

Multilocus phylogenetic and geospatial analyses illuminate diversification patterns and the biogeographic history of Malagasy endemic plated lizards (Gerrhosauridae: Zonosaurinae)

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Abstract

Although numerous studies have attempted to find single unifying mechanisms for generating Madagascar's unique flora and fauna, little consensus has been reached regarding the relative importance of climatic, geologic and ecological processes as catalysts of diversification of the region's unique biota. Rather, recent work has shown that both biological and physical drivers of diversification are best analysed in a case-by-case setting with attention focused on the ecological and life-history requirements of the specific phylogenetic lineage under investigation. Here, we utilize a comprehensive analytical approach to examine evolutionary drivers and elucidate the biogeographic history of Malagasy plated lizards (Zonosaurinae). Data from three genes are combined with fossil information to construct time-calibrated species trees for zonosaurines and their African relatives, which are used to test alternative diversification hypotheses. Methods are utilized for explicitly incorporating phylogenetic uncertainty into downstream analyses. Species distribution models are created for 14 of 19 currently recognized species, which are then used to estimate spatial patterns of species richness and endemism. Spatially explicit analyses are employed to correlate patterns of diversity with both topographic heterogeneity and climatic stability through geologic time. We then use inferred geographic ranges to estimate the biogeographic history of zonosaurines within each of Madagascar's major biomes. Results suggest constant Neogene and Quaternary speciation with divergence from the African most recent common ancestor ~30 million years ago when oceanic currents and African rivers facilitated dispersal. Spatial patterns of diversity appear concentrated along coastal regions of northern and southern Madagascar. We find no relationship between either topographic heterogeneity or climatic stability and patterns of diversity. Ancestral state reconstructions suggest that western dry forests were important centres of origin with recent invasion into spiny and rain forest. These data highlight the power of combining multilocus phylogenetic and spatially explicit analyses for testing alternative diversification hypotheses within Madagascar's unique biota and more generally, particularly as applied to phylogenetically and biologically constrained systems.

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Introduction

The search for mechanisms that drive biological diversification in the world's biodiversity hotspots has fuelled decades of research. Time, climate, geographic heterogeneity and physical isolation have been invoked as the prime abiotic drivers of organismal speciation, with the Neotropics serving as the classic test system for investigating their relative importance (e.g. Haffer, 1969; Patton & da Silva, 1998). Increasingly, these studies from the Neotropics have served as a foundation for similar investigations of speciation in Madagascar (e.g. Goodman & Ganzhorn, 2004; Wilmé *et al.*, 2006; Craul *et al.*, 2007; Dewar & Richard, 2007). Madagascar's exceptionally high levels of diversity and endemism must certainly relate to the island's long separation from Africa ~165 million years ago (Ma) and absolute geographic separation ~88 Ma (Hay *et al.*, 1999; Yoder & Nowak, 2006; Vences *et al.*, 2009), yet the factors regulating the diversification and maintenance of high species diversity in some groups (e.g. primates) and low diversity in others (e.g. birds) remain disputed (Vences *et al.*, 2009; Crottini *et al.*, 2012). Most recently, a geospatial modelling approach has shown that mixed models that simultaneously incorporate multiple hypotheses of causality are best able to explain the diversification history of Madagascar's biota. However, a 'one-size-fits-all' model does not exist (Brown *et al.*, 2014). Thus, phylogenetic groups must be examined on a case-by-case basis. Here, we apply this approach to the zonosaurine lizards of Madagascar.

A variety of hypotheses have been proposed to explain diversity patterns in the Malagasy biota, many of which rely on climate change during the Pleistocene (Vences *et al.*, 2009). Although numerous studies of temperate zone speciation for a variety of organisms have supported an association with Quaternary glacial cycles (e.g. Carstens & Knowles, 2007; Mila *et al.*, 2007), similar studies of Neotropical organisms (e.g. Ribas *et al.*, 2005) and subboreal latitudinal comparisons (e.g. Weir & Schluter, 2004) suggest a dominance of pre-Pleistocene diversification (Rull, 2006). Recent estimates of divergence times for Malagasy clades also provide evidence for pre-Quaternary speciation (e.g. Horvath *et al.*, 2008; Townsend *et al.*, 2009; Chan *et al.*, 2012; Wood *et al.*, 2015), with a recent decline in diversification rate for many vertebrate clades (Scantlebury, 2013). However, relatively few studies to date have utilized multilocus coalescent species tree methods to estimate phylogenetic relationships and divergence times within Malagasy clades, which may prove useful for recent diversification events and/or short internodes. Adequately assessing divergence times and relationships is also vital in a biogeographical context to understand how and when endemics colonized each of Madagascar's major biomes (Cornet, 1974).

Recent studies have suggested that specific regions of Madagascar (particularly the north) may be particularly

important centres of diversification (e.g. Boumans *et al.*, 2007; Townsend *et al.*, 2009), but the causes of such patterns remain elusive. As noted by Vences *et al.* (2009), aside from studies focusing on the time of origin (arrival) of Malagasy lineages, relatively little effort has been directed at quantifying spatial and temporal patterns of diversification within these radiations (but see Yoder & Yang, 2004; Poux *et al.*, 2008; Raxworthy *et al.*, 2008; Townsend *et al.*, 2009; Chan *et al.*, 2012; Shi *et al.*, 2013; Wood *et al.*, 2015). Some evidence suggests that altitude and topographic heterogeneity may contribute to high levels of speciation and endemism throughout Madagascar (Wollenberg *et al.*, 2008; Wood *et al.*, 2015). Correlative patterns of endemism with contemporary climatic variables have also been investigated (e.g. Pearson & Raxworthy, 2009). However, less effort has focused on combining multilocus phylogenetic and spatially explicit methods to test for the effects of climatic stability or instability through geologic time on patterns of historical diversification within Madagascar. Arguments for these contrasting processes have been as follows: first, areas of higher instability throughout glacial–interglacial cycles may lead to population fragmentation and allopatric speciation (i.e. a standard refugia model). Conversely, areas experiencing higher climatic stability would likely have lower extinction rates and would allow speciation to progress in a continual fashion through multiple evolutionary processes. Thus, areas experiencing higher stability would also harbour higher species richness and endemism. Indeed, it has been suggested that global levels of diversity are inversely related to the degree to which climatic variables fluctuate over orbital timescales (10–100 kyr Milankovitch cycles), and nowhere is the glacial–interglacial mean annual air temperature difference less than the area around Madagascar, the African Cape and the Atlantic Coastal Forest of Brazil (Dynesius & Jansson, 2000).

Herein we combine multilocus coalescent and geospatial analyses to test a suite of evolutionary hypotheses using one of the more comprehensively sampled (both taxonomically and geographically) groups of Malagasy vertebrates, the endemic plated lizards (Gerrhosauridae: Zonosaurinae). We are particularly interested in elucidating the historical processes important for shaping diversification patterns through space and time. Combining multilocus phylogenetic and geospatial analyses in a single unified framework provides a powerful approach for testing alternative diversification hypotheses throughout Madagascar. For example, although phylogenetic analyses may suggest some degree of Pleistocene speciation in these lizards, geospatial analyses may find weak correlations between Quaternary climate change and spatial patterns of species richness and endemism suggesting that diversification rate may be relatively constant and independent of climate. We address three primary questions in this study:

(1) How are rates of diversification structured through time, and are divergence times solely pre-Quaternary? (2) How and when did the ancestor of zonosaurines colonize Madagascar, and how did dispersal and speciation progress spatially through Madagascar's primary biomes? (3) How does climatic stability through Quaternary glacial maxima and topographic heterogeneity correlate with spatial patterns of diversity throughout Madagascar?

Materials and methods

Taxonomic sampling

Our phylogenetic sampling included a subset of 65 individuals from 17 of the 19 recognized zonosaurine species (Glaw & Vences, 2007) selected from a larger data set of 123 individuals (Raselimanana *et al.*, 2009). No data were currently available for *Z. maximus* or *Z. maramaintso* for the loci used. Although a recent study utilized more genes to elucidate zonosaurine relationships, sampling within taxa was limited (Recknagel *et al.*, 2013). As species tree methods generally perform better with at least two individuals per species (Heled & Drummond, 2010), we chose to utilize genes containing a more comprehensive sampling of individuals. Sequences of the mtDNA cytochrome *b* gene (~1142 bp) and subsets of the nDNA loci NT3 (419 bp) and *C-mos* (542 bp) were employed to assess divergence times of the zonosaurine radiation (Table S1). Details of all molecular laboratory work were reported in Raselimanana *et al.* (2009). Multiple sequence alignments were performed using MAFFT v7.017 (Katoh *et al.*, 2002) implemented through GENEIOUS PRO 6.1.2 (Drummond *et al.*, 2011). No recombinant break-points were detected for the two nuclear loci as inferred through RDP (Martin & Rybicki, 2000), MAXCHI (Maynard-Smith, 1992), and GENECONV (Padidam *et al.*, 1999) analyses implemented in RDP3 (Martin *et al.*, 2010).

Species tree inference and divergence time estimation

We first used our multilocus data to infer both the phylogenetic relationships and divergence times within zonosaurines using the program BEAST v.1.7.5 (Drummond *et al.*, 2012). To incorporate the stochasticity of the coalescent process into parameter inference, we utilized the multispecies, multi-individual algorithm provided in *BEAST (Heled & Drummond, 2010). A maximum of six individuals per species were used – a number shown to be acceptable in multilocus species tree estimation (Heled & Drummond, 2010). To calibrate our analysis and infer divergence times, we included several outgroup taxa to take advantage of currently available fossil information (Table S1). For all constraints, we placed hard minimum bounds on divergence times (using the 'offset' parameter) representing the putative earliest age of the

respective fossil using lognormal priors. First, Estes (1962) described a fossil *Gerrhosaurus* from the Miocene (Burdigalian), which was putatively referred to as *Gerrhosaurus major*. A subsequent study identified additional fossils of Miocene age attributed to *Gerrhosaurus* (Van Couvering, 1979). Thus, we calibrated the most recent common ancestor (MRCA) of Gerrhosauridae (*Zonosaurus/Tracheloptychus* + *Gerrhosaurus*) with a lognormal distribution (mean = 10, log(stdev) = 1), offset = 15). Second, Nydam & Fitzpatrick (2009) described several fossils of Cenomanian-middle Palaeocene age, which they cluster into the taxonomic identity Contogeniidae. Phylogenetic analyses of these fossil taxa show close affinity with contemporary Xantusiidae. Thus, we followed other recent phylogenetic studies (Noonan *et al.*, 2013) and used these data to calibrate the MRCA of Cordyliformes and xantusiids (mean = 10, log(stdev) = 1), offset = 89). All lognormal parameters were chosen to encompass a temporal range (95% CI) consistent with the estimated dates of all fossils used. We ran three sets of analyses using different combinations of these two priors to see how divergence times were affected. For all calibrations, we followed recent estimates of squamate relationships to place our calibrations (Wiens *et al.*, 2012).

We used the program PARTITIONFINDER v.1.0 (Lanfear *et al.*, 2012) to determine both the optimal partition strategy and substitution models for the species tree analysis including all ingroup and outgroup taxa (Table 1). We then used BEAUTI v.1.7.5 to specify species sets to utilize our fossil calibration information. An uncorrelated relaxed clock (lognormal) was specified for each locus, and a Yule tree prior was selected. Each analysis was run for 200 million generations sampling every 20 000 to obtain a posterior sample of 10 000. Multiple chains were run to maximize ESS values (target > 200). Due to suboptimal mixing of GTR model parameters within codons for *cytb*, we specified a single GTR+I+G model for this gene, which greatly improved overall mixing. Parameter estimates and trees were combined in LogCombiner, and a maximum clade

Table 1 Data partitions and corresponding substitution models selected for all phylogenetic analyses.

Partition	Base pairs	Model
Cytochrome <i>b</i> position 1	381	GTR+H+G
Cytochrome <i>b</i> position 2	381	GTR+H+G
Cytochrome <i>b</i> position 3	381	GTR+H+G
Cmos position 1	139	K80+G
Cmos position 2	139	SYM+H+G
Cmos position 3	139	K80+H
Cmos position 1+position 2	278	NA
NT3 position 1+position 2	360	HKY
NT3 position 3	180	K80+H

NA, not applicable.

credibility tree was generated in TreeAnnotator. All *BEAST analyses were run on the Duke Shared Cluster Resource (DSCR). Adequate convergence of all population size parameters was assessed through Perl scripts using biopy (J. Heled, pers. comm.).

Diversification analysis

As previous research has suggested recent declines in diversification rates in the Malagasy herpetofauna (Scantlebury, 2013), we first used the R package APE (Paradis *et al.*, 2004) to construct lineage through time (LTT) plots for the species trees. We removed all outgroup taxa prior to analysis. We then used the MCC tree (with outgroups removed) to calculate the gamma statistic (γ) to test for diversification rate constancy (Pybus & Harvey, 2000). The null distribution and critical value of γ were determined using the `mccrTest` in LASER (Rabosky, 2006) assuming an incompletely sampled phylogeny and 5000 replicates. We also calculated γ for a sample of 10 000 trees from the posterior distribution to explicitly incorporate phylogenetic uncertainty. We then used the mean γ from all trees in a second `mccrTest` to calculate a new critical value of γ and test for significance.

We also tested the fit of alternative diversification models to the data. Specifically, we compared the likelihoods of the following models: pure birth (Yule), birth–death (bd), diversity-dependent diversification with no extinction (DDL–E) and diversity-dependent diversification with extinction (DDL+E). All analyses were performed in the R package `DDD` v.2.4 (Etienne *et al.*, 2012) using the `dd_ML` function to estimate relevant parameters (e.g. lambda, mu and carrying capacity). Because of computational limitation, all analyses were based on a random sample of 100 trees and we set the number of missing species to two to account for incomplete taxon sampling (Glaw & Vences, 2007). We then used AIC to compare alternative models and calculated AIC weights in the R package `MUMIN` v. 1.9.13. We followed Burnham & Anderson (2002) to interpret AIC values.

Species distribution models, species richness and endemism

Because simple point data may not accurately reflect full geographic ranges of species (Graham & Hijmans, 2006), we first created species distribution models (SDMs) using the program `MAXENT` v 3.3.3k (Phillips *et al.*, 2006; Phillips & Dudik, 2008). We chose the maximum entropy method in `MaxEnt` to model species distributions as previous studies have suggested the method to be robust, particularly with rare species encompassing small sample sizes (e.g. Elith *et al.*, 2006; Pearson *et al.*, 2007; Wisz *et al.*, 2008). Georeferenced locality data for each taxon were obtained from personal (M. Vences, A. Raselimanana) and public

databases (e.g. HerpNet, www.herpnet.org). All locality data were plotted in ArcMap v.10.1 (ESRI) to check for potentially erroneous records that differ from the presently known distribution of species (Glaw & Vences, 2007). A total of 19 current bioclimatic (BIOCLIM) variables were obtained from the WorldClim database for all modelling (Hijmans *et al.*, 2005). These data were combined with vegetation and geology layers obtained with the permission of the Trustees of the Royal Botanic Gardens, Kew. A digital elevation model (dem) layer was also used for all modelling (USGS, 2004). Prior to model fitting, all layers were projected to a Tananarive/Laborde projection in ArcMap at a resolution of 919 m², which was designed specifically for Madagascar.

We created SDMs for 14 of the 19 currently recognized zonosaurine species. All remaining species had been sampled from fewer than five unique points, which may preclude accurate inference of SDMs (Pearson *et al.*, 2007). The total number of points used to train models ranged from 5 to 66 depending on the species (duplicate locality records were removed prior to model training). We used the default regularization multiplier of 1 for all modelling (Anderson & Gonzalez, 2011; Cao *et al.*, 2013). The default of 10 000 background points was used to train models. Because of our relatively small number of points for many species, we selected the bootstrap method of replication using 100 replicates. To convert logistic probabilities to binary presence–absence data, we applied the maximum training sensitivity plus specificity threshold on the average raster file from the 100 replicates for each species as this threshold has recently been shown to be preferable over alternative methods (Cao *et al.*, 2013; Liu *et al.*, 2013). To further minimize the risk of overprediction, we used `SDMTOOLBOX` v1.0 (Brown, 2014) to clip each SDM by creating minimum convex polygons around sample points for each species with a buffer of 100 km. All post-processing of SDMs was performed in ArcMap.

To estimate both spatial patterns of species richness and endemism, we combined the clipped binary SDMs for each species with point data from species with too few records to model. For the latter, we created 25 km buffers around each point to approximate geographic distribution. Species richness was estimated by summing all binary layers and counting the number of species present in a specific cell. Because some estimates of endemism can be highly correlated with species richness (e.g. weighted endemism), we utilized corrected weighted endemism (CWE) as a metric to control for the number of species present in a given cell. CWE can be defined as weighted endemism (a metric based on weighting each species by the inverse of its cell range for all species in a particular cell) divided by the total number of species in a cell. For example, weighted endemism can be calculated as

$$WE = \sum_{i=1}^n 1/C$$

where n represents the total number of endemics and C represents the number of grid cells each endemic occurs in. CWE can then be calculated simply as

$$CWE = WE/K$$

where K is the total number species in a grid cell.

As CWE is generally related to, but uncorrelated with species richness, this estimate provided a fully independent comparison to examine spatial patterns of diversity (Crisp *et al.*, 2001). Both species richness and CWE were estimated using the `SDMTOOLBOX` in `ARCMAP`.

Climatic stability and topographic heterogeneity

We created a set of climatic stability layers spanning four periods through geological time (i.e. 120, 21, 6 and 0 kya) (climate data from: Hijmans *et al.*, 2005; Braconnot *et al.*, 2007). Three different stability layers were constructed, including temperature, precipitation, and combined temperature and precipitation. Prior to constructing our stability layers, we tested for collinearity among all 19 BIOCLIM layers and excluded variables from further analysis if the resulting Pearson coefficient exceeded 0.5. The following variables were excluded from further consideration: Bio3, Bio7, Bio9 and Bio13–Bio18. For each remaining BIOCLIM layer, we calculated the standard deviation of cell values over the four time periods. The resulting standard deviation layer was then standardized to 1 to account for different ranges of variation among layers. Once a standardized standard deviation layer was calculated for each variable, variables within each class (temperature or precipitation) were summed to create a single final stability layer, with lower cell values representing areas of higher stability. A topographic heterogeneity layer was generated by calculating cell-specific (10 km²) standard deviation values for altitude based on digital elevation data from the NASA Shuttle Radar Topographic Mission (SRTM; <http://www.cgiar-csi.org/data/srtm-90m-digital-elevation-database-v4-1#citation>).

Spatial and ancestral state analyses

To estimate ancestral distributions and to elucidate the biogeographic history of Malagasy plated lizards, we used the S-DIVA method implemented in `RASP v2.1b` (Reconstruct Ancestral States in Phylogenies; Yu *et al.*, 2010, 2013). We were particularly interested in determining how many times zonosaurines colonized each of Madagascar's major biomes. Contemporary distribution was classified into major habitat types (i.e. dry forest, spiny forest, rain forest and subhumid forest) following Cornet (1974). Due to the low support for

many nodes in the MCC tree (see Results), all ancestral state reconstructions were based on 10 000 trees from the posterior distribution of our combined *BEAST analyses to explicitly incorporate phylogenetic uncertainty. We used spatially explicit conditional (CAR) and simultaneous (SAR) autoregression models to test for correlations between the climate stability and topographic heterogeneity layers and our observed biodiversity patterns in the program Spatial Analysis in Macroecology (`SAM v.4.0`; Rangel *et al.*, 2010). Additional details of all spatial and ancestral state analyses can be found in the Supplementary Materials.

Results

Species trees and divergence time estimation

All ESS values from the combined *BEAST runs were > 100 with the majority > 200 indicating adequate sampling of the posterior. All individual and combinations of calibrations suggested a Cenozoic origin for zonosaurines (see Table S2), with 95% HPDs overlapping substantially among different calibration schemes. However, using only *Gerrhosaurus major* for calibration resulted in an unrealistically young divergence time for the split between Xantusiidae and Cordyliformes (~45 Ma). Thus, for the remainder of the study, we focus on the results using both calibration points.

Posterior probabilities for relationships were generally low to moderate, although there was high support for several nodes (Fig. 1). Monophyly of both *Zonosaurus* and *Tracheloptychus* was strongly supported. Relatively low support was found for the species groups previously defined in Recknagel *et al.* (2013), although our analysis did recover a monophyletic *Z. rufipes* group. Our analysis also provided strong support for the sister relationships of the following taxa: *Z. trilineatus* + *Z. quadrilineatus*; *Z. laticaudatus* + *Z. anelanelany*; *Z. madagascariensis* + *Z. haraldmeieri*. Divergence of Zonosaurinae from African Gerrhosauridae (represented here by the genus *Gerrhosaurus*) occurred approximately 30 Ma. Cladogenesis within the Malagasy clade began in the Miocene at about 20 Ma and proceeded gradually through the Neogene and Quaternary (Fig. 1). Three species pairs showed divergence times during the Pleistocene.

Diversification analyses

We obtained a γ value of -1.020035 based on the outgroup-pruned MCC species tree, which was not significant based on simulating 5000 incompletely sampled phylogenies (γ critical = -1.771847 ; $P = 0.1834$), indicating a constant diversification rate. In addition, we calculated γ for 10 000 trees from the posterior distribution and obtained a mean value of -0.864566 (Fig. S1). Using this value in a second `mccrTest` also failed to

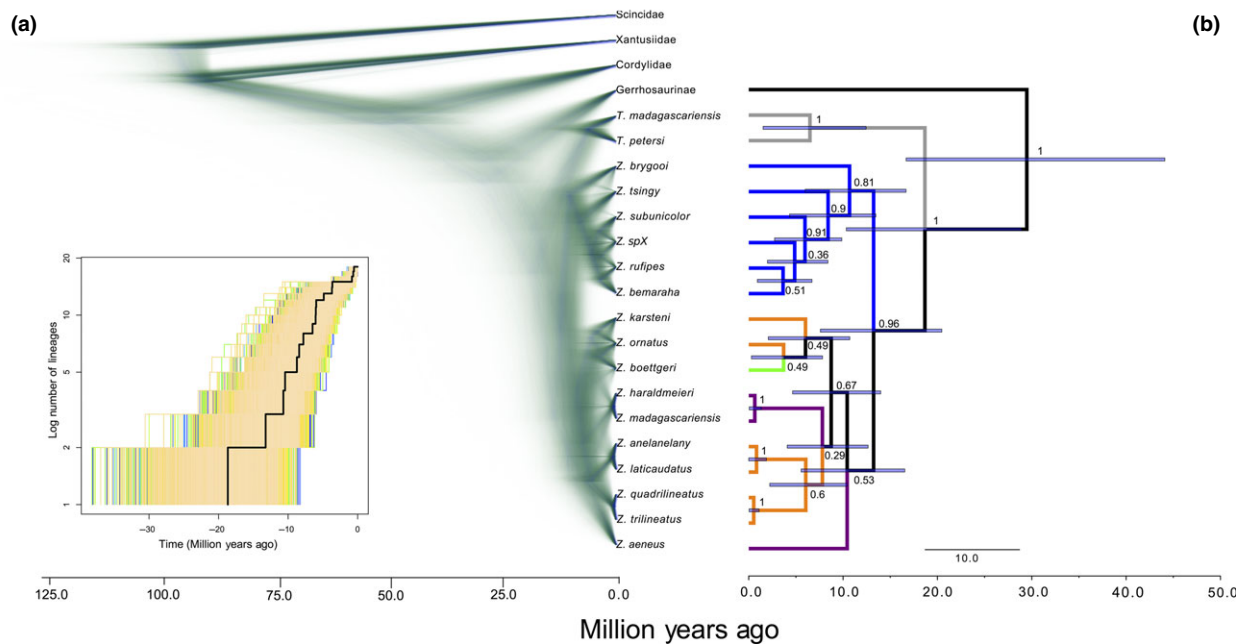


Fig. 1 Species tree results from *BEAST using all three loci. (a) ~35 000 trees from the posterior distribution of multiple independent runs and visualized using the software DENSITREE (Bouckaert, 2010). LTT plot is based on 10 000 trees within the 95% HPD with the MCC tree indicated by the solid black line. (b) MCC tree from the entire posterior distribution colour-coded by species groups defined in Recknagel *et al.* (2013).

reject a constant diversification rate (γ critical = -1.78515 ; $P = 0.2278$). We then compared a suite of alternate diversification models and found the strongest support (based on AIC weights) for a pure birth (Yule) model (Table 2). Although a diversity-dependent model without extinction had some support, the average carrying capacity value for this model (based on 100 trees) included infinity.

Ancestral state reconstruction and biogeographic patterns

Our biogeographic analyses based on 10 000 species trees (to explicitly incorporate phylogenetic uncertainty) in S-DIVA suggested that the majority of

zonosaurine ancestors were concentrated throughout the western dry forests (Fig. 2; see Fig. S2 for levels of uncertainty in ancestral reconstructions). Ancestral distributions for both the MRCA of *Zonosaurus* and for the MRCA of all Malagasy plated lizards were ambiguous. However, results suggested that the subarid spiny forests were colonized twice: once in the *Tracheloptychus* lineage and once in the lineage leading to a clade containing *Z. anelanelany*, *Z. laticaudatus*, *Z. quadrilineatus* and *Z. trilineatus*. Dispersal (represented by blue circles; Fig. 2) occurred multiple times through the evolutionary history of the group, with invasion into the eastern rain forests occurring relatively recently for most species. An extinction event was suggested for the MRCA of *Zonosaurus* (yellow circle; Fig. 2).

Table 2 Comparison of alternate diversification models for Malagasy zonosaurine lizards. All statistics represent mean values from 100 random trees from a *BEAST analysis. Parameters were estimated assuming incomplete taxon sampling (two missing species; Glaw & Vences, 2007).

Model	Lambda	mu	K	loglik	d.f.	AIC	Delta AIC	AICw
Pure birth	0.1388	0.0000	Infinity	-48.7135	1	99.42699	0.0000	0.4720
DDL-E	0.1954	0.0000	Infinity	-48.3140	2	100.6279	1.2009	0.2590
Birth-death	0.1395	0.0014	Infinity	-48.7116	2	101.4233	1.9963	0.1740
DDL+E	0.1959	0.0014	Infinity	-48.3123	3	102.6246	3.1976	0.0950

DDL-E, diversity-dependent diversification without extinction; DDL+E, diversity-dependent diversification with extinction.

Carrying capacity values (K) for pure birth and birth-death models were fixed to infinity, whereas a mean value of infinity was estimated from both diversity-dependent models.

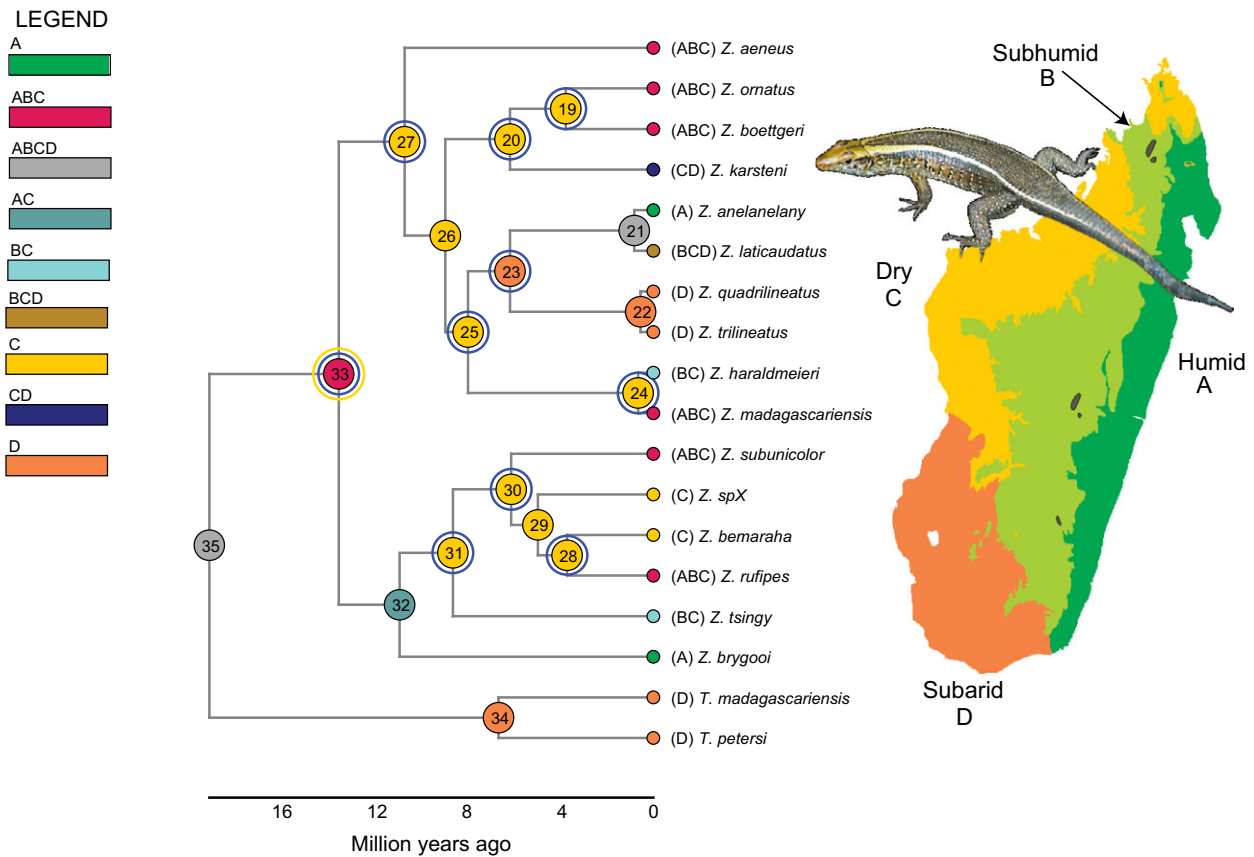


Fig. 2 Biogeographic history for Malagasy zonosaurines inferred through the S-DIVA method in RASP. Ancestral states were inferred from 10 000 species trees from *BEAST to explicitly account for both biogeographic and phylogenetic uncertainty. Results are summarized on the *BEAST MCC tree. For clarity only the most likely ancestral states are shown. Blue circles surrounding nodes represent inferred dispersal events, whereas the yellow circle represents an extinction event. Coloured circles at tips represent present ranges for each species as inferred through our clipped species distribution models. Map is redrawn from Cornet (1974).

Environmental predictors of spatial patterns of diversity

Summing binary SDMs with buffered ranges from unmodelled species indicated that certain regions of Madagascar harbour slightly higher levels of richness and endemism (Fig. 3a,b). Richness was predominantly concentrated in both coastal northern and southern Madagascar. Similar patterns were recovered for CWE, with the north and south showing higher values. However, exceptionally high endemism was observed for central-western Madagascar. Results from SAR and CAR analyses indicated no significant relationship between topographic heterogeneity and climatic stability with species richness (Table S3) or CWE (Table S4). The only models with a significant overall *P*-value were SAR models for richness vs. temperature stability and SAR and CAR models for topographic heterogeneity. Though significant, focal environmental predictors (betas) for these models did not show significant *P*-values, suggesting that

the significance of these models was driven primarily by space (i.e. geographic proximity). AICc scores were substantially lower (and R^2 values higher) for all models that included a spatial component, further indicating that space was the primary driver of patterns of diversity (see Supplementary Material).

Discussion

Rates of diversification and speciation

Divergence time estimates of zonosaurines indicate speciation events occurring gradually throughout the Neogene and the Quaternary. This finding conflicts with findings from other studies of Malagasy species/species pairs of vertebrates. Virtually all such studies have found that the divergence age of species pairs occurred considerably before the Quaternary period (e.g. Yoder & Yang, 2004; Raxworthy *et al.*, 2008; Wollenberg *et al.*, 2011; Chan *et al.*, 2012; Wood *et al.*, 2015); but see

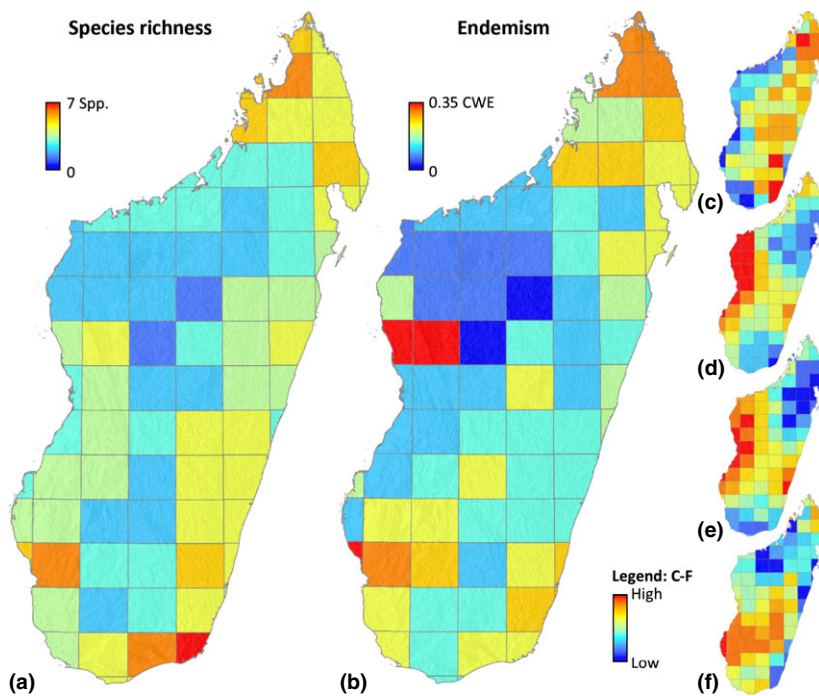


Fig. 3 (a) Estimates of spatial patterns of zonosaurine species richness. (b) Estimates of spatial patterns of zonosaurine corrected weighted endemism (CWE). (c) Topographic heterogeneity. Estimates of climate stability since the last interglacial: (d) Total climate stability, (e) Temperature stability and (f) Precipitation stability. CWE was calculated by dividing weighted endemism (a composite metric based on the inverse of a species geographic range for all species in a given cell) by the total number of species in the cell.

(Russell *et al.*, 2007). In part, these contradictory observations could relate to methodological if not taxonomic bias. Whereas we employ coalescence-based analytical methods to infer divergence times, other studies have relied primarily on more traditional phylogenetic (concatenation) approaches. Further, of the three youngest species pairs of zonosaurines, two differ consistently by colour pattern only and require taxonomic revision (*Z. madagascariensis*/*Z. haraldmeieri* and *Z. quadrilineatus*/*Z. trilineatus*), but the third pair (*Z. anelanelany*/*Z. laticaudatus*) differs in numerous morphological characters and thus appears to represent an unequivocal Pleistocene divergence, thus validating our finding.

Our estimated phylogenetic relationships also differ from those of previous studies (Raselimanana *et al.*, 2009; Recknagel *et al.*, 2013). Although we utilize similar data to Raselimanana *et al.* (2009), we obtain several conflicting relationships with relatively high support. For example, *Zonosaurus* is recovered as monophyletic with strong support in our analysis. Further, in our analysis *Z. trilineatus* and *Z. quadrilineatus* form a clade sister to *Z. laticaudatus* and *Z. anelanelany*, whereas in the previous analysis (Raselimanana *et al.*, 2009) the latter clade is hypothesized to fall at the base of all zonosaurines. In both analyses, *Z. laticaudatus* and *Z. anelanelany* are sister with strong support. Our results also differ in some aspects from a more recent study of zonosaurine phylogeny that includes several nuclear and mitochondrial genes and utilizes similar coalescent methods (Recknagel *et al.*, 2013). Differences regard the placement of the *Z. madagascariensis* group and of *Z. bo-*

ettgeri within the *Z. ornatus* group, whereas the two analyses are concordant in recovering a monophyletic *Z. rufipes* group (to the exclusion of *Z. aeneus*), and suggesting monophyly of *Zonosaurus* (strongly supported in our analysis). Unfortunately, support for deeper nodes in the present study as well as all previous studies remains weak. Thus, it appears that additional data are required to fully resolve some deeper nodes within *Zonosaurus*. Finally, our results contrast with recent analyses that suggest rates of diversification in several Malagasy reptile and amphibian clades have declined closer to the present day (Scantlebury, 2013). Although the inability of our diversification analyses to reject a pure birth model may be due to the small number of species of zonosaurines, previous diversification analyses on other Malagasy clades of equal size were able to reject models of constant diversification (Scantlebury, 2013). This suggests that bias due to sample size may be minimal in our data set.

Spatial characteristics of the zonosaurine radiation

Our results suggest that Malagasy zonosaurines diverged from their African relatives ~30 Ma, a timing which corresponds with dispersal in many other vertebrate groups (e.g. Townsend *et al.*, 2011; Crottini *et al.*, 2012; Tolley *et al.*, 2013). During this time the position of Madagascar was south of its present location, and both ocean currents and palaeorivers were conducive to dispersal from the mainland (Markwick & Valdes, 2004; Ali & Huber, 2010). Although the ancestral distribution of the MRCA of all zonosaurines remains ambig-

uous (Fig. 2), our results in conjunction with the direction and magnitude of ocean currents in the Eocene–Oligocene suggest that the MRCA may have inhabited the dry deciduous forest of northwest Madagascar. Indeed, our results suggest that dry forest was most likely the preferred habitat of Malagasy plated lizards throughout much of their evolutionary history with two independent invasions into the spiny dry forests of the south. Subsequent invasion to the south was also recently inferred for frogs of the genus *Gephyromantis* (Kaffenberger *et al.*, 2012). Interestingly, dispersal into eastern rain forest has occurred independently several times and only recently for most species. These results contrast with a recent study of Malagasy spiders, which finds that eastern rain forests were the most likely ancestral habitat with subsequent invasions into western deciduous and southern spiny forest (Wood *et al.*, 2015). Additional studies focused on Malagasy endemics are needed to test the relative importance of deciduous vs. rain forest habitat as centres of diversification.

Environmental predictors of spatial patterns of diversity

Our estimates of spatial patterns of species richness and endemism are similar to those reported for many vertebrate clades including cophyline frogs (Wollenberg *et al.*, 2008), with moderate values of both richness and endemism in the north. We also find high levels of endemism in western Madagascar, due predominantly to two range-restricted species. However, our results also suggest relatively high levels of richness and endemism in southern Madagascar, a finding that has yet to be recovered in many other taxonomic groups. Although our phylogenetic analyses suggest that three species pairs diverged during the Quaternary, our spatial analyses suggest no correlation between climatic instability through glacial maxima and patterns of species richness and endemism. Our results also show no relationship between topographic heterogeneity and species richness or endemism, in contrast with previous studies (Wollenberg *et al.*, 2008).

The lack of significant correlations observed may be biological or methodological. For example, our modelling approach was complex and relied on diverse types of data and assumptions. How our stability layers were generated, how the SDMs were created and processed, the resolution of analysis, and methods of statistical analysis are all factors that may influence our results. Further, the climate data represent a limited temporal scope (spanning back to *ca.* 120 kya), and the climatic stability beyond this period is not explicitly incorporated. The historic climate data do, however, represent known climatic extremes and may serve as proxies for deeper climatic oscillations. In addition, our lack of correlation may be a result of the number of species used for modelling. Although zonosaurines represent one of

the more speciose endemic reptile clades, the small difference in richness between species rich and species poor areas may simply result in low power once spatial autocorrelation is accounted for.

Alternative diversification hypotheses

Proposed links between Quaternary glacial cycles and the origin of species level diversity in Madagascar (e.g. river-refuge hypothesis – Wilmé *et al.*, 2006) are generally not supported by climate records nor the ages of most species divergences examined to date (e.g. Horvath *et al.*, 2008; Poux *et al.*, 2008; Raxworthy *et al.*, 2008; Townsend *et al.*, 2009; Chan *et al.*, 2012; Wood *et al.*, 2015). Species distributions for many groups, including zonosaurines, do not fit well with this hypothesis. Other hypotheses, including the generally accepted null hypothesis of a mid-domain effect (Laurie & Silander, 2002), do not appear valid for zonosaurines as diversity appears concentrated in both the north and south. Clearly, models of Malagasy diversification must consider both Quaternary and ancient climate change both within Madagascar and globally. For example, palaeoclimate data suggest that the Oligocene–Miocene boundary was a period of extreme global climate change (Zachos *et al.*, 1997). It is thus highly possible that the older divergence dates for many Malagasy clades, including initial cladogenesis within zonosaurines, was a result of these processes. Finally, the fact that our ancestral state analyses suggest widespread dispersal events indicates that peripatric speciation through founder effects may have been important for the group.

Conclusions

Our results suggest constant diversification rates throughout the Neogene and Quaternary with recent independent invasions into the eastern rain forests. The detection of speciation events in the Quaternary differs from patterns observed for other vertebrate radiations in Madagascar, and at least for one species pair, this young divergence cannot be readily explained by a flawed taxonomy. Assuming that the species in question are valid, ~80% of diversification within zonosaurines occurred prior to the Pleistocene. This contrasts sharply with a recently published large-scale chameleon phylogeny where virtually all speciation events (> 99%) in Malagasy representatives (*Brookesia*, *Calumma*, *Furcifer*) occurred well before the Pleistocene (Tolley *et al.*, 2013). Although we detect no relationship between climate and diversity, additional studies are needed focusing on different groups explicitly linking genetic and geospatial analyses. A recovered linkage between climatic stability and biotic diversity in Madagascar would have profound implications for the future survival of Malagasy biodiversity in the face of

projected climate change. Further, the methods employed in this study can be easily applied to test diversification hypotheses in taxa outside of Madagascar. Explicitly linking genetic and geospatial data in a hypothesis-testing framework provides a powerful approach for understanding the diverse processes governing spatial patterns of biodiversity. Only through synergistic approaches can we begin to mitigate the gathering storm of threats to global biodiversity.

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Author contributions

CB, BPN, JLB, APR and ADY conceived the study. CB, BPN and JLB conducted all statistical analyses. APR and MV collected the majority of zonosaurine material examined. All authors contributed to the writing of the paper.

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Supporting information

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

Figure S1 Frequency histogram of the γ -statistic based on 10 000 multilocus species trees (mean = -0.865).

Figure S2 Biogeographic history for Malagasy zonosaurines inferred through the S-DIVA method in RASP.

Table S1 Species, locality information, and GenBank accession numbers for all individuals used in this study. ND = No Data.

Table S2 Posterior distribution of divergence times for Zonosaurinae plus representative outgroups based on different calibration schemes.

Table S3 Ordinary least-squares (OLS) and spatial autoregression results between species richness, topographic heterogeneity and different measures of climatic stability through geologic time.

Table S4 Ordinary least-squares (OLS) and spatial autoregression results between corrected weighted endemism, topographic heterogeneity and different measures of climatic stability through geologic time.

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