

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

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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 fossilbearing 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

G lobal 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.

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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



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 In BF > 2; ref. 56). Right side (most recent) BF for diversification rate decline into the present is truncated for clarity of presentation (2 In 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.