

Hemisphere-scale differences in conifer evolutionary dynamics

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Fundamental differences in the distribution of oceans and landmasses in the Northern and Southern Hemispheres potentially impact patterns of biological diversity in the two areas. The evolutionary history of conifers provides an opportunity to explore these dynamics, because the majority of extant conifer species belong to lineages that have been broadly confined to the Northern or Southern Hemisphere during the Cenozoic. Incorporating genetic information with a critical review of fossil evidence, we developed an age-calibrated phylogeny sampling ~80% of living conifer species. Most extant conifer species diverged recently during the Neogene within clades that generally were established during the later Mesozoic, but lineages that diversified mainly in the Southern Hemisphere show a significantly older distribution of divergence ages than their counterparts in the Northern Hemisphere. Our tree topology and divergence times also are best fit by diversification models in which Northern Hemisphere conifer lineages have higher rates of species turnover than Southern Hemisphere lineages. The abundance of recent divergences in northern clades may reflect complex patterns of migration and range shifts during climatic cycles over the later Neogene leading to elevated rates of speciation and extinction, whereas the scattered persistence of mild, wetter habitats in the Southern Hemisphere may have favored the survival of older lineages.

biogeography | chronogram | climate change

Attempts to understand the global drivers of terrestrial species diversity and patterns of diversification often have focused on latitudinal gradients and differences between tropical and temperate environments (e.g., 1–3). However, the specific disposition of the Earth's landmasses undoubtedly also has influenced the origin, persistence, and distribution of species at continental scales. In particular, there are fundamental geographic differences between the Northern and Southern Hemispheres in terms of land and ocean area, and these differences might be expected to influence broad evolutionary patterns among the organisms in these regions. The impact of these major geographic differences on the evolutionary history of animals and plants of the Northern and Southern Hemispheres has received relatively little attention (4, 5), perhaps because the complex histories of lineages and the landscapes themselves obscure the deepest imprints of large-scale geographic patterns.

Conifers provide an opportunity to evaluate whether such continental-scale differences are reflected in broad evolutionary patterns because the group is diverse (over 600 living species) and has an extensive and well-documented fossil record, and because ~90% of extant conifer diversity is contained in major clades that generally are confined to one of the two hemispheres. Extant Pinaceae and the diverse Cupressoideae within the Cupressaceae are found primarily in temperate and subtropical regions of the Northern Hemisphere (6, 7), whereas extant species of Araucariaceae, Podocarpaceae, and the Callitroideae (the sister group to Cupressoideae) are found primarily in the Southern Hemisphere (6–8; see *SI Appendix* for a full discussion of geographic ranges).

Notably, these strong biogeographic distinctions appear to have been maintained since the end of the Cretaceous. Fossil evidence suggests that Araucariaceae were restricted to the Southern Hemisphere throughout the Cenozoic, as they are today (9). Podocarpaceae were primarily Southern Hemisphere as well (10), although pollen evidence suggests that some members extended farther north during warm time intervals (11).

To explore the impact of these biogeographic differences on the evolutionary history of conifers, we constructed a dated phylogeny that samples 489 conifer species, ~80% of extant diversity. This phylogeny is based on a molecular dataset including two nuclear genes (18S and a phytochrome gene, *PHYYP*) and two chloroplast genes (*matK* and *rbcL*). A maximum-likelihood (ML) tree topology, which agrees well with previously published phylogenetic studies of conifers (e.g., 12–16), was used as a constraint topology for inferring the dated phylogeny. Divergence times were estimated using a Bayesian implementation of an uncorrelated log-normal relaxed clock model with fossil calibration points used as minimum age constraints (*Materials and Methods*). These analyses demonstrate significant differences in the diversification of conifer clades inhabiting the Northern and Southern Hemispheres over the last 65 million years (My), suggesting that a broad geographic signature is imprinted in the evolutionary history of conifers.

Results

To date the nodes of the phylogeny, we focused on calibration fossils that possessed unambiguous, derived characters shared with extant clades, usually at the generic level or above. For example, the characteristic vegetative shoots of *Phyllocladus*, which consist of flattened photosynthetic branches, are not seen in any other extant genus or species within the Podocarpaceae, including *Lepidothamnus*, the sister taxon of *Phyllocladus*. Therefore, the first fossil appearance of such shoots can be used to set a minimum age constraint for the divergence of the *Phyllocladus* lineage from other conifers. Similar criteria were applied across the conifer tree, because all of the major clades in this study possess a fossil record and contain at least one fossil calibration point (see *SI Appendix* for a full discussion of calibration points). Within this framework we performed two sets of analyses. Each used the same 16 fossil calibrations but differed in the width of the prior age distributions linked with each calibration point (*Materials and Methods*). Absolute and relative ages among clades differed little between the analyses (*SI Appendix, Table S1*); here we present

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. JX559887–JX560092).

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results from the more conservative analysis using wider prior age distributions.

The dated phylogeny shows that most extant conifer species diverged in the Neogene but belong to major lineages that generally diverged much earlier in the Mesozoic (Fig. 1A; see also the expanded version in *SI Appendix*, Fig. S4), a pattern that is similar to those found in analyses of other nonangiosperm seed plant lineages (17–19) and is broadly consistent with the paleobotanical record. However, our analyses indicate that the evolutionary dynamics of the conifer lineages inhabiting mainly Southern Hemisphere environments, including both temperate and tropical habitats, differ from those in the Northern Hemisphere lineages. Specifically, Northern Hemisphere lineages have a greater proportion of very recent divergence times (i.e., within the past 5 My) and fewer deep divergences than Southern Hemisphere clades (Fig. 1B; distributions are significantly different at $P < 0.01$ using Mann–Whitney U test). Median node age, which we use to summarize these distributions, for combined Northern Hemisphere clades is nearly half that of combined Southern Hemisphere clades

(5.2 and 8.7 My, respectively). These differences appear to be robust to sampling; although Southern Hemisphere lineages are more poorly sampled than Northern Hemisphere lineages in our dataset, median node ages remain significantly younger in Northern Hemisphere lineages even after species sampling is reduced to 50% (*SI Appendix*, Table S1). Raw molecular branch lengths also are consistent with results from dating analyses; median molecular branch length in combined Southern Hemisphere clades is more than twice that of Northern Hemisphere clades (Fig. 1C) and also is greater in individual clades (*SI Appendix*, Fig. S3).

These broad differences among northern and southern conifers are driven by the major individual conifer clades. Predominantly Southern Hemisphere lineages such as Araucariaceae, Callitroideae, and Podocarpaceae have significantly different age distributions ($P < 0.01$ using Mann–Whitney U test) and older median node ages than the Pinaceae and Cupressoideae, the major lineages in the Northern Hemisphere (Fig. 1D), even after accounting for differences in sampling (*SI Appendix*, Table S1 and Fig. S1). Within the major Northern Hemisphere clades, the abundance of

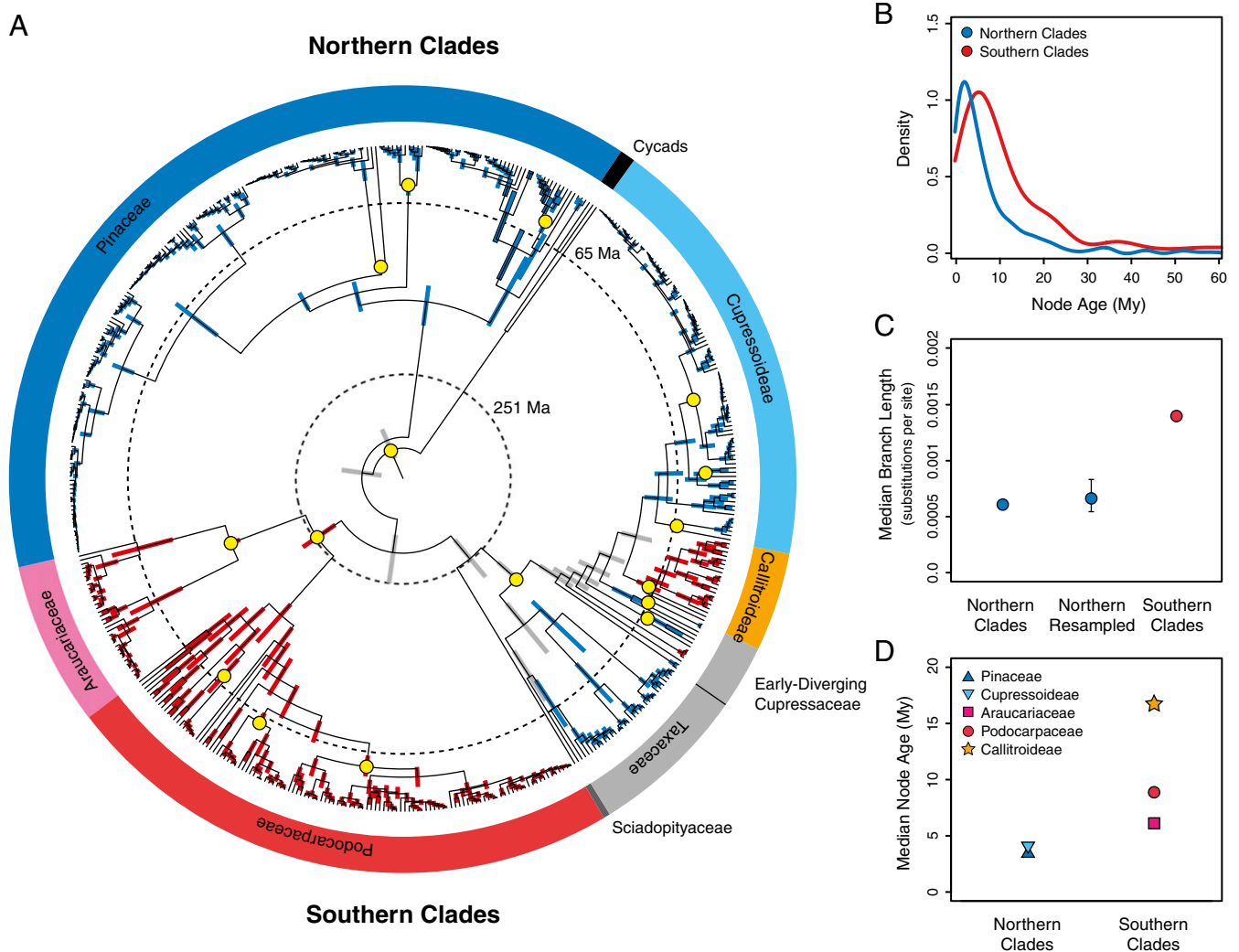


Fig. 1. (A) Dated phylogeny for 489 extant conifer species with cycads as an outgroup. Dashed circles with dates in millions of years ago (Ma) indicate boundaries between the Paleozoic/Mesozoic and Mesozoic/Cenozoic. The 95% confidence intervals on node ages are colored according to the geographic range of extant descendant species. Gray indicates a node that joins extant taxa with both Northern and Southern Hemisphere distributions, red indicates a node joining only extant Southern Hemisphere taxa, and blue indicates a node joining only extant Northern Hemisphere taxa. (B) Scaled density distributions of node ages (in million years; My) for all Northern and Southern Hemisphere clades. (C) Median molecular branch lengths in Northern Hemisphere clades, Southern Hemisphere clades, and Northern Hemisphere clades that were resampled to 50% species sampling; error bars represent 95% confidence intervals based on median node ages for 10,000 resampled trees. (D) Observed median node ages for major Northern and Southern Hemisphere conifer clades.

young divergences is largely the result of several unrelated species-rich subclades that have diversified recently. For example, ~45% of Northern Hemisphere diversity is contained in just two clades, *Pinus* and *Juniperus*, whose median node ages are under 3.5 My. Other large Northern Hemisphere subclades such as *Abies*, *Picea*, and *Cupressus sensu lato* show similarly young ages. Although large radiations are not absent in the Southern Hemisphere, they often show older divergences times. For example, Northern Hemisphere *Pinus* and Southern Hemisphere *Podocarpus* are comparable in terms of species diversity, but the observed median node age of *Podocarpus* is older than that of *Pinus* (5.96 versus 3.21 My), even though the initial divergence of *Podocarpus* from other conifers is more recent based on dating analyses and fossil evidence (10, 13, 20).

The different evolutionary dynamics of Northern Hemisphere clades and Southern Hemisphere clades also are reflected in lineage accumulation plots, which show a sharp increase in the number of Northern Hemisphere lineages that diverge in the last 20 My compared with Southern Hemisphere Callitroideae and Podocarpaceae (Fig. 2A). Sparser sampling in Southern Hemisphere

clades may cause the apparent slowdown in lineage accumulation in the last 5 My in Podocarpaceae and Callitroideae (although not in the better-sampled Araucariaceae), but this effect does not change the general difference between Southern Hemisphere and Northern Hemisphere clades over the Cenozoic. The rapid recent accumulation of lineages in Araucariaceae (and their relatively young divergence ages) primarily reflects a recent burst of speciation within *Araucaria* localized to New Caledonia (21).

To examine further the differences in evolutionary dynamics, tree topology and divergence time estimates in each focal clade were fit to five diversification models reflecting various origination, extinction, and diversification scenarios using a coalescence approach that allows extinction and origination rates to vary through time (22). All focal clades (Northern Hemisphere Cupressoideae and Pinaceae and Southern Hemisphere Podocarpaceae, Araucariaceae, and Callitroideae) were best fit by a model in which species diversity remained roughly constant through time, but species turnover rates in Northern Hemisphere clades were high, whereas those of Southern Hemisphere clades were variable but often lower (Fig. 2B; see also *SI Appendix*, Table S3).

Discussion

Our estimated divergence ages generally agree with results from published studies focusing on Podocarpaceae (15) and Pinaceae (23; in the conservative version of their calibrations), although they are considerably younger than a recently published study of Cupressaceae (24). The discrepancy in age estimates most likely results from differences in the implementation of prior age distributions, because both studies used a similar set of fossils as minimum age constraints. Mao et al. (24) generally used extremely wide uniform priors on age distributions associated with each fossil, potentially biasing posterior densities toward older divergence ages (25). Our conservative use of fossils as stem taxa with narrower lognormal prior age distributions potentially may bias estimates toward younger ages, but the marked relative differences between Northern and Southern Hemisphere clades that are the primary focus of this study remain unaffected.

The distribution and abundance of recently diverged species in Northern Hemisphere lineages is not related directly to latitude or specific habitat type within the Northern Hemisphere (*SI Appendix*, Fig. S2); they are broadly concentrated in regions of high conifer diversity such as mountainous areas of western North America and southern China. Southern Hemisphere conifers likewise show similar age patterns across wide geographic and environmental ranges; species inhabiting open, dry habitats in Australia and Argentina, temperate rainforests in New Zealand, and tropical montane forests in New Guinea all have generally older estimated divergence times than species in Northern Hemisphere clades. The process or processes underlying these differences therefore appear to operate on regional and continental scales, and an explanation for these patterns must encompass a wide variety of specific habitats and environments.

In the Northern Hemisphere, falling global temperatures over the Cenozoic are associated with a general shift in the middle- and high-latitude landmasses from warm subtropical and temperate climates in the Eocene to colder, drier, and more strongly seasonal climates from the Oligocene onwards (26–30), especially in the later Neogene with the onset of extensive Northern Hemisphere ice cover and glaciations (31–33). Increasingly extreme climatic shifts through time, especially in the later Neogene, may have favored the replacement of older lineages with those better adapted to cooler and/or drier conditions, resulting in high turnover rates and the disproportionate loss of ancient lineages. In addition, climatic and landscape history may have contributed to the abundance of very recent species divergences in Northern Hemisphere clades. Repeated instances of species migration, range contraction, and range expansion in response to glacial cycles can lead to isolated populations and speciation,

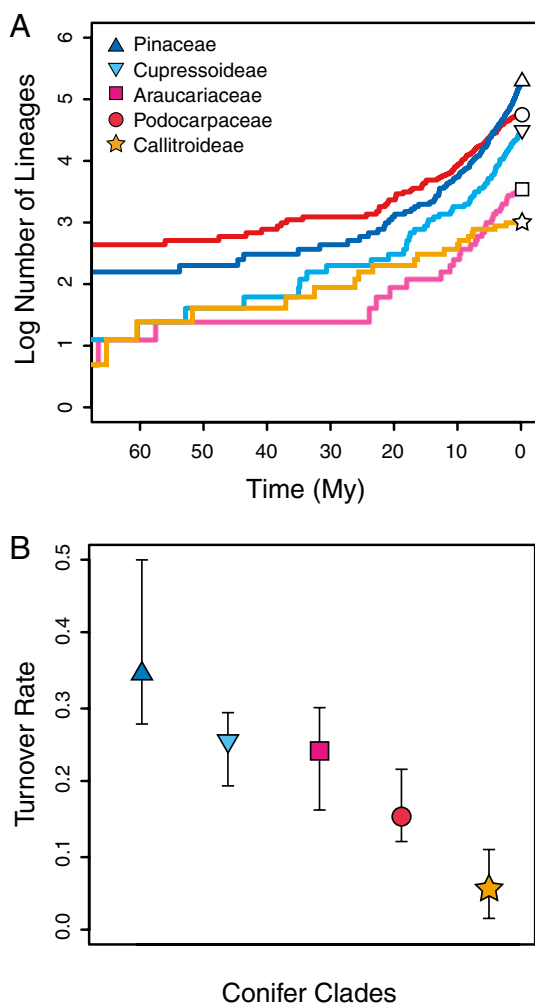


Fig. 2. Diversification dynamics of Northern and Southern Hemisphere conifer clades. (A) Lineage accumulation over the Cenozoic in Northern Hemisphere clades (Pinaceae, Cupressoideae) and Southern Hemisphere clades (Araucariaceae, Podocarpaceae, and Callitroideae). (B) Estimated turnover rates for Northern and Southern Hemisphere clades. Error bars represent 95% confidence intervals derived from fitting a constant diversity with turnover model to 1,000 different dated trees sampling the posterior age distributions from the BEAST analysis.

especially in the types of mountainous environments where conifer diversity is high (34–36). In a general sense, the structure of the Northern Hemisphere conifer clades may record the complex history of climate-driven turnover and range shifts taking place within the large midlatitude land areas of the Northern Hemisphere.

In contrast, southern landmasses with high conifer diversity, such as Australia, New Zealand, and South America, have drifted apart and northwards from the Cretaceous to the recent (37, 38). These movements have led to the scattered persistence of relatively warm or wet habitats, often moderated by oceanic climates (4), which appear to be reflected in aspects of modern vegetation. Trees living in Southern Hemisphere temperate forests are not as cold tolerant as many Northern Hemisphere temperate species (4, 39), and most southern conifers prefer relatively wet environments (10, but also see ref. 40). The Southern Hemisphere clearly experienced major climatic shifts over the Cenozoic, particularly the spread of open, dry environments in the late Neogene (41–43). However, temperate rainforests and broadleaf evergreen forests were common before the Pleistocene (44, 45) and still are present in New Zealand and in parts of Australia and South America. These now fragmented habitats are among those in which older conifer lineages adapted to warmer or wetter climates have survived at high diversity.

The evolutionary history of conifers undoubtedly reflects complex interactions among a suite of factors ranging from global-scale climatic and geographic patterns to smaller-scale attributes of specific lineages and their environmental settings. Nevertheless, this study suggests that there are large-scale and consistent differences between the diversification of southern and northern conifer clades. General differences in climatic and landscape history resulting from the dispositions of landmasses in the hemispheres appear to have left a distinct imprint on conifer evolutionary history. More work is needed to understand how these factors may have affected the biogeographic history of other clades.

Materials and Methods

We inferred a large DNA sequence-based phylogeny for conifers using the PHYLogeny Assembly with Databases pipeline (PHLAWD, <http://code.google.com/p/phlawd/>) (46). PHLAWD uses a “baited” sequence-comparison approach in which a small subset of sequences for a clade of interest provided by the user is used to filter GenBank sequences and to determine whether these sequences are homologous to the gene regions of interest. The program then conducts saturation analyses on these sequences by comparing uncorrected genetic distances with corrected distances based on a Jukes-Cantor (JC) substitution model. If a gene alignment is saturated, the alignment is broken up according to the National Center for Biotechnology Information classification system, and the blocks are aligned separately. We used this approach to search GenBank for three genes that generally have been used to construct phylogenetic relationships both within and among the major conifer clades, including two regions of chloroplast genome DNA, *matK* (sampled for 446 taxa in our dataset) and *rbcl* (437 taxa), and one nuclear coding region, 18S (97 taxa). We then added newly generated sequences from another nuclear gene, *PHYYP* (212 taxa), which were contributed by S.M. Our total species list was compared with conservative taxonomic treatments (6–8, 47), especially for Northern Hemisphere conifers, to avoid biasing our results by oversplitting particular clades. Species varieties generally were excluded from the analysis unless recent phylogenetic studies suggest they should be considered as separate species [e.g., in *Hesperocyparis*

(48)]. Three cycad genera (*Cycas*, *Zamia*, and *Encephalartos*) were used as an outgroup for this analysis because cycads have a more definitive early (Paleozoic) fossil record than *Ginkgo*, another potential outgroup, and can be used more reliably to date the divergence between conifers and other seed plant groups. Gnetales were not included in this analysis because our primary focus was on the evolution of extant conifers, and the phylogenetic placement of Gnetales remains uncertain, with different analyses and data sets providing high support for conflicting topologies (49).

Initial sequence alignments were estimated using MAFFT (v.6) (50), and profile alignments were estimated using MUSCLE (v.3.6) (51). The combined sequence matrix of 6,436 sites for 489 ingroup taxa and three outgroup taxa contained 40% gaps or missing sequence data, primarily because of incomplete sampling for 18S (known in only 20% of species). This gene was retained in the analysis because it is important for resolving and dating deep nodes within the conifer tree. An ML phylogenetic analysis of the total sequence alignment was performed using RAXML (v.7.2.6) (52), and tree searches were conducted under the GTR+CAT approximation of rate heterogeneity, partitioned by gene region. A criterion based on the Robinson-Foulds (RF) distance also was used to assess asymptotic convergence of the log likelihood score over time, where the ML search was stopped if the RF distance between two consecutive runs was smaller than 1% (53). The phylogenetic analysis was restarted 100 times to obtain a set of 100 ML trees, and each was scored under GTR+ Γ to estimate branch lengths and the likelihood score. A 50% majority rule consensus of the 100 ML trees showed that relationships were nearly identical among the different trees, with the exceptions relating to minor differences in relationships among very closely related species. The single best ML tree was used to carry out further analyses.

We estimated divergence times in conifers using Markov chain Monte Carlo (MCMC) methods implemented in BEAST (v.1.6.1) (54). BEAST allows for uncertainty in divergence time estimation by simultaneously incorporating uncertainty in both tree topology and the age of multiple fossil calibrations. Age estimates for fossil calibrations are treated as probabilistic priors rather than as point estimates (55; see also discussion of priors in *SI Appendix, SI Materials and Methods*). BEAST also employs an uncorrelated relaxed-clock (UCLN) model to estimate divergence times (56). For each branch, the UCLN independently draws substitution rates from a lognormal distribution, allowing substitution rates to be either autocorrelated (i.e., inherited from parent node to child node) or uncorrelated across the phylogeny, depending on the data.

For the BEAST analysis, a separate GTR+ Γ substitution model was applied to each gene region, and the topology was constrained to reflect the best tree from the ML analysis of the data in RAXML. The branch lengths of the constraint tree also were smoothed to time in r8s (57) to overcome the problem of inferring a zero probability during the initial parameter search in BEAST. We ran four independent MCMC runs of 50 million generations, sampling every 1,000 generations. To ensure that the posterior distribution of topologies and branch lengths came from the target distribution, convergence and proper sampling of the likelihood surface (effective sample size >200) of the chains was assessed using Tracer v. 1.5, with the first 10 million generations discarded as burn-in for each run. Post burn-in samples from the marginal posterior distribution were combined using Log-Combiner v. 1.6.1, and trees were summarized with TreeAnnotator. The results represent the maximum clade credibility trees with the consensus ages being the median estimate.

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