SHORT COMMUNICATION

Body size evolution in South American *Liolaemus* lizards of the *boulengeri* clade: a contrasting reassessment

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Abstract

Bergmann's rule predicts larger body sizes in species living in higher latitudes and altitudes. This rule appears to be valid for endotherms, but its relevance to ectotherm vertebrates has largely been debated. In squamate reptiles (lizards and snakes), only one study, based on *Liolaemus* species of the *boulengeri* clade, has provided phylogenetic evidence in favour of Bergmann's clines. We reassessed this model in the same lizard clade, using a more representative measure of species body size and including a larger number of taxa in the sample. We found no evidence to support Bergmann's rule in this lineage. However, these non-significant results appear to be explained only by the inclusion of further species rather than by a different estimation of body size. Analyses conducted on the 16 species included in the previous study always revealed significant relationships between body size and latitude–altitude, whereas, the enlarged sample always rejected the pattern predicted by Bergmann's rule.

Introduction

Much of the variation in the ecological and life-history patterns of animals is attributable to differences in body size (Peters, 1983; Losos, 1990; Blackburn *et al.*, 1999). Therefore, understanding factors affecting specializations in this trait is a major challenge in evolutionary biology (Belk & Houston, 2002).

Bergmann's rule predicts that body size in organisms tends to be positively correlated to latitude and altitude, and therefore, negatively correlated to environmental temperature (Bergmann, 1847; Blackburn *et al.*, 1999). The justification for this relationship is that larger body size reduces rates of heat loss per unit mass. This represents an advantage for endothermic physiologies, but less obvious is its relevance to ectotherms. Indeed, for ectotherm vertebrates, evidence supporting the hypothesis that geographical gradients constrain variation in body size remains elusive and controversial (Blackburn *et al.*, 1999; de Queiroz & Ashton, 2004). Instead, ecological adaptations such as perch preferences appear

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to be better predictors of divergence in this variable (Rummel & Roughgarden, 1985; Glossip & Losos, 1997).

The first non-phylogenetic studies testing Bergmann's rule in ectotherms showed supporting evidence (Ray, 1960; Lindsey, 1966). However, later phylogenetic comparative analyses have suggested that only turtles and salamanders tend to follow patterns predicted by this model (Ashton & Feldman, 2003), whereas fish, anurans, snakes and lizards appear to reverse the trend (Ashton, 2002; Belk & Houston, 2002; de Queiroz & Ashton, 2004). Remarkably, a recent study conducted on South American *Liolaemus* lizards of the *boulengeri* clade showed that these species follow Bergmann's rule (Cruz *et al.*, 2005). This research provided the only known phylogenetic evidence supporting this model in a monophyletic squamate (lizards and snakes) clade. However, two features of Cruz *et al.*'s (2005) study suggest their results should be interpreted with caution. First, several Patagonian species belonging to the *boulengeri* clade were excluded from their analyses. Second, these authors used the maximum recorded snout-vent length (SVL) for each species to estimate the effects of geographical gradients on the evolution of body size. It is unknown whether *Liolaemus* lizards follow asymptotic growth curves, therefore estimations based on the largest recorded individual may bias results (Brown *et al.*, 1999). According to

Brown *et al.* (1999), percentiles lower than the maximum SVL provide more accurate estimates for asymptotic size in lizards that do not follow asymptotic growth curves.

Here we reassess the problem of body size evolution under geographical gradients in the *Liolaemus* species of the *boulengeri* clade, to test for compliance with Bergmann's rule. We use a larger sample of species belonging to this clade, and a different methodology to estimate SVL. Our aim is not to challenge the work of Cruz *et al.* (2005), but to clarify the importance of latitude and altitude in their influence on body size in lizards.

Materials and methods

We gathered data on body size with respect to latitude and altitude from 319 individuals representing 26 species of the *boulengeri* clade (Cruz *et al.*, 2005; Supplementary Material). The sample comprises individuals from both sexes studied alive in the field and specimens housed in the institutions detailed in the Appendix. Museum specimens only include lizards preserved in the field immediately after collection, whereas those kept in captivity were omitted from these analyses. Additional geographical information was taken from previously published records (Cei, 1993; Etheridge, 1993; Cruz *et al.*, 2005; Pincheira-Donoso & Núñez, 2005). We used SVL as a proxy for body size. We calculated the arithmetic average of the largest two-thirds of the total sample of SVL measurements for each studied species (see Losos *et al.*, 2003). As *Liolaemus* species of the *boulengeri* clade may exhibit sexual size differences, with larger males, larger females or no sexual dimorphism (Cei, 1993; Etheridge, 1993, 2000; Pincheira-Donoso & Núñez, 2005; Pincheira-Donoso *et al.*, 2007), and because Cruz *et al.* (2005) did not restrict their analyses to a single sex, these mean values were calculated on the basis of both males and females, in order to estimate a mean value for the species. Whenever possible (23 of 26 species) the two-thirds of the total sample comprised a similar number of males and females.

As environmental temperatures decrease with both increasing latitude and altitude (Lutgens & Tarbuck, 1998; Ashton, 2002), we used the adjusted latitudinal midpoint (ALM) scale recently calibrated by Cruz *et al.* (2005). This scale and similar versions, broadly used for estimations of species' environmental conditions in comparative and multi-taxa studies (e.g. Espinoza *et al.*, 2004; Wiens *et al.*, 2007), combines the effects of latitude and altitude. The ALM scale is based on the assumption that environmental temperature in altitudinal transects declines 0.65 °C each 100 m of increased elevation (see also Lutgens & Tarbuck, 1998). Cruz *et al.* (2005) obtained a corrected latitudinal value for latitude and altitudinal thermal covariation using the formula $y = 0.009x - 6.2627$, where x represents the altitudinal midpoint for each species, and y the corrected tempera-

ture for latitude. Then, the y-value is added to the latitudinal midpoint for each species to give the ALM. We also calculated the adjusted latitudinal range (ALR) for each species, using the extremes of latitudinal and altitudinal distributions. For the regression analyses we used ln(SVL) as dependent variable, and ALM and ALR as predictor variables. Inclusion of ALR was necessary to compare species that shared a geographical centre but differed in their tolerance of ecological extremes.

We analysed data sets using two different methods (see Cruz *et al.*, 2005), including our improved estimates of SVL for each species (see above). First, we used raw data on ln(SVL) and ALM, without explicit phylogenetic control. We analysed the 16 identifiable species of the *boulengeri* clade used by Cruz *et al.* (2005), and then a larger sample of 26 species belonging to this lineage. Second, we used independent contrasts (Felsenstein, 1985) calculated using COMPARE version 4.6b (Martins, 2004), to control for phylogenetic effects. In the first phylogenetic analysis, we re-analysed the relationship between ln(SVL) and ALM for the 16 identifiable species of the *boulengeri* clade used by Cruz *et al.* (2005). We then analysed our whole phylogenetic data set using the same procedure, but including additional species (see Supplementary Material). Variables were evaluated under the phylogenetic hypothesis of Cruz *et al.* (2005) and additional phylogenetic evidence provided by recent studies (Espinoza *et al.*, 2004; Pincheira-Donoso *et al.*, 2007; Fig. 1). As our phylogenetic hypothesis is based on
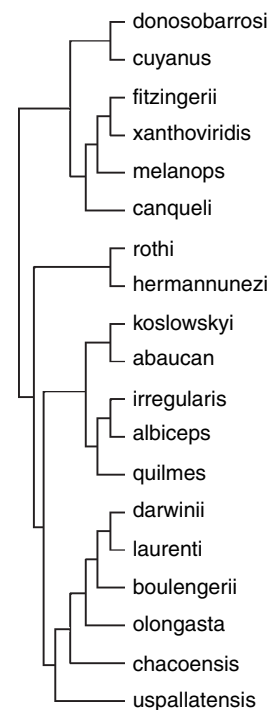
Fig. 1 Phylogenetic relationships between the *Liolaemus* species of the *boulengeri* clade.

different data sets, we applied a speciational model assuming branch lengths equal to 1.0 (Espinoza *et al.*, 2004; Martins, 2004). Following Cruz *et al.* (2005), we checked for the absence of phylogenetic autocorrelation using Abouheif's (1999) test for serial independence (TFSI). Analyses of standardized residuals and Cook's distance values (< 1.0) revealed that the addition of new species did not introduce potential outliers into the regression model (Cook & Weisberg, 1982).
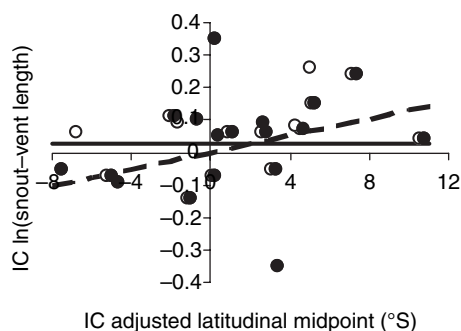
Results

Re-analysis of species used by Cruz *et al.* (2005)

Linear regression of ln(SVL) against ALM for a common subset of 16 species of the *boulengeri* clade supported Cruz *et al.*'s (2005) evidence for Bergmann's rule in these lizards (raw data, $R^2 = 0.364$, $F_{1,15} = 8.002$, $P = 0.013$; independent contrasts, $R^2 = 0.284$, $F_{1,15} = 5.560$, $P = 0.033$, Fig. 2).

Analysis of the expanded clade

Bivariate regression analyses revealed that ALM does not predict increasing variation in species body size when using the entire data set of 26 species of the *boulengeri* clade (see above; also Supplementary Material) and estimating SVL on the basis of the two largest thirds of the total sample (raw data, $R^2 = 0.067$, $F_{1,25} = 1.730$, $P = 0.201$; independent contrast analysis, $R^2 = 0.081$, $F_{1,18} = 1.496$, $P = 0.238$, Fig. 2). Analysis of independent contrasts using this model revealed no contribution of ALR to variation in body size ($F_{1,18} = 0.245$, $P = 0.614$). We found no evidence for phylogenetic autocorrelation after independent contrast analysis (Abouheif, 1999; Cruz *et al.*, 2005) using tests for serial independence (Reeve & Abouheif, 2003) with

1000 random shuffles and 1000 random phylogeny-rotations (independent contrasts, ln(SVL), $P = 0.376$; ALM, $P = 0.076$).

Explanation of results

We consider two possible reasons why the results of our expanded analysis may not be due simply to an improvement of statistical power. First, we used a different methodology for measuring SVL. Second, the species added to the clade sample may have been biased or 'extreme' in some way. We can discount both mechanisms. First, non-significant relationships were also observed if we analysed the maximum recorded SVL for the expanded clade (raw data, $R^2 = 0.091$, $F_{1,25} = 2.389$, $P = 0.135$; independent contrast analysis, $R^2 = 0.085$, $F_{1,18} = 1.585$, $P = 0.225$, see Cruz *et al.*, 2005). Second, the species added to expand the clade sample do not exist outside the body size, altitude and latitude ranges of the original, smaller sample (see Supplementary Material).

Discussion

In agreement with previous studies conducted on ecto-therm vertebrates (e.g. Ashton & Feldman, 2003; de Queiroz & Ashton, 2004), and in contrast to the findings of Cruz *et al.* (2005), our results do not provide support for Bergmann's rule in the *Liolaemus* species of the *boulengeri* clade. Differences in latitude and altitude do not predict evolution of larger body sizes in the species belonging to the studied reptile lineage. Although we controlled the potential statistical bias that analysis of maximum recorded SVL may cause in lineages that do not experience asymptotic growth curves (see above; Brown *et al.*, 1999), the disagreement between Cruz *et al.*'s (2005) study and our results appears to be explained by increasing the number of species in the analyses. Indeed, the analysis of the same 16 species included in the Cruz *et al.*'s (2005) study revealed a significant relation between ALM and increasing body size no matter which method was used to estimate SVL. In contrast, the study of the enlarged species sample weakened the relationship between SVL and ALM to such an extent as to prevent rejection of the null hypothesis. Hence our expanded sample provided no evidence for Bergmann's rule, regardless of the methodology used to estimate body size.

It is not surprising to find that the species of this clade do not follow Bergmann's rule. Smaller body sizes in cooler environments appear to be advantageous for cold-blooded vertebrates, because increasing body mass means that heating rates are slower (e.g. Shine, 1980; Ashton & Feldman, 2003). Therefore, the optimal body temperatures to allow basic biological activities (e.g. feeding, reproduction) can be reached in shorter spans of times (Jaksic, 1998; Mattison, 1999).

Fig. 2 Linear regression of independent contrasts for ln(SVL) against adjusted latitudinal midpoint in the *Liolaemus* species of the *boulengeri* clade, using the species analysed by Cruz *et al.* (2005; see above) (open circles, dashed line $y = 0.013x$) and a new phylogenetic model that includes additional taxa in the phylogeny (filled circles, solid line represents no significant linear relationship).

We conclude that adequately-replicated evidence supporting Bergmann's rule in reptiles is so far restricted to turtle lineages (Ashton & Feldman, 2003). There remains no strong evidence for Bergmann's cline in other reptile groups (de Queiroz & Ashton, 2004). Finally, we suggest the need to test Bergmann's rule in species-rich monophyletic clades of ectotherms with taxa adapted to contrasting environmental conditions, based on samples covering a high proportion of their known diversity.

Acknowledgments

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Summary of the *Liolaemus* species included in this study.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1365–2745.2007.01394.x

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Appendix

Museo Nacional de Historia Natural de Chile (MNHNC), Zoological Museum, Universidad de Concepción, Chile (MZUC), Museo de Historia Natural de Concepción, Chile (MHNC), Institute for Animal Biology, Universidad Nacional de Cuyo, Argentina (IBAUNC), Instituto Argentino de Investigaciones en Zonas Áridas, Argentina (IADIZA), Division of Reptiles and Amphibians, Natural History Museum of London, UK (NHML), and diagnostic collections of J. M. Cei (JMC-DC), J. A. Scolaro (JAS-DC).