Ecology, 91(12), 2010, pp. 3729–3738

Deviations from predictions of the metabolic theory of ecology can be explained by violations of assumptions

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Abstract. The metabolic theory of ecology (MTE) is based on models derived from the first principles of thermodynamics and biochemical kinetics. The MTE predicts that the relationship between temperature and species richness of ectotherms should show a specific slope. Testing the validity of this model, however, depends on whether empirical data do not violate assumptions and are obtained within contour conditions. When dealing with richness gradients, the MTE must be empirically tested only for ectothermic organisms at high organization levels and when their body size as well as abundance does not vary with temperature gradients. Here we evaluate whether the magnitude of the deviations in slope expected from the MTE to empirical data for New World amphibians is due to the violations of model assumptions and to lack of generality due to restricting contour conditions. We found that the MTE correctly predicted biodiversity patterns only at higher levels of organization and when assumptions of the basic model were not violated. Approximately 60% of the deviations from the MTE-predicted slope across amphibian families were due to violations of the model assumptions. The hypothesis that richness patterns are a function of environmental temperature is too restrictive and does not take complex environmental and ecological processes into account. However, our results suggest that it may be possible to obtain multiple derivations of the MTE equation if idiosyncrasies in spatial and biological/ecological issues that are essential to understanding biodiversity patterns are considered.

Key words: body size; diversity gradients; ectotherms; macroecology; spatial autocorrelation; species richness; stationarity; temperature gradients.

Introduction

The decrease in species richness from the tropics toward the poles is one of the oldest and most studied broad-scale biogeographic patterns (Hawkins 2001). The so-called "latitudinal gradients" in species richness occur across various habitats (Rosenzweig 1995, Willig et al. 2003, Hillebrand 2004) and have been known for a long time (Crame 2001, Mittelbach et al. 2007). These gradients have been the subject of many studies since the 19th century, and different hypotheses have been proposed to explain the origins and maintenance of such gradients (e.g., Rohde 1992, Hawkins et al. 2003, Willig et al. 2003, Mittelbach et al. 2007). Recently, Brown et al. (2004) proposed the metabolic theory of ecology (MTE) to explain a priori the allometric scaling of metabolic rates and subsequent mechanisms that may drive many ecological patterns at different geographical scales and levels of biological hierarchy, including the latitudinal gradients of species richness (see Allen et al. 2002, 2006).

According to the MTE, the latitudinal gradients in species richness are due to the increased kinetic energy in the tropics. Therefore, the MTE is part of a more general group of climatic hypotheses proposed to explain this pattern (see Hawkins et al. 2003, Currie et al. 2004). Based on the first principles of thermodynamics and biochemical kinetics, Allen et al. (2002) proposed a model within the framework of MTE that extends the assumption of energetic equivalence (see Damuth 1987) in a local assemblage of ectothermic organisms to include the effects of temperature on individual metabolism. If this pattern is extended along a temperature gradient, the logarithm of species richness should be linear with a slope between –0.6 and –0.7 (where temperature is $1/kT$, T is the temperature expressed in Kelvin, and k is the Boltzmann's constant and is equal to 8.62×10^{-5} eV; see Brown et al. 2004 for details). More recently, Allen et al. (2006) combined the MTE and Kimura's (1983) neutral theory of molecular evolution to develop a more general model in which the higher input of energy in the tropics leads to increased mutation rates that drive evolutionary rates and eventually leads to higher species richness. Recently, Stegen et al. (2009) expanded the scope of the metabolic theory by combining feedbacks among temperature, mutation rate, speciation rate and community structure.

Despite these more complex evolutionary models, recent discussions around the empirical fit of the MTE to latitudinal diversity gradients are still focused on testing the first model (see Gillooly and Allen 2007, Hawkins et al. 2007*a*, *b*, Latimer 2007). The model

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Manuscript received 8 August 2009; revised 16 April 2010; accepted 20 April 2010. Corresponding Editor: N. J. Sanders.

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proposed by Allen et al. (2002), which was incorporated in the MTE, differs from most previous attempts to explain latitudinal gradients in species richness by making precise predictions about the relationship between species richness and the environment (i.e., temperature). This makes the theory, in principle, easily testable and falsifiable (O'Connor et al. 2007, Martínez del Rio 2008). In contrast, tests of the many other available hypotheses developed to explain broad-scale richness patterns are usually based on correlative approaches and try to derive unique predictions for correlations between richness and different components of environmental variation or historical processes (Hawkins et al. 2003, Currie et al. 2004, Mittelbach et al. 2007). Unfortunately, the empirical tests of the MTE applied to richness gradients have been controversial, and the simple equation provided by Allen et al. (2002) does not seem to fit most empirical data (Algar et al. 2007, Hawkins et al. 2007*a*, Kreft and Jetz 2007, Latimer 2007, Sanders et al. 2007; but see Kaspari et al. 2004, Gillooly and Allen 2007).

According to Martínez del Rio (2008), the master equation of the MTE has two components: (1) an allometric component that predicts the relationship between metabolism and body mass and (2) a thermodynamic component that characterizes the effect of metabolism and temperature. Here we will emphasize a basic model of the MTE to explain richness patterns based on its thermodynamic component, as proposed by Allen et al. (2002). Proponents of the MTE see these two components as being linked because they are based on an allometric relationship in which the whole-organism metabolic rate, I, scales as $I = I_0M^{3/4}$ (where I_0 is equal to a normalization constant independent of body size and M is the body mass). Furthermore, metabolism and temperature are linked via the Boltzmann factor, which is used to predict the kinetics of simple biomolecular reactions. More importantly, proponents argue that if the MTE predictions are correct, assumptions at lower levels (e.g., energetic equivalence rule) should be valid as well (Brown et al. 2004, Martínez del Rio 2008).

Similar to the above reasoning, the lack of fit of empirical data to the model of Allen et al. (2002; e.g., Hawkins et al. 2007*a*, *b*) may be caused by a violation of the assumptions and the restrictions of the model, which limit its generality and conceptual domain (there are restricting "contour conditions" for the application of the MTE model). Indeed, as noted by Hawkins et al. (2007*a*, *b*), part of the difficulty in applying the simple equation proposed by Allen et al. (2002) to different data sets is that it is difficult to verify whether fundamental assumptions of the model are violated or if a particular data set falls within the domain of the theory. Although all original applications and tests by Allen et al. (2002) did not explicitly verify these assumptions, they found that particular data sets fit well the predictions. If the assumptions are too unrealistic and are not satisfied, or if data set falls outside the domain of the model, this model will fail to correctly predict the observed empirical pattern, even when basic principles of the model are theoretically correct. Consequently, the MTE will be rejected. This rejection will not be because of fatal conceptual or theoretical flaws in its reasoning but rather because the assumptions and contour conditions make the model extremely restrictive.

Empirical tests of the MTE applied to richness gradients should therefore try to consider different factors about assumptions, generality and conceptual domain that would lead to a false or premature rejection of the model. There are explicit assumptions of the model, and the most important one is that the body size and total abundance do not vary across geographical space (i.e., the energetic equivalence rule). Although it may be difficult to empirically test the MTE assumptions, especially for invariance of mean abundance, Allen et al. (2002) argue that model should be particularly robust against violations of these assumptions. Moreover, these violations are unlikely to occur when dealing with a large number of species. Allen et al. (2002), and more recently Gillooly and Allen (2007), reiterated that the statement above suggests that MTE tests must be conducted for broadly defined groups, unless it is possible to verify that these two basic assumptions are not being violated. Thus, the taxonomic level and species number can be considered a surrogate for testing assumptions of the MTE or at least must be considered when establishing its domain and generality, delimiting its contour conditions (see McCain and Sanders [2010] for an example). Finally, because the model is derived from the effects of temperature in a local assemblage and projected to a broader scale, an implicit statistical assumption is that the same slope (reflecting intrinsic responses to temperature) will appear across the gradient, thus generating a stationary pattern (see Cassemiro et al. 2007*a*). This also suggests that although temperature alone may not be the only explanation for richness gradients, other factors (e.g., productivity, habitat heterogeneity, or historical effects) do interact with temperature and create discontinuities and more complex non-stationary patterns across the climatic gradient (see Hawkins et al. 2003, Whittaker et al. 2007, Wang et al. 2009). For example, if body size and abundance vary with temperature across a latitudinal gradient due to independent ecological and evolutionary processes, it is expected that there are deviations in relationship between species richness and temperature.

In summary, the MTE can be empirically tested only in ectothermic organisms at high organization levels (i.e., large phylogenetic groups and with high taxonomic diversity) as well as when body size and abundance do not vary with temperature gradients. The violations of any of these conditions could result in slope values different from values predicted by the MTE. In this study, we empirically tested which geographical domains and which levels of taxonomic hierarchy the MTE model correctly predicts spatial patterns in species richness of New World amphibians. Moreover, we analyzed whether geographic gradients in body size varied with temperature within each family (as a test of the body size spatial invariance assumption) and evaluated whether the ability of the MTE to correctly predict relationships between temperature and species richness is associated with biological properties (i.e., body size and geographic range characteristics) of the species group.

In general, we examined the relationship between richness and temperature as well as quantitatively evaluated whether the magnitude of the deviations from the slope predicted by the model from Allen et al. (2002) was a function of violations of the conditions required for testing the MTE. Given that we could explain these deviations, our analyses suggest that the MTE may still be a valid model under very specific conditions but may not necessarily be a general conceptual model. Our results also explain why the MTE failed to predict empirical richness patterns across different data sets (e.g., Hawkins et al. 2007*a*), and at the same time indicates that it would be advantageous to develop multiple derivations of the master equation proposed by Allen et al. (2002). For instance, as proposed by Stegen et al. (2009), it would be interesting to consider in the metabolic model the functional heterogeneity, usually found in analyses of different species groups. These derivations should incorporate more complex ecological processes and evolutionary idiosyncratic characteristics of the analyzed organisms in an effort to better describe and understand species richness patterns.

MATERIAL AND METHODS

Species and environmental data

We divided the New World into 4187 grid cells (1° of latitude and longitude). We obtained a temperature map from the Atlas of the Biosphere at a resolution of 0.5° (New et al. 1999; atlas *available online*).[2] We then resampled this map into 1° grid cells, transforming it into Kelvin units. We obtained the distributions of 1344 species of amphibians in the New World from the Global Amphibian Assessment and overlaid these distributions on the same grid system (assessment *available online*).[3] Species were classified into 41 genera, 21 families, 3 orders, and 1 class following the taxonomy available at Amphibiaweb (data *available online*; see also Hillis [2007]).[4] For higher-level groups, the latitudinal and longitudinal range limits were defined by overlapping the geographic distribution of all species in the group.

Body size data for 366 species were also obtained from the Amphibiaweb database (in 2008) and from our own Neotropical database (see also Olalla-Tárraga et al. 2009). We used the maximum snout-to-vent length (SVL, in mm) for Anura and the maximum total length (TL, in mm) for Caudata and Gymnophiona. In cases of sexual dimorphism, we used the female length. However, intraspecific variation is not essential when comparing a broad range of taxa. Because the distribution of animal body sizes is often right-skewed, arithmetic means are strongly influenced by the presence of large-sized species (Olalla-Tárraga et al. 2006). Therefore, we calculated the average body length after a logarithmic transformation (hereafter called mean body size) for all species for which data were available in each taxonomic group.

Testing the MTE model

We independently analyzed the effect of temperature on the species richness of each taxonomic group (class, order, family, and genus) by regressing the natural logarithm of species richness of ectotherms against temperature. The MTE model predicts that when temperature is scaled as $1/kT$ (where T is temperature in Kelvin and k is Boltzmann's constant of 8.62×10^{-5} eV), the slope for the regression should fall between –0.6 and –0.7 (Allen et al. 2002; but see Brown et al. 2004 for a more complete model). Here we used the criteria for acceptance of the MTE (Hawkins et al. 2007*a*) in which nonsignificant relationships (defined as $P > 0.10$) definitely do not support the hypothesis that temperature drives the species richness gradients and statistically significant ($P < 0.05$) negative slopes between –0.60 and –0.70 are fully consistent with MTE model. However, we also adopted a slightly more liberal criteria and considered that marginally significant ($0.05 < P < 0.10$) slopes or slopes between –0.55 and –0.75 could possibly be consistent with the MTE model. Following Brown et al. (2004), we used ordinary least-squares regression (OLS) to find both the coefficient of determination and the empirical slope of the linear relationship. However, notice that although these criteria are useful for establishing if a given data set fits MTE predictions, they will not affect our main analysis (see *Synthesis*), because we did not analyze discrete decisions (support or not) for each data set, but rather the deviations from MTE expectations expressed quantitatively as differences between slopes. Linearity was checked by analyzing OLS residuals and plotting residual vs. estimated values of regressions.

Establishing violation in model assumptions and contour conditions

We analyzed the effect of regions in the New World in which the MTE was supported by calculating the highest, lowest and mean temperature ($1/kT$) within the range of each amphibian group. This allowed us to test whether deviations of slope were related to variance in temperature (i.e., groups inhabiting regions of

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[2] ⟨www.sage.wisc.edu/atlas⟩

[3] ⟨http://www.natureserve.org⟩

[4] ⟨www.amphibiaweb.org⟩

TABLE 1. Explanatory variables of the slope expected under the metabolic theory of ecology (MTE) for 10 amphibian families of the New World.

		$(1/kT) \times$ BS							
Family	$\overline{\text{BS}}$	n	r^2	P	S	Tmean	$\text{AIC}_{\text{OLS}-\text{GWR}}$	b_{obs}	$b_{\text{obs}-\text{exp}}$
Ambystomatidae	146.62	8	0.136	0.369	16	41.82	904.926	−0.132	−0.782
Bufonidae	77.78	41	0.003	0.733	117	40.48	977.13	−0.273	−0.923
Centrolenidae	21.15	17	0.056	0.362	43	39.25	83.873	0.094	−0.556
Hylidae	50.51	110	0.03	0.072	297	39.99	2872.136	−0.615	−1.265
Leptodactylidae	55.82	50	<.001	0.894	433	39.39	1456.485	−0.730	−1.38
Microhylidae	37.93	14	0.104	0.261	44	39.15	770.178	−0.570	−1.22
Plethodontidae	158.89	68	0.13	0.003	113	40.28	782.418	0.123	−0.527
Proteidae	262	5	0.701	0.077	5	40.89	14.162	−0.019	−0.669
Ranidae	85.96	22	0.032	0.428	38	41.23	3066.506	−0.015	−0.665
Salamandridae	157.33	6	0.6	0.07	6	40.93	33.124	−0.015	−0.665
Standardized coefficient	0.312	···	−0.277	···	−0.614	0.123	−0.172	···	···

Notes: Variables are: $\overline{\text{BS}}$, mean body size (mm); BS, body size data for each species per family; n, number of species with body size data; r^2, coefficient of determination showing the body size spatial structure obtained by trend surface analysis (TSA); S, total species richness; Tmean, mean temperature ($1/kT$) within the range of each family; $\text{AIC}_{\text{OLS}-\text{GWR}}$, difference between the Akaike information criterion obtained by ordinary least-squares and geographically weighted regressions; b_{obs}, slope of the relationship between temperature ($1/kT$, where k is Boltzmann's constant and T is the average annual temperature in degrees Kelvin) and species richness. The standardized coefficient is from an OLS regression between explanatory variables and the difference between observed and expected slope values ($b_{\text{obs}-\text{exp}}$).

homogeneous temperature were not expected to fit the MTE model). This also allowed an initial evaluation of geographical effects disturbing the MTE model. For example, it allowed us to determine whether the MTE is applicable only in regions of the Northern hemisphere were temperature is a limiting factor (see Hawkins et al. 2003, Whittaker et al. 2007, Wang et al. 2009).

A more complex analysis of the geographical effects disturbing the basic MTE model was performed following the methods of Cassemiro et al. (2007*a*). In this analysis, the relationship between richness and temperature was analyzed using a geographically weighted regression (GWR; see Fotheringham et al. 2002) for the different amphibian groups. A GWR estimates the regression parameters locally (i.e., at the grid cell level) and allowed us to evaluate the geographic patterns of variation in the slope (non-stationarity) and how they matched the MTE predictions across geographic space. It was predicted that significant non-stationarity would appear if the effects of temperature (a surrogate of energy; Clarke and Gaston 2006) appeared more clearly in northern faunas (Hawkins et al. 2003, Whittaker et al. 2007). The GWR was fitted using a bi-squared kernel and by optimizing the Akaike information criterion (AIC) to establish the bandwidth for defining neighbors. An F value was used to test the null hypothesis that the GWR model represented no improvement over a global OLS regression model. Also, the differences between AIC values of OLS and GWR regressions ($\text{AIC}_{\text{OLS}-\text{GWR}}$) were used to analyze the existence of spatial structure (non-stationarity) in the data.

To determine whether body size was correlated with temperature (i.e., spatial structure of body size), data on the body size of each species of ten amphibian families were regressed against the temperature values ($1/kT$) of the centroid geographical range for each species. We performed the OLS regression with species body size and temperature. The coefficients of determination (r^2) were used as indicators of the spatial structure in body size along the temperature gradients. Thus, the coefficient of determination of the OLS regression performed for each amphibian family (see *Synthesis*) revealed whether there was a high explanation power of temperature on variation of body size.

Synthesis

We used an AIC-based model selection approach to evaluate which correlates (assumptions and contour conditions) best explained the variation in slope predicted by the MTE for the 10 most diversified amphibian families for which body size data was available (see Table 1). The correlates (i.e., predictors) that we used were total richness (S, species pool in the family), mean body size, the coefficient of determination of relationship between body size and temperature (body size climatic trends), $\text{AIC}_{\text{OLS}-\text{GWR}}$ (non-stationarity), and mean temperature within the geographic range of the species of each family. We used the absolute difference between the observed and expected slopes of the relationship between temperatures as a response variable.

The AIC value of each model can be expressed as ΔAIC, which is the difference between the AIC of each model and the minimum AIC of all compared models. A value of ΔAIC higher than 7 indicates that a model has a poor fit relative to the best model, whereas a value less than 2 indicates that a model is equivalent to the minimum AIC model (Burnham and Anderson 2002). The ΔAIC values can also be used to compute Akaike's weighting of each model (w_i), which provides evidence that the selected model is actually the best explanatory

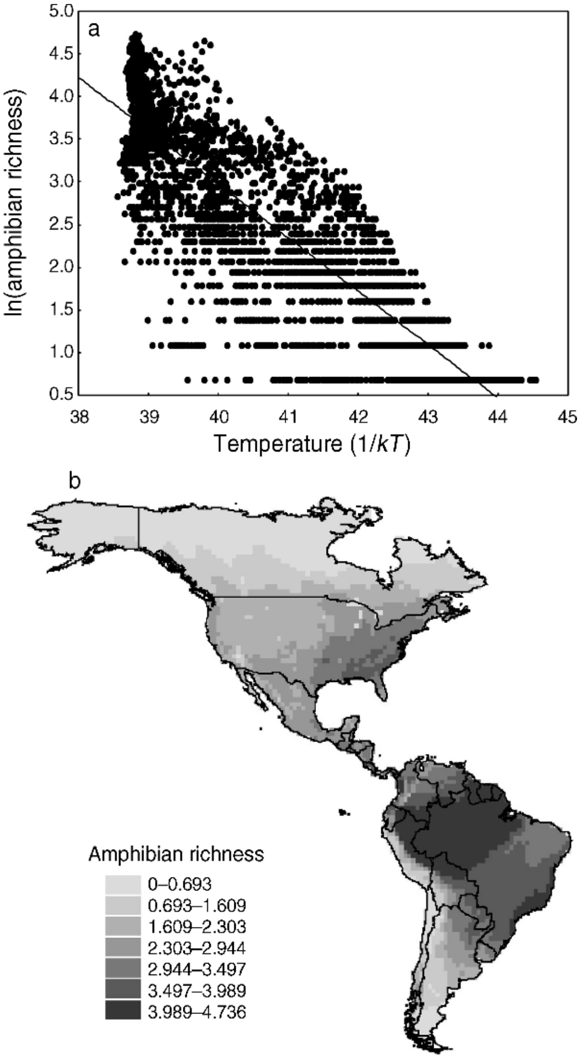

FIG. 1. (a) The relationship between ln-transformed species richness of amphibians and temperature for 4187 cells (1° × 1° latitude–longitude) covering North and South America. Temperature is expressed as $1/kT$, where k is Boltzmann's constant and T is the average annual temperature in degrees Kelvin. (b) Spatial patterns of amphibian richness (ln-transformed) in the New World.

model. Thus, w_i values can also be used to define the relative importance of each predictor across the set of models evaluated by summing w_i values of all models that include the predictor of interest, taking into account the number of models in which each predictor appears (Burnham and Anderson 2002).

Thus, with these analyses we aimed to determine (1) if the estimated slope was statistically related to body size differences and climatic gradients in body size, (2) if the expected MTE slope was more likely to occur when dealing with more diversified taxa, (3) if the MTE predictions could be found in more temperate regions or in regions where the environmental gradients are stronger, and (4) if families for which non-stationarity in richness is detected by GWR tend to deviate from MTE expectations. All analyses were performed in SAM 3.0 (Rangel et al. 2006).

RESULTS

Overall, the richness of amphibians follows the well-known latitudinal diversity gradient, with high-richness cells concentrated in tropical regions (Fig. 1a). The linear regression slope of $\ln(S)$ against $1/kT$ was equal to –0.673 ($\mathrm{CI}_{95\%} = -0.682, -0.663$; Fig. 1b). Temperature alone explained 81% of the variation in the logarithm of species richness. However, a heteroscedastic distribution was observed, forming a constraint envelope common in macroecological data (see Gaston and Blackburn 2000). The correlation (and slope) was significant at $P < 0.043$, according to Dutilleul's (1993) correction for spatial autocorrelation. Because the confidence interval for the slope conservatively includes the values predicted by the MTE (–0.6 to –0.7), our analysis with the entire class Amphibia supports the MTE predictions, even when the effects of spatial autocorrelation are considered.

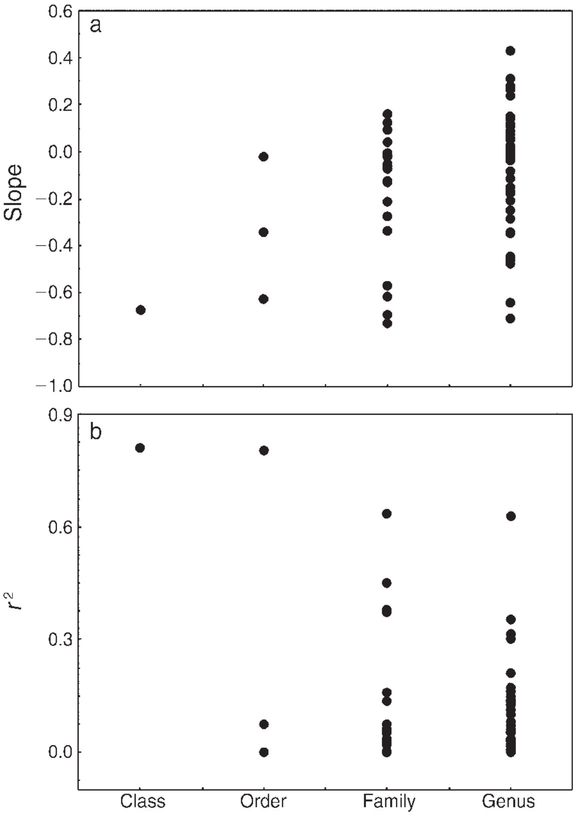

FIG. 2. (a) The relationship between various organizational levels of amphibians and the slope of the relationship between ln(species richness) and temperature ($1/kT$). (b) The relationship between various organizational levels of amphibians and the coefficient of determination (r^2) of the relationship between ln(species richness) and temperature ($1/kT$).

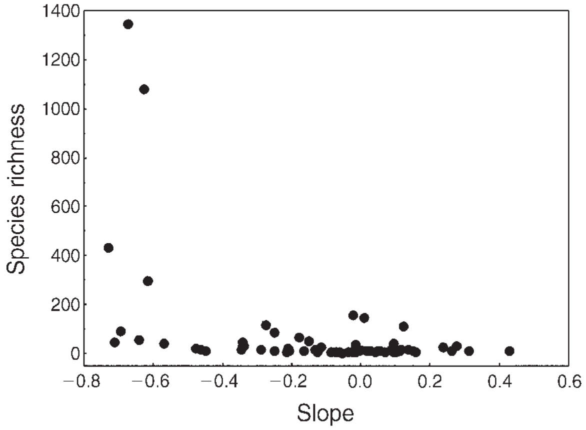

FIG. 3. The relationship between species richness of amphibian groups and the slope of the relationship between ln(species richness) and temperature ($1/kT$) of the ordinary least-squares (OLS) regression.

However, the MTE predictions tend to be correct only for groups of species that are broadly defined (e.g., higher organizational levels; Fig. 2a). The slope value for the entire class Amphibia was equal to −0.67. Slopes at the level of order ranged from −0.627 to −0.022 and only the Anura order fit the MTE predictions well. At the family level, slopes varied between −0.73 and 0.158 and only four out of 21 analyzed families (Dendrobatidae, Hylidae, Microhylidae, and Leptodactylidae) fit the MTE model. Lastly, slopes at the genus level ranged from −0.711 to 0.428 and the predictions of the MTE were met for only two of the 41 genera (see Appendix A; Fig. 2a). Furthermore, the coefficient of determination of the relationship between temperature and species richness was high only in the groups that are broadly defined (e.g., class and order; Fig. 2b). This finding is in accordance with the suggestion of Allen et al. (2002) that the MTE is suitable for higher organizational levels only (also see Hawkins et al. 2007*a*).

The GWR analysis showed that MTE predictions for species richness varied across geographical space (see also Cassemiro et al. 2007a). The GWR values of r^2 (0.95) for the entire class Amphibia showed that there was an improvement of GWR in respect to the OLS model. Furthermore, the high F value (282.673; df = 3889.18, 26.82) indicated highly significant non-stationarity (see Cassemiro et al. 2007*a*). This pattern was mainly observed in groups that can be broadly defined (i.e., class and order; Appendix A).

A map of patterns in amphibian mean body size (see Appendix B) revealed that species found in northern regions tend to be larger. This result is expected under Bergmann's Rule (see Olalla-Tárraga and Rodríguez 2007), but it is important to note that this spatial structures in body size varied across families (Table 1). The mean body size among species was significantly correlated with temperature only for Salamandridae and Proteidae ($r^2 = 0.6$ and 0.73, respectively) and the slopes of the relationship between temperature and species richness for these two families were different than predictions from the MTE. In contrast, the influence of temperature on mean body size of species in other families, such as Hylidae, Leptodactylidae, and Microhylidae, is weak (r^2 = 0.03, >0.001, and 0.104, respectively). Interestingly, for these three families the MTE predicts a significant relationship between species richness and temperature (Table 1). There is no consistent relationship between mean body size and the slope predicted by MTE.

The relationship between overall species richness (species pool) and temperature follows a general pattern. In general, the slope expected by the MTE is found in amphibian groups with a higher species pool ($r^2 = 0.21$; $P \ll 0.001$; Fig. 3). Our analyses also show that groups with correctly predicted slopes are distributed between mean temperature ranging from 38.96 and 40.95 $1/kT$, as found in the tropical climates and mountainous (Andes) regions with mild temperatures (see Appendix A). This is strong evidence that the other potential drivers of diversity patterns in amphibians, besides temperature (as observed in our GWR analyses), should vary in space and may be spatially structured at a regional scale.

Modeling the difference between observed and expected slopes simultaneously against these correlates across the 10 families reinforces that species richness is the predictor variable with the largest effect. The AIC-based model selection shows that the model with the variable "species richness" alone is the most parsimonious. This model had the lowest ΔAIC (=0), with a high value of w_i (=0.658) and the standardized coefficient ($b_{\text{std}} = -0.614$; Table 1).

DISCUSSION

We found support for the thermodynamic model of the MTE (see Allen et al. 2002, Brown et al. 2004) when we analyzed species richness patterns for higher level taxonomic groups, such as class and order, which also show higher species richness. This supports the main contour condition of MTE model for richness gradients, which states that the theory applies better to high taxonomic levels. However, the local regression analysis with GWR revealed considerable non-stationarity at all levels, indicating that the fit of the MTE models for richness are not the same across different regions (see Cassemiro et al. 2007*a*). This result calls attention to an important spatial issue that Brown et al. (2004) did not explicitly take into account. This spatial issue may reflect an overall macroecological pattern in which temperature may be more important in northern temperate regions (Hawkins et al. 2003, Whittaker et al. 2007). However, Wang et al. (2009), testing the metabolic model with trees in North America and eastern Asia (both temperate regions), observed that at higher latitudes, plots of comparable area had higher species richness in North America than in eastern Asia. The authors attributed this difference to regional topography and historical factors (Wang et al. 2009).

Thus, the assumption of spatial stationarity for a robust test of the MTE should be considered. More complex spatial structures were observed when performing GWR analysis or other types of spatial regression (also see Cassemiro et al. 2007*a*). Indeed, spatial autocorrelation is intrinsic to species richness maps (see Cassemiro et al. 2007*b*) and may cause potential problems in testing the MTE. If spatial autocorrelation remains in OLS regression residuals, hypothesis testing may be biased. It then becomes necessary to adopt an explicit spatial regression approach to test hypotheses on particular values of the regression slope. Additionally, both non-spatial (e.g., OLS) and spatial (e.g., autoregressive) regression models assume that the relationship between response and predictor variables applies equally to the entire study area (spatial stationarity) and thus provides an "average" of the entire area. Thus, a violation of the stationarity assumption in spatial regression may create difficulties in generalizing and interpreting the relationship between temperature and species richness. Furthermore, models that incorporate non-stationarity may be useful in revealing more complex spatial structures when testing the MTE predictions for specific groups (see Jetz et al. 2005, Cassemiro et al. 2007*a*). Despite these findings, our multiple regression analysis with New World amphibian families showed that the effect of non-stationarity is not strongly correlated with deviations from the predictions of the MTE, perhaps due to low statistical power of cross-family analyses and high ability of other effects (i.e., species pool) in explaining the pattern.

When deconstructing species richness based on taxonomic levels, we found that the causes of the underlying spatial patterns in amphibian richness may be not as simple as suggested under the MTE. Even so, the best predictor of the correct MTE slope is the overall species richness (species pool) of the studied group. The slope of the relationship between species richness and temperature at higher taxonomic levels is consistent with that predicted by the MTE. Furthermore, when the species pool is large, temperature affects richness as predicted by the MTE, although non-stationarity is still observed (see Cassemiro et al. 2007*a*). In contrast, temperature did not explain species richness patterns for groups with low overall diversity (e.g., most families and genera, since they showed low species richness) and observed slopes diverged widely from the expected slope. However, there should be a smaller variance in the spatial pattern of species richness for species-rich groups than for species-poor groups. Consequently, it is expected that the slope predicted by the MTE fits better for more diversified groups, even at lower taxonomic levels.

It has been suggested by Martínez del Rio (2008) that support for the MTE is found only in higher levels of organization for several reasons. The equation may capture the effect of variation only at lower levels and this may allow simpler models of upper-level phenomena to be built, likely because the metabolic equation of the MTE may be one such sufficient parameter or equation. If sufficient parameters are based on a robust result, they can lead to descriptions of upper-level phenomena that are independent of the fine details of variation at lower levels (Martínez del Rio 2008). However, Brown et al. (2004) warned about the use of correct data to evaluate the MTE and stressed that body size and abundance must be spatially invariable. Likewise, Hawkins et al. (2007*a*, *b*) also stressed the importance of evaluating how violations in underlying assumptions will affect the shape of the relationship between species richness and temperature. Data on abundance at broad spatial scales are difficult to obtain, especially for multiple species (Kaspari 2004, McCain and Sanders 2010), but it is possible to test patterns in body size.

Amphibian body size also varies across temperature gradients, so this tends to violate the assumption of spatial invariance in body size. In general, families that showed a significantly spatial structure in mean body size among their species also had slopes of richness and temperature far from the MTE prediction. However, those families that showed a low spatial variation in body size tended to have slopes that better the predictions of the MTE. In fact, the invariance of body size across geographical space seems to be important in obtaining a good fit of the MTE for the amphibian data analyzed here. For example, the three families with the highest body size showed considerable spatial variation in body size, while families with the smallest body sizes showed the opposite trend. Although AIC-based model selection did not support strong effects of these patterns across families when all variables were added to the model, the body size gradient was the second largest effect in explaining deviations from MTE expectations after richness pool. According to Olalla-Tárraga and Rodríguez (2007), the body size of amphibians increases toward the north, in agreement with Bergmann's Rule. Aside from the violation of the body size invariance assumption, the high richness of small-bodied species in lower latitudes and large-bodied species in higher latitudes probably leads to variation in abundance (Lawton 1990, Srivastava and Lawton 1998). This variation in abundance is not a favorable condition for testing the MTE (Allen et al. 2002).

Our results suggest that understanding how body size varies along temperature gradients will allow a more generalized version of the model that Allen et al. (2002) developed (e.g., Stegen et al. 2009). Based on the equations by Allen et al. (2002), if average body mass changes across a temperature gradient, then non-linear relationships between temperature and species richness are expected. These issues may be critical for understanding how violating the assumptions of body size and density invariance affects the support of the model (Hawkins et al. 2007*a*). For example, Stegen et al. (2009) proposed a more generalized MTE model that relates limiting resource supply, ecological interactions and evolutionary rates to functional trait (body size). Allen et al. (2002) found support for MTE predictions in a study of North American amphibians but they ignored many of the issues we have discussed.

When simultaneously modeling the effects of these variables against observed slopes for the relationship between richness and temperature, we found that approximately 60% of the slope deviations from the predicted slopes of the MTE are a function of violations of the model assumptions or testing the MTE outside ideal contour conditions. Our analyses revealed that the species pool is the most important explanatory variable influencing the slope predicted by the MTE, followed by mean temperature within species ranges, body size patterns and non-stationarity in richness response to temperature. Thus, analyzing poorly diversified groups and groups with clear spatial patterns of variation in body size may provide a lack of fit between observed data and the MTE predictions. In general, when the MTE assumptions are met, the probability of finding the expected slope value is higher. However, 40% of variation in the observed slopes in our analysis was not explained.

Besides the issues discussed above, it is important to emphasize that other factors (e.g., environmental and ecological) may also influence species richness patterns. Even proponents of the MTE do not claim that temperature is the only driver of these patterns (Allen et al. 2002, Wang et al. 2009). Meehan et al. (2004) suggested that a model that includes temperature, body mass and productivity explains patterns of species richness better than a model with only temperature. Indeed, temperature is not a good predictor of species richness in low latitudes where there is a large amount of energy, but energy is a more important driver of diversity in higher latitudes (Hawkins et al. 2003, Whittaker et al. 2007). Thus, temperature is not considered to be a unique explanatory variable or the most important variable for species richness patterns (Hawkins et al. 2007*a*, *b*) and body size (Rodríguez et al. 2008). To explain the spatial patterns in the body size of ectotherms, the "heat balance hypothesis" (sensu Blackburn et al. 1999) takes into account temperature, evapotranspiration and light input in the environment. This seems to be the most suitable hypothesis for amphibians (Olalla-Tárraga and Rodríguez 2007). This may explain why our model to predict deviations in the observed slopes is not fully explained by not meeting the assumptions of the MTE model.

Furthermore, other studies have shown that multiple ecological and evolutionary mechanisms contribute to the latitudinal diversity gradient (Ricklefs et al. 1999, Hawkins et al. 2003, Currie et al. 2004, Cassemiro et al. 2007*b*, Hawkins et al. 2007*a*, *b*, Whittaker et al. 2007, Urbina-Cardona and Loyola 2008). Thus, it is likely that species richness is driven by multiple factors and not solely by temperature and water. For instance, Allen et al. (2006) proposed integrating the evolutionary-rate hypothesis with metabolic theory by using population genetics models to predict how temperature influences rates of genetic divergence among populations and rates of speciation. This combined model predicts that rates of speciation increase toward the tropics and suggests that metabolic rates of individuals are the primary determinant of evolutionary rates (see also Allen and Gillooly 2006; for more recent derivation of the MTE, see Allen and Gillooly 2009, Wang et al. 2009).

In summary, in this study we tested the generality of the MTE by establishing the conditions under which the theory correctly predicts spatial patterns in species richness. We found that the MTE is able to predict species richness patterns for amphibians only at higher levels of taxonomy and when assumptions of the basic model are not violated. This finding supports one of the contour conditions proposed by Allen et al. (2002). However, given that these assumptions of body size and abundance invariance are rarely met in nature, it is clear from our analyses that the hypothesis that richness patterns are a function of the effects of environmental temperature on the energy kinetic of organisms is a simplified assumption that overlooks other operative processes (e.g., environmental, ecological). Our analyses show that proponents of the MTE are paying the cost for establishing restrictive assumptions that are difficult to test in the real world and that make it difficult to test the model. The debate around the empirical validity of the MTE would produce different results if assumptions were strictly met. In these cases, a restrictive model could be applied. However, because violations of the assumptions explained a relatively large amount of the deviation from the expected slope in this study, we suggest that it will be possible to maintain the key principles of the MTE and obtain multiple derivations of the master equation proposed by Allen et al. (2002) to broaden the application of the MTE. These derivations should consider idiosyncrasies in spatial, biological and ecological issues because they are essential to describing and understanding species richness patterns (see Agutter and Wheatley 2004).

Stegen et al. (2009) showed how the MTE could be extended to consider the knowledge about the metabolic basis of speciation, extinction and species richness along environmental gradients and, thus, propose derivations of the original equation of MTE that should include factors such as breadth niche and body size ontogeny. According to Lakatos (1970), refutations and/or derivations do not lead to abandonment of a theory because studies generally develop with problems (or results other than what is predicted). Results that are not predicted are important in order to improve the reasoning behind a theory (Lakatos 1970). Although Martínez del Rio (2008) states that progress of the MTE depends on expanding the family of models that constitute the theory, as much expanding the rigor with which we contrast its predictions with data and experimental results, he emphasizes that these derivations may eliminate the strength of the theory. We agree with Martínez del Rio (2008) that if the proponents of MTE had adopted a model view (i.e., a fragmented approach to investigate a question, without generality), the controversy surrounding MTE would have been much less discordant.

Acknowledgments

We thank Andrew Clarke, David Currie, Thiago F. Rangel, Paulo De Marco, Jr., and three anonymous reviewers for useful comments. Work by F. A. S. Cassemiro is supported by a CAPES doctoral fellowship. J. A. F. Diniz-Filho was continuously supported by several CNPq and FUNAPE/UFG grants.

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APPENDIX A

Ordinary least-squares regressions and geographically weighted regressions of the relationship between temperature and ln-transformed species richness of amphibians of the New World (*Ecological Archives* E091-262-A1).

APPENDIX B

Body size means of 1344 amphibian species in the New World (*Ecological Archives* E091-262-A2).