A global phylogeny of turtles reveals a burst of climate-associated diversification on continental margins

Robert C. Thomson[a,1], **Phillip Q. Spinks**[b,c], **and H. Bradley Shaffer**[b,c]

[a]School of Life Sciences, University of Hawaiʻi, Honolulu, HI 96822; [b]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, CA 90095; and [c]La Kretz Center for California Conservation Science, Institute of the Environment and Sustainability, University of California, Los Angeles, CA 90095

Edited by David B. Wake, University of California, Berkeley, CA, and approved December 30, 2020 (received for review June 12, 2020)

Living turtles are characterized by extraordinarily low species diversity given their age. The clade's extensive fossil record indicates that climate and biogeography may have played important roles in determining their diversity. We investigated this hypothesis by collecting a molecular dataset for 591 individual turtles that, together, represent 80% of all turtle species, including representatives of all families and 98% of genera, and used it to jointly estimate phylogeny and divergence times. We found that the turtle tree is characterized by relatively constant diversification (speciation minus extinction) punctuated by a single threefold increase. We also found that this shift is temporally and geographically associated with newly emerged continental margins that appeared during the Eocene–Oligocene transition about 30 million years before present. In apparent contrast, the fossil record from this time period contains evidence for a major, but regional, extinction event. These seemingly discordant findings appear to be driven by a common global process: global cooling and drying at the time of the Eocene–Oligocene transition. This climatic shift led to aridification that drove extinctions in important fossil-bearing areas, while simultaneously exposing new continental margin habitat that subsequently allowed for a burst of speciation associated with these newly exploitable ecological opportunities.

turtle tree of life | divergence time estimation | GeoSSE | HiSSE | global climate change

Global patterns of species diversity vary markedly across segments of the tree of life. Environmental changes, across spatial and temporal scales, are expected to influence patterns of species richness and distribution, and documenting these macroevolutionary effects can aid our understanding of historical and current environmental impacts on biodiversity (1). This is particularly important for clades of high conservation concern that are declining due to human-mediated climate change. While conservation actions for these clades must necessarily operate on local scales to protect individual species, a more global understanding of how past climate changes have impacted their biodiversity may inform more coordinated management efforts in a rapidly advancing Anthropocene.

Turtles (Testudines) are an evolutionarily unique and morphologically distinctive clade of vertebrates that are imperiled on a global scale (2, 3). Characterized by the presence of a bony carapace and plastron that house both the pectoral and pelvic girdles, turtles possess one of the most derived tetrapod morphologies known. They are also an ancient clade, with stem fossils dating to the Late Permian or Triassic and a crown age estimated at 220 million years ago (mya) (4–6). Turtles enjoy an essentially global distribution spanning an ecologically diverse set of terrestrial, marine, and freshwater habitats, and have invaded each multiple times during their long history (7, 8).

After they appear in the fossil record near the time of the earliest dinosaurs, testudines rapidly expanded in geographic range and ecological diversity (6, 9). The speed with which this diversity built up suggests that turtles may have undergone a rapid, early adaptive radiation (10). However, this early diversification was not sustained, and living turtle diversity is low (348 extant species; ref. 11). Testudine diversity is strikingly depauperate relative to other, much younger crown amniote clades such as birds (>10,000 species with a crown age 111 mya), mammals (>5,000 species, 177 mya), and squamate reptiles (>10,000 species, 201 mya; refs. 12–15).

At present, turtles are also one of the most endangered clades of vertebrates on the planet. More than one-third of chelonian species fall in the International Union for Conservation of Nature (IUCN) Endangered, Critically Endangered, or Extinct categories, and more than three-quarters of species are at serious risk (IUCN Red List categories of Near Threatened, Vulnerable, or greater; see ref. 12). In addition to well-documented losses from human exploitation, several novel, climate-associated conservation challenges have recently emerged, ranging from increasing incursions of sea water into freshwater habitats (16), to sex ratio shifts associated with temperature-dependent sex determination that most turtles employ (17), to the wholesale loss of critical nesting areas (18).

Thus, from both macroevolutionary and conservation biology perspectives, turtles represent a biodiversity conundrum. As a lineage, they are long lived, and morphologically and ecologically

Significance

Biodiversity is unevenly distributed across the tree of life. Understanding the factors that led to this unevenness can illuminate how macroevolutionary processes have interacted with changing global environments to shape patterns of biodiversity. By developing a comprehensive phylogeny for extant turtles and analyzing the diversification dynamics of the group, we show that species-level diversity is strongly associated with historical climate shifts. Our findings indicate that newly exposed continental margins created during a period of cooling and drying are important evolutionary cradles for turtle speciation, explain why turtle biodiversity is orders of magnitude more depauperate than the remaining major lineages of amniotes, and reconcile the seemingly contradictory insights that fossils and extant species suggest into a single picture of evolutionary diversification.

Author contributions: R.C.T., P.Q.S., and H.B.S. designed research; R.C.T., P.Q.S., and H.B.S. performed research; R.C.T. and P.Q.S. analyzed data; and R.C.T., P.Q.S., and H.B.S. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

Published under the PNAS license.

[1]To whom correspondence may be addressed. Email: thomsonr@hawaii.edu.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2012215118/-/DCSupplemental.

Published February 8, 2021.

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

conservative, suggesting evolutionary resilience. However, their low species richness and high degree of endangerment point to the fragility of the lineage in the human-dominated Anthropocene, rendering them an important conservation target. Understanding the history of diversification responsible for the modern diversity of turtles may hold important clues to their future survival, but has been hindered by the lack of a robust, well-sampled, time-calibrated phylogeny. A growing number of molecular phylogenetic studies have targeted the deep family-level relationships within the group (5, 19–24), or more narrowly focused within-family or within-genus relationships with more complete species level sampling (25–42). Despite these efforts, a well-sampled multilocus phylogeny for the clade has remained elusive, in large part because of the difficulty of obtaining tissue samples from the many rare/endangered taxa. Several studies have assembled existing data from GenBank to increase the number of species included in a tree. While informative, this approach necessarily comes with the cost of large blocks of missing data which can decrease accuracy in estimates of both phylogeny and divergence times (43–46). As a consequence, studies of turtle diversification have largely focused on limited geographic, taxonomic, or temporal scales, with little synthesis of the global evolutionary mechanisms responsible for chelonian species richness. Our goal is to provide a synthetic, global phylogeny of turtles, infer historical changes in speciation and extinction rates, and begin a conversation using those insights to improve conservation and management. Given the current conservation crisis faced by the group, addressing this knowledge gap is both timely and critical.

Most studies of turtle diversification have focused on patterns observed in the fossil record. Some paleontological analyses have reported an association between global climate patterns and increases in species richness (10, 47), whereas others have found little evidence for such an association (48). Nicholson et al. (10) analyzed genus-level richness of fossils throughout the Mesozoic. After correcting for sampling biases, they found a period of increasing diversity beginning in the Late Jurassic and continuing into the Cretaceous that coincided with global climate warming (including the Cretaceous Thermal Maximum, ∼85 mya to 90 mya). This study also documented a synchronous global range expansion into higher paleolatitudes, raising the possibility that increased diversity was associated with greater habitat availability in temperate regions (10). Efforts to model environmental suitability envelopes for fossil taxa have similarly suggested climate as a key component of habitat suitability (47). However, at the morphological level, Foth et al. (48) analyzed cranial disparity (essentially a measure of variability across morphospace that serves as a proxy for diversity) among fossil turtles. Foth et al. (48) recovered a period of increasing disparity throughout the Jurassic and Cretaceous, followed by a plateau after the Cretaceous–Paleogene boundary, and, finally, a rapid increase beginning in the Miocene. Foth et al. (48) found, at most, weak evidence for a correlation between global climate and diversity, and instead concluded that disparity (and thus diversity) is linked to biogeographic processes associated with continental breakups and reaggregation.

At more regional scales, the fossil record indicates marked decreases in turtle species richness associated with periods of cooling and drying, especially in western North America across the Eocene−Oligocene transition roughly 34 mya (49, 50). This area formerly included extensive warm wetlands that supported a diverse chelonian community (50). As western North America dried, this fauna disappeared from the fossil record (49, 50). Taken together, the paleontological record suggests a global pattern of gradually increasing diversity through the Cretaceous, with a potential acceleration beginning in the Miocene. However, evidence for the climatological, biogeographic, or other drivers of these patterns are not well understood, and potentially operate differentially depending on geographic scale and context.

To better understand these processes, we investigated the global diversification history of turtles using molecular phylogenetic data. To do so, we developed a well-sampled, nearly complete phylogeny for the world's extant turtles and used it to estimate divergence times and quantify the temporal and geographic patterns of diversification that gave rise to present-day diversity. We found relatively steady rates of diversification throughout most of the group's history, followed by a sharp increase in diversification rates within the last 50 million years that coincides with a long period of global cooling, large scale sea level declines (>100 m), and the consequent availability of extensive, previously unavailable habitat. These rate increases are geographically concentrated along coastal margins and low-lying areas that were exposed as sea levels fell, suggesting that climate change has been an important driver in patterns of both local extinction and speciation during the evolution of turtles.

Results and Discussion

Phylogeny. We developed a well-sampled phylogenetic hypothesis for the extant Testudines. Our taxon sampling includes all 14 living families, 90 of the 92 living genera (98%), and 279 of the 348 living species (80%) of turtles (Table 1). When possible, we included two (occasionally more) individuals per species to allow an assessment of species-level monophyly, resulting in a full dataset of 591 individual turtles.

The tree recapitulates several previously identified relationships in the deep history of the group (Figs. 1 and 2 and *SI Appendix*, Fig. S1) (5, 19–23). We recovered the early divergence between the Cryptodira (hidden-necked turtles) and Pleurodira (side-necked turtles), with maximal support values (Bayesian posterior probabilities = 1); within Cryptodira, we recovered softshell turtles (Trionychia) as the sister group to Durocryptodira (hard-shelled cryptodires); and, within Pleurodira, Chelidae and Pelomedusoides are reciprocally monophyletic (Figs. 1 and 2 and *SI Appendix*, Fig. S1). We recovered all 14 currently recognized families as monophyletic, with interfamilial relationships that are congruent with recent molecular analyses. At more recent levels of divergence, we recovered several notable relationships that we discuss in greater detail in the *SI Appendix*.

Divergence Times and Diversification. Crown turtles likely originated in the Late Triassic ∼210 mya (4, 5). The paleontological record for the group is rich and one of the best known of any major vertebrate lineage (10, 51, 52). Using a series of well-characterized fossil calibrations from Joyce et al. (4) and a version of the molecular dataset that was reduced to one individual per species, we coestimated phylogeny and divergence times for the living turtles (Figs. 1 and 2). This analysis indicates that the two major lineages within the clade, Cryptodira and Pleurodira, diverged in the Late Triassic (median 208 mya; 95% highest posterior density [HPD] 183 mya to 236 mya), followed by the Early Jurassic divergence between the softshell Trionychia (Carettochelyidae and Trionychidae) and the remaining cryptodires 182 mya (95% HPD 161 mya to 200 mya). Most recognized families diverged in the Late Cretaceous or Early Paleogene (4, 5, 46, 53, 54).

Some earlier analyses of the fossil record suggest that climate has played a role in shaping diversity through geological time (10, 47, 49, 50), albeit in different ways and at different scales (also see ref. 48). The number of fossil genera increased in both number and total occupied latitudinal range in the lower Cretaceous (145 mya to 100 mya) and again following the Cretaceous Thermal Maximum in the Late Cretaceous 85 mya to 90 mya (10), raising the possibility that diversification was mediated by an increase in climatically suitable geographic area. Cranial disparity of turtles also accumulated gradually across this time

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

Table 1. Genus and species level sampling used in this study, organized by family

	Genera			Species			
Family	Recognized	Sampled	Proportion sampled	Recognized	Sampled	Proportion sampled	Proportion monophyletic
Carretochelyidae	1	1	1.00	1	1	1.00	1.00
Chelidae	14	13	0.93	58	43	0.74	0.85
Cheloniidae	5	4	0.80	6	4	0.67	1.00
Chelydridae	2	2	1.00	5	4	0.80	1.00
Dermatemydidae	1	1	1.00	1	1	1.00	1.00
Dermochelyidae	1	1	1.00	1	1	1.00	1.00
Emydidae	10	10	1.00	53	51	0.96	0.65
Geoemydidae	19	19	1.00	71	64	0.90	0.84
Kinosternidae	4	4	1.00	27	23	0.85	0.76
Pelomedusidae	2	2	1.00	27	14	0.52	0.80
Platysternidae	1	1	1.00	1	1	1.00	1.00
Podocnemidae	3	3	1.00	8	7	0.88	1.00
Testudinidae	16	16	1.00	58	43	0.74	0.76
Trionychidae	13	13	1.00	31	22	0.71	0.88
Totals	92	90	0.98	348	279	0.80	0.80

Recognized counts of genera and species follow the taxonomy from ref. 11 and have changed in a few cases since that time. Sampled counts are the number that has at least one representative in the phylogeny. The proportion of monophyletic species is the fraction of sampled species that both have at least two samples in the tree (allowing monophyly to be assessed) and are monophyletic.

period and accelerated toward the present, although this analysis did not detect a particularly strong correlation with climate (48).

These paleontological results provide an interesting comparison to the timing of diversification we found in the phylogeny of extant turtles and highlight the significant role that extinction has played in shaping their present-day diversity. Our analysis identified no detectable change in diversification rate during the Cretaceous (Fig. 2*B*). While the fossil record documents a buildup in diversity during the Middle Cretaceous, our divergence time analysis indicates that most living turtle diversity is much younger, with most speciation events occurring within the last 50 mya, well after the end of the Cretaceous (65 mya). The increased diversification observed in the fossil record by Nicholson et al. (10) is at least partly associated with lineages that have no living descendants and therefore do not register in the molecular phylogeny of extant diversity. Taken together, these results document a dramatic turnover in turtle diversity through geological time, with both recent speciation and more ancient extinction contributing to the tree we observe today.

The new chronogram provides evidence for an increasing diversification rate beginning roughly 50 mya during the Eocene (Fig. 2 *B* and *C*). The estimated net diversification rate (speciation minus extinction) roughly triples beginning in the Eocene and remains elevated toward the present day (Fig. 2*B*). Given recent concerns about the identifiability of diversification shift models (55), we also confirmed that a qualitatively similar, but perhaps somewhat earlier, shift was recovered using a state-dependent modeling framework (Fig. 2*D*). Bayes factors (BF) among alternative rate shift models indicate positive (2 ln BF greater than 2 and less than 6; ref. 56) support for a shift that occurs at the Eocene−Oligocene transition 34 mya (Fig. 2*C*). This period coincides with large-scale declines in global sea level, which began falling from a peak of more than 150 m above modern levels ∼50 mya, and experienced a dramatic decline by the end-Eocene (57–60). It also raises the possibility that the increasing availability of suitable habitat along continental margins contributed to increased diversification of modern turtles. As lineages expanded into newly exposed continental margins, they may have rapidly built up species numbers through allopatric isolation in low-lying areas or among new isolated river basins. These environmental changes have been shown to be an important force in structuring diversity of other organisms (61). Previous work has also implicated incised river basins and sea level changes as a potential factor that drives a region of high endemicity in turtles (38, 62).

To test this hypothesis, we asked whether rates of diversification were faster on newly exposed continental margins, as would be expected if declining sea levels created ecological opportunity that led to speciation. Using the most recently available estimates of species ranges (11) and reconstructed paleocoastlines for the Eocene−Oligocene transition (59, 63), we classified each species as currently occupying 1) low-lying coastal areas that had not yet emerged before the Eocene−Oligocene transition ("coastal"), 2) inland habitats exposed and available prior to the transition ("inland"), or 3) both ("widespread") (Fig. 3). We then fit a series of 10 models of geography-dependent (or independent) speciation and extinction using the new chronogram and range data (Table 2). These models vary from scenarios where diversification rate changes may occur, but are independent of geographical range, to scenarios where diversification rates depend on geographical range either alone or in combination with other unobserved ("hidden") traits.

We found that the best-fitting models were those where geographic range was linked to variation in diversification rates (Table 2). Parameter estimates from these models indicate that speciation rates were higher in coastal compared to inland or widespread range lineages (Fig. 4 and *SI Appendix*, Fig. S2). The two best-fitting models together make up 0.951 of the Akaike information criterion (AIC) weight (0.535 and 0.416, respectively) and vary only in whether extirpation is modeled independently and whether jumps in ranges are allowed (i.e., from "coastal" to "inland," rather than evolving through a "widespread" intermediate ancestor). The best-fitting model is the simpler of the two and includes neither separate extirpation nor jumps. Conversely, all the models we tested that treat range evolution and diversification as independent processes were much poorer fits. The best of this group had an AIC 4.79 units lower than the pair of best-fitting models, and an AIC weight of 0.048. All remaining models in the set had AIC weights below 0.0001. These results are not sensitive to uncertainty in topology or divergence time estimates, as repeated analyses across a sample of 100 chronograms drawn from the posterior recovered qualitatively similar results (Table 2).

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

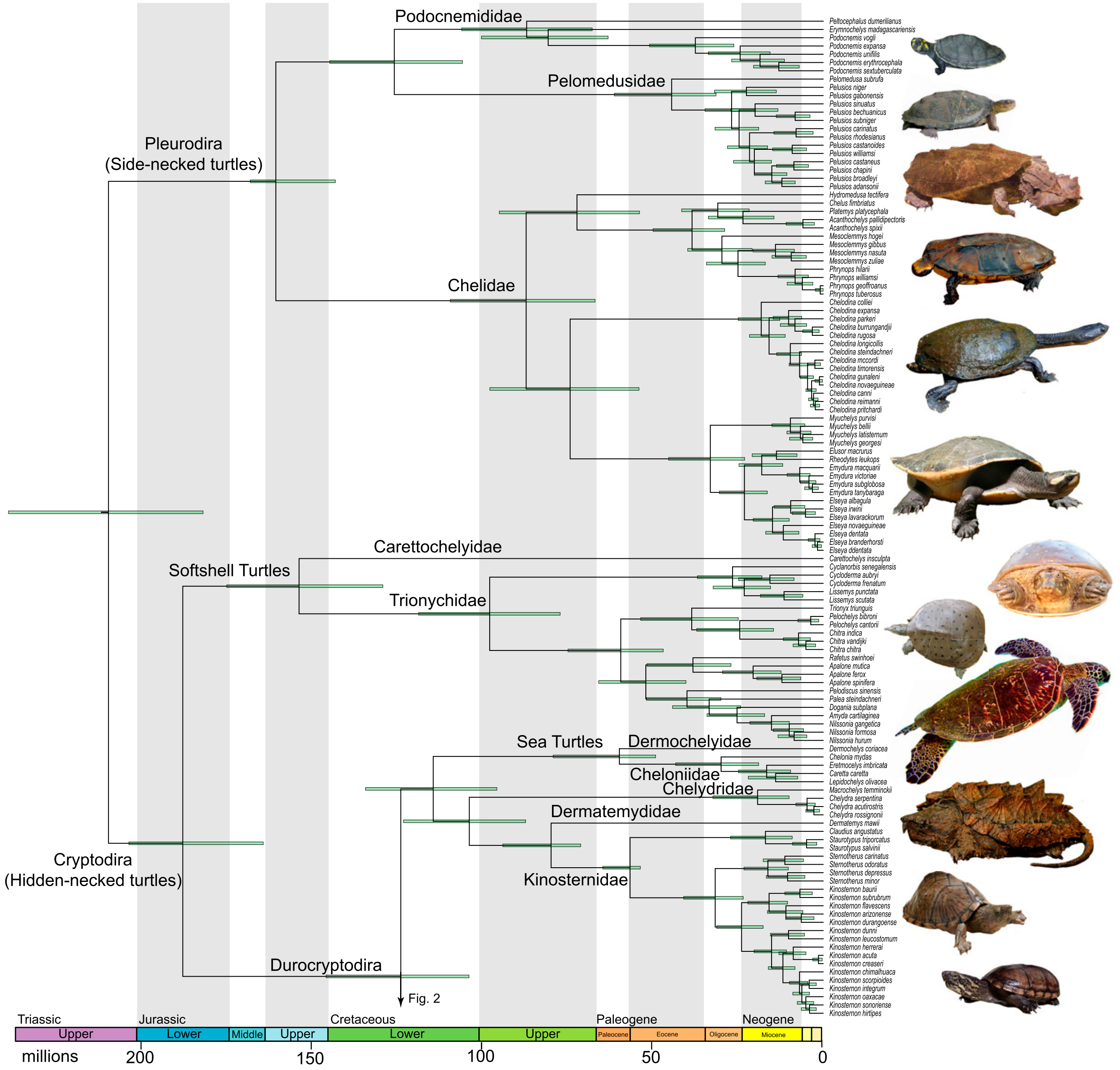

Fig. 1. Evolutionary history of the Testudines. Maximum clade credibility chronogram estimated from the relaxed clock analysis. Bars on nodes indicate 95% HPD of node ages. Families and selected clades are labeled. Images not to scale. Continued in Fig. 2.

While models that link geographic range to diversification fit the data much better than those that do not, we also found substantial model uncertainty between the two best-fitting models. Rather than rely on results from only the single "best" model, we averaged rates across all 10 models using their AIC weights. The weighted average parameter estimates again indicate speciation rates that are threefold higher in coastal regions than inland regions (Fig. 4) and increase through time regardless of the modeling framework (compare Fig. 2 *B* and *D*). Ancestral range estimates (as weighted averages across 10 models) indicate that nearly all coastal species transitioned to coastal areas after the end of the Eocene (dashed line in Fig. 4*A*), and that this pattern is common across families and global regions. If newly exposed land areas served as new habitat for chelonian range expansion and speciation, it stands to reason that this signature may be detectable in ancestral range estimates. Here, the models are agnostic about timing of transitions among areas, but they still recover transitions into and speciation within newly exposed continental margins during this time period. The exceptions to this are the four lineages (within the Carretochelyidae, Geoemydidae, Podocnemidae, and Dermatemydidae) that have moderate weight for coastal ranges well before the Eocene–Oligocene transition (Fig. 4*A*). Three of these are single-taxon-long branches where the reconstructions are necessarily equivocal. The remaining lineage, the diverse Geoemydidae, had equivocal support for coastal ranges before the Eocene–Oligocene transition, which increases to strong support at the time of the transition (Fig. 4*A*, nodes 1 to 8). The model-averaged reconstructed state probabilities for these nodes are provided in Table 3. This uncertainty could arise from the geographic distribution of

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

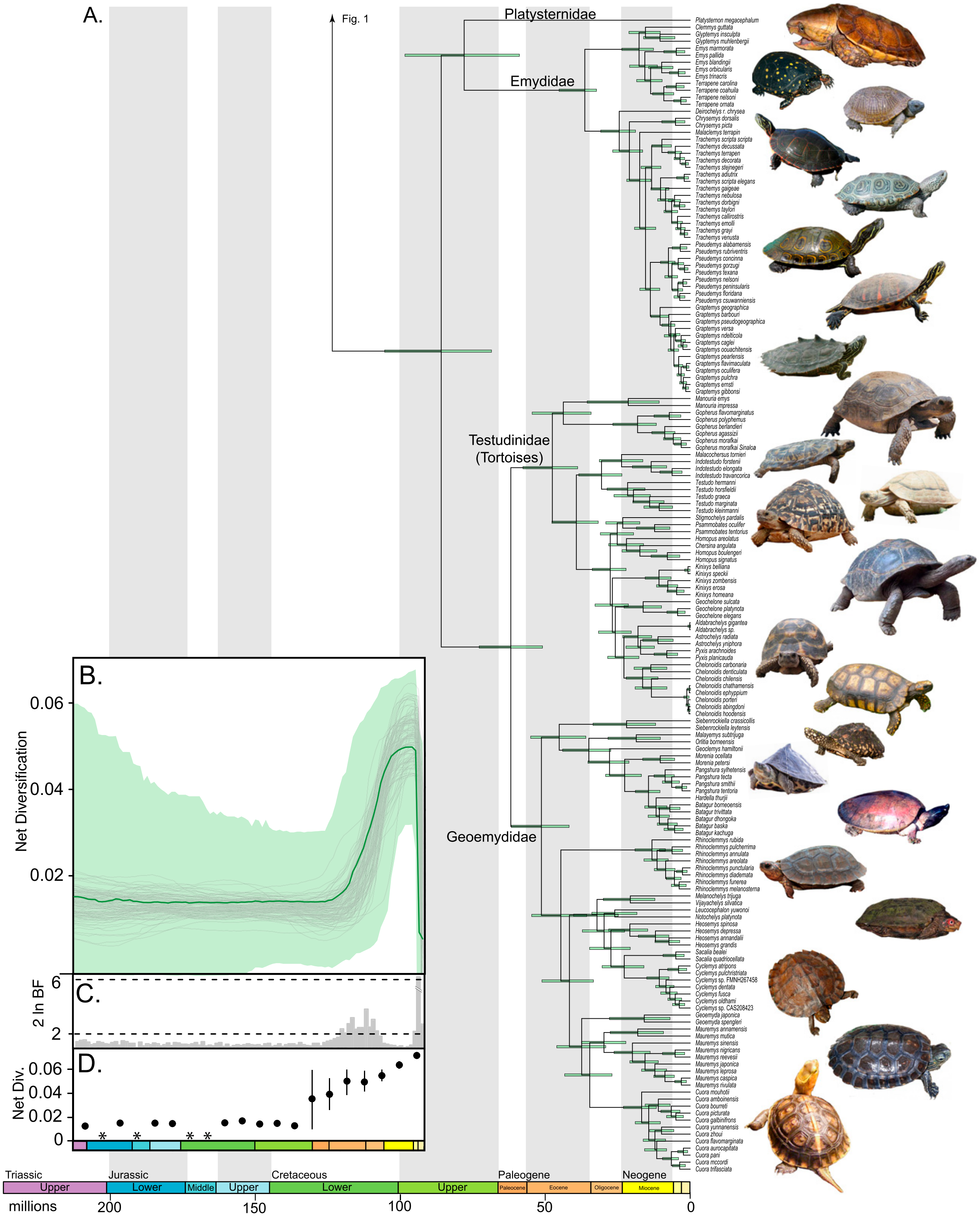

Fig. 2. Evolutionary history of the Testudines. *(A)* Continued from Fig. 1. Maximum clade credibility chronogram estimated from the relaxed clock analysis. Bars on nodes indicate 95% HPD of node ages. Families and selected clades are labeled. Images not to scale. *(B)* Tree-wide diversification rate through time from CoMET analysis. Green depicts diversification rate (mean and 95% HPD) estimated from the tree depicted in A and B. Gray lines depict mean rates from a sample of 100 chronograms from the posterior distribution. *(C)* BF (Bayes factor) for tree-wide shift in speciation. Dashed line corresponds to the threshold for "positive" support (2 ln BF > 2; ref. 56). Right side (most recent) BF for diversification rate decline into the present is truncated for clarity of presentation (2 ln BF = 12.1). *(D)* Average net diversification rate through time summarized from the GeoHiSSE analysis (also see Fig. 4). Asterisks indicate time bins that contain no nodes to summarize. Error bars indicate SD of rates within time bins, and are too small to visualize for several bins.

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

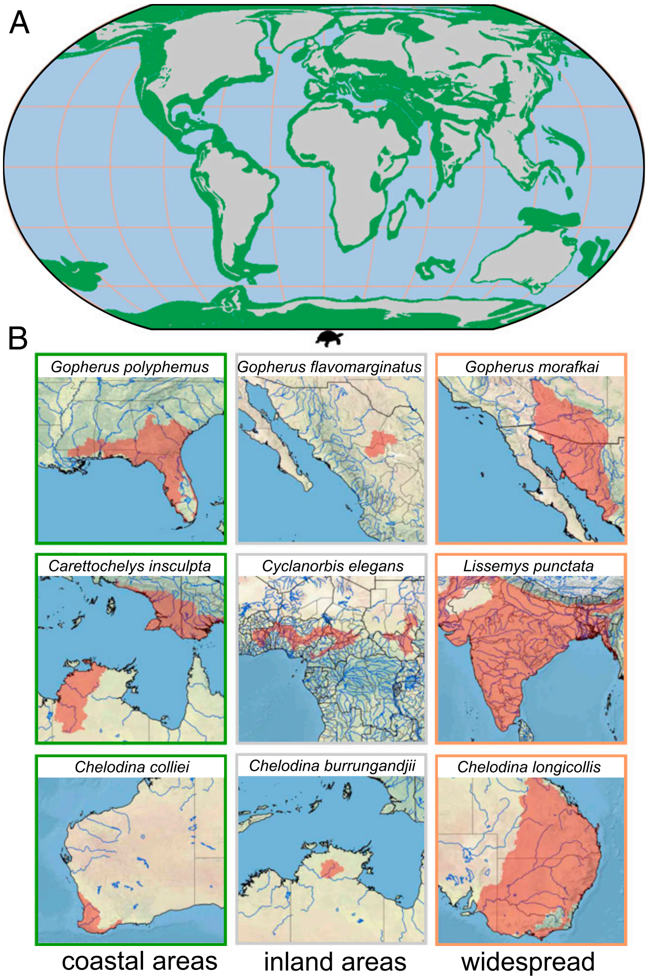

Fig. 3. (*A*) Paleogeographic reconstruction for the time of Eocene–Oligocene transition (34 mya) from refs. 59, 63. Gray areas depict exposed land masses from the time period. Green depicts shallow marine environments that would have been inaccessible to nonmarine turtles before the Eocene–Oligocene transition. (*B*) Example species ranges coded as "coastal," "inland," or "widespread." Ranges reprinted with permission from ref. 88.

the Geoemydidae. Most of the family's diversity occurs on the continental mainland and the islands of Southeast Asia, an area with an extremely complicated geological history. This may lead to a relatively greater level of uncertainty in paleogeographic reconstructions within the region, which would then propagate as error in our categorization of present-day species ranges. Because of this, reconstructions for these early geoemydid nodes could reasonably be interpreted as either modest support against our hypothesis or the result of error in classifying the ranges of species, given the complexity and uncertainty surrounding the area's geology. Whichever the explanation, if we restrict our attention to range reconstructions where the data provide a strong statistical signal, all lineages including Geoemydidae conform to the hypothesis.

Reconciling Fossils and Phylogenies. Some paleontological studies of turtle diversity have recovered an association between climate and diversity, with warm wet regions supporting higher diversity. The fossil record also documents a consistent decrease in diversity over the Eocene–Oligocene transition coincident with global cooling, at least at regional scales in western North America (50), a trend that sharply contrasts with the increasing rate of speciation and accumulation of diversity that we recover during the same period globally. The resolution of this contrast lies in the differences in geographic focus between approaches, and highlights the value of large-scale diversification analyses. In particular, some of the most influential paleontological studies have focused on the rich fossil beds that occur in western North America (in the vicinity of present-day Wyoming), an area that underwent aridification across the Eocene–Oligocene transition as sea levels declined. Given this, the paleontological findings actually accord well with our findings. Declining sea levels drove climate-associated habitat loss in some areas, including western North America where particularly significant fossil beds for turtles now occur. However, it expanded habitat in other regions, including present-day coastal margins that contain far fewer significant fossil-bearing sites. In accordance with these results, the paleontological studies also suggest that differences in patterns of diversification are regional. The same drying that resulted in aridification and extinction in western North America resulted in exposed continental margins and speciation in other areas of the world. As a case in point, southeastern North America, which is now a global hotspot of turtle diversity (64), is partly assembled from the same lineages (e.g., *Apalone*, *Chelydra*, *Chrysemys*, and other Emydidae) that disappeared from western North America during the Eocene–Oligocene transition. Relying on fossils or molecular information alone would miss these different regional signals, yielding an incomplete picture of diversification dynamics at odds with the more complex pattern that they jointly reveal.

Conclusions

We reconstructed a well-sampled and well-supported species-level phylogeny for the majority of extant turtles based on a new, relatively complete molecular dataset. With 591 individuals sampled from all families, 98% of genera, and 80% of the extant species, this analysis provides a global view of evolutionary history for this morphologically and ecologically unique vertebrate clade. The divergence time and diversification analyses identify a major increase in the rate of diversification that is coincident in time and place with patterns of global cooling, sea level change, and range expansion. Our results indicate that the ecological opportunities presented by newly exposed, unoccupied but appropriate habitat has been a key to turtle speciation and persistence, and suggest that its loss may equally contribute to their demise. Given their precarious current status as a globally endangered taxon and the sensitivity of coastal margin habitats to changing climate, our analysis suggests that the effects of climate change on the turtle extinction crisis may be even more extreme than previously thought (65), and that proactive measures, including assisted migration and repatriation into novel habitats, may be required to prevent further losses of chelonian biodiversity. These phylogenetic results, combined with inferences from the fossil record, provide a unified view of the global processes associated with diversification in turtles, and suggest that climate change may have devastating impacts on this globally threatened clade.

Materials and Methods

Taxon and Data Sampling. We follow the taxonomy from the most recent compilation from the Turtle Taxonomy Working Group, which recognizes 348 extant species organized into 92 genera and 14 families (11). Our taxon sampling comprises 279 of these 348 species or 80% of the extant turtles of the world (Table 1). We include representatives of all 14 families and 90 of the 92 genera (98%). When possible, we included two (occasionally more) individuals/species to allow a minimal assessment of species-level monophyly, which led to a full dataset of 591 individual turtles (Dataset S1). We also included GenBank sequences from chicken (*Gallus gallus*) and alligator (*Alligator mississippiensis*) as outgroups (66, 67). We generated new nuclear DNA (nuDNA) sequence data from 15 nuclear loci (AHR, AIING, BDNF, BMP2, HMGB2, HNFL, NB22519, PSMC1, PAX1P1, R35, RAG-1, TB01, TB29, TB73, ZFHX1B; refs. 22, 26, 37, 64, 66–72). To do so, we extracted DNA from blood or soft tissue samples using a salt extraction protocol (73) and amplified PCR products for all loci in 20-μL-volume reactions using the locus-specific

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

Table 2. Diversification rate models investigated in the GeoHiSSE framework

No.	Description	Range effect	Separate extirpation	Hidden states	No. Parameter	AIC	ΔAIC	Model weight	Percent posterior sample
1	GeoSSE, no range effect, only dispersal varies among areas	No	No	No	4	2887.42	59.24	7.30×10^{-14}	0
2	GeoSSE with separate extirpation, only dispersal varies among areas	No	Yes	No	6	2877.11	48.93	1.27×10^{-11}	0
3	GeoHiSSE, no range effect, hidden state effect	No	No	Yes	7	2891.60	63.42	9.03×10^{-15}	0
4	GeoHiSSE with separate extirpation, no range effect, hidden state effect	No	Yes	Yes	9	2832.97	4.79	0.048	3
5	GeoHiSSE, no range effect, four hidden states effect diversification	No	No	Yes	13	2845.49	17.31	9.32×10^{-5}	0
6	GeoHiSSE with separate extirpation, no range effect, four hidden states	No	Yes	Yes	15	2846.50	18.32	5.62×10^{-5}	3
7	GeoSSE, range effect (original GeoSSE model)	Yes	No	No	7	2869.54	41.36	5.58×10^{-10}	0
8	GeoSSE with separate extirpation, range effect	Yes	Yes	No	9	2864.16	35.98	8.21×10^{-9}	0
9	GeoHiSSE, both range and hidden state effects	Yes	No	Yes	15	2828.18	0	0.535	67
10	GeoHiSSE with separate extirpation, both range and hidden state effects	Yes	Yes	Yes	19	2828.68	0.5	0.416	27

Models vary according to whether geographic range and diversification rate are linked (range effect = Yes), whether extirpation is treated as a process separate from range contraction (separate extirpation = Yes), and whether the model employs hidden states to allow for additional diversification rate variation that is not linked to geographic range (hidden states = Yes). Percentage of posterior sample refers to the fraction of 100 chronograms drawn from the posterior distribution for which that model was the best fitting.

annealing temperatures, extension times, and primers for these markers developed in our earlier studies (23, 30, 74). PCR products were checked on 1% agarose gels and sequenced in both directions using Sanger sequencing by Beckman Coulter Genomics (https://www.beckman.com).

Data Matrix. We assembled a data matrix containing 6,029 new sequences generated for this study (68%), 2,004 additional sequences from GenBank (~23%, most of which were submitted previously by our group), and 832 missing sequences (~9.4%). The 15 nuDNA markers ranged in size from 593 bp to 1,488 bp (mean = 904 bp), and totaled 13,559 bp of aligned sequence data for 593 individuals, including outgroups. All sequences are available in GenBank (see Dataset S1 for accession numbers), and the data matrix is available in the Dryad repository associated with this study (https://doi.org/10.5061/dryad.jh9w0vt8w) (75).

Phylogenetic Analyses. We used Geneious v5.1 (76) to translate exons and check for the presence of unexpected stop codons, frame shifts, and other indicators that pseudogenes or other unwanted products may have been unintentionally sequenced. Alignments were carried out using the MAFFT software (77) plugin for Geneious (76). We used jModelTest (78) to select models of nucleotide substitution for each locus prior to the phylogenetic analyses. For the 593-taxon dataset (591 turtles plus two outgroups), we performed Bayesian phylogenetic analyses on the entire 15-locus concatenated and partitioned dataset using MrBayes v3.2 (79). Bayesian analyses consisted of 10 independent runs, each comprising one cold chain and three incrementally heated chains (temperature parameter = 0.1), that ran for up to 10,000,000 generations each, recording the state of the cold chain every 1,000 generations. We examined the Markov chain Monte Carlo (MCMC) samples in Tracer (80) and Are We There Yet? (81) to ensure that all chains appeared to be mixing adequately, that all were sampling from the same target distribution, and that a large sample of all parameters (Effective Sample Size > 200 after burn-in) in the analysis had been obtained. We discarded the first 25% of samples as burn-in, provided that the chains had apparently reached stationarity prior to that point, and combined and summarized the posterior samples using LogCombiner and TreeAnnotator v1.8.2 (82).

Divergence Time Estimates. We estimated divergence times with BEAST v.1.8.2 (82) using the uncorrelated lognormal relaxed clock model (83) with a diffuse gamma prior on both the mean clock rate and SD, the birth−death stochastic branching process prior, and default priors for remaining parameters of the model. We reduced the dataset to one representative per species or subspecies (288 total) to provide a better match to the birth−-death prior (which does not account for extensive intraspecific sampling) and used 22 uniform fossil calibration priors that have been previously defined and justified (4) to calibrate the clock. We estimated divergence times under the settings and priors described above using independent Hasegawa-Kishino-Yano substitution models for each partition (which was necessary to obtain adequate MCMC performance). We performed nine replicated analyses each using a single MCMC chain that was allowed to run for at least 50,000,000 generations, recording the state of the chain every 5,000 generations. We examined the MCMC samples as above to check for adequate mixing, convergence, and sampling, removed the first 25% of samples as burn-in, and combined results from the nine runs. We then calculated the mean divergence time and corresponding 95% HPD interval for each node in the phylogeny across the replicates.

Diversification Analysis. We investigated the role that changing rates of speciation and extinction may play in explaining the standing diversity of turtles. Some previous studies that focus on fossil species have found increased periods of diversification in association with changes in global temperatures (10, 47). We investigated whether this signal emerged in the phylogenetic tree of extant species by investigating models of tree-wide shifts in diversification rate. Using reversible jump MCMC (rjMCMC), we averaged over models of temporal shifts in speciation and extinction rate using the maximum clade credibility time-calibrated phylogeny. These analyses were implemented using the CoMET functions of the R package TESS (84, 85). We used empirical hyperpriors on the rates of speciation and

Table 3. Model-averaged reconstructed node probabilities corresponding to labeled nodes in Fig. 4

Node	Coastal	Inland	Widespread
1	0.54	0.01	0.46
2	0.65	0	0.35
3	0.67	0	0.33
4	0.48	0	0.52
5	0.75	0	0.24
6	0.83	0	0.17
7	0.73	0.01	0.26
8	0.69	0	0.31

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

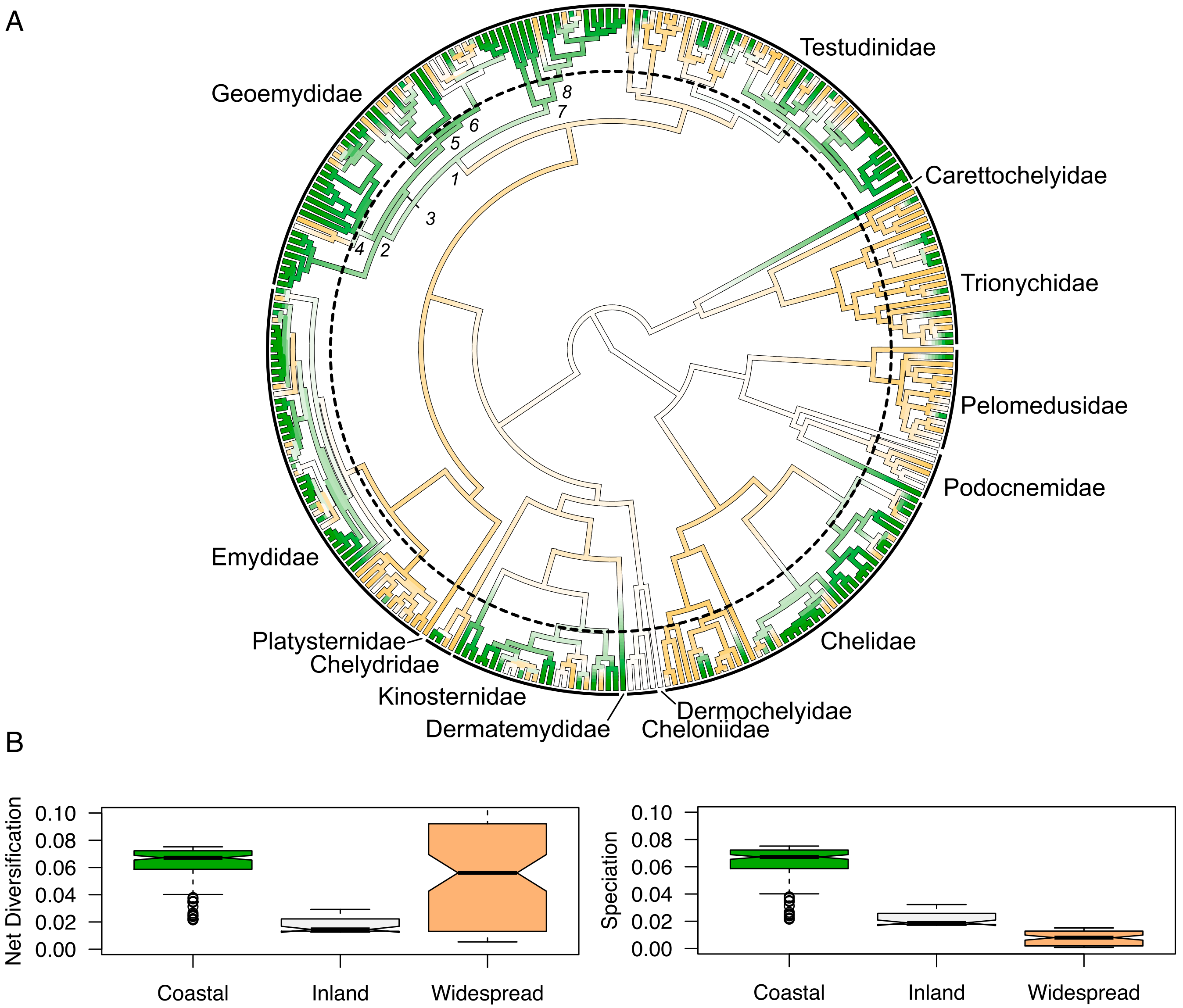

Fig. 4. Diversification and range evolution results from analysis of 10 geographic diversification rate models (Table 2) averaged by model weight. (*A*) Ancestral range estimates reconstruct nearly all transitions to coastal areas occurring after the Eocene–Oligocene (dashed line) when these areas would have been exposed. Green indicates coastal, white indicates inland, and orange indicates widespread. Labeled nodes 1 to 8 correspond to those in Table 3, which provides estimated state probabilities. See text for further detail. (*B*) Estimates of net diversification (*Left*) and speciation (*Right*) indicate higher rates in coastal than inland areas, particularly so for speciation. Fig. 2*D* depicts net diversification rate through time (*Left*) for this analysis.

extinction, set the prior on the expected number of diversification rate shifts to 1, and assumed uniform taxon sampling for the 80% of species that we sampled. We started the MCMC using the software's autotuning and autostopping rules to terminate the chain after 500 effective samples had been achieved, repeated the analysis four times, and checked that each individual run had large effective sample sizes, a stable Geweke statistic near zero across all time periods in the analysis, and the replicated analyses all converged to similar results. We ran additional analyses varying the lognormal priors on speciation and extinction rates, the expected number of rate shifts, and the taxon sampling probabilities to ensure that our qualitative results were not sensitive to these settings. We repeated these analyses targeting the joint prior probability distribution to ensure that the posterior samples strongly diverged from the prior and that the data (the tree and divergence times) were informative with respect to the parameters of the model. We then allowed the rjMCMC to explore models that allowed for mass extinction events, identifying no strong evidence for these under any settings. We repeated these analyses on a sample of 100 trees drawn randomly from the posterior distribution of chronograms to check that the results were not sensitive to uncertainty in topology or divergence times. Finally, because recent work has raised concerns about the reliability of analyses under such models (55), we treated these results cautiously and rechecked them using the Geographic Hidden State Speciation and Extinction (GeoHiSSE) modeling framework.

We further investigated whether diversification dynamics are associated with geographic ranges by fitting a set of 10 models from the GeoSSE and GeoHiSSE modeling framework (86) to the tree and range data for turtles, assuming uniform taxon sampling for the 81% of turtles that we sampled in coastal areas, 90% in widespread areas, and 77% for inland areas. These models vary according to whether diversification is linked to range, whether range contractions are treated separately from extirpation, and whether the models allow diversification rate variation associated with unobserved (or hidden) states (Table 2). We fit the models to the tree and range data, reconstructed ancestral range under each model, and calculated AICs to identify the best supported models in the set using the R package HiSSE (87). We then averaged estimates of diversification parameters and ancestral ranges across the set of models using AIC weights following Caetano et al. (86). We repeated these analyses across a posterior sample of 100 chronograms to

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

check that the results were robust to uncertainty in topology and timing of divergence. To check the results of the CoMET analysis, we also examined mean diversification rate variation across time by dividing the tree into 20 equally sized time bins and calculating the average net diversification rate across the nodes in each bin.

Data Availability. DNA sequences have been deposited in Genbank (see *SI Appendix*, S2 for accession numbers) and the Dryad digital repository (doi.org/10.5061/dryad.jh9w0vt8w) (75).

ACKNOWLEDGMENTS. This project would not have been possible without the invaluable assistance of a large global network of dedicated biologists, conservation professionals, and natural history collections that donated or helped us source tissue samples for the many rare and endangered species included here. We gratefully acknowledge the support of the following individuals and institutions (current institutional affiliations have changed in some cases): Alan Resetar (Field Museum of Natural History); Andrew Snider (Fresno-Chaffee Zoo); Anne Meylan (Florida Fish and Wildlife Commission); Arthur Georges (University of Canberra); Brice P. Noonan; Bryan Stuart (North Carolina Museum of Natural Sciences); Carla Cicero, Jim McGuire, Carol Spencer, and Ted Papenfuss (Museum of Vertebrate Zoology, University of California, Berkeley); Cesar Ayres (University of Vigo); Charles Innis (New England Aquarium); Chris Austin and Donna Dittmann (Louisiana State University Museum of Natural Science Collection of Genetic Resources); Chris Phillips (Illinois Natural History Survey); Dan Holland; Dave Collins and Bill Hughes (Tennessee Aquarium); David Cannatella and Travis LaDuc (Texas Natural History Collections, University of Texas at Austin); David Emmett; David H. Nelson; Dennis Uhrig; Dirk Stevenson; Dwight Lawson (Zoo Atlanta); Ed DeGrauw; Elmar Meier; Eric Munscher; Erin Jenkins (National Aquarium Baltimore); Eskandar Pouyani; Frank Slavens; Gary Crow and Paul Crow (Kadoorie Farms and Botanical Gardens); Gerry Dorrestein; Glenn Lubcke; Greg Pauly (Natural History Museum of Los Angeles County); Hans-Dieter Philippen; Harald Artner; Henk Zwartepoorte (Rotterdam Zoo); Hugh Quinn (Cleveland Metroparks Zoo); Ian Ives; Indraniel Das; James F. Parham; Jamie Bettaso; Janet Braun and Laurie Vitt (Oklahoma Collection of Genomic Resources, University of Oklahoma, Sam Noble Oklahoma Museum of Natural History); Jeanette M. McQuire; Jennifer Smith; Jens Vindum (California Academy of Sciences); John Carr; John Iverson; John Jensen, Eric Johansen, Jerry Johnston, Kenny Krysko and Pam Soltis (Florida Museum of Natural History—Genetic Resources Repository); Jonathan Campbell and Carl Franklin (University of Texas, Arlington Collection of Vertebrates); Jonathan Fong; Jose Rosado (Museum of Comparative Zoology, Harvard University); K. Stohlgren; Kat Caldera; Kate Slavens; Kevin de Queiroz (United States National Museum); Levi Gray; Malgosia Nowak-Kemp; Marcie Tarvid (Tulsa Zoo); Mark P. Wallace Sr.; Matt Aresco; Matt Osentoski and Chris Tabaka (Binder Park Zoo); Merijn Kerlen; Mike Forstner (Texas State University-San Marcos); James Godwin (Alabama Natural Heritage Program); David Hall and Toby Hibbitts (Texas Cooperative Wildlife Collections); Minh Le and David Kizirian (American Museum of Natural History, Ambrose Monell Cryo Collection); Müge Gidiş; Paul Gibbons (Turtle Conservancy); Paul Moler; Paul Stone; Paul Vander Schouw; Penny Danielewicz (Buffalo Zoo); Peter C. H. Pritchard (Chelonian Research Institute); Peter Dutton; Peter Meylan; Peter Paul van Dijk; Peter Scott; James Barzyk, Rich Glor, and Rafe Brown (University of Kansas Natural History Museum); Raymond Farrell and Robert Zappalorti (Herpetological Consultants, Inc.); Raymond Saumure; Richard Vogt; Rick Haeffner (Denver Zoo); Rick Hudson (Fort Worth Zoo); Ross Kiester; Rudolf Wicker; Russell Burke; Scott Smith; Scott Symers; Sharon Chancellor; Steve Freedberg; Steve Mockford; Sue Angus and Chris Banks (Melbourne Zoo); Suzanne McGaugh; T. G. Jackson; Tag Engstrom; Tamás Molnár; Taylor Edwards; Teri Herman; Thomas Owens (San Diego Zoo); Thomas Ziegler (AG Zoologischer Garten Köln); Tibor Kovács; Tom French and Michael Amaral (US Fish and Wildlife Service); Tomas Diagne; Wayne King (Florida Museum of Natural History); Will Selman (Louisiana Department of Wildlife and Fisheries); William McCord; and Wolgang Böhme (Zoologisches Forschungsmuseum Alexander Koenig). We thank Anthony Barley, Müge Gidiş, Geoffrey Lovely, Genevieve Mount, and Cathy Newman for their assistance with molecular data collection. Anthony Barley, Luke Campillo, Rebecca Chong, and Michael Landis provided insightful advice and comments. We acknowledge the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway and the University of Hawaii Cyberinfrastructure Group for providing access to high-performance computing resources. Support for this work was provided by funding from the US NSF (Awards DEB 0817042 and DEB 1239961).

1. F. L. Condamine, J. Rolland, H. Morlon, Macroevolutionary perspectives to environmental change. *Ecol. Lett.* **16**, 72–85 (2013).
2. C. J. Butler, A review of the effects of climate change on Chelonians. *Diversity (Basel)* **11**, 138 (2019).
3. A. G. J. Rhodin *et al*., Global conservation status of turtles and tortoises (order Testudines). *Chelonian Conserv. Biol.* **17**, 135 (2018).
4. W. G. Joyce, J. F. Parham, T. R. Lyson, R. C. M. Warnock, P. C. J. Donoghue, A divergence dating analysis of turtles using fossil calibrations: An example of best practices. *J. Paleontol.* **87**, 612–634 (2013).
5. H. B. Shaffer, E. McCartney-Melstad, T. J. Near, G. G. Mount, P. Q. Spinks, Phylogenomic analyses of 539 highly informative loci dates a fully resolved time tree for the major clades of living turtles (Testudines). *Mol. Phylogenet. Evol.* **115**, 7–15 (2017).
6. T. R. Lyson, G. S. Bever, Origin and evolution of the turtle body plan. *Annu. Rev. Ecol. Evol. Syst.* **51**, 143–166 (2020).
7. A. L. Jaffe, G. J. Slater, M. E. Alfaro, The evolution of island gigantism and body size variation in tortoises and turtles. *Biol. Lett.* **7**, 558–561 (2011).
8. G. S. Ferreira, A. D. Rincón, A. Solórzano, M. C. Langer, The last marine pelomedusoids (Testudines: Pleurodira): A new species of *Bairdemys* and the paleoecology of Stereogenyina. *PeerJ* **3**, e1063 (2015).
9. R. B. J. Benson, G. Domokos, P. L. Várkonyi, R. R. Reisz, Shell geometry and habitat determination in extinct and extant turtles (Reptilia: Testudinata). *Paleobiology* **37**, 547–562 (2011).
10. D. B. Nicholson, P. A. Holroyd, R. B. J. Benson, P. M. Barrett, Climate-mediated diversification of turtles in the Cretaceous. *Nat. Commun.* **6**, 7848 (2015).
11. Turtle Taxonomy Working Group, *Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status* (Chelonian Research Monographs, ed. 8, 2017).
12. J. F. Clements *et al*., The eBird/Clements Checklist of Birds of the World. https://www.birds.cornell.edu/clementschecklist/download/. Accessed 12 June 2020.
13. S. Kumar, G. Stecher, M. Suleski, S. B. Hedges, TimeTree: A resource for timelines, timetrees, and divergence times. *Mol. Biol. Evol.* **34**, 1812–1819 (2017).
14. P. Uetz, P. Freed, J. Hošeck, The Reptile Database. www.reptile-database.org. Accessed 12 June 2020.
15. D. E. Wilson, D. A. M. Reeder, *Mammal Species of the World: A Taxonomic and Geographic Reference* (Johns Hopkins University Press, 2005).
16. M. Agha *et al*., Salinity tolerances and use of saline environments by freshwater turtles: Implications of sea level rise. *Biol. Rev. Camb. Philos. Soc.* **93**, 1634–1648 (2018).
17. F. J. Janzen, Climate change and temperature-dependent sex determination in reptiles. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7487–7490 (1994).
18. Papahānaumokuākea Marine National Monument, Assessing the damage: The first step after Hurricane Walaka. https://www.papahanaumokuakea.gov/new-news/2018/11/21/ffs-walaka/. Accessed 17 April 2019.
19. J. G. Krenz, G. J. P. Naylor, H. B. Shaffer, F. J. Janzen, Molecular phylogenetics and evolution of turtles. *Mol. Phylogenet. Evol.* **37**, 178–191 (2005).
20. A. J. Barley, P. Q. Spinks, R. C. Thomson, H. B. Shaffer, Fourteen nuclear genes provide phylogenetic resolution for difficult nodes in the turtle tree of life. *Mol. Phylogenet. Evol.* **55**, 1189–1194 (2010).
21. N. G. Crawford *et al*., A phylogenomic analysis of turtles. *Mol. Phylogenet. Evol.* **83**, 250–257 (2015).
22. H. B. Shaffer, P. Meylan, M. L. McKnight, Tests of turtle phylogeny: Molecular, morphological, and paleontological approaches. *Syst. Biol.* **46**, 235–268 (1997).
23. R. C. Thomson, A. M. Shedlock, S. V. Edwards, H. B. Shaffer, Developing markers for multilocus phylogenetics in non-model organisms: A test case with turtles. *Mol. Phylogenet. Evol.* **49**, 514–525 (2008).
24. A. Georges, J. Birrell, K. M. Saint, W. P. McCord, S. C. Donnellan, A phylogeny for side-necked turtles (Chelonia: Pleurodira) based on mitochondrial and nuclear gene sequence variation. *Biol. J. Linn. Soc. Lond.* **67**, 213–246 (1998).
25. B. W. Bowen, W. S. Nelson, J. C. Avise, A molecular phylogeny for marine turtles: Trait mapping, rate assessment, and conservation relevance. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5574–5577 (1993).
26. T. N. Engstrom, H. B. Shaffer, W. P. McCord, Multiple data sets, high homoplasy, and the phylogeny of softshell turtles (Testudines: Trionychidae). *Syst. Biol.* **53**, 693–710 (2004).
27. C. R. Feldman, J. F. Parham, Molecular phylogenetics of emydine turtles: Taxonomic revision and the evolution of shell kinesis. *Mol. Phylogenet. Evol.* **22**, 388–398 (2002).
28. M. K. Fujita, T. N. Engstrom, D. E. Starkey, H. B. Shaffer, Turtle phylogeny: Insights from a novel nuclear intron. *Mol. Phylogenet. Evol.* **31**, 1031–1040 (2004).
29. J. B. Iverson, M. Le, C. Ingram, Molecular phylogenetics of the mud and musk turtle family Kinosternidae. *Mol. Phylogenet. Evol.* **69**, 929–939 (2013).
30. P. Q. Spinks, R. C. Thomson, M. Gidiş, H. Bradley Shaffer, Multilocus phylogeny of the New-World mud turtles (Kinosternidae) supports the traditional classification of the group. *Mol. Phylogenet. Evol.* **76**, 254–260 (2014).
31. M. Le, C. J. Raxworthy, W. P. McCord, L. Mertz, A molecular phylogeny of tortoises (Testudines: Testudinidae) based on mitochondrial and nuclear genes. *Mol. Phylogenet. Evol.* **40**, 517–531 (2006).
32. H. Li *et al*., Phylogenetic relationships and divergence dates of softshell turtles (Testudines: Trionychidae) inferred from complete mitochondrial genomes. *J. Evol. Biol.* **30**, 1011–1023 (2017).
33. E. Naro-Maciel, M. Le, N. N. FitzSimmons, G. Amato, Evolutionary relationships of marine turtles: A molecular phylogeny based on nuclear and mitochondrial genes. *Mol. Phylogenet. Evol.* **49**, 659–662 (2008).
34. J. M. Seddon, A. Georges, P. R. Baverstock, W. McCord, Phylogenetic relationships of chelid turtles (Pleurodira: Chelidae) based on mitochondrial 12S rRNA gene sequence variation. *Mol. Phylogenet. Evol.* **7**, 55–61 (1997).
35. P. Q. Spinks *et al*., Misleading phylogenetic inferences based on single-exemplar sampling in the turtle genus *Pseudemys*. *Mol. Phylogenet. Evol.* **68**, 269–281 (2013).
36. P. Q. Spinks, R. C. Thomson, H. B. Shaffer, A reassessment of *Cuora cyclornata* Blanck, McCord and Le, 2006 (Testudines, Geoemydidae) and a plea for taxonomic stability. *Zootaxa* **2018**, 58–68 (2009).

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021

37. P. Q. Spinks *et al*., Cryptic variation and the tragedy of unrecognized taxa: The case of international trade in the spiny turtle *Heosemys spinosa* (Testudines: Geoemydidae). *Zool. J. Linn. Soc.* **164**, 811–824 (2012).
38. R. C. Thomson, P. Q. Spinks, H. B. Shaffer, Molecular phylogeny and divergence of the map turtles (Emydidae: *Graptemys*). *Mol. Phylogenet. Evol.* **121**, 61–70 (2018).
39. P. Q. Spinks *et al*., Species boundaries and phylogenetic relationships in the critically endangered Asian box turtle genus *Cuora*. *Mol. Phylogenet. Evol.* **63**, 656–667 (2012).
40. P. Q. Spinks, R. C. Thomson, E. McCartney-Melstad, H. B. Shaffer, Phylogeny and temporal diversification of the New World pond turtles (Emydidae). *Mol. Phylogenet. Evol.* **103**, 85–97 (2016).
41. P. Q. Spinks, H. B. Shaffer, J. B. Iverson, W. P. McCord, Phylogenetic hypotheses for the turtle family Geoemydidae. *Mol. Phylogenet. Evol.* **32**, 164–182 (2004).
42. M. Le, W. P. McCord, J. B. Iverson, On the paraphyly of the genus *Kachuga* (Testudines: Geoemydidae). *Mol. Phylogenet. Evol.* **45**, 398–404 (2007).
43. J. B. Iverson *et al*., "In search of the tree of life for turtles in defining turtle diversity" in *Proceedings of a Workshop on Genetics, Ethics, and Taxonomy of Freshwater Turtles and Tortoises*, H. B. Shaffer, N. N. FitzSimmons, A. Georges, A. G. J. Rhodin, Eds. (Chelonian Research Foundation, 2007), pp. 85–106.
44. R. C. Thomson, H. B. Shaffer, Sparse supermatrices for phylogenetic inference: Taxonomy, alignment, rogue taxa, and the phylogeny of living turtles. *Syst. Biol.* **59**, 42–58 (2010).
45. J. M. Guillon, L. Guéry, V. Hulin, M. Girondot, A large phylogeny of turtles (Testudines) using molecular data. *Contrib. Zool.* **81**, 147–158 (2012).
46. A. G. Pereira, J. Sterli, F. R. R. Moreira, C. G. Schrago, Multilocus phylogeny and statistical biogeography clarify the evolutionary history of major lineages of turtles. *Mol. Phylogenet. Evol.* **113**, 59–66 (2017).
47. A. M. Waterson *et al*., Modelling the climatic niche of turtles: A deep-time perspective. *Proc. Biol. Sci.* **283**, 1839 (2016).
48. C. Foth, E. Ascarrunz, W. G. Joyce, Still slow, but even steadier: An update on the evolution of turtle cranial disparity interpolating shapes along branches. *R. Soc. Open Sci.* **4**, 170899 (2017).
49. H. J. Hutchison, "Testudines" in *The Terrestrial Eocene-Oligocene Transition in North America*, D. R. Prothero, R. J. Emry, Eds. (Cambridge University Press, 1996), pp. 337–353.
50. J. H. Hutchison, "Western North American reptile and amphibian record across the Eocene/Oligocene boundary and its climatic implications" in *Eocene-Oligocene Clmatic and Biotic Evolution*, D. R. Prothero, W. A. Berggren, Eds. (Princeton University Press, 1992), pp. 451–463.
51. W. G. Joyce, Phylogenetic relationships of Mesozoic turtles. *Bull. Peabody Mus. Nat. Hist.* **48**, 3–102 (2007).
52. E. S. Gaffney, P. A. Meylan, A. R. Wyss, A computer assisted analysis of the relationships of the higher categories of turtles. *Cladistics* **7**, 313–335 (1991).
53. R. C. M. Warnock, J. F. Parham, W. G. Joyce, T. R. Lyson, P. C. J. Donoghue, Calibration uncertainty in molecular dating analyses: There is no substitute for the prior evaluation of time priors. *Proc. Biol. Sci.* **282**, 20141013 (2015).
54. T. J. Near, P. A. Meylan, H. B. Shaffer, Assessing concordance of fossil calibration points in molecular clock studies: An example using turtles. *Am. Nat.* **165**, 137–146 (2005).
55. S. Louca, M. W. Pennell, Extant timetrees are consistent with a myriad of diversification histories. *Nature* **580**, 502–505 (2020).
56. R. Kass, A. E. Raftery, Bayes factors. *J. Am. Stat. Assoc.* **90**, 773–795 (1995).
57. B. U. Haq, J. Hardenbol, P. R. Vail, Chronology of fluctuating sea levels since the Triassic. *Science* **235**, 1156–1167 (1987).
58. K. Miller, G. Mountain, J. Wright, J. Browning, A 180-million-year record of sea level and ice volume variations from continental margin and deep-sea isotopic records. *Oceanography (Wash. D.C.)* **24**, 40–53 (2011).
59. R. D. Müller *et al*., Ocean basin evolution and global-scale plate reorganization events since Pangea breakup. *Annu. Rev. Earth Planet. Sci.* **44**, 107–138 (2016).
60. R. D. Müller, M. Sdrolias, C. Gaina, B. Steinberger, C. Heine, Long-term sea-level fluctuations driven by ocean basin dynamics. *Science* **319**, 1357–1362 (2008).
61. R. M. Bonett, A. L. Trujano-Alvarez, M. J. Williams, E. K. Timpe, Biogeography and body size shuffling of aquatic salamander communities on a shifting refuge. *Proc. Biol. Sci.* **280**, 20130200 (2013).
62. J. R. Ennen *et al*., Hierarchical, quantitative biogeographic provinces for all North American turtles and their contribution to the biogeography of turtles and the continent. *Herpetol. Monogr.* **31**, 142 (2017).
63. R. D. Müller *et al*., GPlates: Building a virtual Earth through deep time. *Geochem. Geophys. Geosyst.* **19**, 2243–2261 (2018).
64. R. A. Mittermeier, P. P. van Dijk, A. G. J. Rhodin, S. D. Nash, Turtle hotspots: An analysis of the occurrence of tortoises and freshwater turtles in biodiversity hotspots, high-biodiversity wilderness areas, and turtle priority areas. *Chelonian Conserv. Biol.* **14**, 2–10 (2015).
65. C. B. Stanford *et al*., Turtles and Tortoises Are in Trouble. *Curr. Biol.* **30**, R721–R735 (2020).
66. International Chicken Genome Sequencing Consortium, Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature* **432**, 695–716 (2004).
67. J. A. St John *et al*., Sequencing three crocodilian genomes to illuminate the evolution of archosaurs and amniotes. *Genome Biol.* **13**, 415 (2012).
68. N. Backström, S. Fagerberg, H. Ellegren, Genomics of natural bird populations: A gene-based set of reference markers evenly spread across the avian genome. *Mol. Ecol.* **17**, 964–980 (2008).
69. R. T. Kimball *et al*., A well-tested set of primers to amplify regions spread across the avian genome. *Mol. Phylogenet. Evol.* **50**, 654–660 (2009).
70. B. P. Noonan, P. T. Chippindale, Vicariant origin of Malagasy reptiles supports Late Cretaceous Antarctic land bridge. *Am. Nat.* **168**, 730–741 (2006).
71. C. R. Primmer, T. Borge, J. Lindell, G.-P. Saetre, Single-nucleotide polymorphism characterization in species with limited available sequence information: High nucleotide diversity revealed in the avian genome. *Mol. Ecol.* **11**, 603–612 (2002).
72. T. M. Townsend, R. E. Alegre, S. T. Kelley, J. J. Wiens, T. W. Reeder, Rapid development of multiple nuclear loci for phylogenetic analysis using genomic resources: An example from squamate reptiles. *Mol. Phylogenet. Evol.* **47**, 129–142 (2008).
73. J. Sambrook, D. W. Russell, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, ed. 3, 2001).
74. P. Q. Spinks, R. C. Thomson, A. J. Barley, C. E. Newman, H. Bradley Shaffer, Testing avian, squamate, and mammalian nuclear markers for cross amplification in turtles. *Conserv. Genet. Resour.* **2**, 127–129 (2010).
75. R. C. Thomson, P. Spinks, H. B. Shaffer, Data from: A global phylogeny of turtles reveals a burst of climate-associated diversification on continental margins. *Dryad digital repository*. https://datadryad.org/stash/dataset/doi:10.5061/dryad.jh9w0vt8w. 19 January 2021.
76. M. Kearse *et al*., Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).
77. K. Katoh, K. Misawa, K. Kuma, T. Miyata, MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **30**, 3059–3066 (2002).
78. D. Posada, jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**, 1253–1256 (2008).
79. F. Ronquist *et al*., MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542 (2012).
80. A. Rambaut, A. J. Drummond, D. Xie, G. Baele, M.A. Suchard, Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* **67**, 901–904 (2018).
81. J. A. A. Nylander, J. C. Wilgenbusch, D. L. Warren, D. L. Swofford, AWTY (are we there yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* **24**, 581–583 (2008).
82. A. J. Drummond, M. A. Suchard, D. Xie, A. Rambaut, Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**, 1969–1973 (2012).
83. A. J. Drummond, S. Y. W. Ho, M. J. Phillips, A. Rambaut, Relaxed phylogenetics and dating with confidence. *PLoS Biol.* **4**, e88 (2006).
84. M. R. May, S. Höhna, B. R. Moore, A Bayesian approach for detecting the impact of mass-extinction events on molecular phylogenies when rates of lineage diversification may vary. *Methods Ecol. Evol.* **7**, 947–959 (2016).
85. S. Höhna, M. R. May, B. R. Moore, TESS: An R package for efficiently simulating phylogenetic trees and performing Bayesian inference of lineage diversification rates. *Bioinformatics* **32**, 789–791 (2016).
86. D. S. Caetano, B. C. O'Meara, J. M. Beaulieu, Hidden state models improve state-dependent diversification approaches, including biogeographical models. *Evolution* **72**, 2308–2324 (2018).
87. J. M. Beaulieu, B. C. O'Meara, Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Syst. Biol.* **65**, 583–601 (2016).
88. Turtle Taxonomy Working Group, *Turtles of the World, 7th Edition: Annotated Checklist of Taxonomy, Synonymy, Distribution with Maps, and Conservation Status* (Chelonian Research Monographs, 2014).

Downloaded at UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL on June 15, 2021