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### **ORIGINAL ARTICLE**

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# Testing the role of climate in speciation: New methods and applications to squamate reptiles (lizards and snakes)

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### Abstract

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Climate may play important roles in speciation, such as causing the range fragmentation that underlies allopatric speciation (through niche conservatism) or driving divergence of parapatric populations along climatic gradients (through niche divergence). Here, we developed new methods to test the frequency of climate niche conservatism and divergence in speciation, and applied it to species pairs of squamate reptiles (lizards and snakes). We used a large-scale phylogeny to identify 242 sister species pairs for analysis. From these, we selected all terrestrial allopatric pairs with sufficient occurrence records (n = 49 pairs) and inferred whether each originated via climatic niche conservatism or climatic niche divergence. Among the 242 pairs, allopatric pairs were most common (41.3%), rather than parapatric (19.4%), partially sympatric (17.7%), or fully sympatric species pairs (21.5%). Among the 49 selected allopatric pairs, most appeared to have originated via climatic niche divergence (61-76%, depending on the details of the methods). Surprisingly, we found greater climatic niche divergence between allopatric sister species than between parapatric pairs, even after correcting for geographic distance. We also found that niche divergence did not increase with time, further implicating niche divergence in driving lineage splitting. Overall, our results suggest that climatic niche divergence may often play an important role in allopatric speciation, and the methodology developed here can be used to address the generality of these findings in other organisms.

#### KEYWORDS

climatic niche, lizards, niche conservatism, niche divergence, snakes, speciation

### 1 | INTRODUCTION

Speciation is a fundamental topic in evolutionary biology (Futuyma, 2013), and species' climatic niches may be crucial for this process (e.g., Hua & Wiens, 2013; Moritz, Patton, Schneider, & Smith, 2000). The realized climatic niche is the set of large-scale temperature and precipitation conditions where a species occurs (e.g., Soberón, 2007). The climatic niche (along with other factors) may help determine where species are distributed geographically (Soberón, 2007), which may have important consequences for speciation. The climatic niche can promote speciation via two main mechanisms, which are potentially related to different geographic modes (Hua & Wiens, 2013;

Moritz et al., 2000). The first mechanism, climatic niche divergence, is the most intuitive. Populations of a species may occur under divergent climatic conditions (e.g., different elevations), and if conditions are mutually unsuitable for adjacent populations, this could reduce gene flow between these populations and thereby facilitate reproductive isolation and speciation. This mechanism can be considered a subset of the general process of ecological speciation (e.g., Nosil, 2012; Schluter, 2001, 2009), in which adaptation to divergent ecological conditions reduces gene flow and drives speciation. The most obvious geographic mode of speciation through climatic niche divergence involves populations distributed parapatrically along a climatic gradient.

The second mechanism, speciation via climatic niche conservatism (Hua & Wiens, 2013: Wiens, 2004a), is considerably less obvious. Under this scenario, different populations of a species become separated by a zone of unsuitable climatic conditions, either through dispersal across this zone or vicariance (i.e., populations that formerly occurred within the zone of now unsuitable conditions go locally extinct). For example, a pair of montane, mesic-adapted species may be separated by warmer, drier lowland conditions. This pattern presumably arises because their ancestral species was widespread in the lowlands during periods of cooler, wetter climate but could not adapt quickly enough to lowland climatic conditions when climate warmed (e.g., Hua & Wiens, 2013; Wiens, 2004a). In general, niche conservatism (climatic or not) may be the primary driver of allopatry and thus the initial stage of allopatric speciation (with subsequent evolution of intrinsic reproductive isolation, depending on one's species concept; Wiens, 2004b). Intrinsic isolation between populations in similar habitats could evolve through both genetic drift and selection (e.g., via Dobzhansky-Muller incompatibilities or mutation-order speciation; Coyne & Orr, 2004; Schluter, 2009). The process of populations becoming allopatric is important because allopatry may be the most common geographic mode of speciation (e.g., Barraclough & Vogler, 2000; Coyne & Orr, 2004). Niche conservatism should be an essential component of allopatry and therefore allopatric speciation, based on first principles (i.e., with unlimited niche evolution, species would rarely be allopatric; Wiens, 2004a). However, allopatry need not be caused by unsuitable climatic conditions. Instead, allopatric populations may be separated by unsuitable ecological conditions unrelated to climate (e.g., island populations separated by marine habitat). Consideration of niche conservatism suggests that speciation is not necessarily synonymous with evolutionary divergence and reinforces the importance of geographic modes (i.e., allopatric, parapatric, sympatric) for understanding speciation. Importantly, to our knowledge, niche conservatism is only a potential mechanism of allopatric speciation and not other geographic modes (Wiens, 2004b).

The roles of climatic niche divergence and conservatism in speciation have been explored in numerous studies, but many studies had important limitations. First, many studies simply compared climatic niches of sister species (e.g., niche overlap; Warren, Glor, & Turelli, 2008), without testing if climatically unsuitable habitat separates the geographic ranges of allopatric sister species. In these cases, species ranges may be separated by nonclimatic factors (e.g., barriers of unsuitable microhabitat, such as rivers or marine habitat), making their climatic niche similarly irrelevant to their allopatry and lineage splitting. These studies could also underestimate the importance of niche conservatism, if species diverged climatically over time after their original splitting through niche conservatism. Other studies have compared niches of sister species with respect to the niches of the background area, that is, a larger area surrounding the current species ranges (e.g., Loera, Sosa, & Ickert-Bond, 2012; McCormack, Zellmer, & Knowles, 2010). However, this approach could also falsely infer that climatic niche conservatism caused allopatric speciation when it did not. The climatic niches of the two species may be more - MOLECULAR ECOLOGY -WII

similar to each other than to climatic conditions from random locations from the background area, but their climatic niches might not differ significantly from the area of allopatric habitat that separates them (e.g., if they are separated by nonclimatic factors). Conversely, species ranges might be more similar to the background area than to the intervening habitat between them, which could cause niche conservatism to be rejected when it actually occurred. The approach of Glor and Warren (2011) is intended to identify climatic barriers, but requires that at least one species be present in the habitat between the ranges of the compared species, making it "not applicable" for testing if allopatric species are separated by climatically unsuitable habitat (p. 678). We argue that species niches must be compared with respect to each other and to the area of intervening habitat that is potentially causing their allopatric distribution (e.g., Hua & Wiens, 2010; Kozak & Wiens, 2006). Furthermore, we argue that sister species are the species pairs that should be compared to address speciation (contra Glor & Warren, 2011). Some studies have also compared climatic niche models of putative species for species delimitation (e.g., Bond & Stockman, 2008), but without an explicit methodology for testing speciation mechanisms.

Herein, we build on the methodological frameworks of Kozak and Wiens (2006) and Hua and Wiens (2010) by comparing climatic niches of allopatric species pairs with the climate of the intervening area that separates them. However, we advance their methodology in several ways. First, we use the n-dimensional hypervolume (Blonder, Lamanna, Violle, & Enguist, 2014) to estimate climatic niches. This method can quantify niches in high dimensions, which may be preferable to low-dimensional approaches (e.g., Broennimann et al., 2012), especially when the niche is described by many variables (Blonder et al., 2014). Further, hypervolume overlaps can estimate the proportion of the entire climatic niche shared between species. In comparison, Kozak and Wiens (2006) and Hua and Wiens (2010) used mean distances between niches of sister species (and the intervening area), an approach that cannot assess whether niches of two species intersect (a potential problem when testing between niche conservatism and divergence, see Section 4). As in these previous studies, we also use Maxent niche models (Phillips & Dudik, 2008) to assess the climatic suitability of the area separating the ranges of allopatric species pairs (and assess whether each species' range is suitable for its sister species). We also developed a framework to assign a single speciation mechanism (niche conservatism vs. divergence) to each species pair for both methods, as opposed to estimating a mechanism for each species separately (as in Hua & Wiens, 2010). Finally, unlike most previous studies, we test whether climatic niches of allopatric species may have diverged over time after an initial origin through niche conservatism by testing for a correlation between ages of species pairs and their level of niche divergence. Overall, we develop a methodology to test the roles of climatic niche conservatism and divergence that can be applied to most organisms.

Herein, we use this new methodology to conduct the first largescale analysis of climate and speciation in squamate reptiles (lizards and snakes). We also estimate the frequency of different geographic modes of speciation in this group, based on hundreds of sister WII FY-MOLECULAR ECOLOGY

species pairs. Several previous studies have addressed climaterelated speciation mechanisms in squamates. However, these studies all suffered from the issues described above. Furthermore, they were restricted to groups of closely related species (e.g., Ahmadzadeh et al., 2013; Gonçalves et al., 2012; Knouft, Losos, Glor, & Kolbe, 2006), rather than analysing patterns across squamates. Some studies found evidence for climatic niche conservatism (e.g., Gonçalves et al., 2012), whereas others found niche divergence (e.g., Ahmadzadeh et al., 2013), and others found both modes within the same group (Knouft et al., 2006; Pyron & Burbrink, 2009; Rato et al., 2015; Raxworthy et al., 2008). Thus, both mechanisms appear to drive allopatric speciation in squamates. The open question is as follows: How frequent is each mode?

In this study, we test the relative frequency of these two climatic modes across a large number of allopatric sister species pairs, using our new approach. We first identify the most common geographic modes among 242 pairs of squamate sister species, the largest analysis of geographic modes in squamates. We then focused on all allopatric species pairs separated by land and with adequate occurrence records (n = 49 pairs) to test whether speciation is more frequently caused by climatic niche conservatism or divergence. If allopatric speciation is driven by climatic niche conservatism, the climatic niches of sister species will be significantly more similar to each other than to the intervening habitat. Further, climatic niche similarity between sister species should be negatively correlated with their time since splitting (i.e., very similar immediately after splitting but potentially diverging over time, like any other trait). Alternatively, if allopatric speciation is mostly driven by climatic niche divergence, climatic niches of sister species will be more similar to the intervening habitat than to each other, and niches should already be divergent at the time of their initial separation (and not necessarily diverging over time). Furthermore, niche divergence of allopatric species pairs should be similar to that of parapatric species pairs (which are often thought to arise through ecological divergence). Our results suggest that allopatry is the most common geographic mode in squamates and that climatic niche divergence is the most common climatic mechanism in allopatric pairs. To our knowledge, our study represents the largest analysis of the relative frequencies of niche conservatism and divergence in allopatric speciation for any group of organisms.

#### 2 | MATERIALS AND METHODS

#### 2.1 | Selecting species pairs

We started with a time-calibrated phylogeny of 4,162 extant squamate species (Zheng & Wiens, 2016), containing 1,342 potential sister species pairs. Given ~10,000 described squamate species (Uetz & Hošek, 2016), some sister species in the tree would not be sister species if all species were included. To increase the likelihood that selected pairs actually represent sister species, pairs were only included if they: (i) diverged <20 Ma (including 1,094 pairs), and (ii) belonged to a genus in which  $\geq$ 75% of currently recognized species were included in the tree (Uetz & Hošek, 2016; last queried December 2016). This second threshold excluded all but 351 pairs.

We acknowledge that without complete taxon sampling (or even with it) it is not guaranteed that all sampled species pairs are actually sister species. However, we show that our conclusions would be the same if we excluded all species pairs from incompletely sampled genera.

### 2.2 | Obtaining locality data

We queried the VertNet online database (http://www.vertnet.org/in dex.html) to obtain a list of catalogued museum specimens and their georeferenced localities for the selected species. For each species, we examined all known variations in their taxon names to maximize localities found. Species pairs for which one or both species lacked georeferenced localities on VertNet were excluded. We initially used only georeferenced localities from VertNet. However, for allopatric pairs for which only one or two unique georeferenced localities were available, we georeferenced any additional records lacking coordinates but having detailed locality data in the VertNet database. We included these species if we could obtain at least three unique georeferenced localities. Species with few localities usually had small geographic ranges, so greatly increasing their number of localities would be difficult (and should have little impact on their estimated climatic niche). Furthermore, excluding species with small ranges could strongly bias the estimated frequencies of speciation modes. A total of 242 species pairs passed all criteria, with a mean of 115 localities/species (median = 18). Localities are given in Supporting Information Data S1.

#### 2.3 Developing range maps for sister species

We next estimated range maps for each species to classify their geographic modes. We plotted all occurrence records for each species on a map using the R package *raster* (Hijmans & van Etten, 2012; R Development Core Team, 2015) and compared them to a distribution map for that species. Distribution maps were obtained from The Australian Reptile Online Database (AROD.com.au) for Australian species and from The Reptile Database (Uetz & Hošek, 2016) for all others. For species lacking detailed distribution maps in these databases (e.g., outside Australia, Europe and North America), we searched for range maps in the primary literature (using Google Scholar). Specimen records outside the known range were excluded. These erroneous records seemed to represent either: (i) human introductions, (ii) misidentified specimens or (iii) incorrect coordinates. We then created a minimum convex polygon (MCP) around the final set of localities for each species (Supporting Information Appendix S1).

# 2.4 | Determining geographic overlap of sister species

We used the range maps to classify the level of geographic overlap of each sister species pair (i.e., allopatric, parapatric, partially sympatric or sympatric). Allopatric pairs had no overlap in their MCPs, and all localities were at least 20 km from those of their sister species. Thus, there was an uninhabited area between the ranges of sister species. Parapatric pairs had no overlap in their MCPs but at least one locality of one species was <20 km from the nearest locality of its sister species. We recognize that 20 km is arbitrary, and a pair separated by a smaller distance could be considered allopatric. We used this distance as a buffer, in case the sampled localities did not reach the most extreme limits of the actual species distributions. Sympatric pairs were those in which the MCP of one species was entirely nested within that of its sister species. Finally, partially sympatric pairs were those in which their MCPs partially overlapped. All geographic modes inferred from the MCPs were confirmed with species' range maps (from the sources listed above) to ensure that few localities for some species did not lead us to infer an erroneous geographic mode.

We used these patterns of geographic overlap of species to make tentative inferences about geographic modes of speciation. Thus, the simplest explanation for currently allopatric species pairs is that they originated through allopatric speciation. However, we recognize that geographic ranges can change considerably after speciation (e.g., Barraclough & Vogler, 2000). Therefore, we conducted a regression analysis to test whether geographic overlap increased with time since the species split (i.e., crown-group age). We did this for sympatric and partially sympatric species pairs and then for all species pairs (with range overlap for allopatric and parapatric pairs set to 0). If currently nonallopatric pairs originated via allopatric speciation, range overlaps should generally increase with time (e.g., Barraclough & Vogler, 2000). Range overlap was calculated as the area (km<sup>2</sup>) over which species MCPs intersect. Divergence times were obtained from the tree of Zheng and Wiens (2016).

# 2.5 | Testing the role of climate in allopatric species pairs

For each allopatric species pair, we tested whether speciation occurred via climatic niche conservatism or divergence. As noted above, allopatric speciation is presumably the only geographic mode related to climatic niche conservatism (i.e., conservatism causing allopatry due to a barrier of climatically unsuitable habitat). Under this model, the geographic range of each species should be more climatically suitable for its sister species than the area separating their geographic ranges. If not, then factors other than unsuitable climatic conditions should explain the absence of both species in the intervening area. We excluded species separated by marine habitat, as they are almost certainly isolated by nonclimatic factors.

For each allopatric species pair, we first defined the intervening area. We generated a MCP around the combined occurrence records of both species from which we subtracted the MCP of each species. The remaining polygon represented the area between the species ranges, constrained by the minimum and maximum coordinate values of each species. This polygon was further buffered inwards (i.e., its extent was reduced) to avoid selecting random intervening points (see below) that were too close to known localities. The intervening area was buffered by 0.1 degrees (~11 km at equator) when the minimal

distance between the two species was >100 km, and by 0.01 degrees (~1.1 km at equator) when the distance was <100 km. These values were chosen to compensate for possible sampling bias (i.e., if known localities were not at the edge of the actual species range) while still leaving the intervening area large enough for drawing random points.

For all localities, we extracted values from 19 bioclimatic variables from the widely used WorldClim dataset (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) using *raster*. This dataset is based on average values from weather stations from 1950 to 2000, spatially interpolated to span areas lacking weather stations. We used the finest spatial resolution available (30 s, ~1 km<sup>2</sup>).

For each species pair, we first estimated the climatic niche of each species using the n-dimensional hypervolume (Blonder et al., 2014). The hypervolume method creates an n-dimensional space reflecting the species' niche. We obtained climatic data from the intervening area using a number of random localities equal to the number of summed localities for both species. We then extracted climatic data from these localities as described above. The number of climate variables was reduced using principal components analysis (PCA) based on a correlation matrix in R (the correlation matrix was used rather than the covariance matrix as temperature and precipitation variables are on different scales). We used PCA rather than raw variables given potential correlations among bioclimatic variables. Following Blonder et al. (2014), the number of principal components (PCs) initially retained equalled the number of localities (for the sister species with fewer localities) divided by ten. From this initial set of PCs, we then retained only PCs with eigenvalues >1 (Kaiser, 1960). For example, if species A had 200 records and species B had 30, we would retain three PCs, assuming both had eigenvalues >1. We calculated the hypervolume for each species' niche and the niche of the intervening area, and calculated hypervolume intersections (i.e., climatic niche overlap) between the two species and between each species and the intervening area. The hypervolume was calculated using the R package hypervolume (Blonder et al., 2014) with the following recommended settings: bandwidth estimated from the data, quantile of 0.1, and 1,000 replicates. The entire process was replicated 100 times, with different random localities from the intervening area selected in each replicate. For each species, we inferred the climatic speciation mechanism (i.e., conservatism or divergence) by comparing climatic niche intersection between the sister species' niches and the niche of the intervening area. We defined  $H_A$  as the hypervolume of species A,  $I_{A,B}$ is the hypervolume intersection between species A and species B, and  $I_{AC}$  is the hypervolume intersection between species A and the intervening area. Given these definitions, climatic niche conservatism was inferred for species A when

$$I_{A,B}/H_A > I_{A,C}/H_A$$

and climatic niche divergence was inferred when

#### $I_{A,B}/H_A < I_{A,C}/H_A$

In other words, conservatism was inferred for species A when the relative hypervolume intersection between A and B (i.e., intersection of A and B divided by hypervolume of A) was larger than WILEY-MOLECULAR ECOLOGY

the relative intersection between A and the intervening area. Likewise, divergence was inferred for species A when the relative hypervolume intersection between A and B was smaller than the relative intersection between A and the intervening area. The support for a given speciation mechanism in a given species (i.e., climatic niche conservatism vs. climatic niche divergence) was considered significant if the same mechanism was inferred in 95 of 100 replicates.

The overall niche-related mechanism for each species pair was inferred as follows. Note that our goal here was to infer the relative frequency of these mechanisms, and so we considered the relative support for each mechanism rather than simply whether there was strong support for one mechanism in both species in a pair. When the same mechanism was significantly supported for both species (or if the same mechanism was significant for one species and nonsignificant for the other), we inferred the significant mechanism as the mechanism for that pair. When the same mechanism was supported in the majority of replicates but was still nonsignificant for both species, we inferred that mechanism. When one mechanism was significant for one species and the alternative mechanism was nonsignificant for the other, we considered the significant mechanism the most likely explanation. When each species supported a different mechanism (significant or nonsignificant), we considered the results in conflict and did not assign a mechanism to that pair. We also tallied results that were only significant for both species in a pair. However, there can only be one mechanism for each pair. Therefore, we generally prefer the less conservative approach even if the support for the same mechanism was not significant in both species.

For our second approach, we used Maxent with the R package dismo (Hijmans, Phillips, Leathwick, & Jane, 2015). We used logistic probabilities from Maxent niche models to compare the niches of sister taxa and the climate of the intervening area. We selected 500 random localities from the intervening area, extracted bioclimatic variables from localities and intervening points and reduced the number of variables with PCA. We kept all PCs with eigenvalues >1 (Kaiser, 1960). Maxent was run with default parameters (all features included, beta multiplier = 1). For each species, logistic probability values were extracted from all its localities, all randomly selected intervening points, and all localities of its sister species. We calculated the mean, standard deviation, 90th percentile and 10th percentile of probabilities for the localities from the species of interest, its sister species, and intervening area. The entire process was replicated 10 times with a different set of 500 random points from the intervening area selected in each replicate.

From these analyses, we first determined whether the suitable climatic niche of one species also occurred within the distributional area of its sister species and within the intervening area between them. The climatic niche of species A was considered suitable for its sister species B (or the intervening area) if the 10th percentile of the logistic probabilities extracted from localities for species A was smaller than the 90th percentile of logistic probabilities extracted from localities for species B (or the random points of the intervening area). In other words, the niche of species A was considered suitable

for species B when at least 10% of localities of species B had higher logistic probability values than the 10% of localities of species A with the lowest logistic probability values. Otherwise, the niche was considered unsuitable. Use of the 10th percentile is arbitrary. However, standard deviations of logistic probabilities were generally low (Supporting Information Appendix S6), yielding very similar values using the 5th and 15th percentiles. The final suitability or unsuitability between the two species and the intervening habitat was assigned based on the majority of the 10 replicates.

Niche conservatism was strongly supported when each species' geographic range was climatically suitable for its sister species, but the intervening habitat was unsuitable for both. Niche divergence was strongly supported when each species' range was climatically unsuitable for its sister species, but the intervening habitat was suitable for one or both. However, many pairs showed different combinations of suitability and unsuitability (Table 1). Therefore, we also compared the mean logistic probability values predicted from a model for species A for the intervening area and the range of species B (and vice versa). For example, if the model for species A predicted the intervening area and the range of B to be unsuitable, but the mean logistic probabilities for species B were higher than the mean logistic probabilities for the intervening area, we tentatively inferred niche conservatism. This allowed for the possibility that the species niches were originally suitable for each other, but later diverged and became unsuitable. This possibility was further tested by examining niche overlap over time (see below). A key to determining the mechanism given all possible combinations of suitability and unsuitability is listed in Table 1. The mechanism was considered significant, if it was inferred in at least nine of the 10 replicates. For both assessments (hypervolume and Maxent), we conducted a regression analysis between niche divergence and the number of localities to ensure that our results were not affected by different numbers of localities for different species pairs.

# 2.6 | Relationship between niche similarity and time in allopatric species pairs

Climatic niches of sister species may diverge over time, especially if they originated by niche conservatism. Thus, simply finding niche divergence between allopatric pairs may not fully rule out niche conservatism. Therefore, we tested whether the climatic niche similarity between sister species is correlated with the time since they split (i.e., divergence time). If allopatric species arose predominantly via niche conservatism (niches that were initially very similar), we expect a negative correlation between niche similarity and time across species pairs. Divergence times were obtained from the tree of Zheng and Wiens (2016). We assessed niche similarity from niche overlap values calculated from the n-dimensional hypervolumes as

$$(I_{A,B}/H_A + I_{A,B}/H_B)/2$$

where  $H_A$  is the hypervolume of species A,  $H_B$  is the hypervolume of species B and  $I_{A,B}$  is the intersection between species A and species B.

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**TABLE 1** A key to distinguishing niche conservatism and niche divergence in allopatric species pairs using Maxent niche modelling. First, for species A, we determined whether suitable climatic conditions were present within the geographic range of its sister species B (column 1) and in the intervening area between the ranges of the two species (column 2). Next, for species B, we determined whether suitable climatic conditions were present within the geographic range of species A (column 3) and in the intervening area (column 4). Suitability was inferred from the logistic probabilities of a Maxent model (see Section 2.5 for details). The last column explains the conditions under which conservatism or divergence was inferred for a given species pair, based on comparing the mean logistic probabilities between sister species and the intervening area. For example, in the first row, if the geographic ranges of both species were unsuitable for the other and the intervening area was unsuitable for both species, conservatism was supported if (a) for the niche model built for species A, the mean logistic probability of the intervening area was lower than the mean logistic probability of the range of species B, the mean logistic probability of the range of species B, the mean logistic probability of the range of species B, the mean logistic probability of the range of species B, the mean logistic probability of the range of species A (i.e., SpB-Intv < SpB-SpA). Some outcomes gave conflicting results ("Conflict"), and in these cases, a mechanism was not inferred for that species pair

Species A–Species B	Species A–Intervening area	Species B–Species A	Species B–Intervening area	Climatic speciation mechanism
Unsuitable	Unsuitable	Unsuitable	Unsuitable	$\label{eq:conservatism} Conservatism if SpA-Intv < SpA-SpB and SpB-Intv < SpB-SpA; Divergence if SpA-Intv > SpA-SpB and SpB-Intv > SpB-SpA$
Suitable	Suitable	Suitable	Suitable	$\label{eq:conservatism} Conservatism \mbox{ if SpA-Intv} < \mbox{SpA-SpB} \mbox{ and SpB-Intv} < \mbox{SpB-SpA}; \mbox{ Divergence} \mbox{ if SpA-Intv} > \mbox{SpA-SpB} \mbox{ and SpB-Intv} > \mbox{SpB-SpA} $
Unsuitable	Suitable	Unsuitable	Suitable	Divergence
Unsuitable	Suitable	Suitable	Suitable	Divergence if SpB-Intv > SpB-SpA
Unsuitable	Suitable	Unsuitable	Unsuitable	Divergence if SpB-Intv > SpB-SpA
Suitable	Unsuitable	Suitable	Unsuitable	Conservatism
Suitable	Unsuitable	Suitable	Suitable	Conservatism if SpB-Intv < SpB-SpA
Suitable	Unsuitable	Unsuitable	Unsuitable	Conservatism if SpB-Intv < SpB-SpA
Unsuitable	Suitable	Suitable	Unsuitable	Conflict
Suitable	Unsuitable	Unsuitable	Suitable	Conflict
Suitable	Suitable	Unsuitable	Unsuitable	Conservatism if SpA-Intv < SpA-SpB and SpB-Intv < SpB-SpA; Divergence if SpA-Intv > SpA-SpB and SpB-Intv > SpB-SpA

We also used Maxent models to calculate mean niche similarity of species pairs:

$$(P_{AB}/P_{AA} + P_{BA}/P_{BB})/2$$

where  $P_{AB}$  is the mean logistic probability among localities for species B extracted from the Maxent model built from localities for species A,  $P_{AA}$  is that among species A localities from the model for species A,  $P_{BA}$  is that for species A localities from the model for species B, and  $P_{BB}$  is that among species B localities from the model for species B. We then performed Spearman's rank correlation between these two measures of niche similarity and time. Normality was rejected based on Shapiro–Wilk tests and Q–Q plots.

# 2.7 | Comparison of niche similarity in allopatric and parapatric species pairs

If allopatric species pairs generally originate via climatic niche conservatism, then we expect their niche similarity should exceed that of parapatric species pairs, given that niche divergence is expected to drive parapatric speciation (e.g., Coyne & Orr, 2004; Moritz et al., 2000). Conversely, if both allopatric and parapatric species generally originated via climatic niche divergence, then their mean niche similarity should be similar. We calculated the two measures of niche similarity described above for all parapatric species pairs with at least three localities (n = 44). We then conducted a Wilcoxon rank-sum test to compare mean niche similarity between allopatric (49) and parapatric (44) species pairs. However, we might expect allopatric species pairs to have lower similarity than parapatric pairs, simply because their geographic ranges are further apart. We therefore performed a second test that corrected for geographic distance. Specifically, we divided estimates of niche divergence (1-overlap for hypervolume, and 1-suitability for Maxent) by the In-transformed distance (in km) between the range centroids. R scripts for all analyses are available as Supporting Information Data S2 (hypervolume analyses) and Supporting Information Data S3 (Maxent analyses).

#### 2.8 | Major assumptions

We acknowledge that our analyses depend on many assumptions (e.g., Merow, Smith, & Silander, 2013; Phillips & Dudik, 2008). First, we assume that the included localities adequately reflect the geographic range and climatic niche of each species. Second, we assume that species' present-day distributions and climatic niches are relevant to the ranges and climatic conditions when speciation occurred. Nevertheless, we do conduct analyses that address how ranges and niches changed over time. We did not conduct analyses using paleoclimatic data because such datasets are generally only available for younger timescales (e.g., Last Glacial Maximum) that would be irrelevant for the species analysed here (which split >1 million years ago). Third, we assume that the climatic variables analysed are actually WII FY-MOLECULAR ECOLOGY

relevant to determining the geographic distributions of these species. However, these analyses should also reveal if climate was irrelevant in determining their distributions (e.g., if niche models predict both the sister species' ranges and intervening area as climatically suitable). Finally, our overall goal was to estimate the relative frequencies of climatic niche conservatism versus divergence in speciation. Thus, unless there are systematic biases that favour one mechanism over the other, our overall conclusions should be robust, despite potential errors associated with some species pairs. We address these and other assumptions further in the Discussion.

### 3 | RESULTS

#### 3.1 Frequency of geographic modes in squamates

We identified 242 species pairs with adequate distributional data (Supporting Information Appendix S1). Pairs were broadly distributed among clades and regions, including 59 pairs of snakes and 183 of lizards. Lizard pairs were broadly distributed among major clades (80 = Scincoidea, 64 = Iguania, 25 = Gekkota, 13 = Lacertoidea, 1 = Anguimorpha). Further, 114 pairs were tropical and 128 temperate: pairs were considered tropical if the latitudinal mid-point of their combined localities was between 23.5°S and 23.5°N (following Hua & Wiens, 2010). Many pairs were Australian (91) but others were broadly distributed among biogeographic regions (35 Ethiopian, 55 Nearctic, 37 Neotropical, 13 Oriental, 11 Palearctic; regions from Kreft & Jetz, 2010).

Among the 242 species pairs, 100 were allopatric, 47 parapatric, 43 partially sympatric and 52 fully sympatric (Supporting Information Appendix S1). Thus, based on current overlap alone, allopatric speciation appeared to be the most common mode, but other modes might also be relatively common. The 100 allopatric pairs included 69 separated by terrestrial habitat and 31 separated by marine habitats (i.e., both insular species or one insular and one mainland). The degree of range overlap in sympatric and partially sympatric species pair is not significantly correlated with time since the species split ( $r^2 = 0.01$ , p = 0.351; Supporting Information Appendix S2). However, range overlap was significantly correlated with time using all species pairs, albeit weakly ( $r^2 = 0.02$ , p = 0.043).

# 3.2 | Testing climatic mechanisms in allopatric species pairs

We calculated hypervolumes for all allopatric species pairs separated by terrestrial habitat and having at least three localities/species. Forty-nine pairs met these criteria, including nine snakes and 40 lizards, and 20 tropical and 29 temperate (Table 2). Some phylogenetically well-studied genera were represented by multiple pairs (e.g., *Lerista, Sceloporus*), but this need not be problematic for our analyses. Hypervolumes and their overlaps for pairs and intervening area are given in Supporting Information Appendix S3. Mean values across 100 replicates are given in Supporting Information Appendix S4.

On average, hypervolume overlaps were higher between allopatric species pairs and their intervening areas (46%) than between sister species (26%; Figure 1a). This difference was significant (p < 0.0001; Wilcoxon rank-sum test). Thus, climatic niches occupied by allopatric species were less similar to each other than to the intervening area between them. Following our criteria (Table 1), four pairs originated through climatic niche conservatism and an additional four most likely originated this way (Table 2). Conversely, 32 pairs originated through climatic niche divergence and an additional five most likely originated this way (Table 2). Four pairs showed conflicting results between sister species. Two showed conflicting nonsignificant results and both had small sample sizes (3-4 localities/species). The other two had significant but conflicting results. In both, the species with the larger range size supported conservatism and that with the smaller range size supported divergence (see Section 4). When we considered only pairs for which the same mechanism was significantly supported for both species, 22 pairs supported divergence and two supported conservatism (Table 2).

We used Maxent to determine the suitability or unsuitability of each species' geographic range relative to its sister species and to the intervening area for these 49 allopatric pairs. The mean suitability of the intervening area across all species pairs (0.37) was higher than the mean suitability for sister species (0.30; Figure 1b), but not significantly (p = 0.091; Wilcoxon rank-sum test). Thus, climatic niches occupied by allopatric sister species were less similar to each other than to the habitat separating them. Using Maxent, 10 pairs supported niche conservatism, 30 supported niche divergence and nine could not be assigned to either mechanism (Table 2). Considering only pairs with significant support, 23 supported divergence and four supported conservatism (Table 2). Full results are given in Supporting Information Appendices S5 and S6.

In summary, results from the niche hypervolume and Maxent approaches were generally similar. However, for several pairs, a different mechanism was supported by each method (Table 2). In these cases, there were either few localities for one or both species (e.g., small range sizes), the two species had very different range sizes, or species niches were not very different from the intervening area. Additionally, out of these pairs, only one pair (pair 124) exhibited significant results for both methods. Considering only species pairs from genera with complete species sampling gave similar frequencies for each mechanism (hypervolume: 82% divergence, 18% conservatism, 11 pairs; Maxent: 77% divergence, 23% conservatism, 13 pairs; Table 2).

# 3.3 | Relationship between niche similarity and divergence times among allopatric species pairs

We found no relationship between the two measures of niche similarity and the time since sister species split among the 49 allopatric pairs (hypervolume: rho = 0.083; p = 0.569; Figure 2a; Maxent: rho = -0.075; p = 0.606; Figure 2b). Under niche conservatism, climatic niches should be similar initially but might diverge over time.

the ranges of each species and their intervening area. The eleventh column shows the mean logistic probabilities of localities for one species based on the niche of the second species (averaged species pair number and names of both species. The fourth column shows the time since the two species split (i.e., divergence time) in millions of years ago (Ma). The fifth column indicates the divergence, unk = the mechanism could not be inferred (see Section 2 for details). The eleventh to thirteenth columns summarize results from the Maxent analyses of the climatic suitability of indicates the final mechanism inferred from this approach: C = climatic niche conservatism, D = climatic niche divergence, unk = no mechanism could be inferred. Full results are in Supporting inferred speciation mechanism from this approach: C = climatic niche conservatism, D = climatic niche divergence, (C) = most likely climatic niche conservatism, (D) = most likely climatic niche column shows the percentage overlap between the species niche and the climate of the intervening area between the species' ranges (averaged for both species); the tenth column shows the **TABLE 2** Climatic mechanisms inferred for 49 allopatric species pairs using two methods: n-dimensional hypervolume and Maxent logistic probabilities. The first three columns show the proportion of species in the genus that were sampled in the tree. The sixth and seventh columns list the number of georeferenced localities for each species. The eighth to tenth columns summarize results from the hypervolume analyses: The eighth shows the percentage of the niche of one species that is shared with its sister species (averaged for both species); the ninth for both species). The twelfth column shows the mean logistic probability of random localities drawn from the intervening area based on the niche of each species. The thirteenth column Information Appendices S3–S6. Significant results based on resampling of the intervening habitat are indicated by\*

							Hypervolu	me (%)	Maxent			
Allop	batric species pair		Divergence time (Ma)	Genus completeness	Localities Species 1	Localities Species 2	Overlap with the sister species niche	Overlap with the intervening niche	Speciation mechanism	Suitability of the sister species niche	Suitability of the intervening niche	Speciation mechanism
8	Aprasia parapulchella	A. pseudopulchella	4.94	0.77	28	38	11	31	D*	0.02	0.06	D*
10	Arrhyton dolichura	A. tanyplectum	3.58	0.88	ю	ო	41	44	unk	0.51	0.47	unk
11	Arrhyton redimitum	A. vittatum	9.23	0.88	10	10	30	26	(C)	0.08	0.19	D
14	Aspronema cochabambae	A. dorsivittatum	4.24	1.00	с	4	38	26	U	0.30	0.37	unk
16	Bogertophis rosaliae	B. subocularis	13.68	1.00	21	185	42	96	D*	0.06	0.14	D*
17	Bothriechis aurifer	B. rowleyi	4.24	0.80	с	с	24	48	D	0.34	0.36	unk
19	Bothriechis marchi	B. thalassinus	3.32	0.80	7	ю	45	25	(C)	0.24	0.09	U
22	Brasiliscincus caissara	B. heathi	3.49	1.00	с	12	16	38	D	0.02	0.16	D*
27	Cerastes cerastes	C. gasperettii	2.94	0.75	10	13	45	57	D	0.29	0.30	unk
34	Coeranoscincus frontalis	Coggeria naufragus	17.76	1.00	44	11	49	26	(D)	0.21	0.08	U
35	Colobodactylus dalcyanus	C. taunayi	17.69	1.00	з	ю	31	34	(D)	0.42	0.50	D
40	Crotaphytus antiquus	C. reticulatus	5.29	0.89	4	17	0	88	D*	0.03	0.12	D*
44	Ctenophorus femoralis	C. fordi	6.38	0.76	110	84	0	19	D*	0.00	0.07	D*
45	Ctenophorus gibba	C. salinarum	18.51	0.76	43	170	30	53	D*	0.08	0.13	D*
48	Ctenophorus tjantjalka	C. vadnappa	8.64	0.76	29	104	42	47	D	0.18	0.14	U
51	Dierogekko kaalaensis	D. thomaswhitei	7.37	0.89	с	6	0	35	D*	0.31	0.07	U
63	Ephalophis greyae	Parahydrophis mertoni	5.46	1.00	26	6	36	53	D	0.14	0.36	D*
68	Gambelia copeii	G. sila	3.30	1.00	59	134	ო	47	D*	0.10	0.22	*0
82	Lacerta schreiberi	L. strigata	17.64	1.00	7	17	61	77	D	0.33	0.41	D
90	Leptodeira maculata	L. rubricata	4.73	0.83	138	4	4	89	D*	0.12	0.20	D*
67	Lerista borealis	L. walkeri	11.26	0.77	50	20	14	32	D*	0.02	0.18	D*
												(Continues)

							Hypervolu	me (%)	Maxent			
Allop	tric species pair		Divergence time (Ma)	Genus completeness	Localities Species 1	Localities Species 2	Overlap with the sister species niche	Overlap with the intervening niche	Speciation mechanism	Suitability of the sister species niche	Suitability of the intervening niche	Speciation mechanism
98	Lerista bougainvillii	L. viduata	10.33	0.77	519	ო	49	54	(D)	0.46	0.48	unk
111	Lerista ingrami	L. zonulata	3.56	0.77	ю	37	0	70	D*	0.17	0.28	D*
112	Lerista kendricki	L. yuna	4.12	0.77	52	17	0	49	D*	0.01	0.21	D*
113	Lerista kennedyensis	L. onsloviana	6.10	0.77	12	33	0	24	D*	0.00	0.14	D*
115	Lerista simillima	L. vermicularis	1.02	0.77	80	20	36	53	D*	0.27	0.36	D
116	Lerista terdigitata	L. tridactyla	2.56	0.77	81	16	57	50	unk	0.14	0.20	D
122	Liopholis kintorei	L. pulchra	12.22	0.91	29	47	0	36	D*	0.00	0.10	D*
123	Liopholis margaretae	L. modesta	17.69	0.91	ю	25	12	44	D*	0.02	0.16	D*
124	Lioscincus maruia	L. tillieri	18.29	1.00	80	18	58	25	ť	0.06	0.10	D*
125	Lioscincus steindachneri	L. vivae	14.22	1.00	5	9	51	20	(C)	0.35	0.21	* Ŭ
142	Nephrurus amyae	N. sheai	6.63	1.00	37	70	0	42	D*	0.00	0.10	D*
143	Nephrurus deleani	N. laevissimus	6.14	1.00	17	8	38	73	D	0.34	0.35	unk
161	Paralaudakia himalayana	P. stoliczkana	14.97	0.75	7	13	5	71	D*	0.11	0.36	D*
180	Plestiodon ochoterenae	P. parvulus	6.55	0.76	4	з	45	38	unk	0.18	0.29	D*
182	Pogona barbata	P. nullarbor	2.10	0.75	757	55	51	48	unk	0.27	0.09	ť
183	Pogona henrylawsoni	P. minima	4.33	0.75	28	13	6	40	D*	0.03	0.10	D*
189	Rhinechis scalaris	Zamenis situla	8.42	0.83	9	8	67	61	(C)	0.54	0.47	U
190	Saltuarius cornutus	S. salebrosus	13.88	0.86	45	21	24	31	(D)	0.03	0.15	D*
192	Saproscincus hannahae	S. tetradactylus	17.91	0.83	43	46	11	С	ť	0.22	0.04	ť
199	Sceloporus arenicolus	S. graciosus	3.21	0.76	108	1864	35	53	D*	0.19	0.21	unk
203	Sceloporus consobrinus	S. woodi	3.29	0.76	489	201	5	56	D*	0.09	0.14	D*
204	Sceloporus cozumelae	S. smithi	7.15	0.76	38	13	50	57	(D)	0.14	0.09	unk
205	Sceloporus cryptus	S. subpictus	0.77	0.76	9	4	32	13	U	0.25	0.28	unk
207	Sceloporus gadoviae	S. maculosus	19.82	0.76	23	13	6	42	D*	0.10	0.06	U
209	Sceloporus heterolepis	S. palaciosi	8.07	0.76	15	ю	5	28	D	0.07	0.12	D
211	Sceloporus jalapae	S. ochoterenae	17.47	0.76	37	6	52	77	D	0.45	0.28	* Ŭ
231	Typhlosaurus braini	T. meyeri	14.95	1.00	4	ю	18	40	D	0.20	0.25	D
233	Uma exsul	U. paraphygas	7.39	0.83	22	2	0	55	۵	0.15	0.23	*

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TABLE 2 (Continued)

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**FIGURE 1** Boxplot showing niche similarity across 49 allopatric species pairs. We compared niche similarity between each species' geographic range and the intervening area (left) and between the geographic ranges of sister species (right). (a) Niche similarity is calculated from niche hypervolume overlap ranging from 0% (the hypervolumes of sister species do not overlap) to 100% (the hypervolumes are identical). (b) Niche similarity is calculated from Maxent logistic probabilities ranging from 0 (the geographic range of the sister species is unsuitable for the modelled species) to 1 (the geographic range of the sister species is as suitable as the range of the modelled species). Boxes show the median, the 25th and 75th percentiles, and the range

These results do not support this prediction, further implicating niche divergence as the most common mechanism here.

# 3.4 Comparison of niche similarity in allopatric and parapatric species pairs

Allopatric species pairs exhibited significantly lower niche similarity than parapatric pairs (Supporting Information Appendix S7, S8). Mean hypervolume overlap was 26% for allopatric pairs and 45% for parapatric pairs (Figure 3a; p < 0.0001; Wilcoxon rank-sum test). Similarly, mean suitability of sister species niches from Maxent was 0.17 for allopatric pairs and 0.42 for parapatric pairs (Figure 3b; p < 0.0001). Niche similarity was uncorrelated with geographic distance among allopatric species pairs (hypervolume: rho = 0.081, p = 0.582; Maxent: rho = -0.100, p = 0.497). Niche similarity in allopatric species pairs was significantly lower even after correcting for geographic distance



**FIGURE 2** Relationship between climatic niche similarity and time since splitting (crown-group age) among 49 allopatric species pairs. (a) Niche similarity is calculated from niche hypervolume overlap ranging from 0% (the hypervolumes of sister species do not overlap) to 100% (the hypervolumes are identical). (b) Niche similarity is calculated from Maxent logistic probabilities ranging from 0 (the geographic range of the sister species is unsuitable for the modelled species) to 1 (the geographic range of the sister species is a suitable as the range of the modelled species). Each circle represents an average value for the two species in each pair

(hypervolume: allopatric = 11.9; parapatric = 8.7; p = 0.0002; Maxent: allopatric = 11.1; parapatric = 9.2; p = 0.029).

### 4 | DISCUSSION

Climatic niches may play an important role in speciation, as climatic niches may determine (to a large extent) where species and populations can and cannot occur. There are two major hypotheses for how climate drives speciation (e.g., Hua & Wiens, 2013; Moritz et al., 2000; Schneider, Smith, Larison, & Moritz, 1999). Under climatic niche divergence, the geographic range of each species is unsuitable for its sister species. Under climatic niche conservatism, incipient allopatric species pairs are separated by a barrier of climatically unsuitable habitat. Many previous studies addressed the prevalence of these mechanisms (e.g., Graham, Ron, Santos, Schneider, & Moritz, 2004; Hua & Wiens, 2010; Kozak & Wiens, 2006, 2007; McCormack et al., 2010; Rato et al., 2015; Raxworthy et al., 2008). However, we argue that most studies used approaches that suffered from various limitations, especially ignoring the suitability of -WILEY-MOLECULAR ECOLOGY



**FIGURE 3** Boxplot comparing niche similarity in allopatric (n = 49) and parapatric (n = 44) species pairs. (a) Niche similarity is calculated from the hypervolume overlap, ranging from 0% (the hypervolumes do not overlap) to 100% (the hypervolumes are identical). (b) Niche similarity is calculated from Maxent logistic probabilities ranging from 0 (the geographic range of the sister species is unsuitable for the modelled species) to 1 (the geographic range of the sister species is as suitable as the range of the modelled species). Boxes show the median, the 25th and 75th percentiles, and the range

intervening habitats for allopatric species pairs. Here, we developed new methods to address these limitations. We then applied these approaches to many species pairs of squamate reptiles. We also provide the first large-scale analysis of the frequency of geographic speciation modes in squamates. Overall, our results suggest that allopatric speciation is the most widespread geographic mode in squamates (although other modes seem to be common also). We also find that allopatric speciation occurs most frequently through climatic niche divergence, rather than climatic niche conservatism. Nevertheless, we supported speciation through climatic niche conservatism in ~20% of the allopatric species pairs examined. Below we discuss our conclusions about mechanisms of speciation and then discuss our methodological conclusions.

#### 4.1 | New insights on climate and speciation

Climate may drive speciation through two main mechanisms: niche conservatism and niche divergence. Climatic niche conservatism has

been suggested as a potential driver of allopatric speciation (i.e., causing the initial allopatry that splits populations), as when two sets of populations of a species become separated by a zone of climatically unsuitable habitat (Hua & Wiens, 2013; Wiens, 2004a). This initial lineage splitting may be considered speciation, or there may be subsequent change within allopatric populations leading to intrinsic reproductive isolation (Hua & Wiens, 2013; Wiens, 2004a,b). Conversely, niche divergence is often hypothesized to be associated with parapatric speciation, where niche dissimilarity between populations in different parts of the species range impedes gene flow between them and promotes speciation (e.g., Coyne & Orr, 2004; Hua & Wiens, 2013; Moritz et al., 2000; Rundle & Nosil, 2005; Schluter, 2001, 2009). More specifically, theoretical studies suggest that speciation via climatic niche divergence will occur in parapatric taxa with low niche lability when climate varies over space but not time or in species with high niche lability under temporally fluctuating climate (Hua & Wiens, 2013).

In contrast to these expectations, we found that most allopatric species pairs supported the niche divergence model (Table 2). Moreover, allopatric pairs were more climatically divergent (on average) than parapatric ones. Despite these overall results, ~20% of the 49 allopatric species pairs supported climatic niche conservatism. This is consistent with other analyses (using appropriate methods that analyse intervening regions) finding strong support for climatic niche conservatism in some cases (e.g., 75% of eight allopatric species pairs in salamanders; Kozak & Wiens, 2006). On the other hand, other analyses have found little evidence for speciation via climatic niche conservatism (e.g., 0% of nine allopatric pairs in frogs; Hua & Wiens, 2010). Also, we found similar frequencies of niche conservatism (i.e., cases supported by at least one method) among tropical (n = 6; 30%) and temperate (n = 8; 28%) species pairs, rather than finding more frequent conservatism in the tropics (per Cadena et al., 2012; see also Hua & Wiens, 2010). Our results here represent the largest survey so far (49 species pairs) and show both modes occur but that niche divergence is more frequent (~60-80%). Using more stringent statistical criteria for each pair yields fewer pairs but even stronger support for niche divergence (92% of 24 pairs and 85% of 27 for each method; Table 2).

Intriguingly, our results are consistent with macroevolutionary studies showing that climate niche divergence drives large-scale patterns of diversification (speciation–extinction) in other groups, including amphibians (Gómez-Rodríguez, Baselga, & Wiens, 2015; Kozak & Wiens, 2010; Moen & Wiens, 2017) and birds (Cooney, Seddon, & Tobias, 2016). Although comparable analyses have not been carried out in squamates, new climatic datasets (e.g., Pie, Campos, Meyer, & Duran, 2017) should facilitate this.

Our results raise a particularly perplexing question: How exactly does climatic niche divergence drive allopatric speciation? We suggest two initial possibilities. First, it is intuitive that speciation should be accelerated between allopatric species occurring under divergent climatic conditions, analogous to the scenario of ecological speciation proposed for parapatric species (see above). But then why are these lineages allopatric in the first place? For example, allopatric species may be separated by areas of unsuitable microhabitats (e.g., rivers), rather than unsuitable climatic conditions. But if this were the case, it is unclear why their climatic niches should be significantly divergent so often. One possibility is that many lineages might initially become allopatric through nonclimatic factors (e.g., unsuitable microhabitats, biotic interactions, stochastic long-distance dispersal or extinction of intervening populations [as in Ahmadzadeh et al., 2013]). However, these cases of allopatry may be more likely to lead to the formation of permanently isolated allopatric species when the allopatric populations occur under divergent climatic conditions. We note that if these nonclimatic factors were the only drivers of allopatry, then climatic niche divergence might be expected to increase over time or be greater in parapatric pairs. Our results do not support either prediction. We speculate that climatic niche divergence might help ensure the successful speciation of populations that became allopatric through nonclimatic factors, and which otherwise might simply merge without speciating. Some authors have suggested that such "ephemeral" speciation events might be very widespread (Rosenblum et al., 2012).

A similar scenario is that many climatically divergent allopatric pairs arose through parapatric speciation but local extinctions (e.g., due to nonclimatic factors) resulted in their current allopatric distributions. This scenario is consistent with our findings that parapatric species are younger than allopatric species pairs (mean divergence time between parapatric sister species = 6.89 Ma; allopatric = 9.04 Ma; p = 0.014).

Unexpectedly, we found that climatic niche divergence is higher in allopatric species pairs than parapatric ones. Even if some allopatric species originated in parapatry (as suggested above), parapatric species are expected to originate exclusively via niche divergence and should therefore exhibit lower niche similarity than allopatric species. Yet, we found that niche similarity between allopatric species pairs was significantly lower than between parapatric pairs, even after correcting for greater geographic distance between allopatric pairs.

Why should parapatric species have lower climatic niche divergence? One possibility is that parapatric species pairs are not always driven by niche divergence. Remarkably, many parapatric species pairs exhibited large climatic niche overlap and high mutual suitability (Supporting Information Appendix S7, S8), in stark contrast to the standard scenario of parapatic speciation through niche divergence. One potential explanation is that many parapatric species arose as allopatric species (possibly even through niche conservatism), but later came into secondary contact, with their current geographic ranges limited by competition with their sister species (Weir & Price, 2011). If this were the case, parapatric pairs should be older than allopatric pairs. Our results show just the opposite (see above). Another explanation is that parapatric species pairs may diverge along gradients related to microhabitats (e.g., rocky vs. sandy), with climatic niches being generally conserved. Conversely, some parapatric species may occur in similar microhabitats but have patchy distributions due to microhabitat specialization, leading to microallopatry and eventually to speciation, again with climatic niches being conserved.

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Overall, our results provide surprising observations relevant to both allopatric speciation (given that the exact mechanism by which niche divergence drives allopatry is difficult to explain) and parapatric speciation (given that parapatric species are more climatically similar than expected, making their parapatry difficult to explain). We suggest that understanding these speciation modes in the future may require new analyses that incorporate microhabitat and other factors. They may also require new theoretical work on climate and speciation.

#### 4.2 | Speciation in squamate reptiles

Our study represents the first large-scale analysis of geographic modes of squamate speciation. We recognize that current geographic distributions of sister species may not always reflect the geographic mode by which they originated (e.g., Pontarp, Ripa, & Lundberg, 2015), but the impossibility of inferring geographic modes from present-day range overlaps may be overstated (see Cardillo, 2015; Phillimore et al., 2008). Furthermore, there are methods for inferring the potential impact of postspeciational dispersal on the inference of geographic modes, such as testing for increasing range overlap over time (e.g., Barraclough & Vogler, 2000).

Overall, we found that allopatry was the most frequent mode among squamates (~41% of 242 species pairs), supporting the widespread view that allopatric speciation is the most prevalent geographic mode (e.g., Barraclough & Vogler, 2000; Coyne & Orr, 2004; Hua & Wiens, 2010; Kozak & Wiens, 2006; Lieberman, 2000; Phillimore et al., 2008). Nevertheless, we found that parapatric and sympatric pairs were also common (~20% each). Of course, currently, sympatric and parapatric pairs may have originated via allopatric speciation, followed by secondary contact (e.g., Barraclough & Vogler, 2000). In this case, we would expect allopatric pairs to be younger than parapatric and sympatric species. We found that allopatric species (9.04 Ma) were not significantly younger than sympatric pairs (9.64 Ma; p = 0.068) nor parapatric pairs (6.89 Ma; p = 0.014; Supporting Information Appendix S1). We also found that range overlap of partially sympatric species not correlated with time (Supporting was Information Appendix S2) although range overlap was significantly correlated with time across all species (mainly driven by parapatric pairs being younger than allopatric and sympatric pairs). Furthermore, we found several very young pairs with completely or predominantly overlapping ranges which likely did not originate in allopatry (e.g., pairs 102, 128, 234 in Supporting Information Appendix S1). These cases could be interesting subjects for future research on nonallopatric speciation.

Finally, among all 100 allopatric species pairs, 31% were associated with islands, strongly supporting the importance of islands in speciation (Lomolino, Riddle, & Brown, 2006). Interestingly, these results suggest that niche conservatism in microhabitat (i.e., aquatic vs. terrestrial) plays a frequent role in allopatric speciation, rather than climate.

# 4.3 | Methodology for testing the role of climate in speciation

Studies that have tested whether climatic niche conservatism or divergence drives speciation have used a variety of methods to address this question (e.g., Graham et al., 2004; Hua & Wiens, 2010; Kozak & Wiens, 2006; Loera et al., 2012; McCormack et al., 2010; Pyron & Burbrink, 2009). We believe that a crucial part of any methodology to address this question is to address the climatic suitability of the area separating the ranges of sister species (Hua & Wiens, 2010; Kozak & Wiens, 2006). Approaches that fail to include this component may give misleading results, because (to our knowledge) the only mechanism by which niche conservatism may drive speciation is by causing allopatry. Species climatic niches might be similar to each other or relatively divergent, but what is critically important for speciation via climatic niche conservatism is whether they are separated by a barrier of unsuitable climatic conditions (Hua & Wiens, 2010; Kozak & Wiens, 2006).

Kozak and Wiens (2006) and Hua and Wiens (2010) both included tests of whether the climatic niches of sister species were significantly different from each other and from the area separating them. We advanced the methodology of these earlier studies in several ways. First, we used the multidimensional hypervolume (Blonder et al., 2014) to define climatic niches of species and niche similarity was calculated from niche overlap. The hypervolume overlap estimates the proportion of the niche that is similar between the two species and therefore seems appropriate even for species with broad, overlapping niches. In comparison, Kozak and Wiens (2006) and Hua and Wiens (2010) compared climatic niches of sister species (and intervening areas) by calculating distances between multivariate distributions. This can be problematic in some cases. For example, two species may have broad niches that partially overlap. The two species may occupy identical climatic conditions in the areas adjacent to the intervening area (in support of conservatism) but the two species might still exhibit large mean distance between their niches (because their niches are broad with only a partial overlap), which may incorrectly lead to inferring niche divergence.

Like Kozak and Wiens (2006) and Hua and Wiens (2010), we also used Maxent niche modelling to compare niche suitability of each species' range to the other and to the area separating them. We assigned a single speciation mechanism to each species pair (as in Kozak & Wiens, 2006), as opposed to assigning a speciation mechanism to individual species (as in Hua & Wiens, 2010). We think that there should be a single mechanism driving each splitting event, not a different mechanism for each species.

There are also ways that our analyses could yield incorrect results. However, we think that these issues are more related to data than to our methodology and would also mislead other methods. First, if only a small portion of the niche of a wide-niched species is captured with the sampled localities, the estimated niche similarity between species may be inaccurate. Additionally, following Blonder et al. (2014), the number of hypervolume dimensions used was based on the number of localities. Thus, for species with <10 localities, only a single PC was used. The hypervolume overlap might therefore be larger for pairs with fewer localities. Indeed, Qiao, Escobar, Saupe, Ji, and Soberón (2017) suggested that the hypervolume methodology may overestimate niche volumes in low dimensions and constrict niche volume estimates in high dimensions. Fortunately, we found no significant negative relationship between the summed number of localities per pair and niche overlap (Spearman's rho = -0.13, p = 0.37). Additionally, as each species pair was analysed separately, the niches of the two species and of the intervening habitat should be affected in a similar way (all three may be overestimated or underestimated), which should not affect the relative suitability of the species pair with respect to the intervening habitat. The problematic case would be if each species had very different numbers localities. Indeed, we found inconsistent results in such cases (see below).

Second, results could be biased by incomplete sampling of localities within the intervening habitat (e.g., if one or both species actually occur in portions of the intervening area). Our approach might infer the intervening area to be suitable (or more suitable than the niche of the sister species) and we could incorrectly conclude niche divergence to be the mechanism of speciation. We attempted to ameliorate this problem by including a buffer in the intervening area that prevented random points from being too close to known localities. Similarly, biased sampling of occurrence records (e.g., high sampling in some parts of the species range and sparse sampling in others) can cause overfitting in Maxent (i.e., model fits too tightly to calibration data) as this method assumes that all locations are equally likely to be sampled (Merow et al., 2013). The hypervolume, on the other hand, is much less sensitive to sampling bias (Blonder et al., 2014).

Third, an unequal number of localities caused by differences in geographic range sizes could result in different mechanism being inferred for each species in a species pair (i.e., conservatism for one, divergence for the other). When the ranges (and niches) have very different sizes, the species with a bigger range and larger niche will often predict the niche of the sister species as suitable but not vice versa, resulting in conservatism being inferred for the larger ranged species and divergence for the smaller ranged species. We found some evidence for this possibility (pairs 98, 143, 199). However, most pairs (~80%) were unambiguously assigned to one mechanism by both methods.

Our analyses were based on species' realized climatic niches (i.e., conditions where they occur), not fundamental niches (i.e., considering only conditions they can physiologically tolerate). Thus, we may sometimes underestimate the climatic suitability of species ranges and intervening areas, if nonclimatic factors are limiting the ranges of many species. However, this scenario seems unlikely to explain our overall results, which show that most allopatric pairs have mutually unsuitable ranges separated by suitable intervening habitats. If our results were primarily driven by errors related to underprediction, we would not expect a strong pattern across most species pairs.

Our analyses do not identify the specific mechanisms that might prevent sister species from occurring in each other's ranges or in the areas that separate them. Thus, the proximate range-limiting factors might be related to physiological tolerances to climate, or instead to effects of climate on the distribution of relevant biotic factors (e.g., prey, predators, pathogens or vegetation important for microhabitat). The specific factors might also vary considerably among the dozens of species analysed. Nevertheless, our results set the stage for future analyses of these factors. Importantly, the fact that range limits might be set by interactions between climate and biotic factors rather than physiology alone does not invalidate our results. Our main goal was to test the general mechanisms by which climate is

related to speciation (i.e., divergence vs. conservatism), and if it is

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related at all.

In conclusion, we developed methods to test two competing mechanisms of climate-driven speciation (niche conservatism vs. niche divergence). These methods can be applied to any allopatrically distributed sister species. We applied these methods to a large number of sister species of squamate reptiles (lizards and snakes), yielding the most extensive comparison of these climatic mechanisms to date. We also provide the first large-scale assessment of the frequency of different geographic modes in squamate reptiles. Our results revealed that climatic niche divergence is the most common mechanism of allopatric speciation (~60–80%), although climatic niche conservatism was supported for several species pairs (~20%). Overall, our results raise many important questions for future empirical and theoretical research, such as how exactly climatic niche divergence drives allopatric speciation, and why do parapatric species pairs have such similar climatic niches.

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### DATA ACCESSIBILITY

Data archiving: All data and scripts are included as Supporting Information, Data S1–S3 are also available on Dryad (https://doi.org/10. 5061/dryad.78s7g21).

#### AUTHOR CONTRIBUTIONS

The study was designed by T.J. and J.J.W. The analyses were performed by T.J. The manuscript was written by T.J. and J.J.W.

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#### REFERENCES

- Ahmadzadeh, F., Flecks, M., Carretero, M. A., Böhme, W., Ilgaz, C., Engler, J. O., ... Rödder, D. (2013). Rapid lizard radiation lacking niche conservatism: Ecological diversification within a complex landscape. *Journal of Biogeography*, 40, 1807–1818. https://doi.org/10.1111/jbi.12121
- Barraclough, T. G., & Vogler, A. P. (2000). Detecting the geographical pattern of speciation from species-level phylogenies. *American Naturalist*, 155, 419–434.
- Blonder, B., Lamanna, C., Violle, C., & Enquist, B. J. (2014). The n-dimensional hypervolume. *Global Ecology and Biogeography*, 23, 595–609. https://doi.org/10.1111/geb.12146
- Bond, J. E., & Stockman, A. K. (2008). An integrative method for delimiting cohesion species: Finding the population-species interface in a group of California trapodoor spiders with extreme genetic divergence. Systematic Biology, 57, 624–646.
- Broennimann, O., Fitzpatrick, M. C., Pearman, P. B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., ... Guisan, A. (2012). Measuring ecological niche overlap from occurrence and spatial environmental data. *Global Ecology and Biogeography*, 21, 481–497. https://doi.org/10.1111/j. 1466-8238.2011.00698.x
- Cadena, C. D., Kozak, K. H., Gómez, J. P., Parra, J. L., McCain, C. M., Bowie, R. C. K., ... Graham, C. H. (2012). Latitude, elevational climatic zonation and speciation in New World vertebrates. *Proceedings* of the Royal Society of London, 279, 194–201. https://doi.org/10. 1098/rspb.2011.0720
- Cardillo, M. (2015). Geographic range shifts do not erase the historic signal of speciation in mammals. *American Naturalist*, 185, 343–353. https://doi.org/10.1086/679663
- Cooney, C. R., Seddon, N., & Tobias, J. A. (2016). Widespread correlations between climatic niche evolution and species diversification in birds. *Journal of Animal Ecology*, 85, 869–878. https://doi.org/10. 1111/1365-2656.12530
- Coyne, J. A., & Orr, H. A. (2004). Speciation. Sunderland, MA: Sinauer Associates.
- Futuyma, D. J. (2013). Evolution, 3rd ed. Sunderland, MA: Sinauer Associates.
- Glor, R. E. & Warren, D. (2011). Testing ecological explanations for biogeographic boundaries. *Evolution*, 65, 673–683. https://doi.org/10. 1111/j.1558-5646.2010.01177.x
- Gómez-Rodríguez, C., Baselga, A., & Wiens, J. J. (2015). Is diversification rate related to climatic niche width? *Global Ecology and Biogeography*, 24, 383–395. https://doi.org/10.1111/geb.12229
- Gonçalves, D. V., Brito, J. C., Crochet, P.-A., Geniez, P., Padial, J. M., & Harris, D. J. (2012). Phylogeny of North African Agama lizards (Reptilia: Agamidae) and the role of the Sahara desert in vertebrate speciation. *Molecular Phylogenetics and Evolution*, 64, 582–591. https://doi. org/10.1016/j.ympev.2012.05.007
- Graham, C. H., Ron, S. R., Santos, J. C., Schneider, C. J., & Moritz, C. (2004). Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution*, 58, 1781–1793. https://doi.org/10.1111/j.0014-3820.2004.tb00461.x
- Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978. https://doi.org/10.1002/(ISSN)1097-0088
- Hijmans, R. J., Phillips, S., Leathwick, J., & Jane, E. (2015). dismo: Species distribution modeling. R package version 1.0-12. http://CRAN.R-pro ject.org/package=dismo.
- Hijmans, R. J., & van Etten, J. (2012). raster: Geographic analysis and modeling with raster data. R package version 2.0-12. http://CRAN.Rproject.org/package=raster.
- Hua, X., & Wiens, J. J. (2010). Latitudinal variation in speciation mechanisms in frogs. Evolution, 64, 429–443. https://doi.org/10.1111/j. 1558-5646.2009.00836.x

II FV-MOLECULAR ECOLOGY

- Hua, X., & Wiens, J. J. (2013). How does climate influence speciation? American Naturalist, 182, 1–12. https://doi.org/10.1086/ 670690
- Kaiser, H. F. (1960). The application of electronic computers to factor analysis. Educational and Psychological Measurement, 20, 141–151. https://doi.org/10.1177/001316446002000116
- Knouft, J. H., Losos, J. B., Glor, R. E., & Kolbe, J. J. (2006). Phylogenetic analysis of the evolution of the niche in lizards of the Anolis sagrei group. Ecology, 87, S29–S38. https://doi.org/10.1890/0012-9658(2006)87[29:PAOTEO]2.0.CO;2
- Kozak, K. H., & Wiens, J. J. (2006). Does niche conservatism promote speciation? A case study in North American salamanders. *Evolution*, 60, 2604–2621. https://doi.org/10.1111/j.0014-3820.2006.tb01893. x
- Kozak, K. H., & Wiens, J. J. (2007). Climatic zonation drives latitudinal variation in speciation mechanisms. *Proceedings of the Royal Society* of London, 274, 2995–3003. https://doi.org/10.1098/rspb.2007. 1106
- Kozak, K. H., & Wiens, J. J. (2010). Accelerated rates of climatic-niche evolution underlie rapid species diversification. *Ecology Letters*, 13, 1378–1389. https://doi.org/10.1111/j.1461-0248.2010.01530.x
- Kreft, H., & Jetz, W. (2010). A framework for delineating biogeographical regions based on species distributions. *Journal of Biogeography*, 37, 2029–2053. https://doi.org/10.1111/j.1365-2699. 2010.02375.x
- Lieberman, B. S. (2000). Allopatric speciation and vicariance. Paleobiogeography (pp. 63–71). Boston, MA: Springer. https://doi.org/10. 1007/978-1-4615-4161-5
- Loera, I., Sosa, V., & Ickert-Bond, S. M. (2012). Diversification in North American arid lands: Niche conservatism, divergence and expansion of habitat explain speciation in the genus *Ephedra*. *Molecular Phylogenetics and Evolution*, 65, 437–450. https://doi.org/10.1016/j.ympev. 2012.06.025
- Lomolino, M. V., Riddle, B. R., & Brown, J. H. (2006). *Biogeography*. Sunderland, MA: Sinauer Associates.
- McCormack, J. E., Zellmer, A. J., & Knowles, L. L. (2010). Does niche divergence accompany allopatric divergence in *Aphelocoma* jays as predicted under ecological speciation? Insights from tests with niche models. *Evolution*, 64, 1231–1244.
- Merow, C., Smith, M. J., & Silander, J. A. (2013). A practical guide to MaxEnt for modeling species' distributions: What it does, and why inputs and settings matter. *Ecography*, 36, 1058–1069. https://doi. org/10.1111/j.1600-0587.2013.07872.x
- Moen, D. S., & Wiens, J. J. (2017). Microhabitat and climatic niche change explain patterns of diversification among frog families. American Naturalist, 190, 29–44. https://doi.org/10.1086/692065
- Moritz, C., Patton, J. L., Schneider, C. J., & Smith, T. B. (2000). Diversification of rainforest faunas: An integrated molecular approach. Annual Review of Ecology, Evolution, and Systematics, 31, 533–563. https:// doi.org/10.1146/annurev.ecolsys.31.1.533
- Nosil, P. (2012). Ecological speciation. Oxford, UK: Oxford University Press. https://doi.org/10.1093/acprof:osobl/9780199587100.001.0001
- Phillimore, A. B., Orme, C. D. L., Thomas, G. H., Blackburn, T. M., Bennet, P. M., Gaston, K. J., ... Owens, I. P. F. (2008). Sympatric speciation in birds is rare: Insights from range data and simulations. *American Naturalist*, 171, 646–657. https://doi.org/10.1086/587074
- Phillips, S. J., & Dudik, M. (2008). Modeling of species distributions with Maxent: New extensions and a comprehensive evaluation. *Ecography*, 31, 161–175. https://doi.org/10.1111/j.0906-7590.2008. 5203.x
- Pie, M. R., Campos, L. L. F., Meyer, A. L. S., & Duran, V. (2017). The evolution of climatic niches in squamate reptiles. *Proceedings of the Royal Society of London, 284*, 20170268. https://doi.org/10.1098/rspb. 2017.0268

- Pontarp, M., Ripa, J., & Lundberg, P. (2015). The biogeography of adaptive radiations and the geographic overlap of sister species. *American Naturalist*, 186, 565–581. https://doi.org/10.1086/ 683260
- Pyron, A. R., & Burbrink, F. T. (2009). Lineage diversification in a widespread species: Roles for niche divergence and conservatism in the common kingsnake, *Lampropeltis getula*. *Molecular Ecology*, 18, 3443–3457. https://doi.org/10.1111/j.1365-294X.2009.04292.x
- Qiao, H., Escobar, L. E., Saupe, E. E., Ji, L., & Soberón, J. (2017). Using the KDE method to model ecological niches: A response to Blonder et al. (2017). *Global Ecology and Biogeography*, 26, 1076–1077. https://doi.org/10.1111/geb.12610
- R Development Core Team (2015). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available at: http://www.R-project.org/
- Rato, C., Harris, D. J., Perera, A., Carvalho, S. B., Carretero, M. A., & Rödder, D. (2015). A combination of divergence and conservatism in the niche evolution of the Moorish gecko, *Tarentola mauritanica* (Gekkota: Phyllodactylidae). *PLoS ONE*, 10, e0127980. https://doi. org/10.1371/journal.pone.0127980
- Raxworthy, C. J., Pearson, R. G., Zimkus, B. M., Reddy, S., Deo, A. J., Nussbaum, R. A., & Ingram, C. M. (2008). Continental speciation in the tropics: Contrasting biogeographic patterns of divergence in the *Uroplatus* leaf-tailed gecko radiation of Madagascar. *Journal of Zoology*, 275, 423–440. https://doi.org/10.1111/j.1469-7998.2008. 00460.x
- Rosenblum, E. B., Sarver, B. A. J., Brown, J. W., Des Roches, S., Hardwick, K. M., Hether, T. D., ... Harmon, L. J (2012). Goldilocks meets Santa Rosalia: An ephemeral speciation model explains patterns of diversification across time scales. *Evolutionary Biology*, *39*, 255–261. https:// doi.org/10.1007/s11692-012-9171-x
- Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, 8, 336–352. https://doi.org/10.1111/j.1461-0248.2004.00715.x
- Schluter, D. (2001). Ecology and the origin of species. Trends in Ecology and Evolution, 16, 372–380. https://doi.org/10.1016/S0169-5347(01) 02198-X
- Schluter, D. (2009). Evidence for ecological speciation and its alternative. *Science*, 323, 737–741. https://doi.org/10.1126/science. 1160006
- Schneider, C.J., Smith, T.B., Larison, B., & Moritz, C. (1999). A test of alternative models of diversification in tropical rainforests: Ecological gradients vs. rainforest refugia. *Proceedings of the National Academy* of Sciences USA, 96, 13869–13873. https://doi.org/10.1073/pnas.96. 24.13869
- Soberón, J. (2007). Grinnellian and Eltonian niches and geographic distributions of species. *Ecology Letters*, 10, 1115–1123. https://doi.org/ 10.1111/j.1461-0248.2007.01107.x
- Uetz, P., & Hošek, J. (2016). The Reptile Database. Electronic Database. Available at: http://www.reptile-database.org/
- Warren, D. L., Glor, R. E., & Turelli, M. (2008). Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. Evolution, 62, 2868–2883. https://doi.org/10.1111/j.1558-5646.2008.00482.x
- Weir, J. T., & Price, T. D. (2011). Limits to speciation inferred from times to secondary sympatry and ages of hybridizing species along a latitudinal gradient. *American Naturalist*, 177, 462–469. https://doi.org/10. 1086/658910
- Wiens, J. J. (2004a). Speciation and ecology revisited: Phylogenetic niche conservatism and the origin of species. *Evolution*, 58, 193–197. https://doi.org/10.1111/j.0014-3820.2004.tb01586.x
- Wiens, J. J. (2004b). What is speciation and how should we study it? American Naturalist, 163, 914–923. https://doi.org/10.1086/ 386552

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Zheng, Y., & Wiens, J. J. (2016). Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Molecular Phylogenetics and Evolution*, 94, 537–547. https://doi.org/ 10.1016/j.ympev.2015.10.009

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