

Cross-species and assemblage-based approaches to Bergmann's rule and the biogeography of body size in *Plethodon* salamanders of eastern North America

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Over the past few years, there has been a resurgence of interest in the investigation of spatial patterns in body size over large-scales to explore Bergmann's rule at different phylogenetic scales across a range of taxa (Blackburn et al. 1999, Gaston et al. 2008). In its original formulation Bergmann's rule predicts that among closely related endotherms those living in colder regions tend to be larger than those in warmer environments as a result of their reduced surface-to-volume ratios and, hence, better heat conservation (Bergmann 1847). Remarkably, the debate around the validity of this ecogeographical "rule" has long been fostered by the conflation of pattern (increasing body size towards colder regions) and mechanism (heat conservation) (Blackburn et al. 1999). However, there is increasing recognition that the geographical distribution of body size is markedly idiosyncratic and Bergmann's physiological mechanism cannot explain the observed size clines everywhere (Rodríguez et al. 2008, Diniz-Filho et al. 2009). Accordingly, a differentiation has to be made between pattern and process, so that the latter is not explicitly inherent to the former. In doing such distinction we do not only spur the scientific debate around the validity of the "rule", but the search of alternatives to Bergmann's original mechanism that are able to account for the observed size clines.

Part of the controversy around Bergmann's rule stems from the seminal papers of Ray (1960) and Lindsey (1966) suggesting that some ectothermic organisms display intra and interspecific body size variation as a response to environmental gradients, which apparently requires alternative explanations to the ones offered for endotherms (Cushman et al. 1993, Ashton and Feldman 2003, Olalla-Tárraga and Rodríguez 2007). Since then, workers have tried to identify ecological or evolutionary mechanisms accounting for body size clines in ectotherms, but we are far from a consensus on a unifying mechanism.

A critical step before searching for underlying mechanisms is indeed examining what the patterns look like in nature. Because Bergmann's rule was originally formulated for endothermic vertebrates, an abundant number of studies have reported the existence of body size gradients in mammals and birds (Ashton et al. 2000, Ashton 2002a, Rodríguez et al. 2008, Diniz-Filho et al. 2009, Olson et al. 2009), whereas the geographical variation of body size for many ectothermic organisms remains mostly unknown (but see below). Several authors have documented the patterns and explored the causes of body size variation in ectotherms at different levels of biological organization (Ashton 2002b, Belk and Houston 2002, Ashton and Feldman 2003, Blanckenhorn and Demont 2004, Olalla-Tárraga et al. 2006, Olalla-Tárraga and Rodríguez 2007, Adams and Church 2008). Notwithstanding, the variety of methods and terminologies involved not only prevent a comparison of results from different studies but also indicate the lack of agreement on a single standard approach to investigate Bergmann's rule. Gaston et al. (2008) recently attempted to clear up part of the confusion by identifying three kinds of approaches to studying spatial patterns in biological traits in general and Bergmann's rule in particular: intraspecific, interspecific and assemblage-based. They stressed that the distinction between intra- and interspecific approaches seems to be clear, but there is some confusion on the methodological differences between interspecific (hereafter "cross-species") and assemblage-based studies. Beyond semantic considerations (e.g. the assemblage-based approach has also been termed as the "community", "interspecific" or "grid-based" approach) (Blackburn and Hawkins 2004, Olalla-Tárraga et al. 2006), the methodological disparities between cross-species and assemblage-based approaches may not be trivial in terms of interpreting patterns and processes (Ruggiero and Hawkins 2006). In our view, this requires particular attention.

Here, we first highlight the conceptual basis of cross-species and assemblage-based approaches to outline the advantages and limitations of each of them. We then illustrate the differences by using distributional and body size data for *Plethodon* salamander species in eastern North America as a case study. Recently, Adams and Church (2008) used a cross-species analysis to evaluate Bergmann's rule in this group. From their results, complemented with a meta-analysis of the intraspecific relationship between body size and temperature for 59 amphibians (including 38 *Plethodon* species), they concluded that amphibians do not follow Bergmann's rule and discarded the classical heat conservation hypothesis as a valid explanation in this taxon. We conduct complementary analyses to show how the combination of an assemblage-based approach with phylogenetic eigenvector regression (PVR) (Diniz-Filho et al. 2007) can be useful to generate further insights into potential evolutionary and ecological mechanisms. In the light of our findings, we argue that it is premature to dismiss the existence of geographic body size gradients in amphibians, much less the search for causal factors.

Cross-species vs assemblage-based approaches

Cross-species approaches treat each species as an independent datum and use bivariate scatter-plots to examine covariation of body size and latitude (or temperature) across species. This method has also been termed as the "midpoint approach": it involves obtaining a single spatial measure for each species (usually the latitudinal midpoint of its geographic range) and then plotting these mid points against the species' body sizes (see Blackburn and Hawkins 2004, and references therein). In contrast, assemblage-based studies explore geographical patterns within grids covering the study region and combine the species' presences/absences in the cells with their body sizes to obtain cell-mean body size values (usually log-transformed geometric means) (Ruggiero and Hawkins 2006 and Gaston et al. 2008). Blackburn and Hawkins (2004) termed this the "community approach", as such investigations examine the spatial distribution of summary statistics for body size across faunal assemblages (grid cells) of a particular biogeographic region. Therefore, the units of analysis in cross-species approaches are single species, whereas in assemblage-based methods are measures of average body size of all the species occurring within grid-cells.

The pros and cons of cross-species and assemblage-based approaches have been discussed elsewhere (Blackburn and Hawkins 2004, Ruggiero and Hawkins 2006, Meiri and Thomas 2007). Notably, both methods need to circumvent potential problems associated with different sources of pseudoreplication. In assemblage-based studies, the varying proximity among units of analysis (i.e. grid cells) makes them have different levels of spatial autocorrelation. This causes degrees of freedom estimated in the usual way to be inflated, which will lead to increased type I error if ignored. Spatial statistical techniques allow tackling this (Diniz-Filho et al. 2003). In contrast, in cross-species analyses, inflated type I errors associated to pseudoreplication may arise from the phylogenetic non-independence of the data, which

requires the use of phylogenetic comparative methods. Assemblage-based studies may also suffer from phylogenetic autocorrelation effects (Olalla-Tárraga and Rodríguez 2007), but it is only recently that workers have found methods to jointly deal with spatially and phylogenetically structured data (Diniz-Filho et al. 2007, Kühn et al. 2009, see below).

According to several authors (Blackburn and Hawkins 2004, Ruggiero and Hawkins 2006), the main advantage of assemblage approaches is that they allow a direct evaluation of the environmental structure underlying broad-scale geographical patterns, a feature that is severely limited in the case of cross-species analysis as environmental gradients are reduced to a single point in geographical or environmental space. That is, cross-species methods ignore the geographical structure that exists in the data by reducing the multidimensional nature of geographic ranges to single values. This can have serious implications for the interpretation of ecological and evolutionary patterns and processes such as those associated to Bergmann's rule, even leading to incorrect or equivocal conclusions (Blackburn and Hawkins 2004, Ruggiero and Hawkins 2006). Because ecological and evolutionary processes usually take place in a geographical context, spatially explicit approaches are necessary to gain a multidimensional perception of species' trait gradients (such as body size gradients) and to make explicit their links to environmental variation (Ruggiero and Hawkins 2006).

While Meiri and Thomas (2007) also stressed the above-mentioned limitations of cross-species studies, they paid special attention to those of assemblage-based approaches. Specifically, they pointed towards the potential sensitivity of the analyses to the uneven variation of species richness across cells and the use of mean log masses (or lengths) within each cell as a measure of body size. They emphasized the need to control for the effects of richness (because species are not added to cells at random) and claimed that measures of central tendency other than the mean are preferred (because body size distributions are often right-skewed at broad spatial scales) (Brown 1995). As a solution, they have suggested including species richness as a covariate in multiple regression models and using median body size rather than the mean (Olson et al. 2009). However, far from being a pervasive problem with the assemblage-approach, it should be noted that these concerns only make sense in the absence of log-normal size distributions and the existence of species-poor cells. The solution to both potential problems is straightforward since we can evaluate to what extent the use of means or the spatial distribution of species richness affects the results.

Phylogenetic components of body size and the assemblage approach in *Plethodon*

We followed the methods described in Diniz-Filho et al. (2007). Initially, we created a 110 × 110 km equal area grid of 319 cells and used geographic range maps (IUCN, Conservation International and NatureServe 2006) for each of the 44 *Plethodon* salamanders described in eastern North America together with body size data for each species (kindly provided by Adams and Church 2008) to calculate

average \log_{10} body length (hereafter mean body size) in each grid cell (see Olalla-Tárraga et al. 2006, Olalla-Tárraga and Rodríguez 2007 for details). A single body size estimate was used for each species assuming that intraspecific spatial variation in body size is small relative to the interspecific variation (Gaston et al. 2008, Olalla-Tárraga et al. 2009). Almost 80% of the *Plethodon* salamanders in eastern North America show no relationship between temperature and body size at the intraspecific level, whereas >15% display a significant positive correlation (Adams and Church 2008). Because only three species display significant negative relationships between temperature and intraspecific size variation, contrary to the observed assemblage (interspecific) level pattern (see below), there are strong arguments to believe that our analyses are not only unaffected, but could be reinforced by the inclusion of intraspecific geographic size trends.

Analyses based on \log_{10} medians generated similar estimates to those obtained for \log_{10} means (Supplementary material Table S1). Because species of *Plethodon* have been extensively used in behavioral, ecological and evolutionary studies, their ranges (extents of occurrence) are well known (see Highton 1995 and Lannoo 2005 and references therein).

We then used phylogenetic eigenvector regression (PVR) (Diniz-Filho et al. 1998; see also Desdevises et al. 2003, Kühn et al. 2009). PVR estimates the phylogenetic signal in body size data by regressing this trait against a set of orthogonal eigenvectors extracted from a pairwise phylogenetic distance matrix that describes phylogenetic relatedness among species. Hence, we can partition the total body size of each species (T) into a phylogenetic component (P), which represents its predicted body size value according to the phylogeny, and a specific or “ecological” component (S), which corresponds to the model residuals and can be interpreted as the independent response of the species (see also Diniz-Filho et al. 2009 for a detailed explanation). Rohlf (2001) criticized the PVR method because all eigenvectors would be necessary to represent the entire phylogeny and, thus, estimates of P and S could be biased by eigenvector selection. Nonetheless, simulation studies have revealed correct type I error rates when estimating phylogenetic correlations using PVR (Martins et al. 2002, Diniz-Filho and Tôrres 2002). Moreover, using a few eigenvectors is enough to take phylogenetic autocorrelation into account and ensure species’ independence in respect to the analysed trait for further analyses. To check this assumption, we used Moran’s I autocorrelation coefficients in the S-component (see also Diniz-Filho and Tôrres 2002).

We based our PVR analysis on a species-level phylogenetic tree for *Plethodon* (Wiens et al. 2006) and selected the first five eigenvectors for the analysis, by successively adding eigenvectors to remove residual autocorrelation (Diniz-Filho and Tôrres 2002). These eigenvectors described 95.3% of the variation in phylogenetic structure and were subsequently used as predictors of body size. A multiple regression model of body size against the selected eigenvectors explained 63.2% of the variance in size, with significant contribution of all eigenvectors, thus indicating the existence of phylogenetic signal in the data. Also, it is worthwhile noting that there is no autocorrelation

in model residuals (Moran’s $I = -0.07$; $p = 0.486$), so that the set of eigenvectors we used were appropriate to describe phylogenetic patterns in body size and ensure species’ independence in respect to trait variation. Using the predicted and residual values of this regression (i.e. the P and S component values obtained for each species), we generated mean-P and mean-S values for each cell (Fig. 1a and b respectively). Finally, we built regression models to evaluate the relationships between the spatial patterns observed for mean-P and mean-S components and environmental variation.

We processed three environmental variables in ArcGIS 9.2 at the resolution of the equal area grid cells to incorporate them as predictors into the analyses: mean annual temperature, annual precipitation, and the Global Vegetation Index. Following a standard procedure in macroecology, the original data in raster format were upscaled by averaging all 0.5 degree pixels to the resolution of our grid cells (110×110 km) (see Olalla-Tárraga et al. 2006 and Olalla-Tárraga and Rodríguez 2007, for further details on data description and sources). These variables were selected on the basis that they are related to three hypotheses proposed for explaining body size gradients in amphibians, heat balance, water availability and primary productivity, respectively (Olalla-Tárraga and Rodríguez 2007). Because most *Plethodon* species occur in forested areas of the Appalachian and Ouachita mountains (Highton 1995), we also added to our models range in elevation within each cell as a measure of mesoscale climatic variation (Olalla-Tárraga et al. 2006, see also Rodríguez et al. 2008). Statistical analyses were performed with SAM (Spatial Analysis in Macroecology; Rangel et al. 2006), STATISTICA (StatSoft 2003) and PDAP (Phenotypic Diversity Analysis Programs; Garland et al. 1993).

In contrast to Adams and Church’s (2008) cross-species analysis, our assemblage approach identified a strong gradient of body size variation in *Plethodon* assemblages in geographical space, with decreasing size northwards. Thus, *Plethodon* salamanders in eastern North America follow the converse to Bergmann’s rule. This pattern is partly influenced by the occurrence of *Plethodon cinereus*, a small-bodied species, towards the northern distributional limits of this clade. A large proportion of the current distribution range of *P. cinereus* lies in northern latitudes that were covered by the Laurentide ice sheet in the Pleistocene and could only be colonized after the glacials retreated. Hence, a third of the data consist of monospecific assemblages of *P. cinereus* which may lead to the suggestion that the extreme northerly distribution of *P. cinereus* is an artifact and the species should be removed from the analysis. Even though we favor the view that there are no obvious biological reasons for the exclusion of *P. cinereus*, we conducted separate analyses excluding the species (Supplementary material Table S2). By doing so, despite an extreme reduction in statistical power, the model remained significant (although GVI and precipitation become more important than temperature – perhaps because the range of variation for this variable was also extremely reduced, with minimum average temperature increasing from -2°C to 4°C).

We found that our three-variable models including mean annual temperature, annual precipitation and the

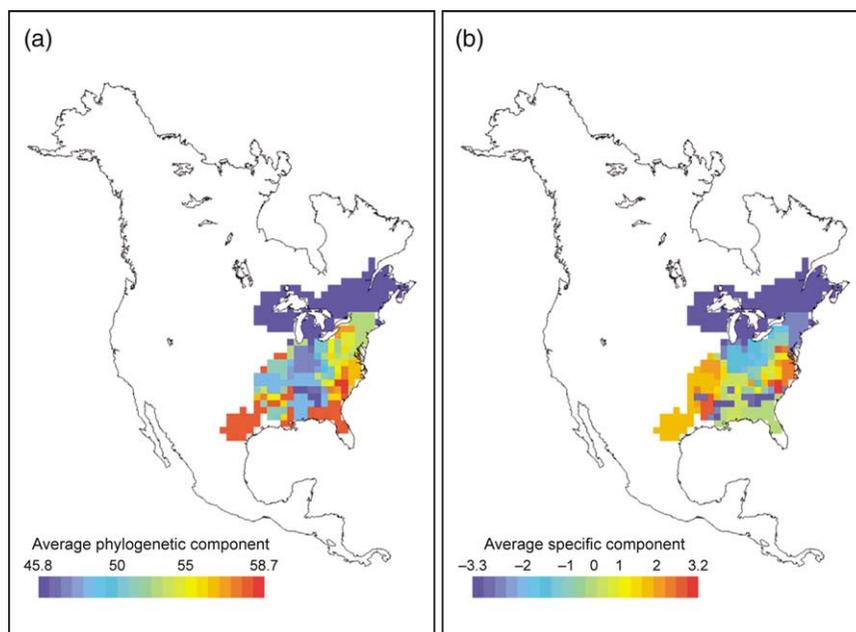


Figure 1. Geographical patterns of the average phylogenetic component of body size (a) and the specific component (b) resulting from PVR for all the 44 *Plethodon* salamander species in eastern North America. Numbers included in the legend of each map are snout-to-vent length values in millimeters. Overall, there are marked gradients in latitudinal space, but local geographic mismatches between phylogenetic and specific components.

Global Vegetation Index explained 53.9 and 70.6% of the variation in mean-P and mean-S, respectively (Table 1). Mean annual temperature was the primary predictor of the spatial pattern of mean-P and mean-S components, being positively correlated with both. To adjust mean body sizes in grids by the number of species (Meiri and Thomas 2007), we repeated these analyses using weighted-Least Square regression. Specifically, we used species richness values (S) (Supplementary material Fig. S1) and $(1 - \text{Log}_{10}[1/S^2])$ as weighting factors to recalculate standardized regression coefficients (Olalla-Tárraga and Rodríguez 2007). Following Olson et al. (2009) we also built models using species richness as a covariate. Our results remained robust in all cases and mean annual temperature consistently had the highest standardized coefficient in the models for mean-P and mean-S (Table 1).

Additionally, to address the statistical problems associated with spatial autocorrelation in the model residuals, we included eigenvector-based spatial filters as predictors in

the regression models (Olalla-Tárraga et al. 2009), an approach analogous to PVR in a spatial context. Also, we built spatial correlograms based on Moran's I coefficients (not shown) to evaluate if this approach removed the spatial autocorrelation of the residuals of our 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). The inclusion of spatial filters to remove spatial autocorrelation in the residuals of our regression models did not change the relative importance of the environmental predictors as indicated by the standardized partial regression coefficients (Table 1). Range in elevation contributed little to the increase of the explanatory power (adjusted R^2) of the models (e.g. between 0.7 and 1.5%), hence we did not include this variable in our models.

Although our results support Adams and Church's (2008) contention that this group does not follow Bergmann's rule, it contradicts their conclusion that there

Table 1. Multiple regression models of environmental variables against average cell values of phylogenetic (P) and specific (S) components obtained from PVR analysis for *Plethodon* species in eastern North America. For each model we show their corresponding coefficients of determination (R^2) and standardized partial regression coefficients of the predictors. Additionally, we provide results from weighted-LS regression analyses using species richness in each cell (S) and $(1 - \text{Log}_{10}[1/S^2])$ as weighting factors. Env+Spa models include non-redundant spatial filters as predictors, whereas Env+Rich models include species richness. Predictor variables are: Temp = mean annual temperature; Precip = annual precipitation; GVI = global vegetation index. An asterisk means that the standardized regression coefficients were significant at a probability level $p < 0.001$.

Model	Weighting variable	Standardized coefficients (P component)				Standardized coefficients (S component)			
		Temp	Precip	GVI	R^2	Temp	Precip	GVI	R^2
Env	None	0.868*	-0.250*	-0.03	0.539	0.823*	-0.03*	0.064*	0.706
Env+Spa	None	0.851*	-0.064	-0.150*	0.715	0.717*	0.119	-0.01	0.746
Env+Rich	None	0.756*	-0.230	-0.031	0.478	0.825*	0.014	0.077	0.743
Env	$1 - \text{Log}_{10}(1/SR^2)$	0.777*	-0.380*	0.047	0.458	0.776*	-0.11*	0.108*	0.610
Env	Species richness	0.684*	-0.430*	0.117*	0.364	0.746*	-0.16*	0.116*	0.523

is no spatial pattern in body size at any level of biological organization. Moreover, our results reveal a strong positive association between the geographical patterns in the phylogenetic (P) and ecological (S) components of body size for species assemblages and mean annual temperature. This also runs counter to Adams and Church's (2008) analysis that was unable to detect a signal of mean annual temperature in body size variation, but it is similar to an assemblage-based study for the complete urodele fauna of Europe and eastern North America (Olalla-Tárraga and Rodríguez 2007).

The heat balance hypothesis

Olalla-Tárraga and Rodríguez (2007) proposed the heat balance hypothesis to account for body size gradients exhibited by both endotherms and ectotherms at an assemblage level. For ectothermic groups with thermoregulating abilities, whose body size is not large enough to limit heat gain in low energy environments (e.g. snakes), this hypothesis parallels the traditional heat conservation mechanism originally conceived by Bergmann for endotherms. In contrast, for thermoconformers, which have limited abilities to control heat exchange and depend more closely on the thermal conditions of the environment, the hypothesis predicts a reversed Bergmann pattern as animals would minimize body size in colder regions to reduce heating times and maximize the time available for foraging and reproduction (Olalla-Tárraga and Rodríguez 2007). Hence, the heat balance hypothesis stems from older ideas presented by Cowles and Bogert (1944) and espoused by Ashton and Feldman (2003) that took into account the importance of surface-to-volume ratios for the thermal adjustments and activity times in reptiles (Olalla-Tárraga et al. 2006), but complementary develops expectations for ectotherms with a high degree of thermoconformism (Olalla-Tárraga and Rodríguez 2007). By doing the distinction between behavioral thermoregulators and thermoconformers, the heat balance hypothesis does not only consider the role of body size for the thermal ecology of ectotherms, but indirectly incorporates the importance of factors that constrain thermoregulatory behaviors in these organisms. For instance, the common absence of behavioral thermoregulation in salamanders is likely due to hydric limitations on heat exchange with the environment resulting from high rates of cutaneous evaporative water loss (Feder 1982, Feder and Lynch 1982, see also Olalla-Tárraga et al. 2009).

Consistent with the predictions of the heat balance hypothesis, Olalla-Tárraga and Rodríguez (2007) found that anurans (behavioral thermoregulators) follow Bergmann's rule in Europe and North America, whereas urodeles (mostly thermoconformers) do the opposite. In the latter case, the clear converse Bergmann's gradients may be also determined by the fact that the elongate body plans of urodeles generate a larger surface to volume ratio, which also contributes to increased heating rates in low energy environments.

Our results for *Plethodon* salamanders are also consistent with the heat balance hypothesis. The pattern of the specific component (S) is strongly related to thermal availability in

the environment, which suggests independent adaptive responses of species to current climatic conditions (Diniz-Filho et al. 2007). That is, species smaller than expected by their phylogenetic relatedness are more frequent in cold environments, whereas species larger than expected are mostly distributed in warmer areas.

However, the individual responses of species to temperature appear not to be the only cause for the overall gradient in body size, as we also found a climatic signal in the phylogenetic component (P). This is likely to be related to the particularities of recent diversification in this woodland-adapted salamander group since the early Pliocene. Several authors have suggested that most extant species of *Plethodon* salamanders in eastern North America originated as a result of rapid speciation events during the last five Mya following a history of shifts in forest cover along the Appalachian mountains associated to climatic changes (Highton 1995, Kozak et al. 2006, Wiens et al. 2006). According to this, continuous altitudinal shifts in forest cover through the Pliocene and Pleistocene may have favoured allopatric diversification events that generated many morphologically-similar cryptic species that, at the same time, tended to conserve their ancestral climatic niches (Highton 1995, Kozak and Wiens 2006, Wiens et al. 2006). Because rapid evolutionary radiation usually involves marked changes in the diversity of morphological and ecological characters, *Plethodon* appears to be a particular case of rapid speciation (Kozak et al. 2006).

Under this hypothesis, the observed relationship between the spatial pattern in the phylogenetic component of body size and mean annual temperature may simply reflect the interplay between rapid diversification and a structured trend across the phylogeny to maintain ancestral temperature-body size relationships. Even if true, this pattern in the phylogenetic structure of the data is not strong enough to obscure the signal of unique responses of each species to current climate variation found in the specific component.

Bearing in mind the above, why do we find a pattern that Adams and Church (2008) did not detect? Statistically, Adams and Church's (2008) cross-species analyses are properly done. However, we favor the view that cross-species analyses provide different information and are not as useful as the assemblage approach under almost all possible scenarios. We would expect both approaches to converge into similar results only when distribution ranges for the whole set of analysed species within the clade are either extremely restricted or mostly unknown. On the contrary, when species ranges are well known and comprise enough environmental variation, we suggest examining the occurrence of interspecific body size gradients using spatially-explicit assemblage approaches (i.e. using complete geographic information, see Blackburn and Hawkins 2004, Ruggiero and Hawkins 2006 and above). Of course, this is not to say that a cross-species approach is not helpful in detecting correlated evolution between traits across species. For instance, cross-species analyses conducted in a phylogenetic framework have proven to be useful to detect lineage-level patterns in the relationships between a number of life history attributes and body size (see e.g. Freckleton et al. 2002 and references therein). The problem arises when we use as a trait a variable that does not actually represent the

whole geographic range of a species (either the latitudinal midpoint or an overall measure of temperature as Adams and Church did). In doing so, we are restricting a multi-dimensional trait (i.e. a species distribution) to a single point. Therefore, a more in depth approach to study Bergmann's rule requires employing alternative protocols that simultaneously consider the distribution of species into a geographic and phylogenetic framework, such as the assemblage approach we have described here.

Concluding remarks

We have shown how an assemblage approach complemented with phylogenetic eigenvector regression can be useful to explore both the ecological and evolutionary mechanisms associated with Bergmann's rule in a spatially explicit context. Contrary to a cross-species approach, our assemblage-based analysis detected strong geographical trends in interspecific variation of body size of *Plethodon* salamanders in eastern North America. These arguments lead us to call for caution in making generalizations on the validity of Bergmann's rule or its reverse in particular taxa, unless we take into account the limitations of our database, the clade we are using as model system and the statistical techniques we employ to analyze the data. This is especially true if we are also interested in examining potential explanations. Although our analyses favor the heat balance hypothesis as a mechanism, considering the idiosyncratic patterns detected for different taxa, the disparities between phylogenetic and non-phylogenetic studies and the apparent discrepancies at different taxonomic scales, a general explanation for Bergmann's rule still remains elusive.

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