



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

João Fabrício Mota Rodrigues

**ECOLOGIA GEOGRÁFICA E EVOLUÇÃO DE QUELÔNIOS**  
**CONTINENTAIS**

Orientador: José Alexandre Felizola Diniz Filho

Goiânia

Maio 2017

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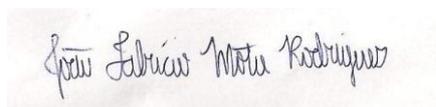
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**Orientador: José Alexandre Felizola Diniz Filho**

Tese apresentada à Universidade Federal de Goiás como parte das exigências do Programa de Pós-Graduação em Ecologia e Evolução para obtenção do título de Doutor em Ecologia e Evolução

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Aos três dias do mês de maio de 2017 (03/05/2017), às quatorze horas (14h), no Auditório do ICB V, UFG, reuniram-se os componentes da banca examinadora: **Prof. Dr. José Alexandre Felizola Diniz Filho, ICB-UFG; Profa. Dra. Levi Carina Terribile, ICB-UFG; Prof. Dr. Nelson Jorge da Silva Jr., PUC-GO; Prof. Dr. Franco Leandro de Souza, UFMS; Dr. Leo Caetano Fernandes da Silva, IBAMA-GO**; para, em sessão pública presidida pelo (a) primeiro(a) examinador(a) citado(a), procederem à avaliação da defesa de tese intitulada: **"Ecologia geográfica e evolução de quelônios continentais"**, em nível de doutorado, área de concentração em Ecologia e Evolução, de autoria de **João Fabrício Mota Rodrigues**, discente do Programa de Pós-Graduação em Ecologia e Evolução da Universidade Federal de Goiás. A sessão foi aberta pelo(a) presidente(a), que fez a apresentação formal dos membros da banca. A palavra, a seguir, foi concedida a(o) autor(a) da tese que, em cerca de 40 minutos, procedeu à apresentação de seu trabalho. Terminada a apresentação, cada membro da banca arguiu a(o) examinada(o), tendo-se adotado o sistema de diálogo sequencial. Terminada a fase de arguição, procedeu-se à avaliação da tese. Tendo-se em vista o que consta na Resolução nº 1127 de dezembro de 2012 do Conselho de Ensino, Pesquisa, Extensão e Cultura (CEPEC), que regulamenta o Programa de Pós-Graduação em Ecologia e Evolução, a tese foi aprovada, considerando-se integralmente cumprido este requisito para fins de obtenção do título de Doutor(a) em Ecologia e Evolução pela Universidade Federal de Goiás. A conclusão do curso dar-se-á quando da entrega da versão definitiva da tese na secretaria do programa, com as devidas correções sugeridas pela banca examinadora, no prazo de trinta dias a contar da data da defesa. Cumpridas as formalidades de pauta, às 17 h e 00 min.,

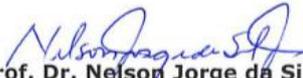
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**ECOLOGIA GEOGRÁFICA E EVOLUÇÃO DE QUELÔNIOS  
CONTINENTAIS**

Tese apresentada à Universidade Federal de Goiás como parte das exigências do Programa de Pós-Graduação em Ecologia e Evolução para obtenção do título de Doutor em Ecologia e Evolução

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**Mai 2017**

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## **RESUMO**

Compreender os processos responsáveis pelos padrões de distribuição atuais das espécies é um dos principais objetivos da Ecologia. Nesta tese, visamos entender quais fatores estão relacionados à distribuição da diversidade de quelônios, grupo de animais ectotérmicos cujos padrões de diversidade ainda são pouco conhecidos, ao longo do tempo e do espaço. Para esse fim, usamos dados de mapas de distribuição das espécies de quelônios continentais, além de informações de história de vida (tipo de hábitat e tamanho corporal), e reconstruímos uma hipótese filogenética para o grupo usando dados moleculares. O grupo dos quelônios apresentou uma explosão de diversificação durante a irradiação da família Emydidae, o que está provavelmente ligado a um evento de oportunidade ecológica. Além disso, animais aquáticos apresentaram taxas de diversificação mais elevadas que os animais terrestres, o que ajuda a explicar a maior diversidade de animais aquáticos atuais. A distribuição da riqueza de quelônios ao longo dos continentes é influenciada principalmente por variáveis climáticas tais como temperatura e precipitação, porém o intervalo de tempo desde o qual as áreas foram colonizadas também influencia nesse padrão. A diversidade beta entre as comunidades de quelônios é influenciada principalmente pela distância geográfica entre as comunidades, e comunidades de diferentes domínios biogeográficos são estruturadas de modo diferenciado. Finalmente, a diversidade de tamanhos corporais dos quelônios também é influenciada pela temperatura, de modo que animais menores são mais comuns em áreas mais frias.

**Palavras-chave:** Diversidade Beta; Diversificação; Métodos Filogenéticos

Comparativos; Oportunidade Ecológica; Quelônios; Riqueza de Espécies; Tamanho Corporal.

## **ABSTRACT**

Understanding the processes that shape the current distribution patterns of species is one of the main goals of Ecology. In this thesis, we aimed to understand which factors are related to the distribution of the turtle diversity, a group of ectothermic animals whose diversity patterns are still little known, over time and space. To that end, we used distribution data from continental chelonian species, as well as life history information (habitat type and body size), and reconstructed a phylogenetic hypothesis for the group using molecular data. Turtles had a burst of lineage diversification during the irradiation of the Emydidae family, which is probably linked to an event of ecological opportunity. In addition, aquatic animals had higher rates of diversification than terrestrial animals, which helps to explain the current greater diversity of aquatic animals. Turtle richness distribution along the continents is mainly influenced by climatic variables such as temperature and precipitation, but the time when lineages first colonized the continental regions also influences this pattern. Beta diversity among chelonian communities is mainly influenced by the geographical distance between communities, and communities from different biogeographic realms are structured by different drivers. Finally, body size diversity of turtles is also influenced by temperature, and small animals are more common in cold areas.

**Keywords:** Beta Diversity; Body Size; Diversification; Ecological Opportunity; Phylogenetic Comparative Methods; Species Richness; Turtles.

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## INTRODUÇÃO GERAL

Compreender os processos responsáveis pelos padrões de distribuição atuais das espécies é um dos principais objetivos da Ecologia. Entretanto, a compreensão desses processos requer um estudo em conjunto de processos atuando em múltiplas escalas espaciais e temporais, enfocando mecanismos de ação mais recentes, como clima atual, mas também mecanismos mais antigos ligados à história evolutiva dos organismos (Wiens & Donoghue, 2004). Desse modo, a conciliação de processos ecológicos e evolutivos permite a compreensão de padrões de diversidade atuais que não seriam compreendidos usando apenas uma das duas abordagens.

A quantidade de espécies presentes numa região é uma função primeiramente das taxas de especiação, extinção e dispersão (Ricklefs, 1987; Wiens, 2011). Assim, compreender a distribuição dos organismos requer investigar onde o balanço entre essas taxas é positivo, permitindo assim um acúmulo de espécies. Diversos estudos atuais têm usado métodos filogenéticos para estimar essas taxas (Nee *et al.*, 1994; Pyron & Burbrink, 2013; Morlon, 2014), e, com isso, a importância das variações nas taxas de diversificação sobre a diversidade de diferentes grupos de organismos tem sido avaliada e corroborada. Esses novos métodos também permitiram avaliar a influência de características dos organismos sobre suas taxas evolutivas (especiação, extinção e transição de caracteres), tais como ocorrência na região tropical (Pyron & Wiens, 2013; Pyron, 2014; Rolland *et al.*, 2014), tamanho corporal (Fitzjohn *et al.*, 2009), dieta (Tran, 2014), habitat (Bloom *et al.*, 2013), dentre outros, reforçando que traços de história de vida podem estar ligados ao processo de diversificação dos seres vivos.

Apesar da importância bem estabelecida das taxas evolutivas sobre a distribuição da diversidade, diversos estudos apontam para a importância de outros fatores que também poderiam estar relacionados aos padrões de riqueza. Características

climáticas atuais, por exemplo, apresentam forte relação com a distribuição das espécies (Hawkins *et al.*, 2003b, 2005, 2007), porém muitos autores caracterizam esse efeito como indireto, via influência do clima sobre as taxas de especiação e extinção (Wiens, 2011). O tamanho das regiões ou efeito da área também tem sido apontado como um importante fator indireto, visto que áreas maiores apresentam mais oportunidade de especiação (Fine, 2015). Finalmente, outro fator importante é o tempo, tendo em vista que áreas colonizadas mais antigamente tiveram mais tempo para que o processo de especiação acontecesse em relação a áreas recentemente ocupadas (Stephens & Wiens, 2003; Wiens, 2011). Compreender a importância relativa de cada um desses componentes é um dos principais objetivos dos estudos macroecológicos atuais.

Embora as taxas de especiação e extinção possuam grande importância na formação do *pool* regional de espécies, em escala local as características do clima e a capacidade de dispersão são importantes para entender como essas espécies encontram-se distribuídas no espaço. Comunidades locais podem ser estruturadas com base em processos ecológicos ligados a nicho (conjunto de características que permitem que um grupo de espécies sobreviva num local), assim como por processos históricos ligados à capacidade de dispersão e características da região (a composição de espécies encontrada num local depende principalmente da capacidade das espécies de superar barreiras geográficas e dispersarem) (Leibold *et al.*, 2004). Enquanto as comunidades de alguns grupos de organismos são estruturadas por processos de nicho (Kraft *et al.*, 2008; Siefert *et al.*, 2013), outras comunidades, compostas principalmente por organismos com capacidade de dispersão limitada, são estruturadas predominantemente por limitações de dispersão (Beaudrot & Marshall, 2011; Hájek *et al.*, 2011). Compreender como esses processos agem sobre os organismos das diferentes regiões biogeográficas é um importante passo para entender como as mudanças antrópicas, as quais podem

causar isolamento de populações e mudanças no clima, podem afetar a diversidade atual.

As características ou atributos das espécies também são importantes para compreender a composição das comunidades ecológicas. O tamanho corporal é uma característica muito estudada, e sabe-se que em muitos animais essa característica apresenta uma variação latitudinal, padrão conhecido como Regra de Bergmann (Ashton & Feldman, 2003; Diniz-Filho *et al.*, 2009; Olalla-Tárraga *et al.*, 2009; Olson *et al.*, 2009). Entretanto, o padrão latitudinal esperado pela Regra de Bergmann (espécies maiores nas regiões mais frias) não é tão amplamente recorrente nos ectotérmicos quanto é nos endotérmicos, sendo comum a ausência de padrão ou mesma a inversão da regra (espécies menores nas regiões mais frias) (Ashton & Feldman, 2003; Olalla-Tárraga *et al.*, 2006; Olalla-Tárraga & Rodríguez, 2007), reforçando a importância de mais estudos para compreender os processos responsáveis pela ausência do padrão.

Os quelônios correspondem a um grupo com aproximadamente 340 espécies amplamente distribuídas (Buhlmann *et al.*, 2009; van Dijk *et al.*, 2014). Apesar da grande distribuição do grupo, pouco se sabe sobre os padrões e processos ligados à distribuição atual desses animais (Iverson, 1992; Buhlmann *et al.*, 2009; Angielczyk *et al.*, 2015; Ennen *et al.*, 2016). Além dessa lacuna de conhecimento, os quelônios podem representar modelos interessantes para estudos histórico-ecológico-evolutivos dado que possuem capacidade de dispersão limitada [área de vida pequena em relação aos outros vertebrados e limitações de dispersão dentro de rios (Souza, 2005; Slavenko *et al.*, 2016)] e são animais ectotérmicos e dependentes de temperaturas ambientais para manterem seu padrão de atividade. Desse modo, esta tese usa esses animais como modelos para avaliar quatro grandes questões em ecologia e evolução, assim como para

compreender mais sobre os processos responsáveis pelos padrões de diversidade dentro do grupo.

1) No primeiro capítulo, realizamos um estudo macroevolutivo, avaliando a diversidade dos quelônios numa longa escala temporal, de modo a compreender como as taxas de diversificação variaram dentro do grupo ao longo de sua existência. Dado que o hábitat (aquático ou terrestre) é uma característica que influencia outras características dentro do grupo (Jaffe *et al.*, 2011; Slavenko *et al.*, 2016) e dado o histórico de discussões sobre as diferenças de diversidade entre o ambiente aquático e terrestre (Grosberg *et al.*, 2012; Wiens, 2015), o primeiro capítulo busca responder a seguinte pergunta: o hábitat das espécies de quelônios, mais especificamente, a transição entre o hábitat terrestre e o aquático, é capaz de influenciar as taxas de especiação? Nesse capítulo, reconstruímos uma filogenia usando dados moleculares e realizamos análises bayesianas com essa filogenia para compreender se os padrões de diversificação dentro de quelônios estavam relacionados às mudanças de hábitat entre ambiente aquático e terrestre. Capítulo publicado na revista *Molecular Phylogenetics and Evolution*.

2) No capítulo 2, o foco das nossas análises passou da escala temporal para a escala espacial, de modo a entender os fatores responsáveis pela distribuição da riqueza atual de quelônios. Tendo em vista as diferentes hipóteses existentes para explicar diferenças de riqueza entre locais (Wiens, 2011; Fine, 2015), este capítulo busca responder à pergunta: qual conjunto de hipóteses (ecológico, histórico ou evolutivo) possui maior habilidade de explicar o padrão de riqueza de espécies de quelônios? Nesse capítulo, usamos dados de mapas de distribuição das espécies de quelônios continentais do mundo todo, assim como dados de clima e filogenéticos para compreender a

importância relativa de cada uma dessas hipóteses. Capítulo publicado na revista *Ecography*.

3) No capítulo 3, o foco das análises deixa de ser a quantidade de espécies num local para ser a composição de espécies desses locais. Dado que os diferentes domínios zoogeográficos possuem diferentes histórias evolutivas e são em geral isolados geograficamente entre si (Hawkins *et al.*, 2003a; Holt *et al.*, 2013), torna-se mais interessante avaliar os processos responsáveis pela diferença de composição entre as comunidades dentro de cada domínio zoogeográfico. Desse modo, este capítulo visa responder a seguinte pergunta: as comunidades de quelônios nos diferentes domínios zoogeográficos são estruturadas por processos de nicho ou pela distância geográfica? Nesse capítulo, usamos os dados de distribuição das espécies para avaliar a relação entre a diversidade beta de quelônios nos diferentes domínios zoogeográficos e as distâncias ambientais e geográficas. Capítulo aceito para publicação na revista *Journal of Biogeography*.

4) No capítulo 4, o foco das análises passa a ser o tamanho corporal das espécies e a sua variação ao longo do espaço geográfico. Dado que os padrões de variação geográfica no tamanho corporal em ectotérmicos não formam uma regra geral tal como observado nos endotérmicos (Ashton & Feldman, 2003; Olalla-Tárraga *et al.*, 2006; Pincheira-Donoso *et al.*, 2007; Meiri, 2011), este capítulo objetiva compreender o padrão de variação geográfica no tamanho corporal dos quelônios e quais fatores determinam essa variação. Neste capítulo, coletamos dados de tamanho corporal e distribuição para as espécies atuais e utilizamos análises incorporando componentes espaciais e filogenéticos para compreender a variação do tamanho corporal e seus determinantes nos quelônios.

Finalmente, além desses quatro capítulos buscando entender aspectos mais teóricos ligados a fatores possivelmente relacionados à diversidade do grupo dos quelônios, também foram desenvolvidos dois estudos com enfoque mais prático ou de conservação, avaliando a mudança de nicho durante o processo de invasão de duas espécies de quelônios de água doce - *Trachemys scripta* e *Trachemys dorbigni* (Rodrigues *et al.*, 2016a,b). No estudo envolvendo *T. scripta*, avaliamos se o processo de mudança de nicho durante a invasão influenciava a qualidade dos modelos de distribuição correlativos gerados para a espécie na área invadida. No estudo de *T. dorbigni*, avaliamos se as condições ambientais invadidas por uma espécie pode ser mais bem explicada pela análise em conjunto das condições ambientais de suas diferentes subespécies. Esses dois manuscritos podem ser encontrados nos anexos 1 e 2 desta tese.

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## Capítulo 1

Rodrigues, J.F.M., Diniz-Filho, J.A.F. (2016): Ecological opportunities, habitat, and past climatic fluctuations influenced the diversification of modern turtles. *Molecular Phylogenetics and Evolution* **101**: 352–358.

**Ecological opportunities, habitat, and past climatic  
fluctuations influenced the diversification of modern turtles**

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## **Abstract**

Habitat may be viewed as an important life history component potentially related to diversification patterns. However, differences in diversification rates between aquatic and terrestrial realms are still poorly explored. Testudines is a group distributed worldwide that lives in aquatic and terrestrial environments, but until now no-one has evaluated the diversification history of the group as a whole. We aim here to investigate the diversification history of turtles and to test if habitat influenced speciation rate in these animals. We reconstructed the phylogeny of the modern species of chelonians and estimated node divergence dates using molecular markers and a Bayesian approach. Then, we used Bayesian Analyses of Macroevolutionary Mixtures to evaluate the diversification history of turtles and evaluate the effect of habitat on this pattern. Our reconstructed phylogeny covered 300 species (87% of the total diversity of the group). We found that the emydid subfamily Deirochelyinae, which forms the turtle hotspot in south-eastern United States, had an increase in its speciation rate, and that Galapagos tortoises had similar increases. Current speciation rates are lower in terrestrial turtles, contradicting studies supporting the idea terrestrial animals diversify more than aquatic species. Our results suggest that habitat, ecological opportunities, island invasions, and climatic factors are important drivers of diversification in modern turtles and reinforce the importance of habitat as a diversification driver.

**Key-words:** Biodiversity hotspots, Deirochelyinae, freshwater turtles, macroevolution, speciation, tortoises

## **1. Introduction**

Nowadays, terrestrial environments have a higher number of species than aquatic environments, although the latter has greater area and volume, contradicting species-area relationship expectations (Grosberg et al., 2012; May, 1994). Differences in diversity between aquatic and terrestrial realms have been studied for a long time, and many hypotheses have been suggested to explain this pattern, including effects of productivity, complexity, differences in environmental biophysical properties, and biological interactions, among others (Benton, 2001; Grosberg et al., 2012; May, 1994; Vermeij and Dudley, 2000). Environments without competitive species, for example, may represent ecological opportunities for enhanced diversification, which may appear in phylogenetic analyses as an early burst in diversification (Betancur-R et al., 2012; Losos, 2010).

In recent years, many methodological advances have allowed the use of molecular phylogenies to better understand macroevolutionary patterns and processes (Morlon, 2014; Nee et al., 1994; Pybus and Harvey, 2000; Pyron and Burbrink, 2013; Rabosky, 2014). These analyses have been used to detect slowdowns and shifts in diversification rates along the evolutionary history of clades, to estimate speciation and extinction rates, to evaluate the influence of traits on these rates, and to understand the current diversity of many groups of organism. Some studies, for example, have used these phylogenetic methods to evaluate the influence of habitat on diversification rates (Betancur-R et al., 2012; Bloom et al., 2013; Carrete Vega and Wiens, 2012; Santini et al., 2013; Wiens, 2015a). These studies showed that freshwater systems have a higher speciation rate than marine systems (Bloom et al., 2013), whereas others found higher speciation in a marine system is also possible (Santini et al., 2013). However, only two recent papers evaluated the differences in diversification rates between aquatic and

terrestrial clades, revealing that terrestrial groups may have a higher diversification rate (Wiens, 2015a, 2015b).

Turtles are a tetrapod group distributed worldwide comprised of approximately 340 species (Uetz and Hosek, 2015). However, until now, the lack of densely sampled and dated molecular phylogeny for the group precluded the use of phylogenetic methods to understand the macroevolutionary processes related to their current diversity and distribution (but see Thomson and Shaffer (2010) for a large phylogeny covering almost two thirds of current species). Besides, turtles are an interesting model group to study the question related to the difference in terrestrial and aquatic realm diversities, because they are found in both realms (Bonin et al., 2006). Current turtles have a common aquatic ancestor (Jaffe et al., 2011; Joyce and Gauthier, 2004) and terrestrial lineages of these animals are densely concentrated in the family Testudinidae, a monophyletic clade including only terrestrial species (Bonin et al., 2006). This pattern may raise questions regarding whether this permanent change in habitat has led to an increase in speciation rate in this terrestrial clade, characterizing an ecological opportunity. Therefore, the study of diversification patterns in turtles may allow a better understanding of the macroevolutionary processes that generated the current diversity of these animals and how this diversity is affected by ecological opportunities and habitat shifts, helping to improve our understanding about questions regarding the differences in diversity between aquatic and terrestrial habitats.

We aimed to reconstruct phylogenetic relationships and divergence dates among living turtles using molecular data and use them to evaluate diversification patterns in the group. More specifically, we aimed to test whether habitat influences speciation rates in chelonians and whether the arrival in the terrestrial realm of the Testudinidae clade corresponded to a burst in speciation. To the best of our knowledge, this is the

first study to use molecular phylogenies to understand macroevolutionary patterns and processes related to the diversity of turtles.

## **2. Materials and methods**

### *2.1. Phylogeny and Ancestral State Reconstruction*

We reconstructed the relationships among living turtles (the term “turtles” is used in this text herein as a general designation covering all chelonians) using molecular data from five different molecular markers (See Tables S1 and S2 in Supplementary Information). The topology and divergence times of the phylogeny were estimated using BEAST v1.8 using a lognormal relaxed clock (Drummond and Rambaut, 2007) and fossils (Joyce et al., 2013) were used to calibrate the branch lengths (see Supplementary Methods in Supplementary Information for a complete description of the methods used to reconstruct the phylogeny).

To explore habitat shifts during the evolutionary history of turtles, we used stochastic character mapping to reconstruct ancestral states of habitat for the turtles present in our phylogeny (Bollback, 2006). Ancestral states were reconstructed using the MCC phylogeny and 100 simulations. Turtles were classified as terrestrial or aquatic based on the amount of their life time that they spend in each habitat, following Jaffe et al. (2011). For example, semiaquatic species spending more life time on land than in water were classified as terrestrial (see Table S3 for a list containing the species and their habitats). Stochastic character mapping analysis was performed in the R package Phytools (Revell, 2012).

### *2.2. Diversification analyses*

First, we evaluated the diversification patterns in turtles using Bayesian Analyses of Macroevolutionary Mixtures (BAMM), which uses Reversible Jump Markov Chain Monte Carlo (rjMCMC) searches for detecting the number and position of events of change in diversification rate in the whole group (Rabosky et al., 2014a). Since our phylogeny has missing species, we used the BAMM feature that takes non-random missing species into account in the analyses. We used the traditional turtle families and their richness according to Uetz & Hosek (Uetz and Hosek, 2015) to account for non-random sampling by taking into account the number of sampled species in each family in the analysis (Rabosky et al., 2015). We ran four chains for 2,000,000 generations, sampling from the chains every 1000 generations. Then, we used the R package BAMMtools (Rabosky et al., 2014b) to remove a 10% burning-in and to evaluate the BAMM results. We also inspected the convergence in the distribution of number of rate shifts in the MCMC results of BAMM evaluating the effective sample size (ESS) using the package coda (Plummer et al., 2006). All these analyses were performed in our Maximum Clade Credibility (MCC) tree.

To evaluate the existence of an increase in speciation rate due to the change of an aquatic to a terrestrial habitat that occurred in the Testudinidae clade, we identified the node corresponding to this family, which covers almost all terrestrial turtles, and observed whether this clade had a speciation rate higher than the other turtles using BAMMtools or whether it was identified as a rate shift event with high posterior probability. We focused all our BAMM analyses in speciation rates because of the problems related to estimating extinction rates (Rabosky, 2010; Rabosky et al., 2015). We used phylorate plots and cohort matrices to represent the tempo of diversification along the phylogeny and lineages that commonly share the same evolutionary regime (Rabosky et al., 2014a).

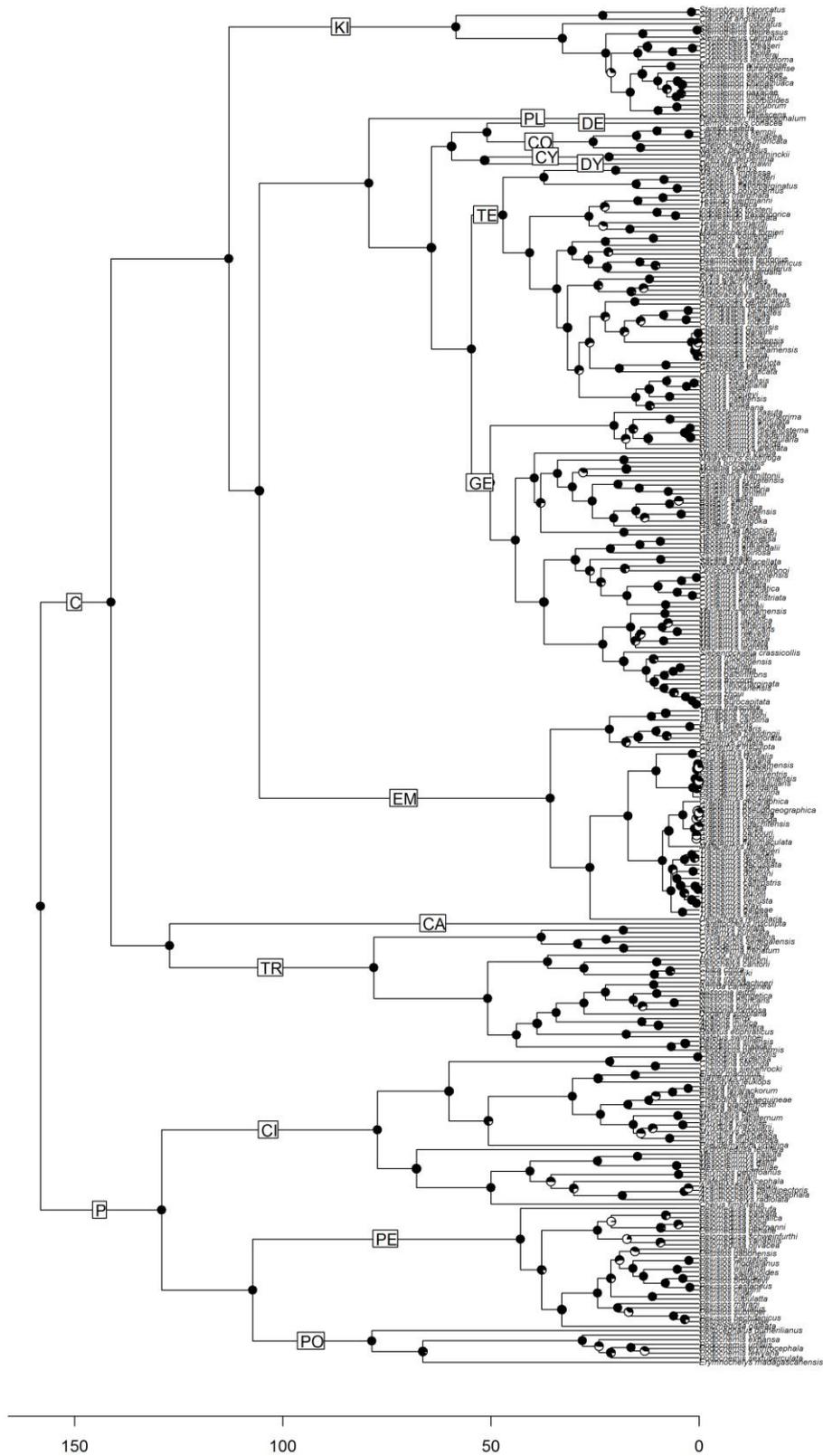
To further evaluate if habitats influence the current speciation of turtle species, we obtained speciation rates for each species from BAMM analyses (Rabosky et al., 2015), and compared whether these rates were different between aquatic and terrestrial turtles using a Wilcox sign test. The BAMM approach estimates a posterior distribution of speciation rates that are able to change through time along the branch lengths of the phylogeny; then, in order to obtain a single speciation rate for each species, we averaged the values of the posterior distribution for each species at the present, allowing the accommodation of different diversification histories in the group (e.g. some species are always found in high speciation regimes, while others are found in such regimes fewer times) (Rabosky et al., 2015, 2014a). This analysis allowed us to evaluate all terrestrial species, including those outside the family Testudinidae, such as some *Terrapene*, *Geoemyda*, and *Heosemys* species. Habitat data were the same used for the ancestral state reconstructions described early (see Table S3). We followed this approach because analyses that use traits to estimate speciation and extinction rates (e.g. Maddison et al., 2007; Goldberg et al., 2011) may have a high type I error, mainly when there are multiple diversification regimes in a phylogeny (Rabosky and Goldberg, 2015) (which was found in our phylogeny – see Results). However, in order to reinforce our results, we also evaluated if speciation rate was related to habitat using Binary-State Speciation and Extinction Models (BiSSE) (Maddison et al., 2007), and the results were qualitatively similar to those found using BAMM (see Supplementary Methods and Supplementary Results for a description of these analyses using BiSSE).

### **3. Results**

#### *3.1. Phylogeny and Ancestral State Reconstruction*

Our reconstructed phylogeny included 87% of all the modern turtles and had high posterior probability (> 95%) for the majority of its nodes (Fig. 1). All the parameters established as priors had an effective sample size (ESS) higher than 200. The median node height and the highest probability density (HPD) interval of Testudines (whole group) was 158.17 Mya (Million years ago) (152.24–169.60), Cryptodira 141.23 Mya (132.11–153.18), Pleurodira 129.06 Mya (112.38–149.23), and of Testudinidae, 47.13 Mya (40.21–54.05). Testudinidae had posterior probability of 1 and was monophyletic.

The ancestral state reconstruction of habitat in turtles was very similar to reconstructions performed in previous studies (Jaffe et al., 2011; Joyce and Gauthier, 2004). We also found that the common ancestor of living chelonians was aquatic and that a terrestrial habitat appeared in an ancestor of the family Testudinidae and in few events along the history of the group (Fig. S1).

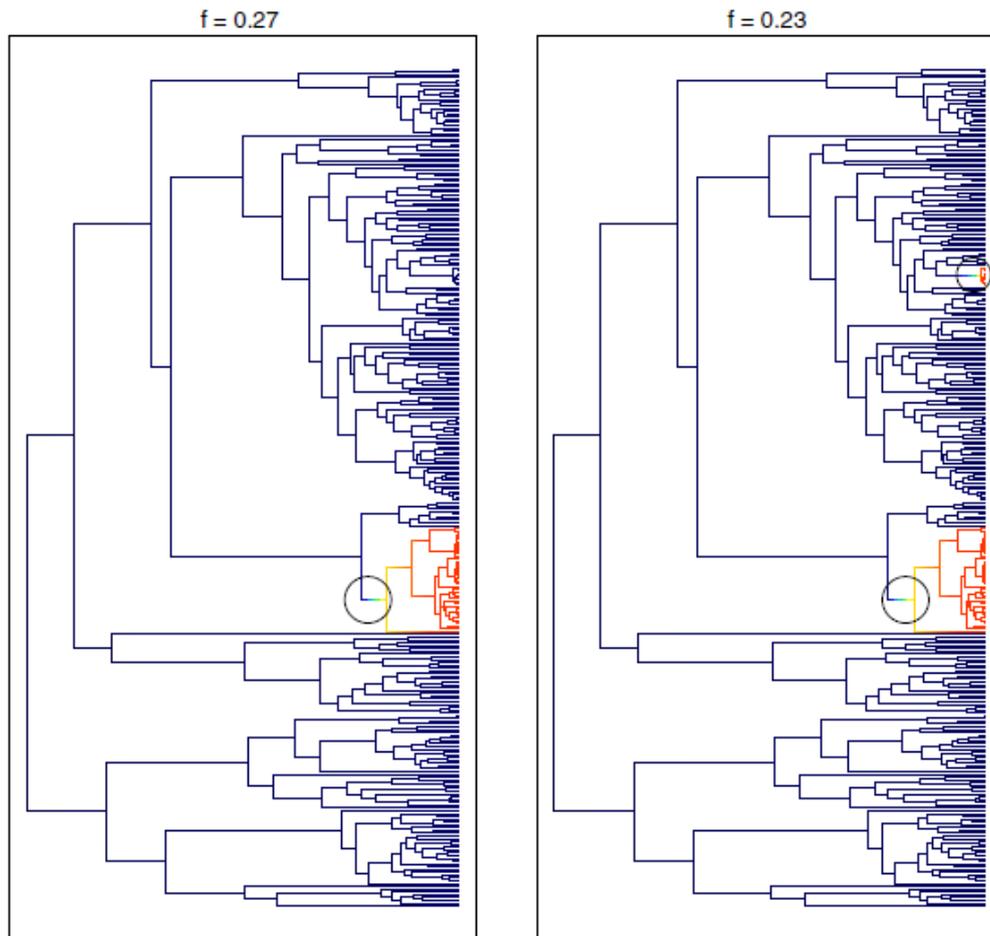


**Fig. 1.** Maximum Clade Credibility (MCC) tree containing the 300 species of turtles and tortoises. Pie charts represent the posterior probability of each node, and filled pies

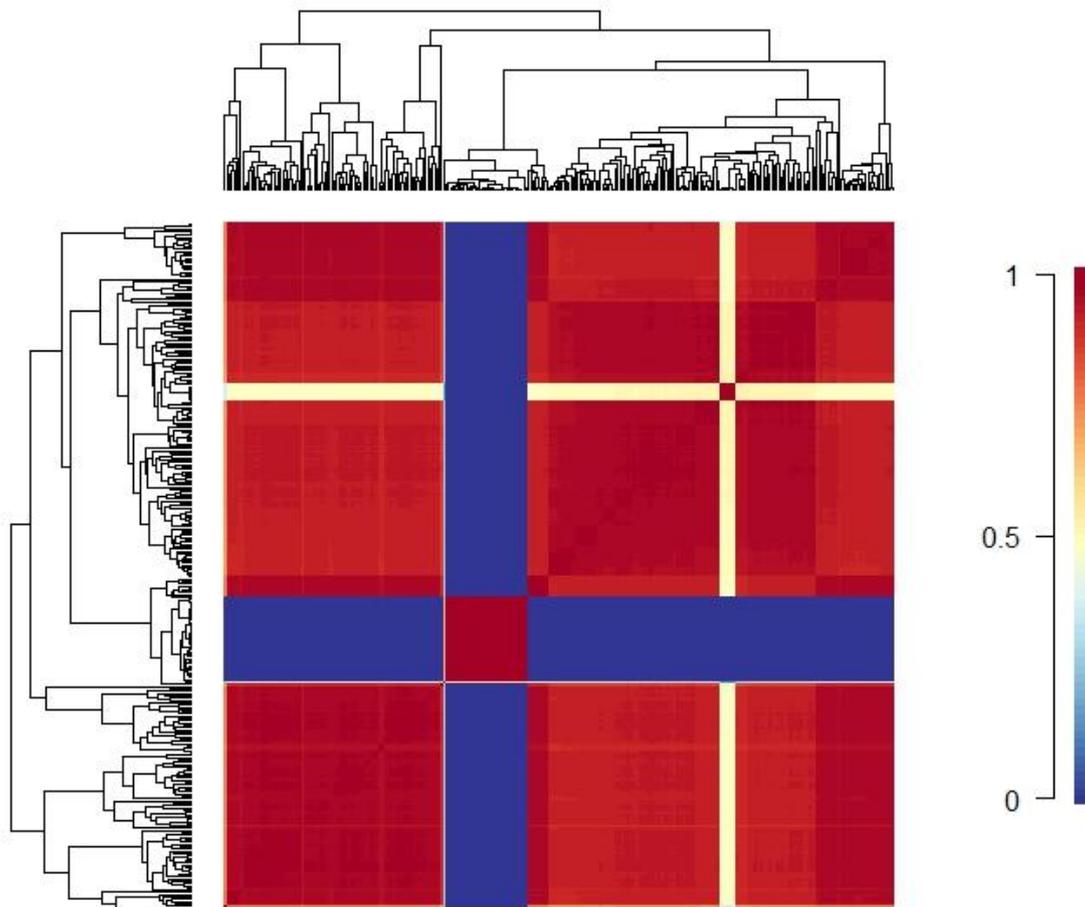
are nodes with posterior probability = 1. C = Cryptodira, P = Pleurodira, KI = Kinosternidae, PL = Platysternidae, DE = Dermochelyidae, CO = Cheloniidae, CY = Chelydridae, DY = Dermatemydidae, TE = Testudinidae, GE = Geoemydidae, EM = Emydidae, CA = Carettochelyidae, TR = Trionychidae, CI = Chelidae, PE = Pelomedusidae, PO = Podocnemididae.

### 3.2. Diversification analyses

The Bayesian analyses using BAMM suggest the occurrence of multiple shifts in the speciation rates along the phylogeny (Fig. 2) and that some groups are hardly found in the same diversification regime (Fig. 3). We found high ESS values (> 500) for the number of rate shifts in our BAMM analysis. In the reconstructed phylogeny, the emydid subfamily Deirochelyinae (*Chrysemys* + *Deirochelys* + *Graptemys* + *Malaclemmys* + *Pseudemys* + *Trachemys*) had a high increase in speciation rate, which was found in both more frequent shift configurations (Fig. 2). The Galapagos species of tortoises also represented an important rate shift, but it was found in only one of the two most probable scenarios (Fig. 2). These groups are also hardly found in the same regime (Fig. 3), emphasizing that they may be two independent events of an increase in diversification.



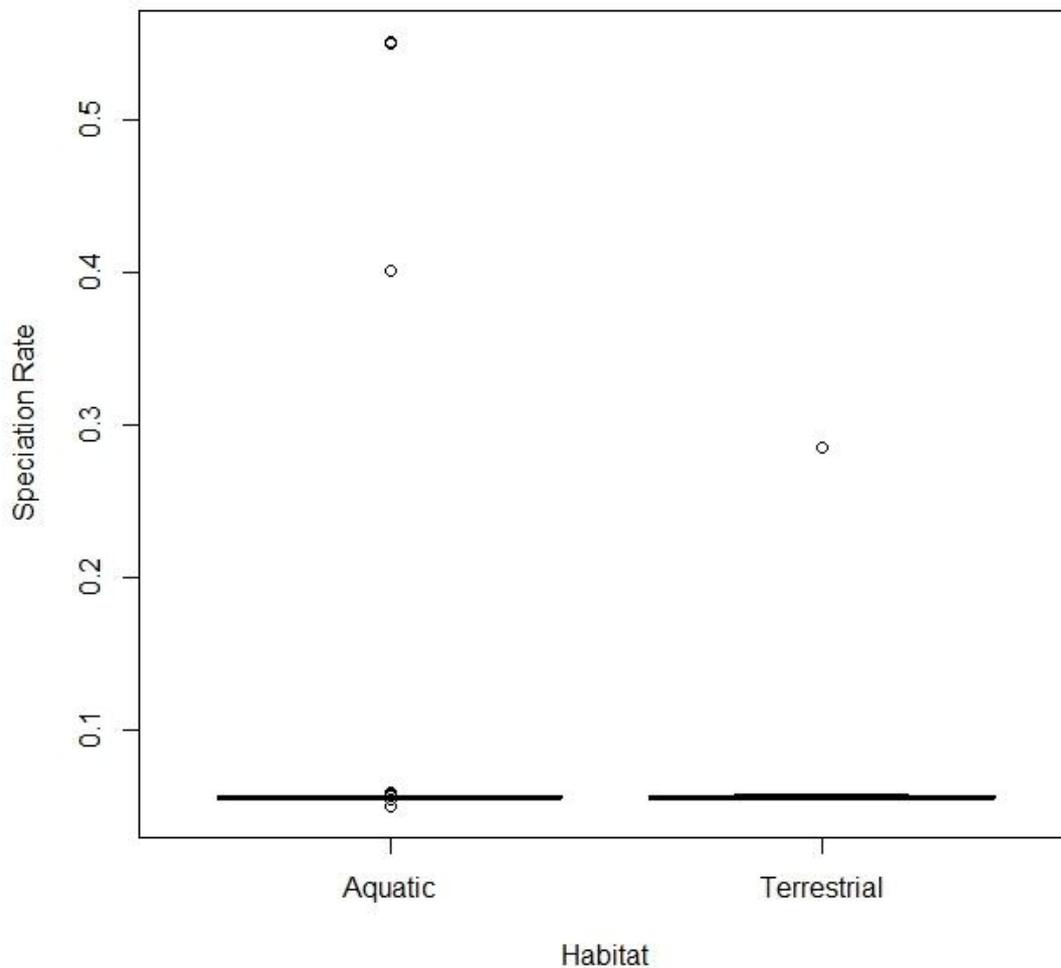
**Fig. 2.** The two most frequent rate shift configurations in the Testudines phylogeny reconstructed in our study. Warm colours represent an increase in speciation rate when compared to the ancestral lineage, while the cold ones are reductions. The circles represent the shifts in speciation, and their size is proportional to the marginal probability of the change in the specific branch.  $f$  = frequency or the posterior probability of the rate shifts. The lower circle is the emydid subfamily Deirochelyinae (*Chrysemys* + *Deirochelys* + *Graptemys* + *Malaclemmys* + *Pseudemys* + *Trachemys*), and the upper one represents the Galapagos tortoises. See Figure S2 in the Supplementary Material to see other rate shift scenarios.



**Fig. 3:** Macroevolutionary cohort matrix for speciation in Testudines. Warm colours represent pairs of species that commonly share a same macroevolutionary rate regime. Observe that groups with range shifts observed in Figure 2 do not share macroevolutionary rate shifts with the other species (Deirochelyinae and Galapagos tortoises).

There was no difference in speciation between the family Testudinidae and the rest of the turtles (Testudinidae (mean speciation rate and its 95% quantile) = 0.059 and 0.050–0.071, the rest of the turtles = 0.069 and 0.058–0.081), which contradicts our

expectation that speciation would have increased in this clade due to the transition to terrestrial habitats. Finally, we found that speciation rate was higher in aquatic species than in terrestrial species ( $W = 10318$ ,  $P < 0.001$ ; Fig. 4). We reran this analysis after removing the taxa with disproportionately high speciation rates such as the subfamily Deirochelyinae (aquatic species) and the Galapagos tortoises (terrestrial species), but the result was qualitatively the same (higher speciation in aquatic species:  $W = 7734$ ,  $P < 0.001$ , Fig. S3). Analyses using BiSSE also found that aquatic species had slightly higher speciation rates (see Supporting Results, Fig. S4).



**Fig. 4.** Speciation rate for aquatic and terrestrial turtles. The extreme outliers for the aquatic group are species from the subfamily Deirochelyinae (*Chrysemys* + *Deirochelys* + *Graptemys* + *Malaclemmys* + *Pseudemys* + *Trachemys*) and the Galapagos species are the ones for the terrestrial group.

#### **4. Discussion**

We found that habitat is an important speciation driver and terrestrial turtle species have a lower current speciation rate than aquatic ones, reinforcing the influence of habitat on diversification processes found in other studies. We did not find support for a higher speciation rate in the Testudinidae clade in comparison to the rest of the group, contradicting our hypothesis that reaching the terrestrial realm could have represented an ecological opportunity among turtles. However, evolutionary radiations non-related to habitat seem to have occurred in the subfamily Deirochelyinae, which forms one of the current hotspots of turtle diversity, as well as in the Galapagos tortoises.

Habitat shifts may influence the speciation/diversification rates in many animal species (Bloom et al., 2013; Hollingsworth et al., 2013; Santini et al., 2013; Wiens, 2015a, 2015b), but until now only two studies have evaluated the effect of changes between aquatic and terrestrial systems on these rates (Wiens, 2015a, 2015b). In our study, we found that aquatic species had higher speciation rates than terrestrial species. This pattern may be driven by higher probability of allopatric speciation among freshwater specialist species (Grosberg et al., 2012), which has already been used to explain the high diversity of map turtles in south-eastern USA (Mittermeier et al., 2015). Wiens (2015b) already highlighted the high diversity in freshwater systems considering their very small area on Earth and the need for explanations for this

diversity. Considering that 97% of the aquatic turtle species sampled in our phylogeny are freshwater species, the high speciation rate found in our study is in accordance with this high diversity found in freshwater systems.

Our results suggest that turtles seem to not follow the general pattern found for vertebrates and for the other animals, where diversification rates are higher in the terrestrial realm (Wiens, 2015a, 2015b). It is important to highlight that our study focused on speciation rates and not on diversification rates, and higher extinction rates in aquatic turtles could reduce the diversification rate in this group. However, it is well-known that estimating extinction rates based on phylogenetic methods is very problematic (Davis et al., 2013; Rabosky, 2010; Rabosky et al., 2015), and only new methods will help us to improve these findings.

Although Testudinidae overall had lower rates of speciation than most lineages of the other turtles, the Galapagos tortoises showed an increase in speciation rate in one of the two most frequent rate shift scenarios (Fig. 2). The increase in diversification after reaching new habitats, such as islands, is common in many animal groups (Harmon et al., 2003; Losos, 2010), including recently extinct tortoises (Austin and Arnold, 2001), suggesting the importance of ecological opportunities in the diversification of these terrestrial animals. Such diversification in island tortoises is not limited to species richness, but also occurs in morphological variation, since island turtles are generally larger than mainland turtles (Itescu et al., 2014; Jaffe et al., 2011).

Previous studies suggest that time-for-speciation and niche conservatism are important factors influencing the diversity and distribution of Deirochelyinae (Stephens and Wiens, 2009, 2003a). However, no study so far has evaluated speciation rates in the group. The rate shift in the speciation rate of the group Deirochelyinae (*Chrysemys* + *Deirochelys* + *Graptemys* + *Malaclemmys* + *Pseudemys* + *Trachemys*) is an interesting

topic to discuss, because these animals form the second richest turtle hotspot on Earth (Mittermeier et al., 2015). This high speciation rate may be related to past environmental conditions. The divergence age of Deirochelyinae, which has a posterior probability of 1 (see Fig. 1 and 2), was estimated as 26.24 Mya (HPD 95% = 18.80–33.42) which is coincident with a late Oligocene warming and increase in wet conditions (Zachos et al., 2001). Fossils of aquatic turtles are highly absent in the Early Oligocene of North America, probably due to the severe dry conditions found in this region (Corsini et al., 2011; Hutchison, 1982), which could have allowed Deirochelyinae turtles to diversify in an environment with low competition. Deirochelyines are aquatic turtles with aquatic ancestors and present a broad range of diet strategies (herbivores, omnivores, and carnivores) (Stephens and Wiens, 2004, 2003b), such high functional variation reinforces the hypothesis of ecological opportunity and adaptive radiation. Besides, many species of this group belonging to the genera *Trachemys*, *Graptemys*, and *Pseudemys* had divergence ages estimated earlier than 2 Mya, highlighting the possible influence of the recent Quaternary Glaciations on the diversification of the group too. *Graptemys* species, for example, are aquatic specialists whose speciation dynamics seemed to be highly influenced by changes in watershed courses during the Pleistocene (Mittermeier et al., 2015). Indeed, some authors suggest that speciation rates should be high in freshwater systems due to dispersal limitation promoting isolation and diversification (Grosberg et al., 2012), which could be amplified in the changes in watersheds in the Pleistocene. Such recent speciation events are reinforced by low range overlap among sister species of the family Emydidae, which include the subfamily Deirochelyinae (Stephens and Wiens, 2003a).

Despite the interesting results regarding the Deirochelyinae subfamily and Galapagos tortoises, we would like to report that these results may be driven by

taxonomic bias, causing an oversplit in these groups. There is much taxonomic controversy regarding the division of species in the genus *Pseudemys* (Spinks et al., 2013; Wiens et al., 2010) and Galapagos tortoises are also considered a species complex (van Dijk et al., 2014). In order to account for such possible bias, mainly among Deirochelyinae, we reran the BAMM analyses after leaving only a single Galapagos species and only three species of *Pseudemys* (*P. alabamensis*, *P. gorzugi*, *P. peninsularis*), whose differences seem to be more strongly supported (Spinks et al., 2013). However, even after excluding species, the increase in speciation rate in the Deirochelyinae subfamily remained (see Fig. S5). Unfortunately, since Galapagos tortoises were reduced to a single species, the speciation burst of the group obviously disappeared, but considering that current taxonomic revision of turtles and tortoises by several specialists (van Dijk et al., 2014) consider them as different species, we are confident of their speciation burst. Finally, despite possible taxonomic bias problems, our sensitivity analysis supports our initial results.

We conclude that habitat influences speciation rates in turtles and that current aquatic species have higher speciation rates than terrestrial species. Other types of ecological opportunity and processes may have also influenced the diversity of these animals, such as island invasions and variation in climatic conditions. Finally, our analyses suggest that the turtle hotspot found in south-eastern North America was driven by high speciation rates in the subfamily Deirochelyinae. As far as we know, this is the first study to provide macroevolutionary evidence supporting the existence of this turtle hotspot.

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# **Ecological opportunities, habitat, and past climatic fluctuations influenced the diversification of modern turtles**

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## **Supplementary information**

### **Appendix A – Supplementary methods and supplementary results**

#### **A1 – Supplementary Methods.**

**Table S1:** Nucleotide substitution models used for each marker

**Table S2** – GenBank accession numbers of the sequences used in this study for each species.

**Table S3** – Habitat data and references for the turtles sampled in the phylogeny.

#### **A2 – Supplementary Results.**

**Fig S1:** Ancestral state reconstruction of habitat using stochastic character mapping along the maximum clade credibility phylogeny of turtles.

**Fig S2:** The four more frequent rate shift configurations in the Testudines phylogeny reconstructed in our study.

**Fig S3:** Speciation rate for aquatic and terrestrial turtles after removing the extreme outliers of the aquatic group and terrestrial group.

**Fig S4:** Posterior distribution of speciation rate of aquatic and terrestrial species of turtles obtained from BiSSE model.

**Fig S5:** Diversification regimes in turtles using BAMM after leaving only a single species of Galapagos tortoises and three species of *Pseudemys* strongly supported.

## A1 - Supplementary methods

### *Phylogenetic reconstruction*

We collected molecular data of five different molecular markers, being three mitochondrial (Cytochrome b - CytB, 12S rRNA, and NADH dehydrogenase subunit 4 - ND4) and two nuclear (Recombination Activating Gene - Rag 1 and Rag 2) of 300 species of turtles from GenBank (see Table S2 in Supporting Information for a complete list of markers collected for each species and their reference numbers). These markers are commonly used in phylogenetic studies of turtles and are good descriptors for estimating relationships among different hierarchical levels in the group (Iverson et al., 2013; Jaffe et al., 2011; Vargas-Ramírez et al., 2008). The sequences for each marker were aligned using the algorithm ClustalW in BioEdit 7.2.5 (Hall, 1999) and later edited by eye. We used JModelTest v2.1.3 to evaluate which nucleotide evolutionary model was most fitted to each data set, using AICc to compare the models (Darriba et al., 2012). The substitution models used for each marker are listed in Table S1. Finally, we concatenated all the sequences of the five markers in a single dataset which had 5786 bp (CytB = 1129bp, 12S = 411bp, ND4 = 769bp, rag1 = 2818bp, and rag2 = 659bp).

**Table S1** – Nucleotide substitution models used for each marker. The models were selected based on AICc in the software JModelTest v2.1.3 (Darriba et al., 2012)

| <b>Marker</b> | <b>Substitution model</b> |
|---------------|---------------------------|
| CytB          | TVM + I + G               |
| 12S           | TIM2ef + G                |
| ND4           | GTR + I + G               |

|      |             |
|------|-------------|
| Rag1 | GTR + I + G |
| Rag2 | HKY + G     |

Then, we used BEAST v1.8 and a lognormal uncorrelated molecular clock to reconstruct the phylogeny and estimate the divergence times of the clades (Drummond and Rambaut, 2007). In order to provide reliable calibrations points to our phylogeny and provide a good integration with the information available in the fossil record, we followed calibration data provided by Joyce et al. (2013) who carefully evaluated many fossil evidences for different groups of turtles following protocols to avoid the inclusion of problematic fossils with uncertain data. We used a lognormal distribution using the minimal age as the offset of the distribution and adjusting mean and standard deviation to ensure that the maximum age was the 97.5% of the distribution because the maximum ages could not be determined as accurately as minimum ages. We used all the minimum and maximum calibration dates provided by Joyce et al. (2013), except the node ages related to divergence of species and the ages of the group Americhelydia, covering a total of 18 fossil calibration points. We also defined Testudines, Cryptodira and Pleurodira clades as monophyletic because of the great amount of paleontological evidences supporting these hypotheses (Joyce et al., 2013). In BEAST, we used a unique uncorrelated lognormal molecular clock for all the sites, Yule tree priors, linked tree topologies and unlinked substitution models among the sites (each site has its own substitution model. See Table S1). We ran 100,000,000 Markov Chain Monte Carlo (MCMC) generations, sampling from the chain every 10,000 generations. We inspected if the chain has reached stationarity after discarding a burning of 10% in Tracer v1.6 (Drummond and Rambaut, 2007). We ran three chains of 100,000,000 and other of 96,000,000 generations. In the latter chain, we removed 30,000,000 generations as a

burning (it was also sampled every 10,000 generations). All the chains converged to the same values, and they were combined after removing their burning in LogCombiner v1.8.2 (Drummond and Rambaut, 2007). We checked the Effective Sample Sizes (ESS) of the combined file in Tracer. We also combined the tree files using the same procedure explained before. LogCombiner was also used to obtain the Maximum Clade Credibility (MCC) tree.

### *Binary-State Speciation and Extinction analyses*

We used Binary-State Speciation and Extinction (BiSSE) models to further evaluate the influence of habitat on speciation rates. We used the habitat classification provided in Table S3 and Markov Chain Monte Carlo (MCMC) sampling with exponential priors to explore the speciation rate parameter (FitzJohn, 2012). We developed a BiSSE model in our Maximum Clade Credibility (MCC) phylogeny allowing all the parameters to vary and implemented MCMC searches with 10000 iterations to investigate whether speciation rate was different between aquatic and terrestrial species.

BiSSE models and MCMC searches were implemented in the R package Diversetree (FitzJohn, 2012), and we used the sampling fraction argument for account for missing species (88% for both terrestrial and aquatic species). Some authors suggest that BiSSE analyses may lack power when phylogenies have less than 300 tips and when there is a high imbalance among characters in the tips (a character with frequency lower than 10%) (Davis et al., 2013), but these problems were not found in our data (number of species = 300; approximately 75% aquatic and 25% terrestrial species).

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**Table S2.** GenBank accession numbers of the sequences used in this study for each species. The symbol “\_” indicates that we found no sequence for that molecular marker for the species.

| <b>Species</b>                       | <b>CytB</b> | <b>12S</b>  | <b>ND4</b>  | <b>Rag1</b> | <b>Rag2</b> |
|--------------------------------------|-------------|-------------|-------------|-------------|-------------|
| <i>Acanthochelys macrocephala</i>    | EF535282.1  | _           | EF535294.1  | _           | _           |
| <i>Acanthochelys pallidipectoris</i> | EF535283.1  | U40392.1    | EF535295.1  | _           | _           |
| <i>Acanthochelys radiolata</i>       | EF535289.1  | _           | EF535302.1  | _           | _           |
| <i>Acanthochelys spixii</i>          | EF535288.1  | _           | EF535300.1  | _           | _           |
| <i>Actinemys marmorata</i>           | EU787063.1  | U81321.1    | AY905210.1  | _           | _           |
| <i>Aldabrachelys gigantea</i>        | AY678336.1  | AY081779.1  | _           | _           | DQ497362.1  |
| <i>Amyda cartilaginea</i>            | AY259550.1  | LM537461.1  | AY259600.1  | _           | _           |
| <i>Apalone ferox</i>                 | AY259555.1  | NC_014054.1 | AY259605.1  | JQ950729.1  | JQ950717.1  |
| <i>Apalone mutica</i>                | AY259556.1  | _           | AY259606.1  | _           | _           |
| <i>Apalone spinifera</i>             | AY259557.1  | U81319.1    | AY259607.1  | JQ950728.1  | JQ950718.1  |
| <i>Astrochelys radiata</i>           | AY678359.1  | _           | AY673595.1  | JQ073222.1  | DQ497373.1  |
| <i>Astrochelys yniphora</i>          | AF020896.1  | _           | AY673541.1  | _           | DQ497375.1  |
| <i>Batagur affinis</i>               | FN313568.1  | AY434638.1  | _           | _           | _           |
| <i>Batagur baska</i>                 | FN313567.1  | EU030185.1  | _           | FN256245.1  | FN256247.1  |
| <i>Batagur borneoensis</i>           | _           | EU030186.1  | _           | EU030234.1  | EU030251.1  |
| <i>Batagur dhongoka</i>              | AY434569.1  | AY434631.1  | HM040931.1  | EU030239.1  | EU030256.1  |
| <i>Batagur kachuga</i>               | EU030215.1  | HM921178.1  | HM040934.1  | _           | _           |
| <i>Batagur trivittata</i>            | AM691758.1  | EU030192.1  | _           | EU030240.1  | EU030257.1  |
| <i>Caretta caretta</i>               | AY678314.1  | FJ009027.1  | AY673559.1  | JF415121.1  | FJ009033.1  |
| <i>Carettochelys insculpta</i>       | AY259546.1  | U40632.1    | _           | JQ950730.1  | JQ950719.1  |
| <i>Centrochelys sulcata</i>          | AY081793.1  | AF175334.1  | AY673532.1  | _           | DQ497374.1  |
| <i>Chelodina expansa</i>             | _           | _           | KJ469937.1  | _           | _           |
| <i>Chelodina longicollis</i>         | U81356.1    | U40633.1    | KM581420.1  | AY687921.1  | _           |
| <i>Chelodina novaeguineae</i>        | KC755182.1  | JN188821.1  | KC755123.1  | _           | _           |
| <i>Chelodina oblonga</i>             | NC_015986.1 | U40635.1    | _           | KC753120.1  | KC753126.1  |
| <i>Chelodina siebenrocki</i>         | _           | GU477771.1  | _           | _           | _           |
| <i>Chelonia mydas</i>                | EU918367.1  | FJ039948.1  | NC_000886.1 | FJ039953.1  | FJ039954.1  |
| <i>Chelonoidis abingdoni</i>         | AF192932.1  | _           | _           | _           | _           |
| <i>Chelonoidis becki</i>             | AF192939.1  | _           | AF351762.1  | _           | _           |
| <i>Chelonoidis carbonarius</i>       | AF192928.1  | AF175337.1  | AY673449.1  | EU930790.1  | AF175337.1  |
| <i>Chelonoidis chathamensis</i>      | _           | _           | AF351673.1  | _           | _           |
| <i>Chelonoidis chilensis</i>         | AF192929.1  | HQ289809.1  | AY673451.1  | EU930791.1  | DQ497366.1  |
| <i>Chelonoidis darwini</i>           | AF192940.1  | _           | AF351681.1  | _           | _           |
| <i>Chelonoidis denticulatus</i>      | AY678316.1  | AF175336.1  | AY673539.1  | EU930792.1  | DQ497367.1  |
| <i>Chelonoidis hoodensis</i>         | AF192933.1  | _           | AF351723.1  | _           | _           |

| <b>Species</b>                  | <b>CytB</b> | <b>12S</b>  | <b>ND4</b>  | <b>Rag1</b> | <b>Rag2</b> |
|---------------------------------|-------------|-------------|-------------|-------------|-------------|
| <i>Chelonoidis porteri</i>      | AF192934.1  | –           | –           | –           | –           |
| <i>Chelonoidis vicina</i>       | AF192935.1  | –           | AF351770.1  | –           | –           |
| <i>Chelus fimbriatus</i>        | HQ172156.1  | U40636.1    | –           | AY687918.1  | KC753127.1  |
| <i>Chelydra serpentina</i>      | JN860671.1  | FJ230852.1  | NC_011198.1 | JN654851.1  | HQ260656.1  |
| <i>Chersine angulata</i>        | –           | DQ497248.1  | AY673444.1  | –           | DQ497361.1  |
| <i>Chitra chitra</i>            | AY259562.1  | –           | AF414364.1  | –           | –           |
| <i>Chitra indica</i>            | JQ406951.1  | NC_026028.1 | AF494493.1  | JQ950731.1  | JQ950720.1  |
| <i>Chitra vandijki</i>          | AY259563.1  | –           | –           | –           | –           |
| <i>Chrysemys dorsalis</i>       | –           | HE590227.1  | HE590369.1  | HE590526.1  | HE590556.1  |
| <i>Chrysemys picta</i>          | FJ770587.1  | –           | KC688173.1  | –           | –           |
| <i>Claudius angustatus</i>      | –           | KF301255.1  | –           | KF301393.1  | KF301222.1  |
| <i>Clemmys guttata</i>          | AJ131429.1  | –           | –           | –           | –           |
| <i>Cryptochelys acuta</i>       | KF301367.1  | KF301264.1  | –           | KF301401.1  | KF301231.1  |
| <i>Cryptochelys creaseri</i>    | KF301373.1  | KF301269.1  | –           | KF301406.1  | KF301236.1  |
| <i>Cryptochelys dunni</i>       | KF301374.1  | KF301270.1  | –           | KF301407.1  | KF301237.1  |
| <i>Cryptochelys herrerae</i>    | KF301378.1  | KF301274.1  | –           | KF301411.1  | KF301241.1  |
| <i>Cryptochelys leucostoma</i>  | KF301383.1  | KF301279.1  | –           | KF301416.1  | KF301246.1  |
| <i>Cuora amboinensis</i>        | AY434580.1  | GU477768.1  | AY364609.1  | EU930787.1  | HQ260653.1  |
| <i>Cuora aurocapitata</i>       | AY434626.1  | GU477765.1  | AY572867.1  | HQ442389.1  | JN994096.1  |
| <i>Cuora bourreti</i>           | JN020145.1  | –           | AY364624.1  | JN994074.1  | JN994090.1  |
| <i>Cuora flavomarginata</i>     | AY434606.1  | GU477776.1  | GQ895899.1  | JN808216.1  | JN994083.1  |
| <i>Cuora galbinifrons</i>       | AJ564448.1  | AF043395.1  | AY364617.1  | EU930788.1  | JN994094.1  |
| <i>Cuora mccordi</i>            | AY434568.1  | –           | AY364608.1  | –           | –           |
| <i>Cuora mouhotii</i>           | DQ659152.1  | AF043404.1  | AY699016.1  | JN808219.1  | –           |
| <i>Cuora pani</i>               | AY434574.1  | JN860621.1  | AY590461.1  | JN808214.1  | JN994085.1  |
| <i>Cuora picturata</i>          | AY434576.1  | –           | AY364631.1  | JN994075.1  | JN994081.1  |
| <i>Cuora trifasciata</i>        | AY434627.1  | AB090032.1  | AF348297.2  | –           | –           |
| <i>Cuora yunnanensis</i>        | –           | –           | AY572868.1  | –           | –           |
| <i>Cuora zhoui</i>              | AY434584.1  | AB090033.1  | AY572866.1  | –           | –           |
| <i>Cyclanorbis elegans</i>      | AY259570.1  | –           | AY259615.1  | –           | –           |
| <i>Cyclanorbis senegalensis</i> | AY259569.1  | FR850553.1  | FR850604.1  | AY687903.1  | –           |
| <i>Cyclemys atripons</i>        | AY434617.1  | EU930721.1  | NC_010970.1 | EU930789.1  | AM931605.1  |
| <i>Cyclemys dentata</i>         | AY434579.1  | AF043402.1  | NC_018793.1 | JQ406647.1  | AM931609.1  |
| <i>Cyclemys enigmatica</i>      | AM931645.1  | –           | –           | –           | AM931611.1  |
| <i>Cyclemys fusca</i>           | AM931647.1  | –           | NC_026038.1 | JQ406645.1  | AM931613.1  |
| <i>Cyclemys gemeli</i>          | AM931656.1  | –           | –           | –           | FM877762.1  |
| <i>Cyclemys oldhamii</i>        | AM931665.1  | –           | NC_023220.1 | JQ406648.1  | AM931617.1  |
| <i>Cyclemys pulchristriata</i>  | –           | –           | NC_026027.1 | JQ406649.1  | AM931620.1  |
| <i>Cyclemys tcheponensis</i>    | AY434577.1  | –           | –           | –           | –           |
| <i>Cycloderma aubryi</i>        | AY259566.1  | FR850555.1  | FR850606.1  | –           | –           |
| <i>Cycloderma frenatum</i>      | AY259565.1  | –           | AY259610.1  | –           | –           |
| <i>Cylindraspis indica</i>      | AF371245.1  | –           | –           | –           | –           |
| <i>Cylindraspis inepta</i>      | AF371250.1  | –           | –           | –           | –           |
| <i>Cylindraspis peltastes</i>   | AF371255.1  | –           | –           | –           | –           |

| <b>Species</b>                  | <b>CytB</b> | <b>12S</b>  | <b>ND4</b> | <b>Rag1</b> | <b>Rag2</b> |
|---------------------------------|-------------|-------------|------------|-------------|-------------|
| <i>Cylindraspis vosmaeri</i>    | AF371260.1  | –           | –          | –           | –           |
| <i>Deirochelys reticularia</i>  | FJ770592.1  | HE590228.1  | HE590370.1 | HE590527.1  | DQ497394.1  |
| <i>Dermatemys mawii</i>         | AY678313.1  | KF301254.1  | AY673524.1 | AY687910.1  | KF301221.1  |
| <i>Dermochelys coriacea</i>     | U81363.1    | FJ039913.1  | JX454989.1 | FJ039918.1  | FJ039919.1  |
| <i>Dogania subplana</i>         | AF366350.1  | NC_002780.1 | AY259601.1 | –           | –           |
| <i>Elseya albagula</i>          | KC755168.1  | –           | KC755109.1 | –           | –           |
| <i>Elseya branderhorsti</i>     | KC755169.1  | JN188814.1  | KC755111.1 | KC753121.1  | KC753128.1  |
| <i>Elseya dentata</i>           | KC755171.1  | U40637.1    | KF255950.1 | –           | –           |
| <i>Elseya irwini</i>            | KC755173.1  | –           | KF255958.1 | –           | –           |
| <i>Elseya lavarackorum</i>      | KC755174.1  | –           | KF255953.1 | –           | –           |
| <i>Elusor macrurus</i>          | –           | U40639.1    | KC755125.1 | –           | –           |
| <i>Emydoidea blandingii</i>     | AJ131432.1  | –           | –          | –           | –           |
| <i>Emydura macquarii</i>        | KC755183.1  | U40640.1    | –          | –           | –           |
| <i>Emydura subglobosa</i>       | KC755190.1  | –           | KF255961.1 | KC753122.1  | KC753129.1  |
| <i>Emydura tanybaraga</i>       | KC755186.1  | –           | KC755130.1 | –           | –           |
| <i>Emydura victoriae</i>        | KC755189.1  | –           | KF255960.1 | –           | –           |
| <i>Emys orbicularis</i>         | HQ681920.1  | HQ681908.1  | KJ580956.1 | –           | –           |
| <i>Emys trinacris</i>           | AJ131416.1  | –           | –          | –           | –           |
| <i>Eretmochelys imbricata</i>   | L12718.1    | FJ039970.1  | KP221806.1 | FJ039975.1  | FJ039976.1  |
| <i>Erymnochelys</i>             |             |             |            |             |             |
| <i>madagascariensis</i>         | AM943834.1  | AM943824.1  | FM165619.1 | JQ073220.1  | AM943835.1  |
| <i>Flaviemys purvisi</i>        | KC755193.1  | AF095893.1  | KC755136.1 | –           | –           |
| <i>Geochelone elegans</i>       | –           | HM040909.1  | AY673465.1 | –           | DQ497368.1  |
| <i>Geochelone platynota</i>     | AY678412.1  | DQ497253.1  | AY673554.1 | –           | DQ497372.1  |
| <i>Geoclemys hamiltonii</i>     | AY434573.1  | AY434632.1  | –          | EU030235.1  | EU030252.1  |
| <i>Geoemyda japonica</i>        | AY434602.1  | EU030188.1  | –          | EU030236.1  | EU030253.1  |
| <i>Geoemyda spengleri</i>       | AY434586.1  | AY434634.1  | AY562186.1 | EU030237.1  | EU030254.1  |
| <i>Glyptemys insculpta</i>      | AJ131428.1  | DQ497265.1  | –          | EU930786.1  | DQ497393.1  |
| <i>Gopherus agassizii</i>       | AY434562.1  | AY434630.1  | AY673591.1 | –           | –           |
| <i>Gopherus berlanderi</i>      | AY678345.1  | –           | AY673482.1 | –           | –           |
| <i>Gopherus flavomarginatus</i> | AY678346.1  | –           | AY673475.1 | –           | –           |
| <i>Gopherus polyphemus</i>      | AY678356.1  | –           | AY673485.1 | EU930793.1  | DQ497376.1  |
| <i>Graptemys barbouri</i>       | GQ896190.1  | HE590229.1  | KC688174.1 | HE590528.1  | HE590558.1  |
| <i>Graptemys flavimaculata</i>  | GQ896192.1  | –           | –          | –           | –           |
| <i>Graptemys geographica</i>    | FJ770598.1  | –           | –          | –           | –           |
| <i>Graptemys gibbonsi</i>       | GQ896194.1  | HE590230.1  | HE590372.1 | HE590529.1  | HE590559.1  |
| <i>Graptemys nigrinoda</i>      | GQ896195.1  | HE590231.1  | DQ646420.1 | –           | HE590560.1  |
| <i>Graptemys oculifera</i>      | GQ896196.1  | –           | GQ253573.1 | –           | –           |
| <i>Graptemys ouachitensis</i>   | FJ770599.1  | –           | DQ646421.1 | –           | –           |
| <i>Graptemys</i>                |             |             |            |             |             |
| <i>pseudogeographica</i>        | FJ770600.1  | HE590232.1  | HE590374.1 | HE590531.1  | HE590561.1  |
| <i>Graptemys pulchra</i>        | GQ896199.1  | –           | –          | –           | –           |
| <i>Graptemys versa</i>          | GQ896200.1  | –           | DQ646422.1 | –           | –           |
| <i>Hardella thurjii</i>         | AM495275.1  | AB090025.1  | –          | EU030238.1  | EU030255.1  |

| <b>Species</b>                  | <b>CytB</b> | <b>12S</b> | <b>ND4</b>  | <b>Rag1</b> | <b>Rag2</b> |
|---------------------------------|-------------|------------|-------------|-------------|-------------|
| <i>Heosemys annandalii</i>      | AY434598.1  | AF043408.1 | NC_020668.1 | JN808213.1  | _           |
| <i>Heosemys depressa</i>        | AY434607.1  | EU930722.1 | NC_026024.1 | EU930794.1  | _           |
| <i>Heosemys grandis</i>         | AY434566.1  | AF043400.1 | _           | _           | _           |
| <i>Heosemys spinosa</i>         | AY434578.1  | U81339.1   | _           | AY687913.1  | AM931621.1  |
| <i>Homopus aerolatus</i>        | AY678318.1  | _          | AY673589.1  | _           | _           |
| <i>Homopus boulengeri</i>       | AY678329.1  | DQ497254.1 | AY673433.1  | _           | DQ497377.1  |
| <i>Homopus femoralis</i>        | AY678328.1  | _          | AY673435.1  | _           | _           |
| <i>Homopus signatus</i>         | AY678324.1  | DQ497255.1 | AY673429.1  | _           | DQ497378.1  |
| <i>Hydromedusa tectifera</i>    | _           | U62017.1   | _           | AY988104.1  | _           |
| <i>Indotestudo elongata</i>     | AY434643.1  | AF175338.1 | AY673555.1  | EU930795.1  | DQ497379.1  |
| <i>Indotestudo forsteni</i>     | AJ888372.1  | _          | AY673565.1  | _           | _           |
| <i>Indotestudo travancorica</i> | AY434644.1  | DQ497257.1 | AY673528.1  | _           | DQ497380.1  |
| <i>Kinixys belliana</i>         | AY678404.1  | HE662156.1 | AY673583.1  | _           | DQ497381.1  |
| <i>Kinixys erosa</i>            | AY678413.1  | HE662202.1 | AY673553.1  | _           | _           |
| <i>Kinixys homeana</i>          | AY678395.1  | HE662215.1 | AY673562.1  | _           | DQ497382.1  |
| <i>Kinixys lobatsiana</i>       | _           | HE662219.1 | HE662305.1  | _           | _           |
| <i>Kinixys natalensis</i>       | AY678397.1  | HE662221.1 | AY673582.1  | _           | _           |
| <i>Kinixys nogueyi</i>          | _           | HE662180.1 | HE662266.1  | _           | _           |
| <i>Kinixys spekii</i>           | AY678398.1  | HE662234.1 | AY673581.1  | _           | _           |
| <i>Kinixys zombensis</i>        | _           | HE662182.1 | HE662268.1  | _           | _           |
| <i>Kinosternon alamosae</i>     | KF301368.1  | KF301265.1 | _           | KF301402.1  | KF301232.1  |
| <i>Kinosternon arizonense</i>   | KF301370.1  | KF301266.1 | _           | KF301403.1  | KF301233.1  |
| <i>Kinosternon baurii</i>       | KF301371.1  | KF301267.1 | _           | KF301404.1  | KF301234.1  |
| <i>Kinosternon chimalhuaca</i>  | KF301372.1  | KF301268.1 | _           | KF301405.1  | KF301235.1  |
| <i>Kinosternon durangoense</i>  | KF301375.1  | KF301271.1 | _           | KF301408.1  | KF301238.1  |
| <i>Kinosternon flavescens</i>   | KF301376.1  | KF301272.1 | _           | KF301409.1  | KF301239.1  |
| <i>Kinosternon hirtipes</i>     | KF301379.1  | KF301275.1 | _           | KF301412.1  | KF301242.1  |
| <i>Kinosternon integrum</i>     | KF301380.1  | KF301278.1 | _           | KF301415.1  | KF301244.1  |
| <i>Kinosternon oaxacae</i>      | KF301384.1  | KF301280.1 | _           | _           | KF301247.1  |
| <i>Kinosternon scorpioides</i>  | KF301388.1  | KF301284.1 | _           | KF301420.1  | KF301251.1  |
| <i>Kinosternon sonoriense</i>   | KF301389.1  | KF301285.1 | _           | KF301421.1  | _           |
| <i>Kinosternon subrubrum</i>    | KF301391.1  | KF301287.1 | _           | KF301423.1  | KF301253.1  |
| <i>Lepidochelys kempii</i>      | AY678399.1  | FJ039991.1 | AY673520.1  | FJ039996.1  | FJ039997.1  |
| <i>Lepidochelys olivacea</i>    | L12764.1    | FJ039984.1 | DQ486893.1  | FJ039982.1  | FJ039983.1  |
| <i>Leucocephalon yuwonoi</i>    | AY434608.1  | _          | _           | _           | AM931622.1  |
| <i>Lissemys punctata</i>        | AY259568.1  | FR850548.1 | FR850598.1  | AY687902.1  | _           |
| <i>Lissemys scutata</i>         | AY259567.1  | FR850552.1 | FR850603.1  | JQ950732.1  | JQ950721.1  |
| <i>Macrochelys temminckii</i>   | JN860670.1  | FJ230859.1 | NC_009260.1 | FJ230864.1  | FJ230865.1  |
| <i>Malaclemys terrapin</i>      | FJ770602.1  | HE590234.1 | DQ646423.1  | HE590533.1  | HE590563.1  |
| <i>Malacochersus tornieri</i>   | DQ497314.1  | DQ497260.1 | AY673530.1  | _           | DQ497383.1  |
| <i>Malayemys subtrijuga</i>     | _           | AF043398.1 | _           | EU030241.1  | EU030258.1  |
| <i>Manouria emys</i>            | AY434563.1  | DQ497261.1 | AY673499.1  | _           | DQ497384.1  |
| <i>Manouria impressa</i>        | EF661586.1  | GU477773.1 | AY673500.1  | _           | DQ497386.1  |
| <i>Mauremys annamensis</i>      | AY434564.1  | AB090041.1 | AY337338.2  | HQ442407.1  | _           |

| Species                         | CytB       | 12S        | ND4         | Rag1       | Rag2       |
|---------------------------------|------------|------------|-------------|------------|------------|
| <i>Mauremys caspica</i>         | AY434594.1 | AB090043.1 | AY337340.1  | EU930796.1 | AM905436.1 |
| <i>Mauremys japonica</i>        | AY434587.1 | HQ442365.1 | AY337341.1  | HQ442406.1 | –          |
| <i>Mauremys leprosa</i>         | AY434592.1 | AY434635.1 | DQ902330.1  | EU930797.1 | –          |
| <i>Mauremys mutica</i>          | AY434628.1 | GU477766.1 | JX394176.1  | –          | HQ260652.1 |
| <i>Mauremys nigricans</i>       | –          | JN860616.1 | EF034111.1  | JN808196.1 | –          |
| <i>Mauremys reevesii</i>        | AY434567.1 | GU477764.1 | GQ259441.1  | HQ442404.1 | HQ260651.1 |
| <i>Mauremys rivulata</i>        | AY434623.1 | AY434641.1 | AY337344.1  | EU930798.1 | AM905440.1 |
| <i>Mauremys sinensis</i>        | AY434615.1 | –          | GQ259449.1  | JN808197.1 | –          |
| <i>Melanochelys trijuga</i>     | AY434588.1 | AF043405.1 | HM040936.1  | –          | –          |
| <i>Mesoclemmys dahli</i>        | JX139063.1 | JX139086.1 | JX139071.1  | –          | JX139073.1 |
| <i>Mesoclemmys gibba</i>        | U81348.1   | JX139088.1 | EF535304.1  | AY687919.1 | JX139075.1 |
| <i>Mesoclemmys nasuta</i>       | –          | U40645.1   | –           | –          | –          |
| <i>Mesoclemmys zuliae</i>       | JX139067.1 | JX139087.1 | JX139070.1  | –          | JX139074.1 |
| <i>Morenia ocellata</i>         | AY434605.1 | EU030194.1 | –           | EU030242.1 | EU030259.1 |
| <i>Morenia petersi</i>          | –          | HM040922.1 | –           | –          | –          |
| <i>Myuchelys bellii</i>         | KC755191.1 | –          | KC755134.1  | –          | –          |
| <i>Myuchelys georgesi</i>       | KC755192.1 | AF095894.1 | KC755135.1  | –          | –          |
| <i>Myuchelys latisternum</i>    | U81354.1   | U40638.1   | –           | –          | –          |
| <i>Natator depressus</i>        | AF385674.1 | FJ039955.1 | NC_018550.1 | FJ039961.1 | FJ039962.1 |
| <i>Nilssonia formosa</i>        | AY259547.1 | HE801638.1 | AY259597.1  | –          | –          |
| <i>Nilssonia gangetica</i>      | AY259549.1 | HM040913.1 | AY259599.1  | –          | –          |
| <i>Nilssonia hurum</i>          | AY259548.1 | HE801667.1 | AY259598.1  | –          | –          |
| <i>Nilssonia leithii</i>        | AM495225.1 | HE801669.1 | HE801722.1  | –          | –          |
| <i>Nilssonia nigricans</i>      | AM495227.1 | HE801685.1 | HE801738.1  | –          | –          |
| <i>Notochelys platynota</i>     | AY434613.1 | JN860629.1 | NC_020665.1 | JN808211.1 | –          |
| <i>Orlitia borneensis</i>       | AY434619.1 | AF043399.1 | –           | EU030243.1 | EU030260.1 |
| <i>Palea steindachneri</i>      | AY743417.1 | AY743419.2 | AY259602.1  | KC668144.1 | JQ950716.1 |
| <i>Pangshura smithii</i>        | AY434589.1 | EU030195.1 | –           | EU030244.1 | EU030261.1 |
| <i>Pangshura sylhetensis</i>    | AM495296.1 | –          | JN621107.1  | –          | –          |
| <i>Pangshura tecta</i>          | AY434583.1 | AY434633.1 | HM040933.1  | EU030245.1 | EU030262.1 |
| <i>Pangshura tentoria</i>       | AM495326.1 | AY434639.1 | HM040932.1  | EU030246.1 | EU030263.1 |
| <i>Pelochelys bibroni</i>       | AY259559.1 | –          | AF414361.1  | –          | –          |
| <i>Pelochelys cantorii</i>      | AY259560.1 | JN016746.1 | AF414360.1  | –          | JQ950713.1 |
| <i>Pelodiscus maackii</i>       | –          | FM999003.1 | FM999019.1  | –          | –          |
| <i>Pelodiscus parviformis</i>   | –          | HQ116623.1 | –           | –          | –          |
| <i>Pelodiscus sinensis</i>      | AY583692.1 | AY304497.1 | AY259603.1  | FJ230871.1 | JQ950715.1 |
| <i>Pelomedusa barbata</i>       | –          | HG973063.1 | –           | –          | –          |
| <i>Pelomedusa galeata</i>       | HG973216.1 | HG973127.1 | HG973265.1  | –          | –          |
| <i>Pelomedusa gehafie</i>       | HG973223.1 | HG934010.1 | HG973300.1  | –          | –          |
| <i>Pelomedusa kobe</i>          | HG973227.1 | HG973140.1 | HG973304.1  | –          | –          |
| <i>Pelomedusa neumanni</i>      | –          | HG934020.1 | –           | –          | –          |
| <i>Pelomedusa olivacea</i>      | –          | HG934005.2 | –           | –          | –          |
| <i>Pelomedusa schweinfurthi</i> | –          | HG973150.1 | –           | –          | –          |
| <i>Pelomedusa somalica</i>      | HG973228.1 | HG973151.1 | HG973305.1  | –          | –          |

| <b>Species</b>                    | <b>CytB</b> | <b>12S</b> | <b>ND4</b> | <b>Rag1</b> | <b>Rag2</b> |
|-----------------------------------|-------------|------------|------------|-------------|-------------|
| <i>Pelomedusa subrufa</i>         | AF039066.1  | FJ230873.1 | HG973316.1 | JQ073217.1  | FN645376.1  |
| <i>Pelomedusa variabilis</i>      | –           | HG934014.1 | –          | –           | –           |
| <i>Peltocephalus dumerilianus</i> | AM943833.1  | AM943823.1 | FM165622.1 | AY988101.1  | AM943837.1  |
| <i>Pelusios adansonii</i>         | –           | FR716833.1 | –          | –           | –           |
| <i>Pelusios bechuanicus</i>       | –           | FR716835.1 | FR716946.1 | –           | FR717087.1  |
| <i>Pelusios broadleyi</i>         | FR716896.1  | JQ352029.1 | JQ352049.1 | –           | JQ352078.1  |
| <i>Pelusios carinatus</i>         | FR716902.1  | FR716845.1 | FR716955.1 | –           | FR717097.1  |
| <i>Pelusios castaneus</i>         | KC692463.1  | FR716854.1 | FR716964.1 | KC753123.1  | KC753130.1  |
| <i>Pelusios castanoides</i>       | FR716920.1  | JQ352030.1 | JQ352050.1 | JQ073219.1  | JQ352079.1  |
| <i>Pelusios chapini</i>           | FR716922.1  | FR716863.1 | FR716973.1 | –           | FR717112.1  |
| <i>Pelusios cupulatta</i>         | FR716926.1  | FR716867.1 | FR716977.1 | –           | FR717114.1  |
| <i>Pelusios gabonensis</i>        | JQ352041.1  | JQ352031.1 | JQ352052.1 | AY988103.1  | JQ352081.1  |
| <i>Pelusios marani</i>            | JQ352042.1  | JQ352034.1 | JQ352056.1 | –           | JQ352085.1  |
| <i>Pelusios nanus</i>             | –           | FR716870.1 | FR716980.1 | –           | FR717117.1  |
| <i>Pelusios niger</i>             | –           | FR716872.1 | FR716981.1 | –           | FR717118.1  |
| <i>Pelusios rhodesianus</i>       | FR716936.1  | FR716874.1 | FR716983.1 | –           | FR717120.1  |
| <i>Pelusios sinuatus</i>          | FR716938.1  | FR716877.1 | FN645332.1 | FN645349.1  | FR717123.1  |
| <i>Pelusios subniger</i>          | HE979988.1  | FR716879.2 | FR716989.2 | AY487412.1  | FR717126.1  |
| <i>Pelusios upembae</i>           | –           | FR716883.1 | FR716992.1 | –           | FR717129.1  |
| <i>Pelusios williamsi</i>         | JQ352046.1  | JQ352036.1 | JQ352058.1 | AY687923.1  | JQ352087.1  |
| <i>Phrynops geoffroanus</i>       | JX139069.2  | U40647.1   | JX139072.1 | –           | JX139076.1  |
| <i>Phrynops hilarii</i>           | JN999705.2  | –          | –          | –           | JX139077.1  |
| <i>Platemys platycephala</i>      | EF535285.1  | U40648.1   | EF535299.1 | KC753124.1  | KC753131.1  |
| <i>Platysternon megacephalum</i>  | JN860672.1  | GU477772.1 | DQ016387.1 | KC683666.1  | KC683679.1  |
| <i>Podocnemis erythrocephala</i>  | AM943832.1  | AM943822.1 | FM165621.1 | –           | AM943841.1  |
| <i>Podocnemis expansa</i>         | AM943830.1  | AM943820.1 | FM165620.1 | JQ073221.1  | AM943839.1  |
| <i>Podocnemis lewyana</i>         | AM943827.1  | AM943817.1 | FM165617.1 | –           | AM943825.1  |
| <i>Podocnemis sextuberculata</i>  | –           | AM943819.1 | FM165616.1 | –           | AM943840.1  |
| <i>Podocnemis unifilis</i>        | JF802204.1  | AM943818.1 | FM165623.1 | KC753125.1  | AM943842.1  |
| <i>Podocnemis vogli</i>           | AM943828.1  | AM943821.1 | FM165618.1 | –           | AM943838.1  |
| <i>Psammobates geometricus</i>    | AY678375.1  | –          | AY673580.1 | –           | –           |
| <i>Psammobates oculiferus</i>     | AY678377.1  | –          | AY673575.1 | –           | –           |
| <i>Psammobates tentorius</i>      | AY678382.1  | DQ497264.1 | AY673572.1 | –           | DQ497387.1  |
| <i>Pseudemydura umbrina</i>       | –           | U40650.1   | –          | –           | –           |
| <i>Pseudemys alabamensis</i>      | GQ395715.1  | –          | KC688180.1 | –           | –           |
| <i>Pseudemys concinna</i>         | FJ770603.1  | HE590235.1 | –          | HE590534.1  | HE590564.1  |
| <i>Pseudemys floridana</i>        | FJ770604.1  | HE590236.1 | KC688216.1 | HE590535.1  | HE590565.1  |
| <i>Pseudemys gorzugi</i>          | GQ395700.1  | –          | KC688238.1 | –           | –           |
| <i>Pseudemys nelsoni</i>          | –           | HE590237.1 | KC688242.1 | –           | –           |
| <i>Pseudemys peninsularis</i>     | FJ770607.1  | –          | KC688248.1 | –           | –           |
| <i>Pseudemys rubriventris</i>     | GQ395708.1  | HE590238.1 | KC688252.1 | –           | –           |
| <i>Pseudemys suwanniensis</i>     | GQ395710.1  | –          | KC688229.1 | –           | –           |
| <i>Pseudemys texana</i>           | GQ395713.1  | –          | KC688255.1 | –           | –           |
| <i>Pyxis arachnoides</i>          | AY678415.1  | –          | AY673556.1 | JQ073223.1  | DQ497388.1  |

| <b>Species</b>                      | <b>CytB</b> | <b>12S</b>  | <b>ND4</b>  | <b>Rag1</b> | <b>Rag2</b> |
|-------------------------------------|-------------|-------------|-------------|-------------|-------------|
| <i>Pyxis planicauda</i>             | AY834930.1  | –           | AY673549.1  | –           | DQ497389.1  |
| <i>Rafetus euphraticus</i>          | AY259554.1  | FM999033.1  | AY259604.1  | –           | –           |
| <i>Rafetus swinhoei</i>             | HQ709384.1  | NC_017901.1 | KJ482684.1  | –           | JQ950714.1  |
| <i>Rheodytes leukops</i>            | KC755194.1  | U40651.1    | KF255962.1  | –           | –           |
| <i>Rhinoclemmys annulata</i>        | –           | EU930726.1  | –           | EU930801.1  | –           |
| <i>Rhinoclemmys areolata</i>        | –           | EU930727.1  | –           | EU930802.1  | –           |
| <i>Rhinoclemmys diademata</i>       | AY434616.1  | AY434640.1  | –           | EU930803.1  | KC352578.1  |
| <i>Rhinoclemmys funerea</i>         | AY434599.1  | –           | –           | EU930804.1  | KC352579.1  |
| <i>Rhinoclemmys melanosterna</i>    | AY434590.1  | KC352570.1  | –           | EU030247.1  | DQ497395.1  |
| <i>Rhinoclemmys nasuta</i>          | DQ497324.1  | DQ497268.1  | –           | EU030248.1  | DQ497396.1  |
| <i>Rhinoclemmys pulcherrima</i>     | AY434597.1  | –           | –           | EU930806.1  | –           |
| <i>Rhinoclemmys punctularia</i>     | AY434595.1  | KC352571.1  | –           | EU930809.1  | KC352580.1  |
| <i>Rhinoclemmys rubida</i>          | AY434625.1  | GU477774.1  | –           | EU930810.1  | HQ260655.1  |
| <i>Sacalia bealei</i>               | AY434585.1  | HQ442364.1  | NC_016691.1 | HQ442391.1  | –           |
| <i>Sacalia quadriocellata</i>       | AY434618.1  | EU930736.1  | NC_011819.1 | EU930811.1  | –           |
| <i>Siebenrockiella crassicollis</i> | –           | EU030198.1  | –           | EU030249.1  | EU030264.1  |
| <i>Staurotypus salvinii</i>         | KF301359.1  | KF301256.1  | –           | KF301394.1  | KF301223.1  |
| <i>Staurotypus triporcatus</i>      | U81349.1    | KF301257.1  | –           | KF301395.1  | KF301224.1  |
| <i>Sternotherus carinatus</i>       | JN860673.1  | KF301258.1  | NC_017607.1 | KF301396.1  | KF301225.1  |
| <i>Sternotherus depressus</i>       | KF301362.1  | KF301259.1  | –           | KF301397.1  | KF301226.1  |
| <i>Sternotherus minor</i>           | KF301364.1  | KF301261.1  | –           | KF301399.1  | KF301228.1  |
| <i>Sternotherus odoratus</i>        | GQ896189.1  | KF301262.1  | HQ709263.1  | AY687911.1  | KF301229.1  |
| <i>Stigmochelys pardalis</i>        | –           | AF175335.1  | AY673533.1  | JQ073224.1  | DQ497370.1  |
| <i>Terrapene carolina</i>           | FJ770615.1  | EU930737.1  | KC688256.1  | EU930812.1  | –           |
| <i>Terrapene nelsoni</i>            | AF258873.1  | –           | –           | HQ266660.1  | –           |
| <i>Terrapene ornata</i>             | AJ131427.1  | –           | AY673566.1  | –           | –           |
| <i>Testudo graeca</i>               | HE588138.1  | AF175331.1  | HE585812.1  | –           | DQ497390.1  |
| <i>Testudo hermanni</i>             | AJ888357.1  | AF175327.1  | AY673514.1  | –           | AM491038.1  |
| <i>Testudo horsfieldii</i>          | –           | AF175328.1  | AY673551.1  | –           | DQ497391.1  |
| <i>Testudo kleinmanni</i>           | AJ888370.1  | AF175332.1  | AY673567.1  | –           | DQ497392.1  |
| <i>Testudo marginata</i>            | AJ888319.1  | AF175333.1  | AY673519.1  | –           | AM491037.1  |
| <i>Trachemys adiutrix</i>           | HE590312.1  | HE590241.1  | –           | HE590537.1  | HE590567.1  |
| <i>Trachemys callirostris</i>       | HE590329.1  | HE590259.1  | DQ338507.1  | HE590540.1  | HE590570.1  |
| <i>Trachemys decorata</i>           | –           | –           | JN707379.1  | –           | –           |
| <i>Trachemys decussata</i>          | HE590332.1  | HE590261.1  | JN707397.1  | HE590537.1  | HE590571.1  |
| <i>Trachemys dorbigni</i>           | HE590341.1  | HE590270.1  | DQ338514.1  | HE590540.1  | HE590573.1  |
| <i>Trachemys emolli</i>             | HE590349.1  | HE590278.1  | FR874843.1  | HE590544.1  | HE590574.1  |
| <i>Trachemys gaigeae</i>            | GQ896204.1  | HE590279.1  | JN707419.1  | HE590545.1  | HE590575.1  |
| <i>Trachemys grayi</i>              | HE590365.1  | HE590294.1  | DQ338508.1  | HE590553.1  | HE590582.1  |
| <i>Trachemys ornata</i>             | HE590355.1  | HE590284.1  | –           | HE590547.1  | HE590577.1  |
| <i>Trachemys scripta</i>            | AF207750.1  | HE590290.1  | JN707418.1  | HE590550.1  | HQ260654.1  |
| <i>Trachemys stejnegeri</i>         | FJ770620.1  | –           | JN707417.1  | –           | –           |
| <i>Trachemys taylori</i>            | FJ770623.1  | –           | JN615118.1  | –           | –           |
| <i>Trachemys terrapen</i>           | –           | –           | JN707355.1  | –           | –           |

| <b>Species</b>           | <b>CytB</b> | <b>12S</b>  | <b>ND4</b> | <b>Rag1</b> | <b>Rag2</b> |
|--------------------------|-------------|-------------|------------|-------------|-------------|
| <i>Trachemys venusta</i> | HE590366.1  | HE590295.1  | JN707428.1 | HE590554.1  | HE590583.1  |
| <i>Trachemys yaquia</i>  | –           | –           | DQ338512.1 | –           | –           |
| <i>Trionyx triunguis</i> | AY259564.1  | NC_012833.1 | AY259609.1 | –           | –           |

**Table S3.** Habitat classification of the species included in our phylogeny. Species marked with a \* were very recently separated from *Pelomedusa subrufa* (Petzold et al., 2014). Considering that this species is broadly known as an aquatic species, all the new species were also classified as aquatic.

| <b>Species</b>                       | <b>Habitat</b> | <b>Source</b>              |
|--------------------------------------|----------------|----------------------------|
| <i>Acanthochelys macrocephala</i>    | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Acanthochelys pallidipectoris</i> | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Acanthochelys radiolata</i>       | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Acanthochelys spixii</i>          | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Actinemys marmorata</i>           | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Aldabrachelys gigantea</i>        | Terrestrial    | Bonin <i>et al.</i> (2006) |
| <i>Amyda cartilaginea</i>            | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Apalone ferox</i>                 | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Apalone mutica</i>                | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Apalone spinifera</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Astrochelys radiata</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Astrochelys yniphora</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Batagur affinis</i>               | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Batagur baska</i>                 | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Batagur borneoensis</i>           | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Batagur dhongoka</i>              | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Batagur kachuga</i>               | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Batagur trivittata</i>            | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Caretta caretta</i>               | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Carettochelys insculpta</i>       | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Centrochelys sulcata</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Chelodina expansa</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Chelodina longicollis</i>         | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Chelodina novaeguineae</i>        | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Chelodina oblonga</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Chelodina siebenrocki</i>         | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Chelonia mydas</i>                | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Chelonoidis abingdoni</i>         | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Chelonoidis becki</i>             | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Chelonoidis carbonarius</i>       | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Chelonoidis chathamensis</i>      | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Chelonoidis chilensis</i>         | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Chelonoidis darwini</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |

| <b>Species</b>                  | <b>Habitat</b> | <b>Source</b>                 |
|---------------------------------|----------------|-------------------------------|
| <i>Chelonoidis denticulatus</i> | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Chelonoidis hoodensis</i>    | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Chelonoidis porteri</i>      | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Chelonoidis vicina</i>       | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Chelus fimbriatus</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Chelydra serpentina</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Chersine angulata</i>        | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Chitra chitra</i>            | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Chitra indica</i>            | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Chitra vandijki</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Chrysemys dorsalis</i>       | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Chrysemys picta</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Claudius angustatus</i>      | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Clemmys guttata</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cryptochelys acuta</i>       | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Cryptochelys creaseri</i>    | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Cryptochelys dunni</i>       | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Cryptochelys herrerae</i>    | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Cryptochelys leucostoma</i>  | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Cuora amboinensis</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cuora aurocapitata</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cuora bourreti</i>           | Terrestrial    | Bonin <i>et al.</i> (2006)    |
| <i>Cuora flavomarginata</i>     | Aquatic        | Bonin <i>et al.</i> (2006)    |
| <i>Cuora galbinifrons</i>       | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Cuora mccordi</i>            | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cuora mouhotii</i>           | Terrestrial    | Ji-Chao <i>et al.</i> (2011)  |
| <i>Cuora pani</i>               | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cuora picturata</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Cuora trifasciata</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cuora yunnanensis</i>        | Aquatic        | van Dijk <i>et al.</i> (2010) |
| <i>Cuora zhoui</i>              | Terrestrial    | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclanorbis elegans</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclanorbis senegalensis</i> | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys atripons</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys dentata</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys enigmatica</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys fusca</i>           | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys gemeli</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys oldhamii</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys pulchristriata</i>  | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cyclemys tcheponensis</i>    | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cycloderma aubryi</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cycloderma frenatum</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)    |
| <i>Cylindraspis indica</i>      | Terrestrial    | Jaffe <i>et al.</i> (2011)    |

| <b>Species</b>                       | <b>Habitat</b> | <b>Source</b>              |
|--------------------------------------|----------------|----------------------------|
| <i>Cylindraspis inepta</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Cylindraspis peltastes</i>        | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Cylindraspis vosmaeri</i>         | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Deirochelys reticularia</i>       | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Dermatemys mawii</i>              | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Dermochelys coriacea</i>          | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Dogania subplana</i>              | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Elseya albagula</i>               | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Elseya branderhorsti</i>          | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Elseya dentata</i>                | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Elseya irwini</i>                 | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Elseya lavarackorum</i>           | Aquatic        | Wells (2007)               |
| <i>Elusor macrurus</i>               | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Emydoidea blandingii</i>          | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Emydura macquarii</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Emydura subglobosa</i>            | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Emydura tanybaraga</i>            | Aquatic        | Cann (1997)                |
| <i>Emydura victoriae</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Emys orbicularis</i>              | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Emys trinacris</i>                | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Eretmochelys imbricata</i>        | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Erymnochelys madagascariensis</i> | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Flaviemys purvisi</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Geochelone elegans</i>            | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Geochelone platynota</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Geoclemys hamiltonii</i>          | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Geoemyda japonica</i>             | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Geoemyda spengleri</i>            | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Glyptemys insculpta</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Gopherus agassizii</i>            | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Gopherus berlanderi</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Gopherus flavomarginatus</i>      | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Gopherus polyphemus</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys barbouri</i>            | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys flavimaculata</i>       | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys geographica</i>         | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys gibbonsi</i>            | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys nigrinoda</i>           | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys oculifera</i>           | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys ouachitensis</i>        | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys pseudogeographica</i>   | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys pulchra</i>             | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Graptemys versa</i>               | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Hardella thurjii</i>              | Aquatic        | Jaffe <i>et al.</i> (2011) |

| <b>Species</b>                  | <b>Habitat</b> | <b>Source</b>              |
|---------------------------------|----------------|----------------------------|
| <i>Heosemys annandalii</i>      | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Heosemys depressa</i>        | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Heosemys grandis</i>         | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Heosemys spinosa</i>         | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Homopus aerolatus</i>        | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Homopus boulengeri</i>       | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Homopus femoralis</i>        | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Homopus signatus</i>         | Terrestrial    | Bonin <i>et al.</i> (2006) |
| <i>Hydromedusa tectifera</i>    | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Indotestudo elongata</i>     | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Indotestudo forsteni</i>     | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Indotestudo travancorica</i> | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Kinixys belliana</i>         | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Kinixys erosa</i>            | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Kinixys homeana</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Kinixys lobatsiana</i>       | Terrestrial    | Bonin <i>et al.</i> (2006) |
| <i>Kinixys natalensis</i>       | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Kinixys nogueyi</i>          | Terrestrial    | Bonin <i>et al.</i> (2006) |
| <i>Kinixys spekii</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Kinixys zombensis</i>        | Terrestrial    | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon alamosae</i>     | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon arizonense</i>   | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon baurii</i>       | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon chimalhuaca</i>  | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon durangoense</i>  | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon flavescens</i>   | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon hirtipes</i>     | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon integrum</i>     | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon oaxacae</i>      | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon scorpioides</i>  | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon sonoriense</i>   | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Kinosternon subrubrum</i>    | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Lepidochelys kempii</i>      | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Lepidochelys olivacea</i>    | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Leucocephalon yuwonoi</i>    | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Lissemys punctata</i>        | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Lissemys scutata</i>         | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Macrochelys temminckii</i>   | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Malaclemys terrapin</i>      | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Malacochersus tornieri</i>   | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Malayemys subtrijuga</i>     | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Manouria emys</i>            | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Manouria impressa</i>        | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Mauremys annamensis</i>      | Aquatic        | Jaffe <i>et al.</i> (2011) |

| <b>Species</b>                  | <b>Habitat</b> | <b>Source</b>               |
|---------------------------------|----------------|-----------------------------|
| <i>Mauremys caspica</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mauremys japonica</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mauremys leprosa</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mauremys mutica</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mauremys nigricans</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mauremys reevesii</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mauremys rivulata</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mauremys sinensis</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Melanochelys trijuga</i>     | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mesoclemmys dahli</i>        | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Mesoclemmys gibba</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Mesoclemmys nasuta</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Mesoclemmys zuliae</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Morenia ocellata</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Morenia petersi</i>          | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Myuchelys bellii</i>         | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Myuchelys georgesi</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Myuchelys latisternum</i>    | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Natator depressus</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Nilssonina formosa</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Nilssonina gangetica</i>     | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Nilssonina hurum</i>         | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Nilssonina leithii</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Nilssonina nigricans</i>     | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Notochelys platynota</i>     | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Orlitia borneensis</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Palea steindachneri</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pangshura smithii</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pangshura sylhetensis</i>    | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pangshura tecta</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pangshura tentoria</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pelochelys bibroni</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pelochelys cantorii</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pelodiscus maackii</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelodiscus parviformis</i>   | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelodiscus sinensis</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pelomedusa barbata</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Pelomedusa galeata</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Pelomedusa gehafie</i>       | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Pelomedusa kobe</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Pelomedusa neumanni</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Pelomedusa olivacea</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Pelomedusa schweinfurthi</i> | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Pelomedusa somalica</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)* |

| <b>Species</b>                    | <b>Habitat</b> | <b>Source</b>               |
|-----------------------------------|----------------|-----------------------------|
| <i>Pelomedusa subrufa</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pelomedusa variabilis</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)* |
| <i>Peltocephalus dumerilianus</i> | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pelusios adansonii</i>         | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios bechuanicus</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios broadleyi</i>         | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios carinatus</i>         | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios castaneus</i>         | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios castanoides</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios chapini</i>           | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios cupulatta</i>         | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios gabonensis</i>        | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios marani</i>            | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios nanus</i>             | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios niger</i>             | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios rhodesianus</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios sinuatus</i>          | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios subniger</i>          | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios upembae</i>           | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pelusios williamsi</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Phrynops Geoffroanus</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Phrynops hilarii</i>           | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Platemys platycephala</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Platysternon megacephalum</i>  | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Podocnemis erythrocephala</i>  | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Podocnemis expansa</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Podocnemis lewyana</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Podocnemis sextuberculata</i>  | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Podocnemis unifilis</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Podocnemis vogli</i>           | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Psammobates geometricus</i>    | Terrestrial    | Jaffe <i>et al.</i> (2011)  |
| <i>Psammobates oculiferus</i>     | Terrestrial    | Jaffe <i>et al.</i> (2011)  |
| <i>Psammobates tentorius</i>      | Terrestrial    | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemydura umbrina</i>       | Aquatic        | Bonin <i>et al.</i> (2006)  |
| <i>Pseudemys alabamensis</i>      | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys concinna</i>         | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys floridana</i>        | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys gorzugi</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys nelsoni</i>          | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys peninsularis</i>     | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys rubriventris</i>     | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys suwanniensis</i>     | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pseudemys texana</i>           | Aquatic        | Jaffe <i>et al.</i> (2011)  |
| <i>Pyxis arachnoides</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011)  |

| <b>Species</b>                      | <b>Habitat</b> | <b>Source</b>              |
|-------------------------------------|----------------|----------------------------|
| <i>Pyxis planicauda</i>             | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Rafetus euphraticus</i>          | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rafetus swinhoei</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Rheodytes leukops</i>            | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Rhinoclemmys annulata</i>        | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rhinoclemmys areolata</i>        | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rhinoclemmys diademata</i>       | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rhinoclemmys funerea</i>         | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rhinoclemmys melanosterna</i>    | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rhinoclemmys nasuta</i>          | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rhinoclemmys pulcherrima</i>     | Terrestrial    | Bonin <i>et al.</i> (2006) |
| <i>Rhinoclemmys punctularia</i>     | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Rhinoclemmys rubida</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Sacalia bealei</i>               | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Sacalia quadriocellata</i>       | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Siebenrockiella crassicollis</i> | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Staurotypus salvinii</i>         | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Staurotypus triporcatus</i>      | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Sternotherus carinatus</i>       | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Sternotherus depressus</i>       | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Sternotherus minor</i>           | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Sternotherus odoratus</i>        | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Stigmochelys pardalis</i>        | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Terrapene carolina</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Terrapene nelsoni</i>            | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Terrapene ornata</i>             | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Testudo graeca</i>               | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Testudo hermanni</i>             | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Testudo horsfieldii</i>          | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Testudo kleinmanni</i>           | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Testudo marginata</i>            | Terrestrial    | Jaffe <i>et al.</i> (2011) |
| <i>Trachemys adiutrix</i>           | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys callirostris</i>       | Aquatic        | Bock <i>et al.</i> (2010)  |
| <i>Trachemys decorata</i>           | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys decussata</i>          | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys dorbigni</i>           | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys emolli</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys gaigeae</i>            | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Trachemys grayi</i>              | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys ornata</i>             | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys scripta</i>            | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Trachemys stejnegeri</i>         | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Trachemys taylori</i>            | Aquatic        | Jaffe <i>et al.</i> (2011) |
| <i>Trachemys terrapen</i>           | Aquatic        | Bonin <i>et al.</i> (2006) |

| <b>Species</b>           | <b>Habitat</b> | <b>Source</b>              |
|--------------------------|----------------|----------------------------|
| <i>Trachemys venusta</i> | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trachemys yaquia</i>  | Aquatic        | Bonin <i>et al.</i> (2006) |
| <i>Trionyx triunguis</i> | Aquatic        | Jaffe <i>et al.</i> (2011) |

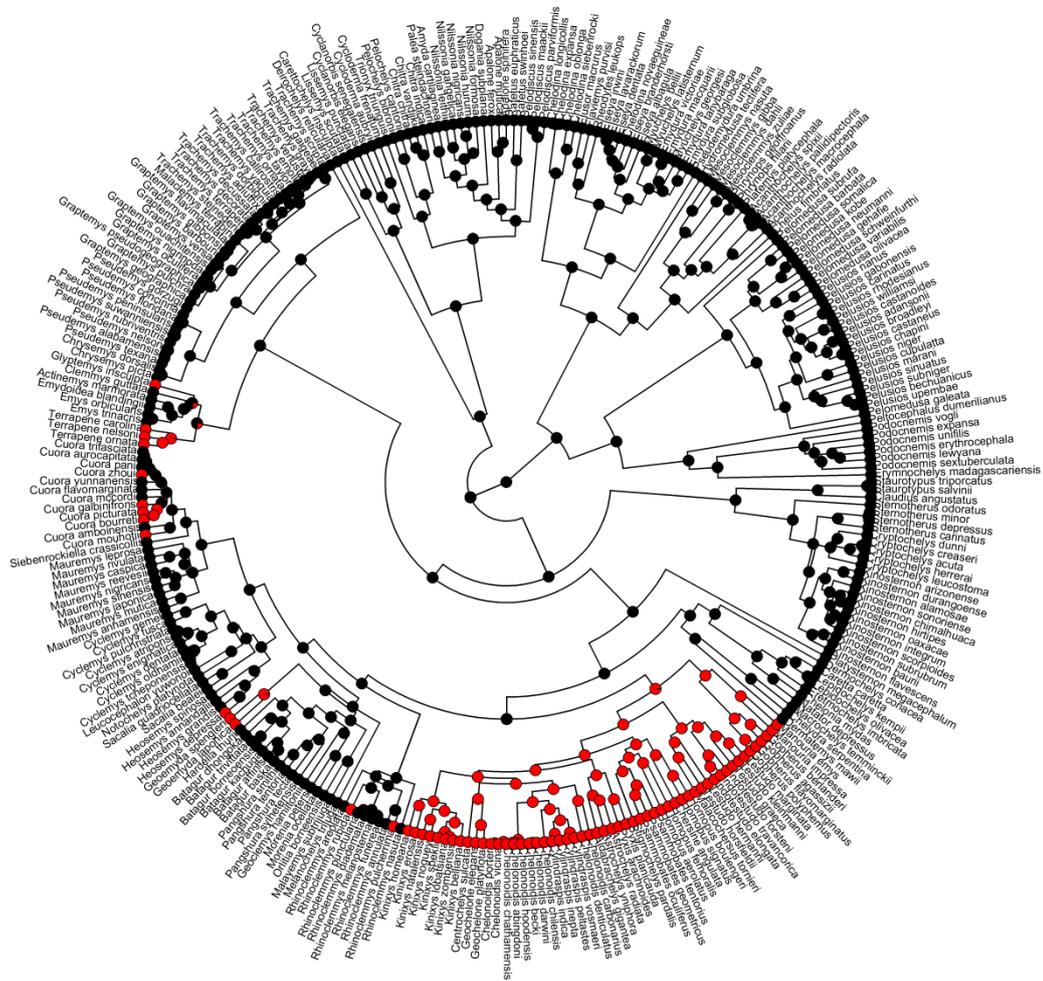
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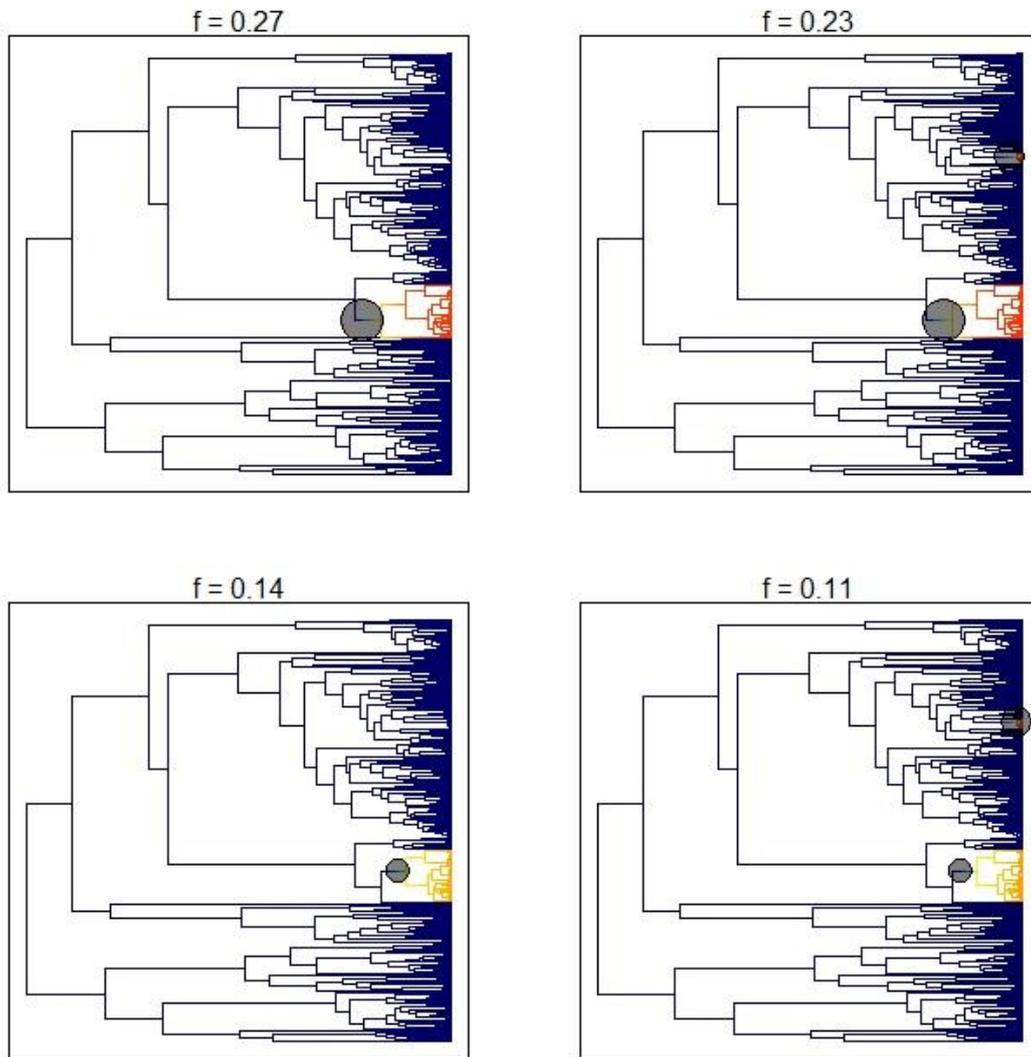
## **A2 - Supplementary Results**

### *BiSSE analyses*

The posterior distribution of speciation rates of aquatic species was slightly higher than the distribution of terrestrial species (Fig. S4).

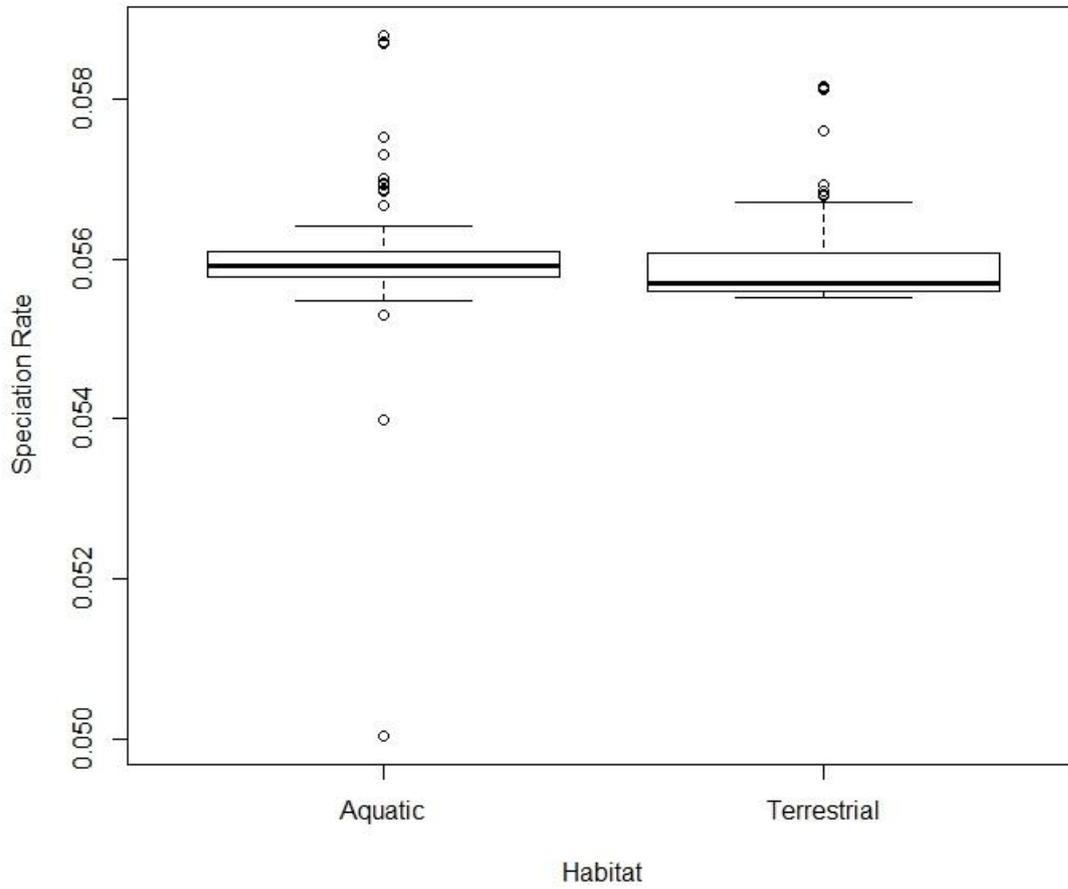


**Fig S1.** Ancestral state reconstruction of habitat using stochastic character mapping along the maximum clade credibility phylogeny of turtles. Aquatic habitat = black; terrestrial habitat = red. Pie charts represent posterior probabilities from the 100 simulations of the stochastic character mapping.

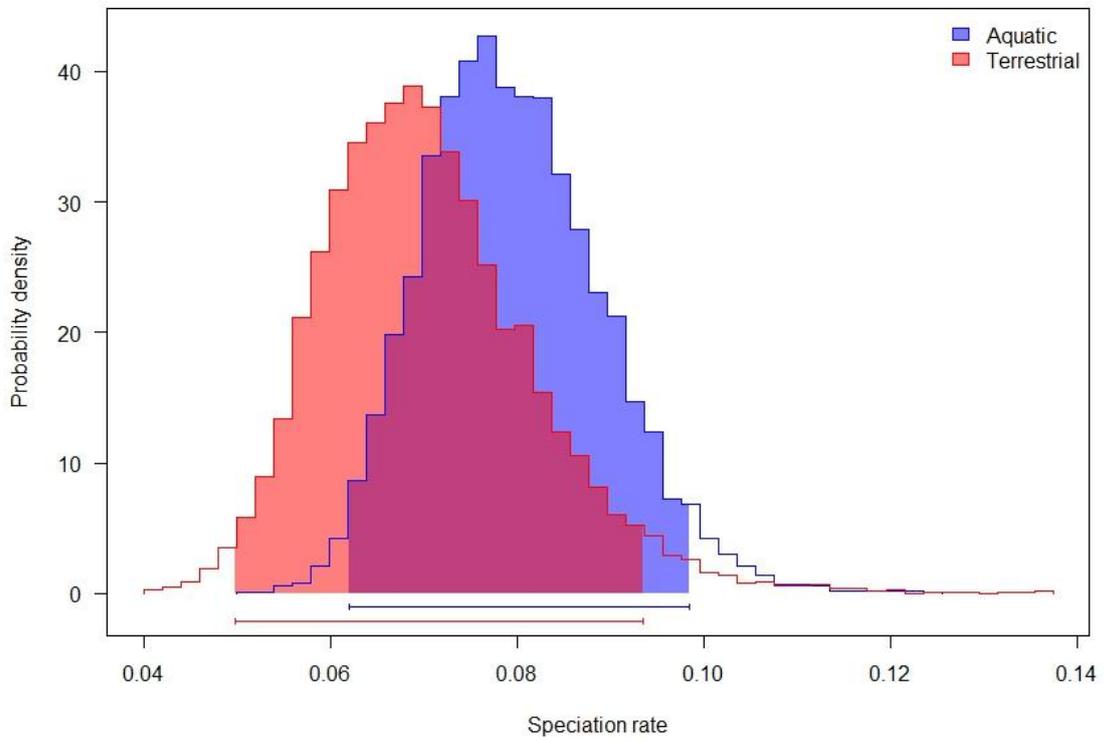


**Fig S2.** The four more frequent rate shift configurations in the Testudines phylogeny reconstructed in our study. Warm colours represent an increase in speciation rate when compared to the ancestral lineage, while the cold ones are reductions. The circles represent the shifts in speciation, and their size is proportional to the marginal probability of the change in the specific branch.  $f$  = frequency or the posterior probability of the rate shifts. The lower circle is the emydid subfamily Deirochelyinae (*Chrysemys* + *Deirochelys* + *Graptemys* + *Malaclemmys* + *Pseudemys* + *Trachemys*), and the upper one represents the Galapagos tortoises. Note that the rate shift in the

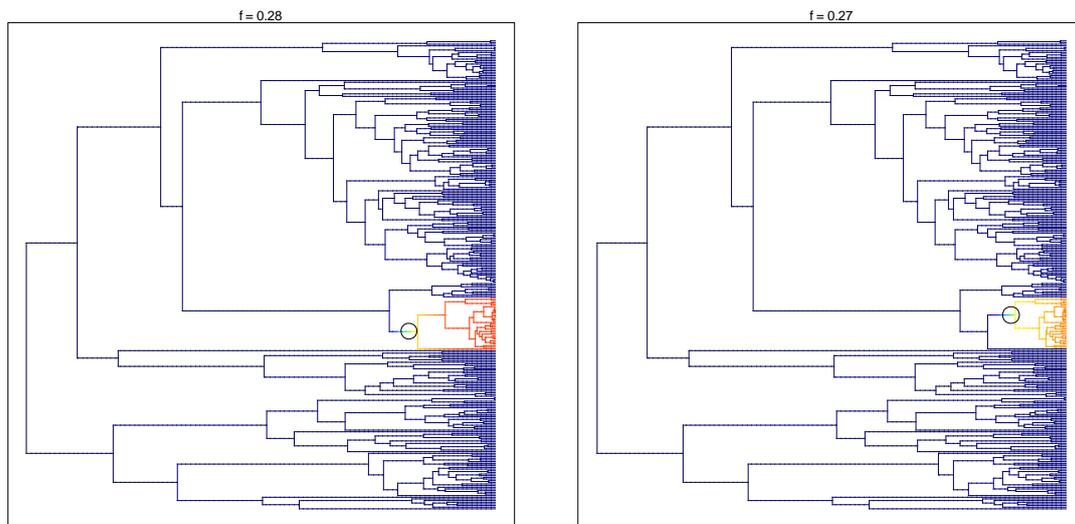
Deirochelyinae includes the species *Deirochelys reticularia* in the two most frequent regimes, but this species is not included in the others.



**Fig S3.** Speciation rate for aquatic and terrestrial turtles after removing the extreme outliers of the aquatic group (subfamily Deirochelyinae) and terrestrial group (Galapagos species).



**Fig S4.** Posterior distribution of speciation rate of aquatic and terrestrial species of turtles obtained from BiSSE model.



**Fig S5:** Diversification regimes in turtles using BAMM after leaving only a single species of Galapagos tortoises and three species of *Pseudemys* strongly supported. Note that the increase in speciation rate in Deirochelyinae remained. Warm colours represent an increase in speciation rate when compared to the ancestral lineage, while the cold ones are reductions. The circle represents the shift in speciation, and its size is proportional to the marginal probability of the change in the specific branch.  $f$  = frequency or the posterior probability of the rate shifts.

## Capítulo 2

Rodrigues, J.F.M., Olalla-Tárraga, M.Á., Iverson, J.B., Akre, T.S.B.,  
Diniz-Filho, J.A.F. (2017): Time and environment explain the current  
richness distribution of non-marine turtles worldwide. *Ecography*. doi:  
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***Time and environment explain the current richness distribution of non-marine turtles worldwide***

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*Time and environment explain the current richness distribution of non-marine turtles worldwide*

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Running title: Global Turtle Macroecology

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## **ABSTRACT**

Ecological, historical, and evolutionary hypotheses are important to explain geographical diversity gradients in many clades, but few studies have combined them into a single analysis allowing a comparison of their relative importance. This study aimed to evaluate the relative importance of ecological, historical, and evolutionary hypotheses in explaining the current global distribution of non-marine turtles, a group whose distribution patterns are still poorly explored. We used data from distribution range maps of 336 species of non-marine turtles, environmental layers, and phylogeny to obtain richness estimates of these animals in  $2^{\circ} \times 2^{\circ}$  cells and predictors related to ecological, evolutionary and historical hypotheses driving richness patterns. Then we used a path analysis to evaluate direct and indirect effects of the predictors on turtle richness. Ancestral area reconstruction was also performed in order to evaluate the influence of time-for-speciation in the current diversity of the group. We found that environmental variables had the highest direct effects on non-marine turtle richness, whereas diversification rates and area available in the last 55 million years minimally influenced turtle distributions. We found evidence for the time-for-speciation effect, since regions colonized early were generally richer than recently colonized regions. In addition, regions with a high number of colonization events had a higher number of turtle species. Our results suggested that ecological processes may influence non-marine turtle richness independent of diversification rates, but probably related to dispersal abilities. However, colonization time was also an important component that must be taken into account. Finally, our study provided additional support for the importance of ecological (climate and productivity) and historical (time-for-speciation and dispersal) processes in shaping current biodiversity patterns.

**Keywords:** climate, diversification rates, freshwater turtles, geographic diversity gradients, macroecology, temperature, time-for-speciation, tortoises

## **Introduction**

Although it is widely known that species richness patterns are the sum of the effects of speciation, extinction and dispersal (Ricklefs 1987, Wiens 2011), understanding why some clades and regions are richer in species than others has been one of the main goals of ecology for centuries (Hawkins 2001). Geographic diversity gradients are well-known worldwide and many hypotheses have been proposed since the 18<sup>th</sup> Century (particularly after the 1960's) to explain these patterns (Hawkins and Porter 2001, Stephens and Wiens 2003, Hawkins et al. 2003b, Mittelbach et al. 2007, Wiens 2011, Brown 2014). Hypotheses explaining richness patterns can be broadly classified into ecological, evolutionary or historical, which are mainly related to differences in environmental factors, diversification rates (speciation minus extinction) and time/area/dispersal, respectively. Despite the fact that the set of potential underlying mechanisms are well established, empirical tests of the hypotheses and the estimation of model parameters to describe such patterns are still challenging (Wiens 2011, Fine 2015).

Many macroecological studies have highlighted the importance of current climate in the context of ecological hypotheses to explain diversity gradients (Hawkins et al. 2003a). The energy hypothesis predicts that areas with high environmental temperatures should support a high diversity, whereas under the productivity hypothesis areas with high primary productivity are expected to harbour high diversity (Hawkins et al. 2003a, b, Brown 2014). Other ecological hypotheses consider climatic stability through time and predict that areas with stable climatic conditions relative to the Last

Glacial Maximum (LGM) are also expected to maintain many species, because few species were able to reach previously glaciated areas since the climatic conditions became warmer (Araújo et al. 2008). Recently, a general consensus is emerging that argues for the need to integrate ecological and evolutionary processes to better account for geographic diversity gradients (Wiens and Donoghue 2004, Fine 2015). For instance, the observed effect of climatic factors on diversity patterns are mediated by changes in diversification and dispersal rates (Wiens 2011, Fine 2015).

Diversification rates (speciation minus extinction) are directly related to the increase and decrease in species number in a community or regional fauna (Ricklefs 1987, Fine 2015). At global scales, these rates are the primary factors determining species diversity and many studies have highlighted the importance of differences in diversification rates (evolutionary explanations) among lineages to shape current diversity patterns (Pyron and Burbrink 2009, Pyron and Wiens 2013, Pyron 2014, Rolland et al. 2014). However, factors other than geographical differences in diversification rates may also be responsible for broad-scale patterns in species richness (Wiens et al. 2006, 2009).

Historical processes related to area and time, which may act concomitantly or not with geographic variations in diversification rates, may also explain diversity patterns in many groups of organisms (Stephens and Wiens 2003, Mittelbach et al. 2007, Wiens 2011, Jetz and Fine 2012, Belmaker and Jetz 2015). Areas that are older or that were larger in the past had more time and space for speciation to occur, thus enhancing high current richness (although it is not trivial to empirically define the “age” of an area) (Chown and Gaston 2000, Fine 2015). Age and area seem to explain well the geographic diversity gradients for several vertebrate clades (Stephens and Wiens 2003, Jetz and Fine 2012, Belmaker and Jetz 2015). However, the effects of time on diversity,

independent of area (time-for-speciation), are also well-known in many vertebrates (Stephens and Wiens 2003, Wiens et al. 2006, 2009). The time-for-speciation effect may shape current diversity patterns without the need to invoke differences in diversification rates (Wiens et al. 2006, 2009, Wiens 2011).

Although the hypotheses explaining biogeographic patterns in species richness have been discussed for a long time, few studies have tested them all together to compare their relative performance (Pyron and Burbrink 2009, Jetz and Fine 2012, Belmaker and Jetz 2015), and much debate still exists regarding their relative ability to describe the observed gradients (Hawkins and Porter 2001, Mittelbach et al. 2007, Wiens 2011, Rabosky and Hurlbert 2015). On the other hand, while most of these hypotheses have been evaluated in endothermic taxa (Hawkins et al. 2005, 2012, Belmaker and Jetz 2015), studies for ectothermic vertebrates are scarce (but see Buckley & Jetz, 2007; Pyron & Burbrink, 2011; Kozak & Wiens, 2012). As a result, developing new studies using animals that are not birds and mammals is important for evaluating the generality of the processes and patterns observed for endotherms.

Non-marine turtles are distributed nearly worldwide and their current total diversity (richness) is roughly 330 species (van Dijk et al. 2014). Despite their widespread distribution, studies seeking to understand the causes of the global diversity gradient in non-marine turtles are scarce. Most knowledge regarding the processes affecting the distribution of these animals has focused on turtles of the family Emydidae from North America, where time-for-speciation and niche conservatism seem to affect their diversity (Stephens and Wiens 2003, 2009). Some authors also found that species richness patterns in freshwater turtles and tortoises are affected by temperature, precipitation and continental area, but do not follow a clear latitudinal gradient (Iverson 1992, Buhlmann et al. 2009, Angielczyk et al. 2015). However, no study so far has

explicitly tested how ecological, evolutionary, and historical processes have jointly affected the diversity of non-marine turtles on a global scale. This study attempts to correct that deficiency and provides additional information regarding the importance of each of these hypotheses in shaping current diversity.

We aimed here to evaluate the relative importance of ecological, evolutionary, and historical hypotheses in explaining global richness patterns of non-marine turtles. First, we examined how much variation in species richness is explained by each hypothesis. Then we used a structural equation modelling approach to assess direct and indirect (mediated through diversification rates) effects of environmental and historical predictors on turtle diversity. Finally, given the effect of time independent of area already documented for the diversity of turtles of the family Emydidae (Stephens and Wiens 2003), we performed additional comparisons to specifically test the time-for-speciation hypothesis for the whole group of non-marine turtles.

## **Material and methods**

### **Data collection**

We used range maps of 336 species of non-marine turtles (freshwater turtles and tortoises, excluding only the marine species - hereafter referred to as “turtles”), which may be viewed in van Dijk *et al.* (2014). We followed the most recent taxonomy proposed by the Turtle Taxonomy Working Group (van Dijk *et al.* 2014) to avoid synonymy, and, we also followed recent re-evaluations of the species *Macrochelys temminckii* (Folt and Guyer 2015), which was recently divided into two allopatric species. The range maps were rasterized in a grid of 2 x 2° degrees including cells with more than 25% of land area to generate a presence-absence matrix of sites (rows) x species (columns). Additional analyses (not discussed here) using a 50% cutoff were not

qualitatively different (see Fig. A6 and A8 in the supplementary material). This grid resolution was adequate to account for errors related to occurrence on range maps (Hurlbert and Jetz 2007). Then, we calculated the richness of turtles by summing the number of species that co-occurred in each grid cell. Grid cells without species were not used in the analyses. Species range overlay and richness maps were obtained in SAM v. 4.0 (Spatial Analysis in Macroecology) (Rangel et al. 2010).

### **Ecological, evolutionary, and historical hypotheses**

To evaluate the importance of ecological, evolutionary, and historical hypotheses to explain the geographic distribution of species richness, we selected a set of variables related to each hypothesis. Regarding the ecological hypotheses, we compiled data for:

- Mean Annual Temperature (Temperature) (Hijmans et al. 2005), related to the energy hypothesis that predicts higher richness in warmer areas;
- Total Annual Precipitation (Precipitation) (Hijmans et al. 2005), related to the hypothesis that areas with more water availability may harbour higher species richness;
- Annual Actual Evapotranspiration (AET) (Ahn and Tateishi 1994), a productivity measure, related to the hypothesis that areas with high productivity are richer than less productive areas;
- Temperature Anomaly (Araújo et al. 2008) in relation to the Last Glacial Maximum (LGM - 22,000 years) (Temperature anomaly), related to the hypothesis that areas with more stable climates support higher species richness.

Temperature (from the present and at the LGM [MIROC Global Circulation Model]) and precipitation data were obtained from WorldClim (<http://www.worldclim.org/>) (Hijmans et al. 2005). Temperature anomaly was calculated as the difference between current temperatures and the values during the

LGM (Araújo et al. 2008). We downloaded data at 10-arc minutes resolution and calculated temperature anomaly at this resolution. Then, we averaged the values for the 2 degree cells used in our study.

We incorporated two variables to test the evolutionary hypothesis. On one hand, we calculated the diversification rate (DR) (Jetz et al. 2012, Belmaker and Jetz 2015) for each species using a posterior sample of 500 time-calibrated phylogenies covering 300 turtle species. These phylogenies were estimated using a Bayesian approach in BEAST (Drummond and Rambaut 2007) on three mitochondrial and two nuclear molecular markers under a lognormal relaxed molecular clock and with fossil data to estimate the divergence times of branch lengths (Rodrigues and Diniz-Filho 2016). DR was calculated only for the species available in our phylogeny, all of which had range maps (281 species). After calculating DR for each species in each phylogeny, we calculated mean DR for each species across all 500 phylogenies. Our results were qualitatively the same when we used median DR instead of mean (see Fig. A5 and A9 in supplementary material). The mean DR for each grid cell was then calculated considering species occurrences. We also calculated the root distance (RD = number of nodes between the tip and the root of the tree) for each species present in our phylogeny and also the mean root distance (MRD) (Kerr and Currie 1999) for each cell as a measure of “diversification rate” using the same routine described above for calculating DR in the 500 phylogenies. We repeated the analyses using MRD in order to evaluate the influence of this metric of diversification rate and because MRD is commonly used in macroecological studies evaluating evolutionary effects on diversity patterns (Hawkins et al. 2005, 2012). The results for the MRD analyses were qualitatively the same as using DR (see supplementary materials). We acknowledge that using incomplete phylogenies may have potential biases due to non-random sampling of taxa

(Heath et al. 2008), but it is not likely a problem for this study because the phylogeny used has a high sampling fraction for all the turtles families, and especially the rich ones.

Finally, to evaluate the historical hypothesis, we used the data of Area x Time (AREATIME) for different bioregions provided in recent publications (Jetz and Fine 2012, Belmaker and Jetz 2015). This measure is based on the amount of area that some bioregions had over the last 55 million years (see Jetz & Fine, 2012 for more details regarding how these measures were estimated). The classification of these bioregions is based on vegetation/biomes (e.g., Tropical Moist Forests, Boreal Forests, Temperate forests) and biogeography (e.g., South America, North America, Africa, Eurasia) (Jetz and Fine 2012). Considering that area availability is an important surrogate of species richness and is related to more opportunities for speciation (Chown and Gaston 2000), it is expected that locations with greater total area over the last 55 million years would have higher species richness. Since measures of area x time are allocated to each bioregion, we obtained this measure for our  $2^{\circ} \times 2^{\circ}$  grid cells by overlapping the bioregions map (Olson et al. 2001) on our grid and collecting the area x time measure for each cell.

To evaluate the time-for-speciation hypothesis without possible confounding effects of area, we reconstructed ancestral areas of the species present in our phylogeny and used the age of the oldest endemic lineage in a region as an estimate of its potential colonization time (Wiens et al. 2006, 2009, Wiens 2011). Besides using this traditional approach, we also used an approach recently described as Colonization Based on Reconstructed Range Size and Location (CRRL), which takes range size into account (Wu et al. 2014). In this method, we used the ages of the oldest lineages occurring in more than one ecoregion to determine the colonization times of each single region. For

example, if there are three areas, A, B and C, and the oldest ancestor for an area AC occurred at 40Mya and the oldest ancestor for an area AB occurred at 30Mya, the first colonization in area A would be estimated as 40Mya (maximum among AB and AC). Besides, it is also possible to calculate two other age measures of colonization time, which include additional biogeographic interpretations: the sum of colonization times, which is 70Mya (30 + 40) in our example and takes into account the number of colonization events, an important component of the historical biogeography that is also related to the diversity of a clade in a given area; and the mean colonization time, 35Mya  $((30 + 40) / 2)$ , which takes into account the uncertainty in the first colonization event (see Wu *et al.*, 2014 for more details).

For this specific analysis of time-for-speciation, we chose to work on a regional scale instead on a grid scale, because reconstructing species origin based on grid cells is computationally infeasible and biologically meaningless. Then, we assigned a region for each species in the phylogeny following the classification proposed by Buhmann *et al.* (2009), who divided non-marine turtles into seven different major biogeographic regions: A = Africa Sub-Saharan, B = Asia (including Indonesia and Philippines), C = Australasia (Australia + New Guinea + islands east of Weber's line), D = Central America (Panama to Mexico, including Caribbean; northern Neotropical), E = Mediterranean, F = North America (Canada and United States; Nearctic), G = South America (southern Neotropical) (Buhmann *et al.* 2009). Some species occur in more than a region, and so they received a code corresponding to a combination of both regions (e.g., DF = species occurring in Central and North America). Ancestral state reconstruction was performed in RASP (Reconstruct Ancestral State in Phylogenies) (Yu *et al.* 2015) version 3.2. We used the S-DEC model (Statistical-Dispersal-Extinction-Cladogenesis or Bayes-Lagrange) which allows accounting for phylogenetic

uncertainty in the ancestral state reconstruction (Yu et al. 2015). The results of the ancestral reconstruction were mapped in the Maximum Clade Credibility (MCC) phylogeny of the group. In the ancestral reconstruction, we allowed a maximum of two ancestral regions for each node because this is the maximum number of regions occupied by current species. Ancestral ranges were restricted to both currently adjacent regions and past continental bridges (e.g., South America – Africa and North America – Asia connections were allowed). Considering that each node receives probabilities of having occurred in a given area according to the S-DEC model, we used three threshold values to determine the ancestral range of each node (Probability > 0.75, 0.85 and 0.95). Results using these three thresholds were similar, so only results of  $P > 0.95$  are shown to conserve space (see Table A1 in supplementary material for results using the other thresholds).

## **Analyses**

First, we performed a partial regression to evaluate the amount of variation in richness explained by each group of hypotheses (Ecological, Evolutionary, and Historical) as measured by the corresponding set of associated variables. Because the residuals of our regressions were strongly spatially structured according to Moran's I spatial correlograms, we generated spatial filters (Spatial Eigenvector Mapping) (Diniz-Filho and Bini 2005, Griffith and Peres-Neto 2006) to explicitly account for spatial processes not included in explanatory variables. Spatial filters were selected on the basis of statistically significant reductions in Moran's I autocorrelation index in the residuals of the regression when the filters were included in the analysis (Griffith and Peres-Neto 2006). These spatial filters were grouped in a fourth group named "space" and were obtained using the *spdep* package in R.

We then used Structured Equation Modelling (SEM) to evaluate the indirect and direct effects of each explanatory variable on turtle species richness. Our path analyses had three paths. The first one was  $AET \sim \text{Temperature} + \text{Precipitation}$ , where AET was modelled as a function of temperature and precipitation. The second path was given as  $DR \sim AET + \text{Temperature} + \text{Temperature Anomaly} + AREATIME$ , where diversification rate (DR) was modelled as a function of temperature, AET, temperature anomaly and area x time, considering current hypotheses that temperature positively influences biological rates, such as diversification rates (Brown 2014, Dugo-Cota et al. 2015), that climatic stability through time allows a high diversification rate by having reduced extinction rates (Dynesius and Jansson 2000, Fine 2015), and that larger areas through time may represent more opportunity for allopatric speciation and consequently diversification rates (Chown and Gaston 2000, Fine and Ree 2006). The third path was  $\text{Richness} \sim AET + \text{Temperature} + \text{Precipitation} + \text{Temperature Anomaly} + AREATIME + \text{Area} + DR$ , where ecological (AET, Temperature, Precipitation, Temperature Anomaly), historical (AREATIME) and evolutionary (DR) processes were allowed to directly influence the diversity of turtles. In this last path, we also included the area of each cell in order to account for methodological biases due to differences in this variable among cells. In all the paths of our model, we included a linear combination of filters (the predicted values of the linear regression between the response variable of each path and the filters selected to reduce the spatial autocorrelation of the residuals of the regression between the dependent and independent variables within each path) to explicitly include spatial processes not accounted by the explanatory variables. Path analyses were performed using the package lavaan (Rosseel 2012) in R, and the path diagrams were drawn in the software CmapTools version 6.01.01 (<http://cmap.ihmc.us>).

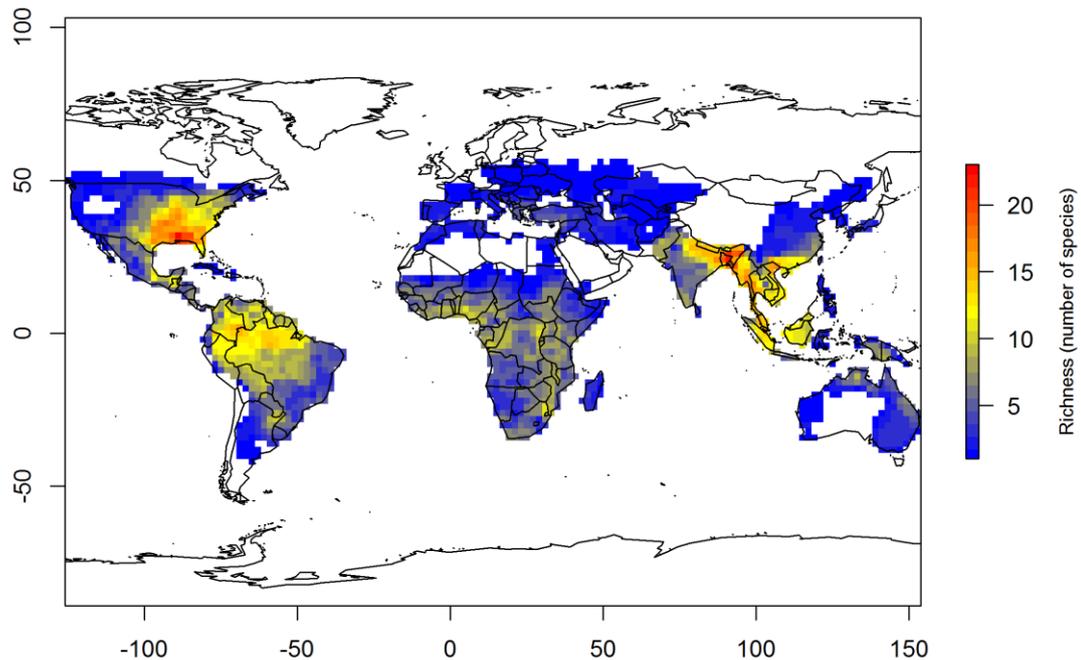
All the variables were standardized to have mean = 0 and variance = 1 prior to all the analyses.

Finally, to properly evaluate the time-for-speciation hypothesis, we regressed the log-transformed ( $\ln$ ) species richness of each of the seven regions used to reconstruct ancestral states against the colonization age of each region, following the approach commonly used in related studies (Wiens et al. 2006, 2009). We also performed simple linear regressions to evaluate how the ages derived from the approach of Wu *et al.* (2014) explain the richness pattern. Since some species occurred in two regions, we generated two vectors of species richness considering these widely distributed species as occurring in only one region (e.g. all species occurring in AB were considered as A in an analysis and as B in the other). However, analyses using both vectors had very similar results, so we present results for only one of the combinations to conserve space (see table A1 in supplementary material for both results). We also ran these time-for-speciation analyses using as response variable the residuals of the regression between richness and area (residual richness) in order to account for differences in area among the regions (see table A2 in supplementary material). Considering that regions close to each other may share similar richness or colonization time due to their proximity and connection, we inspected the existence of spatial correlation in all our variables using Moran's I coefficient under 999 random permutations and the same connectivity matrix used in RASP.

## **Results**

We found a global pattern of turtle richness similar to the one reported in previous studies (Fig. 1) (Buhlmann et al. 2009, Angielczyk et al. 2015). The richest areas are located in the Southeastern United States and the Indo-Malayan region, where cells

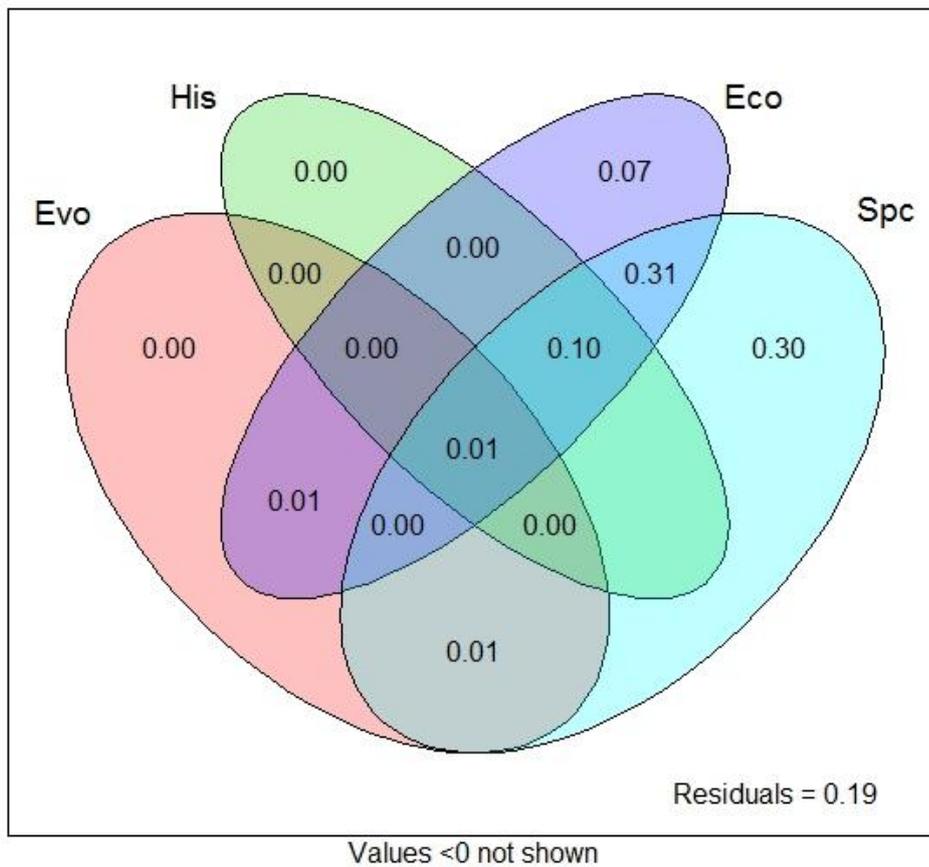
harbour up to 23 and 22 species, respectively. It is not possible to realize a clear latitudinal diversity gradient in turtle richness, although the diversity of these animals is lower at high latitudes than at medium and low latitudes.



**Figure 1:** Species richness of non-marine turtles based on global 2° x 2° grid cells. Note the high species richness in the Southeastern United States and in the Indo-Malay region. Cold colors (blue) represent areas with low richness, while warm colors (red) represent areas with high richness.

Most of the variance in richness was explained by spatial filters and their covariance with ecological factors (Fig. 2, see also Fig. A4, A5 and A6). Among the three groups of hypotheses using DR, ecological hypotheses explained a higher amount

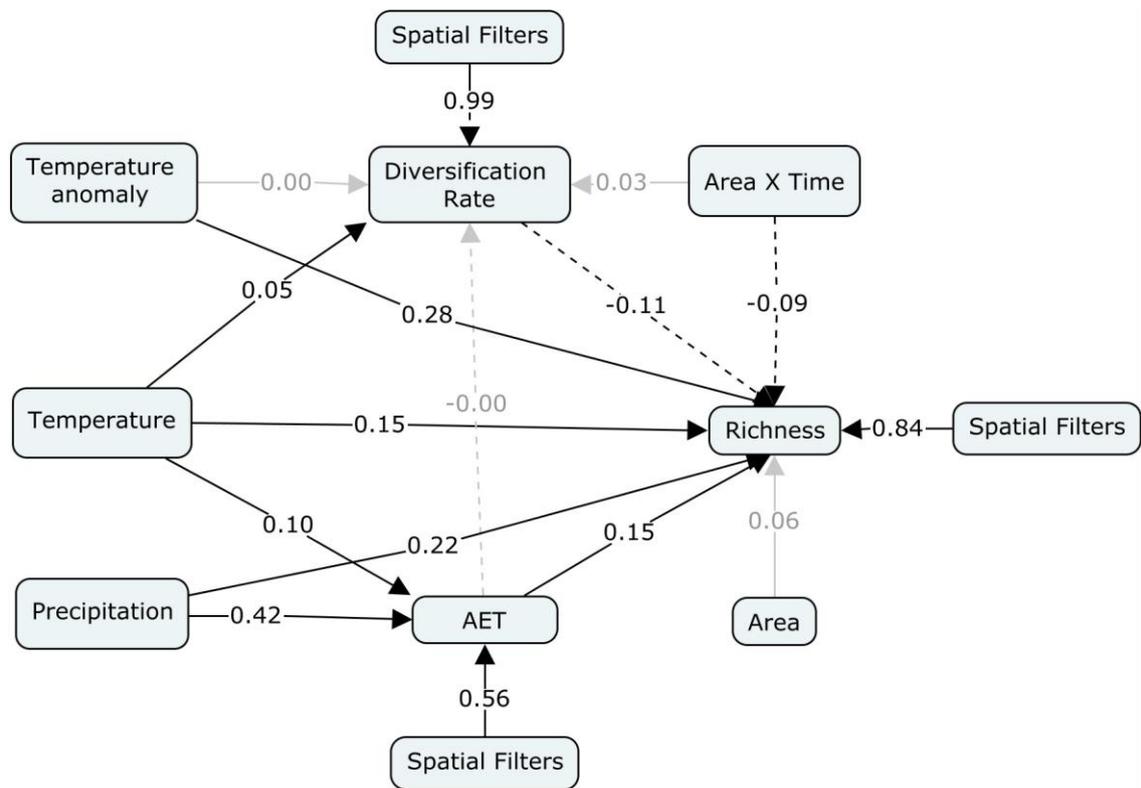
of variance (adjusted  $R^2 = 50\%$ ) than historical (adjusted  $R^2 = 12\%$ ) or evolutionary (adjusted  $R^2 = 3\%$ ) hypotheses.



**Figure 2:** Variance in turtle richness explained by each group of hypotheses. Note the high importance attributed to ecological and spatial components. Evo = Evolutionary hypotheses, explanatory variable = Diversification Rate; His = Historical hypotheses, explanatory variable = Area x Time; Eco = Ecological hypotheses, explanatory variables = Mean Annual Temperature, Total annual precipitation, Annual Actual

Evapotranspiration and Temperature Anomaly in relation to the Last Glacial Maximum;  
 Spc = spatial filters used to account for spatial autocorrelation.

The coefficients of the path analysis using DR are shown in Fig. 3. The highest coefficients are for the linear combination of filters, but the direct effects of temperature anomaly and precipitation had a moderate-weak effect (0.28 and 0.22) on species richness. Area x Time and Diversification rates had effect sizes lower than the environmental variables (Fig. 3, see also Fig. A7, A8 and A9).

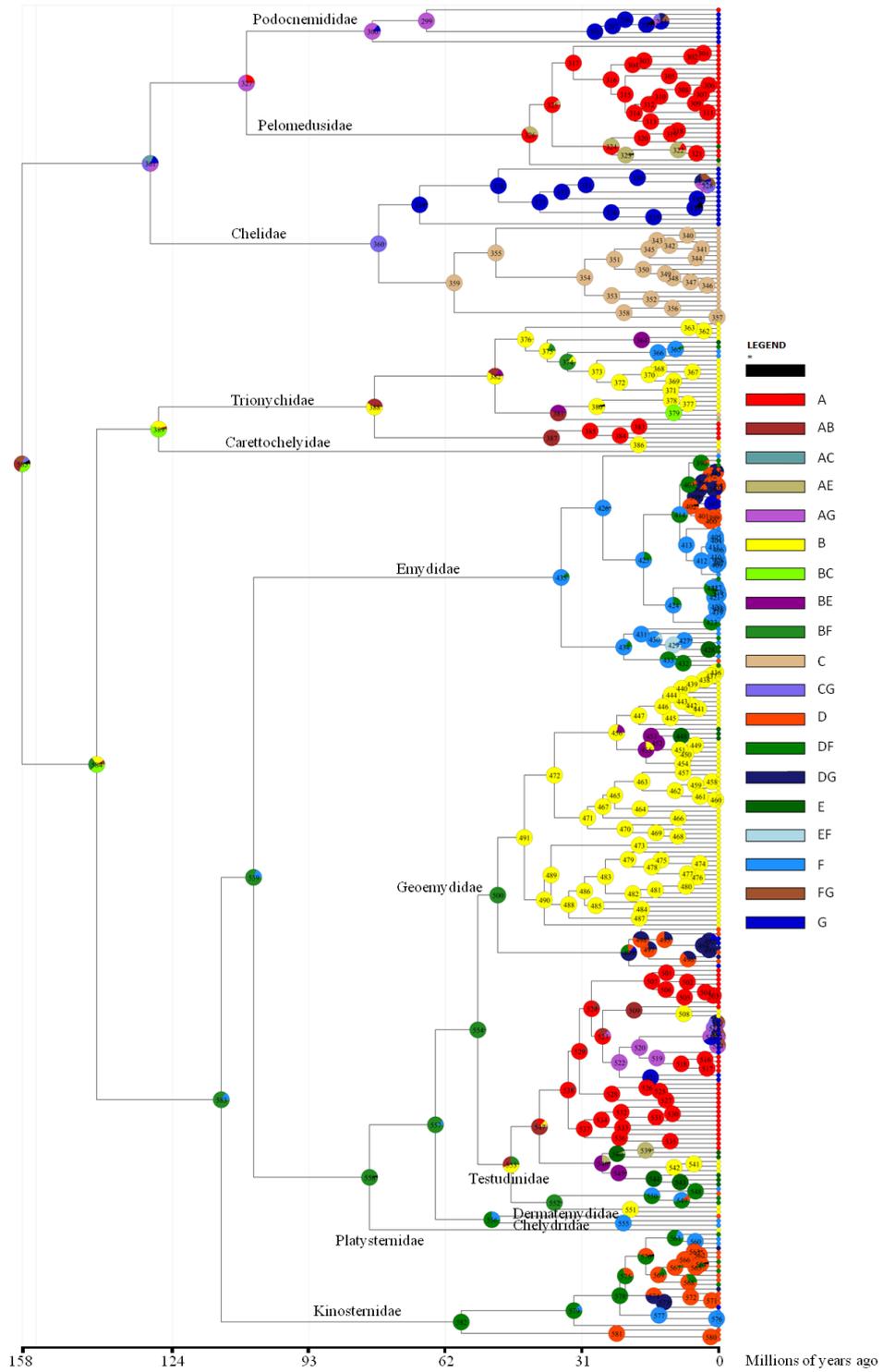


**Figure 3:** Path analysis considering direct and indirect effects of the explanatory variables (using Diversification Rate) on turtle species richness. Temperature anomaly, Total Annual Precipitation, and Annual Actual Evapotranspiration (AET) were the main variables directly influencing species richness. RMSEA = 0.101 and R-squared for the dependent variables AET, Diversification Rate and Richness were 0.917, 0.758 and

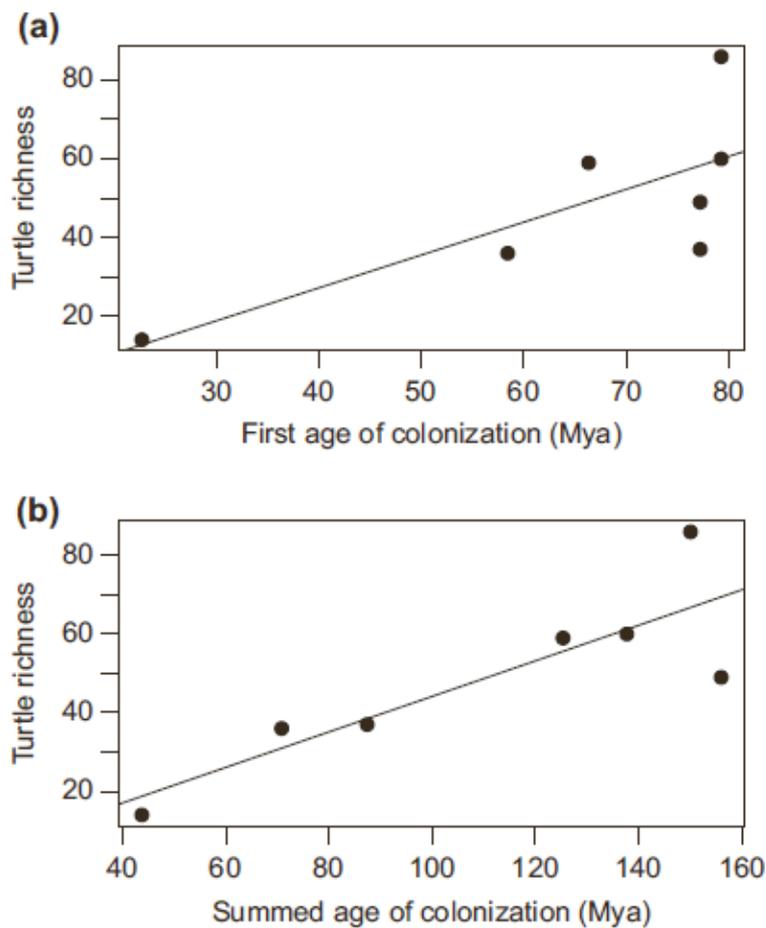
0.760, respectively. Solid black lines are positive effects, dashed black lines represent negative effects, and gray lines (solid or dashed) are non-significant paths. Coefficients equal to 0.00 represent values lower than 0.01.

The reconstruction of ancestral areas for turtles recovered many dispersal events among regions (Fig. 4). The most recent common ancestor of the whole group was assigned to Asia-Australia with the highest probability (31%). Species richness in each ecoregion was not explained by the time-of-colonization of the oldest ancestor ( $F = 1.00$ ,  $df = 1,5$ ,  $P = 0.36$ ) according to the traditional time-for-speciation literature (Wiens et al. 2006, 2009). When we used the colonization times estimated based on the Wu *et al.* (2014) approach, we found that species richness was not explained by the mean colonization time ( $F = 1.26$ ,  $df = 1,5$ ,  $P = 0.31$ ). However, it was explained by the first colonization time ( $F = 6.53$ ,  $df = 1,5$ ,  $P = 0.05$ ) or by the sum of colonization times ( $F = 13.38$ ,  $df = 1,5$ ,  $P = 0.01$ ; Fig. 5; see also Table A1 for results using different threshold probabilities to consider a clade as confidently occurring in an area in the past). When using the residual richness, the colonization times according to Wiens' approach still had no effect on turtle richness, but for Wu's approach colonization time did influence the richness residuals, reinforcing the time-for-speciation effect (Table A2). In addition, the threshold decision used to define ancestral areas impacted which measure of time influenced residual richness patterns: at low thresholds (75% and 85%) mean colonization times influenced richness, while at the high one (95%) the summed colonization times had a significant effect. First colonization time was significant for all the thresholds used. Moran's I coefficients for both response and explanatory variables, based on a connectivity matrix linking the continental areas, were not significant using

999 random permutations, so it is unlikely that spatial autocorrelation is biasing the statistical significance of regression coefficients.



**Figure 4:** Ancestral state reconstruction of occurrence areas of turtle nodes. A = Africa Sub-Saharan, B = Asia (including Indonesia and Philippines), C = Australasia (Australia + New Guinea + islands eastern Weber's line islands), D = Central America, E = Mediterranean, F = North America, G = South America (Buhlmann et al. 2009). Time-scale is in Million years ago. See supplementary material table A3 for a full list of the ancestral occurrence probabilities estimated for each node.



**Figure 5:** The relationship between turtle richness in seven regions and first age of colonization (a) and summed age of colonization (b) of each region. Note that the areas colonized earlier are usually richer than the more recently colonized ones, reinforcing the time-for-speciation effect.

## Discussion

Three main findings emerge from our spatially-explicit and phylogenetically-informed analyses on the causes of the global diversity gradient of turtles: 1) ecological processes may have an effect independent from diversification rates, but probably linked to dispersal, on species richness; 2) historical (as measured through Area x Time) and evolutionary processes had a low effect on the geographic variation in richness of extant turtle species when compared to the ecological processes; 3) time-for-speciation allied with colonization events is a valid historical hypothesis to explain current turtle richness. In other words, we found that ecological and historical (focusing specifically on time rather than on Area x Time) hypotheses are more likely to explain the geographic distribution in species richness of non-marine turtles.

The influence of environmental variables on species richness is commonly detected in macroecological analyses for many vertebrate groups (Hawkins et al. 2003b, 2005, Buckley and Jetz 2007, Araújo et al. 2008), including turtles (Iverson 1992, Ihlw et al. 2012, Angielczyk et al. 2015). However, in recent years, much attention has been directed to the importance of historical (differences in time and area and dispersal) and evolutionary (differences in diversification rates) processes in shaping the current biodiversity patterns, such as latitudinal and altitudinal gradients of biodiversity, or even to understand local community structure (Ricklefs 1987, Wiens 2011, Kozak and Wiens 2012). Our study found that climatic variables commonly linked to ecological hypotheses (AET, temperature anomaly, precipitation and temperature) had much higher effect sizes than historical and evolutionary processes for turtle distribution. We also found that environmental variables are poorly related to diversification rates in turtles (Fig. 3, but see results for MRD and median DR in supplementary material),

which does not support the indirect effect of environmental variables on richness patterns through diversification rates, a mechanism commonly used to explain richness gradients (Wiens 2011, Brown 2014). Even when environmental variables influenced diversification measures, diversification had an effect weaker than the ecological predictors. This unexpected direct effect of ecological variables may be explained by the high influence of environmental variables on the distribution of ectothermic animals and, more specifically, non-marine turtles (Araújo et al. 2008, Bombi et al. 2011, Ihlow et al. 2012). The high degree of explanation shared among ecological factors and spatial filters in variance partitioning analyses and the high coefficients of spatial filters in path analyses suggest the importance of spatial factors not accounted for by our variables, which could be related to dispersal limitations. The many changes in turtle distribution through their evolutionary history (Fig. 4) provide additional insight into the importance of dispersal in shaping current biodiversity in this group. For example, high values of productivity and high values of current temperature compared to the LGM may characterize areas that are more prone to receive migrant species, favouring dispersal to such localities, similar to a species sorting effect (Leibold et al. 2004). Finally, niche conservatism, which has already been raised to explain diversity patterns of the turtle family Emydidae (Stephens and Wiens 2009), could also explain this strong climatic influence on richness patterns of turtles and the lack of relationship with diversification rates (Wiens and Donoghue 2004, Wiens et al. 2010, Wiens 2011).

Our analyses found a weak and negative relationship between diversification rates and extant diversity patterns of turtles (Fig. 3). These rates are directly related to the current diversity of birds and mammals (Belmaker and Jetz 2015), amphibians (Pyron and Wiens 2013), and squamate reptiles (Pyron 2014). However, other studies have already found that differences in diversification rates are not sufficient to explain

some diversity patterns (Wiens et al. 2006, 2009, Jetz et al. 2012). The negative effect of DR on richness is contrary to findings from previous studies (Kozak and Wiens 2016, Scholl and Wiens 2016) and might be explained by the occurrence of young clades with high diversification rates but with low richness (Wiens 2011). Such a scenario might be common in an assemblage-measure of diversification such as the ones we used in our study, because highly diversifying clades may invade new areas, creating patterns of low richness for assemblages with high diversification rates. High dispersal is hypothesized to mask diversification rate effects (Belmaker and Jetz 2015), and this explanation could apply to turtles, considering the high number of regional transitions found in our ancestral range reconstruction. The methods used to estimate diversification rates may also present biases and much debate still exists regarding the ability of recovering evolutionary rates from molecular phylogenies (Pyron and Burbrink 2013, Morlon 2014). This could also mask a relationship between the environmental variables and these evolutionary rates. However, our results are consistent irrespective of the metric used [both Mean Root Distance (MRD) and diversification rate (DR) provided qualitatively similar results]. These metrics have been commonly used in many macroecological studies (Jetz et al. 2012, Hawkins et al. 2012, Belmaker and Jetz 2015), suggesting that our findings are not metric-dependent.

The areas of the various bioregions over the last 55 million years were not important in describing current turtle diversity when compared to other predictor variables. This apparently contrasts with previous findings for mammals, birds, amphibians, and plants (Fine and Ree 2006, Jetz and Fine 2012). Larger areas are expected to allow more speciation events, although this relationship might not be linear (Chown and Gaston 2000). However, there are more uncertainties in measures of area x time than in current environmental variables, which may explain its lack of importance.

Besides, the temporal dynamics of the biomes instead of their total area through time may also influence diversity patterns, as observed for palm species in Africa (Kissling et al. 2012). This represents an interesting area for future evaluations.

Although biomes might also be a good surrogate of habitat diversity for turtles, the very weak influence of area x time on turtle diversity may be explained by the high proportion of aquatic species in the group, which presumably are more directly influenced by rivers and wetlands than for ecoregional terrestrial areas. Considering this possibility and that drainage basins have also changed over the history of the planet (Galloway et al. 2011), distribution patterns in turtles may be more linked to the evolution of drainage basins, and display patterns similar to the ones reported for fishes (Schonhuth et al. 2015). Future studies modelling the evolution of freshwater habitats over the history of the planet could provide interesting insights for understanding turtle diversity. Finally, although we classified climate instability as an ecological driver, it could also be considered a historical one (Kissling et al. 2012), since its effects are dispersed along the last 20,000 years, which would reinforce the importance of history in explaining turtle diversity.

Reconstruction of ancestral ranges in turtles found that the seven major regions where turtles currently occur were colonized several times and at many different time periods across the evolutionary history of the group (Fig. 4). Such multiple colonization events may explain why the summed measure of age of the colonization of each biome was a better predictor of its diversity than the age of the oldest colonization and other metrics. Wu *et al.* (2014) highlighted that this metric also takes colonization events into account, which could explain why it was better than metrics considering only time. However, it is important to highlight that when using residual richness the time predictors derived from Wu *et al.* (2014) explaining residual richness changed

according to the threshold used to define the ancestral age (see Table S2). Using low thresholds increased the uncertainty regarding the ancestral ages estimates, which might explain why mean colonization time (which also reflects uncertainty) had significant effects. The importance of time-for-speciation in turtles, specially for the family Emydidae, is already known (Stephens and Wiens 2003), and our results using the Wu *et al.* (2014) approach, whether using richness or residual richness, also reinforced the importance of colonization time in shaping turtle diversity.

A potential source of bias that could have affected our results is human-caused extinctions, which could mask or obscure relationships between diversity and its drivers (Faurby and Svenning 2015). It is known that some species of turtles were hunted to extinction by humans (Rhodin *et al.* 2015). Future studies covering a broad compilation of turtle fossils could provide interesting additional perspectives on our findings.

To our knowledge, this is the first study to evaluate turtle richness worldwide using grid cells as sample units and testing the importance of the three major hypotheses to explain diversity patterns in these organisms. We conclude that environmental processes do not influence richness through diversification rates, but probably due to dispersal restriction and niche conservatism, and that time-for-speciation is a valid hypothesis to explain some diversity patterns in turtles. Our study also provides additional support for the importance of ecological (climatic instability and productivity) and historical (time-for-speciation and dispersal) processes in shaping the current biodiversity.

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Supplementary material (Appendix EXXXXX at ). Appendix 1

## Supplementary material

### *Time and environment explain the current richness distribution of non-marine turtles worldwide*

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Rodrigues, J. F. M. et al. XXXX. Time and environmental variables explain the current richness distribution of non-marine turtles worldwide. – *Ecography* 000: 000–000.

## Appendix 1

## Supplementary results

Mean Root Distance (MRD) analyses provided qualitatively similar results than diversification rate (DR) regarding the relative importance of ecological, historical, and evolutionary hypotheses (50%, 13%, and 1% respectively, see (Fig. A3). When using median DR instead of mean DR, results were also similar (ecological = 50%, historical = 12%, and evolutionary = 3%, see Fig. A4). Finally, when we included in our analyses only cells with more than 50% of land area, our results still remained the same (ecological = 54%, historical = 12%, and evolutionary = 3%, see Fig. A5).

**Table A1.** Relationship between colonization time and richness under different threshold probabilities used for account for uncertainty in ancestral region reconstruction. “Richness 1” and “Richness 2” are richness values obtained when species occurring in more than one region (21 species occurring in two regions) were classified as occurring in a single region. Thus, in this new classification, if five species occurred in, for example, AB, AB, BC, DE, and EF, they were reclassified as occurring in 1) A, A, B, D, and E; and 2) B, B, C, E, and F. In “First Wiens”, colonization time was the age of the oldest endemic lineage of the region. In “First Wu”, “Sum”, and “Mean”, colonization times were calculated following Wu et al. (2014) (see Material and Methods or Wu et al. 2014 for more details regarding these estimates).

| <b>Model</b>              | <b>F</b> | <b>df</b> | <b>P</b> |
|---------------------------|----------|-----------|----------|
| <i>Richness 1</i>         |          |           |          |
| <i>Probability = 0.75</i> |          |           |          |
| First Wiens               | 0.89     | 1,5       | 0.39     |
| First Wu                  | 22.62    | 1,5       | 0.005    |
| Sum                       | 16.06    | 1,5       | 0.01     |
| Mean                      | 4.21     | 1,5       | 0.09     |
| <i>Probability = 0.85</i> |          |           |          |
| First Wiens               | 0.89     | 1,5       | 0.39     |
| First Wu                  | 7.36     | 1,5       | 0.04     |
| Sum                       | 9.01     | 1,5       | 0.03     |
| Mean                      | 1.76     | 1,5       | 0.24     |
| <i>Richness 2</i>         |          |           |          |
| <i>Probability = 0.75</i> |          |           |          |
| First Wiens               | 1.15     | 1,5       | 0.33     |
| First Wu                  | 8.96     | 1,5       | 0.03     |
| Sum                       | 8.06     | 1,5       | 0.04     |
| Mean                      | 2.89     | 1,5       | 0.15     |
| <i>Probability = 0.85</i> |          |           |          |
| First Wiens               | 1.15     | 1,5       | 0.33     |
| First Wu                  | 7.10     | 1,5       | 0.04     |
| Sum                       | 7.03     | 1,5       | 0.04     |
| Mean                      | 1.80     | 1,5       | 0.24     |

\*Wu, Y. et al. 2014. Understanding historical and current patterns of species richness of babblers along a 5000-m subtropical elevational gradient. - Glob. Ecol. Biogeogr. 23: 1167–1176.

**Table A2.** Relationship between colonization time and residual richness under different threshold probabilities used for account for uncertainty in ancestral region reconstruction. Residual richness values were obtained through a regression between richness and area of the biogeographic regions. “Richness 1” and “Richness 2” represent two richness scenarios when species occurring in more than one region (21 species occurring in two regions) were classified as occurring in a single region. Thus, in this new classification, if five species occurred in, for example, AB, AB, BC, DE, and EF, they were reclassified as occurring in 1) A, A, B, D, and E; and 2) B, B, C, E, and F. In “First Wiens”, colonization time was the age of the oldest endemic lineage of the region. In “First Wu”, “Sum”, and “Mean”, colonization times were calculated following Wu et al. (2014) (see Material and Methods or Wu et al. 2014 for more details regarding these estimates).

| <b>Model</b>              | <b>F</b> | <b>df</b> | <b>P</b> |
|---------------------------|----------|-----------|----------|
| <i>Richness 1</i>         |          |           |          |
| <i>Probability = 0.75</i> |          |           |          |
| First Wiens               | 0.94     | 1,5       | 0.38     |
| First Wu                  | 15.77    | 1,5       | 0.01     |
| Sum                       | 5.55     | 1,5       | 0.06     |
| Mean                      | 11.63    | 1,5       | 0.02     |
| <i>Probability = 0.85</i> |          |           |          |
| First Wiens               | 0.94     | 1,5       | 0.38     |
| First Wu                  | 40.6     | 1,5       | 0.001    |
| Sum                       | 5.58     | 1,5       | 0.06     |
| Mean                      | 8.14     | 1,5       | 0.04     |
| <i>Probability = 0.95</i> |          |           |          |
| First Wiens               | 1.18     | 1,5       | 0.33     |
| First Wu                  | 29.01    | 1,5       | 0.003    |
| Sum                       | 8.04     | 1,5       | 0.04     |
| Mean                      | 3.66     | 1,5       | 0.11     |
| <i>Richness 2</i>         |          |           |          |
| <i>Probability = 0.75</i> |          |           |          |
| First Wiens               | 1.16     | 1,5       | 0.33     |
| First Wu                  | 8.52     | 1,5       | 0.03     |
| Sum                       | 4.23     | 1,5       | 0.09     |
| Mean                      | 6.79     | 1,5       | 0.05     |
| <i>Probability = 0.85</i> |          |           |          |
| First Wiens               | 1.16     | 1,5       | 0.33     |
| First Wu                  | 24.33    | 1,5       | 0.004    |
| Sum                       | 4.95     | 1,5       | 0.08     |
| Mean                      | 6.45     | 1,5       | 0.05     |
| <i>Probability = 0.95</i> |          |           |          |

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|             |       |     |       |
|-------------|-------|-----|-------|
| First Wiens | 1.66  | 1,5 | 0.25  |
| First Wu    | 17.96 | 1,5 | 0.008 |
| Sum         | 7.29  | 1,5 | 0.04  |
| Mean        | 1.80  | 1,5 | 0.24  |

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**Table A3.** Probabilities of the ancestral range reconstruction for each node using S-DEC. A = Africa Sub-Saharan, B = Asia (including Indonesia and Philippines), C = Australasia (Australia + New Guinea + islands eastern Weber's line islands), D = Central America, E = Mediterranean, F = North America, G = South America. Note that combinations of two regions were allowed in the analysis.

| Node | Ancestral states and their corresponding probability |
|------|--|
| 294  | G 39.34 AG 17.80 CG 14.31 DG 14.28 FG 14.27          |
| 295  | G 89.77 AG 3.70 CG 2.18 DG 2.17 FG 2.17              |
| 296  | G 99.94 AG 0.04 CG 0.00 FG 0.00 DG 0.00              |
| 297  | G 99.87 AG 0.12 CG 0.00 FG 0.00 DG 0.00              |
| 298  | G 99.24 AG 0.74 CG 0.00 FG 0.00 DG 0.00              |
| 299  | AG 99.77 G 0.23                                      |
| 300  | AG 86.50 G 13.50                                     |
| 301  | A 100.00   |
| 302  | A 100.00   |
| 303  | A 100.00   |
| 304  | A 100.00   |
| 305  | A 100.00   |
| 306  | A 100.00   |
| 307  | A 100.00   |
| 308  | A 100.00   |
| 309  | A 100.00   |
| 310  | A 100.00   |
| 311  | A 100.00   |
| 312  | A 100.00   |
| 313  | A 100.00   |
| 314  | A 100.00   |
| 315  | A 100.00   |
| 316  | A 100.00   |
| 317  | A 100.00   |
| 318  | A 100.00   |
| 319  | A 100.00   |
| 320  | A 100.00   |
| 321  | A 100.00   |
| 322  | AE 84.01 A 15.99                                     |
| 323  | AE 96.28 E 3.72                                      |
| 324  | A 52.27 AE 47.71 E 0.03                              |
| 325  | A 89.39 AE 10.62                                     |
| 326  | A 64.60 AE 35.40                                     |
| 327  | AG 73.59 A 26.26 G 0.15                              |
| 328  | CG 25.04 AG 24.99 DG 24.99 FG 24.98                  |
| 329  | CG 23.40 AG 23.34 DG 23.34 FG 23.33 G 6.60           |
| 330  | G 98.94 CG 0.27 AG 0.26 DG 0.26 FG 0.26              |
| 331  | G 99.10 CG 0.27 AG 0.22 DG 0.21 FG 0.21              |

| Node | Ancestral states and their corresponding probability |
|------|--|
| 332  | G 95.96 CG 1.02 AG 1.01 DG 1.00 FG 1.00              |
| 333  | G 99.96 CG 0.01 AG 0.01 FG 0.01 DG 0.01              |
| 334  | G 85.77 CG 3.60 AG 3.55 DG 3.54 FG 3.54              |
| 335  | G 98.36 CG 0.42 AG 0.41 DG 0.41 FG 0.41              |
| 336  | G 99.73 CG 0.07 AG 0.07 DG 0.07 FG 0.07              |
| 337  | G 99.91 CG 0.04 AG 0.02 FG 0.02 DG 0.02              |
| 338  | G 99.55 CG 0.29 AG 0.08 FG 0.04 DG 0.04              |
| 339  | G 97.91 CG 1.90 AG 0.19                              |
| 340  | C 100.00   |
| 341  | C 100.00   |
| 342  | C 100.00   |
| 343  | C 100.00   |
| 344  | C 100.00   |
| 345  | C 100.00   |
| 346  | C 100.00   |
| 347  | C 100.00   |
| 348  | C 100.00   |
| 349  | C 100.00   |
| 350  | C 100.00   |
| 351  | C 100.00   |
| 352  | C 100.00   |
| 353  | C 100.00   |
| 354  | C 100.00   |
| 355  | C 100.00   |
| 356  | C 100.00   |
| 357  | C 100.00   |
| 358  | C 100.00   |
| 359  | C 100.00   |
| 360  | CG 99.55 G 0.45                                      |
| 361  | AG 30.82 CG 28.41 AC 22.29 G 18.39 A 0.04 C 0.04     |
| 362  | B 100.00   |
| 363  | B 100.00   |
| 364  | BE 99.65 B 0.35                                      |
| 365  | F 92.77 DF 7.23                                      |
| 366  | F 99.89 DF 0.11                                      |
| 367  | B 100.00   |
| 368  | B 100.00   |
| 369  | B 100.00   |
| 370  | B 100.00   |
| 371  | B 100.00   |
| 372  | B 100.00   |
| 373  | B 100.00   |
| 374  | BF 86.67 B 13.33                                     |
| 375  | B 81.18 BF 18.14 BE 0.68                             |

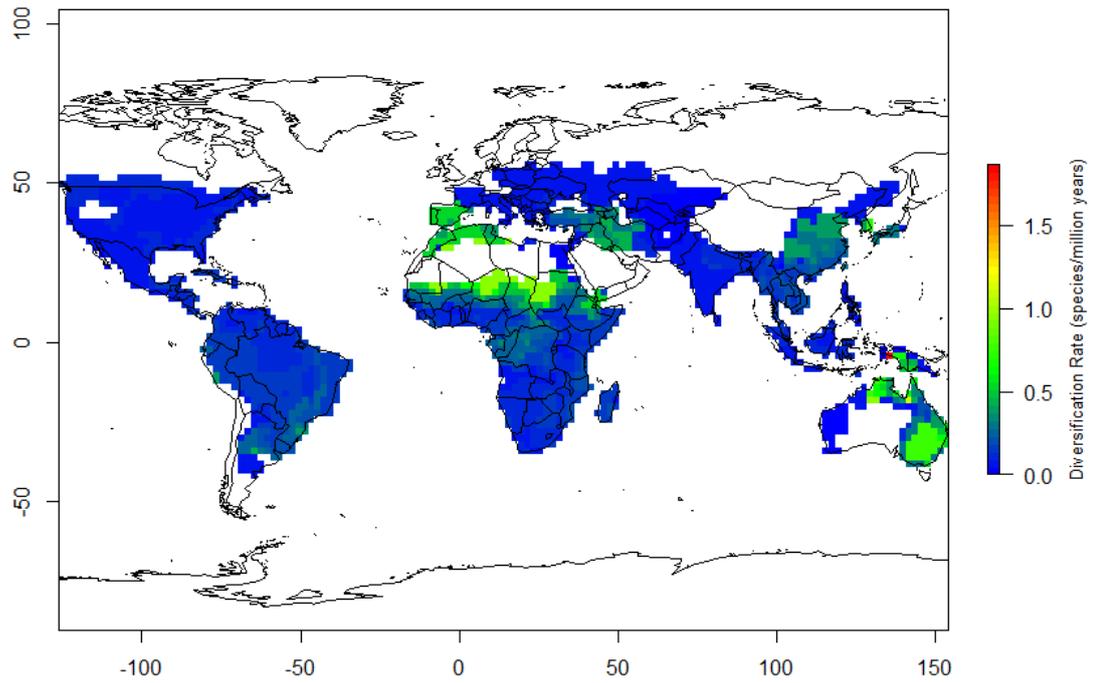
| Node | Ancestral states and their corresponding probability |
|------|--|
| 376  | B 99.38 BF 0.62                                      |
| 377  | B 100.00   |
| 378  | B 100.00   |
| 379  | BC 100.00  |
| 380  | B 95.19 BC 4.81                                      |
| 381  | AB 53.66 BE 45.79 B 0.55                             |
| 382  | B 58.38 AB 25.32 BE 16.22 BF 0.08                    |
| 383  | A 100.00   |
| 384  | A 100.00   |
| 385  | A 100.00   |
| 386  | B 100.00   |
| 387  | AB 100.00  |
| 388  | B 58.70 AB 41.20 BE 0.10                             |
| 389  | BC 56.89 B 36.97 AB 6.14                             |
| 390  | DF 93.71 D 6.29                                      |
| 391  | D 61.56 DG 38.44                                     |
| 392  | D 80.44 DG 19.56                                     |
| 393  | D 100.00   |
| 394  | DG 99.92 D 0.08                                      |
| 395  | DG 84.79 D 15.21                                     |
| 396  | DG 85.76 D 14.24                                     |
| 397  | G 99.68 DG 0.32                                      |
| 398  | DG 98.63 D 1.37                                      |
| 399  | D 100.00   |
| 400  | D 100.00   |
| 401  | D 100.00   |
| 402  | D 95.25 DG 4.75                                      |
| 403  | DF 91.21 D 8.11 DG 0.68                              |
| 404  | F 100.00   |
| 405  | F 100.00   |
| 406  | F 100.00   |
| 407  | F 100.00   |
| 408  | F 100.00   |
| 409  | F 100.00   |
| 410  | F 100.00   |
| 411  | F 100.00   |
| 412  | F 100.00   |
| 413  | F 100.00   |
| 414  | DF 57.30 F 42.62 FG 0.08                             |
| 415  | F 100.00   |
| 416  | F 100.00   |
| 417  | F 100.00   |
| 418  | F 100.00   |
| 419  | F 100.00   |

| Node | Ancestral states and their corresponding probability |
|------|--|
| 420  | F 100.00   |
| 421  | F 100.00   |
| 422  | DF 59.64 F 40.36                                     |
| 423  | DF 58.45 F 41.55                                     |
| 424  | F 75.05 DF 24.95                                     |
| 425  | F 76.43 DF 23.57                                     |
| 426  | F 98.47 DF 1.53                                      |
| 427  | F 98.57 DF 1.43                                      |
| 428  | E 100.00   |
| 429  | EF 91.81 F 8.18 DF 0.01                              |
| 430  | F 86.95 EF 13.05                                     |
| 431  | F 99.38 EF 0.62                                      |
| 432  | DF 99.89 D 0.11                                      |
| 433  | F 51.46 DF 48.54                                     |
| 434  | F 86.43 DF 13.41 EF 0.16                             |
| 435  | F 89.39 DF 10.58 EF 0.03                             |
| 436  | B 100.00   |
| 437  | B 100.00   |
| 438  | B 100.00   |
| 439  | B 100.00   |
| 440  | B 100.00   |
| 441  | B 100.00   |
| 442  | B 100.00   |
| 443  | B 100.00   |
| 444  | B 100.00   |
| 445  | B 100.00   |
| 446  | B 100.00   |
| 447  | B 100.00   |
| 448  | E 100.00   |
| 449  | B 100.00   |
| 450  | B 100.00   |
| 451  | B 100.00   |
| 452  | BE 100.00  |
| 453  | BE 100.00  |
| 454  | B 100.00   |
| 455  | BE 74.11 B 25.89                                     |
| 456  | B 77.71 BE 22.29                                     |
| 457  | B 100.00   |
| 458  | B 100.00   |
| 459  | B 100.00   |
| 460  | B 100.00   |
| 461  | B 100.00   |
| 462  | B 100.00   |
| 463  | B 100.00   |

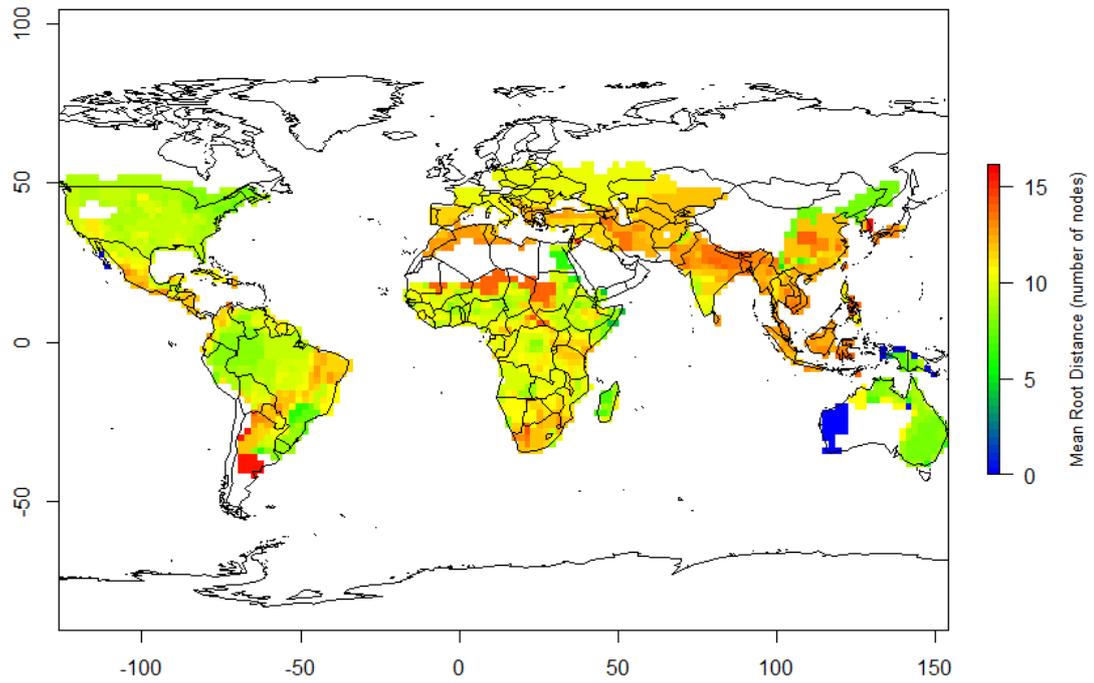
| Node | Ancestral states and their corresponding probability |
|------|--|
| 464  | B 100.00   |
| 465  | B 100.00   |
| 466  | B 100.00   |
| 467  | B 100.00   |
| 468  | B 100.00   |
| 469  | B 100.00   |
| 470  | B 100.00   |
| 471  | B 100.00   |
| 472  | B 100.00   |
| 473  | B 100.00   |
| 474  | B 100.00   |
| 475  | B 100.00   |
| 476  | B 100.00   |
| 477  | B 100.00   |
| 478  | B 100.00   |
| 479  | B 100.00   |
| 480  | B 100.00   |
| 481  | B 100.00   |
| 482  | B 100.00   |
| 483  | B 100.00   |
| 484  | B 100.00   |
| 485  | B 100.00   |
| 486  | B 100.00   |
| 487  | B 100.00   |
| 488  | B 100.00   |
| 489  | B 100.00   |
| 490  | B 100.00   |
| 491  | B 100.00   |
| 492  | DG 80.72 G 18.84 FG 0.44                             |
| 493  | DG 100.00  |
| 494  | DG 100.00  |
| 495  | D 74.34 DG 25.66                                     |
| 496  | D 64.83 DG 35.17                                     |
| 497  | D 71.81 DG 28.16 G 0.02                              |
| 498  | D 54.71 DG 44.81 G 0.48                              |
| 499  | DG 51.19 DF 21.83 D 12.82 G 11.14 FG 3.02            |
| 500  | BF 99.95 AB 0.05                                     |
| 501  | A 100.00   |
| 502  | A 100.00   |
| 503  | A 100.00   |
| 504  | A 100.00   |
| 505  | A 100.00   |
| 506  | A 100.00   |
| 507  | A 100.00   |

| Node | Ancestral states and their corresponding probability |
|------|--|
| 508  | B 100.00   |
| 509  | AB 99.11 A 0.89                                      |
| 510  | AG 40.28 CG 19.91 DG 19.90 FG 19.90                  |
| 511  | AG 39.59 CG 20.14 DG 20.13 FG 20.13                  |
| 512  | AG 48.31 CG 17.24 DG 17.23 FG 17.23                  |
| 513  | AG 55.58 CG 14.73 DG 14.72 FG 14.72 G 0.26           |
| 514  | AG 49.46 CG 16.86 DG 16.84 FG 16.84                  |
| 515  | G 41.59 AG 27.82 CG 10.20 DG 10.19 FG 10.19          |
| 516  | A 100.00   |
| 517  | A 100.00   |
| 518  | A 100.00   |
| 519  | AG 100.00  |
| 520  | AG 99.64 G 0.36                                      |
| 521  | G 99.68 AG 0.32 FG 0.00 DG 0.00 CG 0.00              |
| 522  | AG 99.37 G 0.63                                      |
| 523  | A 64.69 AB 22.49 AG 12.82                            |
| 524  | A 81.57 AB 17.69 AG 0.73                             |
| 525  | A 100.00   |
| 526  | A 100.00   |
| 527  | A 100.00   |
| 528  | A 100.00   |
| 529  | A 89.18 AB 10.71 AG 0.11                             |
| 530  | A 100.00   |
| 531  | A 100.00   |
| 532  | A 100.00   |
| 533  | A 100.00   |
| 534  | A 100.00   |
| 535  | A 100.00   |
| 536  | A 100.00   |
| 537  | A 100.00   |
| 538  | A 89.15 AB 10.85                                     |
| 539  | AE 98.50 E 1.50                                      |
| 540  | E 94.09 AE 5.91                                      |
| 541  | B 100.00   |
| 542  | B 100.00   |
| 543  | E 100.00   |
| 544  | E 100.00   |
| 545  | BE 98.33 E 1.67                                      |
| 546  | BE 74.31 AE 22.31 AB 3.32 E 0.07                     |
| 547  | AB 69.94 A 16.44 B 8.47 AE 5.13 BE 0.02              |
| 548  | DF 100.00  |
| 549  | DF 48.20 F 40.38 D 11.42                             |
| 550  | DF 55.02 F 44.98                                     |
| 551  | B 100.00   |

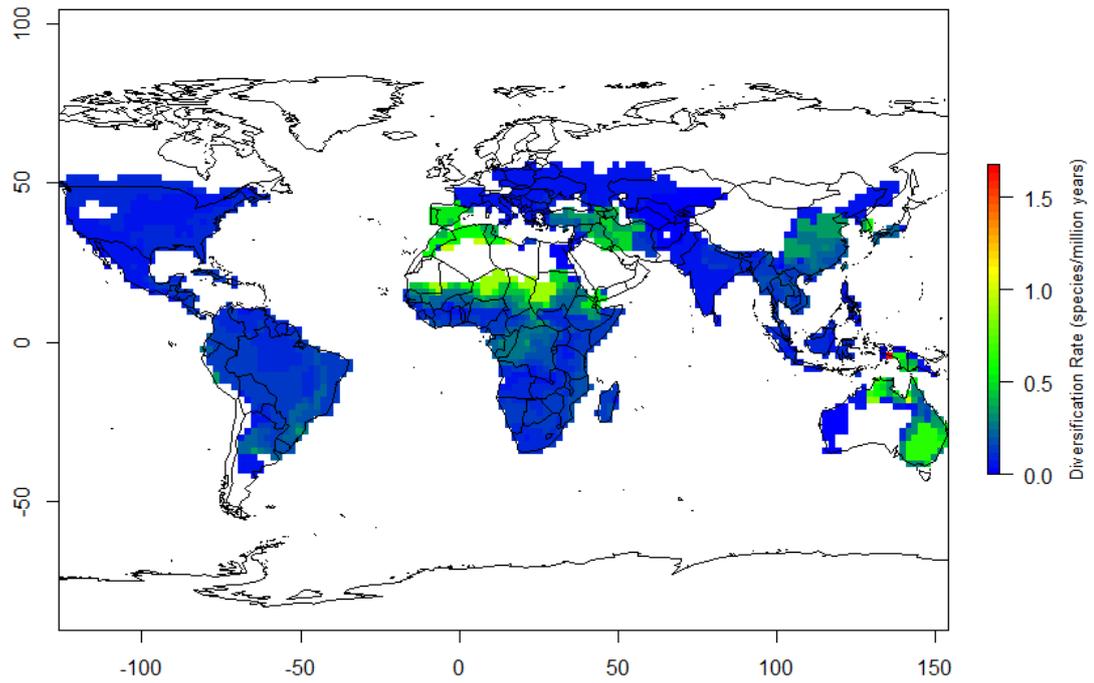
| Node | Ancestral states and their corresponding probability                                    |
|------|---|
| 552  | BF 98.90 B 1.10   |
| 553  | B 45.03 AB 28.72 BF 25.01 EF 1.01 BE 0.24   |
| 554  | BF 99.02 B 0.98   |
| 555  | F 100.00  |
| 556  | DF 74.95 F 25.05  |
| 557  | BF 91.88 F 8.12   |
| 558  | BF 96.33 B 3.67   |
| 559  | BF 80.66 F 19.34  |
| 560  | F 100.00  |
| 561  | DF 81.02 F 18.98  |
| 562  | D 100.00  |
| 563  | D 98.27 DG 1.73   |
| 564  | D 95.27 DF 4.73   |
| 565  | D 88.62 DF 11.38  |
| 566  | D 100.00  |
| 567  | D 94.60 DF 5.40   |
| 568  | D 69.95 DF 30.05  |
| 569  | D 82.32 DF 17.33 F 0.35   |
| 570  | DF 55.30 D 40.00 F 4.71   |
| 571  | D 100.00  |
| 572  | D 100.00  |
| 573  | DG 97.69 D 2.31   |
| 574  | DG 51.93 D 47.31 G 0.75   |
| 575  | DF 69.90 D 30.10  |
| 576  | F 100.00  |
| 577  | F 100.00  |
| 578  | DF 99.31 F 0.58 FG 0.11   |
| 579  | DF 87.78 F 12.22  |
| 580  | D 100.00  |
| 581  | D 100.00  |
| 582  | DF 100.00   |
| 583  | BF 83.28 F 16.72  |
| 584  | BC 38.17 BF 27.35 B 25.72 AB 8.75<br>BC 30.90 AB 28.65 FG 21.44 CG 10.90 AC 4.90 C 2.26 |
| 585  | AG 0.95   |



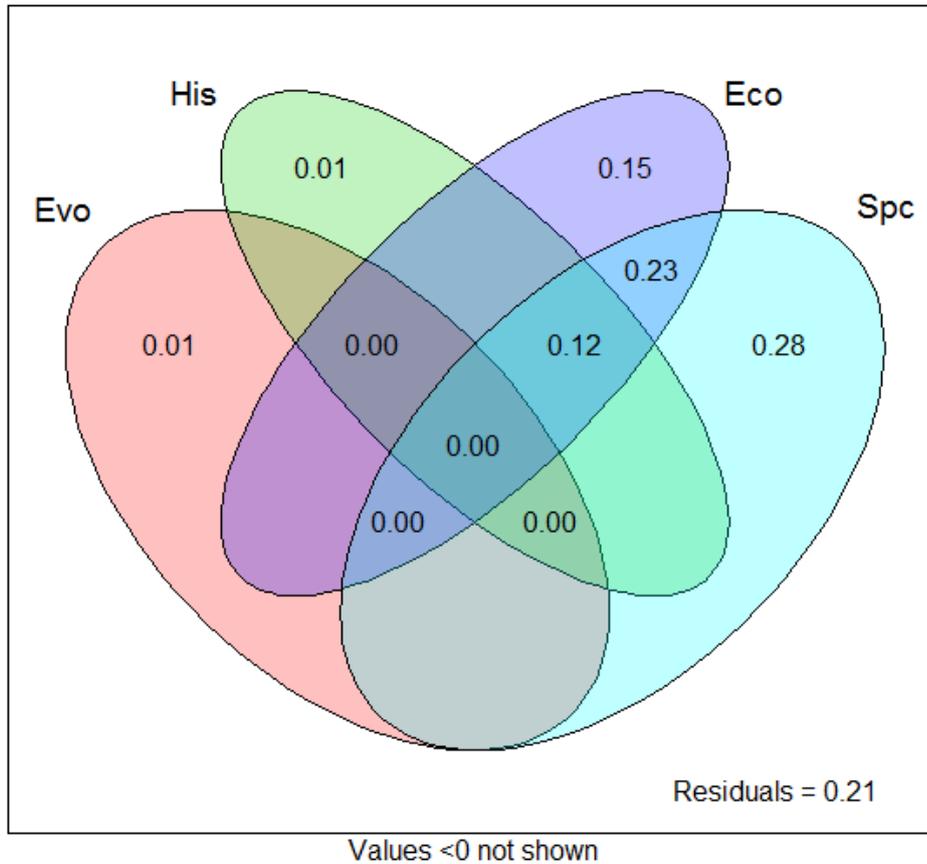
**Figure A1.** Mean diversification rate of non-marine turtles calculated following Jetz et al. (2012). Cold colors (blue) represent areas with low diversification rate, while warm colors (red) represent areas with high diversification rate.



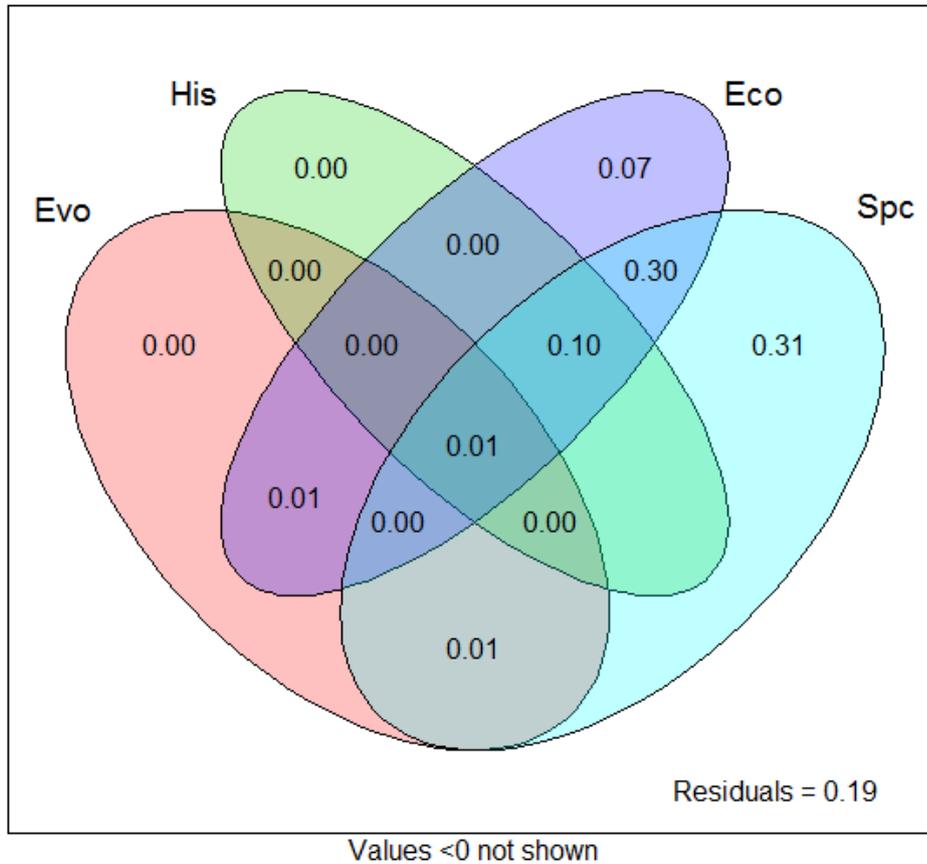
**Figure A2.** Mean Root Distance of non-marine turtles calculated following Kerr & Currie (1999). Cold colors (blue) represent areas with low mean root distance, while warm colors (red) represent areas with high mean root distance.



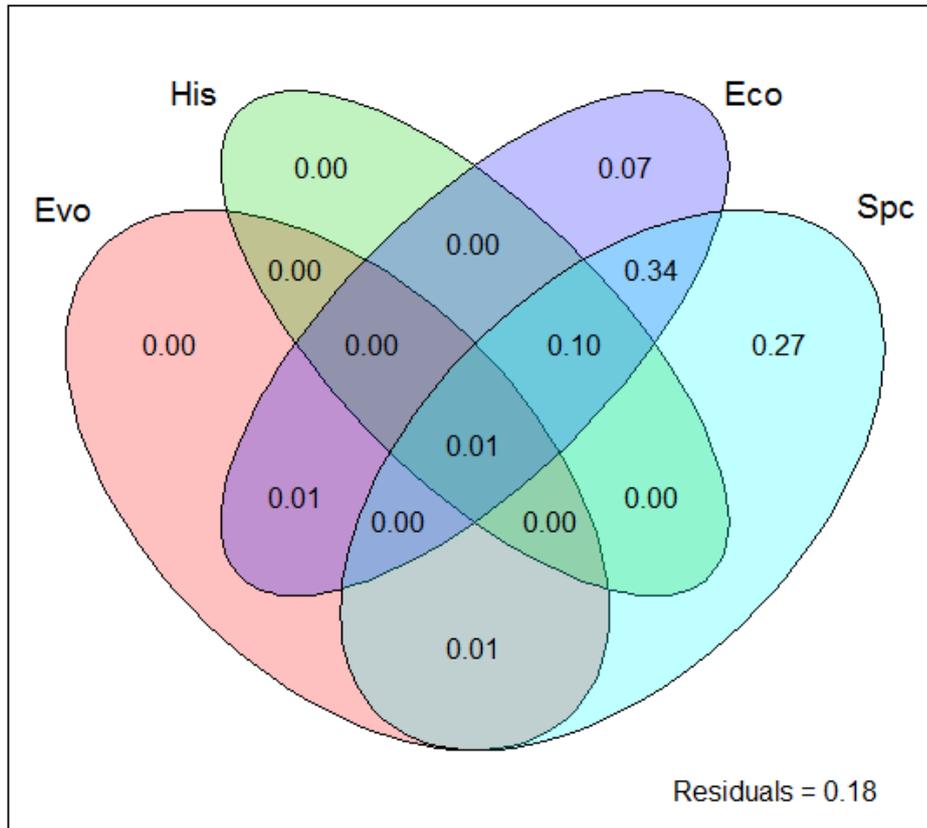
**Figure A3.** Median diversification rate of non-marine turtles calculated following Jetz et al. (2012). Cold colors (blue) represent areas with low diversification rate, while warm colors (red) represent areas with high diversification rate.



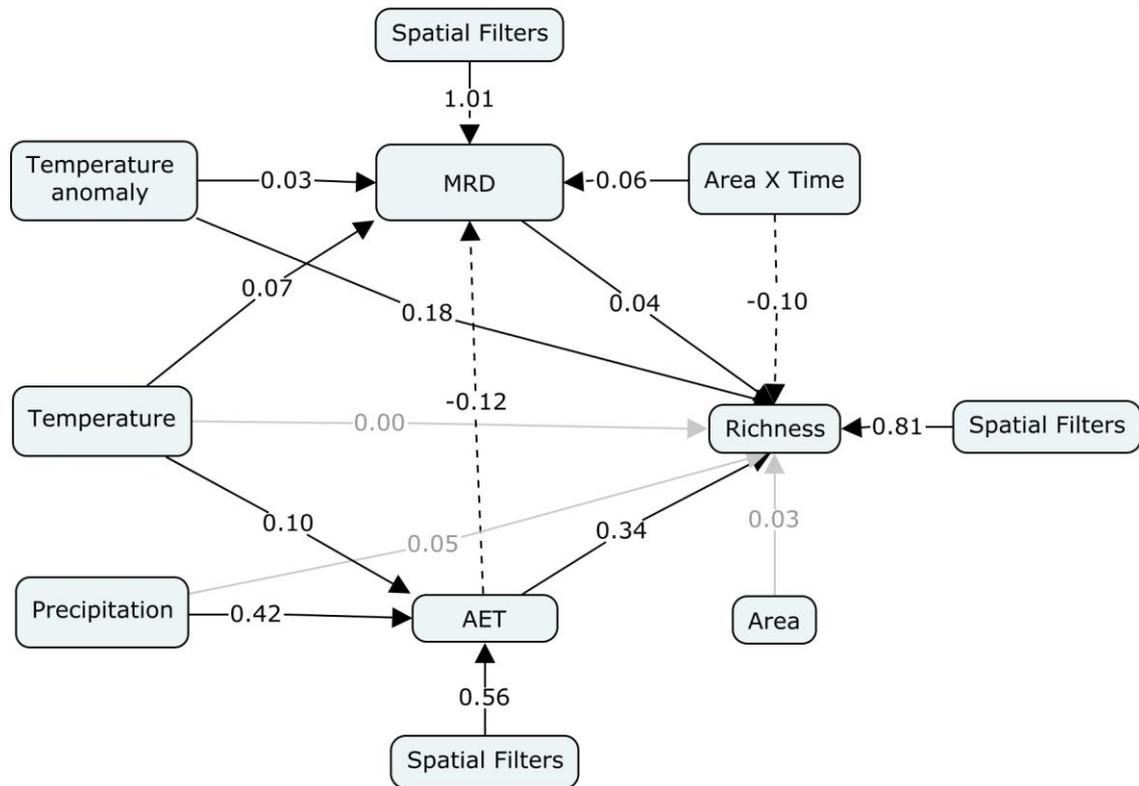
**Figure A4.** Variance in non-marine turtle richness explained by each group of hypotheses. Evo = Evolutionary hypothesis, explanatory variable = Mean Root Distance; His = Historical hypothesis, explanatory variable = Area x Time; Eco = Ecological hypotheses, explanatory variables = Mean Annual Temperature, Total annual precipitation, Annual Actual Evapotranspiration and Temperature Anomaly relative to the Last Glacial Maximum; Spc = spatial filters used to account for spatial autocorrelation.



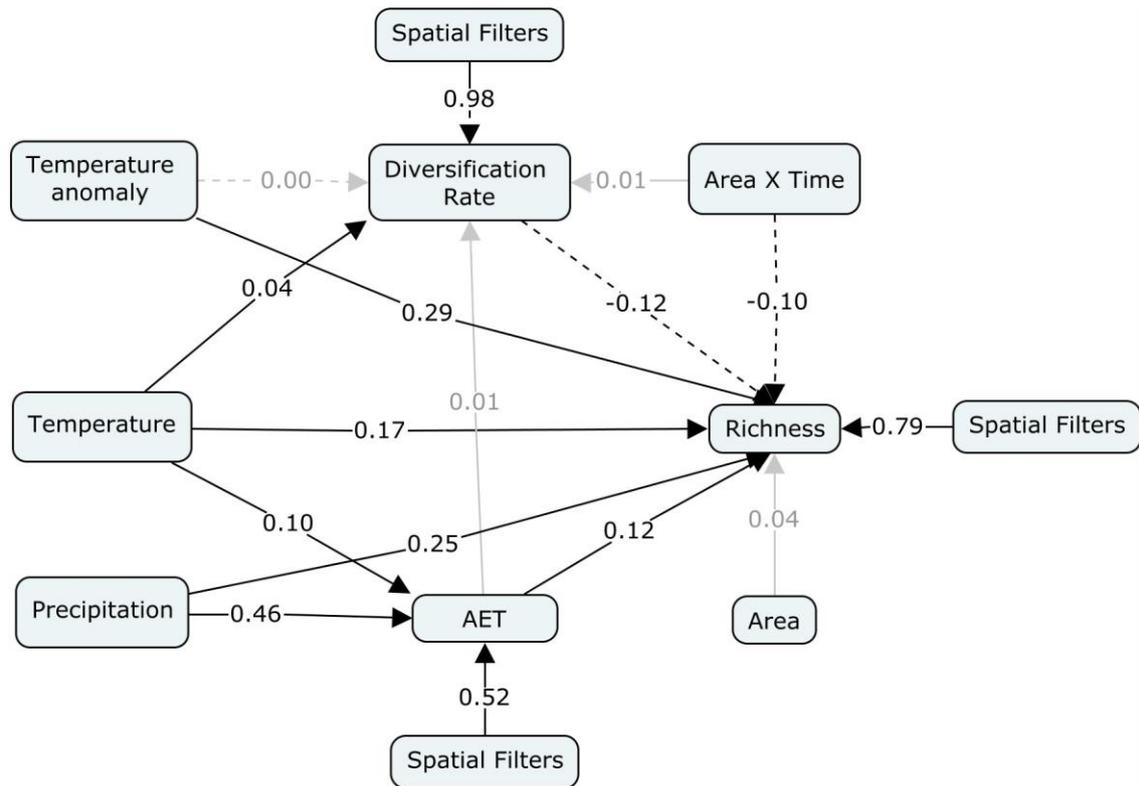
**Figure A5.** Variance in non-marine turtle richness explained by each group of hypotheses. Evo = Evolutionary hypothesis, explanatory variable = Median Diversification Rate; His = Historical hypothesis, explanatory variable = Area x Time; Eco = Ecological hypotheses, explanatory variables = Mean Annual Temperature, Total annual precipitation, Annual Actual Evapotranspiration and Temperature Anomaly relative to the Last Glacial Maximum; Spc = spatial filters used to account for spatial autocorrelation.



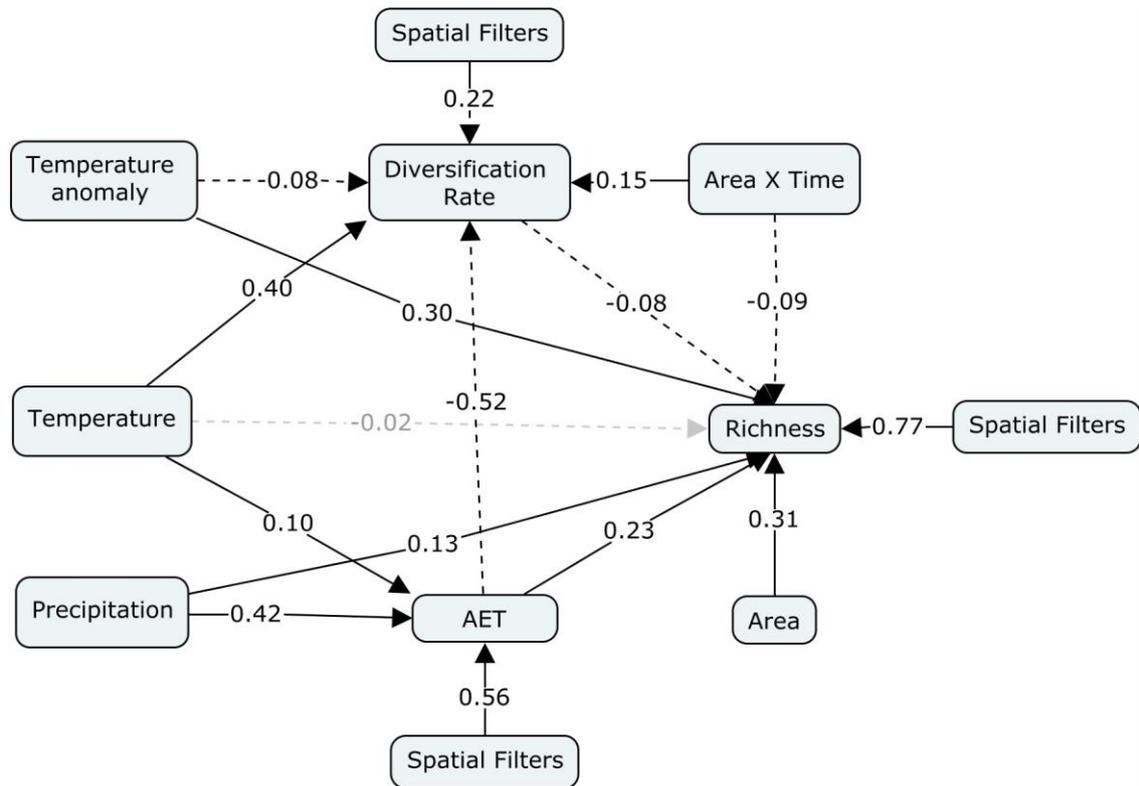
**Figure A6.** Variance in non-marine turtle richness explained by each group of hypotheses when only cells with more than 50% of land area were included in the analyses. Evo = Evolutionary hypothesis, explanatory variable = Mean Diversification Rate; His = Historical hypothesis, explanatory variable = Area x Time; Eco = Ecological hypotheses, explanatory variables = Mean Annual Temperature, Total annual precipitation, Annual Actual Evapotranspiration and Temperature Anomaly relative to the Last Glacial Maximum; Spc = spatial filters used to account for spatial autocorrelation.



**Figure A7.** Path analysis considering direct and indirect effects of the explanatory variables on turtle species richness using Mean Root Distance (MRD) as a diversification measure. Temperature anomaly and Annual Actual Evapotranspiration (AET) were the main variables directly influencing species richness. RMSEA = 0.114 and R-squared for the dependent variables AET, MRD and Richness were 0.917, 0.744 and 0.789, respectively. Solid black lines are positive effects, dashed black lines represent negative effects, and gray lines (solid or dashed) are non-significant paths. Coefficients equal to 0.00 represent values lower than 0.01.



**Figure A8.** Path analysis considering direct and indirect effects of the explanatory variables (using Diversification Rate) on turtle species richness when only cells with more than 50% of land area were included in the analyses. Temperature anomaly, Precipitation and Temperature were the main variables directly influencing species richness. RMSEA = 0.101 and R-squared for the dependent variables AET, Diversification Rate and Richness were 0.920, 0.766 and 0.769, respectively. Solid black lines are positive effects, dashed black lines represent negative effects, and gray lines (solid or dashed) are non-significant paths. Coefficients equal to 0.00 represent values lower than 0.01.



**Figure A9.** Path analysis considering direct and indirect effects of the explanatory variables (using Median Diversification Rate) on turtle species richness. Area, Temperature anomaly, and Annual Actual Evapotranspiration (AET) were the main variables directly influencing species richness. RMSEA = 0.149 and R-squared for the dependent variables AET, Diversification Rate and Richness were 0.917, 0.210 and 0.753, respectively. Solid black lines are positive effects, dashed black lines represent negative effects, and gray lines (solid or dashed) are non-significant paths.

### **Capítulo 3**

Rodrigues, J.F.M., Diniz-Filho, J.A.F. Dispersal is more important than climate in structuring turtle communities across different biogeographic realms. *Journal of Biogeography*. doi: 10.1111/jbi.13003

*Dispersal is more important than climate in structuring turtle communities across different biogeographic realms*

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## Original article

### **Dispersal is more important than climate in structuring turtle communities across different biogeographic realms**

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#### **Abstract**

**Aim:** Ecological communities may be structured by deterministic processes commonly related to climatic conditions or to neutral processes commonly associated to dispersal limitation. This study aims to evaluate the processes responsible for structuring the composition of turtle communities across different biogeographic realms.

**Location:** Global

**Methods:** We used distribution maps of 331 non-marine turtle species to determine the components of beta diversity (turnover and nestedness/richness difference) within the biogeographic realms. We also used a recently published phylogeny to calculate phylogenetic beta diversity. Then, we used partial Mantel tests and multiple regressions on distance matrices (MRM) to evaluate the importance of ecological and spatial factors in determining turtle beta diversity. Besides, we also used multiple regressions to evaluate whether temperature instability since the Last Glacial Maximum (LGM) or topographical heterogeneity was the main driver of beta diversity.

**Results:** The beta diversity of turtles in all realms was mainly influenced by the turnover component. However, the nestedness/richness difference component was also important, mainly in realms subject to large climatic variations since the LGM. Environmental distance was positively related to beta diversity in some realms, but this effect was generally low. Geographical distance explained a higher amount of variance than environmental distances in all the realms. Phylogenetic beta diversity provided similar results to taxonomic beta diversity. Temperature instability since the LGM was a strong driver of taxonomic beta diversity in the Nearctic realm, but its effect on the Neotropical realm was mainly concentrated in the turnover component.

**Main conclusions:** Spatial processes such as dispersal may be more important than climatic differences in structuring the composition of turtle communities in the distinct biogeographic realms. However, when climate is also important, the effect of environmental processes such as temperature instability since the LGM and topographical heterogeneity are not the same in the different biogeographic realms.

**Keywords** Beta diversity; Last Glacial Maximum, Macroecology; Spatial Structure; Testudines; Variance Partitioning

## **Introduction**

For a long time, studies in ecology that try to understand biodiversity patterns have focused their attention on processes driving species richness, i.e. the number of species in a given area (Chown & Gaston, 2000; Hawkins & Porter, 2001; Hawkins et al.,

2003b; Mittelbach et al., 2007). However, more recently attention has increasingly been directed towards understanding patterns in beta diversity as well (i.e. difference in composition between areas). It is now recognised that such knowledge might provide important insights about processes structuring natural communities that are not properly captured in richness studies, since drivers of changes in species composition are more related to community structuring processes than simple changes in the number of species (Graham & Fine, 2008; Beaudrot & Marshall, 2011; Chase & Myers, 2011). Instead, beta diversity studies have helped to understand the importance of deterministic processes related to niche differences and stochastic processes most related to dispersal of natural communities (Kraft et al., 2008; Beaudrot & Marshall, 2011; Chase & Myers, 2011).

Macroecological studies have evaluated potential drivers of beta diversity patterns on large scales in a wide range of vertebrates. However, although these studies covered a few biogeographic realms (Melo et al., 2009; Svenning et al., 2011; Dobrovolski et al., 2012; Siefert et al., 2013) or even global patterns (Buckley & Jetz, 2008; Leprieur et al., 2011; Baselga et al., 2012), they did not consider whether different processes may influence beta diversity in the different realms (but see Qian & Ricklefs, 2012). Biogeographic realms are commonly geographically isolated in relation to each other and also have unique histories and diversities, highlighting the occurrence of independent evolution and different processes shaping their diversity (Hawkins et al., 2003a; Holt et al., 2013). Hence, it would be interesting to evaluate and compare beta diversity patterns and their underlying processes in different biogeographic realms. The change in focus of ecological studies triggered the development of several beta diversity metrics, which improved our ability to identify processes generating differences in composition among communities (Baselga, 2010; Legendre, 2014). Traditional

measures of beta diversity, such as the Sorensen and Jaccard indices, can now be partitioned and used to understand the amount of turnover (beta diversity caused by true species substitution between areas) and the nestedness/richness (beta diversity caused by differences in species richness between areas) components of beta diversity (Baselga, 2010, 2013; Baselga et al., 2012). This approach allows us to more efficiently evaluate the effect of historical factors, such as Pleistocene glaciations, on the current diversity of organisms (Leprieur et al., 2011; Baselga et al., 2012; Baselga, 2013) and also to better explore the relationships between beta diversity and environmental factors because an environmental variable may have opposing effects on each component, hiding a possible effect on the overall beta diversity measure (Lewis et al., 2016). This same partitioning approach may also be applied to other metrics for estimating beta diversity, especially those incorporating the phylogenetic structure of communities (i.e. phylobetadiversity) (Leprieur et al., 2012). However, compared to taxonomic beta diversity, phylogenetic beta diversity studies are still scarce, despite the potential contributions this metric offers for the understanding of biodiversity patterns, such as latitudinal and altitudinal diversity gradients, and processes structuring ecological communities (Graham & Fine, 2008), thus reinforcing the need to include it when phylogenies are available.

Topographic heterogeneity (Melo et al., 2009), current climatic differences (Leprieur et al., 2011), and climatic instability due to Pleistocene glaciations (Leprieur et al., 2011; Baselga et al., 2012; Dobrovolski et al., 2012) have been shown to influence beta diversity of fish, amphibians, birds, and mammals. Turnover components are commonly related to climatic stability through the Pleistocene (mainly inferred from patterns since the Last Glacial Maximum [LGM]), whereas nestedness is more commonly related to climatic instability since the LGM (Dobrovolski et al., 2012).

Climatic stability is assumed to provide time for speciation and the origin of small-range species (Jansson & Dynesius, 2002), both processes increasing turnover components of beta diversity. Topographic heterogeneity is assumed to provide biogeographic barriers that also promote speciation and turnover (Melo et al., 2009). Still, climatic instability should have caused extinctions in some natural communities which were only recently occupied by species with good dispersal rates from neighbouring sites, generating a prevalence of a nestedness component (Baselga, 2010; Dobrovolski et al., 2012).

Processes responsible for shaping beta diversity on large scales are still poorly explored in reptiles, an ectothermic vertebrate group with high beta diversity due to their dispersal limitations and temperature requirements (Qian, 2009; Qian & Ricklefs, 2012). Non-marine turtles (tortoises and freshwater turtles) are a group that is distributed nearly worldwide with approximately 330 species (Turtle Taxonomy Working Group, 2014). However, the macroecological processes responsible for shaping their diversity are still poorly known (Iverson, 1992; Angielczyk et al., 2015; Rodrigues et al., 2016). Recent glaciation events seem to have driven speciation and extinction events in some freshwater turtle genera, suggesting they might be important in explaining beta diversity patterns (Rödder et al., 2013; Mittermeier et al., 2015; Rhodin et al., 2015). Besides, considering that current turtle hotspots are located in regions crossed by hills and mountain ridges, topographical heterogeneity would also be a candidate for explaining beta diversity (Mittermeier et al., 2015). However, since non-marine turtles are animals commonly characterised as poor dispersers, the environmental effects on beta diversity may be weaker (since animals cannot fully track all their best environmental conditions) than geographic distance, causing a distance decay effect independent from environmental variation (Nekola & White, 1999).

Our study aims to understand and compare the processes responsible for shaping the beta diversity patterns of non-marine turtles in the different biogeographic realms. According to previous studies on vertebrates and the available knowledge regarding the processes influencing turtle diversity, we have the following questions and predictions

- 1) How is turtle beta diversity distributed worldwide? Considering that it is a descriptive question, we have no prediction for it;
- 2) Are turtle communities structured predominantly by environmental or geographical processes? We expect that although environment might have some influence on turtle beta diversity, the composition variation between ecological communities of these animals would be more determined by geographical distance (probably related to dispersal limitation) than by environmental effects considering that animals with poor dispersal ability cannot fully track the environmental conditions that are best for them;
- 3) What explains the distance-decay patterns observed for turtle communities in each realm? We expect that distance-decay patterns are due to environmental distances arising from geographical distance or due to differences in area between the regions, because large areas have more different environmental conditions;
- 4) When deterministic processes are important to structure turtle communities, which process is the most important? We expect that the relative importance of the environmental factors is related to the climatic history of the realm. Therefore, where glaciations were stronger in the LGM, we expect a strong effect of temperature variation since this period, while in other realms, topographical heterogeneity might be more important. To the best of our knowledge, this is the first study to evaluate taxonomic and phylogenetic beta diversity of turtles on a large scale.

## **Materials and Methods**

### *Distribution data*

We used updated range maps for 290 species of non-marine turtles (freshwater turtles and tortoises, hereafter only “turtles”, see Table S1.1 in Appendix for a list with the names of the species included), which may be found in Turtle Taxonomy Working Group (2014). These range maps were used to construct presence-absence matrices using a grid of 2° x 2° (local communities), excluding Antarctica, an appropriate grid resolution considering errors existing in range maps (Hurlbert & Jetz, 2007). Only the cells in which more than 50% of their area was continental were kept for analyses. The presence of a species in a given cell was determined if its range map crossed the midpoint of the cell. Considering that different processes may shape biodiversity patterns in different continents and regions (Hawkins et al., 2003b; Beaudrot & Marshall, 2011), we divided the turtle communities into the 11 biogeographic realms defined by Holt et al. (2013), and performed the analyses for each realm separately. Sample sizes for each realm (number of grid cells) may be found in Tables 1 and 2.

### *Beta diversity*

We calculated the beta diversity between pairs of communities (2° x 2° grid cells) for each region following the approach proposed by Baselga (2010). According to current literature, pairwise measures of beta diversity are the best way to avoid biases related to sample size differences when comparing beta diversity between regions (Bennett & Gilbert, 2016). Taxonomic and phylogenetic beta diversity components were calculated using Simpson dissimilarity for the turnover component and a Sorensen-derived formula for the nestedness/richness difference component in the R package ‘betapart’ (Baselga & Orme, 2012) using the functions `beta.pair` and `phylo.beta.pair`, respectively (the equations used in our study are provided in the supplementary material, but see Baselga (2010) for a broad explanation regarding the equations we used for taxonomic

beta diversity and Leprieur et al. (2012) for explanations of the equations used for phylogenetic beta diversity). For phylogenetic beta diversity, we used a maximum clade credibility phylogenetic tree covering 293 non-marine turtle species, estimated using a Bayesian approach, three mitochondrial and two nuclear loci, a relaxed molecular clock, and calibrated using fossil records (Rodrigues & Diniz-Filho, 2016). However, only 250 species in the phylogeny had distribution data, and only these were used in the phylogenetic analyses (see Fig. S1.1 in our supplementary material for a figure of the phylogenetic tree used in our study and the species sampled in the phylogeny). We also calculated standardised effect size values for phylogenetic beta diversity, because phylogenetic beta diversity values may be related to taxonomic beta diversity (Leprieur et al., 2012). We applied a null model where the tips of our phylogeny were randomly shuffled 1000 times and calculated 1000 values of phylogenetic beta diversity for each pair of sites. This null model is interesting because it keeps the richness of each site and the taxonomic beta diversity between sites constant, making it possible to evaluate phylogenetic beta diversity taking into account these patterns (Graham et al., 2009; Leprieur et al., 2012). Finally, standardised effect size values were obtained following the standard formula used to calculate this metric  $[(\text{observed value} - \text{mean expected values}) / \text{standard deviation of expected values}]$ , in which the expected values are obtained from the null models.

In the beta diversity calculations, we did not include communities with less than two species, because low numbers of species produce unstable beta diversity estimates, since the change of a single species could have a strong effect. We did not use a larger threshold because turtle richness is generally low, and higher thresholds would dramatically reduce our sample number (see Rodrigues et al. (2016) and table S1.3 for general information regarding turtle richness in global and biogeographic scale).

After calculating pairwise beta diversity for the communities in the different realms, we also calculated beta diversity (taxonomic and phylogenetic) for each cell of the Neotropical and Nearctic realms in order to investigate drivers of beta diversity for each cell and properly evaluate the effects of areas more affected by climatic instability in relation to the LGM. Beta diversity was calculated as the mean beta diversity between each cell and its neighbours, an approach commonly used in recent studies (Melo et al., 2009; Dobrovolski et al., 2012; Wen et al., 2016). This other metric of beta diversity was calculated only for these two realms because 1) environmental processes were important in shaping beta diversity in these realms (see results); 2) they are large areas, having a large sample size; and 3) they are well-studied realms where beta diversity patterns have already been evaluated for other clades.

#### *Environmental variables*

We obtained data for the mean annual temperature, annual precipitation, and altitude from the Worldclim database (<http://www.worldclim.org/>) at a 10 arc-minute resolution (Hijmans et al., 2005). We also downloaded a raster file with temperatures of the LGM modelled using the MIROC Global Circulation Model from the Worldclim website, and calculated the difference between current and past (LGM) temperatures (temperature anomaly or temperature instability) as a surrogate of the temperature instability effect (Araújo et al., 2008). Finally, these environmental variables were standardised (mean = 0 and standard deviation = 1), and we created an environmental distance matrix between sites using Euclidian distances.

#### *Statistical analyses*

In order to answer question 1 about describing turtle beta diversity patterns worldwide, we mapped the turtle beta diversity for each cell of our 2° x 2° grid using the same approach used to calculate beta diversity for Nearctic and Neotropical cells.

To answer question 2 about the role of environment and geography in structuring turtle communities, since the turnover component represents real species substitution between sites (Baselga, 2010), we used this metric in our pairwise comparisons within regions. First, we used partial Mantel correlations with 999 permutations (Legendre & Legendre, 1998) to evaluate if the turnover component of the beta diversity of turtles in each region was explained by environmental distances after fixing the effect of geographical distances. Geographical distances were obtained using geodesic distance between cells in each region using the function “distGeo” from the R package ‘geosphere’ (Hijmans et al., 2015). These distances were log-transformed ( $\log_e x$ ) prior to analysis, because distance-decay patterns tend to be exponential (Nekola & White, 1999; Tuomisto et al., 2003). We also used multiple regressions on distance matrices (MRM) to evaluate the amount of variance in beta diversity that is due to environmental effects, spatial effects, and shared effects of both components. MRM analyses were performed using the R package ‘ecodist’ (Goslee & Urban, 2007). Since composition and environmental distances may have non-linear relationships, we transformed our turnover estimates using hybrid multidimensional scaling (HMDS) and obtained new compositional dissimilarities using Euclidian distances in the scores of HMDS (Faith et al., 1987). According to Faith et al. (1987), this transformation improves the linear relationship between composition and environmental distances. Then, Mantel tests and MRM analyses were also performed on this new dataset, but the results were qualitatively the same as using the untransformed measure (results not present).

When evaluating question 3 about the causes of distance decay patterns, we performed partial Mantel analyses with 999 permutations, but controlling for environmental distances in these analyses to evaluate whether the distance decay was due to an increase in environmental distance. Finally, we evaluated the relationship between the spatial-only component of variance in the partitioning analysis and the area of each realm using Pearson's correlation coefficient, because large regions or distributions are supposed to harbour higher distance decay effects (Nekola & White, 1999).

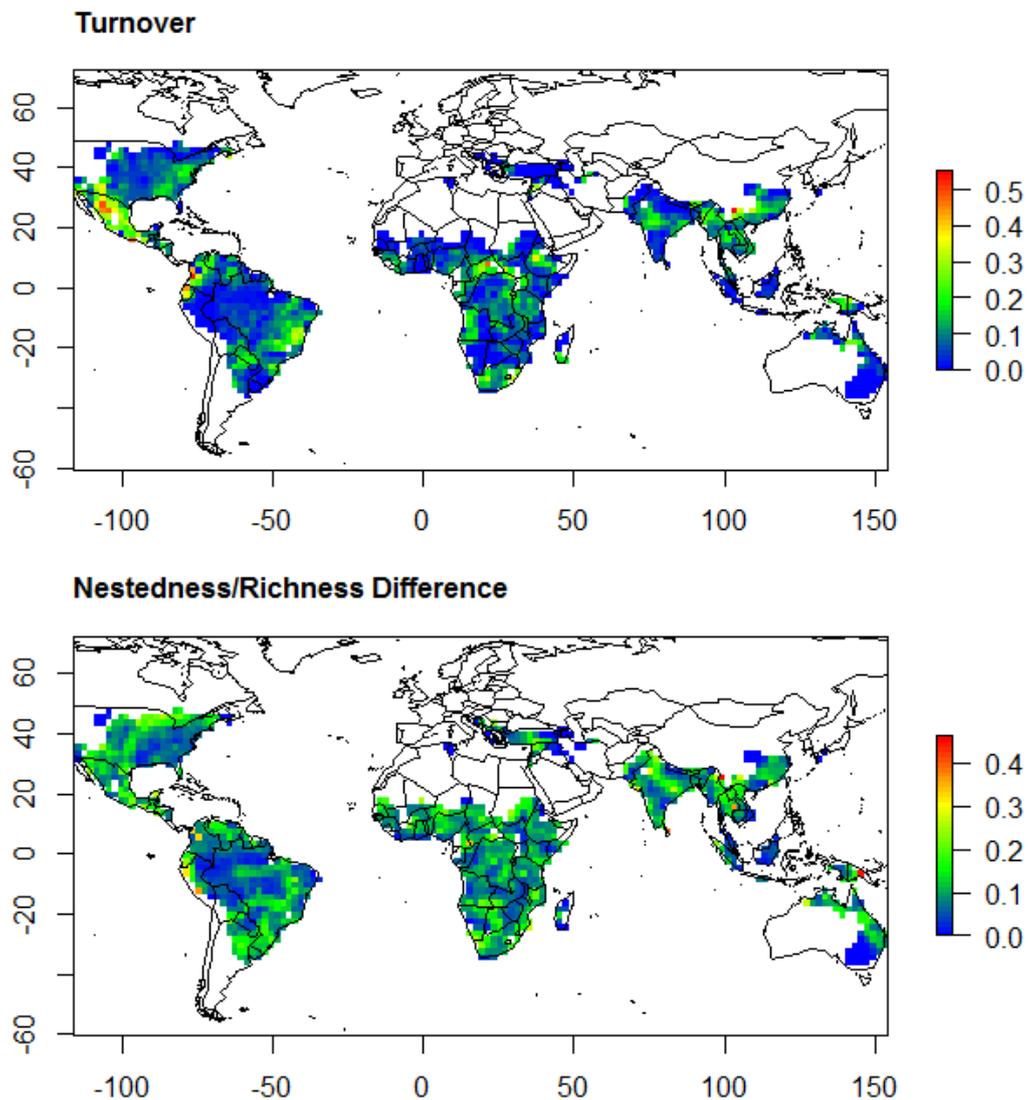
Finally, to evaluate whether temperature anomaly since the LGM or topographical heterogeneity was the main driver of taxonomic and phylogenetic beta diversity (Question 4), we selected two realms in which beta diversity was affected by environmental distances and with a high number of cells, i.e. the Neotropical and Nearctic realms. Altitudinal range was used as our measure of topographical heterogeneity, although it might also represent spatial isolation due to this topographical heterogeneity. We used multiple regressions with spatial filters (Spatial Eigenvector Mapping) (Diniz-Filho & Bini, 2005; Griffith & Peres-Neto, 2006) to account for spatial autocorrelation in our data, retaining for the analyses only those spatial filters that significantly reduced autocorrelation in the data (Griffith & Peres-Neto, 2006). The relative importance of temperature anomaly and topographical heterogeneity was assessed using standardised regression coefficients.

These spatial analyses were performed in the Spatial Analysis in Macroecology – SAM software (Rangel et al., 2010).

## **Results**

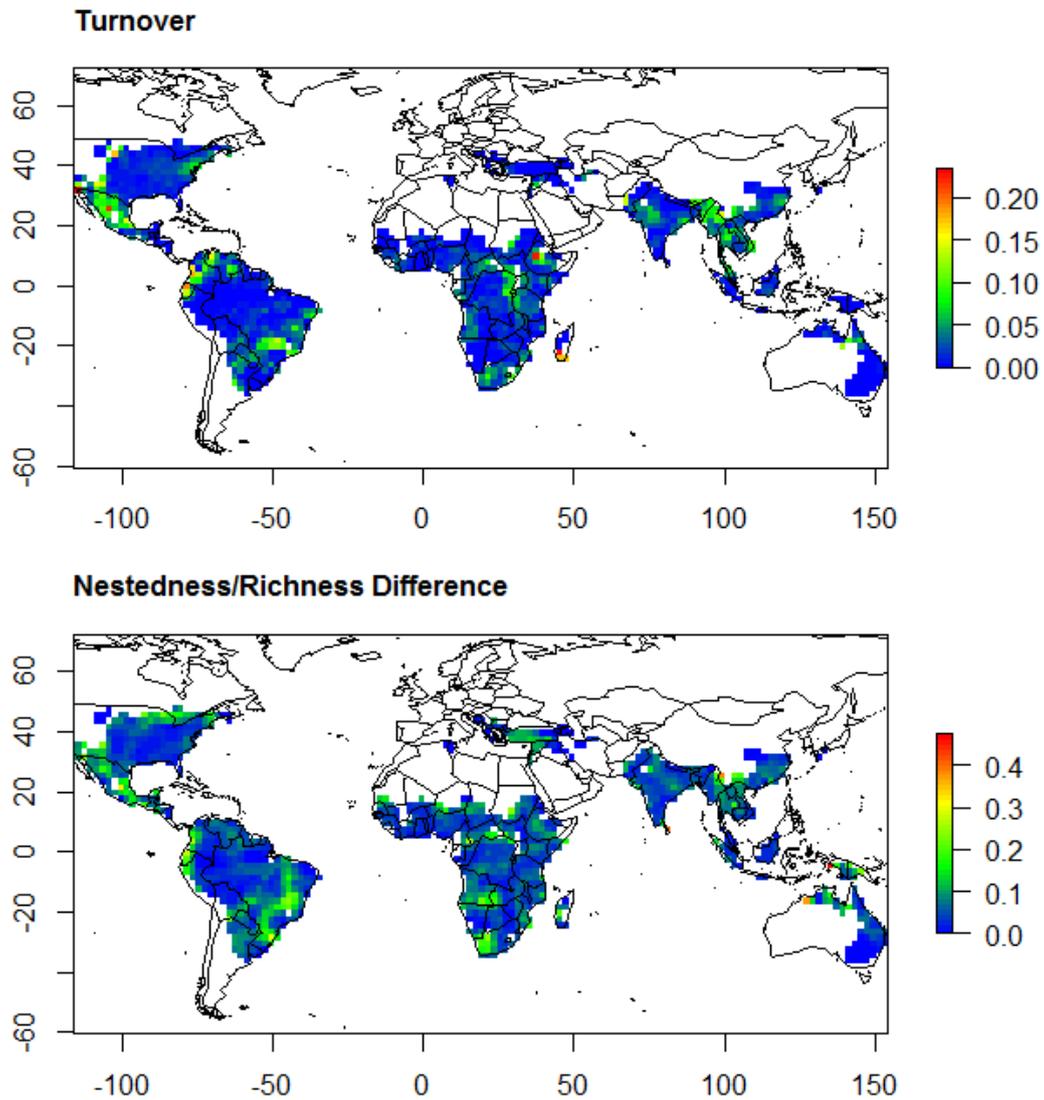
*Question 1: How is turtle beta diversity distributed worldwide?*

Taxonomic turnover was higher in Mexico, Central America, eastern Brazil, and southern China (Fig. 1). The nestedness/richness difference component did not exhibit a clear pattern, but it was consistently higher in the western and northern Neartic (Fig. 1). The phylogenetic beta diversity patterns were similar to the taxonomic ones (Fig. 2).



**Fig. 1.** Taxonomic beta diversity components of turtles worldwide at a 2° x 2° grid.

Warm colours represent areas with high beta diversity, while the cold ones represent areas with low beta diversity.



**Fig. 2.** Phylogenetic beta diversity components of turtles worldwide at a  $2^\circ \times 2^\circ$  grid. Warm colours represent areas with high beta diversity, while the cold ones represent areas with low beta diversity.

*Question 2: Are turtle communities structured predominantly by environmental or geographical processes?*

The turnover component was positively related to environmental distance after controlling for geographic distance in six of the 11 biogeographic realms (Table 1),

suggesting an environmental or deterministic influence on beta diversity. Variance explained by environmental distance was higher in the Sino-Japanese, Panamanian, and Afrotropical realms (Table 1).

The variation in beta diversity was mainly explained by geographical distance in all the realms (Table 1), suggesting the importance of dispersal processes and the strong effect of distance decay in turtle communities. Shared variance explained by environment and space were also important in some realms such as the Australian, Neartic, and Sino-Japanese realms.

Similar results were found when using phylogenetic beta diversity (Table 2). However, standardised effect sizes of phylogenetic beta diversity were not, in general, explained by ecological and spatial factors (See Appendix S1 in Supporting Information, Table S1.4, note the very low R-squared values of the models).

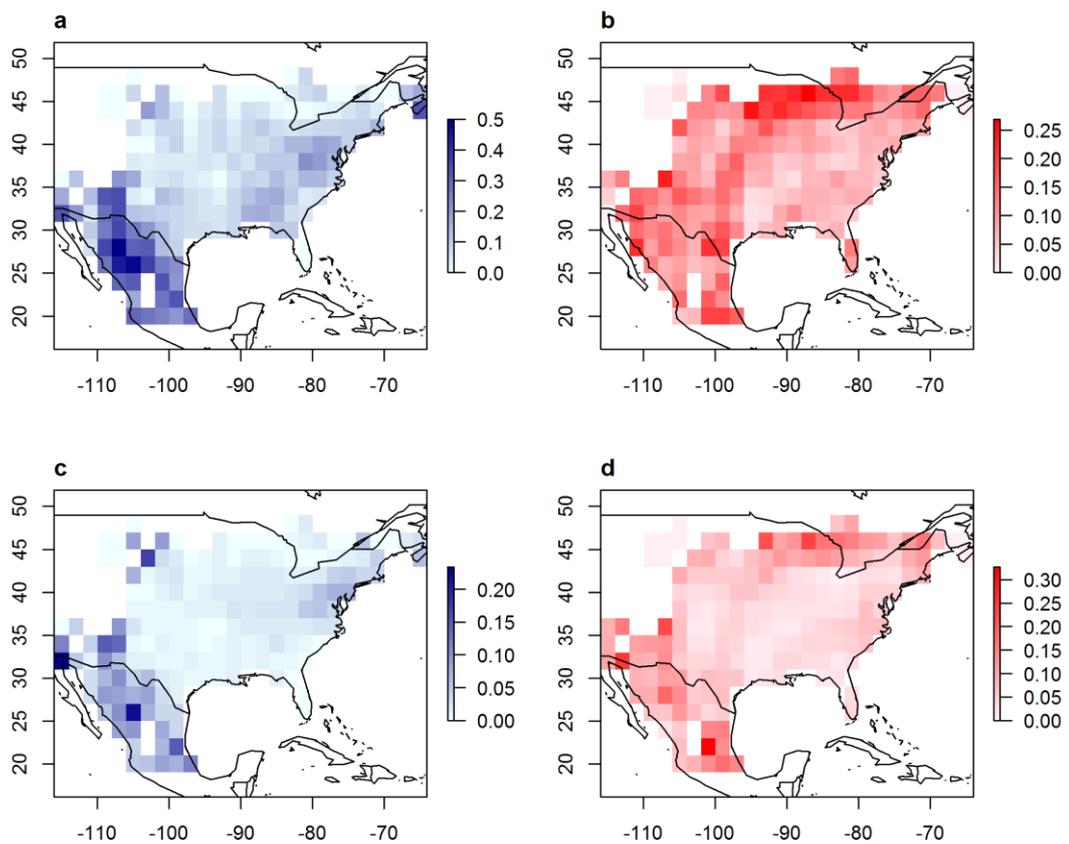
*Question 3: What explains the distance-decay patterns observed for turtle communities in each realm?*

The distance decay in turtle beta diversity remained strong even after we controlled for environmental distances (Table 1). Partial Mantel tests were significant for all the realms, indicating other explanations for distance decay other than just climatic variation (Table 1). Moreover, we found no correlation between the unique spatial components of variance partitioning and realm area ( $r = -0.22$ ,  $df = 9$ ,  $P = 0.51$ ).

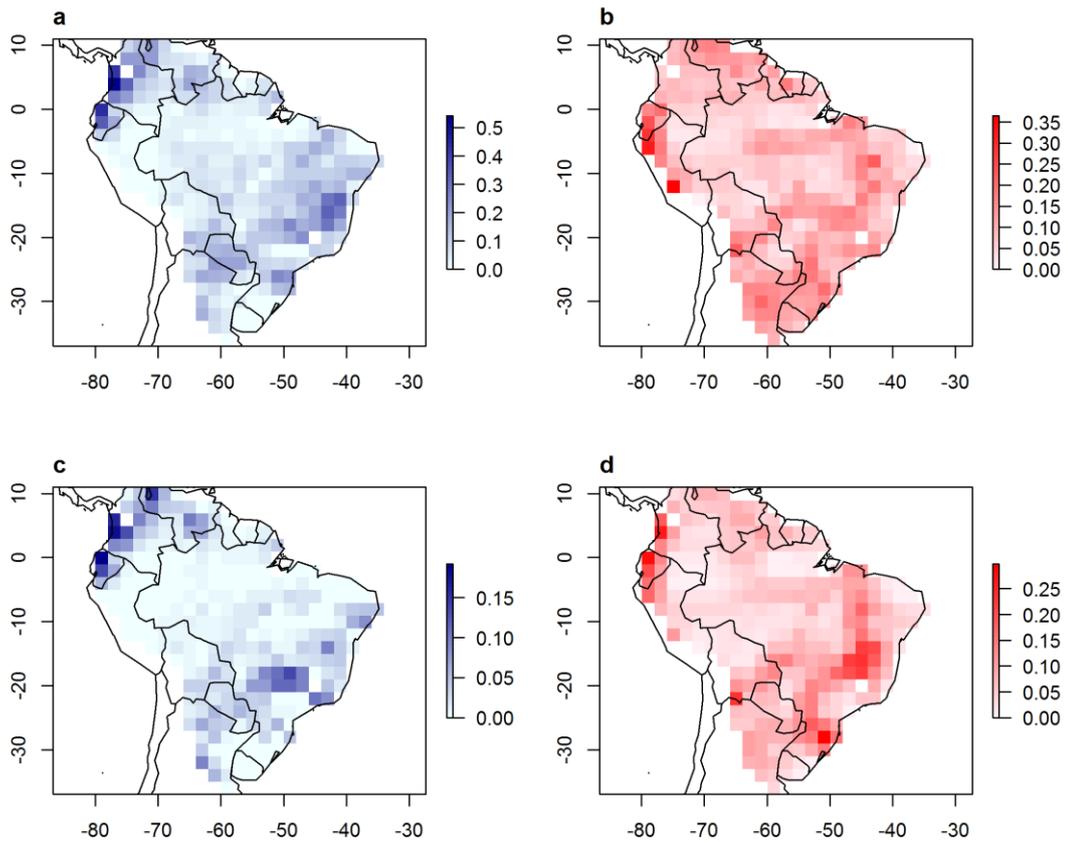
*Question 4: When deterministic processes are important to structure turtle communities, which process is the most important?*

The multiple regression analyses for the Neotropical and Neartic realms found results partially in accordance with our expectations (Table 3). In the Neartic realm, where

glaciations were stronger in the LGM, temperature anomaly was the main driver of taxonomic beta diversity (turnover and nestedness component), but topographical heterogeneity had a stronger effect on phylogenetic beta diversity (Fig. 3 and Table 1). In the Neotropical realm, temperature anomaly was the main driver of turnover components of taxonomic and phylogenetic diversity, but topographical heterogeneity was the main driver of both nestedness components (Fig. 4 and Table 1).



**Fig. 3.** Taxonomic and phylogenetic beta diversity components of turtles in the Nearctic realm. “a” and “b” = taxonomic beta diversity; “c” and “d” = phylogenetic beta diversity. “a” and “c” = turnover component; “b” and “d” = nestedness/richness difference component.



**Fig. 4.** Taxonomic and phylogenetic beta diversity components of turtles in the Neotropical realm. “a” and “b” = taxonomic beta diversity; “c” and “d” = phylogenetic beta diversity. “a” and “c” = turnover component; “b” and “d” = nestedness/richness difference component.

1 **Tables**

2 **Table 1:** Partial Mantel correlation tests (R) associating the turnover component of pair-wise beta diversity in turtles to environmental distance  
 3 after controlling for log-transformed geographical distances within each region and associating pair-wise turnover beta diversity to log-  
 4 transformed geographical distances after controlling for environmental distances and variance explained (R-squared) by environmental, spatial,  
 5 and shared components. \* P < 0.05; \*\* P < 0.01; \*\*\* P = 0.001; NS = non-significant. N = sample size or number of cells of each realm.

| <b>Region (N)</b>   | <b>Partial Mantel (space controlled)</b> | <b>Partial Mantel (environment controlled)</b> | <b>Environment only</b> | <b>Space only</b> | <b>Shared effect</b> |
|---------------------|--|--|-------------------------|-------------------|----------------------|
| Neotropical (297)   | 0.07*                                    | 0.55***  | 0.003594                | 0.286714          | 0.06694              |
| Australian (59)     | 0.12*                                    | 0.55***  | 0.005487                | 0.174786          | 0.412072             |
| Afrotropical (393)  | 0.20***                                  | 0.47***  | 0.026737                | 0.185701          | 0.129589             |
| Madagascan (7)      | -0.25 <sup>NS</sup>                      | 0.58*  | 0.044654                | 0.33107           | -0.03577             |
| Oceanian (11)       | -0.03 <sup>NS</sup>                      | 0.53**   | 0.000767                | 0.2845            | 0.004143             |
| Oriental (162)      | -0.06 <sup>NS</sup>                      | 0.78***  | 0.001367                | 0.56731           | 0.05872              |
| Panamanian (21)     | 0.26**                                   | 0.74***  | 0.028913                | 0.477646          | 0.101442             |
| Saharo-Arabian (27) | 0.04 <sup>NS</sup>                       | 0.69***  | 0.000659                | 0.473755          | -0.00062             |

| <b>Region (N)</b>  | <b>Partial Mantel (space controlled)</b> | <b>Partial Mantel (environment controlled)</b> | <b>Environment only</b> | <b>Space only</b> | <b>Shared effect</b> |
|--------------------|--|--|-------------------------|-------------------|----------------------|
| Nearctic (186)     | 0.16***                                  | 0.40***  | 0.016988                | 0.11469           | 0.252636             |
| Sino-Japanese (40) | 0.51***                                  | 0.62***  | 0.094497                | 0.168127          | 0.473634             |
| Palaearctic (29)   | 0.07 <sup>NS</sup>                       | 0.57***  | 0.00301                 | 0.296803          | 0.07407              |

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1 **Table 2:** Partial Mantel correlation tests (R) associating turnover component of pair-wise phylogenetic beta diversity in turtles to environmental  
2 distance after controlling for log-transformed geographical distances within each region and associating pair-wise turnover phylogenetic beta  
3 diversity to log-transformed geographical distances after controlling for environmental distances and variance explained (R-squared) by  
4 environmental, spatial, and shared components. \* P < 0.05; \*\* P < 0.01; \*\*\* P = 0.001; NS = non-significant. N = sample size or number of cells  
5 of each realm.

| Region (N)         | Partial Mantel      | Partial Mantel           | Environment | Space    | Shared   |
|--------------------|---------------------|--------------------------|-------------|----------|----------|
|                    | (space controlled)  | (environment controlled) | only        | only     | effect   |
| Neotropical (297)  | 0.08*               | 0.40***                  | 0.004726    | 0.154191 | 0.044222 |
| Australian (59)    | 0.04 <sup>NS</sup>  | 0.45***                  | 0.000741    | 0.145353 | 0.274241 |
| Afrotropical (393) | 0.23***             | 0.35***                  | 0.041036    | 0.104419 | 0.107708 |
| Madagascan (7)     | -0.17 <sup>NS</sup> | 0.55*                    | 0.021519    | 0.304586 | -0.02042 |
| Oceanian (11)      | -0.19 <sup>NS</sup> | 0.29*                    | 0.032618    | 0.081072 | 0.008894 |
| Oriental (162)     | -0.04 <sup>NS</sup> | 0.72***                  | 0.000804    | 0.489312 | 0.053973 |
| Panamanian (21)    | -0.14 <sup>NS</sup> | 0.73***                  | 0.009501    | 0.535527 | -0.00048 |

| <b>Region (N)</b>   | <b>Partial Mantel<br/>(space<br/>controlled)</b> | <b>Partial Mantel<br/>(environment<br/>controlled)</b> | <b>Environment<br/>only</b> | <b>Space<br/>only</b> | <b>Shared<br/>effect</b> |
|---------------------|--|--|-----------------------------|-----------------------|--------------------------|
| Saharo-Arabian (27) | 0.05 <sup>NS</sup>                               | 0.58***  | 0.001486                    | 0.34168               | -0.00136                 |
| Nearctic (186)      | 0.22***  | 0.22***  | 0.038552                    | 0.039405              | 0.185425                 |
| Sino-Japanese (40)  | 0.52***  | 0.40***  | 0.142811                    | 0.074808              | 0.390037                 |
| Palaearctic (29)    | -0.01 <sup>NS</sup>                              | 0.40***  | 9.05E-05                    | 0.156634              | 0.021961                 |

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1 **Table 3.** Standardised regression coefficients of environmental effects on beta diversity of turtles in the Neotropical and Nearctic realms. Sample  
 2 sizes in parentheses. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; NS = non-significant.

3

|  |                                      | <b>Taxonomic</b> |  | <b>Phylogenetic</b> |  |
|--|--------------------------------------|------------------|--|---------------------|--|
|  |                                      | <b>Turnover</b>  | <b>Nestedness/<br/>Richness difference</b> | <b>Turnover</b>     | <b>Nestedness/<br/>Richness difference</b> |
| <b>Nearctic</b><br><b>(n = 186)</b>    | <b>Altitudinal</b><br><b>range</b>   | 0.129**          | 0.152**                                    | 0.212***            | 0.501***                                   |
|  | <b>Temperature</b><br><b>anomaly</b> | -0.521***        | 0.223***                                   | -0.205***           | 0.123*                                     |
| <b>Neotropical</b><br><b>(n = 297)</b> | <b>Altitudinal</b><br><b>range</b>   | 0.126***         | 0.343***                                   | 0.352***            | 0.33***                                    |
|  | <b>Temperature</b><br><b>anomaly</b> | 0.235***         | 0.034 <sup>NS</sup>                        | 0.391***            | 0.26***                                    |

## **Discussion**

Geographical distance seems to be the main driver structuring turtle communities in all biogeographic realms. Although environmental processes also influence beta diversity patterns of turtles in some realms, geographical distance was more important than the environmental ones in all realms. Besides, the distance-decay pattern we observed in this study was not influenced by environmental distances, suggesting the importance of dispersal or historical factors in explaining this pattern. Finally, beta diversity patterns in the phylogenetic and taxonomic dimensions were influenced by temperature anomaly since the LGM and topographical heterogeneity in different ways in the Neotropical and Neartic realms.

### *Question 1: How is turtle beta diversity distributed worldwide?*

General patterns of turnover and nestedness/richness difference components of beta diversity were similar between the taxonomic and phylogenetic dimensions, although taxonomic patterns were more evident. Turnover was generally high in areas with moderate to high elevations, such as western North America, north-western South America, and southern continental Asia, reinforcing the importance of altitude in generating turnover among the communities (Melo et al., 2009), which may also affect turtle dispersal (Fritz et al., 2005). Nestedness patterns were less evident, but it is possible to realise that this component was higher in regions affected by LGM in North America, also reinforcing previous findings from the literature which state that glaciated areas were only recently recolonised by a subset of taxa from the non-glaciated regions (Leprieur et al., 2011; Baselga et al., 2012; Dobrovolski et al., 2012; Rödder et al., 2013). These patterns in the Neartic and Neotropical realms are better discussed below.

*Question 2: Are turtle communities structured predominantly by environmental or geographical processes?*

Environmental distances were important in explaining turnover in species composition in turtles in only six realms and their effect was generally low (Table 1; note that the importance of environmental distances are even lower when using phylogenetic beta diversity – Table 2). In these regions, we found a positive relationship between environmental distance and turnover, suggesting that differences in climatic conditions are determining differences in species composition, an ecological processes known as environmental filtering (Keddy, 1992). Environmental processes were most important in large continental areas with relatively high turtle diversity, such as the Nearctic, Australian, Neotropic, and Sino-Japanese regions. These regions do not have strong dispersal barriers, such as those that occur in the Oriental and Oceania (islands) and the Sahara-Arabian (Sahara desert) realms, which could explain the importance of environmental factors there because minimal dispersal limitation may allow species to more readily exploit their optimal ecological requirements (Nekola & White, 1999). In a study covering different zoogeographical realms, Qian & Ricklefs (2012) found that most variation was explained by the combination of environmental and geographical distance, which also occurred for some realms in our study. However, these authors worked with terrestrial vertebrates, including animal groups with higher dispersal capacity such as mammals and birds, which could explain the higher importance they found to environmental distances when compared to our study.

Geographical distance was more important than the environmental ones in explaining turnover in turtle species among the realms. Previous studies also found that

geographical distance was the main factor explaining communities of other terrestrial (Beaudrot & Marshall, 2011; Hájek et al., 2011; Chytrý et al., 2012) and aquatic animals (Beisner et al., 2006), and that this is more common among organisms with poor dispersal ability. Considering the limited dispersal abilities of turtles, this result is not surprising. These animals may not be able to fully exploit the available climatic conditions that are suitable for them and are unable to cross many physical barriers, resulting in a stronger distance decay effect when compared to animals with good dispersal abilities. The low influence of environmental processes may also be due to the environmental variables chosen in our study. However, since we used the same environmental variables that are commonly associated with other diversity patterns in turtles (Iverson, 1992; Angielczyk et al., 2015; Rodrigues et al., 2016), we think this explanation is unlikely. Future studies exploring new environmental variables with biological relevance for turtles might provide interesting results. Finally, considering that spatial scale may also influence beta diversity patterns and their estimated drivers (Mac Nally et al., 2004; Barton et al., 2013), the high importance of geographical distance found in our study may be due to the coarse or global scale used (2° by 2° grid). New studies focusing on beta diversity at fine or local scales may provide new insights regarding the importance of environmental variables.

The similarity found in the correlates of the taxonomic and phylogenetic beta diversity indices reinforces the notion that both metrics could represent similar ecological patterns. This finding has already been reported in other studies employing a phylogenetic beta diversity approach (Leprieur et al., 2012). Although some authors suggest that phylogenetic beta diversity could be more strongly related to environmental variation than the taxonomic dimension (Warren et al., 2014), both beta diversity

dimensions in our study were similarly related to environmental variables. Furthermore, our standardised effect size values of phylogenetic beta diversity were very poorly explained by the predictors used in our study, even the geographical ones. This result suggests that deviations of phylogenetic beta diversity from expected patterns given the taxonomic beta diversity are highly idiosyncratic even at the biogeographic realms level.

*Question 3: What explains the distance-decay patterns observed for turtle communities in each realm?*

The distance decay observed in our study cannot be explained by an increase in environmental distances that are a consequence of geographical distances, even though this explanation is a possible cause of distance decay patterns in some taxa. Other spatial and historical processes could be relevant (Nekola & White, 1999). The distance decay also cannot be explained by the realm's area. However, since this area effect is mainly attributed to increased variation in environmental conditions within larger areas (Nekola & White, 1999), the weak influence of environmental distance on beta diversity patterns and the influence of geographic distance, even after controlling for environmental variation, likely explain the lack of an area effect in our analyses.

*Question 4: When deterministic processes are important to structure turtle communities, which process is the most important?*

The effects of environmental variables on taxonomic beta diversity components in the Nearctic realm followed exactly our initial expectations and results from previous studies evaluating the influence of temperature anomaly on beta diversity, i.e. climatic

instability negatively influenced turnover and positively influenced the nestedness/richness difference components (Leprieur et al., 2011; Dobrovolski et al., 2012). However, the phylogenetic components of beta diversity were more strongly influenced by topography than by temperature anomaly. Since phylogenetic beta diversity, in both components, is more related to speciation and cladogenetic processes than taxonomic beta diversity, altitudinal range representing spatial isolation may have a stronger macroevolutionary effect, which is reflected in phylogenetic beta diversity. Besides, it is possible to observe that phylogenetic beta diversity components are high in the southwestern-western part of the Nearctic realm, where topography is much influenced by mountains. Recent genetic studies have found that mountain chains represent important barriers to gene flow in turtles (Fritz et al., 2005). In the Neotropical realm, temperature instability had an effect inconsistent with previous findings in the literature, as it was positively related to the turnover component and did not even influence the nestedness component in the taxonomic approach. These inconsistencies are in accordance with our previous expectations that realms where glaciations did not have a strong effect should not have similar effects for temperature anomaly when compared to biogeographic realms that were strongly affected (e.g. Nearctic and Palearctic realms). In the Neotropics, areas with climatic instability are related to strong variation in vegetation structure (not to freezing), which may promote diversification and turnover in diversity (Vanzolini & Williams, 1981; Damasceno et al., 2014). These different mechanisms (habitat change VS freezing exclusion) inferred from the same proxy (temperature anomaly) may explain why temperature anomaly since the LGM has different effects in both regions.

We conclude that geographical distance independent of environmental (i.e. climatic) variation is the main driver structuring turtle community compositions across the different biogeographic realms. Besides, environmental processes may have different effects on different biogeographic realms, reinforcing the need to evaluate them independently in future analyses. Thus, by evaluating beta diversity in each realm, it was possible to reinforce the idea that different processes affect the diversity of these large regions, supporting their unique histories.

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### **Supporting information**

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** – Supporting methods and results.

## **Biosketch**

**João Fabrício M. Rodrigues** is a PhD candidate in Ecology and Evolution at Universidade Federal de Goiás and is interested in general macroecological and macroevolutionary questions as well as natural history studies related to amphibians and reptiles, especially freshwater turtles and tortoises.

**José Alexandre F. Diniz-Filho** is a Professor in Ecology and Evolution at Universidade Federal de Goiás. His research is mainly related to ecology and evolutionary biology, with an emphasis in macroecology, geographical ecology, phylogenetic comparative methods, and genetics. He also focuses on statistical methods applied to macroecology, comparative analyses, and population genetics.

**Editor:** Holger Kreft

**Dispersal is more important than climate in structuring turtle communities across different biogeographic realms**

**Appendix S1** – Supporting methods and results

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**Equations used for calculate taxonomic and phylogenetic beta diversity components**

**Table S1.1** - Species used in the taxonomic beta diversity analyses.

**Table S1.2.** Correlation matrix of the environmental variables selected for our study and used for constructing the environmental distance matrix.

**Table S1.3.** Summary of richness values for each cell in different regions.

**Table S1.4.** Variance partitioning results and partial Mantel correlation tests using standardized effect sizes of turnover component of pair-wise phylogenetic beta diversity in turtles.

**Figure S1.1.** Phylogeny of the turtle species used in phylogenetic beta diversity analyses.

## References

### Equations used for calculate taxonomic and phylogenetic beta diversity components

Beta diversity components (turnover and nestedness/richness difference) were calculated using the formulas from Baselga (2010) for taxonomic beta diversity and Leprieur et al. (2012) for phylogenetic beta diversity. These analyses were run in the R package betapart (Baselga & Orme, 2012).

*Taxonomic turnover (Baselga, 2010)*

$$\beta_{turn} = \frac{\min(b, c)}{a + \min(b, c)}$$

*Taxonomic nestedness/richness difference (Baselga, 2010)*

$$\beta_{nes} = \frac{\max(b, c) - \min(b, c)}{2a + \min(b, c) + \max(b, c)} \times \frac{a}{a + \min(b, c)}$$

“a” is the number of species occurring in both communities and “b” and “c” are the number of species occurring only in one of the communities.

*Phylogenetic turnover (Leprieur et al., 2012)*

$$phylo_{turn} = \frac{\min(PD_{Tot} - PD_k, PD_{Tot} - PD_j)}{PD_k + PD_j - PD_{Tot} + \min(PD_{Tot} - PD_k, PD_{Tot} - PD_j)}$$

*Phylogenetic nestedness/richness difference (Leprieur et al., 2012)*

$$phylo_{nes} = \frac{2PD_{tot} - PD_k - PD_j}{PD_k + PD_j} - \frac{\min(PD_{Tot} - PD_k, PD_{Tot} - PD_j)}{PD_k + PD_j - PD_{Tot} + \min(PD_{Tot} - PD_k, PD_{Tot} - PD_j)}$$

“PD<sub>tot</sub>” is the phylogenetic diversity calculated considering both communities together, “PD<sub>k</sub>” and “PD<sub>j</sub>” are the phylogenetic diversities calculated considering each community separately.

**Table S1.1.** Species used in the taxonomic beta diversity analyses.

---

| <b>Species</b>                       |
|--------------------------------------|
| <i>Acanthochelys macrocephala</i>    |
| <i>Acanthochelys radiolata</i>       |
| <i>Acanthochelys pallidipectoris</i> |
| <i>Acanthochelys spixii</i>          |
| <i>Actinemys marmorata</i>           |
| <i>Apalone ferox</i>                 |
| <i>Amyda cartilaginea</i>            |
| <i>Apalone mutica</i>                |
| <i>Astrochelys radiata</i>           |
| <i>Batagur affinis</i>               |
| <i>Batagur baska</i>                 |
| <i>Batagur borneoensis</i>           |
| <i>Apalone spinifera</i>             |
| <i>Batagur dhongoka</i>              |
| <i>Batagur kachuga</i>               |
| <i>Batagur trivittata</i>            |
| <i>Carettochelys insculpta</i>       |
| <i>Chelodina burrungandjii</i>       |
| <i>Centrochelys sulcata</i>          |
| <i>Chelodina canni</i>               |
| <i>Chelodina gunaleni</i>            |
| <i>Chelodina kuchlingi</i>           |
| <i>Chelodina expansa</i>             |
| <i>Chelodina novaeguineae</i>        |
| <i>Chelodina longicollis</i>         |
| <i>Chelodina parkeri</i>             |
| <i>Chelodina reimanni</i>            |
| <i>Chelodina oblonga</i>             |
| <i>Chelodina rugosa</i>              |
| <i>Chelodina walloyarrina</i>        |
| <i>Chelonoidis carbonaria</i>        |
| <i>Chelonoidis chilensis</i>         |
| <i>Chelonoidis denticulata</i>       |
| <i>Chelydra acutirostris</i>         |
| <i>Chelydra rossignonii</i>          |
| <i>Chelus fimbriata</i>              |

---

**Species**

---

*Chersina angulata*  
*Chitra chitra*  
*Chelydra serpentina*  
*Chitra vandijki*  
*Chitra indica*  
*Chrysemys dorsalis*  
*Claudius angustatus*  
*Clemmys guttata*  
*Chrysemys picta*  
*Cuora aurocapitata*  
*Cuora bourreti*  
*Cuora flavomarginata*  
*Cuora mccordi*  
*Cuora galbinifrons*  
*Cuora pani*  
*Cuora picturata*  
*Cuora mouhotii*  
*Cuora yunnanensis*  
*Cuora trifasciata*  
*Cuora zhoui*  
*Cyclanorbis elegans*  
*Cuora amboinensis*  
*Cyclemys atripons*  
*Cyclanorbis senegalensis*  
*Cyclemys dentata*  
*Cyclemys fusca*  
*Cyclemys enigmatica*  
*Cyclemys gemeli*  
*Cyclemys pulchristriata*  
*Cyclemys oldhamii*  
*Cycloderma aubryi*  
*Cycloderma frenatum*  
*Deirochelys reticularia*  
*Dermatemys mawii*  
*Dogania subplana*  
*Elseya albagula*  
*Elseya branderhorsti*  
*Elseya dentata*  
*Elseya irwini*  
*Elseya lavarackorum*  
*Elseya novaeguineae*  
*Elseya rhodini*  
*Elseya schultzei*

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**Species**

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*Emydoidea blandingii*  
*Emydura macquarii*  
*Emydura tanybaraga*  
*Emydura subglobosa*  
*Emydura victoriae*  
*Emydura worrelli*  
*Erymnochelys*  
*madagascariensis*  
*Geochelone platynota*  
*Geochelone elegans*  
*Emys orbicularis*  
*Geoemyda spengleri*  
*Geoclemys hamiltonii*  
*Glyptemys muhlenbergii*  
*Glyptemys insculpta*  
*Gopherus agassizii*  
*Gopherus flavomarginatus*  
*Gopherus berlandieri*  
*Gopherus morafkai*  
*Graptemys barbouri*  
*Graptemys caglei*  
*Graptemys ernsti*  
*Gopherus polyphemus*  
*Graptemys flavimaculata*  
*Graptemys gibbonsi*  
*Graptemys oculifera*  
*Graptemys nigrinoda*  
*Graptemys geographica*  
*Graptemys pearlensis*  
*Graptemys ouachitensis*  
*Graptemys pulchra*  
*Graptemys versa*  
*Graptemys*  
*pseudogeographica*  
*Hardella thurjii*  
*Heosemys depressa*  
*Heosemys annandalii*  
*Heosemys grandis*  
*Homopus areolatus*  
*Homopus boulengeri*  
*Heosemys spinosa*  
*Homopus femoralis*  
*Homopus signatus*  
*Homopus solus*

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**Species**

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*Hydromedusa maximiliani*  
*Hydromedusa tectifera*  
*Indotestudo forstenii*  
*Indotestudo travancorica*  
*Indotestudo elongata*  
*Kinixys belliana*  
*Kinixys erosa*  
*Kinixys lobatsiana*  
*Kinixys homeana*  
*Kinixys natalensis*  
*Kinixys nogueyi*  
*Kinixys spekii*  
*Kinixys zombensis*  
*Kinosternon acutum*  
*Kinosternon arizonense*  
*Kinosternon alamosae*  
*Kinosternon chimalhuaca*  
*Kinosternon baurii*  
*Kinosternon creaseri*  
*Kinosternon dunnii*  
*Kinosternon durangoense*  
*Kinosternon herrerae*  
*Kinosternon hirtipes*  
*Kinosternon flavescens*  
*Kinosternon integrum*  
*Kinosternon leucostomum*  
*Kinosternon oaxacae*  
*Kinosternon sonoriense*  
*Kinosternon scorpioides*  
*Leucocephalon yuwonoi*  
*Lissemys ceylonensis*  
*Kinosternon subrubrum*  
*Lissemys scutata*  
*Macrochelys suwanniensis*  
*Lissemys punctata*  
*Macrochelys temminckii*  
*Malaclemys terrapin*  
*Malacochersus tornieri*  
*Malayemys macrocephala*  
*Malayemys subtrijuga*  
*Manouria emys*  
*Manouria impressa*  
*Mauremys japonica*

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**Species**

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*Mauremys caspica*  
*Mauremys leprosa*  
*Mauremys mutica*  
*Mauremys nigricans*  
*Mauremys rivulata*  
*Mauremys sinensis*  
*Melanochelys tricarinata*  
*Mauremys reevesii*  
*Mesoclemmys dahli*  
*Melanochelys trijuga*  
*Mesoclemmys gibba*  
*Mesoclemmys heliostemma*  
*Mesoclemmys hogei*  
*Mesoclemmys nasuta*  
*Mesoclemmys perplexa*  
*Mesoclemmys tuberculata*  
*Mesoclemmys raniceps*  
*Mesoclemmys zuliae*  
*Mesoclemmys vanderhaegei*  
*Morenia ocellata*  
*Myuchelys bellii*  
*Morenia petersi*  
*Myuchelys latisternum*  
*Nilssonina formosa*  
*Nilssonina gangetica*  
*Nilssonina nigricans*  
*Nilssonina leithii*  
*Nilssonina hurum*  
*Notochelys platynota*  
*Orlitia borneensis*  
*Palea steindachneri*  
*Pangshura smithii*  
*Pangshura sylhetensis*  
*Pangshura tecta*  
*Pangshura tentoria*  
*Pelochelys bibroni*  
*Pelodiscus axenaria*  
*Pelochelys signifera*  
*Pelodiscus parviformis*  
*Pelochelys cantorii*  
*Pelodiscus sinensis*  
*Peltocephalus dumerilianus*  
*Pelomedusa subrufa*

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**Species**

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*Pelusios adansonii*  
*Pelusios bechuanicus*  
*Pelusios carinatus*  
*Pelusios castaneus*  
*Pelusios castanoides*  
*Pelusios chapini*  
*Pelusios cupulatta*  
*Pelusios marani*  
*Pelusios gabonensis*  
*Pelusios niger*  
*Pelusios nanus*  
*Pelusios rhodesianus*  
*Pelusios sinuatus*  
*Pelusios upembae*  
*Pelusios williamsi*  
*Pelusios subniger*  
*Phrynops hilarii*  
*Phrynops tuberosus*  
*Phrynops geoffroanus*  
*Phrynops williamsi*  
*Platysternon megacephalum*  
*Platemys platycephala*  
*Podocnemis erythrocephala*  
*Podocnemis lewyana*  
*Podocnemis expansa*  
*Podocnemis sextuberculata*  
*Podocnemis vogli*  
*Podocnemis unifilis*  
*Psammobates oculifer*  
*Psammobates tentorius*  
*Pseudemys alabamensis*  
*Pseudemys concinna*  
*Pseudemys floridana*  
*Pseudemys gorzugi*  
*Pseudemys nelsoni*  
*Pseudemys peninsularis*  
*Pseudemys rubriventris*  
*Pseudemys texana*  
*Pyxis planicauda*  
*Rafetus euphraticus*  
*Rafetus swinhoei*  
*Rheodytes leukops*  
*Rhinemys rufipes*

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**Species**

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*Rhinoclemmys annulata*  
*Rhinoclemmys areolata*  
*Rhinoclemmys diademata*  
*Rhinoclemmys funerea*  
*Rhinoclemmys melanosterna*  
*Rhinoclemmys nasuta*  
*Rhinoclemmys pulcherrima*  
*Rhinoclemmys rubida*  
*Sacalia bealei*  
*Rhinoclemmys punctularia*  
*Sacalia quadriocellata*  
*Siebenrockiella crassicollis*  
*Staurotypus triporcatus*  
*Sternotherus carinatus*  
*Sternotherus depressus*  
*Sternotherus minor*  
*Sternotherus odoratus*  
*Stigmochelys pardalis*  
*Terrapene mexicana*  
*Terrapene nelsoni*  
*Terrapene carolina*  
*Terrapene yucatana*  
*Terrapene ornata*  
*Testudo hermanni*  
*Testudo graeca*  
*Testudo kleinmanni*  
*Testudo marginata*  
*Trachemys callirostris*  
*Trachemys dorbigni*  
*Trachemys emolli*  
*Trachemys gaigeae*  
*Trachemys grayi*  
*Trachemys nebulosa*  
*Trachemys ornata*  
*Testudo horsfieldii*  
*Trachemys scripta*  
*Trachemys yaquia*  
*Trachemys venusta*  
*Vijayachelys silvatica*  
*Trionyx triunguis*

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**Table S1.2.** Correlation matrix of the environmental variables selected for our study and used to construct the environmental distance matrix. Temperature anomaly corresponds to the difference between present temperature and temperature during the Last Glacial Maximum (22,000 years ago).

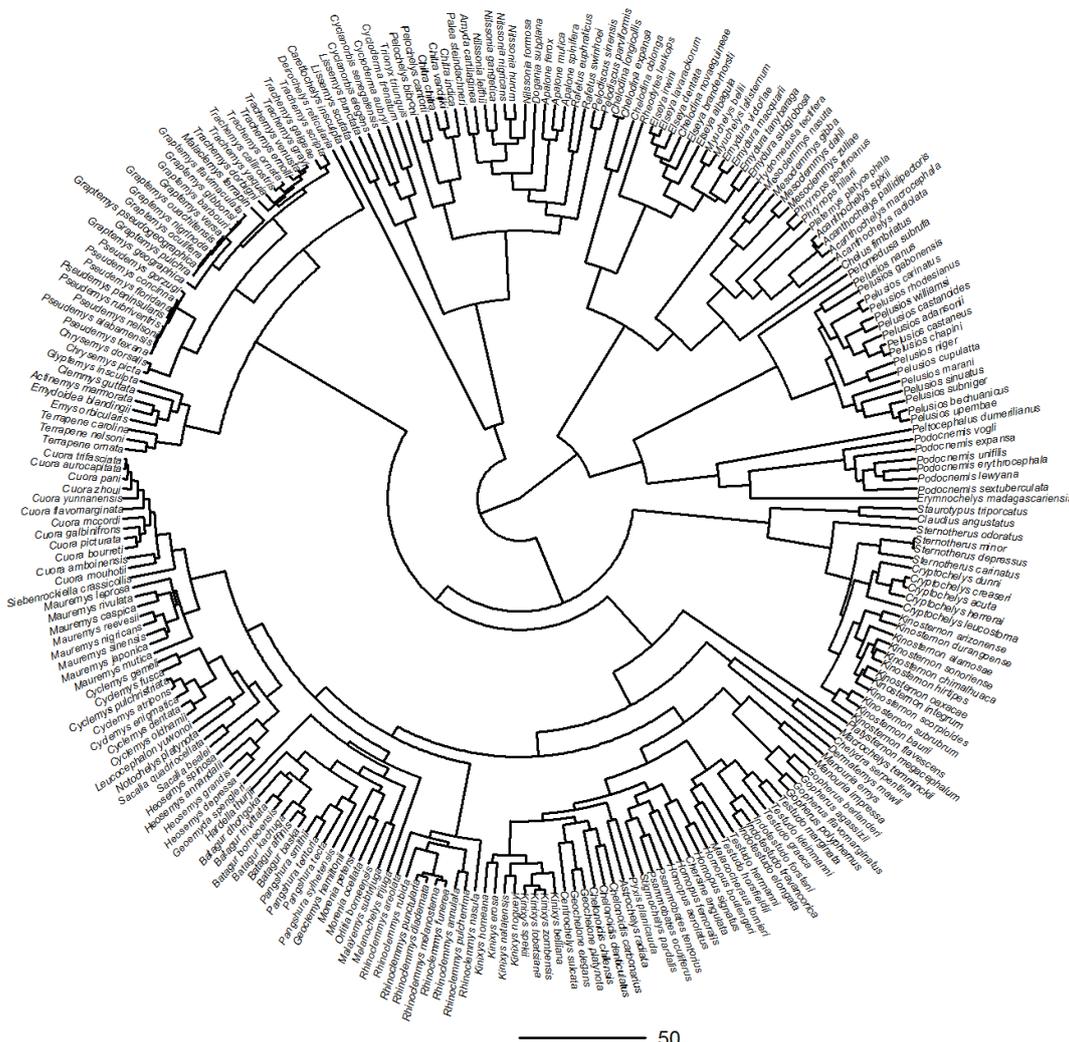
|                            | <b>Altitude</b> | <b>Temperature</b> | <b>Precipitation</b> | <b>Temperature anomaly</b> |
|----------------------------|-----------------|--------------------|----------------------|----------------------------|
| <b>Altitude</b>            | 1               | -0.40835           | -0.24981             | -0.049615403               |
| <b>Temperature</b>         | -0.40835        | 1                  | 0.309293             | -0.699509031               |
| <b>Precipitation</b>       | -0.24981        | 0.309293           | 1                    | -0.162516671               |
| <b>Temperature anomaly</b> | -0.04962        | -0.69951           | -0.16252             | 1                          |

**Table S1.3.** Summary of turtle richness values for each 2° x 2° cell in the different realms. Mean = mean richness; median = median richness; maximum = richness of the cell with the highest richness; minimum = richness of the cell with the lowest richness; Standard Deviation = Standard deviation of richness. N = sample size or number of cells of each realm. Note that sample sizes in this table are different from the values reported in Tables 1, 2 and S1.4 because we did not remove cells with equal or less than two species to calculate summary statistics of richness values in each realm.

| <b>Region (N)</b>    | <b>Mean</b> | <b>Median</b> | <b>Maximum</b> | <b>Minimum</b> | <b>Standard Deviation</b> |
|----------------------|-------------|---------------|----------------|----------------|---------------------------|
| Neotropical (373)    | 6.514745    | 6             | 16             | 0              | 4.272055                  |
| Australian (177)     | 1.819209    | 1             | 9              | 0              | 1.994616                  |
| Afrotropical (510)   | 4.747059    | 5             | 11             | 0              | 2.663281                  |
| Madagascan (12)      | 2.583333    | 3             | 4              | 1              | 0.792961                  |
| Oceanian (17)        | 4.647059    | 5             | 9              | 0              | 2.998774                  |
| Oriental (170)       | 9.764706    | 10            | 22             | 0              | 4.584892                  |
| Panamanian (23)      | 7.304348    | 7             | 12             | 1              | 2.687256                  |
| Saharo-Arabian (249) | 0.907631    | 0             | 8              | 0              | 1.284014                  |
| Nearctic (587)       | 3.103918    | 0             | 23             | 0              | 4.868345                  |
| Sino-Japanese (129)  | 2.48062     | 2             | 16             | 0              | 3.522474                  |
| Palaearctic (1369)   | 0.328707    | 0             | 5              | 0              | 0.741463                  |

**Table S1.4.** Partial Mantel correlation tests (R) associating standardized effect sizes of turnover component of pair-wise phylogenetic beta diversity in turtles to environmental distance after controlling for log-transformed geographical distances within each region and associating pair-wise turnover beta diversity to log-transformed geographical distances after controlling for environmental distances and variance explained (R-squared) by environmental, spatial and shared components. \* P < 0.05; \*\* P < 0.01; \*\*\* P = 0.001; NS = non-significant. N = sample size or number of cells of each realm. NA values found in Oceania are due to the fact that all communities were perfectly nested subsets of the richer one, except two pairs that were totally non-nested. This pattern generates phylobetadiversity values in the null distributions with always the same values.

| <b>Region (N)</b>      | <b>Partial Mantel<br/>(space controlled)</b> | <b>Partial Mantel<br/>(environment<br/>controlled)</b> | <b>Environment<br/>only</b> | <b>Space<br/>only</b> | <b>Shared<br/>effect</b> |
|------------------------|--|--|-----------------------------|-----------------------|--------------------------|
| Neotropical (297)      | 0.006 <sup>NS</sup>                          | 0.10 <sup>***</sup>                                    | 2.39E-05                    | 0.020882              | 0.002019                 |
| Australian (59)        | -0.02 <sup>NS</sup>                          | -0.42 <sup>NS</sup>                                    | 4.14E-05                    | 0.16079               | 0.237509                 |
| Afrotropical (393)     | 0.14 <sup>***</sup>                          | -0.17 <sup>NS</sup>                                    | 0.013127874                 | 0.012778              | -0.00721                 |
| Madagascan (7)         | 0.14 <sup>NS</sup>                           | 0.16 <sup>NS</sup>                                     | 0.000893525                 | 0.063043              | 0.027999                 |
| Oceanian (11)          | NA   | NA   | NA                          | NA                    | NA                       |
| Oriental (162)         | 0.02 <sup>NS</sup>                           | 0.25 <sup>***</sup>                                    | 3.57E-05                    | 0.071237              | 0.01358                  |
| Panamanian (21)        | -0.32 <sup>NS</sup>                          | 0.45 <sup>***</sup>                                    | 0.063046967                 | 0.279574              | -0.02871                 |
| Saharo-Arabian<br>(27) | 0.10 <sup>NS</sup>                           | -0.02 <sup>NS</sup>                                    | 0.009560041                 | 2.42E-05              | 8.89E-05                 |
| Nearctic (186)         | 0.12 <sup>***</sup>                          | -0.02 <sup>NS</sup>                                    | 0.003481393                 | 0.004335              | 0.018966                 |
| Sino-Japanese (40)     | 0.29 <sup>*</sup>                            | 0.12 <sup>NS</sup>                                     | 0.045139326                 | 0.046928              | 0.161392                 |
| Palaearctic (29)       | -0.09 <sup>NS</sup>                          | -0.32 <sup>NS</sup>                                    | 0.033944982                 | 0.077028              | 0.01104                  |



**Figure S1.1.** Phylogeny of the turtle species used in phylogenetic beta diversity analyses. See Rodrigues & Diniz-Filho (2016) for a full version of this phylogeny and for more details about it.

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## **Capítulo 4**

Rodrigues, J.F.M., Olalla-Tárraga, M.Á., Iverson, J.B., Diniz-Filho, J.A.F.

Firing up the shells: temperature is the main correlate of the global  
biogeography of turtle body size.

Submetido para a revista *Global Ecology and Biogeography*

**Firing up the shells: temperature is the main correlate of the global biogeography of turtle body size**

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**Running title:** Body size patterns in turtles

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**Number of references:** 63 references

## **Abstract**

**Aims:** Geographical gradients in body size have been much studied in endotherms, and general rules exist to describe body size variation in these animals. However, the existence of broad-scale patterns in body size variation in ectotherms remains largely undocumented and debated. Turtles (tortoises and freshwater turtles) are ectothermic organisms whose geographical variation in body size has not been examined widely. Here, we aimed to evaluate which of the common hypotheses suggested to explain body size patterns in animals are best suited to this group of reptiles.

**Location:** Global

**Time period:** Current

**Major taxa studied:** Turtles

**Methods:** We gathered distribution, phylogenetic and body size data for 235 species of turtles, which were distributed in a global equal area grid of 200km x 200km. We also obtained predictor variables (mean annual temperature, actual evapotranspiration, temperature variation since the Last Glacial Maximum (LGM) and human foot print) directly associated with the main hypotheses tested in body size studies. Our analyses followed a cross-species and an assemblage-based approach and were performed for all turtles and for terrestrial and aquatic species separately.

**Results:** We found a weak latitudinal gradient in body size of terrestrial turtles, that could not be detected for aquatic turtles and across the whole group. Mean annual temperature was the main correlate of body size for the whole group and for terrestrial turtles in both approaches, while body sizes of aquatic turtles were not influenced by any of the tested variables. In the cross-species approach we also found that temperature variation since the LGM was an important predictor of body size in terrestrial turtles.

**Main conclusions:** Our study reinforces the importance of environmental energy variables and habitat differences in explaining animal body size patterns.

**Keywords:** Carapace length; Ecogeographical rules; Ectotherms; Freshwater turtles; Similarity issue; Tortoises;

## Introduction

Body size is a biological trait commonly associated with many aspects of an animal's life-history. Body size may be related to habitat use (Jaffe, Slater & Alfaro 2011), range size (Gaston & Blackburn 1996), reproductive maturity (Shine & Charnov 1992; Shine & Iverson 1995), and extinction risk (Slavenko *et al.* 2016b) among others. Numerous studies have documented geographical patterns in the distribution of animal body sizes at both intra- and interspecific levels of the biological organization (Ashton 2002; Ashton & Feldman 2003; Meiri & Dayan 2003; Olalla-Tárraga *et al.* 2010; Vinarski 2014). Beyond such a descriptive approach, understanding which mechanisms drive the observed clinal variation in body size has received much attention in recent years.

Many hypotheses have been proposed to explain large-scale body size gradients. According to the classical heat conservation hypothesis, animals should be larger in cold than in warm areas because lower surface to volume ratios reduce heat loss (Blackburn, Gaston & Loder 1999; Salewski & Watt 2016). However, some ectothermic taxa (which by definition rely on external heat sources to increase body temperatures) show a reverse pattern, possibly because small sizes may be advantageous in cold areas to gain heat faster (heat balance hypothesis) (Olalla-Tárraga, Rodríguez & Hawkins 2006; Olalla-Tárraga 2011). Under the primary productivity hypothesis (also referred to as the resource rule), large bodied animals should be more common in productive areas, coincident with high levels of environmental energy and resource availability (Rosenzweig 1968). On the basis of size-dependent dispersal abilities, the migration hypothesis states that larger animals are better represented polewards because these organisms have been able to first recolonize those regions that become available after the ice-sheet retreat following Pleistocene glacial cycles (Blackburn *et al.* 1999;

Olalla-Tárraga *et al.* 2006). Finally, anthropogenic activities such as hunting, harvesting pressure and habitat fragmentation can also affect the geographic distribution of animal body sizes, causing differential extinction levels on large bodied individuals of a population (de Souza Alcântara, da Silva & Pezzuti 2013; Sung, Karraker & Hau 2013; Rhodin *et al.* 2015; Slavenko *et al.* 2016b). Hence, those areas with high human impact levels tend to be coincident with a reduction in the body sizes of populations and species (Diniz-Filho *et al.* 2009; Torres-Romero, Morales-Castilla & Olalla-Tárraga 2016).

Macroecological studies have evaluated body size patterns for different vertebrate taxa, mainly mammals and birds, which are generally larger in temperate areas (Meiri & Dayan 2003; Millien *et al.* 2006; Diniz-Filho *et al.* 2007; Olson *et al.* 2009). This Bergmannian pattern of increasing size polewards is commonly attributed to the heat conservation hypothesis (Blackburn *et al.* 1999; Salewski & Watt 2016). However, it is still unclear the extent to which ectothermic animals display body size gradients as a response to broad-scale environmental variation. While several recent studies have addressed this question over the past few years, ectotherms do not seem to follow a single pattern (Ashton 2002; Ashton & Feldman 2003; Olalla-Tárraga *et al.* 2006; Olalla-Tárraga & Rodríguez 2007; Vinarski 2014), which begs for new analyses across different groups of ectotherms before we can reach a general conclusion.

Turtles are ectotherms distributed nearly worldwide (Iverson 1992a; van Dijk *et al.* 2014; Rodrigues *et al.* 2017). They are an interesting model for studying body size variation due to the facility and confidence of measuring their size because of the shell covering their body. At the intraspecific level, turtles seem to be larger at high latitudes (Ashton & Feldman 2003), but no clear latitudinal body size patterns emerge

interspecifically (Angielczyk, Burroughs & Feldman 2015). No previous study, however, has deeply evaluated which factors are more likely to have influenced geographical patterns in body size in turtles. Here, we aimed to examine the environmental correlates of turtle body size patterns and explicitly test whether the hypotheses usually considered to explain body size variation may be applied to these organisms.

## **Material and Methods**

We used an World Cylindrical equal area grid (200km x 200km) and range maps of 280 turtle species (van Dijk *et al.* 2014) to generate a presence-absence matrix with cells in rows and species in columns. This grid cell resolution is considered adequate to use with range maps, because coarser scales could cause overestimates in species occupancy area (Hurlbert & Jetz 2007).

As in previous turtle studies (Moen 2006; Jaffe *et al.* 2011; Itescu *et al.* 2014), we used maximum carapace length as body size metric, since it is a stable measurement which is not influenced by seasonal variation in reproductive and feeding status. Besides, using this measure avoids biases related to immature individuals influencing body size patterns. We obtained length data for all species whose distribution overlapped at least one grid cell (235 species) mainly from Itescu *et al.* (2014) and complemented the dataset with a literature search. Body size data were log-transformed ( $\log_e x$ ) in order to normalize the typically right-skewed body size frequency distributions.

Assuming that body size usually has a strong phylogenetic component related to the evolutionary history of the group and a specific component commonly associated

with species adaptations after divergence from ancestrals, we used Phylogenetic Eigenvector Regression (PVR) to calculate the variation in body size independent of phylogenetic history (Diniz-Filho, De Sant'ana & Bini 1998; Diniz-Filho *et al.* 2012). To perform PVR, we used a recently published turtle phylogeny, which covers a high number of species, uses data of mitochondrial and nuclear genes and provides branch lengths calibrated with fossil records (Rodrigues & Diniz-Filho 2016). Eigenvectors were selected sequentially until residual phylogenetic autocorrelation (evaluated using Moran's I) of the regression between body size and selected eigenvectors was not significant ( $p > 0.05$ ). The residuals of the regression using body size as the response variable against the final selected eigenvectors were called the specific component (S) and represent a phylogenetically independent body size component. We then calculated median body size and the median component S for turtles co-occurring in each cell.

We then computed a number of environmental variables that have been previously related to each of the tested hypotheses, namely: 1) heat conservation hypothesis and heat balance hypothesis – mean annual temperature (temperature) (Hijmans *et al.* 2005); 2) Productivity hypothesis – Actual Evapotranspiration (AET) (Ahn & Tateishi 1994); 3) Migration hypothesis - temperature variation since the Last Glacial Maximum (LGM), calculated as present temperature minus temperature in the LGM (22,000 years ago) estimated using MIROC-ESM global circulation model (available at [www.worldclim.org](http://www.worldclim.org)); 4) Human impacts hypothesis – Human Footprint, an index of human influence obtained from a combination of spatial data regarding population density, land transformation, human accessibility, and power infrastructure (Sanderson *et al.* 2002). Although some biotic hypotheses such as fecundity and competition have also been suggested to explain body size variations (Iverson & Smith

1993; Iverson *et al.* 1997; Blackburn *et al.* 1999), we focused only on abiotic correlates which tend to become more important in determining animal body size over the large spatial scales we are studying. We calculated mean values of these variables for each grid cell and for each species range. Values for each cell and species were standardized (mean = 0 and sd = 1) prior to statistical analyses in order to allow comparisons among regression coefficients.

Our analyses followed the two interspecific approaches available to explore body size gradients in macroecology: assemblage-based, where grid cells are the sampling units; and cross-species, where species are the sampling units (Gaston, Chown & Evans 2008; Olalla-Tárraga *et al.* 2010). While the cross-species approach allows us to more directly investigate factors influencing species traits, the assemblage-based approach also allows one to evaluate whether the trait may influence the assemblage structure (Millien *et al.* 2006; Bishop *et al.* 2016; Osorio-Canadas *et al.* 2016).

In the assemblage-approach, median log-body size and median specific components for each cell were regressed against the four independent variables described above. We used median assemblage values instead of mean values due to the right-skewed distribution of body size across assemblages (Meiri & Thomas 2007). We used SAR (Simultaneous Autoregressive models) in these analyses in order to account for spatial autocorrelation. SARs are an efficient method to deal with spatial autocorrelation in data sets (Dormann *et al.* 2007; Kissling & Carl 2008). A neighborhood of 1000km was used in SAR analyses because this distance corresponded to the first class of distance in spatial correlograms, where spatial correlation was strongest. We also calculated pseudo- $R^2$  values (hereafter simply “ $R^2$ ”) to our SAR models as a squared Pearson correlation between fitted and observed values (Kissling &

Carl 2008). SARs models were performed in the R package *spdep* (Bivand, Hauke & Kossowski 2013; Bivand & Piras 2015).

However, considering the similarity or co-occurrence issue recently raised in the macroecological literature (Zelený & Schaffers 2012; Hawkins *et al.* 2017), analyses using mean/median species traits obtained through range overlap as response variables may present a high type I error. To account for this possible problem, we created a null model where species identities were randomly shuffled in our body size data before calculating cell values in order to create 1000 values of median body size and specific components for each cell (Zelený & Schaffers 2012; Hawkins *et al.* 2017). Then, we regressed these values against our predictor variables using SARs and created a null distribution of the regression coefficients of each predictor variable. Finally, we compared the observed values of the coefficients (from our real database) with the null distribution in order to evaluate their significance. Z-values were calculated for each coefficient by subtracting the observed coefficient by the mean of the random coefficients, then dividing the result by the standard deviation of the random distribution of coefficient values.

In the cross-species approach, we used the specific component of each species as the response variable and their mean environmental variables as predictors in a SAR analysis in order to account for potential spatial effects on the species values (Freckleton & Jetz 2009; Terribile *et al.* 2012).

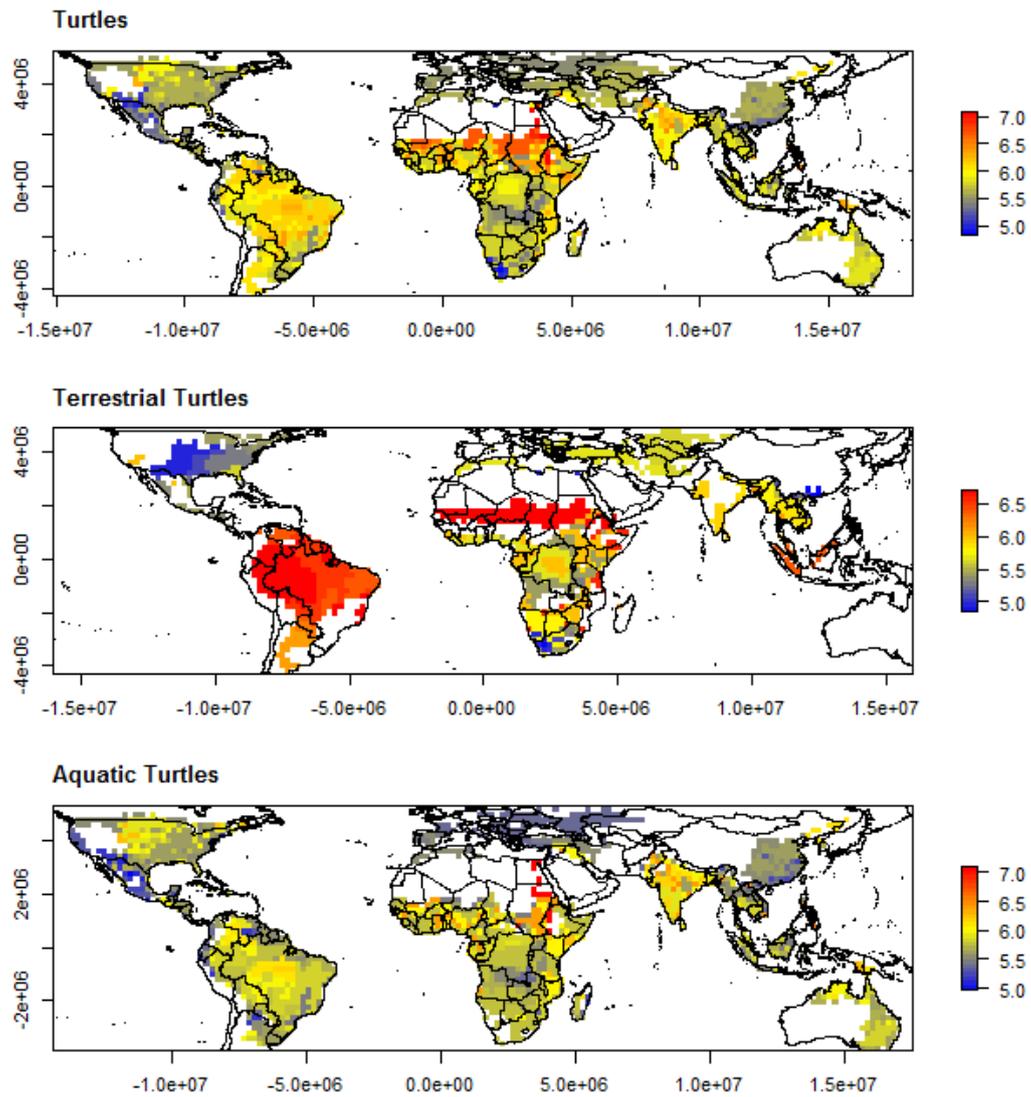
Considering that habitat influences many aspects of turtle life history and evolution, including body size (Jaffe *et al.* 2011; Rodrigues & Diniz-Filho 2016; Slavenko *et al.* 2016a), and that aquatic and terrestrial species are exposed to different environments, we repeated all analyses deconstructing the observed patterns for

terrestrial and aquatic species separately. Terrestrial and aquatic species were defined according to the classification presented in the supplementary material of Rodrigues & Diniz-Filho (2016). All analyses were performed in R ver 3.3.1 (R Core Team 2014).

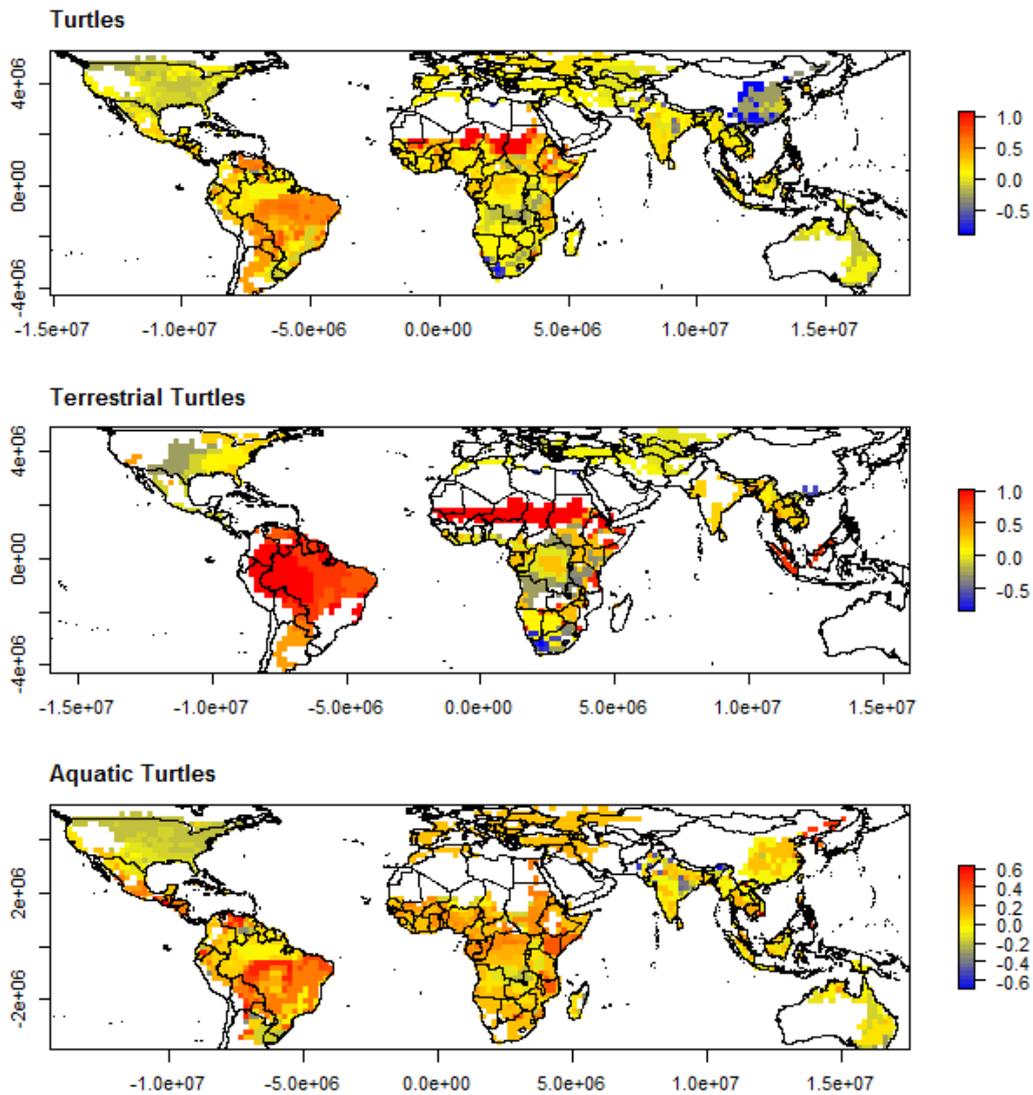
## **Results**

### *Global maps*

Turtles did not follow a clear latitudinal size pattern, but terrestrial species seem to be larger at low latitudes (Fig. 1). Terrestrial turtles in tropical South America and the southern Sahara tend to be larger than in temperate regions. The specific (S) component of body size had a similar pattern of geographic variation (Fig. 2. See also Fig. S1 in Supplementary material for plots of median body size and specific component against latitude of each cell). PVR eigenvectors explained 47% of turtle body size variation, 60% of aquatic turtles body size and 9% of terrestrial turtles body size. The high spatial congruence between patterns in total body size and the specific (S) component in terrestrial species is probably due to this low influence of PVR eigenvectors in body size data.



**Fig. 1.** Turtle body size (ln transformed maximum carapace length) distribution in 200km x 200km grid cells. Body size in each cell is represented by the median body size of all species occurring in the cell. Warm colors represent areas with larger body size, while cold colors represent areas with smaller body size.



**Fig. 2.** Specific component of ln-transformed turtle body size in 200km x 200km grid cells. The specific component for each cell is represented by the median specific component of the species occurring in the cell. Warm colors represent areas where body size is generally larger than expected by the phylogeny, while cold colors represent areas where body size is smaller than expected by the phylogeny.

*Assemblage approach*

SAR analyses found that all variables are correlated with turtle body size, except temperature anomaly and AET for terrestrial and aquatic species, respectively (Table 1). Results for the specific (S) component were similar, but the relationship between body size and human footprint was not significant for all turtles (Table 2). When significant, temperature and temperature anomaly showed positive coefficients, while the relationship with AET and human footprint were negative.

**Table 1:** Results of Spatial Autoregressive models (SARs) using turtle median ln body size as the response variable.

|                            | <b>Coefficients</b> | <b>Z value</b> | <b>P-value</b> | <b>R<sup>2</sup></b> |
|----------------------------|---------------------|----------------|----------------|----------------------|
| <i>Turtles</i>             |                     |                |                | 0.55                 |
| Temperature                | 0.149               | 11.74          | < 0.001        |                      |
| AET                        | -0.056              | -5.35          | < 0.001        |                      |
| Temperature anomaly        | 0.042               | 3.23           | 0.001          |                      |
| Human Foot Print           | -0.030              | -3.85          | < 0.001        |                      |
| <i>Terrestrial turtles</i> |                     |                |                | 0.77                 |
| Temperature                | 0.210               | 12.81          | < 0.001        |                      |
| AET                        | -0.067              | -4.26          | < 0.001        |                      |
| Temperature anomaly        | 0.009               | 0.54           | 0.59           |                      |

|                        |        |       |         |      |
|------------------------|--------|-------|---------|------|
| Human Foot Print       | -0.036 | -3.10 | 0.002   |      |
| <i>Aquatic turtles</i> |        |       |         | 0.41 |
| Temperature            | 0.111  | 8.45  | < 0.001 |      |
| AET                    | 0.001  | 0.05  | 0.96    |      |
| Temperature anomaly    | 0.046  | 3.26  | 0.001   |      |
| Human Foot Print       | -0.019 | -2.37 | 0.02    |      |

**Table 2:** Results of Spatial Autoregressive models (SARs) using specific component of turtle ln body size as the response variable.

|                            | <b>Coefficients</b> | <b>Z value</b> | <b>P-value</b> | <b>R<sup>2</sup></b> |
|----------------------------|---------------------|----------------|----------------|----------------------|
| <i>Turtles</i>             |                     |                |                | 0.54                 |
| Temperature                | 0.119               | 10.56          | < 0.001        |                      |
| AET                        | -0.046              | -4.96          | < 0.001        |                      |
| Temperature anomaly        | 0.038               | 3.30           | 0.001          |                      |
| Human Foot Print           | -0.007              | -1.00          | 0.32           |                      |
| <i>Terrestrial turtles</i> |                     |                |                | 0.72                 |

|                     |        |       |         |
|---------------------|--------|-------|---------|
| Temperature         | 0.194  | 12.48 | < 0.001 |
| AET                 | -0.046 | -3.06 | 0.002   |
| Temperature anomaly | 0.070  | 4.35  | < 0.001 |
| Human Foot Print    | -0.038 | -3.53 | < 0.001 |

*Aquatic turtles*

0.46

|                     |        |       |         |
|---------------------|--------|-------|---------|
| Temperature         | 0.017  | 2.13  | 0.03    |
| AET                 | -0.011 | -1.87 | 0.06    |
| Temperature anomaly | -0.027 | -3.22 | 0.001   |
| Human Foot Print    | 0.020  | 4.03  | < 0.001 |

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The inclusion of null models to account for the similarity issue changed the results and AET, temperature anomaly and human foot print had no longer explanatory power to determining the variation in either total body size or the specific (S) component (Table 3; Fig. S2-S7). Only temperature remained a significant positive predictor for turtle body size in all species and terrestrial ones (Table 3; Fig. S2-S7).

**Table 3:** Z-values representing the effect sizes of the predictors of turtle body size and specific component of turtle body size after accounting for the similarity issue. Specific components are phylogenetically independent body size measures obtained using Phylogenetic Eigenvector Regressions (PVRs).

|                            | Body size |         | Specific component |         |
|----------------------------|-----------|---------|--------------------|---------|
|                            | Z-value   | P-value | Z-value            | P-value |
| <i>Turtles</i>             |           |         |                    |         |
| Temperature                | 2.33      | 0.02    | 2.34               | 0.02    |
| AET                        | -1.08     | 0.28    | -1.20              | 0.23    |
| Temperature anomaly        | 0.52      | 0.60    | 0.56               | 0.58    |
| Human Foot Print           | -1.09     | 0.28    | -0.28              | 0.78    |
| <i>Terrestrial turtles</i> |           |         |                    |         |
| Temperature                | 2.20      | 0.03    | 2.11               | 0.03    |
| AET                        | -0.70     | 0.49    | -0.53              | 0.59    |
| Temperature anomaly        | 0.04      | 0.96    | 0.77               | 0.44    |
| Human Foot Print           | -1.10     | 0.27    | -1.10              | 0.27    |
| <i>Aquatic turtles</i>     |           |         |                    |         |
| Temperature                | 1.39      | 0.17    | 0.34               | 0.73    |
| AET                        | 0.22      | 0.82    | -0.38              | 0.71    |
| Temperature anomaly        | 0.57      | 0.57    | -0.50              | 0.61    |
| Human Foot Print           | -0.792    | 0.43    | 1.32               | 0.19    |

#### *Cross-species approach*

Cross-species results were similar to the results found when using the null models, with turtle body size showing a positive relationship with temperature (Table 4). Terrestrial

turtles followed an analogous pattern, but temperature anomaly was also significant. In aquatic turtles, none environmental variable was related to body size (Table 4).

**Table 4.** Cross-species results of the relationship between the specific component of body size of turtles and their possible predictors.

|                            | <b>Coefficients</b> | <b>z value</b> | <b>P-value</b> | <b>R<sup>2</sup></b> |
|----------------------------|---------------------|----------------|----------------|----------------------|
| <i>Turtles</i>             |                     |                |                | 0.06                 |
| Temperature                | 0.142               | 3.14           | 0.002          |                      |
| AET                        | -0.030              | -0.83          | 0.40           |                      |
| Temperature anomaly        | 0.069               | 1.91           | 0.06           |                      |
| Human Foot Print           | 0.015               | 0.54           | 0.59           |                      |
| <i>Terrestrial turtles</i> |                     |                |                | 0.31                 |
| Temperature                | 0.270               | 2.31           | 0.02           |                      |
| AET                        | -0.078              | -0.87          | 0.38           |                      |
| Temperature anomaly        | 0.171               | 1.99           | 0.05           |                      |
| Human Foot Print           | -0.080              | -0.92          | 0.36           |                      |
| <i>Aquatic turtles</i>     |                     |                |                | 0.02                 |

|                     |        |       |      |
|---------------------|--------|-------|------|
| Temperature         | 0.025  | 0.60  | 0.55 |
| AET                 | -0.008 | -0.24 | 0.81 |
| Temperature anomaly | -0.027 | -0.85 | 0.40 |
| Human Foot Print    | <0.001 | 0.01  | 0.99 |

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## Discussion

Our worldwide analyses did not detect a clear latitudinal gradient in body size for turtles, but temperature emerged as an important variable to account for the observed patterns. Interestingly, this result is consistent across all our analyses (both types of interspecific approaches: assemblage VS cross-species and habitat-based: all VS terrestrial VS aquatic species). AET, temperature anomaly and human footprint which were initially important did not remain significant after computing null models that take into account the similarity issue (Zelený & Schaffers 2012; Hawkins *et al.* 2017). Temperature anomaly had also a positive influence on terrestrial turtles body size at the cross-species approach.

The existence of a general latitudinal pattern in body size of ectothermic animals has been much debated in the recent literature, because a variety of patterns have been reported (Ashton 2002; Ashton & Feldman 2003; Shelomi 2012; Vinarski 2014). Our interspecific analyses do not detect clear latitudinal size clines in turtles, a result that concurs with previous findings (Angielczyk *et al.* 2015). However, a latitudinal pattern was identified when separate analyses were documented for terrestrial species. Larger species (or species with larger body size than expected by the phylogeny) are mainly found in tropical regions. Snakes and urodeles also follow this latitudinal pattern of

increasing size towards low latitudes (Olalla-Tárraga *et al.* 2006; Olalla-Tárraga & Rodríguez 2007; Terribile *et al.* 2009). There is also a small gradient in body size and specific (S) component in the North American aquatic turtles, although they have opposite tendencies. Previous studies with reptiles have already found that some latitudinal rules are only valid above a latitudinal threshold, which is possibly related to an increase in land mass at high latitudes (Hecnar 1999; Reed 2003). Finally, for the specific component maps (Fig. 2), it is noteworthy that turtles from northern Asia are smaller than expected by their phylogenetic history, a pattern that deserves future investigation.

Temperature was the only variable that consistently correlated (positively) with turtle body size across different methods and habitat types. These results were not in accordance with the heat conservation hypothesis, which predicts a negative relationship between temperature and body size. However, they are in accordance with the heat balance hypothesis, which predicts a positive relationship between these variables in large thermoregulating ectotherms (Olalla-Tárraga *et al.* 2006; Olalla-Tárraga 2011). It should be noted that the thermoregulatory mechanism of the heat conservation hypothesis was originally conceived to explain Bergmann's Rule in animals able to produce internal metabolic heat to maintain body temperatures (endotherms) (Salewski & Watt 2016). Large thermoregulating ectotherms, such as snakes (Olalla-Tárraga *et al.* 2006; Terribile *et al.* 2009) have been reported to display patterns as the one reported here for turtles. The positive relationships between body size and temperature in reptiles are commonly explained by the fact that heating rates of large animals are slower, which involves longer periods to warm up, a limiting factor to achieve operative body temperatures under cold environments (Ashton & Feldman

2003; Olalla-Tárraga 2011). This advantage of being small in cold areas is the mechanism suggested by the heat balance hypothesis to explain these positive relationships (Olalla-Tárraga *et al.* 2006; Olalla-Tárraga 2011). Similar to snakes, turtles are active thermoregulators which may expose themselves to sun (basking) to increase their temperature or select microhabitats with their preferred temperature (Boyer 1965; Crawford, Spotila & Standora 1983; Zimmerman *et al.* 1994), reinforcing the importance of warming up faster to save time for other activities when environment is cold. Besides, small turtles may warm up faster than large turtles (Boyer 1965), highlighting a plausible explanation as to why small turtle species are more commonly found in cold temperatures.

The observed positive relationship between temperature and body size in the assemblage-approach reinforces the importance of body size and temperature on turtle community compositions. Small turtles, with faster heating rates and able to more rapidly achieve optimal body temperatures, would be better suited to cope with cold-stressing environments across latitudinal and elevational gradients. Considering that body size distribution in these animals is mainly right-skewed (high number of small species) (Itescu *et al.* 2014), the thermal-dependent pattern documented here might help to explain why turtle diversity is not necessarily highest at the tropics (Iverson 1992b; Angielczyk *et al.* 2015; Rodrigues *et al.* 2017).

Temperature anomaly influenced body size of terrestrial turtles, and marginally influenced turtles generally, in the cross-species analysis. These results support the migration hypothesis, which predicts that large animals with high migration ability are better able to first reach areas strongly influenced by LGM temperature variations. Previous studies have already reported the influence of this temperature variation in

turtle diversity (Rödger *et al.* 2013; Rhodin *et al.* 2015; Mittermeier *et al.* 2015), and our results highlight the importance of considering temperature anomaly in turtle studies at macroscales. The lack of significance of this variable at the assemblage-approach may be due to the scale of our analysis which covers many areas with very small temperature variation since the LGM. Future studies focusing on regions where glaciations had stronger impacts may reveal other results.

In our study, body size patterns in terrestrial species were clearer and more related to our predictor variables than in aquatic species. The greater exposure of terrestrial animals to temperature (the environmental variable that most strongly correlates with turtle body size) when compared to aquatic organisms may explain why the latitudinal gradient was observed only in the terrestrial species. Other explanation to this finding is the relevance of phylogenetic history for the body sizes of aquatic species (PVR eigenvectors explained a high amount of its variance), which seems to dominate the variation in this trait. In snakes of the families Viperidae and Elapidae, the body size of these animals is also strongly influenced by phylogeny, and environment after controlling for phylogeny explained a very low amount of variation in this trait (Terribile *et al.* 2012). The differences we found between the two groups of turtles highlight the importance of performing separate analyses for groups presenting different life-history characteristics (see also Meiri & Thomas 2007).

Although human pressure seems to influence body size variation among turtle populations (de Souza Alcântara *et al.* 2013; Sung *et al.* 2013), this effect was not observed at a species level-analyses. Besides a previous study evaluating geographical patterns in turtle body size using intraspecific analyses of 23 turtle species (Ashton & Feldman 2003) found a positive relationship between body size and latitude in these

animals, which is not in accordance with our results. These differences suggest that different processes may influence body size variation at intra and interspecific analyses. Other possible explanation to these differences is that interspecific studies commonly use a single value of body size across the whole distributional range of the species, which is, to a certain extent, a simplification. Such simplification is currently necessary due to the lack of detailed population data for most species of turtles. With the advance of natural history studies describing body size of more turtle populations, future interspecific studies could try to incorporate intraspecific variation.

We conclude that temperature is the main variable influencing turtle body size and reiterate that ectothermic groups may present body size patterns different from most endothermic animals. We also highlight the importance of separating groups of animals with different habitat characteristics in body size analyses. Our results reinforce the strong role, already found in previous studies, played by temperature in body size gradients. The relevance of temperature identified by all the approaches implemented in our study supports concerns regarding how environmental warming may influence animal distributions and assemblage compositions.

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## Supporting information

**Appendix S1:** Supplementary methods and results

## Biosketch

**João Fabrício M. Rodrigues** is a post-doctoral researcher at the Universidade Federal de Goiás supported by the INCT project. He is interested in general macroecological and macroevolutionary questions as well as natural history studies related to amphibians and reptiles, especially freshwater turtles and tortoises.

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*Global Ecology and Biogeography*

**Supporting information**

**Firing up the shells: temperature is the main correlate of the global biogeography  
of turtle body size**

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**Appendix S1 - Supplementary methods and results**

**Table S1:** Species used in the body size analyses

| <b>Species</b>                       | <b>MCL</b> | <b>Source</b>      |
|--------------------------------------|------------|--------------------|
| <i>Acanthochelys macrocephala</i>    | 295        | Itescu et al. 2014 |
| <i>Acanthochelys pallidipectoris</i> | 175        | Itescu et al. 2014 |
| <i>Acanthochelys radiolata</i>       | 200        | Itescu et al. 2014 |
| <i>Acanthochelys spixii</i>          | 170        | Itescu et al. 2014 |
| <i>Actinemys marmorata</i>           | 210        | Itescu et al. 2014 |
| <i>Amyda cartilaginea</i>            | 830        | Itescu et al. 2014 |
| <i>Apalone ferox</i>                 | 600        | Itescu et al. 2014 |
| <i>Apalone mutica</i>                | 360        | Itescu et al. 2014 |
| <i>Apalone spinifera</i>             | 550        | Itescu et al. 2014 |
| <i>Astrochelys yniphora</i>          | 446        | Itescu et al. 2014 |
| <i>Batagur affinis</i>               | 560        | Itescu et al. 2014 |
| <i>Batagur baska</i>                 | 600        | Itescu et al. 2014 |
| <i>Batagur borneoensis</i>           | 760        | Itescu et al. 2014 |
| <i>Batagur dhongoka</i>              | 480        | Itescu et al. 2014 |
| <i>Batagur kachuga</i>               | 560        | Itescu et al. 2014 |
| <i>Batagur trivittata</i>            | 580        | Itescu et al. 2014 |
| <i>Carettochelys insculpta</i>       | 700        | Itescu et al. 2014 |
| <i>Centrochelys sulcata</i>          | 830        | Itescu et al. 2014 |
| <i>Chelodina expansa</i>             | 480        | Itescu et al. 2014 |
| <i>Chelodina longicollis</i>         | 282        | Itescu et al. 2014 |
| <i>Chelodina novaeguineae</i>        | 300        | Itescu et al. 2014 |
| <i>Chelodina oblonga</i>             | 400        | Itescu et al. 2014 |
| <i>Chelonoidis carbonaria</i>        | 593        | Itescu et al. 2014 |
| <i>Chelonoidis chilensis</i>         | 450        | Itescu et al. 2014 |
| <i>Chelonoidis denticulata</i>       | 820        | Itescu et al. 2014 |
| <i>Chelus fimbriata</i>              | 500        | Itescu et al. 2014 |
| <i>Chelydra serpentina</i>           | 550        | Itescu et al. 2014 |
| <i>Chersina angulata</i>             | 351        | Itescu et al. 2014 |
| <i>Chitra chitra</i>                 | 1400       | Itescu et al. 2014 |
| <i>Chitra indica</i>                 | 1500       | Itescu et al. 2014 |
| <i>Chitra vandijki</i>               | 220        | Itescu et al. 2014 |
| <i>Chrysemys dorsalis</i>            | 160        | Itescu et al. 2014 |
| <i>Chrysemys picta</i>               | 250        | Itescu et al. 2014 |
| <i>Claudius angustatus</i>           | 165        | Itescu et al. 2014 |
| <i>Clemmys guttata</i>               | 125        | Itescu et al. 2014 |
| <i>Cuora amboinensis</i>             | 250        | Itescu et al. 2014 |
| <i>Cuora aurocapitata</i>            | 156        | Itescu et al. 2014 |
| <i>Cuora bourreti</i>                | 180        | Itescu et al. 2014 |
| <i>Cuora flavomarginata</i>          | 195        | Itescu et al. 2014 |
| <i>Cuora galbinifrons</i>            | 198        | Itescu et al. 2014 |
| <i>Cuora mouhotii</i>                | 180        | Itescu et al. 2014 |

| <b>Species</b>                       | <b>MCL</b> | <b>Source</b>      |
|--------------------------------------|------------|--------------------|
| <i>Cuora pani</i>                    | 160        | Itescu et al. 2014 |
| <i>Cuora trifasciata</i>             | 300        | Itescu et al. 2014 |
| <i>Cuora yunnanensis</i>             | 140        | Itescu et al. 2014 |
| <i>Cyclanorbis elegans</i>           | 600        | Itescu et al. 2014 |
| <i>Cyclanorbis senegalensis</i>      | 600        | Itescu et al. 2014 |
| <i>Cyclemys atripons</i>             | 236        | Itescu et al. 2014 |
| <i>Cyclemys dentata</i>              | 210        | Itescu et al. 2014 |
| <i>Cyclemys enigmatica</i>           | 235        | Itescu et al. 2014 |
| <i>Cyclemys fusca</i>                | 242        | Itescu et al. 2014 |
| <i>Cyclemys gemeli</i>               | 232        | Itescu et al. 2014 |
| <i>Cyclemys oldhamii</i>             | 254        | Itescu et al. 2014 |
| <i>Cyclemys pulchristriata</i>       | 227        | Itescu et al. 2014 |
| <i>Cycloderma aubryi</i>             | 610        | Itescu et al. 2014 |
| <i>Cycloderma frenatum</i>           | 560        | Itescu et al. 2014 |
| <i>Deirochelys reticularia</i>       | 260        | Itescu et al. 2014 |
| <i>Dogania subplana</i>              | 350        | Itescu et al. 2014 |
| <i>Elseya albagula</i>               | 450        | Itescu et al. 2014 |
| <i>Elseya branderhorsti</i>          | 500        | Itescu et al. 2014 |
| <i>Elseya dentata</i>                | 400        | Itescu et al. 2014 |
| <i>Elseya lavarackorum</i>           | 320        | Itescu et al. 2014 |
| <i>Emydoidea blandingii</i>          | 274        | Itescu et al. 2014 |
| <i>Emydura macquarii</i>             | 340        | Itescu et al. 2014 |
| <i>Emydura subglobosa</i>            | 260        | Itescu et al. 2014 |
| <i>Emydura tanybaraga</i>            | 280        | Itescu et al. 2014 |
| <i>Emydura victoriae</i>             | 300        | Itescu et al. 2014 |
| <i>Emys orbicularis</i>              | 230        | Itescu et al. 2014 |
| <i>Erymnochelys madagascariensis</i> | 480        | Itescu et al. 2014 |
| <i>Geochelone elegans</i>            | 380        | Itescu et al. 2014 |
| <i>Geochelone platynota</i>          | 300        | Itescu et al. 2014 |
| <i>Geoclemys hamiltonii</i>          | 410        | Itescu et al. 2014 |
| <i>Geoemyda spengleri</i>            | 130        | Itescu et al. 2014 |
| <i>Glyptemys insculpta</i>           | 230        | Itescu et al. 2014 |
| <i>Gopherus agassizii</i>            | 400        | Itescu et al. 2014 |
| <i>Gopherus berlandieri</i>          | 240        | Itescu et al. 2014 |
| <i>Gopherus flavomarginatus</i>      | 400        | Itescu et al. 2014 |
| <i>Gopherus polyphemus</i>           | 387        | Itescu et al. 2014 |
| <i>Graptemys geographica</i>         | 270        | Itescu et al. 2014 |
| <i>Graptemys nigrinoda</i>           | 220        | Itescu et al. 2014 |
| <i>Graptemys oculifera</i>           | 220        | Itescu et al. 2014 |
| <i>Graptemys ouachitensis</i>        | 240        | Itescu et al. 2014 |
| <i>Graptemys pseudogeographica</i>   | 270        | Itescu et al. 2014 |
| <i>Graptemys pulchra</i>             | 273        | Itescu et al. 2014 |
| <i>Graptemys versa</i>               | 214        | Itescu et al. 2014 |

| <b>Species</b>                  | <b>MCL</b> | <b>Source</b>      |
|---------------------------------|------------|--------------------|
| <i>Hardella thurjii</i>         | 610        | Itescu et al. 2014 |
| <i>Heosemys annandalii</i>      | 600        | Itescu et al. 2014 |
| <i>Heosemys depressa</i>        | 263        | Itescu et al. 2014 |
| <i>Heosemys grandis</i>         | 480        | Itescu et al. 2014 |
| <i>Heosemys spinosa</i>         | 230        | Itescu et al. 2014 |
| <i>Homopus areolatus</i>        | 300        | Itescu et al. 2014 |
| <i>Homopus boulengeri</i>       | 110        | Itescu et al. 2014 |
| <i>Homopus femoralis</i>        | 168        | Itescu et al. 2014 |
| <i>Homopus signatus</i>         | 106        | Itescu et al. 2014 |
| <i>Hydromedusa tectifera</i>    | 300        | Itescu et al. 2014 |
| <i>Indotestudo elongata</i>     | 360        | Itescu et al. 2014 |
| <i>Indotestudo travancorica</i> | 331        | Itescu et al. 2014 |
| <i>Kinixys belliana</i>         | 230        | Itescu et al. 2014 |
| <i>Kinixys erosa</i>            | 400        | Itescu et al. 2014 |
| <i>Kinixys homeana</i>          | 223        | Itescu et al. 2014 |
| <i>Kinixys lobatsiana</i>       | 200        | Itescu et al. 2014 |
| <i>Kinixys natalensis</i>       | 160        | Itescu et al. 2014 |
| <i>Kinixys spekii</i>           | 220        | Itescu et al. 2014 |
| <i>Kinosternon acutum</i>       | 120        | Itescu et al. 2014 |
| <i>Kinosternon alamosae</i>     | 136        | Itescu et al. 2014 |
| <i>Kinosternon arizonense</i>   | 152.7      | Itescu et al. 2014 |
| <i>Kinosternon baurii</i>       | 120        | Itescu et al. 2014 |
| <i>Kinosternon creaseri</i>     | 125        | Itescu et al. 2014 |
| <i>Kinosternon dunni</i>        | 175        | Itescu et al. 2014 |
| <i>Kinosternon durangoense</i>  | 144.6      | Itescu et al. 2014 |
| <i>Kinosternon flavescens</i>   | 165        | Itescu et al. 2014 |
| <i>Kinosternon herrerae</i>     | 172        | Itescu et al. 2014 |
| <i>Kinosternon hirtipes</i>     | 185        | Itescu et al. 2014 |
| <i>Kinosternon integrum</i>     | 210        | Itescu et al. 2014 |
| <i>Kinosternon leucostomum</i>  | 175        | Itescu et al. 2014 |
| <i>Kinosternon oaxacae</i>      | 175        | Itescu et al. 2014 |
| <i>Kinosternon scorpioides</i>  | 270        | Itescu et al. 2014 |
| <i>Kinosternon sonoriense</i>   | 175        | Itescu et al. 2014 |
| <i>Kinosternon subrubrum</i>    | 125        | Itescu et al. 2014 |
| <i>Lissemys punctata</i>        | 285        | Itescu et al. 2014 |
| <i>Lissemys scutata</i>         | 230        | Itescu et al. 2014 |
| <i>Macrochelys temminckii</i>   | 800        | Itescu et al. 2014 |
| <i>Malaclemys terrapin</i>      | 230        | Itescu et al. 2014 |
| <i>Malacochersus tornieri</i>   | 180        | Itescu et al. 2014 |
| <i>Malayemys subtrijuga</i>     | 236.7      | Itescu et al. 2014 |
| <i>Manouria emys</i>            | 600        | Itescu et al. 2014 |
| <i>Manouria impressa</i>        | 350        | Itescu et al. 2014 |
| <i>Mauremys caspica</i>         | 250        | Itescu et al. 2014 |

| <b>Species</b>                    | <b>MCL</b> | <b>Source</b>      |
|-----------------------------------|------------|--------------------|
| <i>Mauremys japonica</i>          | 209        | Itescu et al. 2014 |
| <i>Mauremys leprosa</i>           | 250        | Itescu et al. 2014 |
| <i>Mauremys mutica</i>            | 200        | Itescu et al. 2014 |
| <i>Mauremys nigricans</i>         | 269        | Itescu et al. 2014 |
| <i>Mauremys reevesii</i>          | 300        | Itescu et al. 2014 |
| <i>Mauremys rivulata</i>          | 215        | Itescu et al. 2014 |
| <i>Mauremys sinensis</i>          | 271        | Itescu et al. 2014 |
| <i>Melanochelys trijuga</i>       | 385        | Itescu et al. 2014 |
| <i>Mesoclemmys dahli</i>          | 215        | Itescu et al. 2014 |
| <i>Mesoclemmys gibba</i>          | 233        | Itescu et al. 2014 |
| <i>Mesoclemmys nasuta</i>         | 317.1      | Itescu et al. 2014 |
| <i>Morenia ocellata</i>           | 220        | Itescu et al. 2014 |
| <i>Morenia petersi</i>            | 220        | Itescu et al. 2014 |
| <i>Myuchelys bellii</i>           | 300        | Itescu et al. 2014 |
| <i>Myuchelys latisternum</i>      | 280        | Itescu et al. 2014 |
| <i>Nilssonina formosa</i>         | 650        | Itescu et al. 2014 |
| <i>Nilssonina gangetica</i>       | 940        | Itescu et al. 2014 |
| <i>Nilssonina hurum</i>           | 600        | Itescu et al. 2014 |
| <i>Nilssonina leithii</i>         | 640        | Itescu et al. 2014 |
| <i>Nilssonina nigricans</i>       | 910        | Itescu et al. 2014 |
| <i>Notochelys platynota</i>       | 360        | Itescu et al. 2014 |
| <i>Orlitia borneensis</i>         | 800        | Itescu et al. 2014 |
| <i>Palea steindachneri</i>        | 430        | Itescu et al. 2014 |
| <i>Pangshura smithii</i>          | 230        | Itescu et al. 2014 |
| <i>Pangshura sylhetensis</i>      | 200        | Itescu et al. 2014 |
| <i>Pangshura tecta</i>            | 240        | Itescu et al. 2014 |
| <i>Pangshura tentoria</i>         | 271        | Itescu et al. 2014 |
| <i>Pelochelys bibroni</i>         | 1020       | Itescu et al. 2014 |
| <i>Pelochelys cantorii</i>        | 2000       | Itescu et al. 2014 |
| <i>Pelodiscus maackii</i>         | 450        | Itescu et al. 2014 |
| <i>Pelodiscus parviformis</i>     | 120        | Itescu et al. 2014 |
| <i>Pelodiscus sinensis</i>        | 260        | Itescu et al. 2014 |
| <i>Pelomedusa subrufa</i>         | 330        | Itescu et al. 2014 |
| <i>Peltocephalus dumerilianus</i> | 480        | Itescu et al. 2014 |
| <i>Pelusios adansonii</i>         | 238        | Itescu et al. 2014 |
| <i>Pelusios bechuanicus</i>       | 330        | Itescu et al. 2014 |
| <i>Pelusios carinatus</i>         | 300        | Itescu et al. 2014 |
| <i>Pelusios castaneus</i>         | 285        | Itescu et al. 2014 |
| <i>Pelusios castanoides</i>       | 230        | Itescu et al. 2014 |
| <i>Pelusios chapini</i>           | 380        | Itescu et al. 2014 |
| <i>Pelusios cupulatta</i>         | 230        | Itescu et al. 2014 |
| <i>Pelusios gabonensis</i>        | 330        | Itescu et al. 2014 |
| <i>Pelusios marani</i>            | 275        | Itescu et al. 2014 |

| <b>Species</b>                      | <b>MCL</b> | <b>Source</b>      |
|-------------------------------------|------------|--------------------|
| <i>Pelusios nanus</i>               | 120        | Itescu et al. 2014 |
| <i>Pelusios niger</i>               | 350        | Itescu et al. 2014 |
| <i>Pelusios rhodesianus</i>         | 255        | Itescu et al. 2014 |
| <i>Pelusios sinuatus</i>            | 550        | Itescu et al. 2014 |
| <i>Pelusios subniger</i>            | 200        | Itescu et al. 2014 |
| <i>Pelusios williamsi</i>           | 250        | Itescu et al. 2014 |
| <i>Phrynops geoffroanus</i>         | 350        | Itescu et al. 2014 |
| <i>Phrynops hilarii</i>             | 400        | Itescu et al. 2014 |
| <i>Platemys platycephala</i>        | 180        | Itescu et al. 2014 |
| <i>Platysternon megacephalum</i>    | 201        | Itescu et al. 2014 |
| <i>Podocnemis erythrocephala</i>    | 320        | Itescu et al. 2014 |
| <i>Podocnemis expansa</i>           | 890        | Itescu et al. 2014 |
| <i>Podocnemis lewyana</i>           | 463        | Itescu et al. 2014 |
| <i>Podocnemis sextuberculata</i>    | 330        | Itescu et al. 2014 |
| <i>Podocnemis unifilis</i>          | 476        | Itescu et al. 2014 |
| <i>Podocnemis vogli</i>             | 380        | Itescu et al. 2014 |
| <i>Psammobates oculifer</i>         | 147        | Itescu et al. 2014 |
| <i>Psammobates tentorius</i>        | 145        | Itescu et al. 2014 |
| <i>Pseudemys concinna</i>           | 430        | Itescu et al. 2014 |
| <i>Pseudemys floridana</i>          | 400        | Bonin_et_al.,_2006 |
| <i>Pseudemys gorzugi</i>            | 235        | Itescu et al. 2014 |
| <i>Pseudemys nelsoni</i>            | 380        | Itescu et al. 2014 |
| <i>Pseudemys peninsularis</i>       | 403        | Itescu et al. 2014 |
| <i>Pseudemys rubriventris</i>       | 400        | Itescu et al. 2014 |
| <i>Pseudemys texana</i>             | 330        | Itescu et al. 2014 |
| <i>Rafetus euphraticus</i>          | 680        | Itescu et al. 2014 |
| <i>Rafetus swinhoei</i>             | 1800       | Itescu et al. 2014 |
| <i>Rheodytes leukops</i>            | 262        | Itescu et al. 2014 |
| <i>Rhinoclemmys annulata</i>        | 228        | Itescu et al. 2014 |
| <i>Rhinoclemmys areolata</i>        | 239        | Itescu et al. 2014 |
| <i>Rhinoclemmys funerea</i>         | 330        | Itescu et al. 2014 |
| <i>Rhinoclemmys melanosterna</i>    | 290        | Itescu et al. 2014 |
| <i>Rhinoclemmys nasuta</i>          | 220        | Itescu et al. 2014 |
| <i>Rhinoclemmys pulcherrima</i>     | 214        | Itescu et al. 2014 |
| <i>Rhinoclemmys punctularia</i>     | 254        | Itescu et al. 2014 |
| <i>Rhinoclemmys rubida</i>          | 230        | Itescu et al. 2014 |
| <i>Sacalia bealei</i>               | 184        | Itescu et al. 2014 |
| <i>Sacalia quadriocellata</i>       | 145        | Itescu et al. 2014 |
| <i>Siebenrockiella crassicollis</i> | 203        | Itescu et al. 2014 |
| <i>Staurotypus salvinii</i>         | 250        | Itescu et al. 2014 |
| <i>Staurotypus triporcatus</i>      | 380        | Itescu et al. 2014 |
| <i>Sternotherus carinatus</i>       | 160        | Itescu et al. 2014 |
| <i>Sternotherus minor</i>           | 135        | Itescu et al. 2014 |

| <b>Species</b>                | <b>MCL</b> | <b>Source</b>      |
|-------------------------------|------------|--------------------|
| <i>Sternotherus odoratus</i>  | 136        | Itescu et al. 2014 |
| <i>Stigmochelys pardalis</i>  | 720        | Itescu et al. 2014 |
| <i>Terrapene carolina</i>     | 200        | Itescu et al. 2014 |
| <i>Terrapene nelsoni</i>      | 159        | Itescu et al. 2014 |
| <i>Terrapene ornata</i>       | 140        | Itescu et al. 2014 |
| <i>Testudo graeca</i>         | 300        | Itescu et al. 2014 |
| <i>Testudo hermanni</i>       | 250        | Itescu et al. 2014 |
| <i>Testudo horsfieldii</i>    | 280        | Itescu et al. 2014 |
| <i>Testudo kleinmanni</i>     | 144        | Itescu et al. 2014 |
| <i>Testudo marginata</i>      | 400        | Itescu et al. 2014 |
| <i>Trachemys callirostris</i> | 300        | Munera et al. 2004 |
| <i>Trachemys decorata</i>     | 341        | Itescu et al. 2014 |
| <i>Trachemys decussata</i>    | 390        | Itescu et al. 2014 |
| <i>Trachemys dorbigni</i>     | 267        | Itescu et al. 2014 |
| <i>Trachemys emolli</i>       | 372        | Legler 1990        |
| <i>Trachemys gaigeae</i>      | 308        | Itescu et al. 2014 |
| <i>Trachemys grayi</i>        | 600        | Itescu et al. 2014 |
| <i>Trachemys ornata</i>       | 480        | Itescu et al. 2014 |
| <i>Trachemys scripta</i>      | 280        | Itescu et al. 2014 |
| <i>Trachemys venusta</i>      | 424        | Legler 1990        |
| <i>Trachemys yaquia</i>       | 320        | Itescu et al. 2014 |
| <i>Trionyx triunguis</i>      | 1200       | Itescu et al. 2014 |

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## Supplementary figures

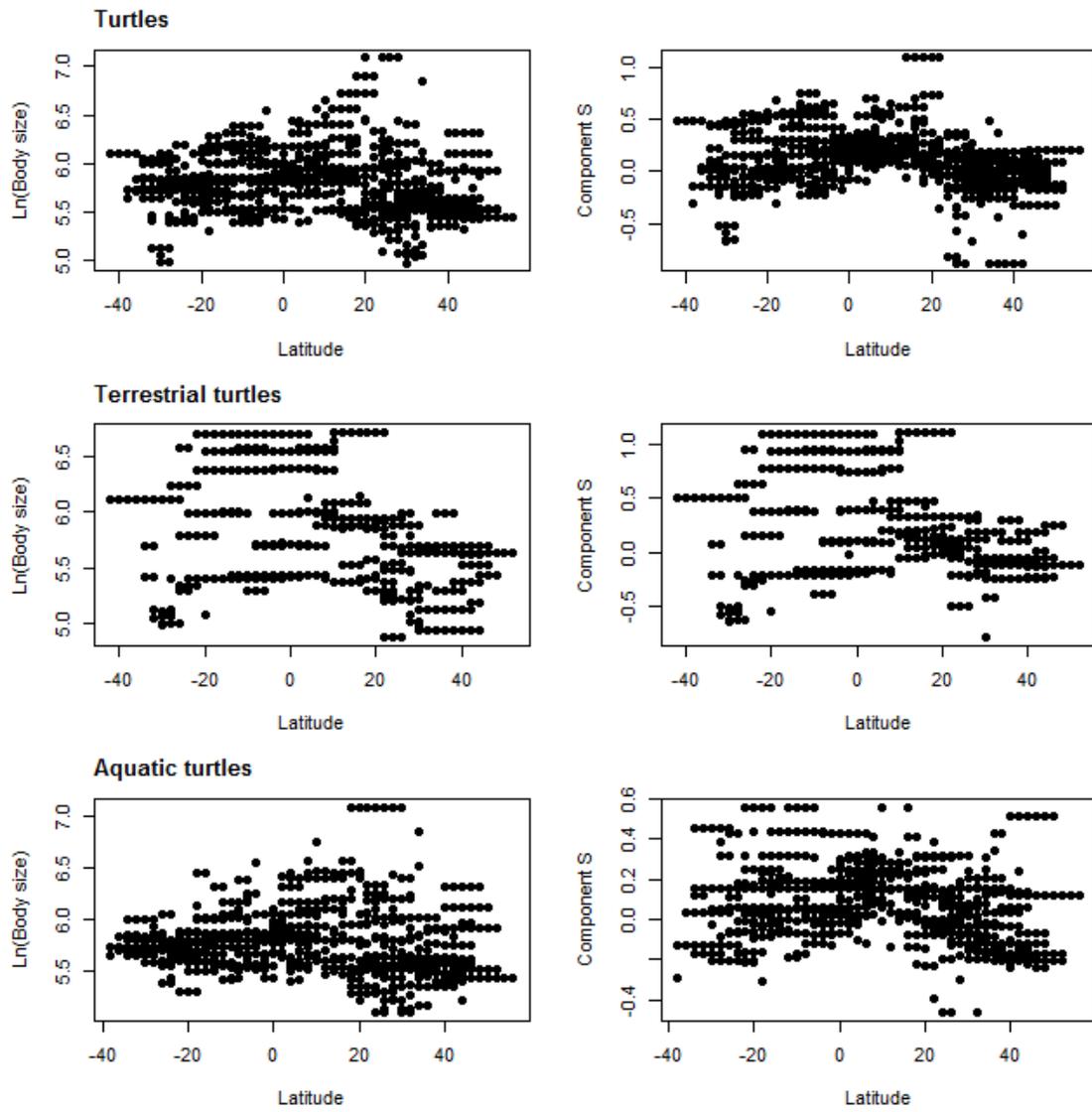


Fig S1: Latitudinal distributions of turtle body size and specific component.

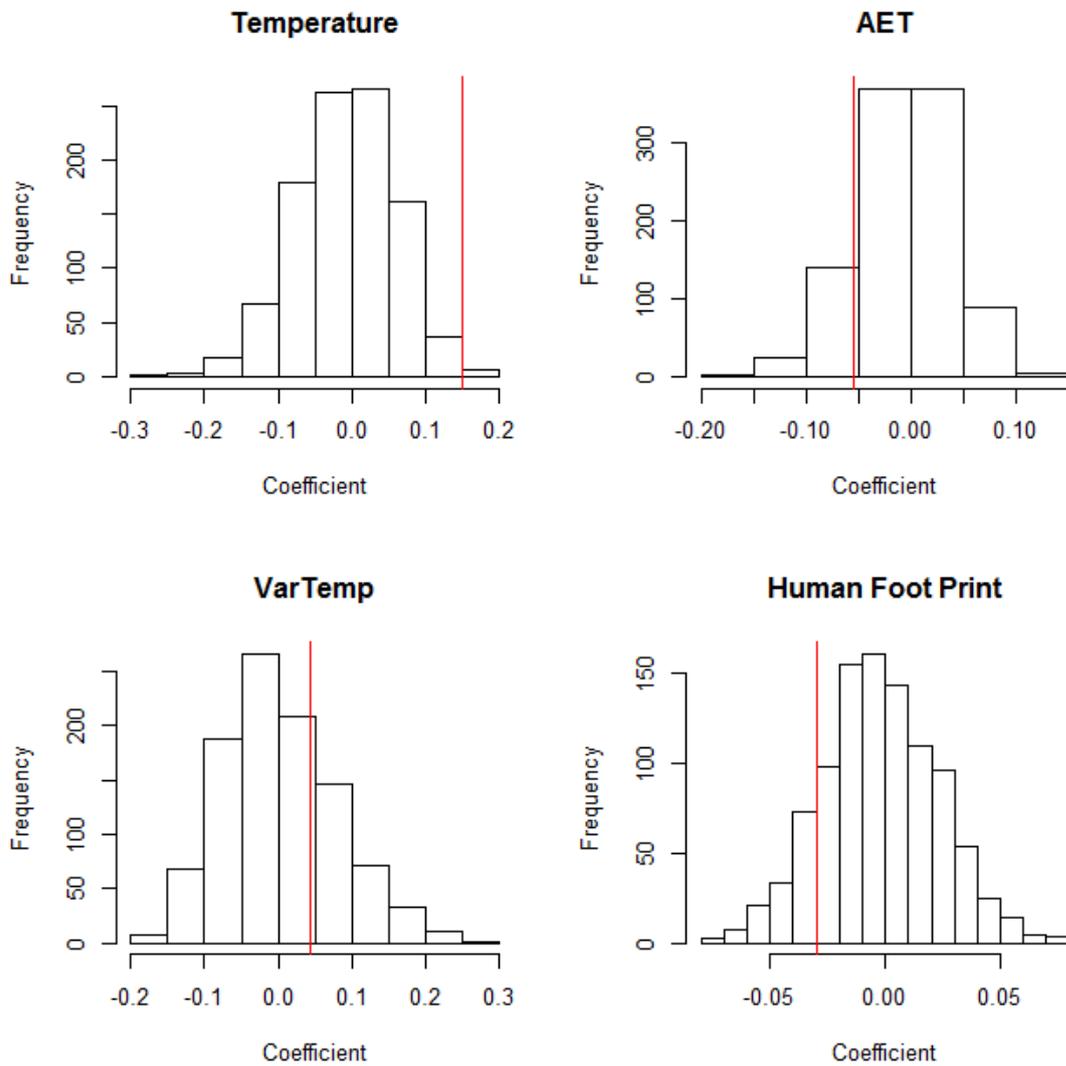


Fig S2: Coefficient distributions for environmental variables (defined in Methods) obtained through the null model for accounting for the similarity issue in analyses of turtle overall body size patterns. Red lines represent the coefficient value obtained when evaluating the real dataset. See table 3 for the Z-values calculated for each variable.

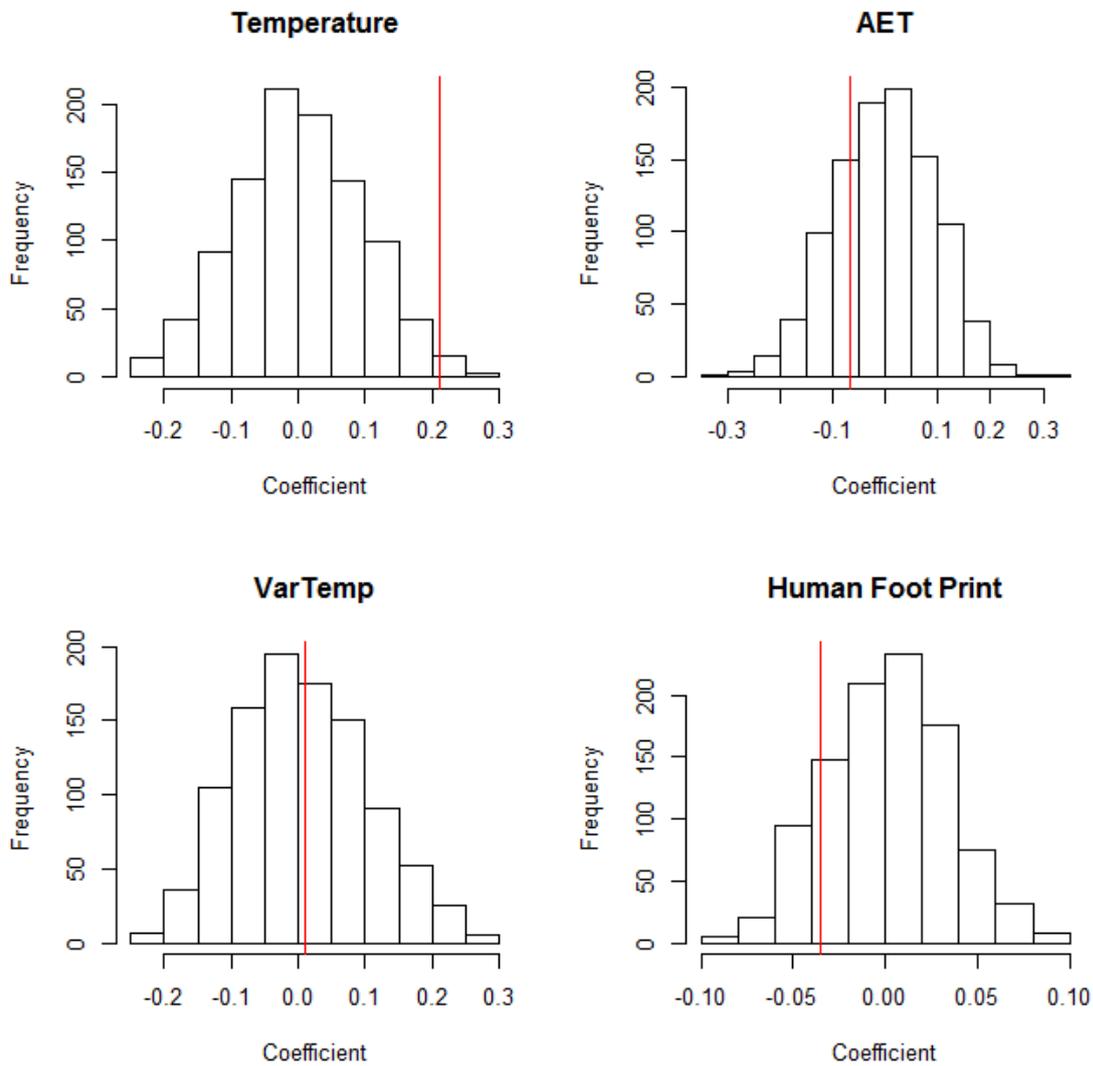


Fig S3: Coefficients distributions for environmental variables (defined in Methods) obtained through the null model for accounting for the similarity issue in analyses of terrestrial turtle body size patterns. Red lines represent the coefficient value obtained when evaluating the real dataset. See table 3 for the Z-values calculated for each variable.

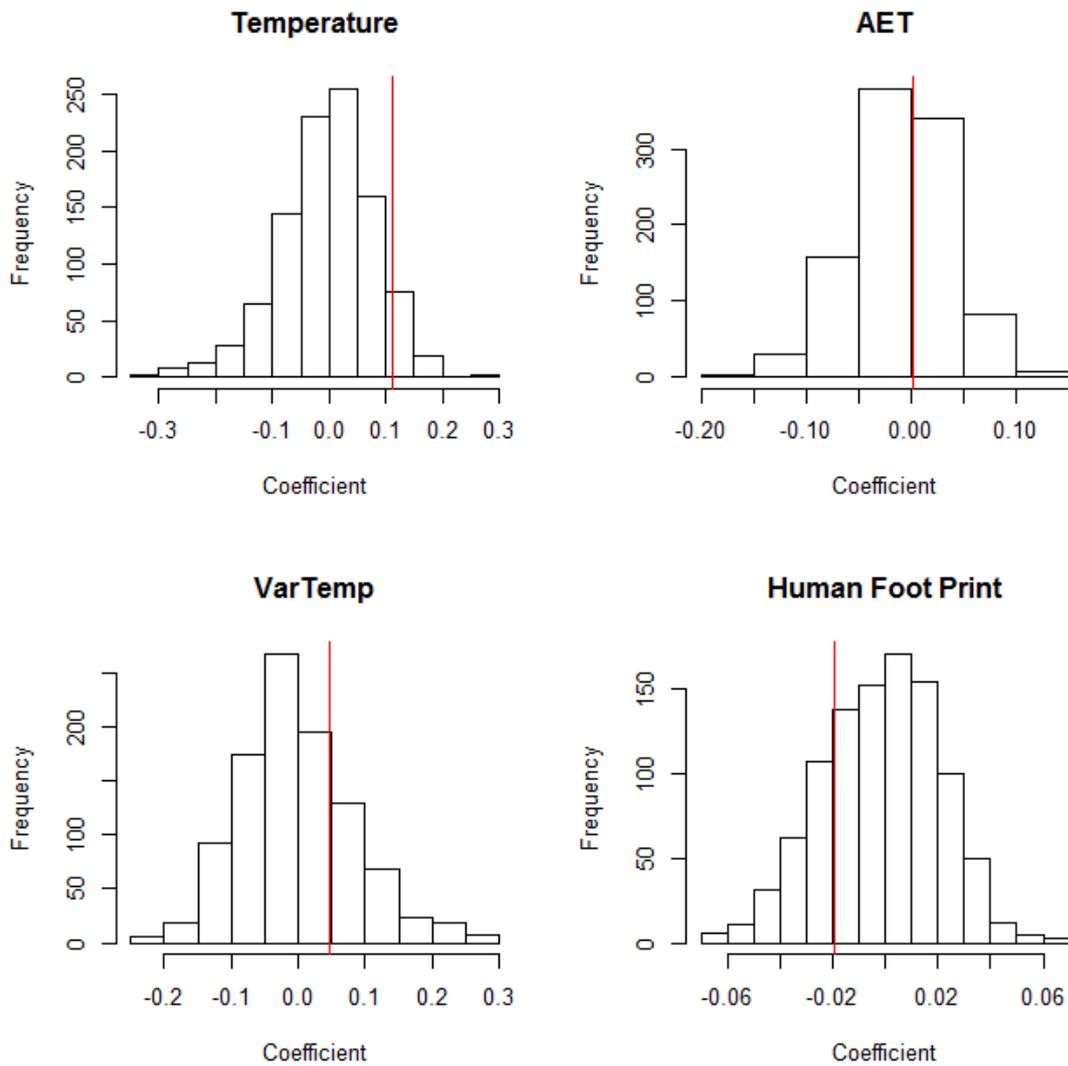


Fig S4: Coefficients distributions for environmental variables (defined in Methods) obtained through the null model for accounting for the similarity issue in analyses of aquatic turtle body size patterns. Red lines represent the coefficient value obtained when evaluating the real dataset. See table 3 for the Z-values calculated for each variable.

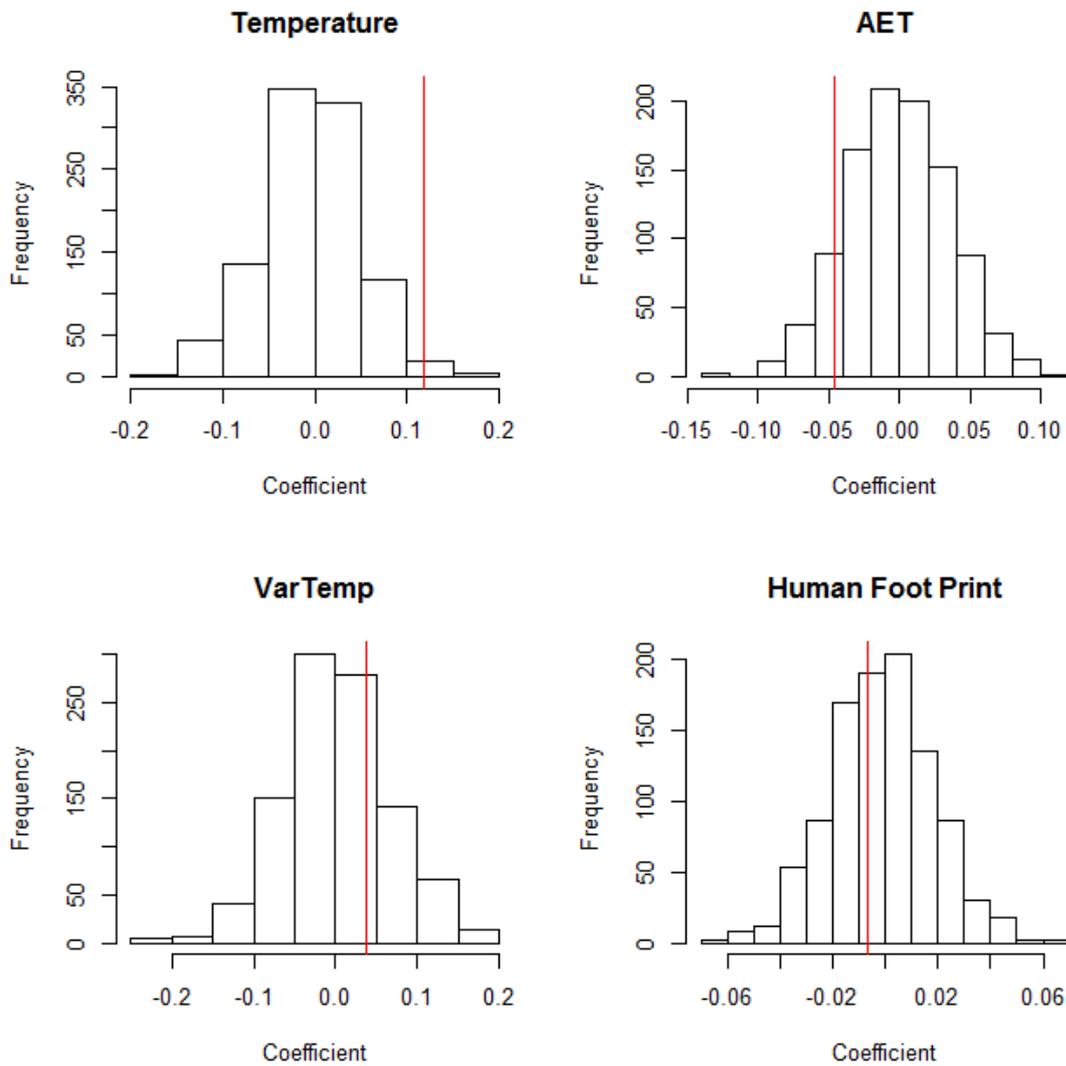


Fig S5: Coefficients distributions for environmental variables (defined in Methods) obtained through the null model for accounting for the similarity issue in analyses of turtle overall specific components obtained from Phylogenetic Eigenvector Regressions. Red lines represent the coefficient value obtained when evaluating the real dataset. See table 3 for the Z-values calculated for each variable.

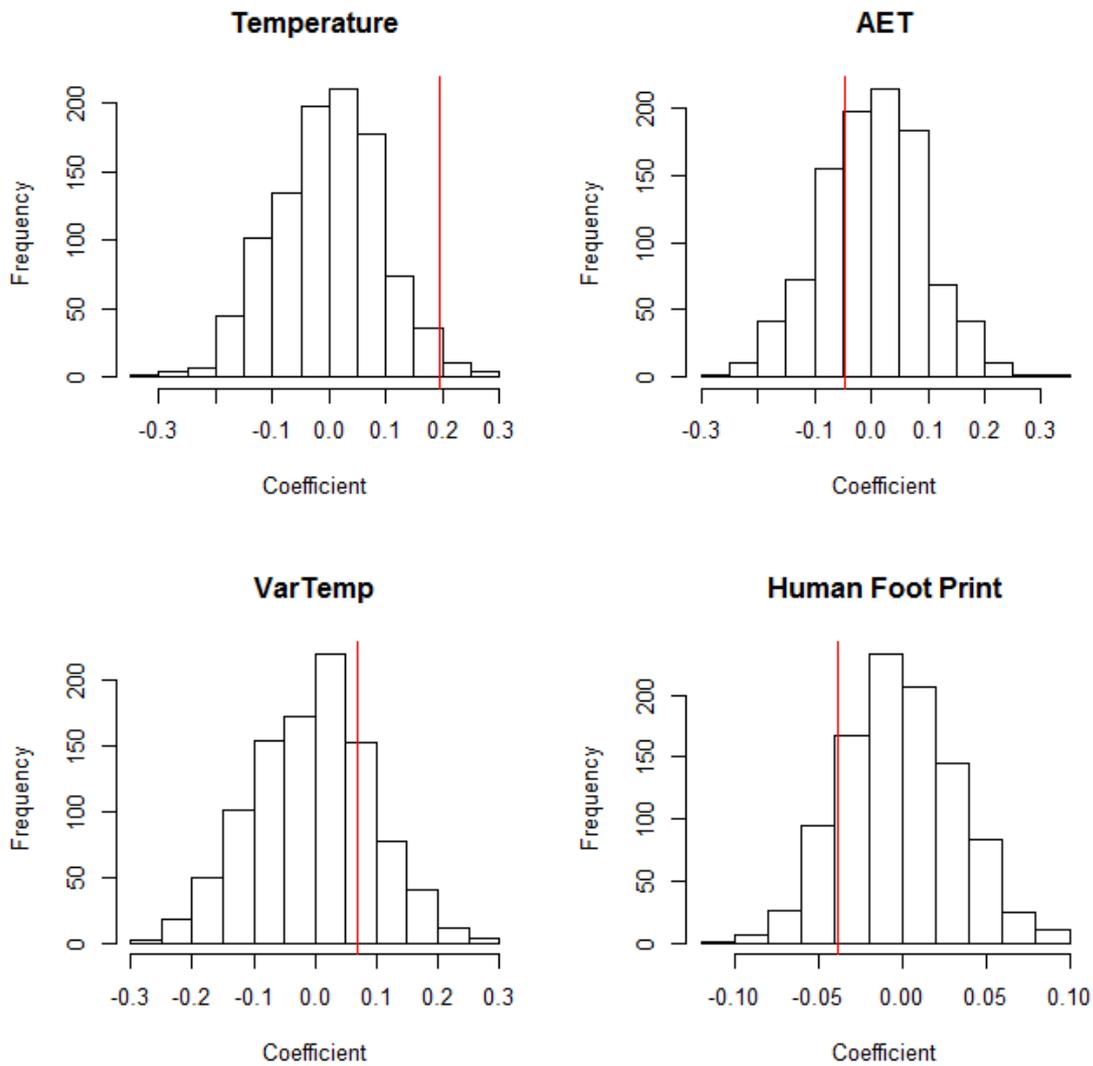


Fig S6: Coefficients distributions for environmental variables (defined in Methods) obtained through the null model for accounting for the similarity issue in analyses of terrestrial turtle specific components obtained from Phylogenetic Eigenvector Regressions. Red lines represent the coefficient value obtained when evaluating the real dataset. See table 3 for the Z-values calculated for each variable.

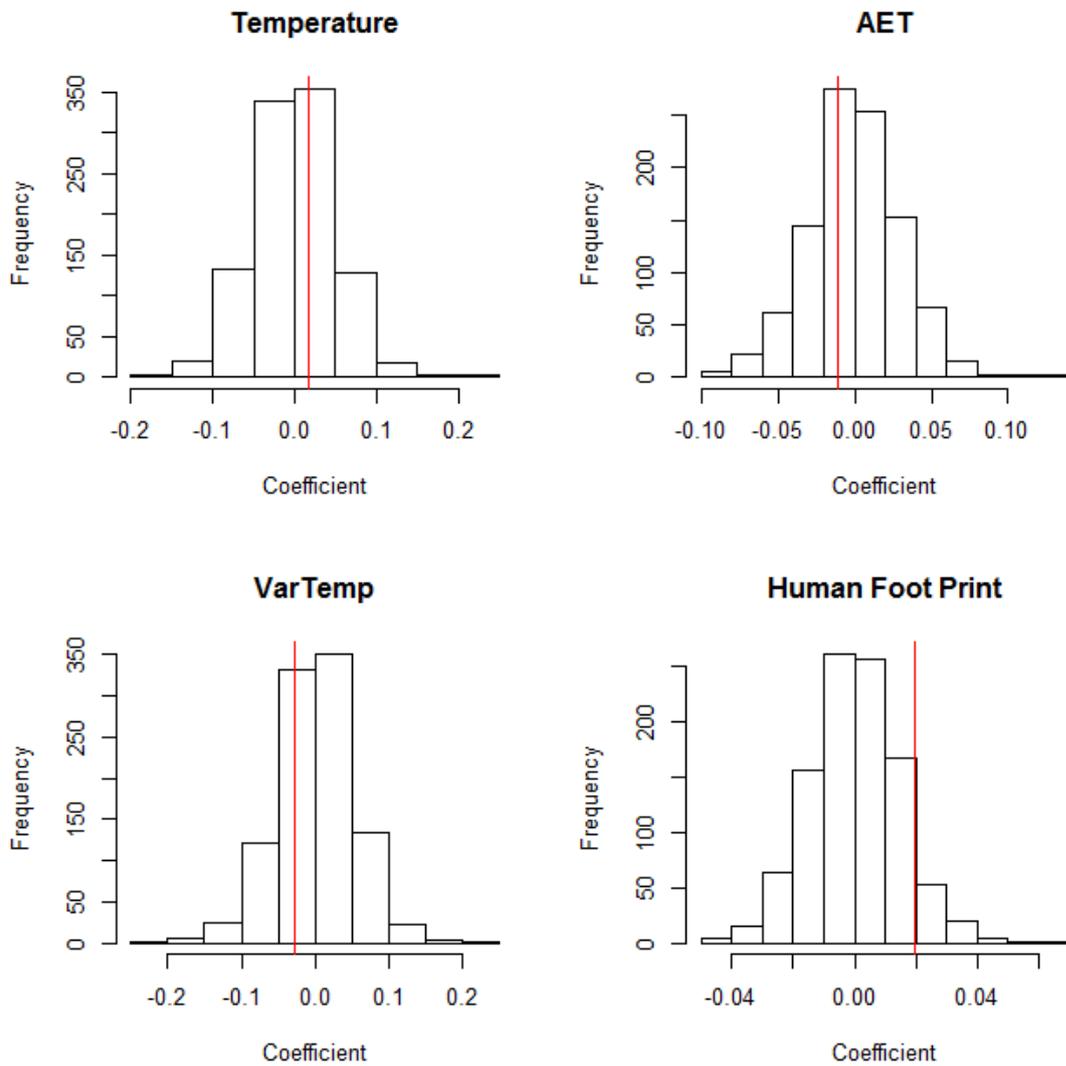


Fig S7: Coefficients distributions for environmental variables (defined in Methods) obtained through the null model for accounting for the similarity issue in analyses of aquatic turtle specific components obtained from Phylogenetic Eigenvector Regressions. Red lines represent the coefficient value obtained when evaluating the real dataset. See table 3 for the Z-values calculated for each variable.

## CONCLUSÕES

Os resultados obtidos nesta tese foram importantes para melhor compreendermos os fatores que podem influenciar a diversificação e os padrões de diversidade em animais, mais especificamente, em quelônios. Encontramos que o hábitat onde os animais vivem pode influenciar suas taxas de especiação e conseqüentemente sua diversidade atual, sendo que espécies de quelônios de água possuem taxas de especiação mais altas que as espécies terrestres. Entretanto o clima e o tempo de colonização também possuem papel importante na variação da riqueza de espécies em diferentes áreas, de modo que áreas mais quentes em relação ao último máximo glacial, com maior taxa de precipitação e de colonização mais antiga apresentaram maior número de espécies de quelônios continentais. Também observamos que a variação em composição entre comunidades pode ser mais influenciada pela distância entre as áreas que por diferenças ambientais, e que diferentes fatores podem influenciar essa variação em composição nos diferentes domínios biogeográficos. Além disso, confirmamos que a temperatura atual é um importante preditor do tamanho corporal em animais ectotérmicos, mas que a variação da temperatura desde o último máximo glacial (22.000 anos atrás) também parece ter influência sobre o tamanho desses animais, de modo que quelônios maiores ocorrem em áreas com temperaturas atuais e variações históricas de temperatura maiores.

Considerando nossos anexos, constatamos que métricas tradicionais de avaliação de modelos de distribuição podem ser problemáticas quando os animais mudam seu nicho nas áreas invadidas e que o nicho de uma espécie na área invadida parece ser mais bem explicado pelo nicho ocupado pelo conjunto de suas subespécies. Finalmente, pudemos reforçar a importância de se considerar a história evolutiva do grupo, fatores ambientais e espaciais para compreender o padrão de diversidade dos quelônios, enfatizando a

interação desses diferentes componentes sobre a distribuição dos organismos. Além disso, nossos resultados sobre a influência de fatores ambientais sobre a riqueza de espécies e o padrão espacial de tamanho corporal sugerem que mudanças climáticas podem afetar a distribuição da biodiversidade desses animais.

# Apêndices

## Apêndice 1

Rodrigues, J.F.M., Coelho, M.T.P., Varela, S., Diniz-Filho, J.A.F. (2016):

Invasion risk of the pond slider turtle is underestimated when niche expansion occurs. *Freshwater Biology* **61**: 1119–1127.

**Invasion risk of the pond slider turtle is underestimated when niche expansion occurs**

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**Abbreviated title:** Niche shift of an invasive turtle

**Keywords:** Ecological niche models; Freshwater turtles; Invasive alien species; Model evaluation; *Trachemys scripta*

## Summary

1. In recent years, changes have been detected in the climatic niches of several non-native species. In spite of this, and although Ecological Niche Models (ENMs) assume species show climatic niche conservatism, here we use ENM to assess risks of invasion by alien species. In this study we tested how niche expansion of the pond slider (*Trachemys scripta*) differs in invaded continents and how the performance of ENMs is affected by different niche shift scenarios.

2. We described niche equivalence (whether native and invaded niches are identical), unfilling (native niche not present in invasive niche), expansion (invasive niche not present in native niche) and stability, based on the pond slider native and invaded occurrence points. We created an ENM using a Maxent method, based on the native occurrences of this turtle, and evaluated the model's performance using invasive records.

3. Our results indicate that the pond slider niche changed when new areas that were either warmer (Asia and Latin America) or colder (Europe) than its native niche were invaded. Processes related to niche shift (stability, unfilling, and expansion) varied between continents. We also found that niche expansion is not a good predictor of ENM

performance, which may indicate that the effects of this process on models performance are more complex than a simple direct effect. Finally, the models had a dramatically poor performance when evaluated for sensitivity (percentage of presence records correctly predicted as presences in the models), reiterating the problems of using ENMs and their traditional evaluation methods when focal species do not conserve their native niche.

4. We draw attention to important mitigatory measures, such as environmental education and strong control of commercialization to manage invasion by the pond slider turtle, since we still lack standard methods to predict the potential invasion risks for new areas when focal species do not conserve their native niche.

## **Introduction**

Managing ecological invasions is one of the most important biological challenges of the XXI century. The key point is anticipating the consequences of biological invasions before they impact native communities. The very first step of a conservation strategy is to map the areas that would be potentially affected by the new species. However, in practise, species invasions are difficult to prevent and manage, mostly because the spatial models that are used to predict risks of invasion fail to correctly estimate the species range in the new habitat (Tingley *et al.* 2014). One of the causes of this failure is that the behaviour of the alien species in the new habitat can be unexpected.

Niche conservatism is the tendency of species to retain their ancestral niches (Wiens *et al.* 2010; Peterson 2011; Pearman *et al.* 2014). Maps of the potential invasive

species distribution in new areas are normally built using Ecological Niche Models (ENM), which rely on the assumption of climatic niche conservatism between native and non-native ranges (Pearman *et al.* 2008; Peterson 2011). Currently, increasing evidence for climatic niche shifts in several non-native species have been detected (Broennimann & Guisan 2008; Beaumont *et al.* 2009; Gallagher *et al.* 2010; Li *et al.* 2014). However, despite this evidence, ENMs are still widely used to assess the invasion risk of many non-native species (Peterson 2011).

Freshwater turtles are among the most traded reptiles of the world (Masin *et al.* 2014), which make them a good model group in which to investigate invasion risks. Most turtle trade involves the pond slider (*Trachemys scripta*: Emydidae), with millions of individuals exported from their native region, the United States of America (Telecky 2001). The pond slider has been able to reproduce in new locations, and currently has a widespread distribution including Central America, South America, Europe and Asia (Rödder *et al.* 2009). It is the only turtle considered to be globally invasive (Masin *et al.* 2014). Observed consequences of the appearance of the pond slider in new communities include strong competition with native turtle species (Cadi & Joly 2004; Polo-Cavia, López & Martín 2008, 2009, 2010). Pond slider may cause weight loss and increased mortality in European pond turtle (*Emys orbicularis*: Emydidae) (Cadi & Joly 2004), displace the Spanish terrapin (*Mauremys leprosa*: Geoemydidae) from its common basking sites and reduce its basking time (Polo-Cavia *et al.* 2010). These negative interactions with the Spanish terrapin are reinforced by aggressive behaviors and high ability of body heat retention of pond slider (Polo-Cavia *et al.* 2008, 2009). Thus, correct management of this species in new locations, in order to prevent its expansion, is highly important for conservation of the local fauna.

Some previous studies have modelled the environmental requirements of the pond slider, and predicted its invasive range using ENMs (Rödder *et al.* 2009; Masin *et al.* 2014). However, this species presents climatic niche shifts in its invaded ranges (Li *et al.* 2014) and, consequently, ENM predictions could fail to correctly estimate the potential invasion risk of this species. Here, we aim to evaluate how niche expansion potentially affects the performance of ENMs. We expected that the accuracy of the model predictions would be related to the observed niche expansion and that models would have less predictive power in continents where the pond slider has a higher niche expansion.

## **Methods**

### *Climate data*

We model the ecological niche of the pond slider using five environmental variables obtained from WorldClim ([www.worldclim.org](http://www.worldclim.org)) at a resolution of 0.16 degrees or 10-arc minutes (Hijmans *et al.* 2005): mean annual temperature (BIO1); maximum temperature of warmest month (BIO5); minimum temperature of coldest month (BIO6); annual precipitation (BIO12); precipitation of driest month (BIO14). These environmental variables are directly related to the natural history of the pond slider and are more able to represent its potential distribution than using a full set of bioclimatic variables (Rödder *et al.* 2009).

### *Occurrence data*

We used 339 occurrence points from native areas of the pond slider in North America and 103 occurrence points from invaded areas of South and Central America (Latin America; 51 records), Europe (38 records) and Asia (14 records) (Rödder *et al.* 2009). This database included all subspecies of pond slider, although most invasion records are from the red-eared slider (*Trachemys scripta elegans*: Emydidae), the most traded freshwater turtle worldwide (Telecky 2001). All the occurrence data were obtained from a previous compilation (Rödder *et al.* 2009) that included data from the Global Diversity Information Facility (GBIF), HerpNet databases, Delivering Alien Invasive Species Inventories for Europe database (DAISE), Brazilian “Instituto Horus”, along with data from other databases and published papers (for a full list references see Text S1 in Rödder *et al.* 2009). All these occurrences represent areas where the pond slider established reproductive populations (Rödder *et al.* 2009). This criterion allows us to use the invasion records to estimate the climatic niche of this species more accurately, because reproduction represents occurrences where the species has found suitable climatic conditions to establish viable populations, characterizing its fundamental niche. Considering that this species is also bred in farms for sale as pets, invasion records without evidence of reproduction could merely represent release points, with no relationship to the species niche requirements. Furthermore, other authors have already found climatic differences between feral (without reproduction record) and established populations of pond slider (Ficetola, Thuiller & Padoa-Schioppa 2009).

### *Evaluating niche conservatism*

Traditional methods of measuring niche conservatism in biological invasions do not consider the extent and variability of the environmental conditions, and thus, they could produce misleading results. Here, we followed the recent approach proposed by Broennimann *et al.* (2012) and Guisan *et al.* (2014). This method creates a global environmental space that covers all the environmental conditions where the species occurs, including both native and invaded areas, and generates occupancy values based on comparisons of the species occurrence data with the global environmental space (Broennimann *et al.* 2012).

We used the first two axis of a Principal Component Analysis (PCA) including the five environmental variables cited above (which explained more than 80% of the total variance), to create an environmental grid of 100 x 100 cells (global environmental space), where species occupancy was allocated (Broennimann *et al.* 2012). We measured niche overlap between native and each invaded range using Schoener's D, an index ranging from 0 to 1, where 0 means no overlap between niches and 1, a total overlap. We also evaluated niche equivalency, which compares the observed Schoener's D with values calculated between two groups of random points extracted from the global environment where the species occurs (including native and invaded ranges) (Warren, Glor & Turelli 2008; Petitpierre *et al.* 2012; Broennimann *et al.* 2012). We used 100 permutations to evaluate the significance level of D.

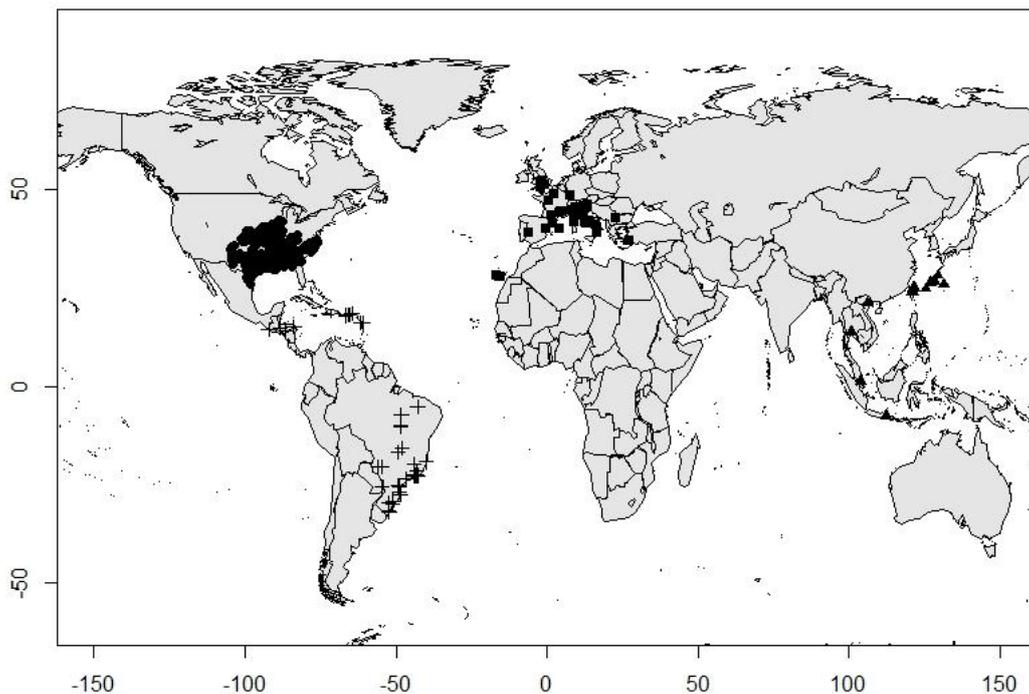
We also evaluated niche stability (proportion of the native niche observed in the exotic niche), unfilling (proportion of the native niche not occupied in the exotic niche) and expansion (new environmental requirements observed in the exotic niche), which allowed us to quantify and discuss different aspects of the species niche (Petitpierre *et al.* 2012; Guisan *et al.* 2014). For measuring niche stability, unfilling and expansion, we

used only 95% of the intersection area between the native and invaded gridded environmental space, in order to control for possible environmental outliers (Petitpierre *et al.* 2012).

#### *Evaluating the effects of niche expansion in model performance*

We created an ENM using the native occurrence records of the pond slider in North America using Maxent, a modelling algorithm that only requires presence records to fit the models (Phillips, Anderson & Schapire 2006). Maxent has a good performance among presence-only ENM algorithms (Elith *et al.* 2006), and it is also commonly used in invasion studies (Rödder *et al.* 2009; Palaoro *et al.* 2013; Masin *et al.* 2014). Prior to these analyses, we used a geographical filter in our full set of occurrence data in native areas, because removing excessive records which are close to each other may reduce sampling bias and improve the predictions of the models (de Oliveira *et al.* 2014; Fig. 1). We evaluated the ENM model using the invasive records (also filtered for geographical proximity) of the pond slider in Latin America (South America + Central America), Europe, and Asia. We used the Area Under the Curve (AUC) of the Receiver Operating Characteristic plot (ROC) of each of these comparisons as a performance value. To make these evaluations, we randomly sampled background points in each invaded area 100 times and used the mean and standard deviation of AUC to evaluate the quality of the model calibrated in the native area. The number of background points was the number of occurrence points of the species in each invaded continent. We performed 100 samplings to take into account the variability of evaluation values that could be achieved in the random sampling and because performing several runs of

pseudoabsences and averaging them provides a better performance in machine-learning methods (Barbet-Massin *et al.* 2012). AUC = 0.5 indicates that the model predictions are equal to a random model (Swets 1988). We investigated differences in this metric among the continents using a Kruskal-Wallis test, and used Mann-Whitney U tests to evaluate *a posteriori* pairwise differences among them. We expected the accuracy of the model's predictions (AUC) to be related to niche expansion measurements.



**Fig. 1:** Filtered occurrence records of the pond slider (*Trachemys scripta*) used in Maxent analyses. We used a geographical filter to remove records which were close to each other in order to increase model performance. Dark circles = native points in North

America; Crosses = invasive records in Latin America; dark squares = invasive points in Europe; and dark triangles = invasive points in Asia.

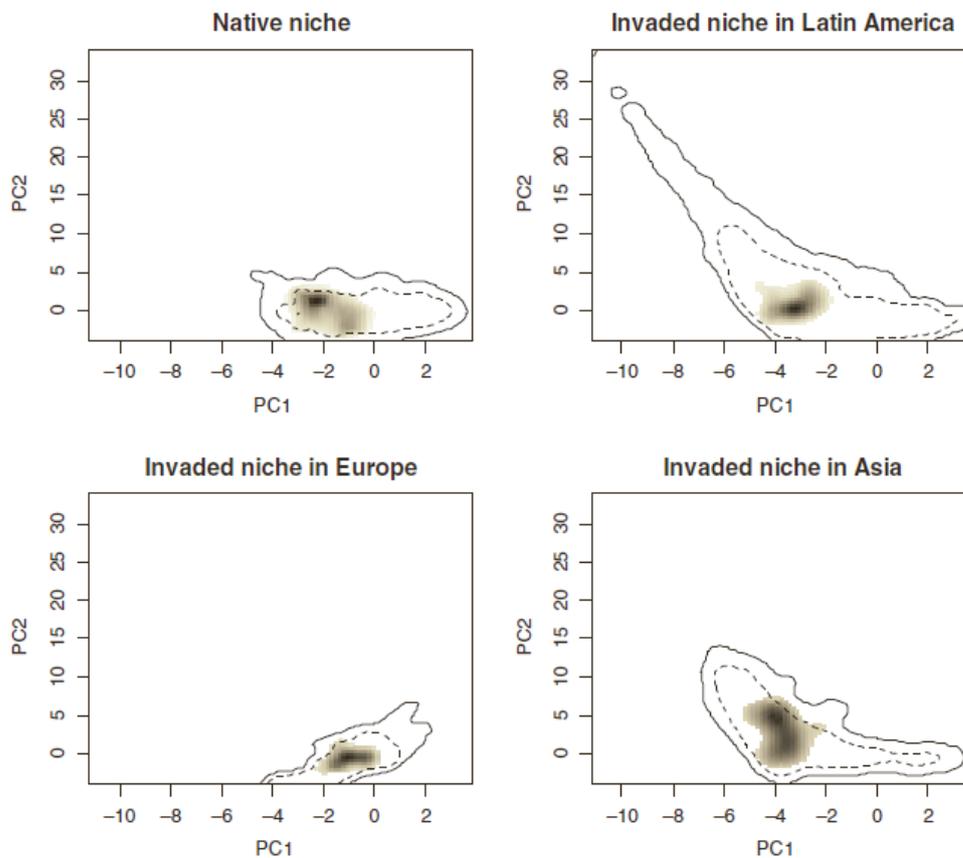
Complementary to using AUC to evaluate the performance of our models in new areas, we also used sensitivity (percentage of presences correctly predicted by the model). We chose to use this further approach because in invaded ranges exotic species have recently arrived and have consequently had insufficient time to fully expand. Therefore, dealing with absences in these situations may be problematic. We used the threshold that maximizes sensitivity + specificity to transform the predictions of our Maxent model of each invaded area into a presence-absence map. This threshold generates adequate Maxent predictions, while the commonly used minimum presence threshold overestimates the areas where the species occurs (Varela *et al.* 2014).

Statistical analyses were performed in R ver. 3.1.2 (R Core Team 2014) using the packages *ecospat* (Broennimann *et al.* 2015) and *dismo* (Hijmans *et al.* 2015) and their dependencies.

## **Results**

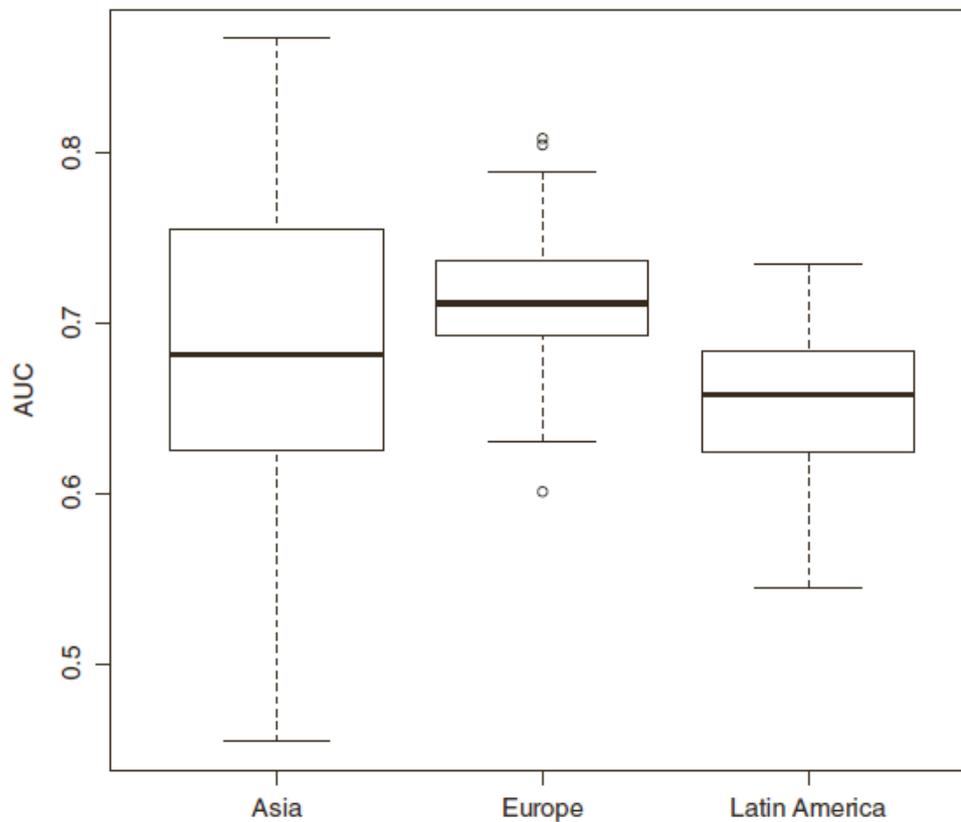
We found no niche equivalency between native and invaded areas of the pond slider. Schoener's D similarity measure was lower than expected by random (Europe:  $D = 0.27$ ,  $p = 0.02$ ; Latin America:  $D = 0.39$ ,  $p = 0.02$ ; Asia:  $D = 0.02$ ,  $p = 0.02$ ), indicating niche shifts in the non-native areas (Fig. 2). In Europe, the pond slider occurs in cooler climatic conditions when compared to the native area, although in Latin America and

Asia it occurs in warmer areas. Regarding precipitation, the species occupies a higher range of precipitation conditions in Asia than in all other continents (Fig. 2). We also found different niche particularities in the different invaded areas with differences in niche unfilling (NU), niche stability (NS) and niche expansion (NE). The European continent had the lowest level of climatic niche shifts (NU = 0.435, NS = 0.951, NE = 0.049). The observed environmental niche in Europe is very similar to the original living conditions of the species, but the native niche is not completely fulfilled. In the same way, the species presented median unfilling and stability and low niche expansion in the Latin American continent (NU = 0.552, NS = 0.622, NE = 0.378). In contrast, we found evidence for great climatic niche shifts in Asia (NU = 0.804, NS = 0.320, NE = 0.680), where the species experienced a great niche expansion and a large unfilling of its original niche. We also performed these analyses using only 75% of the intersection area between the native and invaded gridded environmental space, but the qualitative pattern was the same (results not shown).



**Fig. 2** Niche of the pond slider (*Trachemys scripta*) in native and invaded areas represented using the first two axis of a PCA (Principal Component Analysis) including: mean annual temperature, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation and precipitation of driest month. Grey shading represents the occurrence density of the species in the climatic space. The first axis mainly represents temperature variables and is negatively correlated with them. The second axis mainly represents precipitation variables and is positively correlated with them. Solid contour represents 100% of the available environment, and the dashed one, 50%.

The predictions of the environmental niches of the species in the new areas are not very precise. Europe has the highest accuracy (AUC = 0.71, Standard Deviation or SD = 0.04), Asia has an intermediate accuracy (AUC = 0.68, SD = 0.09), and Latin America has a low accuracy (AUC = 0.65, SD = 0.04). We found differences in the model's performance in the different continents ( $W = 58.80$ ,  $df = 2$ ,  $P < 0.001$ ; Fig. 3). ENMs could explain better the occurrence of the species in invasive areas of the European continent where the species presented more stability. The performance of the model was better in Europe than Asia ( $U = 3984$ ,  $P = 0.01$ ), where there was a high level of niche shift. However, contrary to our expectations, the model's performance was better in Asia than in Latin America ( $U = 6152$ ,  $P = 0.005$ ). Since niche expansion was higher in Asia than in Latin America, we expected the model performance would be lower in Asia.



**Fig. 3** Boxplots with the Area Under the Curve (AUC) of the Receiver Operating Characteristic plot (ROC) values found in the performance evaluation of 100 sets of background data sampled for each invaded area of the pond slider (*Trachemys scripta*).

However, when evaluating the models using only the occurrence points in the invaded areas, the models showed a dramatically poor performance. Sensitivity values using the threshold that maximizes sensitivity/specificity (0.3523) were zero in all the invaded continents, reinforcing the low performance of the models to predict species invasion.

## Discussion

The pond slider has already reached large areas of Asia, Europe and Latin America (Rödder *et al.* 2009; Masin *et al.* 2014). Our results indicate that it changed its original niche when invading these new areas and that it has invaded environments that are both warmer (Asia and Latin America) and colder (Europe) than its native niche (Fig. 2). Processes related to niche shift (stability, unfilling, and expansion) varied between continents, as previously observed for this species (Li *et al.* 2014). We also found that niche expansion is not a good predictor of the performance of the ENM. A logical expectation was that the model performance would be lower in non-native areas with high expansion, than in non-native areas with low expansion. However, we do not confirm this expected pattern, which may indicate that the effects of this process on models performance may be more complex than a simple direct effect. Finally, the models had a dramatically poor performance when evaluated for sensitivity, reinforcing the problems of using ENMs and their traditional evaluation methods when focal species do not conserve their native niche.

Niche stability and expansion, complementary measures of niche shift, are highly related to the similarity between native and invaded areas (Parravicini *et al.* 2015), which was also found in our study, where expansion was higher in areas with climates more different from the native one, being the inverse to the tendency observed for stability. In general, niche shift is related to many processes such as native range size, time since the first introduction and latitudinal location of the invasion (Li *et al.* 2014). Moreover, it is known that niche shifts may be artifacts derived from the choice of environmental variables used in the modeling process (Peterson & Nakazawa 2008; Rödder *et al.* 2009). However, this problem was controlled in our study by using

variables that were related to the animal's life history and that provided the best ENMs in another study (Rödder *et al.* 2009). Understanding species niche shifts is a key step required to accurately model and map a species potential invasive range.

Although our study focuses mainly on abiotic conditions related to niche shifts and distribution changes, biotic variables and dispersal processes could also be responsible for niche shifts in the invasion process of the pond slider. Species distributions are mainly governed by three factors: climatic (abiotic), biological interactions (biotic) and dispersal (migration) (Soberón 2007). However, in human-mediated invasions, some biotic limitations available in the native range, such as competitors, predators and parasites, may be overcome (Hierro, Maron & Callaway 2005; Sax *et al.* 2007), and species could then reach new climatic conditions. The native range of the pond slider is a highly diverse turtle hotspot (Mittermeier *et al.* 2015) which is richer than most communities in the invaded range of this species. Such changes in the biotic conditions suggest a reduction in negative biotic interactions. Dispersal constraints are also removed in human-mediated invasions, and species can reach regions which would not normally be occupied (due to natural dispersal limitations), producing several founder effects (Hierro *et al.* 2005; Sax *et al.* 2007). Such introductions are common in the widely traded pond slider (Telecky 2001), and this may also allow the species to reach climatic conditions not occupied in its native range.

Further, new populations in Europe, Latin America and Asia are not the result of a natural species expansion from its original range towards new areas, being composed by individuals raised and selected in farms. Individuals from farms might be biased samples of the gene pool and the phenotypic plasticity of the original populations. Thus,

humans may be accelerating the processes of selection and dispersal of this species, up to a point where the original pond slider populations may no longer be a good sample for predicting potential distribution in new areas. Our results regarding niche shifts in the invaded areas highlight this possibility. However, there is still no data available on the impact of such human-based introductions, and considering that we used only occurrences of reproductive populations (areas where the invasive species is in equilibrium with their climatic limits), we believe our conclusions on niche dynamics for the pond slider will hold.

Most problems discussed when ENMs are used to predict ecological invasions are related to their assumption of niche conservatism (Guisan *et al.* 2014). It is expected that ENMs will not properly capture the potential areas of invasion when species change their niche in the invaded areas. Our results support this expectation: the model fitted using native points of the pond slider did not predict any occurrence in the new areas. Interestingly, we had intermediate values of AUC for Asia, Latin America and Europe (most of them over 0.6, see Fig. 3). The evaluation process of ENMs of invasive species should also not rely on performance metrics, such as AUC, that commonly use pseudoabsence/background points to evaluate the models. A recent study (Parravicini *et al.* 2015) already recommends caution when using AUC or the Boyce index to evaluate the accuracy of invasion models. In our study, some individual evaluations of the model for Asia and Europe had AUC higher than 0.8 (see Fig. 3). This would indicate a good prediction for these regions. However, what is really happening is that all the pseudoabsences (randomly generated zeros across the target geographic extent) are correctly predicted as zero, while actual occurrences of the species are not necessarily well predicted. The sampling of absence/pseudoabsence points and their influence on

model performance are a highly debated topic in ENM (Chefaoui & Lobo 2008; Wisz & Guisan 2009; VanDerWal *et al.* 2009; Barbet-Massin *et al.* 2012). We suggest that this problem may be even greater for invasive species, which may present niche shifts and have had insufficient time to reach all the areas where they can potentially live. Therefore, using a more simple evaluation method, such as sensitivity, may be a more effective performance value.

The current methods for predicting species invasions do not allow species to adapt to new conditions. This is an important flaw of the ENM, because it is clear that species are able to adapt to new conditions in order to survive (Broennimann & Guisan 2008; Beaumont *et al.* 2009; Gallagher *et al.* 2010; Li *et al.* 2014). This means that the current theoretical and methodological framework for predicting the extent of species invasions needs revising. Future studies may focus on the development of techniques that allow a more efficient and dynamic modeling method which takes into account niche shifts.

Considering the problems of using ENM to predict areas where the pond slider may occur, other measures may be more effective in order to properly manage its invasion in these continents. This species was the most exported turtle from the United States from 1989 to 1997 (5,252,173 individuals) (Telecky 2001). The best way to control the spread of this species is probably by banning its commercialization outside its native range. The European Union, for example, has already legally interrupted the importation of this turtle (see Commission Regulation (EC) No 349/2003), and in Brazil, there are also legal documents that control the importation of exotic animals (see Portaria IBAMA No 93/1998). However, a possible illegal trade or absence of proper regulation may be common, making it difficult to regulate the invasion of this species.

An evaluation of the efficacy of a policy to regulate ballast water in Canada, for example, has reinforced the need for inspection in order to ensure proper compliance with measures against invasive species and to increase the efficiency of those measures (Bailey *et al.* 2011). Investing in environmental education in order to make people aware of the problems of releasing the pond slider, and other invasive species, into the natural environment may be another important measure. Many people do not think that a small pond slider hatchling will become a large adult of approximately 300 mm (Gibbons & Lovich 1990). This large growth is commonly followed by a release of the pet into the environment. Teillac-Deschamps *et al.* (2009), for example, have already shown that integrating different strategies to talk to the public regarding this invasive turtle may have good results.

We conclude that there is no niche conservatism in the invasion processes of the pond slider in different continents and that niche expansion is not a good predictor of performance of ecological niche models for this species. Besides, evaluating model performance for invasion cases using AUC may be problematic. Modelled predictions of the pond slider distribution in invaded areas might misestimate the real potential areas where the species can survive. We still lack a dynamic method to properly predict potential invasion risks in new areas and are unable to accurately manage this worldwide invasive species. Considering all these problems, investing in environmental education and in strong control of the commercialization of this species are probably the best ways to manage its invasion and to avoid problems with it. Where standard/static ENMs are still used, we suggest using sensitivity to evaluate their performance.

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## Apêndice 2

Rodrigues, J.F.M., Coelho, M.T.P., Diniz-Filho, J.A.F. (2016): Exploring intraspecific climatic niche conservatism to better understand species invasion: the case of *Trachemys dorbigni* (Testudines, Emydidae). *Hydrobiologia* **779**: 127–134.

**Exploring intraspecific climatic niche conservatism to better understand species invasion: the case of *Trachemys dorbigni* (Duméril & Bibron, 1835)**

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**Abstract**

Niche conservatism at distinct levels of biological hierarchy is still a highly debated topic in ecology. The general evaluation of niche shifts is mainly addressed to species level, with few explorations at lower or higher hierarchical levels. The freshwater turtle *Trachemys dorbigni* (Black-Bellied Slider) has recently been divided in two subspecies that occur in very different climatic conditions, and is also considered to be an invasive species in parts of eastern and southeastern regions in Brazil. Here, we aimed to explore the effects of evaluating climatic niche conservatism at subspecific levels during the invasive process of *T. dorbigni*. We evaluated niche conservatism based on similarity (whether niches are more similar than expected by chance), and also measured expansion, stability and unfilling in the invaded niche. We found that the climatic niches of the *T. dorbigni* recognized subspecies are very different, but when they are

merged, the environmental condition created is more similar to the invasive niche of the subspecies *T. dorbigni dorbigni*. We also found consistent evidence of niche conservatism in invaded areas, which enables the effective use of ecological niche models to forecast *T.dorbigni dorbigni* invasion in other geographic regions.

Keywords: Biological invasion; Emydidae; freshwater turtles; niche shift; subspecies

## **Introduction**

The discussion regarding the evolutionary rates of niche shift is a highly debated topic in Ecology. Recently, empirical evidences and methodological advances in the evaluation of niche conservatism and niche shifts through comparisons of ecological niche overlap among closely related species (mainly using biological invasions) have opened the gates to a vast literature seeking to understand the evolutionary dynamics of species niche at short time scale (Warren et al., 2008; Broennimann et al., 2012; Guisan et al., 2014; Li et al., 2014; Parravicini et al., 2015). Such comparisons are performed considering the realized Grinnellian niche concept, which covers abiotic dimensions that allow positive growth rates, obtained from current species distributions (Soberón, 2007; Soberón & Nakamura, 2009). For instance, niche shifts, reflecting adaptations to new environments, creates a flaw in Ecological Niche Models (ENMs) that can be used to project species distributions, because such models assume equilibrium of species with climate (Araújo & Pearson, 2005) and, in an evolutionary sense, niche conservatism (Pearman et al., 2008). ENMs have been widely used to predict invasion dynamics, but rarely issues related to niche evolution and violation of ENMs assumptions have been considered (but see Palaoro et al., 2013).

Most recent discussions regarding niche conservatism are focused on niche comparisons among species (Petitpierre et al., 2012; Li et al., 2014; Parravicini et al., 2015). Niche conservatism evaluations in lower level clades, such as subspecies, are not yet a common goal in ecological studies, maybe due to a low availability of occurrence data discriminating subspecies. Ecological studies have found support for niche conservatism in subspecies of a parakeet in different continents (Strubbe et al., 2015b) and among subspecies of Mexican birds (Peterson & Holt, 2003), but they also reported

niche shifts among subspecies of birds and snakes (Peterson & Holt, 2003; Alexander Pyron & Burbrink, 2009). Thus, exploring niche dynamics in subspecies is still critically needed to describe particularities and common responses of different taxa, and invasive species may be an adequate model to understand this dynamic.

The freshwater turtle *Trachemys dorbigni* (Duméril & Bibron, 1835) represents an interesting case to evaluate questions related to niche conservatism at the subspecies level. *Trachemys dorbigni* and *Trachemys adiutrix*, Vanzolini, 1995, were recently considered the same species by molecular evidence (Fritz et al., 2012). Now classified as subspecies, *T. dorbigni dorbigni* occurs in the Rio de la Plata region, covering southern Brazil, Uruguay and northern Argentina, whereas *T. dorbigni adiutrix* occurs in the northern State of Maranhão, Brazil (Fritz et al., 2012) (Fig. 1). Furthermore, *T. dorbigni dorbigni* is also collected for pet trade (Bujes & Verrastro, 2008; Fagundes et al., 2010), and the commercial trade of this turtle has caused its invasion into eastern and southeastern Brazil, as well as other regions (Santos et al., 2009; Santana et al., 2014).

The evaluation of niche conservatism in the invasion process of a species might provide an interesting natural experiment to evaluate how combining occurrence data of subspecies might affect niche conservatism inferences. In this study, we aimed to test (i) if niche conservatism is observed between the two subspecies of *T. dorbigni* and (ii) if the environmental conditions of the invaded areas can be better predicted considering only the native environmental niche of the invasive subspecies (*T. dorbigni dorbigni*) or considering the native environmental niche of the whole species (including both subspecies). We expected that the subspecies have minor overlap among their niches, since they are found in very different climatic conditions. We also expected that the

invaded niche could be better predicted by the whole species' niche (including data of both subspecies) rather than by only using the native occurrences of the invasive subspecies.

## **Methods**

### *Climatic data*

We carefully choose a set of variables to represent the environmental niche of *T. dorbigni* subspecies and used a 'minimalistic set' of variables based on their biological meaning, reinforced by evidences for the species or its genus (see explanations for each variable below in the next paragraph). It is a common and recommended practice in species modeling studies (Hijmans & Graham, 2006; Ficetola et al., 2007).

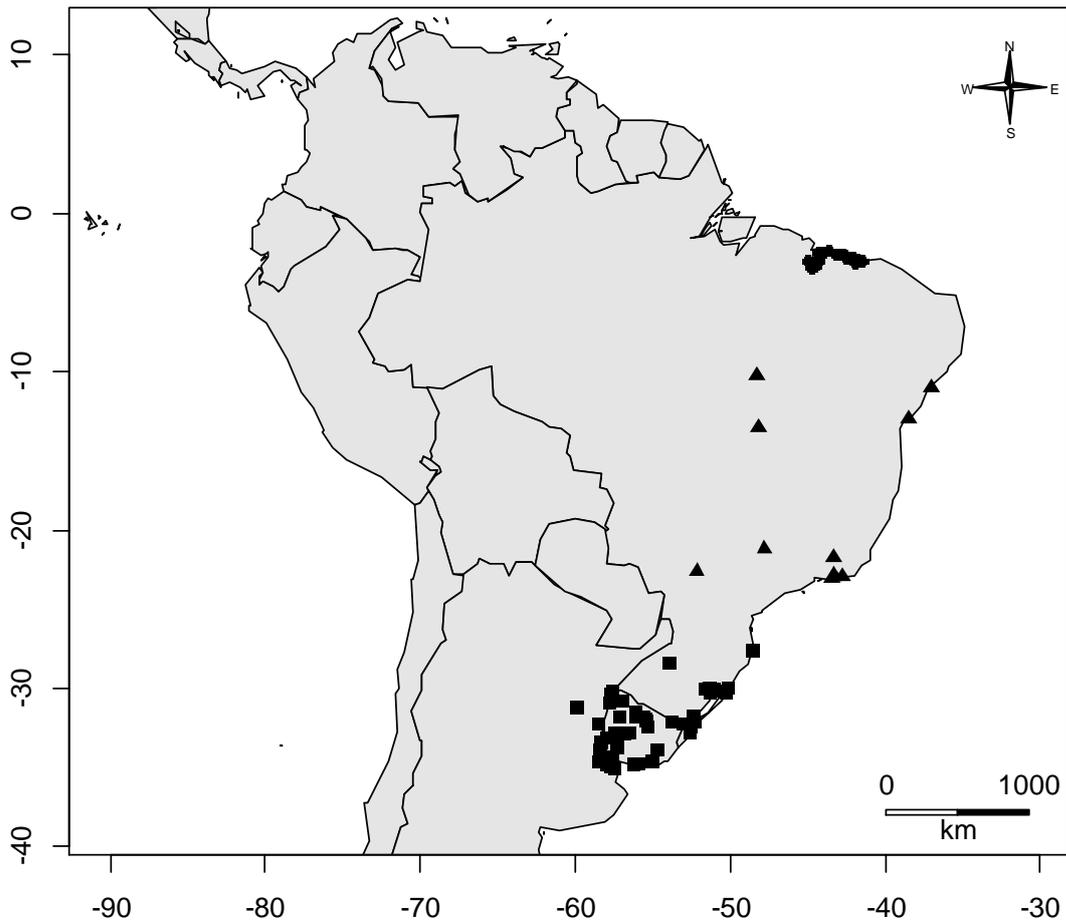
We used six environmental variables in 0.16 degrees or 10-arc minutes resolution available in WorldClim (<http://www.worldclim.org>) (Hijmans et al., 2005) to characterize the environmental niche of *T. dorbigni*: mean annual temperature (BIO1); maximum temperature of the warmest month (BIO5); minimum temperature of coldest month (BIO6); annual precipitation (BIO12); precipitation of driest month (BIO14); and altitude. A resolution of 10-arc minutes was chosen to ensure independence among occurrence data given that aquatic turtles may move broad distances in rivers (Obbard & Brooks, 1980; Pluto & Bellis, 1988). BIO1 and BIO12 are general variables describing temperature and precipitation, which are also much related to productivity and water availability, being commonly used in general studies modelling reptile and turtle distributions (Araújo et al., 2008; Rödder et al., 2009). The variables describing extreme temperatures (BIO5 and BIO6) were used because incubation time and

hatching success in *Trachemys dorbigni* are highly dependent and limited to extreme temperature (Molina & Gomes, 1998). Finally, BIO14 was used because *T. dorbigni* spends most of its lifetime in aquatic habitats (Bager et al., 2007; Bujes & Verrastro, 2008; Bujes, 2010; Quintela et al., 2011) and it might be vulnerable to low precipitation conditions. Altitude was used because the distribution of *T. dorbigni* is mainly concentrated in lowland areas. We also included landcover data representing the distribution of forests and open vegetation in the world in 8km cell resolution (available at <http://glcf.umd.edu/data/landcover/>) because the species of the genus *Trachemys* seem to prefer open vegetation formations, where there are abundant sunny nesting sites (Moll & Moll, 1990). Hence, these seven variables are related to the environmental conditions required for our study species. Although other environmental variables are available, most of them would have the same effect as the variables we selected and would not have as much biological reason as the ones we choose.

#### *Occurrence data*

We used 90 occurrence records of *T. dorbigni* for the analyses: 50 records were *T. dorbigni dorbigni*, 29 were *T. dorbigni adiutrix*, and 11 records represented areas of *T. dorbigni dorbigni* invasions (Fig. 1). Native points of *T. d. dorbigni* and *T. d. adiutrix* were obtained in SpeciesLink website (<http://splink.cria.org.br/>), EMYSsystem website (<http://emys.geo.orst.edu/>) and through an intensive literature search (see Online Resource 1). We collected the invasion points from the literature (see Online Resource 1) and from the database of Instituto Hórus ([http://www.institutohorus.org.br/index\\_eng.htm](http://www.institutohorus.org.br/index_eng.htm)). Since the definition of the status of *T. dorbigni adiutrix* as a subspecies is very recent (Fritz et al., 2012), we considered that

the invasion records that report only “*Trachemys dorbigni*” referred to the subspecies *T. dorbigni dorbigni*.



**Fig. 1** The occurrence records of *Trachemys dorbigni* used in the study. Black squares are *T. dorbigni dorbigni*; black triangles are *T. dorbigni dorbigni* invaded points; and black circles are *T. dorbigni aditrix*

The selection of background areas was based on the watershed where the subspecies and the invasion cases were found, because *T. dorbigni* spends most of its lifetime in the water, leaving only for nesting (Bager et al., 2007; Bujes & Verrastro, 2008; Bujes, 2010; Quintela et al., 2011). Then, the background areas selected in our study were the areas of the watersheds where the occurrence records of the subspecies were found. The background of *T. d. dorbigni* was defined as an area covering the rivers Iguaçú, Paraná, Uruguay and River de la Prata (Rio de la Prata region). For *T. d. adiuatrix*, we used the Atlântico Nordeste Ocidental watershed, and for the invaded points, the São Francisco, Atlântico Leste and Atlântico Sudeste watersheds.

#### *Evaluating niche conservatism in the invasion process*

To evaluate niche conservatism in the species invasion, we analyzed two possible scenarios: 1) only including the native occurrence points of *T. dorbigni dorbigni*; and 2) considering a complex of *T. dorbigni adiuatrix* and *T. dorbigni dorbigni* points as the native range (also referred here as complex *Trachemys dorbigni*). The “invaded niche” was estimated based on invasion occurrence records. We used the Principal Component Analysis (PCA<sub>env</sub>) approach proposed by Broennimann et al. (2012), which allows to describe the environmental space occupied by a species or subspecies, for example, based on its occurrence records, without projecting data in the geographical space. We used the first two axis of the PCA built with the seven environmental variables described earlier to characterize the environmental niche. The environmental space was then divided into a grid 100 x 100, where the species occurrence and environmental densities were calculated following the formulas presented in Broennimann et al. (2012). Then, we used these two measures to calculate

the species occupancy in the environmental space and to evaluate niche conservatism, estimated as niche similarity. We evaluated niche similarity (whether niches are more similar than expected by chance) using permutation analyses of the Schoener's D index, used to evaluate niche overlap (Warren et al., 2008).

We also evaluated niche unfilling (native niche not present in invaded niche), expansion (invaded niche not present in native niche) and stability, after removing the extreme climatic values with densities lower than 5 and 25% in order to reduce the effect of environmental outliers (Broennimann et al., 2012; Petitpierre et al., 2012; Guisan et al., 2014). We only reported the results for 25% because both cutoffs (5 and 25%) produced very similar outcomes. Finally, we used Multivariate Environmental Similarity Surface (MESS) (Elith et al., 2010) to evaluate the availability in environmental conditions of the background area of *T. dorbigni dorbigni* and of complex *T. dorbigni* in South America.

We performed all analyses in R ver. 3.1.2 (R Core Team, 2014). Niche comparisons were performed using *ecospat* package (Broennimann et al., 2015), and MESS analyses were done in *dismo* (Hijmans et al., 2015).

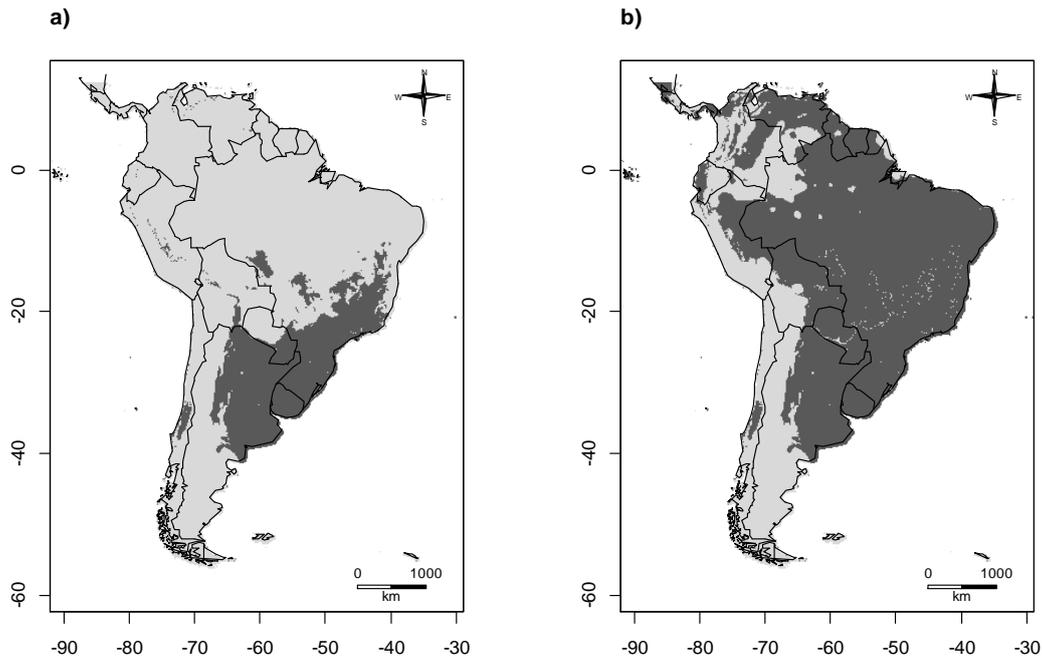
#### *Evaluating niche overlap between subspecies*

We also performed niche similarity analyses to measure climatic niche overlap between the subspecies.

## **Results**

As expected, the subspecies climatic niches are different according to the several metrics used here. They are not more similar than would be expected by chance ( $D = 0$ ,

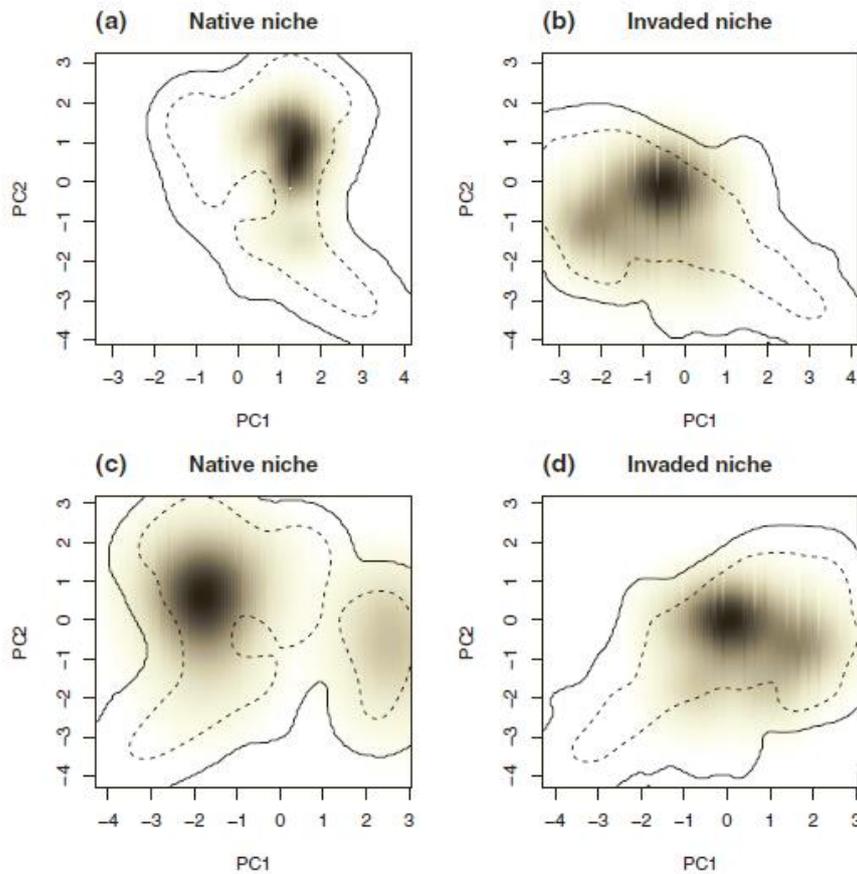
*T. d. dorbigni* – *T. d. adiutrix* and *T. d. adiutrix* – *T. d. dorbigni*,  $p = 1$ ). Besides, the background area of *T. dorbigni adiutrix* has very different environmental conditions when compared to the area of *T. dorbigni dorbigni* (Fig. 2a).



**Fig. 2** Multivariate Environmental Similarity Surface (MESS) analyses highlighting areas in South America which area environmentally similar (there is no extrapolation; black areas) to the background area of a) *Trachemys dorbigni dorbigni*, and b) *T. dorbigni dorbigni* + *T. dorbigni adiutrix*

When the native niche of *T. d. dorbigni* is compared with the invaded niche, the invaded niche is similar to the native niche ( $D = 0.18$ ,  $p = 0.01$ ; Fig. 3a-b). This pattern is highlighted by the high stability when compared to expansion and unfilling components of niche dynamics ( $NS = 0.88$ ,  $NE = 0.12$ ,  $NU = 0.001$ ). The invaded area

also had environmental conditions different from the native area (Fig. 2a). Nonetheless, when we considered the complex *T. dorbigni*, the niche expansion observed in the invaded area decreased to 0% and the stability became 100% (NE = 0, NS = 1, NS = 0.001). This result suggests that *T. d. dorbigni* is occupying parts of the native niche of *T. d. adiutrix* in its invasion process. The evidence remains strong for niche conservatism in the invasion of eastern-southeastern Brazil with invaded niche more similar to the native niche than expected by chance ( $D = 0.33$ ,  $p = 0.01$ ; Fig. 3c-d). The climate of the invaded area was analogous (within the envelope of environmental conditions (Guisan et al., 2014)) to the background of the *T. dorbigni* complex (Fig. 2b).



**Fig. 3** Environmental niche of *Trachemys dorbigni dorbigni* in native (a) and invaded area (b) and the environmental niche of *Trachemys dorbigni dorbigni* + *Trachemys*

*dorbigni adiutrix* (complex *Trachemys dorbigni*) as native area (c) and the invaded niche (d). Solid and dashed lines represent 100% and 50% of the available environment respectively. Gray shading illustrates the density of occurrence of the species. PC1 and PC2 are the first two axis of Principal Component Analyses (PCA) of the environmental variables that represented the environmental niche of the species. In the comparison between native *T. dorbigni dorbigni* and invaded area (a and b), the variances explained by PC1 and PC2 were 38.92% and 31.56% respectively. In the comparison between the complex *Trachemys dorbigni* and invaded area (c and d), PC1 and PC2 explained, respectively, 41.25% and 28.88% of the variance of the niche descriptors.

## **Discussion**

Niche conservatism is not expressed at the subspecies level of *T. dorbigni*, but it occurs in its invasion process. Using an invasion case of the species helped us to better understand the intrinsic differences in the subspecies niches, and the invaded niche is better explained when combining the occurrence records of both subspecies to define its native range. In the invasion process, the species occupies parts of the climatic niche of both subspecies. Moreover, we found strong evidence of niche conservatism in *T. dorbigni* invasion of eastern-southern Brazil, at least as expressed by current occurrences in the native and invaded range.

Niche overlap among species and subspecies has raised questions about species limits (Hill & Terblanche, 2014). Despite the high differences between the environmental niches of *T. dorbigni* subspecies, including both subspecies niches as native niche improved the explanation of the invaded niche, which reinforces the new taxonomic relationship proposed by Fritz et al. (2012). This new classification is not a

consensus among turtle specialists (van Dijk et al., 2014), and future taxonomic studies may provide additional insights regarding the classification of these species. However, currently available occurrence data of native and invasion records of *Trachemys dorbigni* and environmental niches estimated using these records seem to support the new classification.

The lack of niche conservatism among subspecies was also found in other studies (Peterson & Holt, 2003; Alexander Pyron & Burbrink, 2009; Strubbe et al., 2015b). Peterson & Holt (2003) suggest that these differences may have non-adaptive explanations such as sampling bias, environmental variation across the landscape or unevaluated ecological variables, such as biotic interactions. Considering the analyses of the invasion process and the high environmental variation existing between the native areas of both subspecies (the climate of the native area of *T. d. aditrix* is not analogous to the climatic conditions of the native area of *T. d. dorbigni*, compare Fig. 1 and Fig. 2a), the variation in the landscape seems to be a strong and parsimonious hypothesis to explain the lack of niche overlap between *T. dorbigni dorbigni* and *T. dorbigni aditrix*.

According to Fritz et al. (2012), the ancestral of *T. dorbigni* probably colonized South America about 6 million years ago, arriving from Central America. The disjunctive distribution of the subspecies may suggest that the ancestral of *T. dorbigni* had a broad environmental tolerance. However, future studies should explore reasons to explain why this species successfully occupied areas in northern and southern Brazil, without occupying the Brazilian central region, which has large areas with similar environmental conditions to regions where *T. d. dorbigni* occurs (see Fig. 2). Moll & Moll (1990) reviewed evidences from other studies suggesting that *Trachemys* species may not be well adapted in dense forest due to low availability of open, sunny habitats

to nest. While *Trachemys dorbigni* also digs its nests in open areas with none or little vegetation (Bager et al., 2007), rainforest expansions during the Pleistocene may have excluded *T. dorbigni* from the Brazilian central region. Sampling bias may also explain such disjunctive distribution because the majority of chelonian studies are focused on the Amazon region and southern Brazil (Souza & Molina, 2007).

The evidence for niche conservatism in the invasion process of *T. d. dorbigni* reveals that the species might have the tendency to retain its native niche in invaded regions. These conclusions regarding niche conservatism are more robust in the analysis of the complex *T. dorbigni* because current invaded areas are all similar to the native environmental background (see Fig. 3b; Guisan et al. 2014). It is important to clarify that niche conservatism is not a recurrent pattern in biological invasions (Guisan et al. 2014) because there are many evidences of niche shifts in a series of invasive organisms (Strubbe et al., 2013, 2015a, 2015b; Parravicini et al., 2015), even in reptiles (Li et al., 2014). However, in our data, when we used only the bioclimatic data from WorldClim (BIO1, BIO5, BIO6, BIO12, and BIO14), which were already used for describe *Trachemys scripta* niche (Rödder et al., 2009), we found evidence of niche shift (results not reported). This influence of the environmental data used on the results of niche shift evaluations were already highlighted in previous studies (Peterson & Holt, 2003; Warren et al., 2008), reinforcing the importance of selection of the variables used to describe the climatic niche. Confirming that *T. d. dorbigni* conserves its native niche in invaded area is a first step to efficiently try to forecast future invasions using ENMs (Peterson, 2011). It is important to note that our study used a large compilation of occurrence records available in literature. This dataset is the best available to test niche conservatism and expansion of this species. Future studies, when more data might be

available, are interesting to ensure that species invasion could still be forecasted using ecological niche models, if evidences of niche conservatism remain strong.

The recognition of *Trachemys dorbigni* as an invasive species is very recent, and nothing is known about its impact on the native species or local community structure. Masin et al. (2014) evaluated the risk of invasion of some freshwater turtles and *T. dorbigni* was not even cited. Besides, this species is congeneric and has similar natural history traits (body size, clutch size) to *Trachemys scripta elegans*, the most common invasive turtle worldwide (Bager et al., 2007; Masin et al., 2014), reinforcing that *T. dorbigni* may represent a potential environmental risk if not properly controlled. Future studies in the areas where *T. dorbigni* has been recorded as invasive are critical to improve our understanding of its impacts on native turtles and their communities.

Thus, we conclude that the environmental niches of the subspecies of *Trachemys dorbigni* are very different and that the environmental niche obtained when the occurrences of both subspecies are combined is a better predictor of the invasive niche of the subspecies *T. dorbigni dorbigni*. Besides, *T. dorbigni* conserves its native environmental niche in the invasion process, allowing future studies to use ENMs to predict areas with suitable climates for their invasion in other Brazilian regions. The improvement in niche overlap in the invasion process when considering both subspecies reinforces the current classification of them as a single species.

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### **Disclosure of potential conflicts of interest**

Conflict of Interest: The authors declare that they have no conflict of interest.

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### **Electronic Supplementary Material**

**Online Resource 1** Literature containing the occurrence records of *Trachemys dorbigni* used in the study.

**Exploring intraspecific climatic niche conservatism to better understand species invasion: the case of *Trachemys dorbigni* (Duméril & Bibron, 1835)**

**Hydrobiologia**

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**Electronic Supplementary Material**

**Online Resource 1** Literature containing the occurrence records of *Trachemys dorbigni* used in the study. The occurrence records which are not found in the references below may be found in SpeciesLink (<http://splink.cria.org.br/>; nine different occurrence records of *Trachemys dorbigni dorbigni* from the Coleção de Répteis do Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCT-PUCRS) and one occurrence record of *Trachemys dorbigni adiutrix* from Coleção de Répteis do Museu de Zoologia da UNICAMP (ZUEC-REP)), EMYSsystem (<http://emys.geo.orst.edu/>; 23 occurrence records of *T.*

*dorbigni. dorbigni*) and Instituto Horus websites  
([http://www.institutohorus.org.br/index\\_eng.htm](http://www.institutohorus.org.br/index_eng.htm)).

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