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Diversification rates are more strongly related to microhabitat than climate in squamate reptiles (lizards and snakes)

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Patterns of species richness among clades can be directly explained by the ages of clades or their rates of diversification. The factors that most strongly influence diversification rates remain highly uncertain, since most studies typically consider only a single predictor variable. Here, we explore the relative impacts of macroclimate (i.e., occurring in tropical vs. temperate regions) and microhabitat use (i.e., terrestrial, fossorial, arboreal, aquatic) on diversification rates of squamate reptile clades (lizards and snakes). We obtained data on microhabitat, macroclimatic distribution, and phylogeny for >4000 species. We estimated diversification rates of squamate clades (mostly families) from a time-calibrated tree, and used phylogenetic methods to test relationships between diversification rates and microhabitat and macroclimate. Across 72 squamate clades, the best-fitting model included microhabitat but not climatic distribution. Microhabitat explained ~37% of the variation in diversification rates among clades, with a generally positive impact of arboreal microhabitat use on diversification, and negative impacts of fossorial and aquatic microhabitat use. Overall, our results show that the impacts of microhabitat on diversification rates can be more important than those of climate, despite much greater emphasis on climate in previous studies.

KEY WORDS: **Climate, diversification, microhabitat, phylogeny, reptiles, species richness.**

Why do clades differ in their species richness? At any given taxonomic scale, the species richness of clades can vary from a single species to thousands or more. Explaining this variation is an important challenge spanning both evolutionary biology and ecology. In general, there are two basic explanations for why some clades have more species (reviewed in Wiens 2017). First, species-rich clades may be older, and may have more species simply because they have had more time to accumulate richness through speciation events over time. Second, species-rich clades may have faster rates of net diversification (speciation minus extinction over time). Thus, older clades with fewer species will have lower rates of net diversification, whereas younger clades with more species will have faster rates of net diversification. Note that this must be the case regardless of variation in diversification rates over time within these clades, and regardless of variation among their subclades. Recent analyses spanning the Tree of Life suggest that most variation in richness among comparable clades (e.g., same taxonomic rank) is explained by variation in diversification rates, not clade ages (Scholl and Wiens 2016). Given this perspective, an essential part of explaining species richness patterns among clades is to understand why net diversification rates vary (e.g., Ricklefs 2007; Wiens 2017).

Numerous studies have addressed the potential correlates of variation in diversification rates among clades (e.g., Rolland et al. 2014; Weber and Agrawal 2014; Wiens et al. 2015; reviewed in Wiens 2017), many of them focusing on the potential impact of large-scale climatic distributions on the diversification rates of clades. This is a logical hypothesis to test, since it is well

known that tropical regions have higher species richness across most clades (e.g., Hillebrand 2004). Indeed, many studies have found higher rates of diversification in tropical clades, especially in larger scale studies that span multiple families (e.g., Pyron and Wiens 2013; Rolland et al. 2014). Results at smaller phylogenetic scales (e.g., within families) have often been more mixed (e.g., Wiens et al. 2006b, 2009; Soria-Carrasco and Castresana 2012; Jansson et al. 2013). Some studies have also suggested that greater climatic niche divergence within clades explains patterns of diversification among clades (e.g., Kozak and Wiens 2010; Gómez-Rodríguez et al. 2015; Cooney et al. 2016; Moen and Wiens 2017), more so than the climates where these clades occur.

Another ecological factor that might help explain variation in diversification rates among clades is microhabitat. By "microhabitat" we refer to habitat use at small scales, such as being aquatic, arboreal, terrestrial, or fossorial. Relatively few studies have explored the impact of microhabitat on large-scale patterns of clade diversification, or compared the impacts of microhabitat to those of climate. However, a recent study suggested that microhabitat use (i.e., occurring in aquatic vs. terrestrial microhabitats) explains ~65% of the variation in diversification rates among the 12 major clades of vertebrates (Wiens 2015a). Moreover, this study suggested that the positive impact of terrestrial microhabitat on diversification rates and richness patterns might be more important than that of climate, since many species-poor vertebrate clades are both aquatic and tropical (e.g., lungfish, crocodilians). However, that study did not explicitly test the relative importance of microhabitat and climate for explaining variation in diversification rates among clades. A recent study in frogs (Moen and Wiens 2017) found that arboreal microhabitat use and rates of climatic niche change among species were both more important than climatic distributions of species (e.g., occurring in tropical vs. temperate regions) in explaining diversification rates and richness patterns among families.

Here, we test the relative impacts of microhabitat use and climatic distribution on large-scale patterns of clade diversification, using squamate reptiles (lizards and snakes) as a model system. Squamate reptiles include ~10,000 described species (Uetz and Hosek 2015), and include ~40 families of lizards and ~25 families of snakes (exact numbers of families depend on the classification used). Squamates offer an excellent model system for studying this topic for several reasons. First, along with birds, they represent one of the most species-rich tetrapod clades. Second, they show substantial variation in microhabitat use (Pough et al. 2016), including many species that are terrestrial (e.g., tegus and most monitor lizards), arboreal (e.g., many geckoes and *Anolis* lizards), aquatic (e.g., elapid sea snakes), and fossorial (e.g., amphisbaenians, or "worm lizards"). Third, many important resources are available to address the impacts of microhabitat and climate on diversification in squamates, especially at the level of families. These include (1) a time-calibrated phylogeny that includes all families and ~40% of all species (Zheng and Wiens 2016), (2) a taxonomic database with the current richness of described species in each family (the net diversification rate of each clade can then be estimated given its age and richness; Magallón and Sanderson 2001), (3) a database with information on microhabitats of many species (IUCN 2014), which can be used (along with other literature) to estimate the proportion of species in each family that use each microhabitat type, and (4) a recent study (Pyron 2014) summarizing the climatic distributions of many species (i.e., whether they ocur in tropical vs. temperate regions). The latter study suggested that tropical climates positively influenced diversification rates of squamate clades (Pyron 2014). However, the impact of microhabitats on diversification rates among squamate clades has not been addressed, nor compared to the impact of climate. Here, we use phylogenetic regression to test the relative fit of models with different sets of microhabitat and climatic variables, and evaluate whether the best-fitting model (the one that best explains variation in diversification rates among clades) includes microhabitat, climate, or both.

Methods

DIVERSIFICATION RATES

Our primary analyses were based on estimates of net diversification rates for families, using the methods-of-moments estimator for stem-group ages (Magallón and Sanderson 2001). We then tested whether diversification rates of families were related to the proportion of species in each family occurring in particular microhabitats and climates. However, we also performed supplementary analyses using crown-group ages, genera, and an alternative approach to estimating clade-level diversification rates. We also performed MuSSE analyses (described in the final section of the methods), which do not require delimiting clades.

We focused primarily on the stem-group estimator because the stem group incorporates the entire history of a group, and it only requires that at least one species be included in the phylogeny within each clade (for estimation of clade ages). The crown-group estimator is problematic because it can underestimate clade ages (and thereby overestimate diversification rates) if taxon sampling within clades is incompelete. Furthermore, the crown-age estimator cannot be used in groups with only one species (or one species sampled), since the crown-group age is then unclear. The exclusion of the most species-poor clades may then lead to strongly biased conclusions, since these clades may have the lowest diversification rates. Finally, the stem-group age of a clade is older than the crown group age, and simulations show that the accuracy of this net diversification estimator increases with clade age (Kozak and Wiens 2016). This makes sense, since a longer time

scale should make it more likely that a clade will come to have the richness expected given its age and diversification rate.

The methods-of-moments estimator requires an estimated age and species richness for each clade, and a relative extinction fraction (ε; extinction/speciation). The relative extinction fraction is intended to correct for the failure to sample entire clades due to high extinction rates across a large-scale tree, and not as an estimate of extinction rates within extant clades (Magallón and Sanderson 2001). We followed standard practice and estimated diversification rates using three relative extinction fractions: zero, intermediate, and high extinction ($\varepsilon = 0.0$, 0.5, and 0.9, respectively). For brevity, we present only results from the intermediate fraction in the main text, since regression results were largely similar across different values. We also performed a limited set of analyses using diversification rates estimated using crown-group ages (for families), but these were broadly similar to those using stem-group ages, and we do not discuss these results in detail (given our preference for the stem-group estimator, as described above).

To estimate clade ages, we used the phylogeny of Zheng and Wiens (2016), which is based on extensive sampling of genes and species. To quantify species richness of families, numbers of described species were obtained from the Reptile Database (Uetz and Hosek 2015). Note that we treated subfamilies of Colubridae as separate taxa (also treated as separate families by Uetz and Hosek 2015). Furthermore, following the results of Zheng and Wiens (2016), the seemingly nonmonophyletic Colubrinae was treated as two separate clades. The Asian arboreal colubrines (Colubrinae 1: *Ahaetulla, Chrysopelea* and *Dendrelaphis*) are placed as sister group to Grayiinae (separate from all other colubrines: Colubrinae 2) and may represent a distinct subfamily (Pyron et al. 2013; Ahaetullinae: Figueroa et al. 2016). Clade ages, species richness, and estimated diversification rates for families are provided in Supplementary File S1. All Supplementary Files (S1-S13) are presently available as Supporting Information and are available on Dryad (https://doi.org10.5061/dryad.c063r).

We note that some authors have stated that these net diversification estimators require constant rates of diversification within clades over time to be accurate, and should only be used if there is a positive relationship between clade age and richness among clades (e.g., Rabosky and Adams 2012). However, these estimators are agnostic about rates within clades over time (mathematically, they depend only on richness and clade age, not patterns of diversification within the clade over time). Thus, younger clades with many species will have higher net diversification rates, and older clades with fewer species will have lower rates, regardless of the details of variation within these clades. Moreover, simulations have shown that the relationship between age and richness among clades has no impact on the accuracy of these estimators (Kozak and Wiens 2016). Instead, simulations show generally strong relationships between true and estimated diversification rates from this method, and that its accuracy increases with clade age, and decreases with incorrect relative extinction fractions (Kozak and Wiens 2016). Although some studies have used BAMM (Rabosky 2014) to analyze patterns of diversification, recent simulations suggest that this approach may not give accurate estimates of speciation, extinction, and diversification rates (Moore et al. 2016). Therefore, we did not use this approach.

Net diversification rate estimators do not require constant rates within or between clades, but variation in net diversification rates among clades over time could potentially uncouple diversification rates from richness patterns (e.g., faster rates in younger clades with lower richness), which would make diversification rates problematic for explaining richness patterns (Wiens 2011; Kozak and Wiens 2016). Therefore, we also tested the relationship between diversification rates and species richness. Following standard practice (e.g., Scholl and Wiens 2016), richness was ln-transformed to improve normality.

Our main analyses focused on family-level clades using the method-of-moments estimator for stem ages. However, we also performed a series of secondary analyses to explore how different methods impacted the results. First, we performed family-level analyses using crown-group ages. Clades with a single described species were considered to have diversification rates of zero. Those with >1 described species but only a single species in the phylogeny of Zheng and Wiens (2016) lacked data on crown-group ages and were therefore excluded from these analyses (i.e., Anomochilidae, Cadeidae, Xenopeltidae, and Xenophidiidae).

Second, we performed analyses using genera as clades instead of families, using the stem-group method-of-moments estimator. We considered using genera to be potentially problematic overall, since simulations show that diversification rate estimators will be more accurate for older clades (e.g., more likely that the observed richness reflects the true, underlying diversification rate; Kozak and Wiens 2016). Indeed, relationships between diversification rates and richness were much weaker for genera than for families (see Results). Crown-group estimates were not used since many genera had only one species or only one species sampled in the phylogeny. Species richness, clade ages, and estimated diversification rates for genera are provided in Supplementary File S2.

Third, we estimated diversification rates for each family using time-variable likelihood models in the R package RPANDA (Morlon et al. 2011, 2016). The models tested included birth-death models (constant, linear, and exponential changes in speciation and extinction rates) and pure-birth models (no extinction). Detailed methods and results are provided in Supplementary Files S9–S13. This approach often gave highly problematic rate estimates, based on multiple criteria. Furthermore, the approach could not be applied to clades with few species, making it inapplicable

to clades with the lowest diversification rates (a potentially serious source of bias). Therefore, our main results used the method-of-moments estimator and not this approach, and we did not analyze the diversification rates estimated from it.

MICROHABITAT DATA

The overall microhabitat usage of each species was categorized using databases and literature sources (data and references in Supplementary File). We first searched the IUCN database for all species with microhabitat data. We then searched the literature (e.g., field guides, papers on ecology) for additional species not included in the IUCN database but included in the squamate phylogeny of Zheng and Wiens (2016). Microhabitat data were obtained for a total of 4214 squamate species (again, the set of species with microhabitat data was not fully identical to the set of species in the phylogeny, despite considerable overlap).

The species sampled should represent overall squamate diversity. Thus, the number of species sampled from each family should be proportional to that family's richness. We estimed the correlation between the number of species described per family (from the Reptile Database; Uetz and Hosek 2015) and the number of species with microhabitat data per family. The results showed a very strong correlation ($r = 0.98$; degrees of freedom (df) $= 70$; $P < 0.0001$), indicating that our data for microhabitat are distributed among families in proportion to their richness. As far as we know, sampling of species within each family should not be biased toward particular microhabitat categories.

Species were assigned to a microhabitat type based on the primary microhabitat in which they were active, including: (1) terrestrial if on the ground (for example, on rocks, sand and/or soil); (2) arboreal if in trees and/or bushes; (3) semiarboreal if in trees and/or bushes as well as on the ground; (4) fossorial if underground and/or under leaf litter (but burrowing and not simply utilizing burrows made by other species); (5) semifossorial if they were active primarily underground and/or under leaf litter as well as on the ground; (6) aquatic if in marine and/or freshwater environments; and (7) semiaquatic if active in marine and/or freshwater environments and on the ground. We did not consider microhabitats that a species used only occasionally or under duress (e.g., burrowing or diving to escape when threatened). However, for less common species, our inferences of microhabitat were based primarily on where individuals were found, following papers and/or database descriptions. We also performed a small set of supplementary analyses treating saxicoly (occurring on rocks) as a separate category in lizards, given the many rock-dwelling lizard species.

The proportion of species in each microhabitat in each family was calculated using two different approaches (Supplementary File), differing in how these "semi" species were treated. First, in the primary approach (proportional microhabitat), species that use more than one microhabitat type (e.g., semiarboreal, semifossorial, and semiaquatic) were split evenly between the terrestrial category and the other microhabitat they use (arboreal, fossorial, or aquatic). The proportion of each microhabitat category in each family was calculated based on the total number of species that used a given microhabitat, the number in a given "semi" category (divided by two), and then divided by the total number of species for which microhabitat data were available for that family. For example, if a family had 100 species overall, 50 of those species had microhabitat data, and 25 were arboreal and 25 terrestrial, we estimated the family to be 50% arboreal rather than 25% arboreal. Moreover, if data for an additional 10 species were obtained, and these 10 species were semiarboreal (totaling 60 species with microhabitat data), we would consider the family to still be 50% arboreal (i.e., 25+5 divided by 60). For the second approach (strict), only the four strict categories of microhabitat use were included (terrestrial, arboreal, fossorial, and aquatic), and species that used more than one microhabitat type (e.g., semiarboreal) were excluded. However, since this strict approach excluded considerable information, our primary analyses used the proportional approach.

Genus-level analyses used only "strict" microhabitat proportions (File S5). Initial analyses using "proportional" microhabitat often crashed, presumably because many variables had zero (or near zero) variance due the high frequency of taxa with proportions at or close to zero (Kuhn 2008).

We acknowledge that our categorization of microhabitat for some species could be in error (e.g., for poorly known species characterized based on few observations). However, simulations based on similar analyses in frogs suggest that regression analyses of microhabitat and diversification can be robust to high levels of random error (e.g., when microhabitats of 20% of species are characterized incorrectly there is little discernible impact on regression results; Moen and Wiens 2017). Therefore, we suggest that such errors should not overturn our conclusions.

CLIMATIC DISTRIBUTION

We also tested for the possible effects of climatic distribution on diversification rates. Specifically, we tested for a relationship between diversification rates and species climatic distributions (temperate vs. tropical) for 4162 species, all included in the phylogeny of Zheng and Wiens (2016). Data on species distribution were extracted from Pyron (2014), in which species were classified as tropical (1), temperate (2), or both (0) (Supplementary File S6). The only exception was Gerrhopilidae, which was not sampled by Pyron (2014). We obtained data on the climatic distribution of this family from the Reptile Database (Uetz and Hosek 2015). As for microhabitats, species that occurred in both climatic regions were split and added to each category (tropical and temperate). The proportion of temperate and tropical species in each

family was estimated by dividing the number of tropical or temperate species, plus half of those found in both regions, by the total number of species with distributional data available for that family (File S7 for families and S5 for genera). Among the 4162 species in this dataset, 3269 (79%) were tropical, 803 (19%) were temperate, and 90 (2%) occurred in both climatic regimes. Note that we did not perform state-dependent speciation-extinction analyses (SSE) for climatic distributions, given that such analyses have already been performed (Pyron 2014).

As found for microhabitat data, there was a strong correlation between the number of species sampled from each family for climatic data and the total number of species in each family ($r = 0.87$; df $= 70$; $P < 0.0001$). This result provides some evidence that our sampling should be representative of the overall distribution pattern for squamates, even though not all squamate species were included.

TESTING THE RELATIONSHIPS BETWEEN MICROHABITAT USE, CLIMATIC DISTRIBUTION, AND DIVERSIFICATION RATES

The hypothesis that microhabitat use impacts diversification rates was primarily tested using phylogenetic generalized least-squares regression (PGLS; Martins and Hansen 1997). PGLS was also used to test the relative impact of climatic distribution on diversification rates. PGLS was implemented in the R package *caper* (version 0.5.2; Orme 2013) in RStudio (version 0.99.489). Branch lengths were transformed based on maximum-likelihood estimated values of phylogenetic signal (lambda; Pagel 1997, 1999; Freckleton et al. 2002), with kappa and delta each fixed at 1. The time-calibrated phylogeny from Zheng and Wiens (2016) was used, after pruning the tree to include only one arbitrarily chosen species per family (the choice of species has no impact, since each species will have the same branch length to the stem age of the family). Each microhabitat was tested separately as an independent variable (with diversification rate as the dependent variable), as was climatic distribution. Then a series of multiple regression analyses were run, first including all variables and then sequentially excluding the independent variable with the lowest *F*-value, following analyses of variance (ANOVA) model selection (i.e., backward elimination stepwise model selection), a standard approach in multiple regression that is implemented in *caper*. All analyses were conducted including all squamates simultaneously, and then with lizards and snakes treated separately (given their potential for different patterns of microhabitat usage and diversification). Amphisbaenians were classified with lizards in these analyses. We acknowledge that dividing squamates into lizards and snakes is somewhat arbitrary (and that lizards are not monophyletic), but these separate analyses helped to further confirm the robustness of the overall squamate-level analyses. We focused on results from multiple regression analyses as they allowed us to test the effects of multiple microhabitats (and climate) simultaneously. However, results from pairwise comparisons are shown as Supporting Information (i.e., diversification rate vs. each independent variable treated separately). The relationships between microhabitat use and climatic distributions were also tested.

Our conclusions were based primarily on the choice among multivariate models that included or excluded different sets of microhabitat and climatic variables, selecting the model with the lowest AIC (Akaike information criterion) value. An AIC difference of four or more was considered strong support for the best-fitting model over the next best model (Burnham and Anderson 2002). We also present *P*-values for individual variables in the context of each multivariate model (representing the significance of a given variable when all other variables in the model are held constant). However, these particular *P*-values should be interpreted cautiously: variables can still contribute substantially to the overall model even with *P*-values >0.05 (Sokal and Rohlf 1995, p. 632). This is apparent from our results, in which some seemingly nonsignificant variables (by this criterion) strongly impact model fit. Again, our conclusions are based primarily on a model-selection approach (the differences in AIC values of different models that include different sets of predictor variables). We note that some authors might prefer to use the r^2 to choose among competing models rather than the AIC (e.g., following from Shmueli 2010). However, we note that both criteria pick nearly identical models here, and we indicate cases where they do not. Regardless, these differences have no impact on the conclusions.

Given that proportions for microhabitat and climatic distributions were not normally distributed (Table S1), analyses were conducted using logit-transformed proportions (Files S4 and S6). We did not use the traditional arcsine transformation for proportions given the potential problems with this transformation (see Warton and Hui 2011). One problem regarding logit-transformation is that proportions equal to 0 and 1 transform to undefined (infinite) values. For this reason, we added a small value ($e = 0.5$) to both the numerator and denominator of the logit function, as suggested by Warton and Hui (2011).

Note that almost all analyses in this study were merely testing the robustness of the results in Table 1. Therefore, we did not perform a Bonferroni correction for assessing significance across all *P*-values in this study. Furthermore, most results in Table 1 would remain significant using a sequential Bonferroni correction (Rice 1989).

MuSSE ANALYSES

As another set of alternative analyses, we used the MuSSE method (multiple state speciation and extinction) to test the effects of microhabitat use on speciation and extinction rates, as implemented

Table 1. Results from multiple regression analysis of the relationships between proportional microhabitat use and climatic distribution (independent variables) and stem-group diversification rates (dependent variable) estimated for 72 squamate families, based on PGLS and ANOVA model selection.

	Parameter	*P*-value	*F*-value	Adjusted r^2	AIC
Model 1					
div ~ ter + fos + arb + aqua + trop		**<0.0001**	9.143	0.364	668.98
	ter	0.3898	0.7493		
	fos	0.0803	3.1535		
	arb	**0.0114**	2.9400		
	aqua	**<0.0001**	38.6503		
	trop	0.6375	0.2241		
Model 2					
div ~ ter + fos + arb + aqua		**<0.0001**	**11.51**	**0.371**	**667.23**
	ter	0.3870	0.7581		
	fos	0.0785	3.1905		
	arb	0.0892	2.9744		
	aqua	**<0.0001**	39.1031		
Model 3					
div ~ fos + arb + aqua		0.0787	2.364	0.054	692.44
	fos	0.1633	1.9854		
	arb	0.0676	3.4477		
	aqua	0.2020	1.6595		
Model 4					
div ~ fos + arb		0.0718	2.736	0.046	692.07
	fos	0.1439	2.1843		
	arb	0.0741	3.2886		
Model 5					
div ~ arb	arb	**0.0286**	4.992	0.053	690.59

Significant *P*-values (<0.05) and best-fitting model (based on AIC) are boldfaced. Div, diversification rate with $\varepsilon = 0.5$; ter, proportion of terrestrial species; fos, proportion of fossorial species; arb, proportion of arboreal species; aqua, proportion of aquatic species; trop, proportion of tropical species.

in the R package *diversitree* (FitzJohn 2012). We note that BiSSE-type methods have become somewhat controversial for testing correlates of diversification rates (e.g., Maddison and FitzJohn 2015; Rabosky and Goldberg 2015). However, the use of multiple states (microhabitats) and separate analyses of lizards and snakes should greatly reduce the chances that a single clade with high diversification rates for one state will erroneously determine the overall patterns. Nevertheless, this approach has additional disadvantages, in that it does not address how much variation in diversification rates is explained by one or more microhabitat states, nor does it allow straightforward comparison with the variation in diversification rates explained by climate. On the other hand, the approach is potentially advantageous in that it can estimate the relative contributions of speciation and extinction to variation in diversification rates, and does not require defining clades a priori. We note that we could not apply the HiSSE approach of Beaulieu and O'Meara (2016) since it requires that the trait analyzed be binary (two states), unlike the multistate data we analyze here. We also reiterate that the MuSSE analyses used here are only secondary analyses relative to the primary, PGLS analyses.

For the MuSSE analysis, we used the 2175 species included in the phylogeny of Zheng and Wiens (2016) for which microhabitat data were obtained. Aquatic species were excluded, since they represented less than 3% of the total number of included species and because rare states are known to be problematic for BiSSE-class methods (Davis et al. 2013). Similarly, we excluded species that occurred in multiple microhabitats (e.g., semiarboreal, semiaquatic). For MuSSE analyses, each species must be assigned to a single state, and treating "semistates" as distinct states would be problematic since they all have frequencies <10% (Table S2). Therefore, we used three microhabitat states in this analysis: (1) terrestrial; (2) fossorial, and (3) arboreal (File S8). We tested full models in which speciation rates (λ) and extinction rates (μ) were different between microhabitat states as well as constrained models in which speciation or extinction rates were assumed to be constant (e.g., $\mu 1 = \mu 2 = \mu 3$). We tested each model with transition rates (q_{ij}) between states set to be asymmetrical (e.g.,

different transition rate from state *i* to state *j* and from state *j* to state *i*). Preliminary analyses gave problematic results with high rates of transitions from fossorial to terrestrial and arboreal states (transitions that are highly unlikely, given that most fossorial lineages are limbless or limb-reduced; Wiens et al. 2006a). Therefore, the rate of transitions leaving the fossorial state (from fossorial to terrestrial ($q_{F\rightarrow T}$) and fossorial to arboreal ($q_{F\rightarrow A}$)) were set to zero. Given this set of models, we compared the relative fit of the data to each model using the AIC. Specifically, among the models compared for a given set of taxa (e.g., all squamates, lizards, snakes) the model with the lowest AIC was considered to be the best-fitting model for that dataset. Finally, we obtained credibility intervals around parameters estimated by the best model using a Markov Chain Monte Carlo (MCMC) approach, as implemented in the R package *diversitree* (FitzJohn 2012), with each chain run for 10,000 steps, and the first 500 deleted as burn-in. Again, we did not perform SSE analyses for climatic distributions, given that such analyses have already been performed (Pyron 2014).

Results

The phylogeny, diversification rates, microhabitat states, and climatic distributions for squamate families are summarized in Figures 1 and 2. Our survey (Table S2) suggests that almost half of all squamate species occupy terrestrial microhabitats (43%), with fewer species that are fossorial (18%), arboreal (20%), or aquatic (4%). Approximately, 15% of sampled squamate species occupy more than one microhabitat type.

Multiple regression analyses showed a significant association between microhabitat use and diversification rates across Squamata, with no significant effect of climatic distributions (Table 1). The model including all four microhabitat types but excluding climate (tropical vs. temperate distribution) had the best fit (lowest AIC value; Table 1). Models including climate had poorer fit (Table 1; Fig. S1), but with an AIC difference of 1.75. The best-fitting model showed that microhabitat alone explained 37% of the variation in diversification rates among squamate clades, with a particularly strong negative impact of aquatic microhabitat. In contrast, the model including microhabitat and climate explained only 36% of the variation in diversification rates, showing that climate explained little variation not already explained by microhabitat. Diversification rates in turn explained 81% of the variation in species richness among clades ($r^2 = 0.81$; $P < 0.0001$; Table S3). Analyses of each microhabitat category separately (Table S4) suggested a weak negative relationship between diversification and fossoriality (Fig. 3B) and a strong positive relationship with arboreality (Fig. 3C). Results for different extinction fractions (Tables S5–S6) and from strict microhabitat categories (i.e., excluding species that regularly use more than one microhabitat; Tables S7–S9) were generally similar and are not discussed in detail.

Results were broadly similar analyzing lizards and snakes separately (Tables 2 and 3). For lizards (Table 2), the best-fitting model included all four microhabitat types, but not climate. Microhabitat use explained 38% of the variation in diversification rates. Diversification rates were significantly higher in clades with higher proportions of arboreal species and lower proportions of fossorial and aquatic species (Table 2, Fig. 4). Interestingly, the effect of arboreality seemed to primarily stem from the low diversification rates of clades with no or few arboreal species. In contrast, the low diversification rates for aquatic lineages were driven largely by the monotypic families Lanthanotidae and Shinisauridae (note that very low richness is expected given low diversification rates). In a supplementary analysis, we tested for a possible effect of saxicolous microhabitat on diversification rates in lizards, given the many species of rock-dwelling lizards (17% of squamates overall; Table S2). However, no significant associations between saxicoly and diversification rate were detected (Table S10). Similar patterns were found when considering different relative extinction fractions and different ways of treating species that use more than one microhabitat ($\varepsilon = 0.0$ and 0.9: Tables S11–S12; strict microhabitat: Tables S13–S15). Similar patterns were also observed from pairwise relationships between diversification and microhabitat (Table S16). Species richness was strongly predicted by diversification rates in lizards ($r^2 = 0.78$; Table S17).

In snakes (Table 3, Tables S18–S20), the best-fitting model based on the AIC included microhabitat and climate, but the one with the highest r^2 included only microhabitat (microhabitat alone explained 41% of the variation in diversification rates). For both models, the strongest effect was a negative relationship between diversification rates and the proportion of aquatic species (Fig. 5A). The aquatic colubrid subfamily Grayiinae and the predominantly aquatic families Acrochordidae and Homalopsidae strongly contributed to the pattern of lower aquatic diversification rates. There was also a weak positive effect of terrestrial microhabitat on diversification rates that was found only in snakes (Fig. 5D). Furthermore, in snakes, net diversification rates were negatively related to tropical distribution (contrary to the expectation of higher diversification rates in tropical clades; Figs. S1C and S2). However, this negative relationship was not significant. Results from pairwise models for snakes suggested a slightly negative relationship with fossoriality (Fig. 5B), but did not support the negative relationship with aquatic habitat use found in the full multivariate model (Table S21; a pattern also found in squamates overall). Patterns of species richness among snake clades were strongly related to their diversification rates ($r^2 = 0.89$; Table S22).

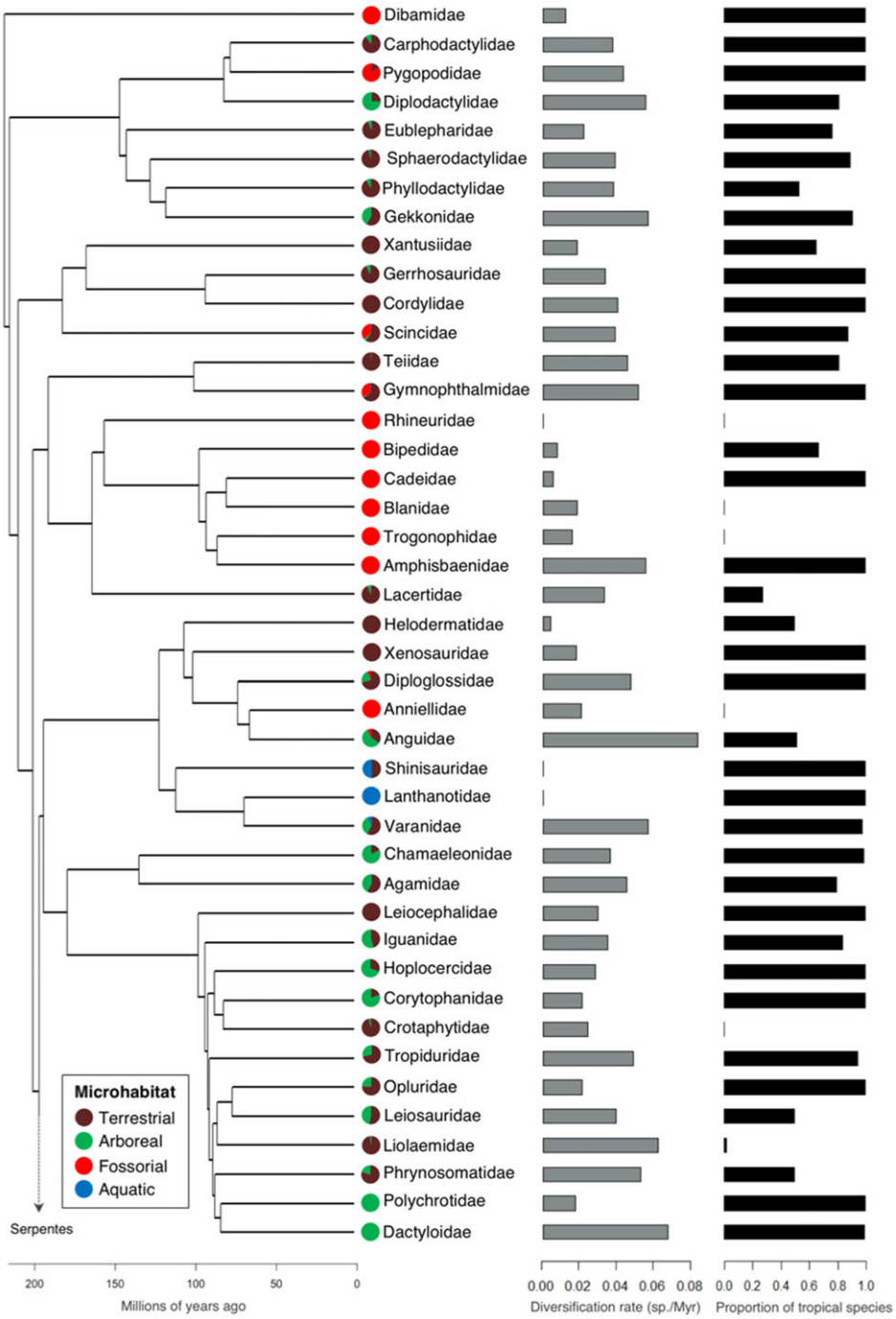

Figure 1. **Microhabitat usage, net diversification rates, and proportions of tropical species among 43 lizard families. Pie charts represent the proportions of microhabitat use within each family, including terrestrial (dark brown), arboreal (green), fossorial (red), and aquatic (blue) species. Bar plots represent diversification rates (in gray) and the proportion of tropical species for each family (in black). Diversification rates were estimated using stem ages and assuming an intermediate relative extinction fraction ($\varepsilon = 0.5$). Phylogeny is from Zheng and Wiens (2016).**

Overall, there were no significant relationships between microhabitat use and climatic distribution (Table S23). However, the relationship between climate and arboreality approached significance across squamates (but not in lizards or snakes separately), as did the relationship between climate and terrestriality in snakes. Importantly, arboreality had the strongest impact on diversification in lizards (Table 2), in which climate and arboreality are unrelated (Table S23).

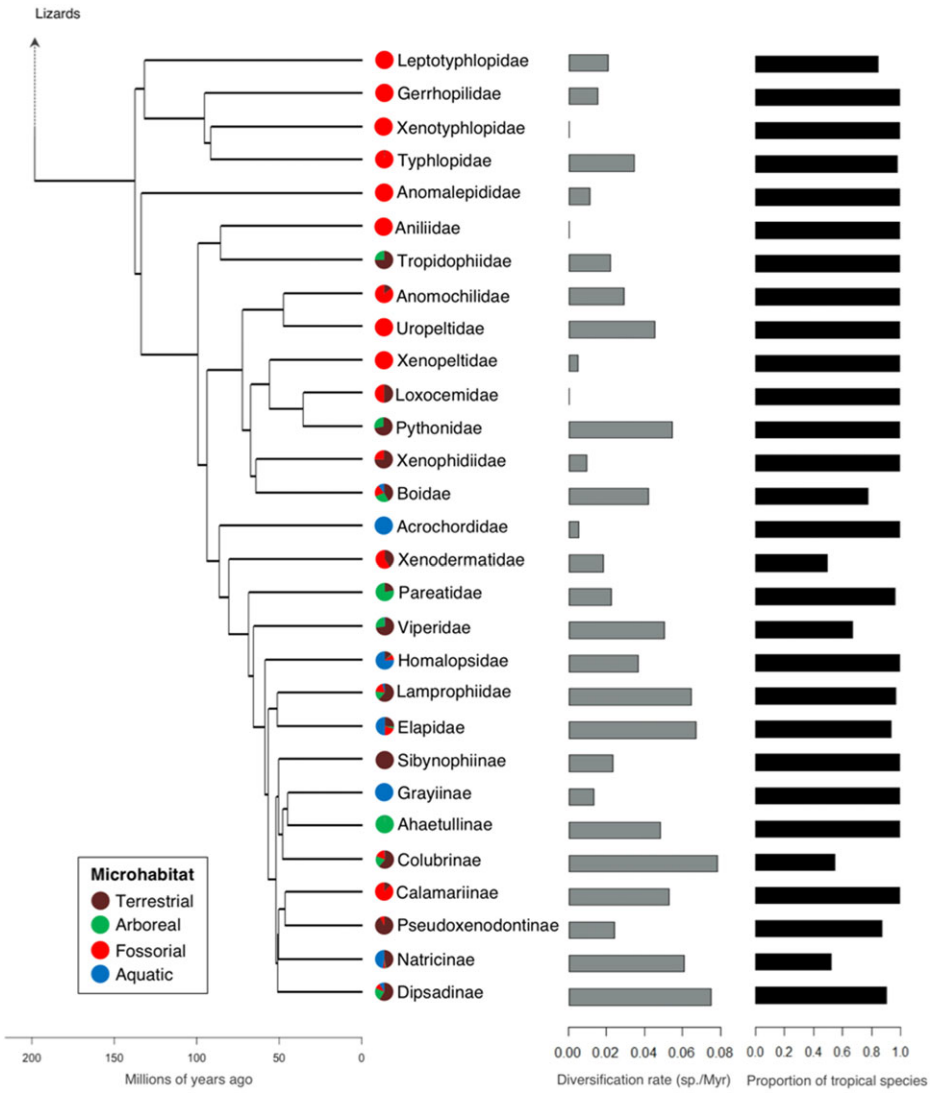

Figure 2. **Microhabitat usage, net diversification rates, and proportions of tropical species among 29 snake clades. Pie charts represent the proportions of microhabitat use within each family including terrestrial (dark brown), arboreal (green), fossorial (red), and aquatic (blue) species. Bar plots represent net stem diversification rates (in gray) and the proportion of tropical species for each family (in black). Diversification rates were estimated using stem ages and assuming an intermediate relative extinction fraction ($\varepsilon = 0.5$). Phylogeny is from Zheng and Wiens (2016).**

Results using crown-group diversification rates (Tables S24–S26) across squamates were broadly similar to those using stem-group rates, including significant impacts of aquatic and arboreal microhabitats on diversification, and negligible impacts of tropical climates. However, crown-group diversification rates were more weakly related to species richness ($r^2 = 0.58$; Table S27), and showed weaker relationships with microhabitat overall.

Genus-level results corroborated some family-level patterns, including lower diversification rates related to fossorial and aquatic species and no significant effect of climate on diversification. For squamates and lizards, diversification rates decreased with the proportion of fossorial (and sometimes aquatic) species (Tables S28–S30 for squamates and S31–S33 for lizards; Figs. S3 and S4, respectively). In snakes, aquatic habitat use strongly decreased diversification (Tables S34–S36; Fig. S5). Climatic distributions showed little relationship to diversification rates (Table S37–S39). Overall, relationships between microhabitat and diversification for genera were weaker than for families. Species richness of genera were significantly related to diversification rates for all squamates, lizards, and snakes (Table S40), but the relationship was substantially weaker than for families (i.e., diversification rates explain only 30–35% of the variation in richness using $\varepsilon = 0.5$).

Finally, we used MuSSE to estimate the effect of microhabitat use on speciation and extinction rates (but again, these were not our main results, especially since they exclude aquatic

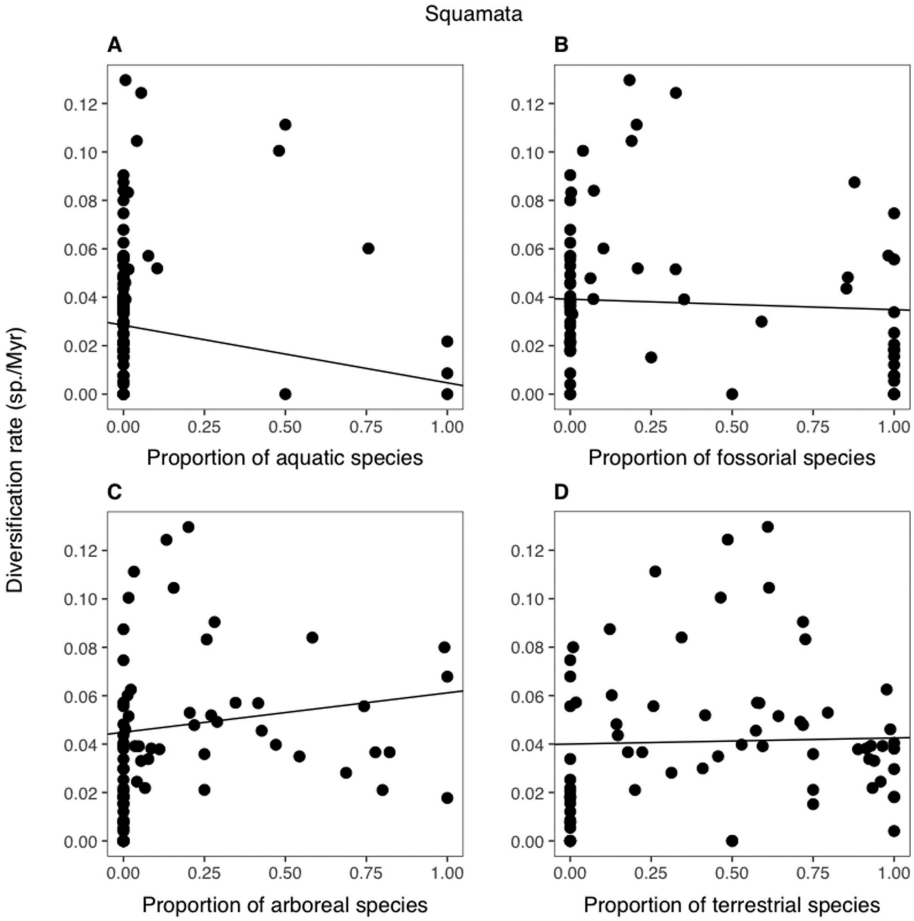

Figure 3. **Relationships between diversification rates of 72 squamate families and their proportions of (A) aquatic, (B) fossorial, (C) arboreal, and (D) terrestrial species (PGLS results in Table S4). Diversification rates were estimated assuming an intermediate relative extinction fraction ($\varepsilon = 0.5$). Similar relationships were found assuming low and high relative extinction fractions ($\varepsilon = 0.0$ and 0.9; Tables S11 and S12). Lines are from standard linear regression showing relationships between each microhabitat and diversification rates. Alternative results for strict microhabitat proportions are shown in Tables S13–15. Note that we show here the actual proportions (from 0.0 to 1.0) of species using each microhabitat, but we analyzed data using logit-transformed proportions to better reflect statistical assumptions (graphs are similar for raw and logit-transformed data; see Fig. S6).**

lineages). For squamates overall, the best model had different speciation rates for different microhabitats, equal extinction rates, and different transition rates between microhabitats. Specifically, speciation rates were lower in fossorial lineages than in arboreal and terrestrial ones (Table 4), consistent with our other results suggesting lower diversification rates in fossorial lineages (but not supporting higher diversification rates in arboreal lineages). For lizards, the best-fitting model had both speciation and extinction rates equal across microhabitats (in contrast to PGLS results; Table S41). However, the fit was nearly equal to one with different speciation rates in different microhabitats, with the highest rates in arboreal microhabitats, lower rates in terrestrial microhabitats, and the lowest rates in fossorial microhabitats (Table S41), broadly concordant with the PGLS results. The model chosen for snakes was the same as for Squamata: different speciation rates combined with equal rates of extinction and different transition rates between states (Table S42). In snakes, speciation rates were highest in terrestrial lineages and lowest in fossorial lineages, broadly consistent with the PGLS results. The credibility intervals around parameter estimates for squamates are provided in Table S43 and Figs. S9–S11.

Discussion

In this study, we tested the relative importance of microhabitat and climate for explaining patterns of diversification among major clades of squamate reptiles (lizards and snakes). We found that microhabitat usage explained ~37% of the variation in diversification rates, with arboreal microhabitat use having a significant positive effect on diversification, and fossorial and aquatic microhabitats having negative effects. In contrast, the climatic distributions of clades did not generally have a significant impact on

Table 2. Results from multiple regression analysis of the relationships between proportional microhabitat use and climatic distribution (independent variables) and stem-group diversification rates (dependent variable) estimated for 43 lizard families, based on PGLS and ANOVA model selection.

	Parameter	*P*-value	*F*-value	Adjusted r^2	AIC
Model 1					
div ~ ter + fos + arb + aqua + trop		0.0004	5.796	0.363	365.20
	ter	0.1077	2.7177		
	fos	**0.0449**	4.3058		
	arb	**0.0013**	12.1171		
	aqua	**0.0034**	9.8359		
	trop	0.9622	0.0023		
Model 2					
div ~ ter + fos + arb + aqua		**0.0001**	**7.439**	**0.380**	**363.20**
	ter	0.1030	2.7910		
	fos	**0.0422**	4.4219		
	arb	**0.0011**	12.4438		
	aqua	**0.0029**	10.1011		
Model 3					
div ~ fos + arb + aqua		**0.0044**	5.12	0.227	371.79
	fos	**0.0212**	5.7660		
	arb	0.0640	3.6353		
	aqua	**0.0193**	5.9594		
Model 4					
div ~ fos + aqua		**0.0031**	6.696	0.213	371.65
	fos	**0.0222**	5.6632		
	aqua	**0.0082**	7.7281		
Model 5					
div ~ aqua	aqua	**0.0273**	5.239	0.092	376.90

Significant *P*-values (<0.05) and best model chosen by AIC are boldfaced. Div, diversification rate with ε = 0.5; ter, proportion of terrestrial species; fos, proportion of fossorial species; arb, proportion of arboreal species; aqua, proportion of aquatic species; trop, proportion of tropical species.

squamate diversification rates. Patterns of diversification among clades were then strongly related to patterns of species richness. Overall, our results support the hypothesis that microhabitat can be more important than climate in determining large-scale patterns of clade diversification. Despite many previous studies that have tested the effects of climatic distributions on diversification rates (e.g., Pyron 2014; Gómez-Rodríguez et al. 2015; Cooney et al. 2016), ours is among the first to explore the impacts of microhabitat usage (e.g., Wiens 2015a; Moen and Wiens 2017). Our results add to the growing list of studies that show that local-scale ecological factors can strongly influence patterns of clade diversification over deep timescales (hundreds of millions of years; e.g., Wiens 2015a; Wiens et al. 2015; Jezkova and Wiens 2017), perhaps even more so than large-scale ecological factors (such as climate; see also Moen and Wiens 2017). More broadly, this result runs counter to the long-standing idea that local-scale ecological factors are primarily important at shallow evolutionary timescales (e.g., Fig. 1 of Cavender-Bares et al. 2009). Below, we discuss how different microhabitats might influence diversification, the potential importance of climate for diversification, what might explain the remaining variation in squamate diversification rates not explained by microhabitat or climate, and potential sources of error in our study.

HOW DOES MICROHABITAT INFLUENCE DIVERSIFICATION?

Our results show that aquatic microhabitat use can have a strong negative impact on diversification rates. These results are concordant with those of Wiens (2015a) across the major clades of vertebrates, but differ from those of Moen and Wiens (2017) for frogs. Here, most lineages of aquatic squamates are freshwater rather than marine. Across all animals, freshwater richness is similar to marine richness, and both marine and aquatic lineages have lower net diversification rates (Wiens 2015b). Our results show that these large-scale patterns also occur at smaller phylogenetic scales (i.e., among squamate families). However, it is not clear why freshwater lineages have lower net diversification rates. One notable pattern is that freshwater lineages appear to have relatively restricted geographic ranges. For example, most low-diversity aquatic clades are largely restricted

Table 3. Results from multiple regression analysis of the relationships between proportional microhabitat use and climatic distribution (independent variables) and stem-group diversification rates (dependent variable) estimated for 29 snake clades, based on PGLS and ANOVA model selection.

	Parameter	*P*-value	*F*-value	Adjusted r^2	AIC
Model 1					
div ~ ter + fos + arb + aqua + trop		**0.0045**	**4.626**	**0.393**	**286.068**
	ter	**0.0320**	5.2078		
	fos	0.1538	2.1748		
	arb	0.1801	1.9109		
	aqua	**0.0012**	13.5755		
	trop	0.6145	0.2606		
Model 2					
div ~ ter + fos + arb + aqua		**0.0018**	5.899	0.412	297.2004
	ter	**0.0290**	5.3733		
	fos	0.1471	2.2440		
	arb	0.1730	1.9716		
	aqua	**0.0010**	14.0070		
Model 3					
div ~ ter + fos + aqua		0.1874	1.726	0.072	295.2457
	ter	0.0743	3.4676		
	fos	0.2401	1.4481		
	aqua	0.3797	0.7995		
Model 4					
div ~ ter + fos		0.1045	2.466	0.095	293.5021
	ter	0.0728	3.4945		
	fos	0.2379	1.4594		
Model 5					
div ~ ter	ter	0.0747	3.436	0.0800	293.7184

Significant *P*-values (<0.05) and best-fitting model (based on AIC) are boldfaced. Div, diversification rate with $\varepsilon = 0.5$; ter, proportion of terrestrial species; fos, proportion of fossorial species; arb, proportion of arboreal species; aqua, proportion of aquatic species; trop, proportion of tropical species.

to southeast Asia (Acrochordidae, Homalopsidae, Lanthanotidae, Shinisauridae) or tropical Africa (Grayiinae). Moreover, the freshwater lineages Lanthanotidae and Shinisauridae have particularly small geographic ranges (Pough et al. 2016). Small geographic ranges might limit speciation rates and increase extinction (e.g., Rosenzweig 1995). It might also be that particular freshwater habitats are relatively unstable over long geological timescales (e.g., lakes fill, rivers change course). Thus, lower aquatic diversification rates in squamates may be related to the long-term effects of extinction in these habitats, as in marine amniotes (e.g., Miller and Wiens 2017). Although resolving why freshwater environments have lower diversification rates is beyond the scope of this study, the results here are nevertheless promising in showing that this pattern occurs among relatively closely related taxa (clades that diverged tens of millions of years ago, instead of hundreds of millions of years ago, as in Wiens 2015a), which should make this pattern more tractable for more mechanistic studies in the future.

Our results also show that arboreal microhabitat use has a positive impact on diversification rates. Intriguingly, arboreality is the only microhabitat type that significantly impacts diversification rates in frogs, where its influence is also positive (Moen and Wiens 2017). We speculate that two main factors might drive the positive impact of arboreality on diversification. First, arboreal microhabitats can extend upwards for tens of meters (i.e., into forest canopies), allowing the potential for numerous species to co-occur without competitive exclusion. For example, we found relatively fast rates of diversification in dactyloid lizards (*Anolis*) which are known to partition arboreal habitats, with different sets of species specialized for tree trunks, tree crowns, and twigs (at least in the Caribbean; Irschick et al. 1997; Losos et al. 1998). Second, rapid diversification in arboreal lineages may reflect the relative recency of modern forests (given that angiosperms are thought to have begun diversifying ~150 Myr ago; see review in Magallón et al. 2015). Indeed, we are unaware of any predominantly arboreal lineages that are older than >150 Myr old. Interestingly, invasion of arboreal microhabitats by frogs is also relatively recent (Moen and Wiens 2017). In fact, despite their rapid diversification rates, arboreal species make up only ~20% of squamate species richness (based on our estimates). Their higher rates of diversification might reflect rapid radiation in a relatively

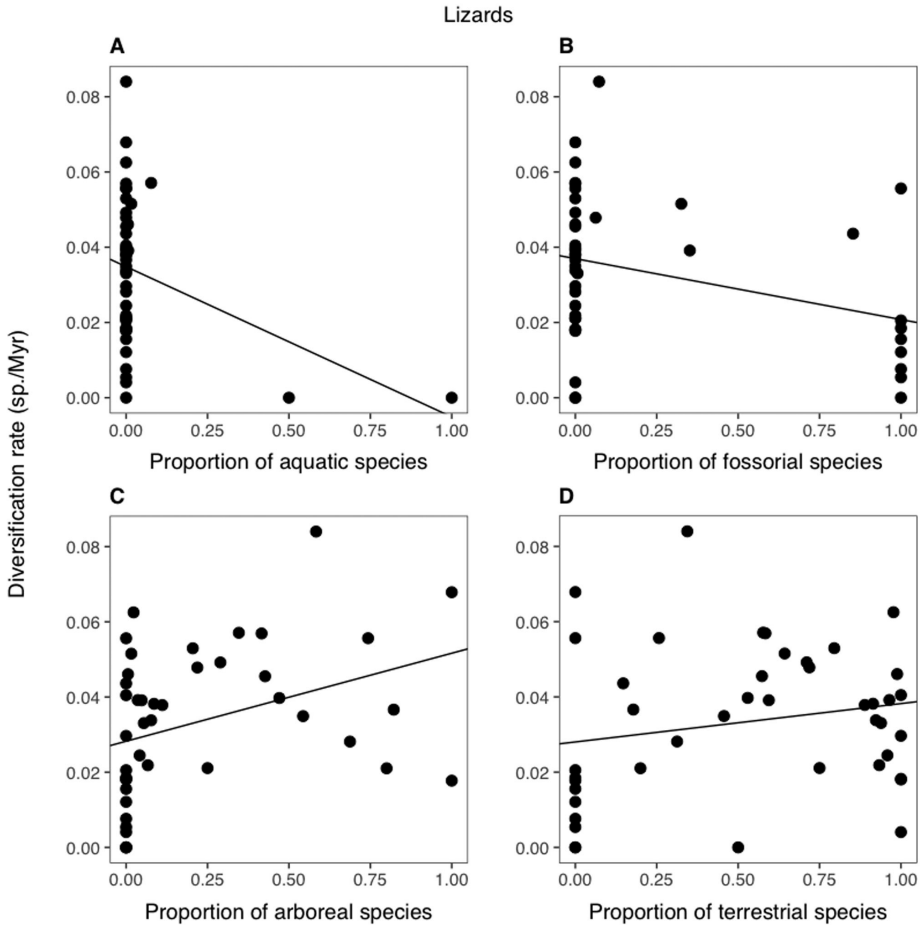

Figure 4. Relationships between diversification rates of 43 lizard families and their proportions of (A) aquatic; (B) fossorial, (C) arboreal, and (D) terrestrial species (PGLS results in Table S16). Diversification rates were estimated assuming an intermediate extinction fraction ($\varepsilon = 0.5$). Similar relationships were found assuming low and high extinction fractions ($\varepsilon = 0.0$ and 0.9; Tables S17 and S18). Lines are from standard linear regression showing relationships between each microhabitat and diversification rates. Alternative results for strict microhabitat proportions are shown in Tables S19–21. Note that we show here the actual proportions (from 0.0 to 1.0) of species using each microhabitat, but we analyzed data using logit-transformed proportions to better reflect statistical assumptions (graphs are similar for raw and logit-transformed data; see Fig. S7).

new environment (i.e., ecological opportunity; see review in Yoder et al. 2010), rather than the ability of arboreal habitats to sustain a larger number of sympatric species. In further support of this idea, we find no clear relationship between arboreality and diversification rates in families with more than ~12.5% arboreal species (Figs. 3 and 4). Of course, these two hypotheses are not mutually exclusive. We also note that families with more species might have more microhabitat types by chance, but this does not explain why arboreality seems to have a positive influence on diversification, whereas other nonterrestrial microhabitats have a negative impact (i.e., aquatic, fossorial).

Our results are also consistent with the idea that fossorial lineages have reduced diversification rates. For example, our PGLS analyses in lizards strongly supported the idea that predominantly fossorial families have reduced diversification rates (e.g., dibamids, rhineurids), with some support also from PGLS analyses in snakes and across squamates, and from MuSSE analyses across squamates and within lizards and snakes. An important question for future research will be to determine how fossorial microhabitats might reduce diversification. One possibility is that fossorial habits might reduce large-scale patterns of dispersal (e.g., as suggested for burrowing lizards by Wiens et al. 2006a). Another possibility is that underground habitats are relatively homogeneous and resource-poor for lizards, and therefore decrease diversification.

CLIMATE, DIVERSIFICATION, AND THE LATITUDINAL DIVERSITY GRADIENT

A particularly surprising result of our study is that occurrence in tropical climates appeared to have little impact on diversification rates in squamates. This is surprising because squamates have higher richness overall in tropical regions

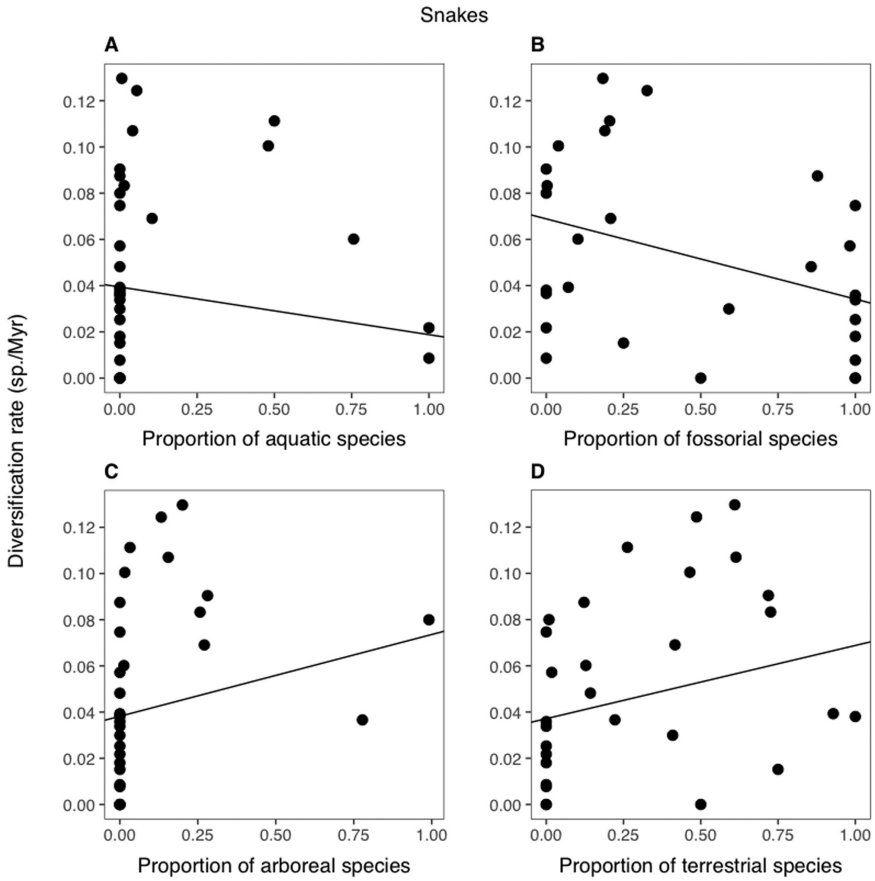

Figure 5. **Relationships between diversification rates of 29 snake clades and their proportions of (A) aquatic, (B) fossorial, (C) arboreal, and (D) terrestrial species (PGLS results in Table S21). Diversification rates were estimated assuming an intermediate extinction fraction ($\varepsilon = 0.5$). Similar relationships were found assuming low and high extinction fractions ($\varepsilon = 0.0$ and 0.9; Tables S25 and S26). Lines are from standard linear regression showing relationships between each microhabitat and diversification rates. Alternative results for strict microhabitat proportions are shown in Tables S27–S29. Note that we show here the actual proportions (from 0.0 to 1.0) of species using each microhabitat, but we analyzed data using logit-transformed proportions to better reflect statistical assumptions (graphs are similar for raw and logit-transformed data; see Fig. S8).**

(Pyron 2014; this study). Various factors might contribute to higher tropical richness that may not be obvious from these analyses. First, higher richness in a given region can be explained by higher diversification rates of lineages in the region, earlier occupation of that region (i.e., allowing more time for richness to build up through speciation, even if diversification rates are similar among regions), or some combination of these factors (reviewed in Wiens 2011). So, time may contribute more significantly to higher tropical richness in squamates than variation in diversification rates. Our results run counter to the widespread idea that higher tropical diversity is explained by factors that increase speciation rates in the tropics (general review in Mittelbach et al. 2007). Second, lower temperate richness might be explained by the absence of clades in temperate regions (due to extinction, failure to colonize, or both), a pattern that may not be obvious from analyses of extant clades alone.

WHAT ABOUT THE REST OF THE VARIATION IN DIVERSIFICATION RATES?

Our analyses show that microhabitat use explains ~37% of the variation in diversification rates among squamate families. This raises the obvious question: what explains the rest? There are several possibilities. In amphibians, changes in climatic distribution among species within clades are more important for explaining patterns of diversification than the climatic distributions themselves (Gómez-Rodríguez et al. 2015; Moen and Wiens 2017; for birds see Cooney et al. 2016). Thus, the clades with the fastest diversification rates are not simply tropical, but instead are clades that seem to have transitioned between (for example) arid and mesic habitats and tropical and temperate regions. Similarly, transitions between microhabitats might help to explain some additional variation in diversification rates among families (but see below). Changes in climatic niches might also be

Table 4. Model fitting and parameter estimates of the three states diversification models in MuSSE for Squamata.

Model													
λ	μ	λT	λF	λA	μT	μF	μA	$q_{T\to F}$	$q_{T\to A}$	$q_{A\to T}$	$q_{A\to F}$	AIC	ΔAIC
F	F	0.0411000	0.0323000	0.0390000	0.0000036	0.0000026	0.0000003	0.0017400	0.0031900	0.0020600	0.0000020	19860.5	4.0
C	F	0.0397000	0.0397000	0.0397000	0.0000062	0.0005500	0.0000147	0.0018500	0.0031800	0.0020400	0.0000002	19867.2	10.8
F	**C**	**0.0411000**	**0.0323000**	**0.0390000**	**0.0000034**	**0.0000034**	**0.0000034**	**0.0017300**	**0.0031800**	**0.0020500**	**0.0000001**	**19856.5**	**0.0**
C	C	0.0394000	0.0394000	0.0394000	0.0000005	0.0000005	0.0000005	0.0017200	0.0031700	0.0020400	0.0000001	19864.4	7.9

Transitions rates from fossorial to terrestrial ($q_{F\to T}$) and arboreal ($q_{F\to A}$) were set as zero. Model specifications are as follows: free to vary (F) and constrained with same rates (C). Microhabitat states are as follows: terrestrial (T), fossorial (F), and arboreal (A) and parameters estimated are: speciation rates (λ), extinction rates (μ), and transitions rates between states (q). Model fit is compared using the AIC and the best AIC model has the lowest AIC, indicated in bold.

related to dispersal and geographic range area of clades. Body size has been shown to have little or no impact on rates of diversification of squamate clades (Feldman et al. 2016), although rates of change in body size should be considered also (e.g., Adams et al. 2009). Rates of change in body shape do not appear to be strongly related to diversification rates across squamate clades (Bergmann and Irschick 2012), which suggests that transition rates in microhabitat might not be important either. However, it might be worth reinvestigating the impacts of body-shape rates with different phylogenetic trees and comparative methods.

POTENTIAL SOURCES OF ERROR

We acknowledge several sources of error that might have influenced our results but should not overturn our major conclusions. First, we note that characterizing microhabitats of species is not always easy, and assessments for some may not be fully accurate (especially for rarer species that have not been well studied ecologically). However, our dataset spans thousands of species, which should reduce the impact of errors on individual species. Furthermore, our results show significant nonrandom associations between microhabitat and diversification. If our microhabitat data were swamped with random errors, then there would be no significant relationships detected with diversification. Indeed, analyses of frogs suggest that even high error rates (20%) in assigning microhabitats should have little impact on microhabitat-diversification relationships (Moen and Wiens 2017). Second, we note that our study is correlative, and it is possible that other variables might have more direct impacts on diversification than microhabitat, but which might be correlated with microhabitat. Although this is a possibility, we do not know of any such variables in squamates, and the general concordance between results after subdividing the data (i.e., lizards and snakes) makes this seem less likely. Errors in estimating diversification rates are also a possibility, but simulations (Kozak and Wiens 2016) show that there can be strong relationships between true and estimated net diversification rates, especially for older clades (e.g., >50 Myr each). Nevertheless, further work on the accuracy of the estimators used here would be useful. Errors in the phylogeny, divergence dates, and species richness of clades are also possible, and might especially impact the estimated diversification rates. Overall, there are many sources of potential error that might influence our results, but these seem most likely to be sources of random error that would weaken observed relationships, rather than create spuriously significant relationships.

On the other hand, future analyses might find a stronger impact of climate on diversification. In our study, we focused on whether species (and clades) were primarily tropical or temperate, treating climatic distribution as a categorical variable (using the same data as a previous study that found a strong impact

of climate on diversification; Pyron 2014). In theory, analyzing climatic data more directly might show a stronger relationship with diversification (e.g., using mean species values for annual mean temperature). It is also possible that considering other aspects of climate (e.g., precipitation) might support a relationship with diversification. For example, many squamate species occur in arid regions. Detailed analyses of the most species-rich group of North and Middle American lizards (Phrynosomatidae), which have higher richness in more arid regions, found no evidence that drier climates promoted diversification (Wiens et al. 2013). On the other hand, an analysis of Australian geckoes found higher diversification rates in arid regions (Brennan and Oliver 2017), but this result may be quite atypical given that Australia is dominated by arid regions (unlike the rest of the world). Nevertheless, this might be a useful topic to explore further in future analyses. Unfortunately, fine-scale climatic data for a representative sampling of thousands of squamate species are not currently available.

CONCLUSIONS

In conclusion, our results show that microhabitat usage can have a significant impact on diversification rates of clades, stronger than that of climate (i.e., tropical vs. temperate distribution). Our results support the idea that aquatic microhabitats reduce diversification rates (as found across major clades of vertebrates), as do fossorial microhabitats, and that arboreal microhabitats increase them. Major challenges for future studies will be to understand the mechanisms behind these patterns (e.g., how do specific microhabitats influence speciation and extinction?), to explain the variation in squamate diversification rates not explained by microhabitat, and to test the generality of these patterns in other groups of organisms.

AUTHOR CONTRIBUTIONS

MBC and JJW designed the study, MBC and DSM performed analyses, MBC and JJW wrote the paper, and all authors contributed to revisions.

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LITERATURE CITED

Adams, D. C., C. M. Berns, K. H. Kozak, and J. J. Wiens. 2009. Are rates of species diversification correlated with rates of morphological evolution? Proc. R. Soc. Lond. B 276:2729–2738.

Bergmann, P. J., and D. J. Irschick. 2012. Vertebral evolution and the diversification of squamate reptiles. Evolution 66:1044–1058.

Brennan, I. G., and P. M. Oliver. 2017. Mass turnover and recovery dynamics of a diverse Australian continental radiation. Evolution 71:1352–1365.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference. A practical information-theoretic approach. Springer, New York.

Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. Ecol. Lett. 12:693–715.

Cooney, C. R., N. Seddon, and J. A. Tobias. 2016. Widespread correlations between climatic niche evolution and species diversification in birds. J. Anim. Ecol. 85:869–878.

Davis, M. P., P. E. Midford, and W. P. Maddison. 2013. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. BMC Evol. Biol. 13:38.

Feldman, A., N. Sabath, R. A. Pyron, I. Mayrose, and S. Meiri. 2016. Body sizes and diversification rates of lizards, snakes, amphisbaenians, and the tuatara. Global Ecol. Biogeogr. 25:187–197.

Figueroa, A., A. D. McKelvy, L. L. Grismer, C. D. Bell, and S. P. Lailvaux. 2016. A species-level phylogeny of extant snakes with description of a new colubrid subfamily and genus. PLoS One 11:e0161070

FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. Am. Nat. 160: 712–726.

Gómez-Rodríguez, C., A. Baselga, and J. J. Wiens. 2015. Is climatic niche width related to diversification rate? Global Ecol. Biogeogr. 24:383–395.

Hillebrand, H. 2004. On the generality of the latitudinal diversity gradient. Am. Nat. 163:192–211.

Irschick, D. J., L. J. Vitt, P. A. Zani, and J. B. Losos. 1997. A comparison of evolutionary radiations in mainland and Caribbean *Anolis* lizards. Ecology 78:2191–2203.

Jansson, R., G. Rodriguez-Castaneda, and L. E. Harding. 2013. What can multiple phylogenies say about the latitudinal diversity gradient? A new look at the tropical conservatism, out of the tropics, and diversification rate hypotheses. Evolution 67:1741–1755.

Jezkova, T., and J. J. Wiens. 2017. What explains patterns of diversification and richness among animal phyla? Am. Nat. 189:201–212.

Kozak, K. H., and J. J. Wiens. 2010. Accelerated rates of climatic-niche evolution underlie rapid species diversification. Ecol. Lett. 13:1378–1389.

———. 2016. Testing the relationships between diversification rates, species richness, and trait evolution. Syst. Biol. 65:975–988.

Kuhn, M. 2008. Building predictive models in R using the caret package. J. Stat. Softw. 28:1–26.

Losos J. B., T. R. Jackman, A. Larson, K. de Queiroz, and L. Rodriguez-Schettino. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279:2115–2118.

Maddison, W. P., and R. G. FitzJohn. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. Syst. Biol. 64:127–136.

Magallón, S., and M. J. Sanderson. 2001. Absolute diversification rates in angiosperm clades. Evolution 55:1762–1780.

Magallón, S., S. Gómez-Acevedo, L. Sánchez-Reyes, and T. Hernández-Hernández. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. New Phytol. 207:437–453.

Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. Am. Nat. 149:646–667.

Miller, E. C., and J. J. Wiens. 2017. Extinction and time help drive the marine-terrestrial biodiversity gradient: is the ocean a deathtrap? Ecol. Lett. 20:911–921.

Mittelbach, G. G., D. Schemske, H. V. Cornell, A. P. Allen, J. M. Brown, M. Bush, S. Harrison, A. Hurlbert, N. Knowlton, H. A. Lessios, et al. 2007.

Evolution and the latitudinal diversity gradient: speciation, extinction, and biogeography. Ecol. Lett. 10:315–331.

Moen, D. S., and J. J. Wiens. 2017. Microhabitat and climatic niche change explain patterns of diversification among frog families. Am. Nat. 190:29–44.

Moore, B. R., S. Höhna, M. R. May, B. Rannala, and J. P. Huelsenbeck. 2016. Critically evaluating the theory and performance of Bayesian analysis of macroevolutionary mixtures. Proc. Natl. Acad. Sci. U. S. A. 113:9569–9574.

Morlon, H., T. L. Parsons, and J. B. Plotkin. 2011. Reconciling molecular phylogenies with the fossil record. Proc. Natl. Acad. Sci. U. S. A. 108:16327–16332.

Morlon, H., E. Lewitus, F. L. Condamine, M. Manceau, J. Clavel, and J. Drury. 2016. RPANDA: an R package for macroevolutionary analyses on phylogenetic trees. Methods Ecol. Evol. 7:589–597.

Orme, D. 2013. The caper package: comparative analysis of phylogenetics and evolution in R. R package version 0.5.2.

Pagel, M. 1997. Inferring evolutionary processes from phylogenies. Zool. Scripta 26:331–348.

———. 1999. Inferring the historical patterns of biological evolution. Nature 401:877–884.

Pough, F. H., R. M. Andrews, M. L. Crump, A. H. Savitzky, K. D. Wells, and M. C. Brandley. 2016. Herpetology. 4th ed. Sinauer, Sunderland, MA.

Pyron, R. A. 2014. Temperate extinction in squamate reptiles and the roots of latitudinal diversity gradients. Global Ecol. Biogeogr. 23:1126–1134.

Pyron, R. A., and J. J. Wiens. 2013. Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. Proc. R. Soc. Lond. B 280:20131622.

Pyron, R. A., F. T. Burbrink, and J. J. Wiens. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evol. Biol. 13:93.

Rabosky, D. L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS One 9:e89543.

Rabosky, D. L., and D. C. Adams. 2012. Rates of morphological evolution are correlated with species richness in salamanders. Evolution 66:1807–1818.

Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. Syst. Biol. 64:340–355.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:222–225.

Ricklefs, R. E. 2007. Estimating diversification rates from phylogenetic information. Trends Ecol. Evol. 22:601–610.

Rolland, J., F. L. Condamine, F. Jiguet, and H. Morlon. 2014. Faster speciation and reduced extinction in the tropics contribute to the mammalian latitudinal diversity gradient. PLoS Biol. 12:e1001775.

Rosenzweig, M. L. 1995. Species diversity in space and time. Cambridge Univ. Press, Cambridge, UK.

Scholl, J. P., and J. J. Wiens. 2016. Diversification rates and species richness across the Tree of Life. Proc. R. Soc. Lond. B 283:20161335.

Shmueli, G. 2010. To explain or to predict? Stat. Sci. 25:289–310.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd ed. W. H. Freeman, New York.

Soria-Carrasco, V., and J. Castresana. 2012. Diversification rates and the latitudinal gradient of diversity in mammals. Proc. R. Soc. Lond. B 279:4148–4155.

Uetz, P., and J. Hošek, eds. 2015. The Reptile Database. Available at http://www.reptile-database.org. Accessed February, 18, 2015.

Warton, D. I., and F. K. Hui. 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3–10.

Weber, M. G., and A. Agrawal. 2014. Defense mutualisms enhance plant diversification. Proc. Natl. Acad. Sci. U. S. A. 111:16442–16447.

Wiens, J. J. 2011. The causes of species richness patterns across space, time, and clades and the role of "ecological limits". Q. Rev. Biol. 86:75–96.

———. 2015a. Explaining large-scale patterns of vertebrate diversity. Biol. Lett. 11:20150506.

———. 2015b. Faster diversification on land than sea helps explain global biodiversity patterns among habitats and animal phyla. Ecol. Lett. 18:1234–1241.

———. 2017. What explains patterns of biodiversity across the Tree of Life? BioEssays 39:1600128.

Wiens, J. J., M. C. Brandley, and T. W. Reeder. 2006a. Why does a trait evolve multiple times within a clade? Repeated evolution of snake-like body form in squamate reptiles. Evolution 60:123–141.

Wiens, J. J., C. H. Graham, D. S. Moen, S. A. Smith, and T. W. Reeder. 2006b. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. Am. Nat. 168:579–596.

Wiens, J J., J. Sukumaran, R. A. Pyron, and R. M. Brown. 2009. Evolutionary and biogeographic origins of high tropical diversity in Old World frogs (Ranidae). Evolution 63:1217–1231.

Wiens, J. J., K. H. Kozak, and N. Silva. 2013. Diversity and niche evolution along aridity gradients in North American lizards (Phrynosomatidae). Evolution 67:1715–1728.

Wiens, J. J., R. T. Lapoint, and N. K. Whiteman. 2015. Herbivory increases diversification across insect clades. Nat. Commun. 6:8370.

Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson, et al. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evol. Biol. 23:1581–1596.

Zheng, Y., and J. J. Wiens. 2016. Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4,162 species. Mol. Phylogenet. Evol. 94:537–547.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

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