



UNIVERSIDADE FEDERAL DE GOIÁS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM
ECOLOGIA E EVOLUÇÃO



Sidney Feitosa Gouveia

**Origem e natureza de padrões macroecológicos
em anfíbios: antigas questões, novas abordagens**

Goiânia – GO
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**Origem e natureza de padrões macroecológicos em
anfíbios: antigas questões, novas abordagens**

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“O limite do saber humano, em qualquer ramo científico, possui um interesse maior, o qual se incrementa sob a influência da proximidade aos domínios da imaginação.”

Charles Darwin.

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Conteúdo

| | |
|---|------------|
| Lista de Figuras | i |
| Lista de Tabelas | iii |
| Resumo | iv |
| Abstract | v |
| Introdução Geral | 6 |
| Referências | 14 |
| | |
| Capítulo 1. Nonstationary effects of productivity, seasonality, and historical climate changes on global amphibian diversity | 20 |
| Abstract | 20 |
| Introduction | 21 |
| Material and Methods | 26 |
| Results | 30 |
| Discussion | 33 |
| References | 38 |
| | |
| Capítulo 2. Environmental steepness, tolerance gradient, and ecogeographical rules in glassfrogs (Anura: Centrolenidae) | 44 |
| Abstract | 44 |
| Introduction | 44 |
| Material and Methods | 47 |
| Results | 52 |
| Discussion | 56 |
| References | 60 |
| Supplementary Material | 65 |
| References | 73 |
| | |
| Capítulo 3. Climatic niche at physiological and macroecological scales: thermal tolerance–geographic range interface and niche dimensionality. | 76 |
| Abstract | 76 |
| Introduction | 77 |
| Methods | 79 |
| Results | 83 |
| Discussion | 86 |
| Concluding Remarks | 90 |
| References | 91 |
| Supplementary Material | 97 |
| | |
| Conclusões Gerais | 99 |
| Referências | 103 |

Lista de Figuras

Capítulo 1

- Figure 1.** Global pattern of amphibian richness, obtained by overlaying species ranges from IUCN (2009) on a $2.0^\circ \times 2.0^\circ$ grid cell system..... **27**
- Figure 2.** Maps of partial coefficients from the partial GWR analyses between predictors of productivity, seasonality and historical climate variability. Individual contributions of each hypothesis (i.e. non-shared variation) are shown in the maps labeled by the related hypothesis and maps of overlapped explanation are positioned between them. P = Productivity; S = Seasonality; H = Historical variability. In abbreviations, colons denote sharing between pairs of predictors alongside, whereas dots indicate that the following predictor was excluded. For example, P:S.H denotes the shared fraction of productivity and seasonality, excluded historical variability. Internal Venn diagram displays regression coefficients (R^2) from partial OLS analyses, and upper codes correspond to the partial GWR equivalent. Gray areas indicate negative R^2 due to opposite signal effects between predictors or among neighbour cells (see Legendre and Legendre 1998) **31**
- Figure 3.** Mean values of the relative contribution (R2) of climatic predictors within biogeographic realms, as drawn from the partial-GWR analyses. The Indomalayan realm was excluded and the Palearctic was divided in its Eastern and Western portions. Note the predominance of productivity over the other predictors in most realms **32**

Capítulo 2

- Figure 1.** Theoretical models of the variation of vertical vs. horizontal range sizes. Model *a* illustrates the null expectation, in which species horizontally large-ranged should also be vertically large-ranged. In such case, models residual can be taken as a proxy of the species tolerance. In model *b*, other factors distort the expectation, preventing the inference of tolerance. **51**
- Figure 2.** Spatial pattern of body size (T; median of snout-vent length midpoints), and Phylogenetic (P) and specific (S) components of body size variation from 148 species of Centrolenidae after overlaying onto a 0.5° cells grid along the Neotropics. Legend scales of P and S maps are missing because mathematical transformation makes their values meaningless, thus being useful only in a geographical comparative context **53**
- Figure 3.** Observed relationship between vertical and horizontal range sizes from a restricted major axis regression (RMA – Model II). The result supports the scenario *a* of the Fig. 1. **54**
- Figure 4.** Correlation of median log body size (a, b), median log vertical range (c), and median log horizontal range (d) against geographical predictors (altitude and latitude) in Centrolenidae. Only the graph (a) with fit line shows a significant correlation. This latter relationship is slightly strengthened if the both leftmost and uppermost outliers are omitted. **55**

Figure S1. Phylogeny of glassfrogs (Anura: Centrolenidae) based on Guayasamin et al. (2009). Black branches and names refer to clades present in the original phylogeny. Red branches and names indicate clades inserted as polytomies according to previous classifications (see Table S1). Quotations denote species considered incertae sedis by Guayasamin et al. (2009) due to unavailability of molecular data. For these species, the authors maintained generic names in order to follow the current taxonomy, until a conclusive placement of them in the proper genera. Species with multiple placements in the Guayasamin's et al. phylogeny (e.g. *C. buckley*, *H. fleischmanni*) had the positions arbitrarily defined in favor of one of the possible positions **72**

Capítulo 3

Figure 1. Phylogenetic Signal Representation (PSR) curves showing the evolutionary rates of critical thermal maximum (CT_{max}), geographical maximum temperature (T_{max}), niche marginality (OMI 1st axis) and niche breadth for 47 anurans. Red and yellow bands are the confidence intervals for the neutral (Brownian motion) and null (random) expectations, respectively (Diniz-Filho et al. 2012). Note that CT_{max} and OMI 1st axis have very similar patterns of evolution (i.e., slightly faster than Brownian motion) **85**

Figure 2. Phylogenetic Generalized models between the anuran larvae CT_{max} and macroecological climatic niche features: a = niche position (OMI 1st axis); b = T_{max} ; c = niche breadth **86**

Figure S1. Spatial distribution and local richness after overlapping the extent of distribution of all 47 anurans onto a cells grid of $1^\circ \times 1^\circ$ resolution **97**

Figure S2 Non-ultrametric phylogeny for 47 anurans, after Frost (2006). Different colours denote different families..... **97**

Figure S3. Interspecific variability of climatic niche traits (CT_{max} ; T_{max} ; niche position and niche breadth) among 47 anurans. Species are ordered alphabetically **98**

Lista de Tabelas

Capítulo 1

Table 1. Predictions for broad-scale relationships between species richness and the variables representing the three climatic hypotheses analysed. Stationary predictions were drawn from selected references and nonstationary predictions are as devised here explicitly for amphibians. LGM stands for Last Glacial Maximum **24**

Table 2. Spatial Eigenvector Mapping regression (SEVM) and Geographically Weighted Regression (GWR) between amphibian species richness and the sets of predictors of the three hypotheses evaluated. F means the F statistic, and AICc the sample size-corrected value of the Akaike Information Criterion. The OLS regression was affected by spatial autocorrelation in the residuals and thus omitted here **30**

Capítulo 2

Table S1. Data and respective reference of body size (BS; midpoint snout-vent length), geographical range (GR) maps, altitudinal range (AR; maximum-to-minimum elevational difference), and phylogenetics relationships for all Centrolenidae frogs used in this study. Quotations denote species considered *incertae sedis* by Guayasamin et al. (2009) due to unavailability of molecular data. For these species, the authors maintained generic names in order to follow the current taxonomy, until a conclusive placement of them in the proper genera **65**

Capítulo 3

Table 1. Statistics of Phylogenetic Generalized Least Squares (PGLS) models between CT_{max} and macroecological niche traits. The λ is the index that transforms the trait phylogeny to fit a Brownian motion model. β = models slope; ε = standard error; AICc = corrected model's score..... **84**

Resumo

Estudos ecológicos envolvendo largas escalas espaciais e temporais têm um histórico respeitável nas ciências naturais. Até a proposição da macroecologia no final dos anos 80, várias fundações teóricas foram estabelecidas a partir dos estudos dos padrões de distribuição de espécies e suas propriedades, e da influência dos processos espaciais e evolutivos na formação e sucessão desses padrões ao longo do tempo. A macroecologia atualmente compreende diversas questões ecológicas de larga escala, tendo inclusive transposto as fronteiras de outros campos de pesquisas. Algumas das suas questões centrais envolvem o papel do espaço geográfico como modulador de processos ecológicos, das relações filogenéticas que conferem dependência morfológica e ecológica entre espécies, problemas de transposição de escalas e níveis de organização ecológicas, passagem da documentação de padrões para a inferência de processos causais e desenvolvimento de procedimentos e ferramentas para atender a essas demandas. Apesar do progresso observado, muitas questões fundamentais da relação entre espécies e seu ambiente em macroescala, ligadas à compreensão da origem e natureza dos padrões macroecológicos, permanecem elusivas. Nesta tese abordamos três questões relacionadas à influência do clima sobre alguns padrões macroecológicos através da variabilidade ecofisiológica das espécies. Estas tratam 1) dos gradientes globais de diversidade; 2) dos gradientes climáticos de tamanho corporal e distribuição geográfica (regra de Bergmann e regra de Rapoport, respectivamente); e 3) da evolução e ocupação do nicho climático por meio da variação nas tolerâncias termais das espécies. Utilizamos dados de diversas fontes, incluindo climáticos e biogeográficos, além de distribuições geográficas, morfologia, fisiologia e filogenias das espécies. Empregamos técnicas de análises espaciais, métodos comparativos filogenéticos e modelos evolutivos, inclusive propondo novas aplicações para alguns dos métodos. Corroboramos alguns padrões reconhecidos e revelamos outros pouco conhecidos. Indicamos prováveis mecanismos para alguns dos padrões, e demonstramos a aplicabilidade de algumas abordagens e ferramentas para a resolução de questões macroecológicas. Por fim, ressaltamos o poder integrador da macroecologia e a necessidade de agregar distintos dados e procedimentos analíticos para avançar na explicação de processos ecológicos que operam em largas escalas, sobretudo aqueles relacionados à interação espécies-clima, uma vez que as atuais mudanças ambientais devem demandar novos desenvolvimentos dessa natureza.

Abstract

Ecological studies involving broad spatial and temporal scales have an honorable history in natural sciences. Until the proposition of macroecology at the later 80's, several theoretical foundations were established upon studies of distributional patterns of species and their properties, and of the influence of spatial and evolutionary processes in the formation and succession of these patterns over time. Currently, macroecology comprises many broad-scale ecological questions, and traversed the boundaries of other research fields. Some of its central questions relate to the role of the geographical space as a modulator of ecological processes, to the phylogenetic relationships that confer morphological and ecological dependence among species, to problems of transposal of ecological scales and levels of organization, to the shifting from pattern documentation to inference of causal processes, and to the development of procedures and tools to address such demands. Despite the progress observed, many key questions of the relationship between the species and their environment at macroscales, which are linked to the origin and nature of macroecological patterns, remain elusive. In this thesis, we address three questions related to the influence of the climate on some macroecological patterns by means of the species' ecophysiological variability. These are related to 1) the global diversity gradient; 2) the climatic gradients in body size and range size (Bergmann's rule and Rapoport's rule, respectively); and 3) the evolution and occupation of the climatic niche by means of the variation of the species' thermal tolerances. We used data from various sources, including climatic and biogeographic data, along with geographical distribution, morphology, physiology and phylogenies of the species. We employed techniques of spatial analyses, phylogenetic comparative methods and evolutionary models, also proposing novel applications to some methods. We corroborate some well known patterns and revealed other less known. We pointed to likely mechanisms to some patterns and showed the applicability of some approaches and tools to solving macroecological questions. Finally, we highlight the integrative power of macroecology and the need for merging distinct data and analytical procedures in order to advance on the explanation of ecological processes that operate at broad scales, particularly those related to the species–climate interaction, as current environmental changes are supposed to demand fresh developments of this kind.

Introdução Geral

Estudos de padrões ecológicos em largas escalas espaciais precedem a concepção da própria ecologia. Questões ainda hoje discutidas têm sua origem em estudos naturalísticos, principalmente do século XVIII e XIX, quando expedições individuais (e.g. Georges-Louis de Buffon, Alexander von Humboldt, Alfred Wallace, Charles Darwin) incluíam, por exemplo, descrições e determinantes dos limites de distribuição de espécies ou diferenças na ‘variedade de animais e plantas’ ao longo do espaço geográfico (Egerton 2012). Já no início século XX, diversas questões de ampla abrangência espacial e temporal já eram investigadas de maneira sistemática (e.g. Grinnell 1917; Arrenius 1921; Willis 1922). Logo, estas estabeleceram algumas das bases conceituais para o que seria sintetizado no atual conhecimento macroecológico (e.g. Hutchinson 1957; Preston 1960; MacArthur & Wilson 1963; Janzen 1967; MacArthur 1972).

Em seu estabelecimento como programa científico, a macroecologia (*sensu* Brown & Maurer 1989) focava na distribuição de recursos espaciais e energéticos entre espécies em largas escalas espaciais e temporais. O objetivo dessa abordagem seria compreender as regularidades dos processos biológicos em níveis continental e global que determinariam os padrões de riqueza e densidade de espécies. A consequência dessa compreensão mais abrangente seria, portanto, a formulação de leis naturais e princípios unificantes subjacentes ao funcionamento da natureza (Blackburn & Gaston 2002). Neste contexto, atributos como o tamanho corporal das espécies foram logo percebidos como fundamentais, já que este estaria de algum modo relacionado com a alocação de espaço e recursos energéticos entre indivíduos (Brown & Maurer 1989; Brown 1995). Atualmente, podemos definir ‘macroecologia’ como um programa científico voltado a questões ecológicas em largas escalas espaciais e em tendências macroevolutivas que influenciam os padrões de distribuição, diversidade e abundância de espécies e seus atributos (Brown 1995; Hawkins & Diniz-Filho 2008).

Rapidamente, em seu desenvolvimento, a macroecologia incorporou (ou pelo menos se sobrepôs a) outros ramos da ecologia que lidavam com questões em macroescala, tais como os gradientes de diversidade, regras ecogeográficas, teoria de biogeografia de ilhas, paleoecologia, entre outros (Kent 2005). Essa sobreposição situou a macroecologia em uma posição importante em relação a outros campos das ciências naturais, já que a perspectiva macroscópica tem permitido algumas sínteses e

generalizações de fatos e ideias que muitas vezes estiveram isolados e desconectados entre si e seus campos de investigação (Brown 1999; Blackburn & Gaston 2001; Beck et al. 2012). A união de métodos filogenéticos à distribuição de comunidades ecológicas ao longo do espaço geográficos (Webb et al. 2002; Cavender-Bares et al. 2006) é um exemplo do poder de integração e generalização da abordagem macroecológica. Outro exemplo é o uso de dados paleontológicos combinados com análises de distribuição geográfica de clados ancestrais para reconstruir condições passadas e inferir gradientes de diversidade no passado (Svenning et al. 2011).

Devido à incorporação dos vários programas de pesquisa que lidavam com o componente espacial (e.g. gradientes de diversidade, determinantes ambientais da distribuição de espécies), a influência da dimensão espacial nos fenômenos tornou-se logo um tema importante em macroecologia (Bini et al. 2009). Essa importância é vista no desenvolvimento de bases conceituais e metodológicas. Por exemplo, levar em conta o efeito da proximidade geográfica, ou autocorrelação espacial, tornou-se essencial para a interpretação das relações entre variáveis espacialmente estruturadas (Diniz-Filho et al. 2003). Entretanto, considerar explicitamente a estruturação espacial de processos ecológicos vai além do controle da autocorrelação espacial. É prática comum ‘usar’ o espaço como um emulador de dinâmicas estocásticas de alguns processos ecológicos como dispersão, estrutura de comunidades e estrutura genética de populações em larga escala (Hubbell 2001; Sexton et al. 2009). Outra condição que deriva da relação entre processos ecológicos e o espaço é a variabilidade das relações entre atributos biológicas e os seus descritores ambientais. Diferentes variáveis podem ser explicadas por diferentes preditores conforme sua posição no espaço e a escala em que o fenômeno se manifesta. Ou ainda, a relação entre uma mesma variável e seu preditor ecológico pode variar ao longo do espaço geográfico. Esta condição é conhecida como não-estacionaridade espacial e é abordada analiticamente através de estatísticas espaciais que admitem essa condição nas relações (Foody 2004; Cassemiro et al. 2007). Apesar de ser reconhecido como um efeito importante para vários padrões macroecológicos e dispor de meios analíticos para acessar seus efeitos, a não-estacionaridade espacial permanece pouco explorada em ecologia em geral. Provavelmente isso acontece por que para se entender a variação espacial e entre escalas das relações ecológicas dependa primeiramente da identificação dos fatores reais subjacentes aos processos ecológicos investigados, o que por vezes não é tão claro.

Além da estruturação espacial dos processos ecológicos, a natureza das questões investigadas (i.e. envolvendo escalas temporais amplas) e a necessidade de acoplar o processo evolutivo aos padrões observados, resultaram na incorporação da dimensão temporal nos estudos macroecológicos. Essa incorporação tem ocorrido através de duas fontes principais e distintas em termos de tradição científica: a filogenética e a paleontologia (Smith & Boyer 2012). Atualmente há um importante movimento de fusão entre métodos filogenéticos e paleontologia para reforçar o suporte às questões macroecológicas (e.g. Butterfield 2007; Verbruggen et al. 2009). Essa fusão busca maximizar a qualidade da informação histórica no sentido de, por exemplo, elucidar o componente evolutivo dos padrões ecológico atuais, fornecer réplicas temporais desses padrões e caracterizar o fluxo de mudanças ambientais e evolutivas (Verbruggen et al. 2009; Smith & Boyer 2012). Uma terceira abordagem é a interpretação de processos históricos (e.g. isolamentos e desenvolvimento de biotas e intercâmbios regionais) através do uso de classificações de regiões biogeográficas (e.g. Kissling et al. 2012). Um exemplo típico é o das ecorregiões e dos domínios ecogeográficos (e.g. Udvardy 1975; Holt et al. 2013) Neste caso, os domínios ecogeográficos caracterizam um conjunto de fenômenos ecológico-evolutivos em uma determinada região, período histórico e clado específicos, quando outras inferências mais detalhadas inexitem ou são de difícil dedução (Beck et al. 2012).

No entanto, a maioria das questões envolvendo o processo evolutivo em macroecologia tem sido abordada através do desenvolvimento e incorporação de métodos comparativos filogenéticos. A crescente disponibilidade de filogenias (moleculares ou combinadas) abrangentes (e.g. Bininda-Emonds 2007; Pyron & Wiens 2011; Jetz et al. 2012) e o incremento na capacidade computacional para analisar extensos bancos de dados têm impulsionado as investigações relacionadas à evolução e distribuição de caracteres biológicos e das relações de parentesco entre as espécies ao longo do espaço geográfico. Neste sentido, uma aplicação central das filogenias em estudos macroecológicos está relacionada com o controle da possível dependência estatística na variabilidade de atributos biológicos entre espécies filogeneticamente próximas, ou autocorrelação filogenética (Diniz-Filho & Bini 2008). Além dessa dependência estatística, o acoplamento de informações filogenéticas às características e condições ambientais experimentadas pelas espécies (atuais e ancestrais) tem sido responsável pelo estabelecimento de um tópico central na ecologia atual, o conservatismo filogenético de

nicho (Cooper et al. 2010; Wiens et al. 2010). Este fenômeno, em que espécies mais próximas filogeneticamente são mais similares do que espécies selecionadas do clado aleatoriamente, estabeleceu-se como uma das principais bases para investigação e explicação para diversos padrões ecológicos, sobretudo de larga escala. Estes incluem, por exemplo, os padrões de distribuição e diversificação de espécies (Buckley et al. 2010; Wiens *et al.* 2006), evolução de atributos biológicos (Ackerly et al. 2006), formação de comunidades ecológicas (Webb et al. 2002), potencial invasivo de espécies (Mata et al. 2011) e respostas das espécies à mudanças climáticas (Parmesan & Yohe 2003).

Outra questão importante do programa macroecológico é identificar os níveis de organização biológica em que os processos se manifestam. Estes podem ser relevantes em nível de populações (e.g. Peterson et al. 2006), entre espécies ou comunidades (e.g. Olalla-Tárraga et al. 2010; Diniz-Filho et al. 2009) ou em níveis organizacionais ainda mais elevados (e.g. Kerr et al. 2007). Evidentemente, a maioria dos fenômenos tem efeito em distintos níveis ecológicos, e a possibilidade de abordá-los simultaneamente permite expandir sua compreensão de forma hierárquica e interligada (e.g. Willig 2003; Diniz-Filho 2004; Hui 2009). No entanto, muitas questões sobre níveis específicos requerem um suporte metodológico próprio, e a fusão de abordagens em distintos níveis implicam, geralmente, em falta de informações de um nível que sejam extrapoladas para outros. Um exemplo é a modelagem de padrões espaciais de assembleias de espécies em larga escala, o SESAM (Guisan & Rahbek 2011). O SESAM integra modelagem macroecológica e modelagem de distribuição de espécies para prever padrões de diversidade de assembleias. Entretanto, as regras de assembleias que definem as interações e co-ocorrências entre espécies nas assembleias raramente estão disponíveis em largas escalas geográficas.

Depois de pouco mais de duas décadas, a abordagem macroecológica permitiu avanços significativos nas suas áreas de atuação, inclusive promovendo integração de campos de investigação que anteriormente se desenvolviam em tradições distintas e pouco comunicantes (e.g. Hawkins 2004; Blackburn & Gaston 2001). De modo geral, a disponibilidade de informações abrangentes, o desenvolvimento de ferramentas analíticas e surgimento de novas questões têm favorecido esse caráter integrador da macroecologia. Atualmente, os estudos macroecológicos têm tentado avançar além da documentação de padrões macroecológicos e direcionado esforços pra elucidar as causas desses padrões, ou

seja, os processos subjacentes. No entanto, a natureza dos fenômenos macroecológicos (sobretudo em função de suas escalas de espaço e tempo) restringe, por exemplo, a execução de manipulações experimentais que poderiam subsidiar inferências causais. Além disso, como referido acima, alguns mecanismos atuam em diferentes escalas e níveis ecológicos, o que torna a identificação desses determinantes uma tarefa difícil (Beck et al. 2012). Uma alternativa seria investigar a dependência de escala das relações ecológicas, de modo a identificar que fatores estão relacionados aos padrões em determinadas escalas (Hendriks et al. 2009). Outra tendência é a utilização de simulações ‘orientadas por processos’, as quais permitem gerar expectativas nulas de padrões macroecológicos que podem ser confrontados com os padrões observados (Gotelli et al. 2009).

No entanto, muitas das questões fundamentais permanecem ainda indefinidas, em parte, pela necessidade de novas integrações entre dados e análises, ou mesmo pela indisponibilidade de informações fundamentais relacionadas aos padrões observados, tais como dados de abundâncias relativas de espécies ao longo de largas escalas geográficas, variabilidade genética, interações bióticas e parâmetros fisiológicos. Os atributos fisiológicos das espécies constitui um grupo de parâmetros que tem se tornado crítico em macroecologia. Apesar de há algum tempo apontado como essencial (Garland & Carter 1994; Chown & Gaston 1999), apenas recentemente esse grupo de informações foi introduzida de maneira mais sistemática na macroecologia, numa disciplina que vem sendo chamado de ‘macrofisiologia’ (Chown et al. 2004; Gaston et al. 2009). Uma razão primordial para a utilização de dados fisiológicos é que vários padrões macroecológicos têm nesses fatores seus pressupostos, hipóteses principais ou mesmo mecanismos subjacentes (Chown & Gaston 1999; Chown et al. 2004). Por exemplo, os tamanhos de distribuição geográfica das espécies têm sido atribuídos à tolerância à variabilidade climática (Janzen 1969; Stevens 1989); gradientes espaciais de tamanho corporal (i.e. regra de Bergmann) têm como principal hipótese a habilidade de conservar o calor corporal (Olalla-Tárraga & Rodríguez 2007); os gradientes globais de diversidade tem como explicação mais amparada a tendência de espécies de manter faixas de tolerância ancestrais relativamente conservadas ao longo do processo evolutivo, i.e. conservatismo de nicho climático (Wiens et al. 2010). De fato, limites e ótimos fisiológicos estão entre as dimensões do nicho ecológico mais características e importantes para as espécies (Kearney & Porter 2009). Portanto, há a necessidade de considerar as implicações da

variabilidade fisiológica das espécies para os padrões macroecológicos e na elucidação dos seus processos subjacentes, sobretudo quando se pretende prever e mitigar possíveis efeitos das mudanças climáticas (Chown & Gaston 2008; Deutsch et al. 2008; Duarte et al. 2012).

Neste contexto, um grupo de variáveis fisiológicas que tem despertado considerável atenção em estudos ecológico em macroescala inclui os valores de tolerância climática. Isso se deve, principalmente, ao fato de que parâmetros específicos do clima (como temperatura e umidade), são intuitiva e prontamente associados ao nicho ecológico das espécies e a seus limites de sobrevivência (Lutterschmidt & Hutchison, 1997). De fato, essas questões de variabilidade e distribuição de tolerâncias fisiológicas em macroescala estão relacionadas à própria origem e natureza dos padrões macroecológicos. Grande parte dos fenômenos ecológicos observados, sobretudo em largas escalas espaciais e temporais é provocada ou modulada pelas respostas das espécies às condições ambientais externas (Hawkins 2004). Evidentemente, outros processos ecológicos estão diretamente ligados aos padrões macroecológicos, incluindo a capacidade de dispersão das espécies (Baselga et al. 2011), suas interações bióticas (Pigot & Tobias 2012), contingências históricas (Hortal et al. 2011) e a estocasticidade ambiental (Pulliam 2000). Contudo, o potencial das espécies de permanecer, dispersar-se ou reagir a mudanças contingenciais do ambiente depende grandemente dos seus níveis de tolerância e capacidade de resposta a variações nas condições ambientais circundantes.

Um passo fundamental em macroecologia, portanto, é buscar entender como as propriedades mais fundamentais das espécies, particularmente relacionadas à variabilidade ecofisiológicas, respondem a condições climáticas atuais e históricas e determinam os padrões ecológicos e evolutivos em larga escala. Essas questões demandam a conjugação de informações das espécies e ferramentas analíticas diversas, ou mesmo de novas aplicações das ferramentas já existentes ou do desenvolvimento de novas abordagens. Valer-se de circunstâncias favoráveis à exploração de algumas das questões abertas, sejam elas disponibilidade de determinados dados ou uma condição ambiental particular, também constitui uma estratégia importante para responder algumas das perguntas.

Historicamente, os anfíbios têm sido importantes como organismos-modelo em pesquisas ecológicas (Hopkins 2007). Por conta de sua condição ectotérmica e pele

permeável, os anfíbios são extremamente sensíveis a variações na temperatura e umidade, e por isso situam entre os organismos mais suscetíveis aos efeitos do clima (Donnelly & Crump 1998). De fato, vários efeitos diretos e indiretos das mudanças no clima sobre esses animais têm sido documentados (Blaustein et al. 2010). Por outro lado, os anfíbios situam-se em um importante debate em macroecologia, relacionado à aplicabilidade dos gradientes de tamanho do corpo com a redução da temperatura, ou regra de Bergmann (e.g. Olalla-Tárraga 2011). Ademais, cresce o volume de informações sobre relações filogenéticas (e.g. Pyron & Wiens 2011), distribuição geográfica (e.g. Grenyer et al. 2006; IUCN 2009) e variabilidade de parâmetros fisiológicos das espécies de anfíbios (e.g. Duarte et al. 2012). Portanto, os anfíbios representam um grupo adequado à investigação de padrões macroecológicos relacionados à variabilidade climática.

Nesta tese utilizamos os anfíbios para investigar algumas questões relacionadas à relação entre clima e atributos ecofisiológicos das espécies na formação de alguns dos principais padrões macroecológicos conhecidos: 1) os gradientes globais de diversidade, 2) as regras ecogeográficas e 3) os padrões de evolução e ocupação de nicho climático em macroescala. Evidentemente, esses temas têm sido debatidos por décadas, de modo que vários aspectos teóricos e metodológicos foram discutidos ao longo do tempo. Entretanto, focamos aqui em algumas questões específicas de cada um desses temas, buscando reunir informações e abordagens analíticas diversas, incluindo dados de distribuição geográfica, parâmetros climáticos, traços morfológicos e fisiológicos, filogenias associadas a modelos evolutivos, empregando novas abordagens de métodos comparativos filogenéticos e análises espaciais para investigar essas questões específicas.

No primeiro capítulo (Gouveia et al. 2012; *Ecography*, *in press*), por exemplo, utilizamos as espécies de anfíbios de todo o mundo pra demonstrar como as relações entre o clima e a riqueza de espécies variam ao longo do espaço (uma condição conhecida como não-estacionaridade espacial) em função dos padrões de evolução das respostas ecofisiológicas das espécies. O estudo testa as hipóteses climáticas mais amplamente defendidas (produtividade, variabilidade climática e mudanças climáticas históricas), utilizando uma nova aplicação de método de regressão ponderada geograficamente (GWR – *geographically weighted regression*). O método leva em conta o efeito da não-estacionaridade espacial e, através da modificação utilizada neste estudo (chamada de ‘GWR parcial’), permite estimar as variações e contribuições relativas de

cada fator climático em cada unidade espacial ao longo do espaço geográfico. Comparamos os resultados do GWR parcial com dois outros métodos de regressão de referência que assumem estacionaridade nos dados, a regressão simples (OLS – *ordinary least square*) e o mapeamento de autovetores espaciais (SEVM – *spatial eigenvector mapping*). Os mapas de contribuições relativas de cada fator climático permite a verificação das predições ecofisiológicas formuladas *a priori*.

No segundo capítulo (Gouveia et al. 2013, *Biological Journal of the Linnean Society; in press*), testamos a presença das duas ‘regras’ ecogeográficas mais debatidas em macroecologia: a regra de Bergmann e a regra de Rapoport. Testamos esses padrões em um clado inteiro de anfíbios anuros, a família Centrolenidae, e ao longo dos gradientes latitudinais e altitudinal. As explicações para ambas as regras ecogeográficas estão diretamente relacionadas à variabilidade na tolerância entre espécies. Uma vez que dados de tolerância são escassos, sobretudo para clados inteiros, propusemos uma decomposição das distribuições horizontal (latitudinal e longitudinal) e vertical (altitudinal) em um componente interespecífico de variabilidade de tolerância. Através desta medida testamos a validade da hipótese de conservação de calor (e consequentemente da hipótese de equilíbrio de calor) para tamanho do corpo. Neste estudo levamos em conta ainda as relações filogenéticas entre as espécies, testando inclusive a variação do componente adaptativo (i.e. controlando o efeito filogenético) ao longo dos gradientes espaciais.

No terceiro capítulo (Gouveia et al., *Global Ecology and Biogeography; in review*), utilizamos um conjunto de dados de tolerância termal reais (temperatura crítica máxima – CT_{max}) de anuros para investigar a relação entre nicho térmico nas escalas fisiológica e macroecológica. Os valores de tolerância foram obtidos experimentalmente a partir de 47 espécies distribuídas nos domínios Neotropical e Paleártico. Aqui estávamos interessados na transferibilidade de parâmetros fisiológicos na manifestação do nicho climático das espécies em macroescala. Testamos a hipótese de que os limites fisiológicos reais das espécies são mais bem descritos por medidas de posição de nicho climático no espaço multivariado do que por limites climáticos geográficos (i.e. temperatura máxima geográfica - T_{max} e largura de nicho multivariado). Isso estaria relacionado a uma maior interferência de fatores externos (interações bióticas e restrições de dispersão) nos extremos do nicho obtidos da distribuição geográfica das espécies,

enquanto medidas posicionais seriam menos afetadas por esses fatores externos. Utilizamos métodos de regressões filogenética e não-filogenética para testar as relações entre as variáveis, e empregamos ainda uma técnica baseada em autovetores (curva PSR) que caracteriza as taxas de evolução dos caracteres em relação ao padrão de ramificação da árvore filogenética das espécies. Esta técnica nos permite comparar as taxas de evolução dos atributos em termos de conservatismo/evolução de nicho. As análises conjuntas do poder explanatório de variáveis do nicho térmico entre diferentes escalas (fisiológica e macroecológica) e dos modelos de evolução desses atributos permitem avaliar a transferibilidade de dados de tolerância termal entre essas diferentes escalas e como os limites fisiológicos se manifestam na distribuição geográfica das espécies e em sua distribuição no espaço climático.

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Capítulo 1: Nonstationary effects of productivity, seasonality, and historical climate changes on global amphibian diversity

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Abstract

Attempts to explain the origin of species diversity gradients often lack generality across geographic regions or taxa. One possible reason for this is that species respond differently to the same environmental descriptors (e.g. climate) across geographical space, i.e. the diversity–environment relationship is spatially nonstationary. Here we evaluate the spatial nonstationarity of the relationships between amphibian species richness and variables representing three primary climatic hypotheses: historical climate variability, seasonality and productivity. We formulated nonstationary explicit predictions for the taxon based on its ecophysiological attributes. We employed two global approaches that assume stationarity – standard non-spatial OLS regression and spatial eigenvector mapping (SEVM) – and compared them with a nonstationary partial GWR (Geographically Weighted Regression), which allows the investigation of the relative contributions of each predictor regionally, helping to portray large-scale patterns. Although productivity was a better correlate to species richness than the other factors in both global and local approaches, no single hypothesis fully explained the worldwide pattern of species richness. Spatial nonstationarity was present in all relationships, and substantial fractions of the variation in the data were unexplained due to collinearity. We discuss some noteworthy regional cases and propose that the history of exposure to specific environmental conditions is responsible for geographical differences in the amphibian–climate relationships, as stated by the niche conservatism hypothesis. Finally, we argue that there is a trade-off in the selection of the spatial scale analysed – regional vs global – regarding the generality vs the explanatory power of the resulting pattern of species richness.

Introduction

The origin of geographical gradients in species richness is one of the most long-standing questions in ecology and biogeography. Despite a few proposals for unified explanations based on historical processes and the effects of current and past climates (Hawkins 2004, Wiens and Donoghue 2004), identifying a general cause for these gradients that is applicable across different taxa, geographic regions or spatial scales has been challenging, resulting in a long discussion in the literature (Willig et al. 2003, Currie et al. 2004, Mittelbach et al. 2007). Multiple causality is now recognised rather than such a universal mechanism, as the processes driving a particular diversity gradient appear to depend on the type of organism and factors such as spatial scale (Field et al. 2009), thus preventing the identification of a general solution. In addition, studies that aim to develop, simultaneously, a general explanatory model and a predictive theory of species richness, have introduced significant confusion to this research agenda (Allen et al. 2002), as attempts to provide a single answer for both goals are doomed to not meet either of them (Brown 1999, see also recent discussion regarding metabolic theory in e.g. Hawkins et al. 2007).

Despite the above discussion, it is generally accepted that climate is a major determinant of the geography of species richness at broad geographical scales, although the nature of such relationship is still controversial (Hawkins 2004, Field et al. 2009). The amounts of available energy and water, for instance, are thought to play a prominent role in driving diversity patterns, operating via environmental productivity, niche conservatism, physiological tolerance, rates of biological activity, rates of diversification or a mixture of these and other mechanisms (Wright 1983, Hawkins et al. 2003a, Hawkins 2004, Wiens and Donoghue 2004, Whittaker et al. 2007, Field et al. 2009, Qian 2010). Two other aspects of climate – seasonality (Klopfer 1959) and historical climate variability (Fischer 1960) – are also thought to affect diversity gradients at large scales (Pianka 1966, Currie 1991, Evans et al. 2005, Araújo et al. 2008, Diniz-Filho et al. 2009, Hortal et al. 2011). It has been argued that the effects of seasonality and temporal variability may be confounded with those of energy or water–energy models because of their collinearity with current climate (Evans et al. 2005). However, hypotheses based on both seasonality and historical variability offer plausible explanations for observed variability in diversity and have received empirical support from correlative analyses.

The seasonality hypothesis, for instance, has some advocates (Wiens et al. 2006) and opponents (Currie 1991, Evans et al. 2005), whereas historical climate variability has a greater (or at least similar) influence on diversity gradients than the current climate in Australia (Hawkins et al. 2005), North America (Montoya et al. 2007) and Europe (Araújo et al. 2008, Hortal et al. 2011, but see Svenning and Skov 2007, Fløjgaard et al. 2011).

Nonetheless, generalisations of individual hypotheses may fail because the strength of the relationships between response and predictor variables varies over geographical space, a condition known as ‘spatial nonstationarity’. In an ecological context, nonstationarity can arise, for example, from different histories of exposure of the regional species pool to particular climatic conditions (Hortal et al. 2011). During the diversification of a clade, some sub-clades are bounded by the group’s original adaptive constraints (e.g. those imposed by climate) whereas others – typically a reduced number – ‘escape’ from those limits and evolve under other conditions (Hortal et al. 2011, Olalla-Tárraga et al. 2011). If some conditions are more restrictive for some sub-clades than for others, then the processes of diversification should differ among them, and different spatial patterns of species richness would arise (Hua and Wiens 2010). Therefore, different patterns of richness involving clades with different relationships with their environment would result in different species–environment relationships as well. From a purely statistical perspective, nonstationarity precludes hypothesis testing and model predictions derived from global statistics, such as traditional linear regression and more sophisticated spatial regression techniques. These techniques assume stationarity in the relationships, and violating this assumption may lead to misleading interpretations of the actual determinants (Fotheringham et al. 2002, Osborne et al. 2007, see also Hawkins 2012 for a recent discussion). A number of statistical methods have been developed to measure and take into account spatial nonstationarity. Among them, Geographically Weighted Regression (GWR) is increasingly used in biogeographical and macro-ecological analyses (Foody 2004, Cassemiro et al. 2007, Svenning et al. 2009, Eiserhardt et al. 2011, Hortal et al. 2011). It incorporates local spatial relationships in a traditional regression framework and calculates regression parameters (e.g. intercept, slope, R^2) for each spatial unit (e.g. grid cells), allowing portrayal of the performance of individual predictors across space and enable testing of model fit to ecological hypotheses at a local scale (Fotheringham et al. 2002, Foody 2004, Cassemiro et al. 2007, Hortal et al. 2011).

Many ecological relationships do not meet the assumption of spatial stationarity, especially at increasing spatial scales (Foody 2004, Svenning et al. 2009, Hortal et al. 2011). Relationships involving broad-scale patterns of species richness are particularly challenging because nonstationarity can arise, for example, from 1) different responses to the same stimulus, 2) interactions of multiple explanatory factors, or 3) nonlinearity and spatially structured relationships between the descriptor used – which is often a proxy – and the actual determinant, which could bias the relationship with the response variable (Cassemiro et al. 2007). Although GWR does not fully address all of these sources of nonstationarity, it can be useful for detecting spatial patterns in the relationships (case 1 above) and identifying interactions among multiple factors (case 2), particularly through the approach proposed here. The third situation, though, is more difficult to address because most of the predictors used in macroecology are, in fact, proxies for the actual determinants and/or processes that drive geographical variations in species diversity. Here we test how the variables representing three climate-related hypotheses – historical climate variability, current seasonality, and current productivity – correlate with the global pattern of amphibian richness. We assess the relationships between species richness and a series of proxy variables for each climatic hypothesis and develop spatially oriented expectations that account for the spatial nonstationarity in these relationships. We also partition the influence of these variables locally as an attempt to untangle their individual and combined effects across space, 1) recognising regions where amphibian diversity responds to particular factors and verifying whether such responses are spatially nonstationary, and 2) identifying areas where the effects of multiple factors overlap, preventing their separation.

The historical climate variability hypothesis provides a good starting point for developing nonstationary counterparts of climatic predictions of species richness gradients. Its traditional formulation predicts a greater accumulation of species in regions that remain weakly disturbed or undisturbed for long periods of time (Fischer 1960, Pianka 1966). Accordingly, most species are removed from regions that are subject to large disturbances (such as glaciations) until either newly adapted forms evolve there or more acceptable conditions are restored, allowing recolonisation. However, this prediction favours the effect of currently experienced conditions, as they determine the maintenance or removal of a species during a given time. It also presupposes that the effect of past conditions fades and then disappears at some point in time (Currie 1991,

Hawkins et al. 2003a, Hawkins 2004). Although this dependence on time may imply a theoretical limitation, as it precludes a predictive theory, it offers a scenario whereby nonstationarity may arise. Therefore, we expect that regions that were affected more strongly by climatic changes during the Last Glacial Maximum (LGM; approximately 21000 yr BP), i.e. the Northern Hemisphere, will retain a stronger imprint of those conditions on the present distribution of species diversity (Table 1). In contrast, the richness pattern in regions with little difference between current and past conditions should be weakly correlated with this climatic anomaly or not correlated at all (but see Hortal et al. 2011, see also Sandel et al. 2011).

Table 1. Predictions for broad-scale relationships between species richness and the variables representing the three climatic hypotheses analysed. Stationary predictions were drawn from selected references and nonstationary predictions are as devised here explicitly for amphibians. LGM stands for Last Glacial Maximum.

| Hypothesis | Predictions | |
|------------------------|---|--|
| | Stationary | Nonstationary |
| Historical variability | Negative (Araújo et al. 2008) | Negative in regions glaciated during LGM, i.e., Northern Hemisphere, and weak or absent elsewhere |
| Seasonality | Negative (Klopfer 1959) | Negative in regions with annual frosts and highly seasonal areas in the tropics and weak or absent elsewhere |
| Productivity (AET) | Positive (Hawkins et al. 2003b; but see Mittelbach et al. 2001) | Positive and stationary |

At the macro scale, seasonality is supposed to affect species richness because it dictates periodic departures from the climatic optima of organisms and thus favours species with more flexible adaptations that enable coping with unevenly distributed resources (Klopfer 1959) or conditions (Stevens 1989). The evolutionary cost of these adaptations would result in seasonality acting as a progressive environmental filter as climate variability increases, hence reducing species number. Climate seasonality can then be expected to affect diversity gradients more strongly 1) in areas where lower extremes cause freezing conditions, and 2) in seasonal areas within the tropics (Table 1). Near-freezing conditions are a critical physiological boundary for most amphibians,

requiring the production of cryoprotectants – a feature known only for few species – and preventing thermal acclimation as seen at higher temperature ranges (Pinder et al. 1992, Rome et al. 1992). Hence, prolonged below-zero Celsius temperatures would prevent many amphibian species from sustaining viable populations in regions of cold climate. In fact, freezing conditions have been argued to have limited anurans from dispersing freely into the Northern Hemisphere (Wiens et al. 2006, Araújo et al. 2008). Therefore, below zero degrees, the more severe the cold, the fewer species may be able to tolerate it. The rationale of the second prediction stems from Janzen's (1967) proposition – formulated for altitudinal barriers to dispersal. Accordingly, tropical species have evolved under a narrower climatic regime and thus fewer of those species would be able to overcome more variable conditions. This should result in a stronger richness–seasonality relationship in climatically variable regions in the tropics.

The effect of current climate could be evaluated by measuring the amount of energy and water directly from, for example, mean annual temperature and precipitation. However, these variables are strongly collinear with past climatic variables (Araújo et al. 2008) and, in addition, they may be related to different mechanistic hypotheses (Hawkins et al. 2003a, b), preventing us from disentangling the independent effects of this factor. Thus, we avoided using these variables to restrict the evaluation of the effects of water and energy to the productivity hypothesis. This predicts that the number of species is a function of the number of individuals, which varies with productivity, meaning that highly productive environments reduce local extinction and thus accommodate more species (Wright 1983, Hawkins et al. 2003b, Buckley and Jetz 2007). Productivity has been represented by different variables such as net primary productivity (NPP), normalised difference vegetation index (NDVI), and actual evapotranspiration (AET) (Woodward 1987, Evans et al. 2005, Fisher et al. 2011). Although it might be worthwhile to evaluate the performances of these surrogates of productivity under a nonstationary viewpoint, we chose AET because it encapsulates both water and energy and is acknowledged as one of the best global predictors of amphibian diversity (Buckley and Jetz 2007, Qian 2010, but see Currie et al. 2004). AET measures the water availability that meets the environmental demand of energy; it is constrained at its upper values by water availability and at its lower bound by energy input, so that both energy and water vary but are always available throughout the full variation of AET (see Fisher et al. 2011 for a recent review). Therefore, for amphibians, for which a liquid water supply is a direct

ecophysiological requirement of nearly all species, a linear correlation with AET should be expected at any scale (Buckley and Jetz 2007, see also Mittelbach et al. 2001 for a review on the shape of energy–richness responses). We therefore expect a stationary relationship between amphibian richness and AET (Table 1), unless it interacts with other factors. Here, we evaluate the importance of the three climate-based hypotheses described above for the geographical gradients of amphibian diversity worldwide, under the assumption that each factor affects species richness at different locations in a particular way. Although through our analyses we are unable to accurately predict the extent (e.g. the number of cells) and boundaries where the effects are independent or overlap each other, the predictions as formulated above allow a direct test of which hypotheses are better supported by nonstationary GWR analyses. This approach also permits us to move from a purely exploratory analysis of nonstationarity in the diversity–climate relationship towards more theory-oriented, albeit correlative, hypothesis testing.

Material and methods

Data origin

We projected the extents of occurrence of all amphibians (Anura, Caudata and Gymnophiona) based on the ‘Global Amphibian Assessment’ database (IUCN 2009) onto a global grid cell system of $2.0^\circ \times 2.0^\circ$ spatial resolution near the equator. The choice of this resolution relies on both the resolution of the palaeoclimatic models used (see below) and the evidence that the effects of climate tend to be maximised at coarser resolution, whereas finer resolutions are more suitable to accounting for factors that operate locally (Belmaker and Jetz 2011). The grid covered terrestrial areas excluding Antarctica, remote islands and continental margins with more than 50% sea surface. The geographic projection that was used leads to a decrease in cell size towards the poles, which could bias the results. However, a test of the sensitivity of species richness to the area of grid cells by including cell area as a covariate in species–climate relationships did not indicate any significant effect of grid cell area (not shown).

We estimated species richness by summing all species’ ranges overlaid onto the grid. To prevent results from being inflated by zeroes, we removed cells from the cores of uninhabited regions (i.e. the northernmost areas of the Holarctic, the Sahara and Middle

East deserts, and some Central Asian regions), retaining zero-richness cells only at their boundaries. A total of 3863 cells remained for the analyses (Fig. 1). The well-known global pattern of amphibian richness is characterised by a higher concentration of species in wet tropical regions such as the Amazon and the Atlantic Forest in South America and the Guinean/Congo rainforests in Africa, and a moderate richness in the eastern coast of both North America and Australia (Buckley and Jetz 2007, Fig. 1).

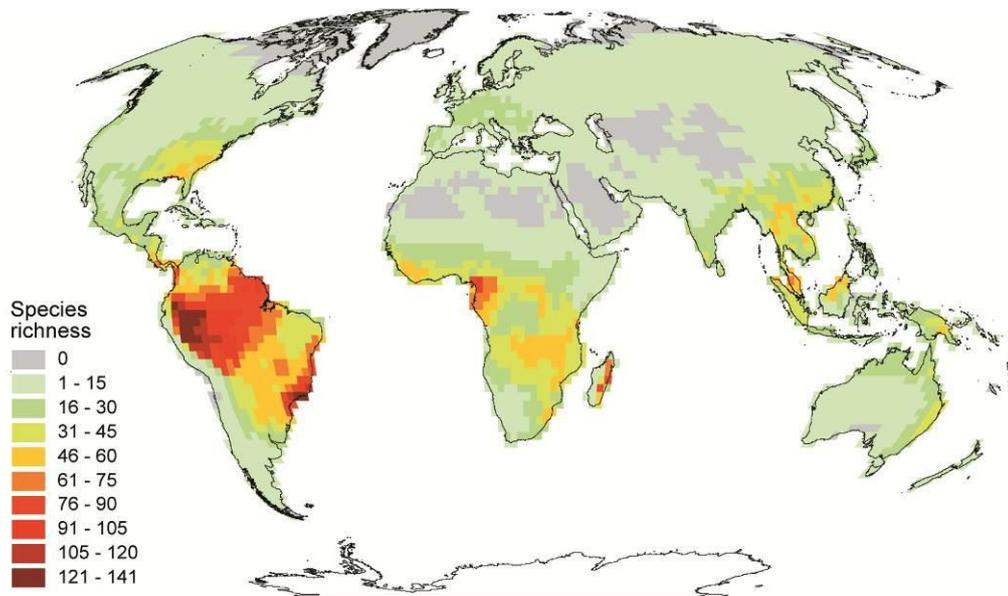


Figure 1. Global pattern of amphibian richness, obtained by overlaying species ranges from IUCN (2009) on a $2.0^{\circ} \times 2.0^{\circ}$ grid cell system.

We used three sets of environmental predictors to account for the three hypotheses addressed: 1) temperature and precipitation anomalies between the Last Glacial Maximum (LGM) and the present were used as a measure of historical climate variability; 2) temperature and precipitation seasonality were used for current climatic variability; while 3) AET accounts for productivity. The historical anomaly of climate was the difference between the current and LGM values of both mean annual temperature and annual precipitation (Araújo et al. 2008, Diniz-Filho et al. 2009, Hortal et al. 2011). The LGM was characterised by strong cooling with minimum temperatures of approximately -30°C over the ice sheets in the Northern Hemisphere and a moderate amount of cooling (22 to 25°C) in tropical and mid-latitude regions (details in Braconnot et al. 2007). Measures of seasonality were the standard deviations of both monthly

temperature and rainfall drawn from a data series of interpolated surfaces (1950–2000; Hijmans et al. 2005). Other measures of seasonality could also be employed, such as the monthly seasonality of AET, as we used the amount of AET as a competing hypothesis. However, the adaptive and range boundaries of amphibians can be best represented by the variations in precipitation and temperature due to their dependence on water availability and high sensitivity to temperature (Pinder et al. 1992), particularly near the colder boundaries of their geographic range (Wiens et al. 2006, Olalla-Tárraga et al. 2011). Additionally, the inclusion of an index of temperature variation is more suitable for accounting for predictions of the response of richness to freezing conditions, as discussed above. AET data were derived from Willmott et al.'s (1985) modification of the Thornthwaite water budget, drawn from a monthly time series (1950–1999; Willmott and Matsuura 2001).

Global models

We first estimated a global model for each predictor through Ordinary Least Squares regression (OLS) to stand as a base-line for subsequent local analyses. Because type I error is usually inflated by spatial autocorrelation (Legendre and Legendre 1998), we also used Spatial Eigenvector Mapping (SEVM) (Diniz-Filho and Bini 2005, Griffith and Peres- Neto 2006) to account for spatial autocorrelation. Briefly, in SEVM, spatial eigenvector filters are incorporated as predictors in the OLS models. These filters describe the multi-scale spatial structure in the data, and are obtained from eigenvectors of truncated pairwise matrices of geographical distances through the principal coordinates of neighbour matrices. Here, filter selection was based on the minimum spatial autocorrelation in the model residuals after adding both environmental predictors and filters, using a Moran's I coefficient lower than 0.05 in the first class of a correlogram as a stopping rule (Griffith and Peres-Neto 2006). We also performed a partial regression analysis involving all three predictors to assess their relative contributions globally (Legendre and Legendre 1998).

Local models

We performed GWR analyses for each set of variables to account for nonstationarity in the species richness–climate relationships. In GWR, an independent

regression is conducted for each data point (here, for each grid cell) where the contribution of each of the samples to the analysis is weighted in relation to its spatial proximity to the specific location under consideration (i.e. focal cell). Here, proximity is defined as the nearby locations within a given bandwidth (h) of pre-set radius and shape (Fotheringham et al. 2002). This allows us to obtain all regression statistics for each focal cell, including partial slopes and coefficient of determination (R^2). We employed a weight matrix (W) derived from the geographical distances between the grid cells' midpoints and a Gaussian decay function at a standardised bandwidth ($h = 1300$ km) for all runs. The bandwidth in GWR is the radius of the extent to which the local calibration results are smoothed (Fotheringham et al. 2002). The bandwidth selected was the one that best removed the spatial autocorrelation in the map of residuals, accounting also for the approximate distance at which regional faunas differ significantly from one another. This procedure optimizes the relationships of the richness values of the focal cells with those of their neighbour cells within areas of relatively similar species pools.

Furthermore, we evaluated the relative contribution of each factor on each grid cell by performing a cell-by-cell variance partitioning among the predictors based on their GWR results (see Eiserhardt et al. 2011 for a recent analogous application). Herein, we refer to this procedure as partial Geographically Weighted Regression (partial GWR). The partial GWR procedure simply mimics a triple partial regression routine (Legendre and Legendre 1998) but replace OLS' global R^2 with the R^2 from the GWR analyses in each grid cell. Briefly, we first computed a GWR analysis with all three groups of variables (e.g. A + B + C), which provided the summed contributions of all predictors plus the fraction shared between them. Then we calculated GWRs for all pairwise combinations of hypotheses (A + B, A + C and B + C), and for each of them alone (A, B and C). A simple set of equations then allows the calculation of the independent and shared components of the covariation between all factors and species richness in each focal cell (Legendre and Legendre 1998).

All of these components can then be mapped to evaluate the relative contributions of each hypothesis in explaining the patterns observed in different regions of the world. All spatial analyses were performed in SAM (Spatial Analyses in Macroecology; Rangel et al. 2010), which is freely available at <www.ecoevol.ufg.br/sam>.

Results

According to the global SEVM models, AET accounted for the largest amount of variation in species richness ($R^2 = 0.618$), although seasonality explained nearly the same amount ($R^2 = 0.608$). Historical climate variability, however, was weakly correlated with amphibian richness worldwide ($R^2 = 0.017$). In the partial OLS regression analyses AET and seasonality shared a large fraction of the variation ($R^2 = 0.569$), whereas each one of them shared negligible fractions with historical variability (Venn diagram in Fig. 2). GWR analyses rendered better model fits than OLS or SEVM for all three hypotheses, although following the overall trend of a larger influence of productivity, followed by seasonality and ultimately by historical climate variability (Table 2, Fig. 2). Nevertheless, the GWR analyses showed a ubiquitous effect of nonstationarity on all species– climate relationships globally.

Table 2. Spatial Eigenvector Mapping regression (SEVM) and Geographically Weighted Regression (GWR) between amphibian species richness and the sets of predictors of the three hypotheses evaluated. F means the F statistic, and AIC_c the sample size-corrected value of the Akaike Information Criterion. The OLS regression was affected by spatial autocorrelation in the residuals and thus omitted here.

| Hypothesis | SEVM | | | GWR | | |
|------------------------|----------|------------|--------------|---------|------------|--------------|
| | F | AIC_c | $R^2_{adj.}$ | F | AIC_c | $R^2_{adj.}$ |
| Productivity | 6251.593 | 2,394.673 | 0.618 | 654.309 | -1,276.840 | 0.856 |
| Seasonality | 1517.260 | 36,958.176 | 0.608 | 407.308 | 34,058.378 | 0.693 |
| Historical variability | 132.617 | 39,013.486 | 0.017 | 256.249 | 35,175.465 | 0.607 |

Nonstationarity was also present in the independent effects of each predictor, which distinguished regions under the prevalence of single factors (maps of productivity, seasonality and historical variability in Fig. 2, 3). Additionally, in many regions, a large amount of variation was inseparable between pairs of predictors (maps P:S.H, P:H.S, and S:H.P in Fig. 2). These results support our prediction of a stronger effect of historical climate variability on the northern hemisphere, particularly in Europe and, to a lesser extent, in North America (Fig. 3). Interestingly, this factor was also an important correlate of amphibian richness in some areas of Central America and north- eastern Brazil (Fig. 2). In all of these areas, the model fit of historical climate variability was low

(R^2 ranging from 0.05 to 0.25) but with a slight, consistent decrease in areas where a lower influence is expected according to our predictions.

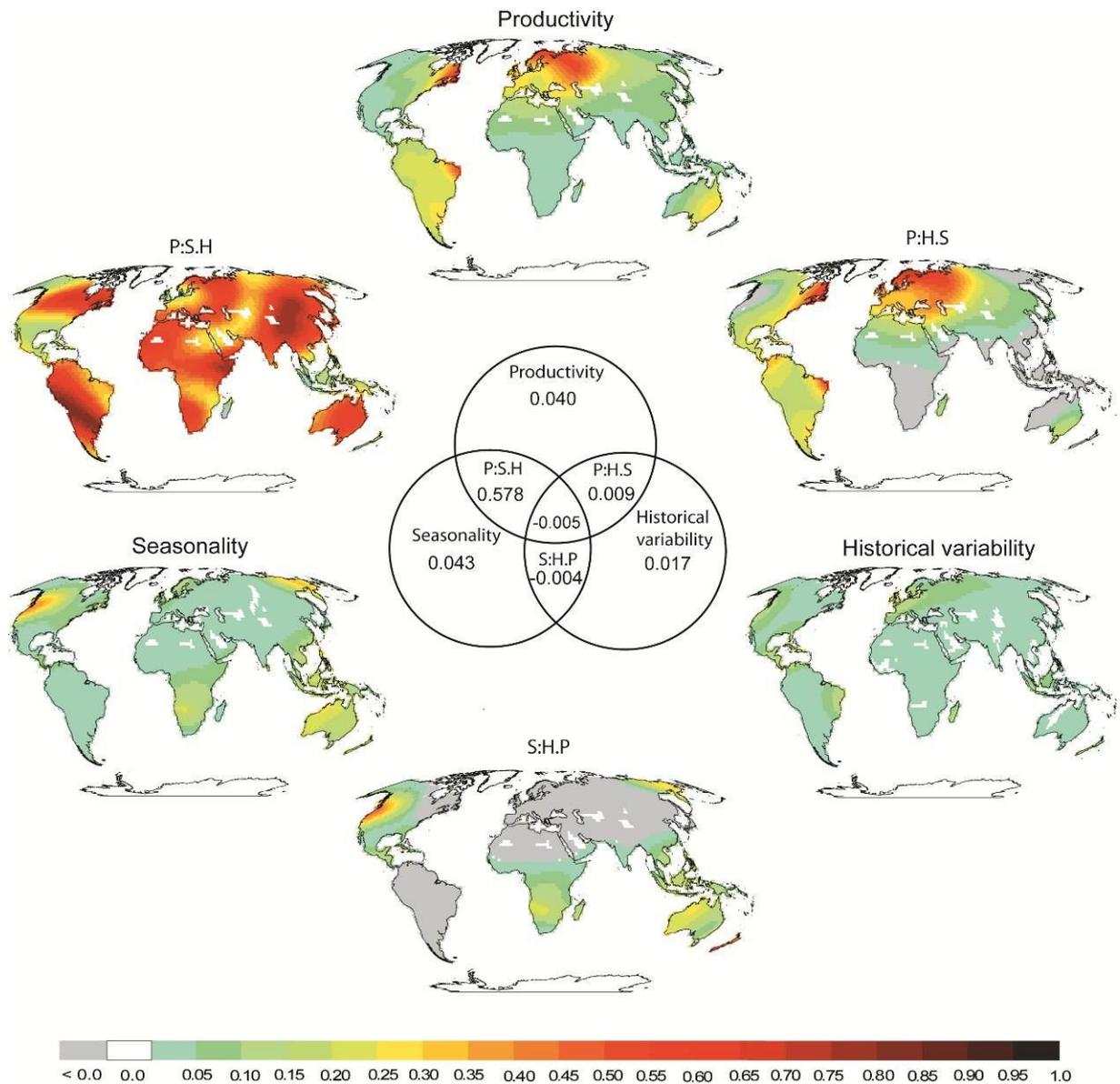


Figure 2. Maps of partial coefficients from the partial GWR analyses between predictors of productivity, seasonality and historical climate variability. Individual contributions of each hypothesis (i.e. non-shared variation) are shown in the maps labeled by the related hypothesis and maps of overlapped explanation are positioned between them. P = Productivity; S = Seasonality; H = Historical variability. In abbreviations, colons denote sharing between pairs of predictors alongside, whereas dots indicate that the following predictor was excluded. For example, P:S.H denotes the shared fraction of productivity and seasonality, excluded historical variability. Internal Venn diagram displays regression coefficients (R^2) from partial OSL analyses, and upper codes correspond to the partial GWR equivalent. Gray areas indicate negative R^2 due to opposite signal effects between predictors or among neighbour cells (see Legendre and Legendre 1998).

Our two predictions for seasonality were also supported, although not fully. The effect of seasonality was substantial in regions with freezing winters in the north-west of North America and Siberia, as well as in strongly seasonal mid-latitude areas such as sub-Saharan Africa and western Australia (Fig. 2). The amphibian richness of tropical and subtropical seasonal areas of South America, together with eastern Australia, however, was more strongly correlated with productivity. This predictor was even more important along the Holarctic, with maximum R^2 values located throughout western Europe and east Asia, from which model fit gradually weakened moving towards the lower latitudes of north Africa and east Asia. In any case, climatic predictors shared a large amount of explained variation. The shared influence of productivity and seasonality was remarkably high and globally widespread, weakening only along Central America and south of North America, and throughout the East Indies (P:S.H in Fig. 2, 3).

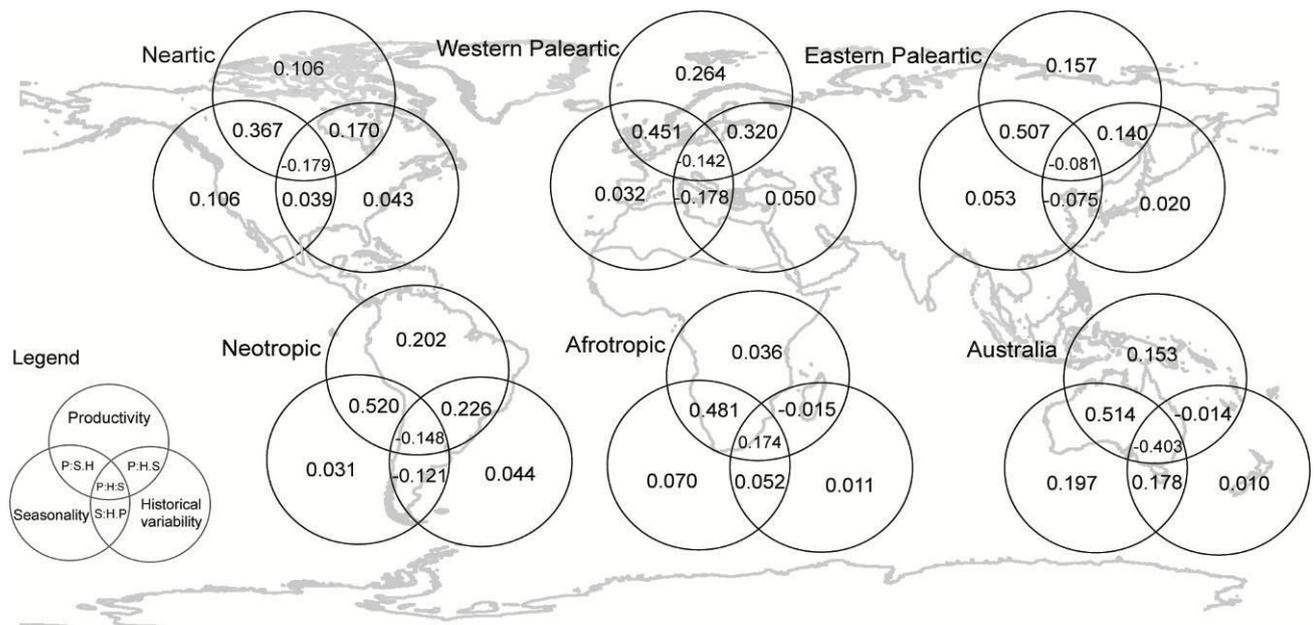


Figure 3. Mean values of the relative contribution (R^2) of climatic predictors within biogeographic realms, as drawn from the partial-GWR analyses. The Indomalayan realm was excluded and the Palearctic was divided in its Eastern and Western portions. Note the predominance of productivity over the other predictors in most realms.

Discussion

Our results support the existing evidence that climate is strongly correlated with the global pattern of amphibian richness, emphasising the relative primacy of water and

energy – most likely through productivity and climate tolerance (Field et al. 2009, Qian 2010, Olalla-Tárraga et al. 2011). This statement is supported by both global (SEVM) and local (GWR) models, which were also qualitatively similar in order of importance of the hypotheses evaluated. The global predominance of productivity identified by SEVM is followed by its geographic prevalence over other predictors in most regions according to the partial GWR analyses. Nevertheless, global models have often been criticised for overlooking the assumption of nonstationarity in large-scale assessments as they may fail to portray the actual effects of the variables involved (Foody 2004, Cassemiro et al. 2007). In fact, when the spatial heterogeneity in relationships with amphibian richness is explicitly examined the universal prevalence of productivity is undermined. The effects of all three of the examined factors show a clear nonstationary spatial arrangement, varying from region to region in both importance and extent.

The substantial amount of variance shared between the predictors deserves further attention. The collinearity between predictors is statistically unsolvable and prevents identifying a single hypothesis as the primary driver of diversity gradients because large fractions of meaningful relationships with species richness remain hidden in data structure. These hidden relationships are partly solvable in our case thanks to the partial GWR analyses, which show that amphibian diversity is to some extent correlated with the independent effect of at least one individual factor in almost all regions. The ability to detect such relationships enables us to identify some possible effects of particular factors in these areas and where these effects decrease.

As expected, the effect of historical climate variability is negligible over most of the world, except in Europe and a small part of North America. Similar to global trends in Carnivora body size (Diniz-Filho et al. 2009), the effects of glacial cycles on diversity gradients are only significant in Holarctic regions. Nonetheless, the influence of climate variability on amphibian diversity in these areas is substantial, particularly in northern Europe (Araújo et al. 2008). The spatial distribution of non-shared influence of historical climate changes aligns with the south-north gradient of ice retraction in Europe (Montoya et al. 2007). In North America, this influence is concentrated along the west coast, perhaps partially due to the late glacial retraction that occurred there (Andrews and Dyke 2007) and to topographic particularities. Interestingly, in the western Palaearctic these areas generally coincide with those hosting amphibian assemblages that are less evolved

in terms of cold tolerance (see Fig. 2b in Olalla- Tárrega et al. 2011). Here it is important to note that if the effects of the last glaciation were assessed through climate- change velocity, a different picture of the influence of past climates on the species richness gradient would perhaps have been formed (Sandel et al. 2011). It is likely that the influence of climate-change velocity was stronger than that of climatic anomaly, particularly across topographically heterogeneous regions. Nonetheless, areas with higher climate change velocity also had larger absolute changes; thus, high spatial congruence between both approaches could be expected.

The effect of paleoclimate observed in Central America and northeastern Brazil was unexpected because those areas were free from LGM glaciers. However, the estimated cooling that occurred in Central America during the LGM ($\sim 25^{\circ}\text{C}$), for instance, was more pronounced than that in other mid-latitude regions, involving even the development of a temperate flora (Bush et al. 2009). This condition may have affected the northward dispersal of amphibians – and thus the distributional pattern of richness – throughout Central America, as suggested for Hylidae, a particularly speciose anuran clade (Wiens et al. 2006). The case of north-eastern Brazil is more tentative. Despite some evidence for dry forest expansions during the late Quaternary (Behling et al. 2000), the low resolution of the palaeoclimatic models prevents us from assessing the consequences of such temporal dynamics on amphibian distribution and diversification in this area. In any case, these local signals of Quaternary climate are of little relevance at the global scale, and may be restricted to a residual variation within the general trend of a large influence of the current climate. It is possible, however, that a significant amount of influence of historical climate changes remains hidden in the variation shared with other factors.

One important finding of this study is the substantial importance of seasonality for amphibian richness gradients. Its influence is apparent across extensive areas of most continents except Europe. These findings contradict former hypotheses, which attributed limited importance to seasonality as a determinant of species richness at large scales (Currie 1991, Evans et al. 2005). Rather, these findings support our initial hypothesis that the effect of seasonality is stronger where amphibians encounter environmental conditions that set adaptive constraints, thus removing more species from a regional species pool than predicted by the average climatic conditions. In Australia, for instance,

seasonality (westward) and productivity (eastward) exhibit opposite patterns along a longitudinal gradient. When Australia is analysed separately, amphibian richness increases exponentially with AET (OLS $R^2 = 0.479$), whereas a higher amount of variation is explained when seasonality is included in the linear model (OLS $R^2 = 0.669$). The westward decrease of species richness in this continent would then correspond to a combination of both lower productivity and higher seasonality. These conditions would set progressively more restricted upper limits to local species richness as both factors change to critical values simultaneously.

Although the strength of the productivity–amphibian richness relationship varies geographically, its dominance should be emphasised. It prevails over the Neotropics, the Holarctic and part of Australia – areas that harbour the greatest fraction of extant amphibians. The unexpected nonstationarity in the richness–AET relationship may be due either to systematic biases in the ability of AET to estimate actual environmental productivity globally (Fisher et al. 2011) or to its interaction with other spatially structured factors, as discussed for Australia. Otherwise, and contrary to our prediction, the spatial pattern of species richness could respond unequally to productivity (see below). Given the primary importance of the effect of productivity both globally and locally, an evaluation of the factor – or factors – behind such nonstationary structure is required for future research.

The influence of different sets of variables on amphibian species pools at different locations may be a consequence of the history of their relationship with the environmental conditions under which their clades have diversified. More specifically, the differences in climate–diversity relationships may depend on the ways in which past environmental conditions affected speciation, extinction, and dispersal rates throughout evolutionary time – the ultimate causes of variation in the number of species across space (Wiens 2011). If clades have evolved under external environmental constraints, in such a way that only some of their sub-clades can escape from those constraints, then insofar as climate changes (not uniformly) across space and time, the resulting pattern of addition and deletion of species in a region would cause different diversity–climate relationships (Hortal et al. 2011). In fact, most amphibian clades (and even more so their sub-clades) are geographically restricted to one or a few continents (the nearly cosmopolitan Bufonidae and Hylidae being the ‘quasi-exceptions’; Pramuk et al. 2008, Wiens et al.

2011); therefore, geographical unevenness in the richness–environment relationship is expected. Following this reasoning, inasmuch as many clades – each with its own adaptive history – occupy the same region, the more intricate and less generalisable the pattern may become and the more residual variation will remain in global environment–richness models.

The above reasoning allows our findings to be linked to the ‘niche conservatism’ hypothesis, explaining diversity patterns at a deeper evolutionary time scale (Wiens et al. 2010). Evidence for climatic niche conservatism is built upon inferences (usually through phylogenies) of niche evolution within the clades in relation to their exposure to environmental factors (Hof et al. 2010, Olalla-Tárraga et al. 2011). It follows that, in our view, niche conservatism cannot be seen as opposed to most climate-based explanations, but rather as a perspective through which we can interpret the history of species–environment relationships over time. We also believe that our results support two points advocated by the niche conservatism hypothesis regarding the shaping of biogeographic patterns of species diversity (Wiens and Donoghue 2004, Wiens et al. 2010): 1) the retention of ancestral niche traits – as some sub-clades conserve their climatic niches due to adaptive constraints; and 2) the duration of stable climatic conditions, which may allow some clades to escape previous constraints if there is enough time (but see Losos 2011).

The above explanation deserves further scrutiny, but external evidence appears to favour it. The strength of the phylogenetic signal of climatic responses among the world’s amphibians is known to vary among biogeographical regions (Hof et al. 2010, Olalla-Tárraga et al. 2011), which implies different clade–climate relationships at different locations. Furthermore, the negative effects of both historical and current climatic variability on the diversification and dispersal of clades is expected only if clades have not evolved enough to endure them (Wiens et al. 2006, Hortal et al. 2011, Sandel et al. 2011); otherwise such effects would be absent. For example, the eastern Palaearctic – which in our analyses appears to be associated with stronger effects of productivity with less importance of historical climatic changes and seasonality – harbours amphibian faunas that have adapted more extensively to life in cold conditions (Olalla-Tárraga et al. 2011, Fig. 2b). In fact, the effect of productivity is more important where neither historical disturbances nor pronounced seasonality have been important for a long time,

such as in many areas in the Neotropics. This suggests that productivity can regulate species richness as has been hypothesised, but only if the clades inhabiting the region have had enough time to adapt to the prevailing climatic conditions; otherwise it would be the latter adaptive constraints that will have a predominant effect on diversity patterns (but see Currie et al. 2004). Additionally, note that our predictions and interpretation of the results take the particular ecophysiological attributes of amphibians into account. Other taxa are expected to show their own unique characteristics (Currie 1991, Hawkins et al. 2003a, Whittaker et al. 2007, Qian 2010), perhaps involving different sets of circumstances related to the region of interest and to how their ecological and life history traits respond to climate change through time.

Our findings reinforce the idea that inferences drawn from global or even regional approaches may fail to offer general explanations of richness patterns. On the one hand, studies conducted on smaller scales are inadequate to fully address the universal drivers of richness gradients due to their narrower environmental and taxonomic scope, hence preventing generalisations. On the other hand, global-scale analyses based on global statistics are unable to detect idiosyncrasies that create nonstationarity in the species–environment relationship, jeopardising the explanatory ability of the models. The root of this trade-off involving global vs local models in evaluating geographical gradients of species diversity may lie in Levins’s (1966) dilemma regarding ‘generality’ and ‘precision’ in ecological models (assuming ‘realism’ for all cases; Levins 1966). The definition of the actual goal of a particular question is central in deciding which method to pursue. If one intends to provide a short explanation for a general question, it is possible that global models, as widely employed, will provide a valid approximation. In that case, productivity is the best candidate for the primary climatic driver of amphibian diversity gradients.

However, if the aim is to understand the specific causes that lead some locations to harbour more species than others, or to determine how environmental changes are going to affect a particular group of species in a particular region (a process that will hardly occur evenly across geographical space), perhaps we should resort to methods that account for both nonstationarity in the processes – and the local variation in the relative contributions of individual predictors – and phylogenetically structured responses to the environment – and hence the effect of evolutionary constraints on regional species pools.

Incorporating these two elements into a single analytical framework appears to be a necessary challenge in the process of understanding the origin and nature of the geographic gradients of diversity.

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Capítulo 2. Environmental steepness, tolerance gradient, and ecogeographical rules in glassfrogs (Anura: Centrolenidae)

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Abstract

Spatial variation in biological traits reflects evolutionary and biogeographical processes of the history of clades, and patterns of body size and range size can be suitable to recover such processes. In the present study, we test for latitudinal and altitudinal gradients in both body and range sizes in an entire family of tropical anurans, Centrolenidae. We partition the species latitudinal, and altitudinal distributions into an indirect measure of tolerance, and then test its effect on the body size gradient. We use an assemblage-based approach to correlate the traits with altitudinal and latitudinal axes, taking into account both phylogenetic and spatial autocorrelation in data. Centrolenids lack any gradient in range size but show a positive cline of both body size and adaptive body enlargement with altitude. This pattern is also positively correlated with an altitudinal gradient of cold tolerance, thus lending support to the heat balance hypothesis as an explanation of the body size cline. By using an entire Neotropical clade of anurans, we add further support for Bergmann's rule in ectotherms, warn for a likely effect of environmental steepness in fashioning the gradient, and offer evidence for an historical scenario (the Oligocene–Eocene Andean uplift) as its likely trigger.

Keywords – Amphibians; assemblage-based approach; Bergmann's rule; comparative methods; Neotropics; Rapoport's rule.

Introduction

Patterns of geographical distributions of species traits reflect ecophysiological and evolutionary processes that have allowed them to endure environmental conditions and diversify in space and time (Gaston, Chown & Evans, 2008). A common way to address

these patterns is by evaluating the so-called ‘ecogeographical rules’ that concisely describe the trait variation in respect to a hypothesized determinant, which is most often linked to climate variation (Gaston & Blackburn, 2000; Gaston et al., 2008). However, despite this conceptual practicality, exceptions to ecogeographical rules are frequent, especially under phylogenetically and geographically inclusive analyses (Meiri & Dayan, 2003; Whitton et al., 2012). Therefore, attempts to identify both the mechanisms behind these ‘rules’ and what causes them to be absent in some circumstances may shed light on how these patterns are shaped (Olalla-Tárraga, 2011).

One of these rules, Bergmann’s rule, states that body size increases towards higher latitudes/altitudes (i.e. colder environments). Its original explanation (offered by Bergmann himself in 1847; for a recent discussion around the original formulation of the rule, see Watt, Mitchell & Salewski, 2010; Meiri, 2011; Olalla-Tárraga, 2011) is that lower temperatures favours large-bodied species because smaller surface-to-volume ratios associated with larger sizes allow species to better conserve heat (James, 1970; Blackburn, Gaston & Loder, 1999). Despite other possible explanations (e.g. correlation of body mass with other cold-benefited trait, migration ability, starvation resistance, resource availability; Blackburn et al., 1999), the ‘heat conservation hypothesis’ has been largely favoured so far (Olalla-Tárraga, 2011). Recently, the ‘heat balance hypothesis’ was suggested as a thermoregulatory unifying explanation for body size gradients in endotherms and ectotherms, which extends and complements Bergmann’s original mechanism (Olalla-Tárraga & Rodríguez, 2007). The heat balance hypothesis takes into account the importance of surface-to-volume ratios for the thermal adjustments, activity times, and operative temperatures in thermoregulating ectotherms and develops complementary expectations for ectotherms with a high degree of ‘thermoconformism’. Anurans, for example, are able to enhance heat gain and conservation, and thus fit in the thermoregulators group, contrasting to urodeles, for example, which lack such ability (Olalla-Tárraga & Rodríguez, 2007).

Although currently well-accepted within the ecological and biogeographical arenas, Bergmann’s rule has been involved in two relevant disputes: the taxonomic resolution to which the rule should apply (i.e. at an intra- or an interspecific level; Ashton & Feldman, 2003; Meiri, 2011), and whether ectotherms fit to its scope. Some studies defend the restriction of the rule only to endotherms (Pincheira-Donoso, 2010; Watt &

Salewski, 2011), whereas others suggest a more inclusive approach (Meiri, 2011; Olalla-Tárraga, 2011). In the present study, we adopt this latter more pluralistic viewpoint, which claims that Bergmann's rules should be investigated in endotherms and ectotherms, and at both intra- and interspecific levels.

Rapoport's rule, in turn, predicts an increase in the species range size with latitude or elevation, and was also proposed as a potential correlate or explanation for latitudinal richness gradients (Stevens, 1989, 1992). The traditional explanation for this rule is the climatic variability hypothesis (Gaston, 2003). The reasoning behind this mechanism is that increasing climatic seasonality towards high latitudes/altitudes selects for species with broad climatic tolerances that can spread their ranges polewards compared to the usually range-restricted species from tropical regions and lower altitudes. The validity of Rapoport's rule has nonetheless been strongly debated (Gaston, Blackburn & Spicer, 1998; Whitton et al., 2012). Even so, despite the lack of support for Rapoport's rule at a global scale, which can be attributed to other factors that affect the species range size (Brown, Stevens & Kaufman, 1996; Gaston, 2003), the climatic variability hypothesis is well supported for many terrestrial and marine organisms (Addo-Bediako, Chown & Gaston, 2000; Sunday, Bates & Dulvy, 2011).

Accordingly, in view of both the mainly advocated explanations for body size and range size gradients [heat conservation and tolerance amplitude (disregarding the weak support for the Rapoport' rule)], we can reasonably link these mechanisms to the species adaptation to colder conditions via tolerance. Indeed, larger bodied organisms are supposed to conserve heat better and, consequently, to better tolerate cold conditions. Similarly, the variability in species range size is related to the amplitude of species tolerance (Addo-Bediako et al., 2000; Sunday et al., 2011). Therefore, if either Bergmann's or Rapoport's rules apply through the aforementioned mechanisms, a given measure of the species' cold tolerance should correlate with both body size or range size, respectively.

Owing to their high susceptibility to environmental variation, amphibians are suited for testing hypotheses on the influence of climate-related factors on adaptive constraints that affect species traits such as body size and range size. In the present study, we used data from an entire, monophyletic clade: the glassfrogs (Anura: Centrolenidae) (148 species; Guayasamin et al., 2009). They are distributed in the Neotropics, at

broadleaf forests and páramo habitats from Central America and Amazonian-Orinoco basins through the Andes to the Atlantic Forest of Brazil and Argentina; from sea level to 3500 m a.s.l. (Cisneros-Heredia & McDiarmid, 2006). We tested whether glassfrogs follow Bergmann's and Rapoport's rules across both latitude and altitude. We formulated a procedure to partition out the occupation of geographical space by species, based on their horizontal and vertical axes of distribution, and extracted a proxy for an among species component of tolerance. We then tested whether this component correlates with the variation in body size, as expected by the heat balance hypothesis. We also tested whether tolerance is related to temperature or to two other possible explanations (aridity and productivity; Blackburn et al., 1999).

Material and Methods

Species and environmental data

We assembled data on body size, range size, and altitudinal range for all species of centrolenids. As a measure of body size, we used the species' midpoint between minimum and maximum snout–vent length (adult SVL in mm). Midpoint SVL was used to reduce the sampling effect of some species that are known only from the holotype. Most of these data were assembled by Guayasamin et al. (2009) based on original references. We also gathered data missing from this source directly from the original description of the species (see Supporting information, Table S1). Range sizes were drawn from the Global Amphibian Assessment (GAA) database (IUCN, 2009). For four recently described species (missing from GAA), we calculated the range size by drawing minimum convex polygons from point locality records provided by specific studies (see Supporting information, Table S1). We overlaid the range sizes onto a grid cell system of $0.5^\circ \times 0.5^\circ$ (latitude \times longitude) spatial resolution, covering the entire distribution of centrolenids, which yielded a presence/absence matrix of all species. We projected the species traits (body size and range size) onto the map according to their geographical distribution. For species known only from type locality, we attributed the minimum range size of our dataset, as provided by the GAA (i.e. approximately 12 square km). As a measure of species altitudinal range, we used the difference between the uppermost and the lowermost altitude (in metres) where the species is known to occur. Most of these

data are provided by Frost (2011). For some species, for which this information was not available, we used the species distributional map to extract these data from an altitude data layer drawn from a global elevation model (HYDRO1k; <http://eros.usgs.gov>).

To estimate the environmental conditions where the species occur, we gathered spatial data of thermal range (i.e. maximum minus minimum annual temperature), aridity (calculated as the precipitation–potential evapotranspiration ratio), and annual precipitation, as a measure of environmental productivity. These data derive from interpolated surface data series. Temperature and precipitation are from Hijmans et al. (2005), and potential evapotranspiration is from Willmott & Matsuura (2001). These data were projected onto the 0.5° cells grid on which species distributions were overlaid.

To account for the effect of phylogenetic structure on species traits, we drew a phylogenetic tree of all centrolenids based on the phylogeny of Guayasamin et al. (2009), which covers 53% of all described species. We inserted the missing species as sister species of those already covered or as polytomies, following the authority of description or other classifications of infrageneric or generic grouping (see Supporting information, Table S1), thus accounting for all 148 known species. Because we added species missing from the original tree, we disregarded the tree branch lengths (originally measured as rate of substitution) in the complete tree in favour of a distance matrix based on the number of tree nodes shared by species. Our goal in adding missing species based on previous taxonomic classification was to allow all species to play their role in the assemblage-level distribution of traits, at the same time as avoiding any sampling bias in the phylogeny construction. However, most previous classifications of Centrolenidae rely on morphological data, and thus species relationships may have undergone different arrangements thereafter, especially after molecular-based assessments. Therefore, to assess the reliability of the results derived from our ‘tapestry’ phylogeny, we rerun all the analyses only with the species present in the Guayasamin et al. (2009) phylogeny (i.e. 79 species), thus taking into account the original tree branch lengths. Also, to assess the pervasiveness of the patterns, we ran the analyses with two most speciose clades of the family: the *Hyalinobatrachium* genus and the clade joining *Centrolene* and *Nymphargus* genera (Guayasamin et al., 2009).

Phylogenetic comparative methods and assemblage-based approach

Because phylogenetic relationships create dependence of trait values and ancestral states, it is always important to test whether species traits constitute independent observation in comparative analyses (Felsenstein, 1985; Harvey & Pagel, 1991). Thus, we tested for phylogenetic signal in body size, range size, and altitudinal range size through Moran's I index in a phylogenetic correlogram based on phylogenetic distances among species (Gittleman & Kot, 1990). We then used a phylogenetic eigenvector regression (PVR; Diniz-Filho, Sant'ana & Bini, 1998) to partition out the positively autocorrelated trait into a phylogenetic (P) and a specific (S) component (for a similar application, see Morales-Castilla, Rodríguez & Hawkins, 2012). We selected a set of eigenvectors following the criterion of significance ($P < 0.05$) of correlations between body size and the eigenvectors (Diniz-Filho et al., 2012a). Briefly, the phylogenetic component (P) translates the trait variation that results from the phylogenetic relatedness among species, whereas the specific component (S) is the fraction that deviates from the phylogenetic model. If correlated with environmental variation or other traits, the S component can be understood as the adaptive response of species (Diniz-Filho et al., 1998; Desdevises et al., 2003). We then used a partial regression analysis to calculate the relative contribution of the P and S components to the total amount of trait variation (T). Although the interpretation of the coefficients of determination, R^2 , in the PVR is now known to be conditional to the eigenvalues of the eigenvectors used in the modelling (Diniz-Filho et al., 2012b), their partial contributions are not studied in this particular context yet. However, using eigenvectors correlated with the response variable and checking for absence of Moran's I in the S component ensures that most of the phylogenetic autocorrelation in data was taken into account (Diniz-Filho et al., 2012a).

We then used an assemblage-based approach (also called community-based or grid-based approach; Blackburn & Hawkins, 2004; Olalla-Tárraga et al., 2010) to map the species body size, including the P and S components (as species traits), onto the grid cells based on the species distribution. In the assemblage-based approach, the trait can be averaged and mapped, allowing portraying the geographical pattern in a bidimensional perspective (for details on how to combine PVR in an assemblage-based approach, see Diniz-Filho et al., 2009; Terribile et al., 2009). We calculated median values, rather than means, of P and S for each cell to take into account the effect of low richness in many cells, which may create non-normal distributions of species values within cells (Meiri & Thomas, 2007).

Partitioning range size components

The major determinants of the range size at macroscale are the differential ability to disperse and to tolerate outermost climates (Brown et al., 1996; Baselga et al., 2011; Whitton et al., 2012). Factors such as interspecific interactions, dietary niche breadth and position, and fine-scale habitat parameters do not substantially affect large-scale patterns of range size. Instead, they are expected to influence range occupancy locally (Baselga et al., 2011; Whitton et al., 2012). Body size can also be rejected as a cause per se of range size variation among small-sized animals. This is because differences in range size of small-sized animals, including glassfrogs (Gouveia, Faria & Rocha, 2012), appear not to arise from summation of individual home ranges (Brown et al., 1996), such as found for large-bodied organisms (Peters, 1983).

We further distinguished the two spatial axes where glassfrogs are distributed, the horizontal (i.e. latitude and longitude) and vertical (altitude), as a means to test each of them independently. To some extent, this distinction assures that we can deal with different degrees of steepness in the climatic gradient: a weaker (horizontal) and a stronger one (vertical). Indeed, the climate range (measured as the mean temperature amplitude) across the altitudinal axis is approximately two-fold wider (47 °C) than that across latitude (28 °C). Following this, we can consider both the horizontal and the vertical axes as referential dimensions of the space that impose different constraints to the tolerance of glassfrogs. Although the climatic variation across latitude is not negligible, it falls within the overall climatic milieu of most of Neotropics, whereas the altitudinal variation is distinctively harsher than anywhere else in the region.

Because dispersal and tolerance act in concert to determine the patterns of species distribution (Baselga et al., 2011), we should predict, as a null expectation, a positive relationship between horizontal and vertical range sizes among closely-related species that are distributed across these two dimensions. Accordingly, species that achieve a higher vertical range than predicted should be somewhat more tolerant relative to average species. In this scenario (Fig. 1, model A), if we plotted the sizes of vertical range against horizontal range on a scatter graph, we should obtain a positive relationship in which, on average, the surplus and shortage of species tolerance would be distributed above and below the model's best fit line, respectively. Alternatively, if other factors played a stronger effect on the overall large-scale pattern of species distribution, we should find

species at all possible conditions (i.e. a random, near-zero correlation) (Fig. 1, model B). In the case of finding the scenario 1A, we could take the model residual as an indirect measure of among-species variability in tolerance. Thereafter, this tolerance component could be separated and assigned to each species, and then be handled in the assemblage-based approach as a species trait.

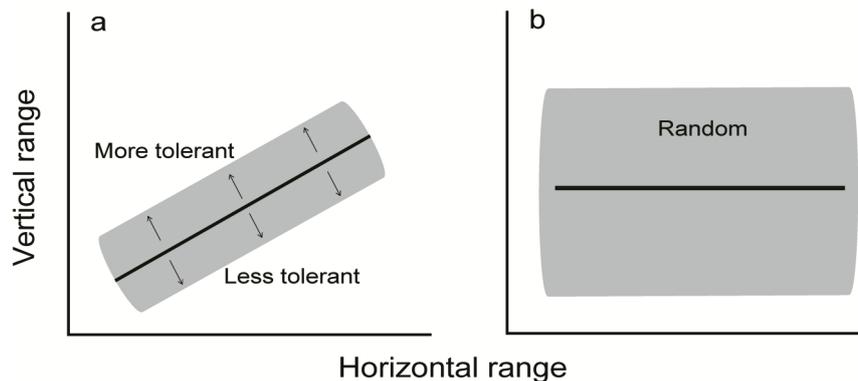


Figure 1. Theoretical models of the variation of vertical vs. horizontal range sizes. Model *a* illustrates the null expectation, in which species horizontally large-ranged should also be vertically large-ranged. In such case, models residual can be taken as a proxy of the species tolerance. In model *b*, other factors distort the expectation, preventing the inference of tolerance.

Because these scenarios do not assume an a priori cause-and-effect state between horizontal and vertical range sizes, and there is measurement error associated to both variables, we performed a cross-species restricted major axis regression (RMA – Model II; Legendre & Legendre, 1998). We ran this analysis without species with zero vertical range size (i.e. known only from a single altitude) because they created a second horizontal (zero) line. Including these species, however, does not significantly alter the statistical result.

As stated above, dispersal and tolerance (and other factors in less extent) can be tightly interrelated when determining the species range sizes, and may vary according to specific circumstances. However, our intention is to summarize a proxy of the interspecific variation in species tolerance that could be used as a comparative descriptor for the spatial asymmetry in body size in the absence of direct measures of tolerance (for a similar approach involving assessment of extinction risk in mammals, see Purvis et al., 2000).

Statistical analysis

We used latitudinal mid-point and maximum altitude of grid cells to test the latitudinal and altitudinal gradients for range size and body size, using Pearson's correlation coefficient. We avoided correlating the trait variations directly with climatic predictors because climatic variables interchange through both latitudinal and altitudinal axes. This caution ensured that we were dealing with both horizontal and vertical predictions, independently (see above). Because centrolenids are distributed northward and southward of the Equator (thus generating a bidirectional prediction along latitudinal gradients), we transformed latitude into absolute values. We tested the vertical gradient of range size by controlling the effect of geometric constraint imposed by the Andean topography on the horizontal range size at high altitudes (Ruggiero & Lawton, 1998). Thus, we extracted the residual of the negative relationship between the horizontal range size and maximum elevation to recalculate the actual available area used by species along the elevational gradient.

We tested the correlation of our proxy for tolerance (i.e. the residual of the horizontal range \times vertical range model) with the variation in the total (T) and the specific (S) components of body size. Also, as a means of identifying the climatic predictor that best explains the variation in our proxy of tolerance, we performed a multiple regression analysis with variance partitioning, involving three predictors that represent alternative climatic hypotheses for Bergmann's clines: temperature, aridity, and productivity (through annual precipitation; Blackburn et al., 1999).

In correlation analyses, the geographical proximity among sample unities (e.g. grid cells) often causes spatial dependence in variables (i.e. spatial autocorrelation) (Legendre & Legendre, 1998). We controlled for this dependence by reducing the degrees of freedom in Pearson's correlation analyses using Dutilleul's (1993) modified *t*-test, at a 5% significance level.

Results

The body size of glassfrogs ranges from 17 to 80 mm, although the size of most species (77%) varies within 20–30 mm. Nevertheless, the trait variation showed a clear spatial pattern: larger species are clustered at elevated zones of Venezuelan, Colombian,

Ecuadorian, and Peruvian forests along the Andes, and also at high altitudes of the northern distribution (Guatemala and Mexico) (Fig. 2, T). Along the Colombian Andes, they show the larger amount of body size variation (20.7–44.3 mm), as well as of altitude (300– 3500 m a.s.l.).

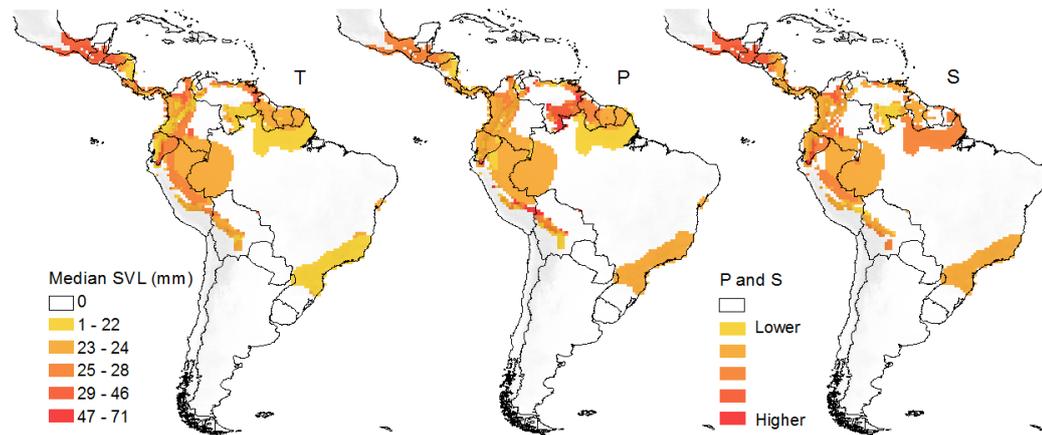


Figure 2. Spatial pattern of body size (T; median of snout-vent length midpoints), and Phylogenetic (P) and specific (S) components of body size variation from 148 species of Centrolenidae after overlaying onto a 0.5° cells grid along the Neotropics. Legend scales of P and S maps are missing because mathematical transformation makes their values meaningless, thus being useful only in a geographical comparative context.

Only body size was phylogenetically autocorrelated, although this effect was relatively weak (Moran's $I = 0.251$ at the first of seven equal distance classes; $P < 0.001$). Phylogenetic autocorrelation persisted up to the third distance unity of the spatial correlogram (not shown), thus expressing a phylogenetic nonindependence approximately at the within-genera level.

Three eigenvectors were retained in the PVR analysis, which completely removed the phylogenetic signal in data (Moran's $I = -0.023$; $P = 0.621$), yielding an S component independent among species. According to the PVR partial regression, P accounted for a relatively small fraction of the interspecific variation in body size (26.7%), coherent with Moran's I and correlograms, whereas the larger amount (73.3%) was related to S.

When P and S components were mapped, the former lacked any clear spatial pattern, except for some scattered locations around Andean foothills of Venezuela, Peru, and Bolivia (Fig. 2, P). Conversely, the S component showed a spatial pattern most

similar to total body size, as expected by the explanation of these components in respect to the original data (Fig. 2, S).

In the spatial occupancy model, altitudinal and horizontal range sizes were positively correlated ($\log \times \log$; RMA *slope* = 0.799, $P < 0.001$; Fig. 3), corresponding to our scenario A of Fig. 1. We found a quite similar pattern when we analyzed only the species from the original molecular tree (*slope* = 0.631, $P < 0.001$). We then took the model residuals as proportional to the interspecific variation in tolerance.

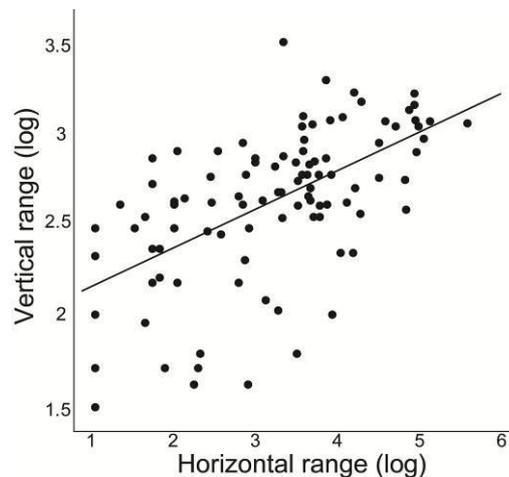


Figure 3. Observed relationship between vertical and horizontal range sizes from a restricted major axis regression (RMA – Model II). The result supports the scenario a of the Fig. 1.

We found a positive, significant correlation of median body size with maximum altitude ($r = 0.609$; $d.f.$ _{Dutilleul} = 49; $P < 0.001$) but not with the latitude ($r = 0.201$; $d.f.$ _{Dutilleul} = 16; $P = 0.419$; Fig. 4B). In addition, we found a positive relationship of the adaptive component (S) of body size with altitude ($r = 0.494$; $d.f.$ _{Dutilleul} = 502; $P < 0.001$; Fig. 4A). Range size correlated neither with latitude ($r = -0.076$; $d.f.$ _{Dutilleul} = 24; $P = 0.714$), nor with altitude ($r = -0.085$; $d.f.$ _{Dutilleul} = 58; $P = 0.518$), after controlling for the Andean geometric constraints.

The results provided by our ‘tapestry’ phylogeny prove to be reliable. The analyses involving only the species included in the molecular tree showed similar results for the relationship of altitude with body size ($r = 0.521$; $d.f.$ _{Dutilleul} = 69; $P < 0.001$) and

the S component ($r = 0.449$; $d.f.$ _{Dutilleul} = 69; $P < 0.001$). Tests of adherence of sub-clades to Bergmann's rule also support the trends. *Hyalinobatrachium* showed a strong relationship for both the body size ($r = 0.793$; $d.f.$ _{Dutilleul} = 13; $P < 0.001$) and the S component ($r = 0.788$; $d.f.$ _{Dutilleul} = 14; $P < 0.001$), and the *Centrolene* + *Nymphargus* complex also conforms to the pattern for both body size ($r = 0.412$; $d.f.$ _{Dutilleul} = 95; $P < 0.001$) and the S component ($r = 0.428$; $d.f.$ _{Dutilleul} = 75; $P < 0.001$).

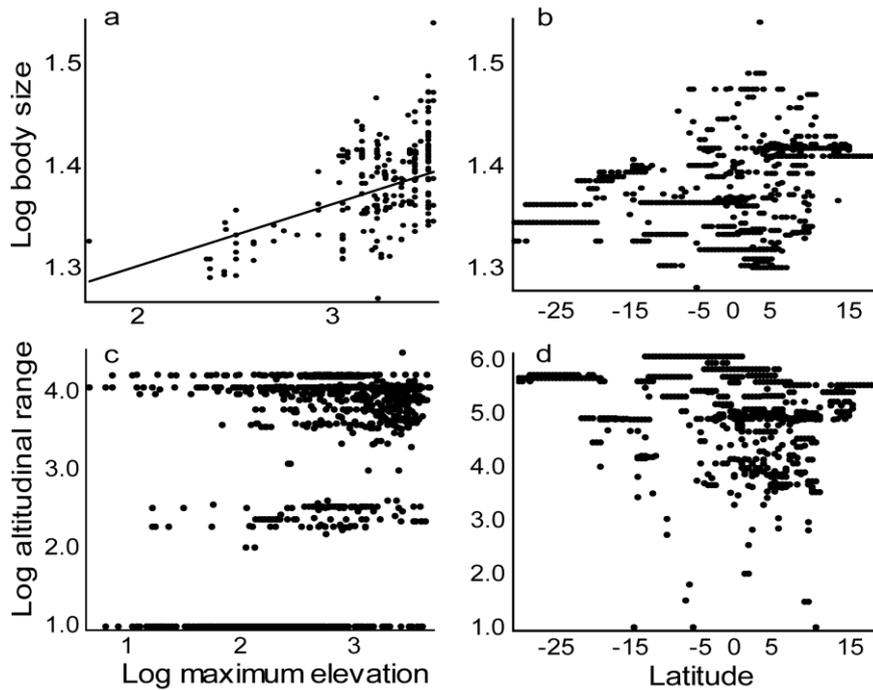


Figure 4. Correlation of median log body size (a, b), median log vertical range (c), and median log horizontal range (d) against geographical predictors (altitude and latitude) in Centrolenidae. Only the graph (a) with fit line shows a significant correlation. This latter relationship is slightly strengthened if the both leftmost and uppermost outliers are omitted.

We also found a positive correlation of our proxy of tolerance with body size ($r = 0.537$; $d.f.$ _{Dutilleul} = 36; $P = 0.001$) and with the S component ($r = 0.489$; $d.f.$ _{Dutilleul} = 32; $P = 0.003$). The same holds for the species from the Guayasamin et al. (2009) tree. Tolerance correlated positively with both body size ($r = 0.530$; $d.f.$ _{Dutilleul} = 53; $P < 0.001$) and the S component ($r = 0.476$; $d.f.$ _{Dutilleul} = 73; $P < 0.001$).

Finally, the multiple regression analysis showed that temperature was the most important predictor of tolerance (partial $R^2 = 0.165$; $P < 0.001$), relative to aridity (partial $R^2 = 0.001$) and productivity (partial $R^2 = 0.021$).

Discussion

We found altitudinal (but not latitudinal) gradients of body size and reject the occurrence of both latitudinal and altitudinal gradients in range size for Centrolenidae. These results agree with most empirical evidence from other taxa and geographical regions, particularly regarding the lack of spatial pattern of range size in the Neotropics. That is, Rapoport's rule is increasingly being alleged as a regional, Holarctic phenomenon, rather than a general rule (Gaston et al., 1998; Whitton et al., 2012). On the other hand, despite some exceptions (Blackburn et al., 1999; Berke et al., 2012), studies of Bergmann's rule on either endotherms or ectotherms vertebrates tend to find clear geographical gradients (Meiri & Dayan, 2003; Olalla-Tárraga & Rodríguez, 2007), including for anurans in tropical regions (Olalla-Tárraga et al., 2009; Bidau, Martí & Baldo, 2011).

It has been recently argued that Bergmann's rule should be strictly considered under its original formulation in the 19th Century (i.e. it should only refer to endotherms; Pincheira-Donoso, 2010; Watt & Salewski, 2011). Indeed, temperature–body size clines in ectotherms are less evident and more complex than in endotherms (Ashton, 2002; Ashton & Feldman, 2003; Olalla-Tárraga & Rodríguez, 2007). However, a lack of support for Bergmann's rule holds for both ectotherms and endotherms (Meiri & Dayan, 2003; Pincheira-Donoso, 2010, Berke et al., 2012); thus, the phenomenon is far from being universal. In view of this debate, we provide further evidence for the adherence of an entire, monophyletic clade of anurans to the pattern. Here, it is worth noting that both the completeness and the monophyly of the clade have been argued as a necessary condition to rigorously test Bergmann's rule (Blackburn et al., 1999).

An important finding is the consistence of the results from comparative analysis, even when adding further uncertainty with both the polytomies and the dismissal of branch lengths, relative to a better resolved phylogeny. This finding may have been benefited by the weak phylogenetic signal in the variation in body size among species.

However, the inclusion of species (for which their taxonomic positions are relatively well known) into a more consistent baseline phylogeny has been shown to provide congruent conclusions between the two approaches (Jetz et al., 2012). Indeed, any phylogeny constructed with a subset of the species gene pool constitute one of many hypotheses on how those species evolved and branched along the evolutionary history (Harvey & Pagel, 1991). Therefore, although complete data can improve the quality and refinement of the conclusions, taking into account the main phylogenetic covariance among species appear to be appropriate for many purposes of large-scale studies.

We have shown that the variation in species body size that is independent from the phylogenetic relatedness among species (i.e. the S component) is positively correlated with the variation in altitude. Indeed, the S component depicts a clear spatial pattern, particularly associated with the Andean slopes. This finding illustrates well the adaptive response expected by Bergmann's rule under an evolutionary perspective. That is, different species may have either smaller or larger bodies, regardless their distribution at the geographical or climatic space. However, this may be related to distinct pressures for body enlargement or reduction along their evolutionary histories that can be unrelated to Bergmann's effect (Diniz-Filho et al., 2009). For closely-related species, however, systematically larger bodies among those experiencing colder conditions, compared to their relatives from warmer zones, constitute strong evidence that body size is being selected for that conditions, and that some underlying mechanism is responsible for such pattern (Diniz-Filho et al., 2009). Interestingly, the overall pattern of body enlargement with altitude among centrolenids holds for the most speciose sub-clades within the family (i.e. *Hyalinobatrachium* and the *Centrolene-Nymphargus* clade). Together, these findings reinforce the reliability of the pattern, and also highlight the applicability of the rule at different taxonomic resolutions, as has been previously advocated (Meiri, 2011; Olalla-Tárraga, 2011).

Besides the heat balance hypotheses (Olalla-Tárraga & Rodríguez, 2007), other mechanisms have been proposed as possible explanations for Bergmann's rule. These include a phylogenetic effect derived from ancestral colonization by larger forms, association of body size with other trait that actually respond to coldness, migration ability, starvation resistance, and resource availability (Blackburn et al., 1999). Our analyses enable us to rule out the phylogenetic contingency hypothesis because the body

size–altitude relationship is independent of the phylogenetic relatedness among species (i.e. through the S component). If we take aridity and precipitation as surrogates for the environmental harshness and productivity, we can also disfavour both the starvation resistance and resource availability hypotheses on the basis of the lack of explanatory ability of those factors. It is yet possible that other features linked to body size actually vary with coldness, such as longevity (Kutrup, Bülbül & Yilmaz, 2005). Given the overall narrow variability in centrolenids body size, this could constitute, in principle, a concurring explanation to the pattern found. We distrust, however, age as a consistent, general explanation for Bergmann’s rule because the pattern holds for several clades (including ecto- and endotherms) and geographical regions.

In addition, both the spatial patterns of body size and S component correlate positively with our proxy of tolerance, either considering or not the presence of polytomies in the phylogeny. These findings allow a linking the increase in average body size to a gradient in cold tolerance towards higher elevations. Cold tolerance, in turn, is directly related to the advantage for thermoregulation, which is (ever since Bergmann himself and as expanded in the heat balance hypothesis) the best candidate to explain body size clines (Olalla-Tárraga & Rodríguez, 2007). This inference departs from the pure documentation of the spatial pattern in body size, and was only possible thanks to our model based on the spatial occupation of species at contrasting spatial axes. Although the reasoning of our model may not apply to other datasets, it may shed light on the dynamic of body enlargement across space. If our proxy for tolerance is in fact correlated to actual climatic tolerance at the physiological level, as we hypothesize, our results support a mechanistic link between species adaptation to cold and body enlargement across large special scales, as first conjectured by Bergmann.

Recently, Olalla-Tárraga (2011) stressed the need to identify the circumstances where Bergmann’s rule does not apply as a means of improving our understanding of the underlying processes. Based on our findings of absence along the horizontal and presence along the vertical gradient of body size, we can suggest that there may be a distinction between the geographical and the environmental gradient as a prerequisite for creating the body size cline. The presence of the body size pattern, at least among glassfrog assemblages, may be independent of the geographical extension that takes place. Instead, a steeper climatic gradient may be a necessary condition to generate the pattern. This may

suggest a minimum threshold of climatic steepness to create body size trends at the assemblage level. Nonetheless, we are unable to determine the minimum slope needed to trigger the pattern. Considering the interference of a number of uncontrolled variables (e.g. the rate of environmental change, the dispersal ability of particular clades), this should be rather difficult, and vary according to the taxon in question.

If linked to the paleoenvironmental history of the Andes (where the body size cline is more conspicuous), together with the spatial distribution of species along the Andean slopes, our results may offer a clue to explain part of the evolutionary history of glassfrogs. Despite its older (Cretaceous) genesis, the Andean uplift first peaked approximately 23 Mya (Oligocene to early Miocene). This period corresponds to the origin of Centrolenidae genera, as inferred from the divergence time based on nuclear and mitochondrial genes (Castroviejo-Fisher, 2009). Indeed, our analyses of phylogenetic autocorrelation of body size showed that the phylogenetic structure in body size persists up to the third distance class (i.e. approximately at the genera level), thus coinciding with the time when both the modern genera and the Andean slopes were being formed. This indicates a pattern of body enlargement that is temporally congruent with the Andean establishment. Further evidence is provided by most speciose (and probably more ancient) genera within Centrolenidae (*Centrolene*, *Nymphargus*, and *Hyalinobatrachium*; Guayasamin et al., 2009). They are distributed at both east and west sides of the Andes, whereas all species belonging to each of these genera are geographically restricted to one or another slope of the mountain ridge. This spatial arrangement indicates an east–west split and further isolated diversification after the Andean uplift, which may have also fashioned their subsequent altitudinal asymmetry in body size.

In summary, we provide evidence for the validity of the altitudinal gradient in body size among centrolenid frogs. This pattern appears to be linked to the variation in tolerance to low temperatures, which is in accordance with the heat balance hypothesis as a likely mechanism underlying Bergmann's rule. We propose that the environmental steepness may be more important than the geographical distance in creating the body size pattern. We also depict a biogeographical scenario that may help to portray the body size gradient of glassfrogs at a subset of their geographical distribution: the uplifting of the Andes and the resulting decrease in temperature with elevation.

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Supplementary Material

Table S1. Data and respective reference of body size (BS; midpoint snout-vent length), geographical range (GR) maps, altitudinal range (AR; maximum-to-minimum elevational difference), and phylogenetics relationships for all Centrolenidae frogs used in this study. Quotations denote species considered *incertae sedis* by Guayasamin et al. (2009) due to unavailability of molecular data. For these species, the authors maintained generic names in order to follow the current taxonomy, until a conclusive placement of them in the proper genera.

| Species | BS (mm) | BS reference | GR (km) | GR reference | AR (m) | AR reference | Phylogenetic relationships |
|---|---------|-------------------------|---------|--------------|--------|--------------|---------------------------------------|
| <i>Celsiella revocata</i> | 23.5 | Guayasamin et al., 2009 | 4585.9 | IUCN, 2009 | 600 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Celsiella vozmedianoi</i> | 27.3 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 30 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>acanthidiocephalum</i> | 31.35 | Guayasamin et al., 2009 | 48.4 | IUCN, 2009 | 350 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Centrolene altitudinale</i> | 23 | Guayasamin et al., 2009 | 108.9 | IUCN, 2009 | 425 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Centrolene antioquiense</i> | 21.5 | Guayasamin et al., 2009 | 3956.7 | IUCN, 2009 | 600 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>audax</i> | 25.2 | Guayasamin et al., 2009 | 859.1 | IUCN, 2009 | 40 | HYDRO1k | Duellman & Schulte, 1993 |
| " <i>Centrolene</i> " <i>azulae</i> | 27.5 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Centrolene bacatum</i> | 20.6 | Guayasamin et al., 2009 | 121 | IUCN, 2009 | 150 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>ballux</i> | 21.25 | Guayasamin et al., 2009 | 229.9 | IUCN, 2009 | 60 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Centrolene buckleyi</i> | 29.85 | Guayasamin et al., 2009 | 93823.4 | IUCN, 2009 | 1200 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Centrolene condor</i> | 27.6 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 100 | Frost, 2011 | Cisneros-Heredia & Morales-Mite, 2008 |
| <i>Centrolene daidaleum</i> | 22.05 | Guayasamin et al., 2009 | 4985.2 | IUCN, 2009 | 430 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>durrellorum</i> | 25.9 | Guayasamin et al., 2009 | 5396.6 | IUCN, 2009 | 350 | Frost, 2011 | Cisneros-Heredia, 2007 |
| " <i>Centrolene</i> " <i>fernanDOI</i> | 24.45 | Guayasamin et al., 2009 | 36.3 | IUCN, 2009 | 0 | Frost, 2011 | Duellman & Schulte, 1993 |
| <i>Centrolene geckoideum</i> | 71.25 | Guayasamin et al., 2009 | 7937.6 | IUCN, 2009 | 410 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Centrolene gemmatum</i> | 24.1 | Guayasamin et al., 2009 | 36.3 | IUCN, 2009 | 300 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>guanacarum</i> | 21.45 | Guayasamin et al., 2009 | 60.5 | IUCN, 2009 | 150 | Frost, 2011 | Ruiz-Carranza & Lynch, 1995b |
| " <i>Centrolene</i> " <i>heloderma</i> | 29.55 | Guayasamin et al., 2009 | 5033.6 | IUCN, 2009 | 500 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Centrolene hesperium</i> | 25.9 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 300 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>huilense</i> | 25.15 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 210 | Frost, 2011 | Ruiz-Carranza & Lynch, 1995 |
| <i>Centrolene hybrida</i> | 21.55 | Guayasamin et al., 2009 | 4852.1 | IUCN, 2009 | 680 | Frost, 2011 | Guayasamin et al., 2009 |

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|---|-------|------------------------------|---------|------------|------|-------------|------------------------------------|
| <i>Centrolene lemniscatum</i> | 27 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Duellman & Schulte, 1993 |
| <i>Centrolene lynchi</i> | 24.95 | Guayasamin et al., 2009 | 3327.5 | IUCN, 2009 | 700 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| " <i>Centrolene</i> " <i>medemi</i> | 34.9 | Guayasamin et al., 2009 | 2323.2 | IUCN, 2009 | 3250 | HYDRO1k | Ruiz-Carranza & Lynch, 1991a |
| <i>Centrolene muelleri</i> | 23.5 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Duellman & Schulte, 1993 |
| <i>Centrolene notostictum</i> | 22.15 | Guayasamin et al., 2009 | 5541.8 | IUCN, 2009 | 710 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Centrolene paezorom</i> | 44.5 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Centrolene peristictum</i> | 19.65 | Guayasamin et al., 2009 | 11543.4 | IUCN, 2009 | 220 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>petrophilum</i> | 28.19 | Ruiz-Carranza & Lynch, 1991b | 314.6 | IUCN, 2009 | 420 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991b |
| <i>Centrolene pipilatum</i> | 21.2 | Guayasamin et al., 2009 | 145.2 | IUCN, 2009 | 440 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Centrolene</i> " <i>quindianum</i> | 25.3 | Ruiz-Carranza & Lynch, 1995b | 677.6 | IUCN, 2009 | 150 | Frost, 2011 | Ruiz-Carranza & Lynch, 1995b |
| " <i>Centrolene</i> " <i>robledo</i> | 22.15 | Ruiz-Carranza & Lynch, 1995b | 7780.3 | IUCN, 2009 | 2000 | Frost, 2011 | Ruiz-Carranza & Lynch, 1995b |
| <i>Centrolene sanchezi</i> | 19.8 | Guayasamin et al., 2009 | 72.6 | IUCN, 2009 | 0 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Centrolene savagei</i> | 21.85 | Guayasamin et al., 2009 | 1306.8 | IUCN, 2009 | 430 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Centrolene scirtetes</i> | 25.25 | Guayasamin et al., 2009 | 193.6 | IUCN, 2009 | 40 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Centrolene solitaria</i> | 19.3 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Centrolene venezuelense</i> | 28.6 | Guayasamin et al., 2009 | 9304.9 | IUCN, 2009 | 100 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Chimerella mariaelenae</i> | 18.8 | Guayasamin et al., 2009 | 13830.3 | IUCN, 2009 | 420 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Cochranella</i> " <i>adenocheira</i> | 22.3 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | HYDRO1k | Cisneros-Heredia & McDiarmid, 2007 |
| " <i>Cochranella</i> " <i>balionota</i> | 20.95 | Guayasamin et al., 2009 | 7804.5 | IUCN, 2009 | 740 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2006 |
| " <i>Cochranella</i> " <i>croceopodes</i> | 24.75 | Guayasamin et al., 2009 | 96.8 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin & Bonnacorso, 2004 |
| " <i>Cochranella</i> " <i>duidaeana</i> | 23.3 | Guayasamin et al., 2009 | 1173.7 | IUCN, 2009 | 0 | HYDRO1k | Savage, 2002 |
| " <i>Cochranella</i> " <i>euhystrix</i> | 31.05 | Guayasamin et al., 2009 | 121 | IUCN, 2009 | 810 | HYDRO1k | Savage, 2002 |
| <i>Cochranella euknemos</i> | 26.5 | Guayasamin et al., 2009 | 40329.3 | IUCN, 2009 | 1180 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Cochranella</i> " <i>geijskesi</i> | 36.1 | Guayasamin et al., 2009 | 121 | IUCN, 2009 | 0 | HYDRO1k | Savage, 2002 |
| <i>Cochranella granulosa</i> | 27.5 | Guayasamin et al., 2009 | 90931.5 | IUCN, 2009 | 1460 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Cochranella litoralis</i> | 19.7 | Guayasamin et al., 2009 | 1439.9 | IUCN, 2009 | 120 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Cochranella mache</i> | 23.75 | Guayasamin et al., 2009 | 399.3 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| " <i>Cochranella</i> " <i>megista</i> | 36.35 | Guayasamin et al., 2009 | 1052.7 | IUCN, 2009 | 700 | Frost, 2011 | Savage, 2002 |
| <i>Cochranella nola</i> | 23.2 | Guayasamin et al., 2009 | 12196.8 | IUCN, 2009 | 1250 | Frost, 2011 | Guayasamin et al., 2009 |

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| <i>“Cochranella” orejuela</i> | 30.5 | Guayasamin et al., 2009 | 810.7 | IUCN, 2009 | 600 | Frost, 2011 | Savage, 2002 |
| <i>Cochranella phryxa</i> | 26.5 | Guayasamin et al., 2009 | 36.3 | IUCN, 2009 | 0 | Frost, 2011 | Aguayo-Vedia & Harvey, 2006 |
| <i>“Cochranella” ramirezi</i> | 26.5 | Guayasamin et al., 2009 | 375.1 | IUCN, 2009 | 810 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Cochranella resplendens</i> | 27.3 | Guayasamin et al., 2009 | 71099.6 | IUCN, 2009 | 560 | Frost, 2011 | Aguayo-Vedia & Harvey, 2006 |
| <i>“Cochranella” ritae</i> | 19 | Lutz & Kloss, 1952 | 2032.8 | IUCN, 2009 | 105 | HYDRO1k | Aguayo-Vedia & Harvey, 2006 |
| <i>“Cochranella” riveroi</i> | 23 | Ayarzagüena, 1992 | 121 | IUCN, 2009 | 0 | HYDRO1k | Savage, 2002 |
| <i>“Cochranella” xanthocheiria</i> | 23.55 | Guayasamin et al., 2009 | 4029.3 | IUCN, 2009 | 1260 | Frost, 2011 | Ruiz-Carranza & Lynch, 1995a |
| <i>Espadarana andina</i> | 24.55 | Guayasamin et al., 2009 | 33904.2 | IUCN, 2009 | 570 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Espadarana callistomma</i> | 29.25 | Guayasamin et al., 2009 | 786.5 | IUCN, 2009 | 201 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Espadarana prosoblepon</i> | 26 | Guayasamin et al., 2009 | 300793.9 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium aureoguttatum</i> | 20 | Guayasamin et al., 2009 | 20848.3 | IUCN, 2009 | 1515 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium bergeri</i> | 24.6 | Guayasamin et al., 2009 | 91972.1 | IUCN, 2009 | 1680 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium carlesvilai</i> | 22.25 | Castroviejo-Fisher et al., 2009 | 34257 | Castroviejo-Fisher et al., 2009 | 900 | Castroviejo-Fisher et al., 2009 | Castroviejo-Fisher et al., 2009 |
| <i>Hyalinobatrachium chirripoi</i> | 25 | Guayasamin et al., 2009 | 8869.3 | IUCN, 2009 | 600 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium colymbiphylum</i> | 26 | Guayasamin et al., 2009 | 119548 | IUCN, 2009 | 950 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium crurifasciatum</i> | 21.5 | Guayasamin et al., 2009 | 142368.6 | IUCN, 2009 | 1176 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium durantei</i> | 22.85 | Guayasamin et al., 2009 | 2250.6 | IUCN, 2009 | 343 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium eccentricum</i> | 24.75 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 300 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium esmeralda</i> | 21.8 | Ruiz-Carranza & Lynch, 1998 | 84.7 | IUCN, 2009 | 50 | Frost, 2011 | Ruiz-Carranza & Lynch, 1998 |
| <i>Hyalinobatrachium fleischmanni</i> | 25.5 | Guayasamin et al., 2009 | 403135.7 | IUCN, 2009 | 1153 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium fragile</i> | 21.7 | Guayasamin et al., 2009 | 6376.7 | IUCN, 2009 | 600 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium guairarepanense</i> | 22.5 | Señaris, 2001 | 411.4 | IUCN, 2009 | 280 | Frost, 2011 | Señaris, 2001 |
| <i>Hyalinobatrachium iaspidiense</i> | 21.25 | Guayasamin et al., 2009 | 19977.1 | IUCN, 2009 | 360 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium ibama</i> | 21.7 | Guayasamin et al., 2009 | 4694.8 | IUCN, 2009 | 450 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium ignioculus</i> | 21.9 | Guayasamin et al., 2009 | 24.2 | IUCN, 2009 | 405 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium lemur</i> | 20.85 | Guayasamin et al., 2009 | 60.5 | IUCN, 2009 | 740 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Hyalinobatrachium mondolfii</i> | 21.4 | Guayasamin et al., 2009 | 40172 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium munozorum</i> | 19.75 | Guayasamin et al., 2009 | 243536.7 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium nouraguense</i> | 19.95 | Guayasamin et al., 2009 | 22397.1 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |

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| <i>Hyalinobatrachium orientale</i> | 21.7 | Guayasamin et al., 2009 | 6497.7 | IUCN, 2009 | 400 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium orocostale</i> | | | | Castroviejo-Fisher et al., | | Castroviejo-Fisher et al., | |
| | 20.1 | Guayasamin et al., 2009 | 895 | 2009b | 300 | 2009b | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium pallidum</i> | 22.15 | Guayasamin et al., 2009 | 677.6 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium pellucidum</i> | 22 | Guayasamin et al., 2009 | 1076.9 | IUCN, 2009 | 740 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium ruedai</i> | 21.4 | Guayasamin et al., 2009 | 11531.3 | IUCN, 2009 | 0 | HYDRO1k | Ruiz-Carranza & Lynch, 1998 |
| <i>Hyalinobatrachium talamancae</i> | 24.5 | Guayasamin et al., 2009 | 6667.1 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium tatayoi</i> | 21.95 | Guayasamin et al., 2009 | 36.3 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium taylori</i> | 19.7 | Guayasamin et al., 2009 | 457283.2 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium valerioi</i> | 22.75 | Guayasamin et al., 2009 | 102499.1 | IUCN, 2009 | 1100 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Hyalinobatrachium vireovittatum</i> | 23.25 | Guayasamin et al., 2009 | 302.5 | IUCN, 2009 | 580 | HYDRO1k | Ruiz-Carranza & Lynch, 1991a |
| <i>Ikakogi tayrona</i> | 29.5 | Guayasamin et al., 2009 | 4065.6 | IUCN, 2009 | 810 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus anomalus</i> | 24.1 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus armatus</i> | 24.05 | Guayasamin et al., 2009 | 48.4 | IUCN, 2009 | 0 | Frost, 2011 | Lynch, & Ruiz-Carranza, 1996 |
| <i>Nymphargus bejaranoi</i> | 24.1 | Guayasamin et al., 2009 | 97864.8 | IUCN, 2009 | 800 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus buenaventura</i> | 21.65 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Cisneros-Heredia & Yáñez-Muñoz, 2007 |
| <i>Nymphargus cariticommatus</i> | 24.6 | Guayasamin et al., 2009 | 60.5 | IUCN, 2009 | 150 | Frost, 2011 | Cisneros-Heredia & Yáñez-Muñoz, 2007 |
| <i>Nymphargus chami</i> | 34.05 | Guayasamin et al., 2009 | 1996.5 | IUCN, 2009 | 480 | Frost, 2011 | Ruiz-Carranza & Lynch, 1995a |
| <i>Nymphargus chancas</i> | 27.9 | Guayasamin et al., 2009 | 48.4 | IUCN, 2009 | 0 | Frost, 2011 | Duellman & Schulte, 1993 |
| <i>Nymphargus cochranae</i> | 27.15 | Guayasamin et al., 2009 | 3956.7 | IUCN, 2009 | 1100 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus cristinae</i> | 28.55 | Guayasamin et al., 2009 | 72.6 | IUCN, 2009 | 160 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus garciae</i> | 26.75 | Guayasamin et al., 2009 | 5263.5 | IUCN, 2009 | 1130 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus grandisonae</i> | 27.9 | Guayasamin et al., 2009 | 16201.9 | IUCN, 2009 | 220 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus griffithsi</i> | 23.25 | Guayasamin et al., 2009 | 8796.7 | IUCN, 2009 | 1190 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus ignotus</i> | 21.9 | Guayasamin et al., 2009 | 3436.4 | IUCN, 2009 | 60 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus laurae</i> | 19.9 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus luminosus</i> | 28.9 | Ruiz-Carranza & Lynch, 1995 | 278.3 | IUCN, 2009 | 290 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus luteopunctatus</i> | 33.1 | Guayasamin et al., 2009 | 108.9 | IUCN, 2009 | 300 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus mariae</i> | 30 | Guayasamin et al., 2009 | 24.2 | IUCN, 2009 | 0 | HYDRO1k | Cisneros-Heredia & McDiarmid, 2007 |

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|---------------------------------|-------|-------------------------------|---------|-------------------------------|------|-------------------------------|------------------------------------|
| <i>Nymphargus megacheirus</i> | 29.95 | Guayasamin et al., 2009 | 665.5 | IUCN, 2009 | 450 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus mixomaculatus</i> | 24.55 | Guayasamin et al., 2009 | 48.4 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Nymphargus nephelophilus</i> | 23.35 | Guayasamin et al., 2009 | 60.5 | IUCN, 2009 | 530 | Frost, 2011 | Savage, 2002 |
| <i>Nymphargus ocellatus</i> | 25 | Guayasamin et al., 2009 | 19299.5 | IUCN, 2009 | 0 | HYDRO1k | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus oreonympha</i> | 25.2 | Guayasamin et al., 2009 | 72.6 | IUCN, 2009 | 230 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Nymphargus phenax</i> | 21.15 | Guayasamin et al., 2009 | 108.9 | IUCN, 2009 | 410 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus pluvialis</i> | 25.7 | Guayasamin et al., 2009 | 544.5 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus posadae</i> | 32.15 | Guayasamin et al., 2009 | 16782.7 | IUCN, 2009 | 1700 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus prasinus</i> | 33.65 | Guayasamin et al., 2009 | 3509 | IUCN, 2009 | 550 | Frost, 2011 | Ruiz-Carranza & Lynch, 1991a |
| <i>Nymphargus puyoensis</i> | 25.6 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 50 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus rosadus</i> | 26.2 | Guayasamin et al., 2009 | 750.2 | IUCN, 2009 | 900 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Nymphargus ruizi</i> | 26.9 | Guayasamin et al., 2009 | 1839.2 | IUCN, 2009 | 660 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus siren</i> | 21.55 | Guayasamin et al., 2009 | 3484.8 | IUCN, 2009 | 400 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Nymphargus spilotus</i> | 26.9 | Guayasamin et al., 2009 | 48.4 | IUCN, 2009 | 90 | Frost, 2011 | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus truebae</i> | 23.7 | Guayasamin et al., 2009 | 133.1 | IUCN, 2009 | 0 | HYDRO1k | Cisneros-Heredia & McDiarmid, 2007 |
| <i>Nymphargus vicenteruedai</i> | 25.55 | Guayasamin et al., 2009 | 217.8 | IUCN, 2009 | 50 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Nymphargus wileyi</i> | 25.2 | Guayasamin et al., 2009 | 24.2 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Rulyrana adiazeta</i> | 25.65 | Guayasamin et al., 2009 | 4162.4 | IUCN, 2009 | 930 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Rulyrana erminea</i> | 24.2 | Guayasamin et al., 2009 | 60.5 | IUCN, 2009 | 230 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Rulyrana flavopunctata</i> | 23.15 | Guayasamin et al., 2009 | 54534.7 | IUCN, 2009 | 1100 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Rulyrana mcdiarmidi</i> | 27.65 | Cisneros-Heredia et al., 2008 | 6562 | Cisneros-Heredia et al., 2008 | 350 | Cisneros-Heredia et al., 2008 | Guayasamin et al., 2009 |
| <i>Rulyrana saxiscandens</i> | 22 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Rulyrana spiculata</i> | 24.7 | Guayasamin et al., 2009 | 17169.9 | IUCN, 2009 | 500 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Rulyrana susatamai</i> | 23.95 | Guayasamin et al., 2009 | 2359.5 | IUCN, 2009 | 750 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Rulyrana tangarana</i> | 22.9 | Guayasamin et al., 2009 | 60.5 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Sachatamia albomaculata</i> | 26.25 | Guayasamin et al., 2009 | 72587.9 | IUCN, 2009 | 380 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Sachatamia ilex</i> | 30.15 | Guayasamin et al., 2009 | 79206.6 | IUCN, 2009 | 1360 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Sachatamia punctulata</i> | 25.9 | Guayasamin et al., 2009 | 2214.3 | IUCN, 2009 | 480 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Teratohyla ameliae</i> | 18.2 | Guayasamin et al., 2009 | 24.2 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |

| | | | | | | | |
|--------------------------------|-------|-------------------------------|----------|-------------------------------|------|-------------------------------|-------------------------------|
| <i>Teratohyla midas</i> | 22.9 | Guayasamin et al., 2009 | 1380090 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Teratohyla pulverata</i> | 27.5 | Guayasamin et al., 2009 | 126178.8 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Teratohyla sornozai</i> | 20.15 | Cisneros-Heredia et al., 2009 | 266 | Cisneros-Heredia et al., 2009 | 550 | Cisneros-Heredia et al., 2009 | Cisneros-Heredia et al., 2009 |
| <i>Teratohyla spinosa</i> | 20.4 | Guayasamin et al., 2009 | 93109.5 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Vitreorana antisthenesi</i> | 24.2 | Guayasamin et al., 2009 | 5045.7 | IUCN, 2009 | 980 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Vitreorana castroviejoi</i> | 23.3 | Guayasamin et al., 2009 | 12.1 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Vitreorana eurygnatha</i> | 20.95 | Guayasamin et al., 2009 | 469274.3 | IUCN, 2009 | 1700 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Vitreorana gorzulae</i> | 20.75 | Guayasamin et al., 2009 | 847 | IUCN, 2009 | 895 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Vitreorana helenae</i> | 19.8 | Guayasamin et al., 2009 | 375.1 | IUCN, 2009 | 0 | HYDRO1k | Guayasamin et al., 2009 |
| <i>Vitreorana oyampiensis</i> | 20.5 | Guayasamin et al., 2009 | 806489.2 | IUCN, 2009 | 0 | Frost, 2011 | Guayasamin et al., 2009 |
| <i>Vitreorana parvula</i> | 17 | Heyer, 1985 | 762.3 | IUCN, 2009 | 405 | HYDRO1k | Ruiz-Carranza & Lynch, 1991a |
| <i>Vitreorana uranoscopa</i> | 22.8 | Guayasamin et al., 2009 | 620209.7 | IUCN, 2009 | 1600 | HYDRO1k | Ruiz-Carranza & Lynch, 1991a |

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Capítulo 3. Climatic niche at physiological and macroecological scales: thermal tolerance–geographic range interface and niche dimensionality

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Abstract

Aim The Hutchinsonian niche–space duality relates to the species realized niche, so its non-abiotic components (e.g. interaction and dispersal) may dissociate tolerance limits from climatic limits at species’ range. This mismatch may reduce the ability of physiological boundaries in predicting geographical range boundaries, which in turn may distort inferences of niche conservatism of adaptive limits. However, niche position in the climatic hyperspace should be less affected by external factors than its boundaries. Here we test the hypothesis that niche position is more reliable to capture the variability and evolutionary pattern of physiological tolerance.

Location Neotropic and Palaeartic.

Methods We used non-phylogenetic and phylogenetic regressions to test relationships between physiological (CT_{\max}) and macroecological niche features (geographical maximum temperature – T_{\max} , and multivariate niche position and breadth) among anurans. We also compare evolutionary rates among these parameters through an eigenvector-based technique, the phylogenetic signal-representation (PSR) curve.

Results CT_{\max} is better related to the multivariate niche position than to T_{\max} , and is unrelated to niche breadth. CT_{\max} and niche position also show similar, high evolutionary rates, i.e. faster than Brownian motion, whereas T_{\max} evolves slower, and niche breadth evolves at random.

Main conclusions Results indicate that the thermal tolerance–range transferability is not straightforward for these anurans. In contrast, a multivariate description of niche

position may suitably account for the variability and evolutionary pattern of the species' adaptive limits. We link these results to the 'niche dimensionality', as manifold interacting dimensions of the niche constrain its boundaries, but succeed in circumscribe its position in the climatic hyperspace.

Keywords Anuran larvae, CT_{\max} , macrophysiology, phylogenetic comparative methods, phylogenetic signal-representation curve, thermal tolerance.

Introduction

After the groundwork laid by Grinnell (1917) and Elton (1927), Hutchinson (1957) formalized the modern idea of the ecological niche, defining it as an n-dimensional hypervolume that encompasses all environmental conditions experienced and relationships played by species. Hutchinson also distinguished the fundamental from the realized niches, to demarcate the conditions that species could live from that they do live, respectively. He further viewed the species realized niche reflected into the geographical space (Hutchinson 1978), a property that would allow addressing major questions in the interface between the ecological requirements and broad-scales patterns of species distributions (Colwell & Rangel 2009; Soberón & Nakamura 2009). These questions include patterns of species distribution, diversity gradients, assembling of ecological communities, trait evolution and speciation, species invasiveness and response to global climate changes (see e.g. Wiens & Donoghue 2004; Wiens et al. 2010; Pearman et al. 2008, and references therein).

The niche–space duality also enabled the field of ecological niche modelling (ENM), which uses environmental information to estimate, reconstruct, and forecast the species niches at distinct spatial and temporal frames (Guisan & Zimmermann 2000). More recently, ENM has involved direct measures of physiological data to account for real constraints to the species niche (Kearney & Porter, 2004, 2009). The technique has been referred to as mechanistic niche modelling, in order to differ from the occurrence-based, correlative niche modelling (Kearney & Porter 2009). Another vein to account for these fundamental niche features as reflected into space is by assessing physiological correlates of the geographical distributions of species, particularly their position and limits (Chown & Gaston, 1999; Calosi et al., 2010; Sunday et al. 2010, 2012; Bozinovic

et al., 2011) This bridging between macroecology and physiology has been termed ‘macrophysiology’ (Chown et al., 2004), a field that aims to describe general properties of the interface between individuals’ endurance to environmental conditions and the patterns of geographical distribution of their species (see also Gaston et al., 2009).

Permeating the niche-space transferability is the environmental changeability in space and time, altering the geography of species, and so (or not) their niches (Pearman et al. 2008; Colwell & Rangel 2009). Whether niches change and to what extent they change have, per se, become a key topic for the modern ecology (Pearman et al. 2008; Losos 2008; Wiens et al. 2010), which is centred around the ‘niche conservatism hypothesis’, or the tendency of closely related species to share more niche similarities among each other than with less related species (Wiens & Donoghue, 2004). Under a phylogenetic framework (Losos 2008; Cooper et al. 2010), this balance between niche evolution and niche conservatism has provided a powerful analytical tool to link evolutionary theory, ecology and biogeography. Most often, these studies have relied on the ‘other way around’ of the tolerance–range transferability. They have used the distributional limits to infer patterns of niche conservatism of species’ tolerance to climate across large spatial and temporal scales (e.g. Roy et al. 2009; Olalla-Tárraga et al. 2011; Nguyen et al. 2011).

However, real limits of climatic tolerance may be loosely defined by- or even divorced from range limits, particularly for terrestrial organisms (Sexton et al. 2009; Sunday et al. 2012; but see Calosi et al. 2010). Multiple factors govern the species range, but how they combine to define range boundaries are poorly understood so far (Pulliam 2000; Gaston 2003; Holt 2003; Sexton et al. 2009). The Soberón & Peterson’s (2005) BAM diagram (from biotic, abiotic, and movement) summarize how different factors affect the species distribution at large spatial scales. Besides tolerance limits (depicted by the abiotic factor), the species distributions undergo several biotic interaction and movement constraints, which may act asymmetrically. If one factor falls short of others, the species will fail to accomplish its potential distribution, and the characterization of any of these factors from the observed distribution of the species will be distorted (see also Soberón & Nakamura 2009).

This multiple interaction of factors in setting the limits of the species range, and therefore their realized niche, is *prima facie* the multidimensional nature of the

Hutchinsonian niche. However, in some way, the species climatic tolerance should be linked to their distribution in its environmental space (otherwise it would perish). Indeed, biophysical tolerance of individuals should evolve according to the position of the species in the (also) multidimensional climatic space. In other words, species tolerances evolve in response to some new condition, after either a spatial or temporal change takes place (Huey & Steverson 1979; Huey & Kingsolver 1993). In this sense, rather than climatic boundaries, a niche description that take into account the position of the species in the multidimensional climatic space should summarize both the variation and the evolutionary pattern of most fundamental features of the species niche, such as biophysical tolerance.

Recently, Hof et al. (2010) used a measure of niche position in the climatic hyperspace to assess the broad-scale patterns of niche conservatism among amphibians. This measurement tentatively characterized the species niche in the absence of true physiological data (Hof et al. 2010). Here, we resort to data on amphibian thermal tolerance to test the hypothesis that niche position in the multidimensional climatic space describes the variation in species tolerance better than climatic niche features drawn from the species niche boundaries. We compare physiological limits characterizing individuals within species to some metrics of species niche, in terms of both explanatory ability and evolutionary rate. This latter describes how traits have evolved along the phylogeny, thus enabling a model-based estimation on niche conservatism/evolution. We use anurans to test which macroecological niche measure best explain the variation in the larval critical thermal maximum (CT_{max}): either maximum temperature of the species' range, multivariate niche position, or niche breadth at the climatic space.

Methods

We used the data of upper thermal limit of physiological tolerance (CT_{max}) of 47 species of anurans. Among them, 42 species are presented by Duarte et al. (2012), and data for other five species were gathered by H. Duarte, M. Tejedo, D. Donaire, D. Baldo and F. Marangoni, following the same protocol (see Duarte et al. 2012). Five species of Caudata analysed by Duarte's et al. (2012) study were excluded. The data employed here comprises species from three communities located at distinct environmental conditions:

the subtropical warm Gran Chaco region, the subtropical Atlantic Forest, and Temperate Europe and northern Africa (see Duarte et al., 2012 for further details). However, each species is distributed at varying geographical positions and climatic conditions, including most of Neotropic and Palearctic (Figure S1).

Also following Duarte et al. (2012), we used the phylogenetic hypothesis for the 47 anurans according to Frost et al. (2006), including branch length estimation based on three nuclear and two mitochondrial genes. Species missing from the phylogeny had their branch lengths inferred from sister-taxa, which has, by definition, the same age of the target species (see details in Duarte et al., 2012, including their electronic Supporting Information).

Physiological data

CT_{max} was estimated from anuran larvae under controlled trials in laboratory, following the Hutchison's dynamic method (Hutchison, 1961). Tadpoles were heated until individuals reach the onset of muscular spasms, which was assumed as maximum thermal limit for species tolerance (see details of laboratorial procedure in Duarte et al., 2012). Fully aquatic, small anuran larvae can be considered isothermal with the environment (Lutterschmidt & Hutchison, 1997a), so they are expected to mirror the environmental tolerance at the species geographical limits better than adults. In addition, the tadpoles of the species studied develop in temporary, shallow ponds, where thermoclines are virtually absent and individuals are fully subject to the actual thermal variation. The ponds temperatures, in turn, are ruled by the outer climate, which is related to the macroclimatic dominion. Adults, in contrast, are capable to circumvent thermal stresses by actively searching for more suitable microhabitats outside the ponds, including fossorial and nightly activity, thus their thermal tolerance limits may not match the environmental limits, as larval tolerance do. Therefore, we can assume that anuran larvae can reasonably represent the susceptibility to thermal variability of the species.

Threshold temperature limits such as the CT_{max} are important parameters related to the concept of fundamental niche as they set hard boundaries for animal survivorship (Huey & Steverson, 1979; Lutterschmidt & Hutchison, 1997b). The earlier definition of CT_{max} (Cowles & Bogert 1944) is "the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death". Nevertheless, it remains challenging to determine, for the

anuran species as a whole, and out of the laboratory controls, the ecological meaning of the CT_{\max} of the larval stage. Indeed, this is a frequent problem among other organisms used as physiological models (e.g. Ribeiro et al., 2012). Probably, in nature, even lower temperatures could cause other sub-lethal distresses, including developmental disorders, or decrease the ability to forage or to evade from predators, thus undermining the viability of the population (Huey & Stevenson 1979). In addition, the CT_{\max} is a complex function of experimental heating rates, and usually information of field heating rates is absent (Ribeiro et al. 2012). In this sense, we should assume that, whichever is the outdoor ecological meaning of acute measures of CT_{\max} , it should be linearly correlated with an actual measure of thermal tolerance to higher temperatures.

Macroecological data

We gathered data of species geographic distribution from the ‘Global Amphibian Assessment’ database (IUCN, 2009). Maximum temperature of species distribution (T_{\max}) was the mean of the maxima within each species’ range. For the multivariate niche measurement (see below), we assembled a set of seven environmental descriptors widely recognized as direct or indirect limiting factors of the climatic niche of amphibians (mean actual evapotranspiration – AET, mean potential evapotranspiration – PET, maximum temperature of the warmest month, minimum temperature of the coldest month, annual range of temperature, mean annual precipitation, and annual range in precipitation). These environmental variables were drawn from interpolated surfaces of time data series (AET and PET from Willmott & Matsuura, 2001; and the others from Hijmans et al., 2005). Both the species’ geographical range and the environmental variables were projected onto a grid cell system of $1^{\circ} \times 1^{\circ}$ (latitude \times longitude) resolution covering the geographic region outlined by the distribution of all 47 species together, i.e. parts of the Neotropic and Palearctic. Each grid cell defined a sample unity for estimating the environmental maximum temperature and the multivariate niche features.

As a measure of multivariate niche position or breadth at the climatic space, we used the Outlying Mean Index (OMI; Doledec et al. 2000). OMI is a multivariate ordination technique that calculates the hypervolumetric space of species niche (i.e. a subset of the Hutchinsonian niche in the multidimensional space) according to the selected factors (e.g., environmental variables). OMI characterizes niche breadth, ‘niche marginality’, ‘inertia’ – which provides an estimate of niche overlap – and ‘residual’ –

which describe the variation in the niche breadth unrelated to the variables of the model. Niche marginality describes the amount of differentiation of the species niche relative to a theoretical, average niche drawn from the environmental data inputted (Doledec et al., 2000), so it can be interpreted as a measure of niche position in the climatic hyperspace (see also Hof et al. 2010). Because niche marginality measures the ecological distance of each species relative to an average, theoretical niche, species similarly distant to this midpoint but at opposite points of a niche axis will have similar values of marginality. Thus we used the species normed scores along the first axis of the OMI ordination (which encompassed 92.35% of the variation among all axes) as a measure of niche position.

We recognize here that the data of both macroclimatic and physiologic features involve measures at two very different scales and levels of biological organization (larvae vs. adults). Therefore, some unavoidable assumptions are required. For instance, the CT_{max} of the individuals should be taken as representative of the entire species. Also, the geographical range of species is assumed to describe the distribution of breeding population, and the climatic variables are good enough and reasonably reflected suitable conditions for them. Although hard for the data at hand, these assumptions are nonetheless common for virtually all broad-scale studies, and putative for our case. This is because we are precisely focused on this possibility of identifying macroscale correlates of the species variability in a physiological property that is shared by individuals of the same species.

Phylogenetic Comparative Analysis

We first ran ordinary least square (OLS) regression of CT_{max} against the macroecological niche features (T_{max} , position, and breadth) independently to test if physiological tolerance and climatic niche parameters at the macroscale are capable to explain each other in a direct way. This could provide, for example, if CT_{max} could systematically approximate the environmental maximum temperature of the species or other niche description. We do not expect, however, CT_{max} and T_{max} to coincide because T_{max} may underestimate the maximum body temperature of individual amphibians. Instead, we question whether CT_{max} and T_{max} are in some way correlated.

Next, we tested if these features are related to each other while accounting for phylogenetic autocorrelation, which can bias tests of significant of standard statistical techniques such as OLS, when applied to cross-species data. We analysed the

phylogenetic signal using phylogenetic signal-representation (PSR) curves (Diniz-Filho et al. 2012) for each trait, as also a means to access their intrinsic evolutionary rate through the phylogeny. PSR curve is built upon the eigenvectors from the phylogenetic eigenvector regression (PVR; Diniz-Filho et al. 1998), in which successive PVR's R^2 of accumulated eigenvectors are plotted against the phylogenetic representation that is given by the accumulated percentage of the corresponding eigenvalues (λ %). The shape of the curve describes the model of evolution of the trait across the phylogeny. A PSR curve near the reference 45° line indicates an evolutionary pattern equivalent to the Brownian motion of trait evolution (Fig. 1), whereas a curve bending below the reference line implies a stronger phylogenetic signal, which can be described by an Ornstein-Uhlenbeck (O-U) process, or by a low lambda model (Freckleton et al. 2002). In contrast, models of accelerated divergence should generate PSR curves traced above the reference line (see Diniz-Filho et al. 2012 for further details). In comparative terms, the trait evolution either slower or faster than an assumed model can be indicative of niche conservatism or niche evolution, respectively (Wiens et al. 2010; Cooper et al. 2010). We used permutations to test the evolutionary model of each trait against a null (random) and a neutral (Brownian motion) model of trait evolution. Departures from these models denote accelerated ($PSR_{area} > 0.0$) or O-U process ($PSR_{area} < 0.0$).

Finally, we tested the associations among traits through Phylogenetic Generalized Linear Models (PGLS), using maximum likelihood estimation for λ (Freckleton et al., 2002). Analyses were run using the PVR and caper packages in R 2.14 (©R Core Development Team).

Results

Species' CT_{max} varied from 35.42 to 44.73°C ($\mu \pm SD = 40.29 \pm 2.20$), whereas geographical T_{max} was considerably lower, as expected, varying between 18.76 and 35.63°C (29.94 ± 4.31). The multivariate niche breadth and position (in terms of departure from the theoretical average niche) were slightly higher for some Neotropical species (e.g. *Dendropsophus minutus*, *Hypsiboas raniceps* and *Trachycephalus venulosus*) (Fig. S2).

According to OLS regressions, the ability of T_{\max} in predicting CT_{\max} was significant, although weak ($R^2 = 0.105$; $F = 6.394$; $P = 0.0150$). In contrast, a significant and substantial amount of variation in CT_{\max} was explained by the multivariate niche position ($R^2 = 0.414$; $F = 31.840$; $P \ll 0.001$), although not by niche breadth ($R^2 = 0.013$; $F = 0.604$; $P = 0.441$). However, these results may be affected by phylogenetic signal in data.

Table 1. Statistics of Phylogenetic Generalized Least Squares (PGLS) models between CT_{\max} and macroecological niche traits. The λ is the index that transforms the trait phylogeny to fit a Brownian motion model. β = models slope; ε = standard error; AICc = corrected model's score.

| CT_{\max} vs. | λ | β | $\pm \varepsilon$ | F-value | p-value |
|-----------------|-----------|---------|-------------------|---------|-----------------------|
| T_{\max} | 0.979 | 0.104 | 0.05 | 4.28 | 0.019 |
| Niche position | 1 | 1.965 | 0.073 | 717.6 | 2.2×10^{-16} |
| Niche breadth | 0.969 | 0.067 | 0.739 | 0.008 | 0.9917 |

The PSR curves showed that CT_{\max} and niche position had evolutionary rates slightly, but significantly faster than Brownian motion (CT_{\max} , $PSR_{\text{area}} = 0.037$; $p < 0.001$; niche position, $PSR_{\text{area}} = 0.026$, $p < 0.001$). In contrast, T_{\max} showed a slower rate, a pattern that can be described by the O-U process (T_{\max} , $PSR_{\text{area}} = -0.196$, $p < 0.001$), whereas the evolutionary pattern of niche breadth did not differ from random ($PSR_{\text{area}} = -0.237$, $p = 0.10$) (Fig. 1). Because of the phylogenetic signal in data, it is worthwhile to apply comparative analyses to test for relationship among variables.

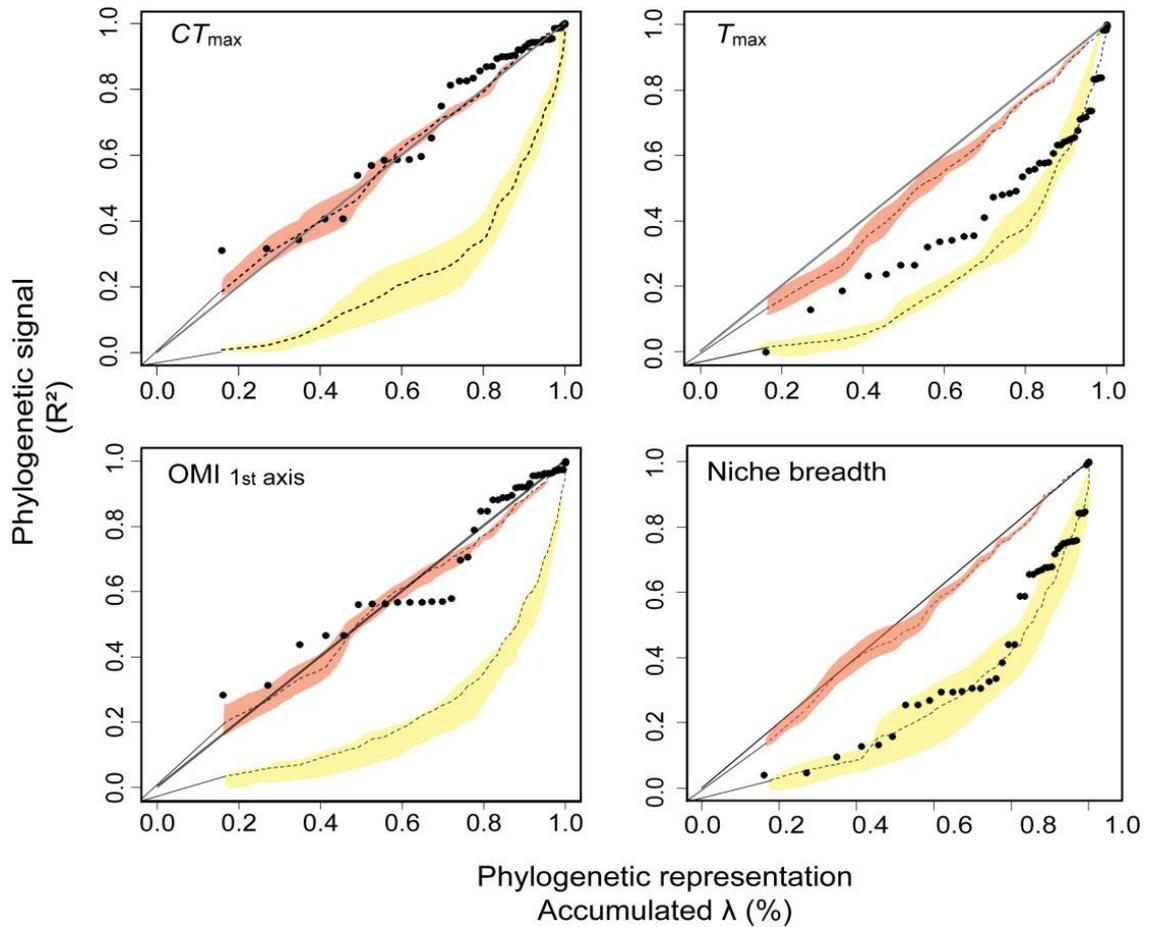


Figure 1. Phylogenetic Signal Representation (PSR) curves showing the evolutionary rates of critical thermal maximum (CT_{max}), geographical maximum temperature (T_{max}), niche marginality (OMI 1st axis) and niche breadth for 47 anurans. Red and yellow bands are the confidence intervals for the neutral (Brownian motion) and null (random) expectations, respectively (Diniz-Filho et al. 2012). Note that CT_{max} and OMI 1st axis have very similar patterns of evolution (i.e., slightly faster than Brownian motion).

PGLS analyses provided similar results to OLS. There is a weak, although significant, relationship between CT_{max} and the T_{max} ($\beta = 0.104 \pm 0.050$; $p = 0.019$), and a non-significant relationship between CT_{max} and niche breadth ($\beta = 0.067 \pm 0.739$; $p = 0.992$). On the other hand, we found a positive, highly significant explanation of the niche position by CT_{max} ($\beta = 1.965 \pm 0.073$; $P \ll 0.001$) (Table 1; Fig. 2).

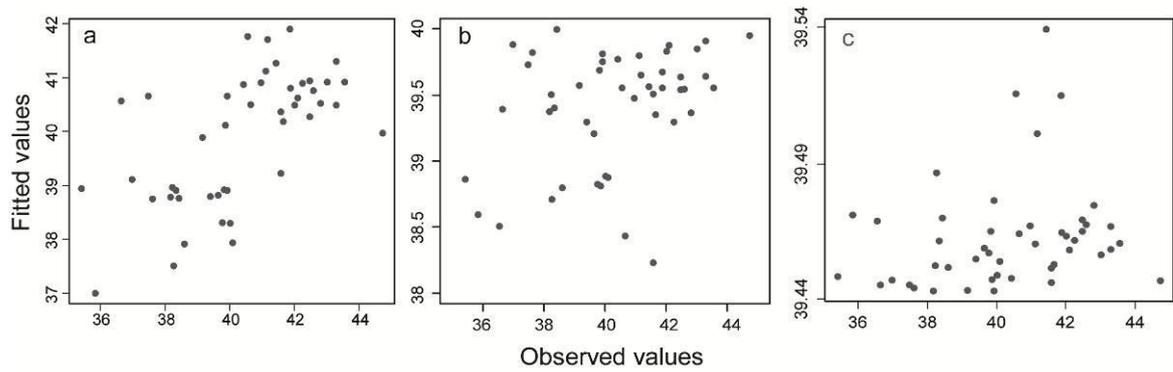


Figure 2. Phylogenetic Generalized models between the anuran larvae CT_{\max} and macroecological climatic niche features: a = niche position (OMI 1st axis); b = T_{\max} ; c = niche breadth.

Discussion

The ability of physiological tolerance limits (i.e. CT_{\max}) in describing geographical range limits (e.g. T_{\max}) – and vice versa – has been a grail for the fields of ecophysiology (and more recently for macrophysiology). In plain words, it would allow one, for example, to use individuals physiological parameters to infer species distributional changes after climatic shifts (reviewed in Bozinovic et al. 2011). At the other way round, it would yield one to infer species average tolerance based on their geographical distribution (a fairly common practice indeed; e.g. Roy et al. 2009; Olalla-Tárraga et al. 2011; Nguyen et al. 2011). In fact, physiological tolerance limits are informative on the susceptibility of species to rapid climatic changes in terms of the maximum tolerable amount of heat (Duarte et al. 2012). However, our results have shown that upper physiological limits alone may fail in characterize species distributional change after climatic shifts.

Of course, other climatic parameters (e.g. T_{\min}) could be more symptomatic of the species thermal limits at the geographical space than T_{\max} (e.g. Addo-Bediako et al. 2000; Sunday et al. 2011). Nonetheless, the rationale of this physiological–geographical transferability of tolerance, as has been applied, builds upon the assumption that tolerance limits define some boundaries of the species fundamental niche and, as species range reflects their niche at the geographical space, tolerance and range boundaries should mirror one another (Calosi et al. 2010). However, besides the various sources of noise in tolerance estimates that can be anticipated (Ribeiro et al. 2012), spatial distribution

actually reflects the climatic niche after it interacts with a sort of other factors, particularly biotic and movement constraints, i.e. the realized niche (Soberón & Peterson 2005; see also Soberón & Nakamura 2009). When these niche dimensions (biotic, abiotic and movement) fail to overlap each other, the species will fulfil only a subset of its suitable area, and hence a single dimension will fail to predict the species' whole distribution. As a result, only in the particular case in which these three factors fit together each of them would be able to describe the others, and so the physiological–geographical transferability would be possible.

Our results also show that the CT_{\max} and T_{\max} may differ in their evolutionary rates. In fact, there is evidence of both faster and slower rates of evolution for either physiological (Huey & Kingsolver 1993; Angilletta et al. 2002; Kellermann et al. 2012a,b) or macroecological (Pearman et al. 2008) niche features. Different features in the same clade may also evolve at specific rates, or yet have varying rates though evolutionary time (Pearman et al. 2008). Therefore, the observed patterns of trait evolution appear to be contingent to the trait and the clade involved. However, different rates – or amounts – of trait change can yield different conclusions on patterns of niche conservatism/evolution for these traits, which in turn can indicate different evolutionary processes (Cooper et al. 2010). Flawed inferences of trait change may therefore misguide conclusions on the evolutionary process of that trait. As the evolutionary pattern observed in T_{\max} should incorporate other factors involved in range determination, T_{\max} alone may lead to inaccurate conclusions on the actual evolutionary pattern of tolerance, if taken as such.

A caveat in our results comes from the quality of macroecological data used. It is possible, for example, that coarse range maps be a poor descriptor of both the species geographical limits (Hurlbert & White 2005) and their climatic limits (which are also coarse). In such case, the poor ability of climatic limits at the geographical space (e.g. T_{\max}) in describing physiological limits would be but a data artefact. The same problem would affect the estimation of the evolutionary rate through the PSR curve. That is, because most species share part of their distribution, it would be possible that an overall low ability in discriminating their climatic boundaries make their T_{\max} to be most similar than expected by chance (i.e. Brownian motion), thus resulting in a more conserved pattern of trait evolution. In fact, some of these caveats are related to the scaling issue

referred before. That is, we are dealing with variables that stand at contrasting spatial scales (pond vs. continent) and levels of organization (individual vs. species). In addition, our physiological data comes from tadpoles, whereas macroecological data characterize terrestrial environments of adult. Although both larvae and adult broad-scale distributions should coincide, we ignore possible, particular developmental modifications in the physiological machinery of each species.

From the physiological standpoint, some important information needed to discuss species limits are also missing. Species limits are defined by the pool of individual limits, including acclimation and inter-individual plasticity of critical temperatures, and are expected to vary according to the conditions of their position in the species range. We only have individual limits under acute change, i.e. a subset of the information of thermal tolerance that can characterize the entire species. From a macroecological stance, there is still the challenge of identifying the species' range limits accurately, even for well-known species. Many factors besides those summarized in the Soberón & Peterson's (2005) BAM scheme are known to cause the species' range to behave dynamically. They include, for example, population's source-sink dynamic (Pulliam 2000), adaptation at peripheral distributions, Allee effect, among many others (see e.g. Sexton et al. 2009). This fluid nature of the range limits is common even during environmentally stable periods, and may often hamper their clear demarcation (Gaston 2003; Holt 2003), especially in the context of the realized niche (i.e. presupposing non-negative population growth rates). In this sense, discriminating between niche conservatism and niche evolution from the species distributional limits may be at least problematic, particularly if inference comes from single variables. This is expected because these inferences may take into account the sort of factors involved in range determination that prevents the species to occupy suitable regions.

We have shown, however, that a multivariate description of the species climatic niche – its position in the climatic space – may provide a reasonable characterization of both the among-species variability and the evolutionary rate of physiological tolerance. Note though that this multivariate niche position also derives from the climatic subspace defined by the species distribution, thus containing the same amount of error of both range maps and climatic data layers. Nonetheless, boundaries of the species range seem to be better described by combined rather than single climatic parameters (Kellermann et

al. 2012b; Smith 2012). Accordingly, combinations of factors (e.g. temperature and humidity) and properties of these factors (e.g. total amounts, variability, range) impose direct and indirect limits to species niche, thus outperforming single parameters in demarcating their position in this climatic hyperspace.

These findings emphasize the importance of taking into account the dimensionality of the niche when attempting to characterize it and assess patterns of niche conservatism or niche evolution. In fact, this issue of niche dimensionality is central in the Hutchinson's (1957) concept. But since then, it has been seen most as a caveat to understand the conservatism/evolution of particular niche dimensions. For instance, Peterson (2011) found dimensionality a problem to manage when defining niche for estimating niche conservatism, although his concern was the likely bias of niche overfitting. Of course, pooling in all factors as possible to define niche may be of little help to understand its evolutionary dynamics, particularly because of data collinearity. Nevertheless, we provide empirical evidence that relying on single dimension may be not only insufficient, but rather misleading. Owing to the intricate and multidimensional nature of ecological niche, taking into account dimensionality in studies of niche dynamic may improve our understanding on the variability and evolution of elementary attributes (e.g. physiological) of the species, which ultimately determine their endurance across temporally and spatially changing conditions. In addition, this approach may circumvent the problem of dealing with variables that are more subject to external constraints, such as climatic boundaries or niche breadth drawn from the species geographical distribution. However, it still remains the question on the number of niche dimensions to be included in the niche description, and it should depend on the taxon involved. If our approach proves useful, then this issue of definition of niche dimensions would be a necessary step for further investigation. Most important, perhaps, is to make available additional data on fundamental properties of physiological endurance of species, together with proper manner to handle them.

According to our results, Hof's et al. (2010) findings of niche conservatism among amphibians with a similar measure of niche position may represent a fine portrayal of the global pattern of variability and evolutionary pattern of species climatic tolerance (despite the admitted limitations in the phylogeny used in that study; see Hof et al. 2010). A direct comparison between Hof's et al. (2010) results and ours is nonetheless

impaired by the differences in both taxonomic resolution and comprehensiveness of the two studies. For instance, we found that CT_{\max} and niche position evolve faster than Brownian motion. These differences may result, for example, from the distinctions in the clades sampled and phylogenetic scale of our study relative to theirs, i.e., including nearly all amphibians.

This issue of phylogenetic scale is a critical one. Depending on the scale investigated, one can draw distinct conclusion on both the species ability to adapt to changing climates and inferences of niche conservatism/evolution (Losos 2008; Pearman et al. 2008; Peterson 2011). Our dataset covers species with varied phylogenetic distances, from deep distances such as more than 200 Myr, between Alytidae/Pelobatidae and the remaining clades, to relatively close such as the species within the *Leptodactylus* genera, separated ca. 5 Myr (Wiens 2011; Figure S3). It is likely that our results on evolutionary rates reflect average large phylogenetic distances among clades (e.g. families or genera), and for this scale, larger amounts of niche change may be the standard expectation. In this case, the term “faster” as employed here is only relative to the other traits investigated and to the evolutionary model of reference, i.e. the Brownian motion model. The term does not imply that anurans are capable to track rapid climatic changes, in shorter time scales (e.g. decades or hundreds of years).

Concluding Remarks

By combining physiological experimental data, macroecological features of species ranges, and phylogenetics data coupled with evolutionary models, we have shown that the variability and the evolutionary pattern of physiological limits, such as CT_{\max} , may be loosely described by variables that characterize environmental limits of the species distribution, such as T_{\max} or niche breadth. These findings challenge the transferability of physiological data into the geographical space, warning for the usage of environmental limits as indicator of tolerance in studies on both the effects of climatic shifts on species distributions and patterns of niche conservatism/evolution. Supporting our claim is the fact that species range limits, and hence their realized niche, are also ruled by other factors (e.g. abiotic, biotic, movement, population dynamic and intraspecific variability). Despite climate change is expected to affect species

survivorship and pattern of distribution, their range boundaries may be unsuited to assess such shifts, unless species distributions are released from stronger external constraints.

In contrast, we show that the among-species variability and the evolutionary pattern of CT_{\max} can be better described by a multivariate measure of the macroecological niche position in the climatic hyperspace. We attribute this result to the interaction of multiple environmental factors in exerting direct and indirect constraints on the species distribution and realized niche, a property that permeates the definition of niche ever since Hutchinson, i.e. the dimensionality of the niche. Further studies involving closely related species – for which fundamental attributes of the niche (e.g. physiology, interaction, and dispersal limitations) are known – are of primer importance to understand their effect on the evolutionary and spatial dynamic of the niche.

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Supplementary Material

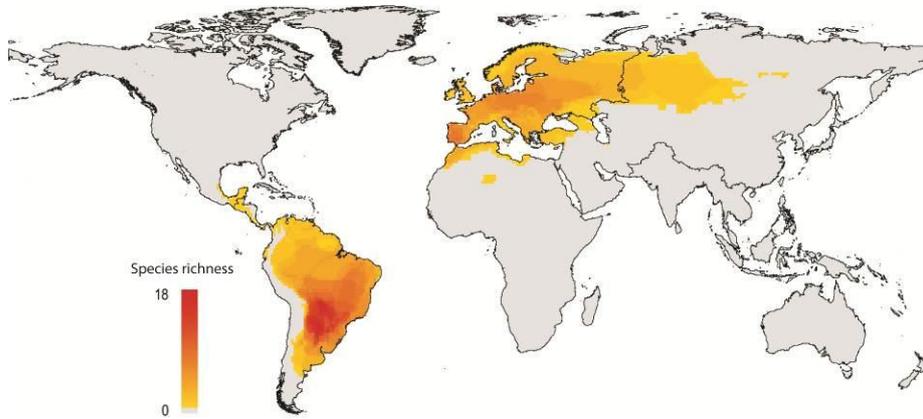


Figure S1. Spatial distribution and local richness after overlapping the extent of distribution of all 47 anurans onto a cells grid of $1^\circ \times 1^\circ$ resolution.

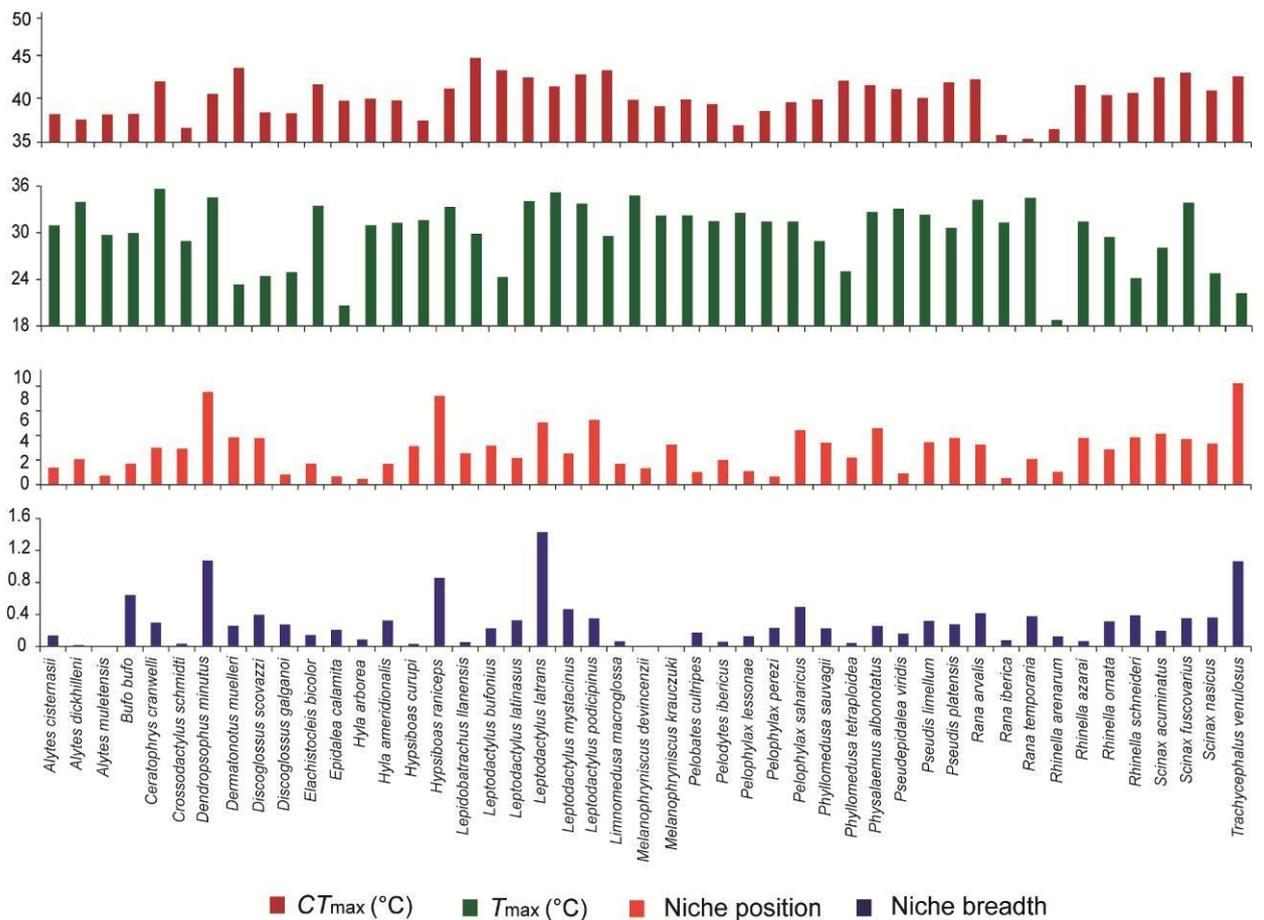


Figure S2. Non-ultrametric phylogeny for 47 anurans, after Frost (2006). Different colours denote different families.

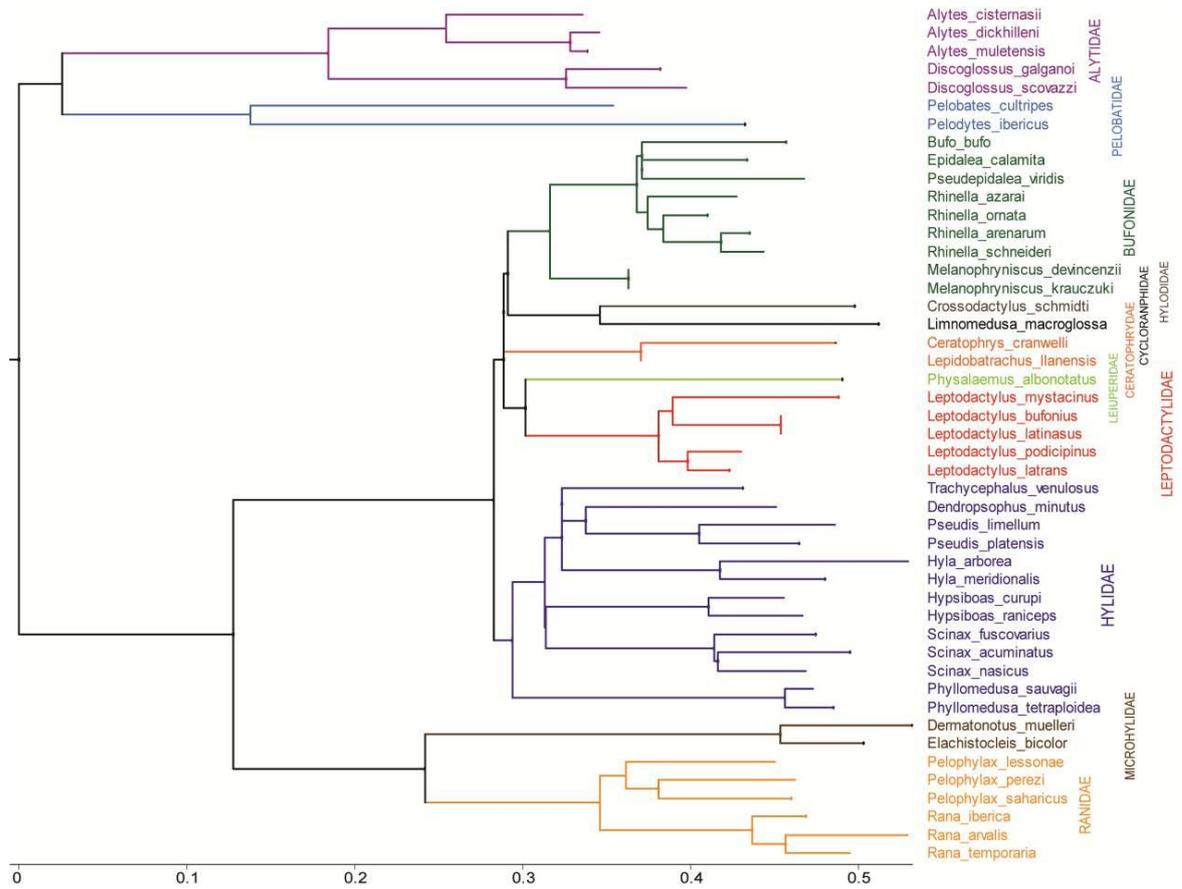


Figure S3. Interspecific variability of climatic niche traits (CT_{max} ; T_{max} ; niche position and niche breadth) among 47 anurans. Species are ordered alphabetically.

Conclusões Gerais

O estabelecimento da macroecologia como programa científico tem promovido progressos significativos no entendimento dos padrões ecológicos em macroescalas, inclusive alguns dos seus mecanismos subjacentes e determinantes históricos (Beck et al. 2012). Esses avanços resultam da busca pela uniformidade de fenômenos ecológicos recorrentes em menor escala e da formulação de inferências, baseadas nas teorias ecológicas, de como determinados padrões emergiriam em certas condições. Esse ambiente de investigação abriu a oportunidade de integrar informações sobre espécies relativamente acessíveis e que refletiam processos ecológicos que deveriam operar nessas escalas macroecológicas (Gaston & Blackburn 2000). Entretanto, muitas das questões centrais são detidas por conjecturas sobre a influência dos parâmetros que atuam em níveis mais fundamentais (biofísicos e bioquímicos) dos indivíduos, os quais geralmente estão em grande parte indisponíveis para inferências nessas escalas (Gaston et al. 2009).

Essa demanda de informações em níveis fundamentais, particularmente fisiológicos, abriu caminho para o campo da macrofisiologia, que busca entender os padrões macroecológicos de atributos fisiológicos e fornecer explicações neste nível para fenômenos em macroescala (Chown et al. 2004). Embora tentativas anteriores (e.g. a teoria metabólica da ecologia; Allen et al. 2002) parecem não ter fornecido uma resposta definitiva dessa ligação entre processos em níveis basais e padrões gerais de diversidade (Isaac et al. 2012), investigações dessa natureza são importantes para o entendimento de como processos ecológicos transcendem entre escalas e níveis organizacionais ecológicos. Enquanto uma teoria unificadora da ecologia entre escalas e níveis de organização não é definitivamente apresentada, permanece a necessidade de entender como determinados componentes interagem para gerar os padrões macroecológicos conhecidos. Esta tese buscou reunir alguns estudos específicos que podem fornecer alguns elementos sobre a integração entre fenômenos que ocorrem em níveis mais fundamentais e consequência que emergem em macroescala.

Os resultados do estudo 1, sobre a influência dos determinantes climáticos sobre gradientes de diversidade de anfíbios, mostram que os fatores afetam a distribuição de riqueza de espécies de forma heterogênea, ou seja, não-estacionária. Essa distribuição espacial dos efeitos individuais de cada fator climático permite verificar como diferentes respostas ecofisiológicas das espécies, em termos de evolução/conservação do nicho

climático, determinam os padrões de distribuição de espécies. De modo geral, os resultados sugerem que o histórico de exposição a determinadas condições climáticas, que por sua vez determina a tolerância climática das espécies em uma região específica, é responsável pela relação entre o ‘*pool*’ local de espécies e os determinantes climáticos em escala regional-continental. Por exemplo, áreas que têm sido climaticamente estáveis por longos períodos tendem a abrigar comunidades de anfíbios em estabilidade com aquelas condições (independente dos valores ambientais médios), e que respondem mais ao gradiente espacial de produtividade. Por outro lado, áreas que sofreram alterações climáticas num período geológico recente (e que, portanto, possui comunidades que sofreram remoções recentes de espécies) respondem, sobretudo, ao gradiente do fator climático associado àquelas alterações predominantes. Por exemplo, o restabelecimento de temperaturas mais elevadas após o último glacial máximo em altas latitudes resulta numa influência mais forte dessas mudanças sobre a riqueza de espécies nessas áreas. Consequentemente, os gradientes de diversidade parecem ser em grande parte definidos pela capacidade de tolerância das faunas regionais, em função do tempo de estabelecimento dos clados e estabilidade do clima nas áreas durante esse período de em estabelecimento.

Com relação ao gradiente espacial de tamanho de distribuição geográfica e de corpo ao longo da latitude e altitude, observamos que o clado estudado, a família Centrolenidae, exibe uma gradiente de tamanho corporal positivo ao longo do eixo altitudinal, mas não os demais gradientes testados. O componente adaptativo da variação do tamanho do corpo também apresentou uma relação positiva com o aumento da altitude. Mais importante, contudo, pudemos dar suporte ao possível efeito da tolerância na geração do gradiente altitudinal de tamanho do corpo, fornecendo assim uma possível primeira evidência do mecanismo subjacente ao padrão como inicialmente proposto. Além disso, fomos capazes de indicar um provável contexto histórico para o aumento do tamanho corporal dos Centrolenidae, particularmente relacionado ao soerguimento dos Andes, há aproximadamente 23 milhões de anos (Oligoceno–Mioceno). Neste estudo, portanto, utilizamos suporte de informações de distribuição das espécies, suas relações filogenéticas e elementos da história geomorfológica da sua área de distribuição que podem ilustrar como as mudanças ambientais afetam a distribuição, a diversidade e os limites de tolerância das espécies, refletindo assim na variação de tamanho corporal, como previsto pela regra de Bergmann.

Referente à questão de transferibilidade entre nicho climático em macroescala e a variabilidade de tolerância termal (CT_{\max}), os resultados mostraram que a variável macroecológica que melhor descreve a variação da tolerância fisiológica é a posição do nicho climático no espaço multivariado, apesar de CT_{\max} ser significativamente relacionado com a temperatura máxima da distribuição das espécies (T_{\max}). Esta relação entre CT_{\max} e posição de nicho se observa levando em conta as relações filogenéticas ou não. Além disso, CT_{\max} e posição do nicho apresentam taxas de evolução semelhantes. Ambas descrevem um padrão ligeiramente mais rápido que o modelo de movimento Browniano, enquanto T_{\max} descreve um padrão de evolução mais lento. Esses resultados chamam atenção para uma relativa discordância entre os limites climáticos em nível fisiológico e limites climáticos no espaço geográfico ou ambiental. Essa disparidade pode afetar inferências de mudanças nos limites de distribuição decorrentes e mudanças climáticas, bem como inferências de padrão de conservatismo/evolução de nicho a partir de variáveis que descrevem limites climáticos na distribuição da espécie. Isso acontece, provavelmente, por que esses limites de distribuição são afetados por uma série de outros fatores, como interações bióticas e restrições de dispersão de espécies, que leva à ausência de ajuste entre os limites fisiológicos e limites climáticos nas espécies.

Cada estudo apresentado limitou-se a questões relativamente específicas de cada tema abordado em macroecologia. Individualmente, eles fornecem evidências de como atributos biológicos elementares, como o histórico de exposição à variação climática e tolerância fisiológica, podem afetar diretamente certos padrões em macroescala. Em conjunto, os resultados podem oferecer uma compreensão mais abrangente e interligada de como características fundamentais da biodiversidade (e.g. riqueza e distribuição de espécies e seus atributos, evolução de nicho e climático e ocupação do espaço geográfico e ambiental) podem ser determinados por processos em níveis básicos da organização biológica. Por exemplo, os estudos 1 e 2 mostram como a duração e a magnitude de mudanças macroclimáticas afetam o padrões de adaptabilidade e diversidade dos clados ao longo de gradientes ambientais. O número de espécies e a distribuição de atributos funcionais, como o tamanho corporal, refletem as restrições que o clima exerce sobre as espécies ao longo desses gradientes, geralmente havendo um papel importante da duração de exposição que permite oportunidades de diversificação e adaptação dos clados a condições distintas. O estudo 3, por sua vez, mostra como se manifestam os limites de tolerância das espécies (que modulam, inclusive, a riqueza local e a distribuição dos

atributos das espécies) ao longo do espaço climático multidimensional. Outra conclusão pode ser obtida a partir dos estudos 2 e 3. Ambos evidenciam que o tamanho da distribuição geográfica das espécies não é definido apenas pelas tolerâncias climáticas no espaço ambiental. Outros fatores como interações bióticas e limites de dispersão podem afetar, seja mais ou menos, a distribuição de determinados grupos de espécies.

O papel do potencial adaptativo das espécies via fisiologia para a ecologia e a biologia é evidente. Entretanto, a fisiologia comparada e a ecologia desenvolveram-se em campos distintos e com pouca comunicação entre si durante boa parte do século XX, após uma origem comum (Gaston et al. 2009). Por esse motivo, tem havido um interesse crescente nos últimos anos do restabelecimento de uma conexão mais próxima entre fisiologia e ecologia. De fato, o desenvolvimento conceitual e metodológico promovido pela disseminação dos estudos macroecológicos abriu um importante caminho para entender as relações entre parâmetros fisiológicos e a distribuição de espécies e seus atributos em larga escala (Chown et al. 2004; Chown & Gaston 2008).

A integração entre ecofisiologia e macroecologia ganha ainda mais proeminência se considerarmos os potenciais efeitos das mudanças climáticas globais sobre as espécies. Isso acontece porque muitos dos efeitos (e.g. alterações nas margens de segurança termal) se manifestam principalmente em nível de distribuição ou mesmo no potencial adaptativo das espécies (i.e. evolução/conservação de nicho), sendo, portanto, mais bem entendidos em escala macroecológica (Deutsch et al. 2008; Chown & Gaston 2008; Duarte et al. 2012). Esforços recentes de integrar dados fisiológicos em inferência de distribuição potencial de espécies e possíveis consequências das mudanças climáticas têm sido cada vez mais comuns (e.g. Kearney & Porter 2009; Higgins et al. 2012). Entretanto, muitos estudos desse tipo (e.g. Gaston et al. 2009; Sexton et al. 2009; Bozinovic et al. 2011; Sunday et al. 2012), incluindo o estudo 3, demonstram que a relação entre os parâmetros fisiológicos e os padrões de distribuição e abundância das espécies ainda não é tão clara.

Algumas das questões na interface entre ecofisiologia e macroecologia são metodológicas, seja num campo ou no outro. Por exemplo, diversas fontes de variação podem afetar, de modo espécie-específico, as estimativas de limites de tolerância como o CT_{max} (Ribeiro et al. 2012), comprometendo análises comparativas. Estas, por sua vez, poderiam ser beneficiadas se os estudos ecofisiológicos focassem em espécies filogeneticamente relacionadas, promovendo uma perspectiva evolutiva dos padrões. Já

no campo da macroecologia, as técnicas de modelagem mecanísticas de distribuição estão ainda em sua infância, e em geral produzem modelos com ajustes variáveis (e.g. Higgins et al. 2012). Outras questões conceituais também deverão receber atenção num futuro próximo. Por exemplo, como interações entre fatores abióticos em macroescala interagem para determinar limites de tolerância e respostas a mudanças ambientais. Ou ainda, como espécies invasoras podem ajudar a entender mudanças nos limites de tolerância em macroescala em termos de plasticidade ou seleção intensa (Chown & Gaston 2008; Mata et al. 2011). Essas e muitas outras questões permanecem abertas em macrofisiologia. Diante dos efeitos das mudanças ambientais em curso, uma pressão cada vez maior tem sido exercida para que seus efeitos reais sejam compreendidos em todos os níveis de organização. Neste sentido, os níveis fisiológicos exercem um papel essencial na determinação dos limitantes e requerimentos das espécies, e conseqüentemente nos seus padrões de abundância, distribuição e diversidade. Portanto, direcionar atenção para essas questões de interface deve preencher uma lacuna importante dos efeitos das mudanças macroclimáticas sobre a biodiversidade, os quais têm uma posição de destaque em macroecologia.

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