently reformulated, suggesting that the positive relationship between body size and environmental evaporative energy in amphibians depends on both the climatic configuration and habitat preferences (see Gouveia and Correia 2016). That is, a positive body size–temperature relationship may exist if the distribution of the species comprises areas of high evaporative potential. In addition, this relationship will exist in clades that experience dehydration, especially arboreal ones, but not in aquatic and semiaquatic species (Gouveia and Correia 2016).

The reasoning behind the relationship between body size and water economy is in fact long-standing in ecophysiology (Jørgensen 1997). However, amphibians can also develop other physiological, behavioral, and morphological traits in response to climatic constraints. Along climatic gradients, a particularly relevant trait for compensating dehydration is the resistance to evaporative water loss (EWL; i.e., total resistance R_t). The trait R_t results from the sum of the skin resistance (R_s) and the boundary layer resistance (R_b). The skin resistance is determined by the structural elements of the animal tegument (Lillywhite 2006), whereas the boundary layer is a layer of air adhering to the animal surface, which creates resistance to evaporation (i.e., R_b; Spotila and Berman 1976; Tracy et al. 2010). Therefore, the emergence of geographical patterns of body size in response to evaporative gradients could be swapped with an increase in R_t for the same function of water conservation. Because body size and R_t affect water economy in different ways (Tracy et al. 2010), learning which circumstance favors one or another specialization should shed light on the generality and significance of geographical gradients of body size in ectotherms, as well as on the evolution of water economy per se.

To address this issue, we built on well-established biophysical equations of hydric relationships in amphibians to propose a state-transition model that links the reduction of EWL to changes in body size and R_t. As data of intraspecific variation of R_t across the distribution of species are lacking or unavailable, thus preventing us from addressing the relationship between R_t and climate, we tested which species should exhibit a geographical gradient of body size and which species should instead show variation of R_t or another process.

A Model of Water Economy in Anurans

The flux of water vapor from the amphibian skin to the atmosphere is determined by the concentration gradient of water vapor between the skin and the air, $\Delta\rho$ (mg cm^{-3}), and the total resistance R_t (s cm^{-1}). It is important to note that although R_s is the organismal trait that is under selective pressure, R_b may be relevant for the animal's endurance (Young et al. 2005) and thus should not be disregarded. Therefore, our model and analyses deal with R_t in all steps.

The evaporative flux, which is typically expressed in relation to the body mass of the animal, can be modeled as

$$\dot{m}_e = \frac{A}{M}\frac{\Delta\rho}{R_t} \tag{1}$$

(Spotila and Berman 1976), where $\dot{m}_e$ is the rate of evaporative water loss per unit of body mass, EWL (mg g^{-1} s^{-1}); A is the surface area of skin exposed to the air (cm^2); and M is the body mass (g). The total surface area of skin is a function of the mass of the animal, that is, $A_{\text{total}} = aM^b$, where the exponent b and the scaling constant a are approximately two-thirds and 10, respectively, for many anurans (Klein et al. 2016). Assuming that the exposed surface area of the skin is two-thirds of the total surface (Tracy 1976), we can substitute the exposed surface area by the mass in equation (1) to obtain

$$\dot{m}_e = \frac{2}{3}aM^{b-1}\frac{\Delta\rho}{R_t}. \tag{2}$$

Equation (2) represents nonlinear decays of EWL in relation to both body mass and resistance to EWL (fig. 1). This means that an increase in body mass reduces EWL to a differ-

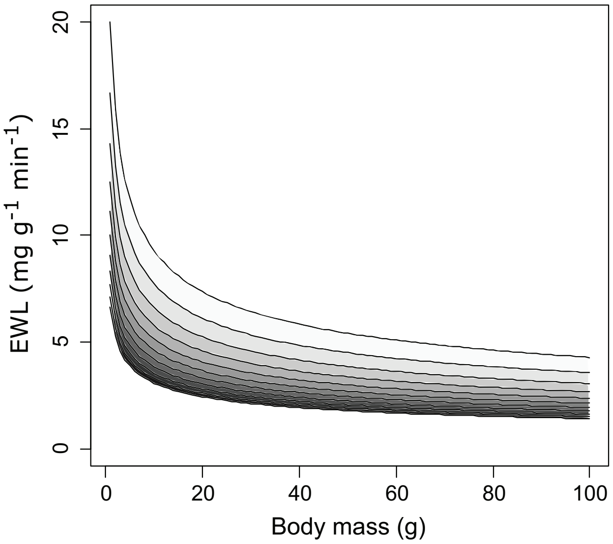

Figure 1: Model predictions for the rate of evaporative water loss (EWL) in relation to body mass and total resistance to water loss. Each line represents increasing values of R_t (eq. [2]), with shading changing from lower (light gray) to higher (dark gray) resistance. Note that the rate of decline in EWL decelerates as both body size and R_t increase. Curves were generated using $\Delta\rho = 0.02$ mg cm^{-3}, which represents a wet-skinned animal at $T_a = 30$°C and 30% relative humidity. Although arbitrary, this $\Delta\rho$ does not affect the conclusions drawn for the sensitivity analysis (i.e., the difference between partial derivatives with respect to mass and total resistance), provided that $\Delta\rho$ is large and has the same value in both partial derivatives.

ent degree in small and large animals, and an increase in resistance reduces EWL to a different degree when resistance is low or high.

Along an environmental gradient that requires the animal to reduce water loss, in addition to adjustments in behavior and activity pattern, selection would favor an increase of either body size or R_t. However, which of the two strategies is more effective depends on the current values of these traits. Increasing body size is more effective if the organism is small, as a result of the reduction of the surface-to-volume ratio as size increases. Alternatively, if the organism is large, increasing R_t may be more effective to reduce EWL. However, if R_t is high, there is little advantage in increasing it further, because of the also nonlinear decay of EWL with R_t. Therefore, one can expect that animals of different sizes and with different resistances undergo different selective regimes (i.e., increase M, R_t, or both) to reduce EWL in a more evaporative environment. To assess the effectiveness of each strategy, we need to quantify how much EWL varies as we increase body mass or resistance, that is, quantify the sensitivity of EWL to M and R_t. This information is provided by the partial derivatives of the water flux in relation to such variables. The partial derivative of EWL in relation to body mass,

$$\frac{\partial}{\partial M}\dot{m}_e = \frac{2}{3}a(b-1)M^{b-2}\frac{\Delta\rho}{R_t}, \tag{3}$$

provides information about the magnitude of change of EWL relative to variations in body mass. Substituting $a = 10$ and $b = 2/3$ (Klein et al. 2016), we obtain

$$\frac{\partial}{\partial M}\dot{m}_e = -\frac{20}{9}M^{-4/3}\frac{\Delta\rho}{R_t}. \tag{4}$$

Equation (4) takes negative values because increasing body mass reduces mass-specific EWL. Moreover, the function approaches zero for large body masses, which means that, all else being equal, EWL is more sensitive to variations in body mass in small compared with large animals (fig. 1).

We can now follow the same procedure to analyze the sensitivity of EWL to variations in resistance. Taking the partial derivative of the flux with respect to resistance to EWL and substituting a and b from equation (3), we obtain

$$\frac{\partial}{\partial R_t}\dot{m}_e = -\frac{20}{3}M^{-1/3}\frac{\Delta\rho}{R_t^{2}}. \tag{5}$$

In this case, EWL decreases in relation to the square of the resistance and approaches zero for large resistances. From equation (4) we know that the sensitivity of EWL to variations in body mass becomes smaller for large body masses. The same question can be addressed for the sensitivity of EWL to variations in resistance (eq. [5]): increasing resistance to EWL may be less effective for large animals, which already have low EWL, than for small animals.

We can thus express the sensitivity of EWL for several combinations of M and R_t. In this case, equations (4) and (5) are represented by two surfaces in a bidimensional plane (fig. 2). These surfaces may intersect (depending on the ranges of body masses and resistances), so that for some combinations of M and R_t, EWL will be more sensitive to the variation of body mass or resistance to EWL (fig. 2). Figure 2 suggests that animals of different body sizes and different resistance to water loss should follow different adaptive pathways to reduce EWL. While small animals with large resistance may increase body size in response to dehydration, large animals with small resistance should increase resistance. The transition from one strategy to the other occurs in the diagonal (fig. 2), and it is more abrupt in the bottom left corner (small animals with low resistance) than in the upper right corner, where the effectiveness of both strategies is low and the difference between them becomes smaller.

It is worth noting that these state transitions should occur only when the organisms undergo a selective pressure to reduce water loss. This means that the demand to reduce water loss is not expected, for example, in aquatic or semiaquatic species, in which water availability is not an issue (Wygoda 1984; Feder and Burggren 1992; Tracy et al. 2010), or where the risk of dehydration is minor, such as in highly wet environments (Young et al. 2006; Gouveia and Correia 2016).

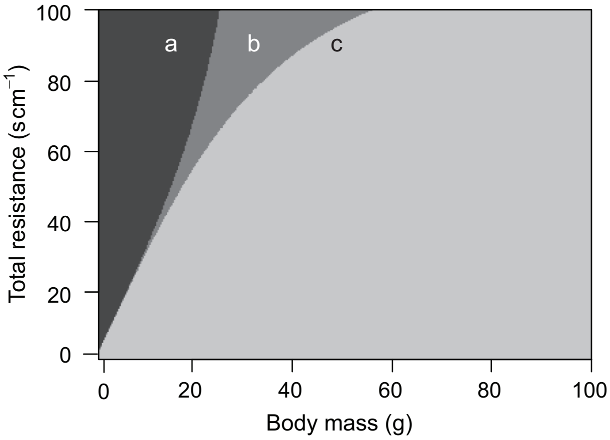

Figure 2: Strategies predicted to reduce evaporative water loss for anurans with different body masses, M, and resistances to water loss, R_t. Each point in the plane represents a combination of M and R_t. Depending on the combination, the organism will be more sensitive to variation body mass (a), will show similar sensitivity to variations in M or R_t (b), or will have greater sensitivity to variations in R_t (c) along a climatic gradient of evaporative energy. Ranges of axes are based on the variation of body size and skin resistance of most known anurans (see, e.g., Tracy et al. 2010).

Following the model, we should expect (i) variations in body size in relation to gradients of evaporative conditions to be more frequent in anurans that are small and resistant to desiccation and (ii) variations in resistance to water loss to be more frequent in anurans with large bodies and low resistance to desiccation. To test these predictions at the intraspecific level, data on the variation of body size and resistance to EWL along the entire geographical distribution of the species would be required. As far as we know, such information is scarce in the literature, especially with respect to resistance to EWL. However, based on the average values of body size and resistance of a species (taken from a set of individuals), we can still predict and test which species should exhibit a gradient in body size.

To assess these predictions, we tested the hypothesis that the body size of anurans relates to the spatial variation of potential evapotranspiration (PET) contingent on their combination of state values of body size and resistance to EWL. Specifically, we predict that small and more resistant anurans will exhibit a positive association between body size and PET across their geographic distribution. By contrast, we predict that both larger species and species with low resistance will not exhibit a significant body size–PET relationship at the macroscale level.

Methods

Studied Species

Our data set includes four species (table 1): two tree frogs (family Hylidae), the lesser tree frog *Dendropsophus minutus* Peters, 1872 and the gladiator tree frog *Boana faber* (Wied-Neuwied, 1821), which represent arboreal species of different sizes; a terrestrial-semiaquatic species, the barker frog *Physalaemus cuvieri* Fitzinger, 1826 (Leptodactylidae); and a terrestrial toad, *Rhinella icterica* Spix, 1824 (Bufonidae). All of these species occur in different habitat types, including closed, open, and disturbed habitats. *Dendropsophus minutus* occurs throughout tropical South America, *B. faber* is typical from Atlantic forest formation, *P. cuvieri* occupies mostly open formations across South America, and *R. icterica* is distributed across closed and open formation of southeastern and south Brazil.

Physiological Measurements

The individuals from which we took physiological measurements of resistance to EWL and body mass were collected at two mountain ranges in southeastern Brazil, Serra do Mar and Serra da Mantiqueira, encompassing an elevational gradient of 1,600 m. Although sampled populations do not cover the entire geographic range of the species, they are representative of a broad environmental variation across the altitudinal range, including tropical and subtropical conditions (Oliveira-Filho and Fontes 2000). After collection, animals were maintained under natural conditions of temperature (22°–27°C), relative humidity (45%–65%), and photoperiod (12L∶12D) in the Laboratório de Fisiologia Animal Comparada, São Paulo State University (UNESP), municipality of Rio Claro, São Paulo state, Brazil (ca. 300 km from the fieldwork).

Measurements were obtained for 54 specimens of *D. minutus*, 44 of *B. faber*, 52 of *R. icterica*, and 33 of *P. cuvieri*. Prior to measuring EWL rates, animals were maintained in individual polyvinyl chloride (PVC) containers filled with 0.5 cm of tap water in a climate-controlled chamber (BOD, 122FC model; Eletrolab) at 25°C for 1 h. They were then carefully blotted with paper tissue, their urinary bladders were emptied by gently pressing on their abdomens, and their body masses were recorded (±0.0001 g), which were considered as the standard masses (hydration level of 100%). Following this, we placed each animal in a circular PVC chamber (8 cm in diameter) connected to an automated open-flow system that measures EWL. For each individual anuran, we measured its EWL, which allowed us to calculate anuran to-

Table 1: Values of average and range (in parentheses) of body mass (M, in g), snout-to-vent length (SVL, in mm) across space, and total skin resistance to water loss (R_t, in s cm^{-1}) of the four species analyzed (*Dendropsophus minutus*, *Boana faber*, *Rhinella icterica*, and *Physalaemus cuvieri*)

Species	SVL (mm)	M (g)	R_t (s cm^{-1})	$\partial \dot{m}_e/\partial M$	$\partial \dot{m}_e/\partial R_t$	Difference	Pearson's r
B. faber	90.8 (77.4–98.9)	56.48 (41.67–89.98)	10.71 (6.88–13.93)	−.04	**−.91**	.87	−.66[**]
D. minutus	21.8 (18.1–24.8)	.59 (.40–.81)	7.59 (4.33–14.68)	**−23.71**	−8.27	−15.44	.62[*]
P. cuvieri	28.0 (25.7–30.4)	1.49 (1.02–1.81)	4.77 (3.41–6.35)	−10.96	**−15.41**	4.45	−.001
R. icterica	104.9 (81.6–149.6)	194.17 (45.17–373.23)	8.36 (5.25–12.11)	−.01	**−.99**	.98	−.08

Note: Also presented are estimates (based on the biophysical model) of sensitivity to variations in M ($\partial \dot{m}_e/\partial M$; eq. [4]) and R_t ($\partial \dot{m}_e/\partial R_t$; eq. [5]), the difference between sensitivities, and the observed Pearson's correlation coefficient between body size × potential evapotranspiration (PET) across geographical space. Values in boldface indicate a larger absolute value of sensitivity (i.e., the trait more sensitive to variation). For the difference, more negative values mean a stronger predisposition to exhibit a positive gradient in body size with PET. Pearson's r and the significance level of the relationship for *D. minutus* account for spatial autocorrelation.

[*] $P < .05$.

[**] $P < .001$.

tal resistance to water efflux (R_t, expressed as seconds per centimeter; Young et al. 2005). All EWL and body mass measurements were performed by the same person (R. P. Bovo).

The trial consisted of providing stable airflow current of 21.66 $cm^3 s^{-1}$, with 30% relative humidity (RH) controlled by an RH/Dewpoint Controller (DG-4; Sable Systems), by a pump combined to a mass flow meter (SS-4 Subsampler; Sable Systems). This flow provided 99% of air turnover for the experimental chamber every 3.5 min (Lasiewski et al. 1966). The airflow was directed into the experimental chamber containing the animal, and the RH of the excurrent air was continuously monitored by a water vapor analyzer (RH-300 RH/Dewpoint Analyzer; Sable Systems), which allowed us to calculate the amount of water lost per animal per unit of time from the increment in water content between the airflow upstream and downstream in the animal chamber. All equipment was maintained inside the climate-controlled chamber at 25°C. Changes in RH were interfaced to a computer by an analog/digital unit (UI-2; Sable Systems) and recorded and analyzed using Expedata software (Sable Systems).

The individual rate of EWL was then corrected for unit area of exposed (i.e., two-thirds) skin surface, which was estimated based on body mass (McClanahan and Baldwin 1969; Young et al. 2005) and the posture assumed by the animals during the experiments. We did not discriminate EWL through the respiratory system in our measurements since it is assumed to be negligible (Spotila and Berman 1976; Bentley and Yorio 1979; Wygoda 1984). Measurements lasted for up to 60 min per individual (usually 30–40 min for *D. minutus* and *P. cuvieri* and 40–60 min for *B. faber* and *R. icterica*). For quantitative estimates, we selected a period of steady-state readings that lasted for at least 10 min, typically during the last half of the measurement period, when the animal adopted a water-conserving posture. To verify the integrity of the system, we ran an empty chamber before and after each individual. Animal sampling was licensed by the environmental regulatory agency (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis; licenses 29703-1 and 22028-1) and the state government of São Paulo (Instituto Florestal; process 26018-013.054/2011). All experimental procedures were approved by the Ethical Committee in Animal Use at the Biosciences Institute affiliated with UNESP, Rio Claro (protocol 0820).

Broadscale Data

Occurrences and measurements of body size of *D. minutus*, *B. faber*, and *P. cuvieri* were obtained from Boaratti and Da Silva (2015), which was obtained from specimens deposited in four different biological collections in the Brazilian state of São Paulo, covering most of the geographical range of each species. To avoid the influence of sexual dimorphism, measurements consisted of the mean snout-to-vent length (SVL) of adult males within each local population (for details, see Boaratti and Da Silva 2015). Occurrence and body size data of *R. icterica* (also given as mean SVL of adult males in millimeters) were obtained by N. M. Maciel at zoological collections from 24 institutions in the United States, Europe, Africa, and South America (see table in the Dryad Digital Repository: https://doi.org/10.5061/dryad.hm22ps1 [Gouveia et al. 2018]). For all species, only localities with five or more sampled individuals were considered in our analyses. The number of sampling localities was 109 for *D. minutus*, 24 for *B. faber*, 34 for *R. icterica*, and 50 for *P. cuvieri*.

To represent the environmental capacity to remove water through evaporation, we used a global data set of annual PET at 30 arcsecs of spatial resolution near the Equator (Trabucco and Zomer 2010). PET describes a trade-off between water availability in the atmospheric and evaporative energy input (here based mainly on thermal energy). We drew the maximum annual PET from the location where the specimens were sampled, as provided by the geographical coordinates from the museum records (see Gouveia and Correia 2016).

Statistical Analyses

We assessed the intraspecific relationships between body size and PET through Pearson's correlation. However, analogous to the effect of phylogenetic autocorrelation on coefficient estimates in interspecific analyses, closely related populations could be more similar to each other due to this kinship rather than due to the environmental factors per se. To account for this effect, we adopted a space-for-time approach (Pickett 1989), in which spatial proximity was assumed to represent temporal relatedness. To this end, we assessed the presence of spatial autocorrelation in body size values of each species with Moran's I test. When spatial autocorrelation was present, we performed Pearson's correlation analysis with Dutilleul's (1993) correction of degrees of freedom. We adopted a 5% significance level for all statistical tests. Data underlying all analyses are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.hm22ps1 (Gouveia et al. 2018).

Results

Values of physiological traits, body size across the geographic range, and sensitivity to trait variation for all four species are summarized in table 1. Based on the combination of body mass and total resistance (i.e., skin resistance + boundary layer resistance) obtained from physiological measurements, *Dendropsophys minutus* was the only species sensitive to an increase in body size rather than resistance as a means to improve water economy (table 1). Other species showed higher sensitivity to variation in resistance to EWL, particularly *Physalaemus cuvieri*. *Rhinella icterica* and *Boana faber* had low EWL as a result of their larger bodies and high R_t; thus, they exhibited low sensitivity to variation in body size and moderate sensitivity to variation in resistance.

The advantage of an increasing body size for *D. minutus* compared with the other species analyzed is evident when we estimate the water economy that resulted from a slight increase in its size. We can derive this estimate from the negative power function described by the relationship between EWL and body mass. An increase in body size of 0.05 g in *D. minutus* (10% of its average size) results in an economy of 1.126 mg h^{-1} g^{-1} of water. This amount is three times greater than that estimated from a proportional increase (10%) in *P. cuvieri* (0.303 mg h^{-1} g^{-1}) and four orders of magnitude higher than that in the large-bodied species *R. icterica* (0.0002 mg h^{-1} g^{-1}) and *B. faber* (0.0008 mg h^{-1} g^{-1}).

Regarding broadscale data, only data on the body size of *D. minutus* were spatially autocorrelated (Moran's I $=$ 0.724; $P < .001$). Still, as predicted, Pearson's correlation analysis of the relationship between PET and body size (SVL) showed a significant positive relationship for *D. minutus* ($r = 0.659$; $P < .05$; table 1) after accounting for spatial autocorrelation. For the semiaquatic and terrestrial species *P. cuvieri* and *R. icterica*, no significant relationship was found, as predicted. For *B. faber*, for which we expected a nonsignificant relationship owing to the relative large body size, we found a significant but negative relationship instead (table 1).

Discussion

We have built on the long-standing knowledge of the biophysical dynamic of water exchange in anurans (Spotila and Berman 1976; Tracy 1976; Tracy et al. 2010) to provide a model that predicts potential pathways to reduce EWL across dehydrating gradients. Even though other attributes might also respond to such gradients (e.g., microhabitat selection and behavioral adjustments), our model provides a basis from which two critical attributes for water economy can be investigated in a geographical context, that is, the increase of either body size or resistance to water loss. In fact, deviations from the prediction of our model could inform the use of alternative strategies to circumvent EWL along a dehydrating gradient. By doing this, we accounted for intraspecific gradients of body size in anurans, a contentious issue in biogeography (e.g., Adams and Church 2008; Pincheira-Donoso 2010; Olalla-Tárraga 2011).

Our model accurately predicted which species did (*Dendropsophus minutus*) and did not (*Rhinella icterica*, *Physalaemus cuvieri*, and *Boana faber*) exhibit a positive body size–PET relationship based on their state values of size and total resistance to EWL. According to our model, species that were predicted not to exhibit the gradient of body size could be more prone to exhibit an increase in R_t as the environmental evaporative energy increases. This is indeed the case for the terrestrial, large-bodied toad *R. icterica* (family Bufonidae). Toads (including *R. icterica*) from semiarid conditions were found to possess greater resistance but not larger bodies than congeners from a mesic biome from the Neotropics (Titon and Gomes 2017). This result closely matches the predictions of our model but in this case involves an interspecific pattern.

In the case of the large-bodied tree frog *B. faber*, our model predicted that this species would not exhibit an increasing body size with PET, because any increase in its size would yield a negligible water economy for this species. According to our model, the sensitivity to neither variations in body mass nor resistance attained large values; thus, changes in these traits would be of limited adaptive value (table 1; see also Tracy et al. 2010). Interestingly, this species showed a negative body size–PET relationship. Although this finding does not contradict the prediction of our model, it suggests that other selective forces rather than water economy are driving the variation of body size in this species. *Boana faber* breeds in small and shallow ponds, which may require a faster development rate of tadpoles under dehydrating conditions, resulting in smaller adults (Gomez-Mestre et al. 2013). If this were the case, smaller sizes of this species in drier environments would result from smaller body sizes at metamorphosis in drying breeding sites (Liao and Lu 2012) and would underscore the prevailing claim that multiple factors are involved in shaping broadscale gradients of animal body size (Blackburn et al. 1999; Blanckenhorn 2000; Gaston et al. 2008).

Among anurans, water economy through increased skin resistance is closely linked to the preferred habitat of species. Aquatic, semiaquatic, and terrestrial species usually possess lower skin resistance than arboreal ones (Wygoda 1984; Feder and Burggren 1992; Young et al. 2005; Lillywhite 2006; Tracy et al. 2010). In fact, as nonarboreal species tend to stay inside or nearby water bodies, they compensate for dehydration by absorbing directly from water sources, including absorbing soil moisture through their permeable skin (Tracy 1976). Our model suggests that most species with low resistance (or high resistance but with large bodies) should not exhibit a geographical gradient of body size. As these species are mostly nonarboreal, we can therefore argue that the tendency of a species to exhibit a cline of body size across an evaporative gradient should be more prevalent in small-sized, skin-resistant, and therefore arboreal anurans. Still, it remains possible that some terrestrial species living under strongly desiccating environments (Thorson and Svihla 1943) are also prone to exhibit this pattern. For these cases, soil moisture could be an appropriate environmental descriptor of evaporative constraint but still operate through the mechanism proposed here.

An outcome of the differential sensitivity of species to water loss should include negative consequences of climate and land use changes on species and assemblages (Albright et al. 2017). If small-sized arboreal species are particularly sensitive to evaporative conditions, as climate change and habitat loss rapidly rearrange geographical patterns of evapotranspiration in many regions (Huang et al. 2015), species from these regions may not adapt fast enough to cope with these changes. At the level of species assemblages, this would affect the frequency distribution of both body size and resistance to EWL and thus their functional structure and diversity. In fact, and in agreement with this rationale, the increase in environmental aridity from anthropogenic impacts has slightly affected macroscale (Rapacciuolo et al. 2017) and local-scale (Pfeifer et al. 2017) patterns of body size distribution by removing small-sized amphibians.

Although only four tropical species belonging to three families were analyzed, our models and results provide a compelling case study of the causality and variation of causal factors underlying gradients of body size in anurans. As in many integrative approaches, ours is constrained by the availability of data from different research fields (Urban et al. 2016)—in our case, ecophysiological parameters and range-wide measures of body size of species. In addition, our model is more suitable to address variability in traits linked to water economy at the intraspecific level. When it comes to an evolutionary scale above species—for example, genera and families—different adaptations can be more likely to occur, such as behavioral adjustment, formation of cocoons, skin secretions, or other physiological traits (Navas et al. 2005). Nonetheless, our model may offer important insights into the evolution of gradients of body size and resistance at the interspecific level (e.g., Titon and Gomes 2015, 2017), as well as on how these features relate to the evolution of arboreality from a physiological standpoint (see Tracy et al. 2010).

In summary, we have provided a mechanistic model that predicts an increase in either body size or total resistance to EWL as possible adaptive pathways to improve water economy in anurans. According to this model, broadscale gradients of body size in anurans should arise under specific combinations of values of body size itself, resistance to EWL, and climatic configuration, thus providing a mechanistic basis to account for gradients of body size in ectotherms. Although all results provided a mechanistic support to the water conservation hypothesis (Gouveia and Correia 2016), we stress that if water conservation is of minor relevance in particular circumstances, other ecological processes that affect variations of body size can hinder the effect of water conservation or even become more important. Still, our model provides a theoretical basis to understand the effect of different processes acting on adaptations linked to water conservation. Finally, advancing and thoroughly testing these ideas will require integration of physiological, morphological, and macroecological data sources.

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