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RUNNING HEAD: LEMUR DIVERGENCE TIMES WITH FOSSIL PRIMATES

TITLE: Phylogeny and divergence times of lemurs inferred with recent and ancient fossils in the tree

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Abstract

Paleontological and neontological systematics seek to answer evolutionary questions with different datasets. Phylogenies inferred for combined extant and extinct taxa provide novel insights into the evolutionary history of life. Primates have an extensive, diverse fossil record and molecular data for living and extinct taxa are rapidly becoming available. We used two models to infer the phylogeny and divergence times for living and fossil primates, the tip-dating (TD) and fossilized birth-death process (FBD). We collected new morphological data, especially on the living and extinct endemic lemurs of Madagascar. We combined the morphological data with published DNA sequences to infer near-complete (88% of lemurs) time-calibrated phylogenies. The results suggest that primates originated around the Cretaceous-Tertiary boundary, slightly earlier than indicated by the fossil record and later than previously inferred from molecular data alone. We infer novel relationships among extinct lemurs, and strong support for relationships that were previously unresolved. Dates inferred with TD were significantly older than those inferred with FBD, most likely related to an assumption of a uniform branching process in the TD compared to a birth-death process assumed in the FBD. This is the first study to combine morphological and DNA sequence data from extinct and extant primates to infer evolutionary relationships and divergence times, and our results shed new light on the tempo of lemur evolution and the efficacy of combined phylogenetic analyses.

Keywords: total evidence, primatology, Bayesian phylogenetics, calibration, chronogram

A primary goal of phylogenetic systematics is discovering and describing species, as well as placing them in the Tree of Life (Felsenstein 2004). One impediment to this goal is extinction: more than 90% of species that ever lived are extinct (Novacek and Wheeler 1992). Understanding the evolutionary history of species can be improved with knowledge of extinct taxa (e.g., Pyron 2011, Pyron 2015). Extinct taxa inform us about the mode of character evolution and transitional forms (Slater et al. 2012; Lihoreau et al. 2015), the timing of species origin and disappearance (Foote 2000), and species distributions in deep time (Patzkowsky and Holland 2012). Unfortunately, biased preservation, incomplete specimens, and the lack of molecular data for comparison to extant species impedes the phylogenetic placement of fossils (Wiens and Morrill 2011; Sansom 2015). Despite these limitations, fossils can give key insights into the phylogenetic placements of living and extinct forms (Wiens et al. 2010; Wiens and Tiu 2012; Pattinson et al. 2015).

Combining morphological and molecular datasets, especially including fossils, can improve phylogenetic inference. Total evidence analyses including extinct taxa have improved resolution for phylogenetic problems as intractable as the relationships of amniotes (Eernisse and Kluge 1993), reptiles (Wiens et al. 2010; Reeder et al. 2015), cetaceans (Spaulding et al. 2009), wasps (Ronquist et al. 2012a), and spiders (Wood et al. 2012). The temporal information captured by fossils is most commonly used to calibrate nodes in a molecular phylogeny based on the assumed position of fossil taxa in extant trees (Parham et al. 2011). Uncertainty in assigning a fossil taxon to nodes in an extant tree may introduce error in divergence time estimation using node calibration. Further, multiple fossil taxa may be associated with a particular node in an extant tree and are reduced to a single calibration point (e.g., 45 fossils could be used for only seven calibration points in Ronquist et al. 2012a). To overcome these limitations, new methods

were designed that infer the topology and divergence times of living and extinct species jointly (Ronquist et al. 2012a) and parameterize the branching process of the phylogeny based on speciation and extinction rates from the fossil record (Heath et al. 2014). The model assumptions differ between these two approaches and the effects of these assumptions on results are becoming clear (e.g., Zhang et al. 2015).

The first method, known as "tip-dating" (hereafter TD), uses total evidence datasets to model the substitution rate of the molecular and morphological data partitions with extant and fossil tips in the phylogeny (Pyron 2011; Ronquist et al. 2012a). The TD method assumes a uniform prior probability on the branching process, such that branching events occur anywhere along internodes according to the branch lengths inferred from the data (Ronquist et al. 2012b, Zhang et al. 2015). Estimating the rate of morphological evolution is difficult with available Markov *k*-state models, however (Beck and Lee 2014), and if evolutionary rates are biased then the uniform branching prior may make divergence time estimation sensitive to the time prior. In contrast to the uniform branching assumption, process-based models such as a birth-death model are especially suitable when the study group has had non-zero extinction (Condamine et al. 2015). The fossilized birth-death process (hereafter FBD), implements a model with a branching process prior based on diversification dynamics (speciation and extinction rates) calibrated with the fossil record (Heath et al. 2014). The utility of fossil dating methods in systematics is evident from the recent surge in publications using them (e.g., Wood et al. 2012; Slater 2013; Arcila et al. 2015) but the efficacy and comparability of the methods have only recently been addressed (Beck and Lee 2014; Grimm et al. 2014, Zhang et al. 2015). In this study, we compare the divergence time estimates inferred from total evidence datasets using the TD and FBD

techniques with extant and extinct primates as an empirical system, focusing on lemurs of Madagascar.

The systematics of fossil and extant primates have been approached from two perspectives: paleontologists with morphological data and extensive sampling of extinct taxa (e.g., Seiffert et al. 2010; Ni et al. 2013; Pattinson et al. 2015), and neontologists with molecular data for nearly all extant species (e.g., Perelman et al. 2011; Springer et al. 2012; Pozzi et al. 2014a,b). Divergence time estimates from molecular data are typically older (60-80 million years ago, Ma, e.g., Perelman et al. 2011) than the appearance of the earliest true primate fossils ~56 Ma (Beard 2008). This discrepancy may be due to convergent slowdowns in molecular rates (Steiper and Seiffert 2012), the fossil record not capturing the timing of emergence (dos Reis et al. 2014a), or limitations of external calibration techniques that cannot use all available fossil information (Pyron 2011). In this study, we focus on the latter possibility.

Even with the extensive primate fossil record, multiple fossils are often reduced to calibrations of a single node; for example, 35 fossil taxa were reduced to 14 node calibrations in Springer et al. (2012). Calibration of the crown primate node has been suggested to be 55-56 Ma (Wilkinson et al. 2011; Kspeka et al. 2015), despite the fact that multiple fossils which may represent the first crown primates are known from a range of ages (e.g., Ni et al. 2013; Seiffert et al. 2015). Among the nodes in the primate tree used for divergence time calibration, the last common ancestor of Lorisiformes has been calibrated based on two key fossils: *Saharagalago* and *Karanisia* (e.g., Horvath et al. 2008; Chatterjee et al. 2009; Pozzi et al. 2014a, see Fig. 1 for taxonomy and simplified phylogeny). Dated at ~37 Ma (Seiffert et al. 2003), these two fossils have only informed a single node – a minimum bound for the divergence between Lorisidae and Galagidae (Springer et al. 2012; Pozzi et al. 2014a,b). The fossil lorisiforms do not represent the

ancestral node themselves, however, because they too share an ancestor with lorises and galagos in the past (Seiffert et al. 2003). Another limitation to node dating is topological uncertainty. The position of *Karanisia*, for example, is not well resolved and it is possibly a stem strepsirrhine, lemuriform or crown lorisid (Seiffert 2012). Given these caveats, calibrating the lorisiform node to the dates of the fossils may be biasing divergence time estimates towards the calibration point.

Other fossils have not been informative at all because stem taxa cannot be assigned to a node for calibration. Plesiadapiformes is possibly a stem primate lineage from the earliest Paleocene/Eocene (Bloch et al. 2007, but see Beard 1990 for the alternative view that plesiadapiforms are sister to Dermoptera), and as such it has had no bearing on the dating of the primate phylogeny because the lineage falls outside the crown group. Eocene crown primates (e.g., Adapiformes, Omomyiforms) and African stem strepsirrhines such as *Djebelemur* have not been informative in divergence time estimation despite their important time periods and geographic locations because they cannot be assigned to nodes for calibration. The lemurs of Madagascar are especially intractable with respect to fossil calibration because there are no true fossil lemurs. There are, however, 17 species of extinct lemurs that are subfossils dating from 400 – 20,000 years ago (Godfrey et al. 2010). Calibrations of lemur divergence times have used multiple primate and nonprimate outgroups (e.g., Yoder and Yang 2000; Horvath et al. 2008). Recently published ancient DNA has allowed some of the subfossils to be placed in the tree with greater precision (Kistler et al. 2015). To close the gap between neontology and paleontology, we focus on the strepsirrhine primates: Lemuriformes from Madagascar and Lorisiformes from Africa and Asia. We include 33 extinct primates, focusing on the earliest possible stem and crown primates, stem strepsirrhines and subfossil lemurs.

Lemurs are a monophyletic radiation of primates that diverged from their closest relatives, the lorisiforms, between 50 and 70 Ma based on node-calibrated molecular divergence times (Yoder and Yang 2000; Horvath et al. 2008; Fabre et al. 2009; Perelman et al. 2011; Pozzi et al. 2014a; Kistler et al. 2015). Living lemurs are species-rich (99 species currently recognized, Schwitzer et al. 2013, IUCN Redlist database www.iucnredlist.org, accessed February 28 2015), in addition to the 17 recently extinct species. It has proven difficult to resolve the lemur phylogeny using molecular data alone (Yoder 1994; Yoder and Yang 2000; Horvath et al. 2008; Perelman et al. 2011; Springer et al. 2012). Molecular analyses conflict regarding the placement of major clades, including the earliest diversification of taxonomic families characterized by short internodes and long branches (Horvath et al. 2008). The placement of the extinct giant lemurs in the phylogeny was originally based on the morphometric affinities of the extinct lemurs to living species (e.g., Jungers et al. 1991; Jungers et al. 1997). Fragments of ancient mitochondrial DNA (Karanth et al. 2005; Orlando et al. 2008) and, more recently, the entire mitochondrial genome for five taxa (Kistler et al. 2015) supported or overturned some of these morphology-based relationships. In this study, we infer near-complete phylogenies of extant and extinct lemurs and their closest relatives with combined morphological and molecular datasets. We date the tree with fossil tips and two different models of the branching process. This study is the first to jointly evaluate the relationships and divergence times of extinct and extant lemurs, and the results change our interpretation of the mode and tempo of lemur diversification.

MATERIALS AND METHODS

The methods follow the schematic given in Figure 2.

Taxonomic Sampling

The taxonomy of lemurs has changed with the increasing use of DNA sequences to delimit many cryptic species that were previously subsumed as single species. The most recent taxonomic compilation recognizes 97 species of living lemurs (Mittermeier et al. 2010), with two new species described since then (Rasoloarison et al. 2013; Thiele et al. 2013) for a total of 99 lemur species (IUCN redlist, accessed April 20 2015). Our dataset included 87 living lemurs (~87.88% of recognized living lemurs), and 14 extinct lemurs (82.35%, Godfrey et al. 2010). We also included a subset of other primates, including the closest extant relatives of lemurs, the Lorisiformes (67.85% of 28 IUCN recognized species), and eight haplorhine primates (< 3% of 294 IUCN recognized species). Fossil taxa included the following: four crown and two potential stem strepsirrhines, five adapiforms, two fossil haplorhines, three early primates of disputed taxonomy, and three stem primates (Fig. 1, Table 1). The complete data matrix included 148 taxa.

Morphological Data

For 47 taxa (16 extinct, 31 extant), we collected morphological data *de novo* from osteological museum specimens, casts and photographs of original specimens, with multiple specimens examined when possible to reflect variation and polymorphisms. The sample size per species varied with the availability of specimens; for example, some species were represented by a single specimen while others were scored for between five and 10 specimens. We supplemented the new dataset with data from the literature for 20 fossil taxa and 19 extant taxa (Ni et al. 2013; Seiffert et al. 2015). The total morphological dataset included 85 taxa.

The starting point for scoring characters was a morphological matrix with 421 characters from previous studies (OSM, Cartmill 1975; Cartmill 1978; Groves and Eaglen 1988; Tattersall and

Schwartz 1974; Tattersall and Schwartz 1991; Yoder 1994; Rasoloarison et al. 2000; Seiffert et al. 2003; Seiffert et al. 2015). Binary and multi-state characters described cranial and long bone features such as crests, processes, bony articulations, and foveae, the presence, number and orientation of foramina. Binary and multi-state dental characters included the presence/absence, relative orientations and development of teeth, cusps, crests, cristae/ids, conules and cingula/ids. We included eight quantitative measurements that were size-adjusted by dividing each variable by the geometric mean of all variables, and then converted to discrete states using gap-coding (Thiele 1993). Polymorphisms were scored as unique states as in Seiffert et al. (2015) to incorporate the polymorphic information in the dataset (Wiens 2000). A complete description of characters and states is given in the Online Supplemental Material (OSM). All characters were treated as unordered. For the taxa scored *de novo*, we were able to collect data on 40 – 60% of the 421 characters, principally cranial and dental characters and postcranial characters of the long bones. Missing data for each species ranged from <1% to 95% (OSM Table S1).

To test the assumption of character independence in the morphological dataset, we converted the original species X character data matrix into a pairwise species matrix for each character in which the values were binary states for the same (1) or different (0) states among pairs of species. These matrices for each character were concatenated, transposed into a pairwise character matrix, and the Gower dissimilarities of characters were calculated (using the *daisy* function in the cluster package, Maechler et al. 2015, for the R statistical environment, R Core Team 2014, and code from Dávalos et al. 2014, OSM File S2). Dissimilarity scores of 0 indicate that the character pair has identical state changes among species; i.e., characters may not be independent. One character of each pair that had 0 dissimilarity was omitted, choosing the character that showed the most 0 dissimilarities with other characters in the dataset. Fifty-one

characters were found to have identical state distributions among species, suggesting they may not be independent, and we excluded those characters in a reduced character dataset (OSM, File S3).

Molecular Data

We compiled published molecular sequences from GenBank using the software Geneious v.7.1.7 (Kearse et al. 2012) or directly from first authors. We selected six protein-coding loci chosen to maximize overlapping coverage among study species, including two mitochondrial loci (mtDNA: *cytochrome b* and *NADH dehydrogenase - 4*) and four nuclear loci (nDNA: *adenosine A3 receptor, cannabinoid receptor 1,* and *recombination activating gene 1* and *2*) for a total of 5767 base pairs. The GenBank accession numbers are available in OSM File S4 (data especially from Yoder et al. 1996; Yoder and Irwin 1999; Pastorini et al. 2001a,b; Murphy et al. 2001; Pastorini et al. 2002; Pastorini et al. 2003; Andriaholinirina et al. 2006; Louis et al. 2006; Andriantompohavana et al. 2007; Craul et al. 2007; Lei et al. 2007; Olivieri et al. 2007; Zaramody et al. 2007; Horvath et al. 2008; Johnson et al. 2008; Orlando et al. 2008; Groeneveld et al. 2010; Weisrock et al. 2010; Perelman et al. 2011; Rumpler et al. 2011; Springer et al. 2012; Markolf et al. 2013; Thiele et al. 2013; Pozzi et al. 2014a,b; Kistler et al. 2015). Sequences for each locus were aligned using amino acid translation alignment in MAFFT (Katoh et al. 2005) as implemented in Geneious v.7.1.7. Alignments were verified and edited manually as necessary (OSM File S5 and TreeBASE submission # 17704).

We analyzed three concatenated molecular matrices: 1) the mtDNA loci, 2) the nDNA loci, and 3) all six loci. For each matrix, the dataset was partitioned to reflect the heterogeneity in substitution rates within the matrix. Finding optimal partitioning schemes and models of sequence evolution for multi-locus datasets is an active area of research. One may chose *a priori*

to partition by each gene, each codon position of each gene, or some combination of these approaches. We conducted searches for the best partitioning scheme using likelihood statistics, as implemented in PartitionFinder software (Lanfear et al. 2012). We first specified each codon of each locus and then used the *greedy* search algorithm to find the partitioning scheme that maximized the fit of the data to the model while minimizing the number of parameters, using the Bayesian Information Criterion (BIC) as well as the second-order Akaike information criterion (AICc) as the measure of model fit. While alternative partitioning approaches are possible, this method is objective, repeatable, and has been used for tree inference and divergence time estimation in previous studies (e.g., Condamine et al. 2015, Lambert et al. 2015). The best-fitting partitioning scheme for the full concatenated dataset was one that included two partitions, each with their own model of sequence evolution: 1) all nuclear genes, 1st and 2nd codon positions of *cytochrome B*, 2nd and 3rd codon positions of *NADH dehydrogenase-4* (GTR+G), 2) 3rd codon position of *cytochrome B*, 1st codon position of *NADH dehydrogenase-4* (SYM+G; OSM Table S2). This partitioning scheme grouped sites in the molecular matrix into slow- and fast-evolving partitions, reflecting differences in the substitution rates and probability of state changes (see Results). Further details on the partitions used for analyses of the mtDNA-only, nDNA-only, and concatenated molecular dataset with reduced taxon sampling (discussed below, *Topology comparison*) are available in the OSM.

Clock Model Comparison

To compare clock models, we used the stepping-stone approach implemented in MrBayes v3.2.6 to calculate the marginal likelihoods of the data under the strict molecular clock model and the following relaxed-clock models: Brownian motion (Thorne and Kishino 2002, TK02), inverse gamma rates (IGR), and Compound Poisson Process (CPP, Ronquist et al. 2012a).

Stepping-stone analysis uses Markov chain Monte Carlo (MCMC) to estimate the likelihood of the given model close to the posterior distribution and at intervals approaching the prior distribution, and is an accurate measure of marginal likelihoods for model comparison using Bayes factors (Xie et al. 2010). Models were compared using Bayes factors by taking the exponent of the difference in marginal log likelihoods between two models. Models with Bayes factors between 3 and 20 were considered moderately supported over alternate models and greater than 20 were considered strong support over alternate models (Kass and Raftery 1995). We ran stepping-stone analyses for 50 steps of 2.5 million generations each, sampling every 2,500 generations and discarding the first step and first 10% of each subsequent step as burn-in.

Phylogenetic Inference

We jointly inferred the phylogeny and divergence times by conducting Bayesian analyses of the total evidence dataset using MrBayes v3.2.6 (Ronquist et al. 2012a; Ronquist et al. 2012b). Additional unconstrained (i.e., non-clock) analyses were conducted on the mtDNA, nDNA, morphology and total evidence datasets to investigate the phylogenetic signal in each dataset, and we compared trees inferred from each dataset based on their marginal likelihoods from stepping-stone analyses (see OSM). With the total evidence datasets, we used both the TD and FBD approaches to estimate the divergence times calibrated with the dates of fossil taxa included in the dataset. The TD analysis parameterizes the substitution rate of the morphological data partition as well as the molecular data and assumes the probability of branching events is uniform (Ronquist et al. 2012a). The FBD analysis estimates speciation, extinction, and preservation parameters from the fossil data to calibrate the diversification rate of the tree and parameterize the branching process (Heath et al. 2014). In the original implementation of the FBD method, the taxonomic association of fossils to living clades is specified *a priori*, similar to

node dating. In MrBayes v3.2.3 and more recent versions, the phylogenetic position of fossils is inferred from the morphological data partition. The fossil dates were taken from the literature (Table 1). For both TD and FBD analyses, we used a uniform prior between the minimum and maximum fossil ages when these dates were available. For the subfossil lemurs, recent dates (i.e., Holocene) are available but setting maximum bounds is not possible. We therefore used a fixed prior because only a point estimate was available with comparatively narrow confidence intervals (a few hundred years, compared to millions of years for other fossil taxa). Dates were first taken from the Paleobiology database (Behrensmeyer and Turner accessed 2015) and verified with primary and secondary literature, especially Hartwig (2002) and references therein, and Godfrey et al. (2010). To evaluate the effects of having a distribution for the calibration priors on divergence time estimates, we ran two FBD analyses: one with the age-range distributions from Behrensmeyer and Turner (accessed 2015, Table 1) and one analysis with only fixed point estimates on divergence dates (the midpoint of the age ranges). Here we focus on the results with distributions on age calibration priors (results from fixed date analyses were similar and are discussed in OSM, see Fig. S3-5 and TreeBASE submission # 17704). We set *Purgatorius* as the outgroup because it is the earliest known possible stem primate or stem euarchontan (Hartwig 2002; Rose 2006).

Model Specifications and Diagnostics

The model of evolution for each data partition was specified *a priori* using the results of the optimal model tests for the molecular dataset and the Markov-*k* model of morphological evolution (standard variable model, Lewis 2001). For the morphological partition, we used the ascertainment bias correction implemented in MrBayes, such that constant characters were removed, while variable and autapomorphic characters were retained. Shapes of the gamma

distributions of rate variation among characters, substitution rates and state frequencies were unlinked among data partitions. We ran two independent Metropolis coupled MCMC (MC3) searches with four chains for 60 - 70 million generations, sampling every 5,000 generations. Three chains were heated (temperature = 0.01) and one was cold which recorded the model parameters. We used the following prior parameter settings: variable rate prior, uniform branching prior for TD and birth-death process prior for FBD, TK02 relaxed clock model with values chosen from an exponential distribution with a rate parameter of 0.1, and a gamma-distributed clock rate. This latter prior defines the prior probability of the evolutionary rate parameter, and dating analyses are sensitive to the clock rate prior, especially when multiple data partitions are defined (dos Reis et al. 2014b). We adjusted the gamma distribution according to the number of data partitions to approximate an independent identically distributed prior by dividing the initial prior shape and rate parameters (2 and 4, respectively) by the number of partitions, such that the shape parameter was 0.666 and the rate parameter was 1.33 (following dos Reis et al. 2014b). This prior placed the highest probabilities on substitution rates in the range of $1X10^{-2}$ to $1X10^{-3}$ substitutions/site/million years, in line with previous studies of primate molecular evolution (Yoder and Yang 2000; Yang 2008). The FBD analysis included additional parameters with the following prior settings: exponentially distributed speciation prior (rate = 20), beta-distributed extinction fraction (extinction rate / speciation rate) and fossilization priors (shape and rate = 1), 'samplestrat' parameter set to 'fossiltip' to indicate the fossil lineages ending in distinct tips rather than as ancestors, and sample probability of 0.25 (approximating the proportion of extant primates in the sample, ~100 sampled / ~400 total extant recognized species, IUCN RedList accessed February 2015). MrBayes v3.2.6 was run on the CIPRES Science Gateway (Miller et al. 2010). Codes are available in OSM File S6.

We verified convergence of the MC3 search by: 1) plotting the time series of parameter values sampled from each chain to assess stationarity; 2) quantifying the effective sample sizes (ESS) for all model parameters, representing the number of independent estimates of the parameter values drawn from the posterior, with ESS values >200 being ideal (quantified in Tracer v1.6, Rambaut et al. 2014); 3) verifying the average standard deviation of split frequencies (ASDSF) were <0.01 and potential scale reduction factor (PSRF) values were stable around 1.00; and 4) examining the split frequencies among chains and generations using the utilities in the online application Are We There Yet (Nylander et al. 2008). For all parameters, independent runs had exhibited mixing and stationarity with ASDSF < 0.01 and PSRF ~1 by ~30 million generations. ESS values combined from the two runs were > 200 for most parameters and the split frequencies of tree comparisons suggested trees converged between runs. We discarded the first 50% of generations as burn-in and summarized the posterior distribution of topologies as the mean clade credibility (MCC) tree (i.e., *contype=allcompat* command in MrBayes v3.2.6).

Table 1. Fossil taxa included in phylogenetic analysis and age-range used for divergence-time estimation, in millions of years ago (Ma).

Genus	Species	Min age (Ma)	Max age (Ma)	Reference
Adapis	*parisiensis*	33.9	41.3	1,2
Aegyptopithecus	*zeuxis*	28.1	33.9	1,2
Altiatlasius	*koulchii*	56	59.2	1,2
Anchomomys	*frontanyensis*	37.2	48.6	1,2
Archaeoindris	*fontoynonti*	0.002149		3
Archaeolemur	*edwardsi*	0.001		3

Archaeolemur	*majori*	0.0014		3
Babakotia	*radafolia*	0.00484		3
Branisella	*boliviana*	26.4		2
Cantius	*abditus*	50.3	55.8	1,2
Carpolestes	*simpsoni*	55.8	56.8	2
Daubentonia	*robustus*	0.001		3
Djebelemur	*martinezi*	41.3	56	1,2
Donrussellia	*provincialis*	48.6	55.8	1,2
Hadropithecus	*stenognathus*	0.0016		3
Karanisia	*clarki*	33.9	38	1,4
Komba	*sp.*	20	22.4	1,2
Leptadapis	*magnus*	33.9	41.3	1,2
Megaladapis	*edwardsi*	0.001		3
Megaladapis	*grandidieri*	0.001		3
Megaladapis	*madagascariensis*	0.00276		3
Mesopropithecus	*pithecoides*	0.0014		3
Mesopropithecus	*dolichobrachion*	0.0014		3
Nycticeboides	*simpsoni*	5.3	11.6	2
Pachylemur	*jullyi*	0.0117		3
Palaeopropithecus	*ingens*	0.001		3
Palaeopropithecus	*maximus*	0.00216		3
Plesiadapis	*tricuspidens*	56	58.7	1,2
Plesiopithecus	*teras*	28.1	33.9	1,2
Pronycticebus	*gaudryi*	38	47.8	2
Purgatorius	*unio*	63.3	66	1,2
Saharagalago	*misrensis*	33.9	38	1,4
Teilhardina	*americana*	50.3	55.8	1,2
Wadilemur	*elegans*	28.1	33.9	1,4

1: Behrensmeyer and Turner accessed 2015, 2: Hartwig 2002, 3: Godfrey et al. 2010, 4: Seiffert et al. 2003

Topology comparison

We investigated the congruence of phylogenetic inferences derived from the total evidence (TE) dataset and the separate analyses of the mtDNA, nDNA and morphological datasets, as well as topologies inferred by maximum likelihood and parsimony (see OSM for details). First, we found the best partitioning schemes for each molecular dataset separately using PartitionFinder (OSM Table S3-S5). Unconstrained phylogenies were inferred for each dataset and the total

evidence dataset using two exponentially distributed priors on branch lengths, such that internal branches had a prior of 0.01 (shape parameter=100) and external branches had a prior of 0.1 (shape parameter=10), since a single prior on branch lengths may be inappropriate when internal branches are shorter than external branches (Yang & Rannala 2005). We then used Bayesian concordance analysis to infer the tree that maximized the relationships in common among trees inferred from separate loci (BUCKy, Larget et al. 2010). The primary concordance tree (PCT) consisted of a reduced set of 36 extant taxa which had data in all three data types. Trees from each dataset and the total evidence dataset were then pruned to this 36 taxon set.

We compared the topological similarity of 1000 trees from the posterior distribution of trees from analyses of each dataset separately and the TE dataset to the PCT by computing the Robinson-Foulds tree distance (Robinson & Foulds 1981) using the *multiRF* function in the R package *phytools* (v0.4-45, Revell 2012). For null distributions, we also generated two sets of 1000 random trees, first simply by randomly shuffling the tip labels on the PCT 1000 times (using the *phyloshuffle* function in the R package *phylotools*, Zhang, Mi & Pei 2010), and also by simulating 1000 trees with 36 tips under a birth-death model (speciation=0.15, extinction=0.05 similar to the results found from the FBD analysis described below, using the *diversitree* package in R, FitzJohn 2012), applying the tip labels from the PCT and randomly shuffling the tip labels again. This allowed us to investigate the differences among trees inferred from different datasets. We used this same methodology to compare the trees inferred from the total evidence dataset with all taxa using Bayesian, maximum likelihood, and parsimony techniques (OSM Methods).

To test for significant differences in the fit of the data to the different tree topology models, the marginal likelihood of the total evidence dataset was compared under the pruned

topology inferences from each separate dataset, the PCT, and the total evidence tree using stepping stone analyses. Topologies in the stepping stone analyses were fixed by specifying node constraints for the nodes from each dataset using the *createMrBayesConstraints* function in the R package *paleotree* (Bapst 2012). Lastly, our analyses suggested two especially surprising results: (1) with the time-calibrated total evidence analysis and the reduced morphological dataset (but not the full dataset), a sister relationship between the African fossil primate *Plesiopithecus tricuspidens* and the extant lemuriform *Daubentonia madagascariensis*, and (2) the extinct lemur genus *Megaladapis* was inferred to be sister to all lemuriforms after the most basal split of *Daubentonia* from other lineages, rather than inferred to be sister to Lemuridae, as was the case with ancient DNA (Orlando et al. 2008, Kistler et al. 2015). We used stepping stone analyses to estimate the marginal likelihood of the total evidence data with four different topology constraints: (1) the FBD tree inferred from the total evidence analysis with the reduced morphological dataset (with the *Plesiopithecus* + *Daubentonia* node), (2) the tree inferred from the total evidence analysis with the reduced morphological dataset but with *Megaladapis* constrained to be sister to Lemuridae, (3) enforcing a negative constraint on the *Daubentonia* + *Plesiopithecus* relationship, such that the probability of a tree containing this relationship was 0, and 4) the tree inferred from the total evidence analysis with the reduced morphological dataset and constraints based on the PCT. Further details are available in the OSM.

Morphological character evolution

To further investigate the surprising sister relationship between *Plesiopithecus tricuspidens* and *Daubentonia madagascariensis*, we mapped synapomorphies on the most parsimonious trees that included the *Plesiopithecus* + *Daubentonia* sister relationship. To further validate the inferences of synapomorphies from the parsimony analysis, we found the posterior probabilities

of character state estimates at the *Plesiopithecus* + *Daubentonia* node for the characters found to be synapomorphies. We used MrBayes to estimate the ancestral states of each morphological character at the node by constraining that node, using the *report* command for the morphological partition and setting *ancstates* to 'yes'. We then ran two MCMC searches for 10 million generations, sampling every 1000 generations, discarded the first 50% as burnin, and combined the post-burnin generations.

Documentation

Additional methods and results, as well as input data matrices and code to run analyses, and output trees are available in the OSM files archived in the Dryad Digital Repository (http://datadryad.org/review?doi=doi:10.5061/dryad.51f00), trees and data matrices are archived in TreeBASE (www.treebase.org, ref. # 17704), and the morphological data are available in MorphoBank (www.morphobank.org, project P2167).

RESULTS

Clock Model Comparison

The relaxed-clock model with the highest marginal likelihood was the TK02 model, with the Bayes factors in support of the TK02 model >$2X10^{23}$, indicating there was strong evidence for the TK02 model being a better fit to the data than other models (Table 2). For all model comparisons, the differences between models were always greater than the differences between runs within analyses (1-10 log likelihood units difference between runs within analyses compared to >50 log likelihood units difference between model comparisons). We therefore chose the TK02 model for divergence time analyses.

Parameter rate estimates

Posterior estimates of model parameters, such as substitution rates for molecular and morphological partitions and base frequencies, were consistent across analyses. The 95% HPD of substitution rates for the molecular partitions were 0.35-0.38 substitutions/site/Ma for the slow-evolving partition (nuclear genes, 1st and 2nd codon positions of *cytochrome B*, 2nd and 3rd codon positions of *NADH dehydrogenase-4*), and 3.99-4.24 sub./site/Ma for the fast-evolving partition (3rd codon position of *cytochrome B*, 1st codon position of *NADH dehydrogenase-4*). The morphological partition substitution rate was intermediate, at 2.22-2.63 sub./character/Ma. The mean TK02 variance parameter of 0.2 (95% HPD 0.08-0.38) and the clock rate parameter of $1.2X10^{-2}$ ($8X10^{-3}$-$1.2X10^{-2}$) indicated low rates of change across the tree. The net speciation rate estimate from the FBD analysis was 0.06 species/Ma (0.03-0.11), the relative extinction fraction (extinction / speciation) was 0.75 (0.40-0.96), and the relative fossilization rate was 0.09 (0.004-0.33).

Phylogenetic Inferences

The data under the FBD model had a higher mean marginal likelihood (-89950.87) than under the TD model (-89984.41), and the difference between models (33.54 log likelihood units) gives a Bayes factor of $3.68X10^{14}$, suggesting the FBD is a better fit to the data than the TD (following Kass and Raftery 1995). The MCC trees generated from the TD and FBD analyses differed in the topology inferred for some fossil taxa, and those nodes had low posterior probabilities (Table 3, Fig. 3, OSM Fig. S1-4, File S7-9, File S16-19, TreeBASE submission # 17704). Especially significant are the placements of the fossil taxa *Plesiopithecus teras* and *Djebelemur martinezi*. Both taxa were suggested to be stem strepsirrhines in previous studies (e.g., Seiffert et al. 2003) but here we inferred *Plesiopithecus* to be sister to *Tarsius* in the FBD analyses with the full morphological dataset (Fig. 3), and sister to *Daubentonia* in the FBD

analyses with the reduced dataset and in all TD analyses (OSM Fig. S2-4). *Djebelemur* was a stem strepsirrhine in the FBD analysis with the full morphological dataset (Fig. 3), while it was a stem lorisiform with the reduced morphological dataset and in the TD analyses (OSM Fig. S2-4).

Table 2. Marginal likelihood of each clock model for divergence-time estimation, calculated as the mean of the summed marginal likelihoods across 50 steps of a stepping-stone analysis. Each model was compared to the model with the lowest marginal likelihood (Thorne-Kishino 2002, TK02) with Bayes factors (TK02 likelihood *versus* alternate model likelihood). The TK02 model had the highest marginal likelihood, exceeding the next-best model (CPP) by ~54 log likelihood units and Bayes factor ~$2X10^{23}$, indicating strong support for the TK02 model over other models.

Model	Marginal likelihood (ln)	Bayes factor (TK02/alternate model)
TK02[a]	-89920.95	-
CPP[b]	-89974.63	$2.1X10^{23}$
IGR[c]	-90030.54	$3.93X10^{47}$
Strict[d]	-90134.51	$5.77X10^{92}$

[a] Thorne-Kishino 2002, [b] Compound Poisson Process, [c] Inverse Gamma Rate, [d] strict molecular clock.

Topology comparisons

We investigated the congruence of tree topologies inferred from the mtDNA, nDNA and morphological datasets separately (OSM Figs S6-S8, Files S10-S12) using a Bayesian concordance analysis (BUCKy). Nodes in the 36-taxon primary concordance tree (PCT, OSM Fig S9, File S13) were supported by concordance factors (CFs) between 0.292 and 1 (median = 0.669), which can be interpreted as the mean proportion of data types for which the same nodes were inferred. CFs in the PCT suggest that 35% of nodes were congruent among all data types, while 56% of nodes were congruent among two data types, and 0.09% of nodes were found in only one data type, on average.

We compared the PCT to the posterior distribution of trees inferred from analysis of the total evidence dataset (TE), mtDNA, nDNA, and morphological data using Robinson-Foulds distances (OSM Figure S10, Table S6). Trees inferred from morphology alone had the greatest distance from the PCT (mean distance=40.75, SE=0.08), indicating the morphological trees were least congruent with the PCT. Trees inferred from the TE had the lowest distance from the PCT (mean=6.04, SE=0.05), followed closely by trees from the mtDNA (mean=7.56, SE=0.04), and the trees inferred from the nDNA had intermediate distance values (mean=20.89, SE=0.04, OSM Table S6). All trees inferred from data were closer to the PCT than random trees (mean=65-68, OSM Figure S10, Table S6).

Topology tests were conducted by comparing the marginal likelihood of the full total evidence dataset with the 36 taxa under topology constraints to match the TE, PCT, mtDNA, nDNA and morphology trees. The marginal likelihood of the data was higher with nodes constrained to match the PCT tree than under topologies inferred from the TE and trees from each dataset separately (Bayes factors=6.66 for the PCT tree compared to TE and $>10^{19}$ compared to alternate topologies, OSM Table S8a). This result suggests that the PCT is a better fit to the data than trees inferred under each dataset separately or all data combined for this subsample of 36 taxa. When comparing the data for the full taxon set, however, we found that enforcing nodes inferred from the FBD analysis with reduced morphological characters is a better fit than alternatives (Bayes factors > $1X10^{90}$ for the FBD tree compared to alternate topologies, OSM Table S8b). These topology tests lend strong support for the relationships inferred from the concatenated dataset over alternatives including constraints based on the primary concordance among data types.

Divergence time inferences

The divergence times estimated from the TD analyses were older than those estimated from FBD (Fig. 4, Table 4), and the TD dates were older than previously inferred using node dating (Table 5). The results of the FBD analysis with wide and fixed date priors were comparable, with a mean difference in the median estimates of 0.53 Ma, and the 95% HPD range was 1 Ma wider on average with fixed dates compared to HPDs estimated using age distributions (OSM Fig. S5).

Table 3. Summary of the phylogenetic placement of taxa in this study compared to previous hypothesized topologies. Results from different analytical techniques are as follows: TD: tip-dating method, FBD 1: fossilized birth-death process with the full 421 character morphological dataset, FBD 2: FBD analysis with the reduced 369 character dataset.

Taxon	Previous placement	Ref	TD placement	FBD placement 1	FBD placement 2
Stem primates, Plesiadapiforms: *Purgatorius*, *Carpolestes*, *Plesiadapis*	Outside crown primates	1	Sister to Crown Primates	Sister to Crown Primates	Sister to Crown Primates
Early primates: *Donrusselia*	Adapiformes	2	Sister to Adapiformes	Sister to Adapiformes	Sister to Adapiformes
Teilhardina	Omomyiformes, Sister to Tarsiidae	3	Sister to Crown Primates	Sister to Crown Primates	Sister to Crown Primates
Altiatlasius	Problematic, possibly Omomyiformes, Sister to Anthropoidea	2,4	Sister to Adapiformes	Sister to Crown Primates	Sister to Adapiformes
Adapiformes	Sister to Strepsirrhini, Sister to Haplorhini	5	Sister to Crown Primates	Sister to Crown Primates	Sister to Crown Primates
Djebelemur	Sister to Strepsirrhini (Stem Strepsirrhini), sister to Lorisiformes (Crown Strepsirrhini)	6-8	Sister to Lorisiformes (Crown Strepsirrhini)	Sister to Strepsirrhini	Sister to Lorisiformes (Crown Strepsirrhini)

Plesiopithecus	Sister to *Daubentonia* (Lemuriformes), Lorisiformes, Stem Strepsirrhini, unresolved	8-11	Sister to *Daubentonia* (Lemuriformes)	Sister to *Tarsius*	Sister to *Daubentonia* (Lemuriformes)
Wadilemur	Crown Lorisiformes, stem galagid	12	Sister to Galagidae	Sister to Galagidae	Sister to Galagidae
Komba	Crown Lorisiformes, stem galagid	12	Sister to *Euoticus* (crown Galagidae)	Sister to *Euoticus* (crown Galagidae)	Sister to *Euoticus* (crown Galagidae)
Nycticeboides	Sister to *Nycticebus* (crown Lorisidae)	12	Sister to *Nycticebus* (crown Lorisidae)	Sister to *Nycticebus* (crown Lorisidae)	Sister to *Nycticebus* (crown Lorisidae)
Saharagalago	Crown Galagidae	11	Sister to Lorisiformes	Sister to Lorisiformes	Sister to Lorisiformes
Karanisia	Crown Lorisidae	11	Sister to Lorisiformes	Sister to Lorisiformes	Sister to Lorisiformes
Lorisidae / Galagidae	Paraphyletic, monophyletic	13, 14	Monophyletic	Monophyletic	Monophyletic
Daubentonia robustus	Sister to *Daubentonia madagascariensis*	15	Sister to *D. madagascariensis*	Sister to *D. madagascariensis*	Sister to *D. madagascariensis*
Megaladapis	Sister to Lepilemuridae Sister to Lemuridae	16-18	Sister to all lemurs *sans Daubentonia*	Sister to all lemurs *sans Daubentonia*	Sister to all lemurs *sans Daubentonia*
Archaeolemuridae	Sister to Palaeopropithecidae + Indriidae	15, 17-19	Sister to Palaeopropithecidae + Indriidae	Sister to Palaeopropithecidae + Indriidae	Sister to Palaeopropithecidae + Indriidae
Palaeopropithecidae	Sister to Indriidae	15, 17-19	Indriidae paraphyletic with *Indri* sister to Palaeopropithecidae	Indriidae paraphyletic with *Indri* sister to Palaeopropithecidae	Indriidae paraphyletic with *Indri* sister to Palaeopropithecidae
Pachylemur	Sister to *Varecia,* Lemuridae	15, 17	Sister to *Varecia,* Lemuridae	Sister to *Varecia,* Lemuridae	Sister to *Varecia,* Lemuridae
Hapalemur simus	*Hapalemur* paraphyletic, *H. simus* sister to *Lemur*	20	*Hapalemur* monophyletic, *H. simus* sister to other	*Hapalemur* monophyletic, *H. simus* sister to other	*Hapalemur* monophyletic, *H. simus* sister to other

	catta		*Hapalemur*	*Hapalemur*	*Hapalemur*
Phaner	Cheirogaleidae, Lepilemuridae	21-23	Sister to Cheirogaleidae	Sister to Cheirogaleidae	Sister to Cheirogaleidae

1: Bloch et al. 2007; 2: Hartwig 2002; 3: Beard 2008; 4: Ni et al. 2013; 5: Gebo 2002; 6: Marivaux et al. 2013; 7: Seiffert 2012; 8: Pattinson et al. 2015; 9: Godinot 2005; 10: Simons & Rasmussen 1994; 11: Seiffert et al. 2003; 12: Seiffert et al. 2005; 13: Yoder et al. 2001; 14: Masters et al. 2005; 15: Godfrey et al. 2010; 16: Tattersall & Schwartz 1974; 17: Kistler et al. 2015; 18: Karanth et al. 2005; 19: Jungers et al. 1991; 20: Pastorini 2000; 21: Tattersall & Schwartz 1991; 22: Horvath et al. 2008; 23: Springer et al. 2012

Table 4. Comparison of the differences in age estimates for 21 nodes in the phylogenies among dating techniques and datasets. "Full" refers to the complete 421 character morphological data matrix, and "reduced" refers to the subset of 369 characters that were found to be independent based on the Gower dissimilarity of state changes among species. Mean difference of age estimates in millions of years ago.

Comparison	**Mean difference**
FBD full vs FBD reduced	-2.55
FBD full vs TD full	-12.10
FBD reduced vs TD reduced	-9.48
TD full vs TD reduced	0.07

Morphological character evolution

We investigated the evolution of morphological characters, focusing on the node that supports the relationship between *Daubentonia* and *Plesiopithecus* to assess what characters support this node in the reduced morphological dataset. The node was present in 36% (20 / 55) of most parsimonious trees (OSM Fig S12, File S15). Across the 20 trees, we found that the synapomorphies linking these taxa included simplification of the lower molar structures (OSM Table S9). This included lower first and second molar cristid obliqua that terminate at the base of the trigonid, lower second molar trigonids and talonids of approximately equal height, weak or rounded cristid obliqua, lower third molars slightly shorter than second molars, and no hypoconulid on the lower third molar (see OSM Table S9). We found that these character states had high posterior probabilities (>0.80) for ancestral estimates at the *Plesiopithecus* + *Daubentonia* node (OSM Table S9), indicating that the parsimony-based synapomorphies are supported in a probabilistic modelling framework. Characters supporting *Plesiopithecus* as a strepsirrhine include mesiodistally compressed lower canines, high crowned and procumbent

lower canines, lower third premolar mesial roots lateral to the distal roots, two lower third premolar roots, and no cristid obliqua on the lower fourth premolar (OSM Table S9). It is important to note that *Plesiopithecus* lacks a toothcomb (procumbent, laterally compressed anterior lower dentition used primarily for grooming fur), which is one synapomorphy of strepsirrhines.

DISCUSSION

This study sought to compare two new methods of inferring phylogeny and divergence times with living and extinct taxa, and to infer the phylogeny and divergence times of primates, focusing on lemurs and their close relatives, lorisiforms. The results revealed striking differences in the divergence time estimates and substantially different statistical support for the two models. The new phylogenies we inferred for lemurs are the most taxonomically complete to date, including representatives of every genus of extant and extinct lemurs as well as inferring the positions of these taxa with strong support. We inferred the split between Haplorhini and Strepsirrhini to be post-Cretaceous, albeit with highest probability density including up to 70Ma, instead of the pre-Cretaceous-Paleogene dates usually inferred from molecular data. Further, we inferred divergence times for lemurs that are more recent than previously estimated from molecular data only. The divergence of the families was concentrated around the Eocene-Oligocene boundary, a geological time period associated with major faunal turnover in many primate clades (Seiffert 2007). These results have implications for the drivers of diversification in primates, especially extant and extinct lemurs.

Differences in divergence times between TD and FBD

The divergence dates for lemurs using TD were ~9-12 My older than those inferred from FBD, on average. While previous node-calibrated estimates of lemur origins suggested the most

recent common ancestors (MRCAs) of all lemurs occurred 41-75 Ma, we estimated the MRCA of lemuriforms to exist 58-72 Ma using TD, and 40-56 Ma from FBD (Table 5). Node-calibrated molecular phylogenies dated the subsequent divergence of lemur families to ~30-40 Ma, and the relationships among families were unresolved (Yoder and Yang 2004; Horvath et al. 2008; Chatterjee et al. 2009; Perelman et al. 2011). Our TD analyses suggested the divergences among families occurred ~50-62 Ma, while the FBD analysis inferred divergence times ~34-49MA (Table 5). Our comparison of the marginal likelihoods for these models suggested that the FBD was a better fit to our data than the TD (Bayes factor > 10^4). The non-overlapping divergence time estimates suggest there are some major differences in the way that time is modelled by these methods.

Recent studies using the TD method have also recovered earlier dates than those inferred using node dating (Ronquist et al. 2012a; Slater et al. 2012; Wood et al. 2012; Slater and Harmon 2013; Beck and Lee 2014). One possible explanation is that the TD method may overestimate divergence times because the Markov-*k* model of discrete morphological evolution may underestimate the morphological rate of change (Beck and Lee 2014). We found that the morphological substitution rate was intermediate between the slowest and fastest evolving molecular partitions. If there is character state saturation in the morphological data, the same character states will appear in different species through homoplasy, leading to underestimated rates of morphological substitution (Wagner 2000), and in turn to overestimated dates.

In conjunction with the potential issues related to estimating rates of morphological evolution, the branching process prior in the TD analysis assumes a uniform prior distribution on branching events, in contrast to the birth-death prior in the FBD (Zhang et al. 2015). The choice of the branching prior in divergence time estimation is not trivial, and a birth-death prior is more

appropriate than a pure-birth prior when extinction is non-zero (Condamine et al. 2015). If the TD method is biased by an underestimated morphological substitution rate and the probability of branching is assumed to be equal through time, then the TD methodology may be prone to pushing nodes deep into the past because the posterior probabilities on node ages are inadequately constrained and sensitive to the time prior. As pointed out by one reviewer of this article, this may result from too much flexibility in the model with the uniform branching prior because node ages are not constrained as they are in node calibration. In contrast, the FBD analysis assumes a birth-death prior for the branching process, such that the probability of a branching event is conditional on the parameterization of the inferred speciation and extinction rates of the tree. This constraint on the branching process means that nodes cannot be pushed too deep in the phylogeny if extinction rates are inferred to be greater than zero because a phylogeny with deep nodes and long external branches indicates low extinction (Pybus and Harvey 2000).

Lastly, the uncertainty in placing fossils on the tree given their patchy and sometimes uninformative character data compounds the uncertainty in branching times and substitution rates. For example, the positions of some fossils breaking up long branches of the tree may draw nodes deeper into the past than if no fossils were considered. The placement of *Megaladapis* as sister to all non-aye-aye lemurs certainly changes our interpretations of crown ages. These methodological considerations may explain the earlier divergence times inferred using TD compared to FBD and previous node-dating techniques.

We argue that the benefits conferred by the ability to place important extinct taxa (e.g., *Djebelemur, Saharagalago, Karanisia, Wadilemur, Komba,* extinct lemurs) outweigh the disadvantages of the artifacts that drive the differences between TD and FBD, especially in comparison to node-based divergence times from extant-only datasets. Previous molecular

analyses could not include calibration information for stem taxa like plesiadapiforms, adapiforms or *Djebelemur*, despite the importance of these fossil taxa in the evolution of primates. Further, the lack of fossils limited node-calibrated molecular analyses of lemurs. The divergence times of extinct and extant lemurs were recently inferred from mitochondrial genomes and the results were similar to those we report, with the exception of the position and divergence time of *Megaladapis* (Kistler et al. 2015) as discussed below. Mitochondrial genomes are known to evolve faster than nuclear genomes, leading to saturation and bias in divergence times towards the calibration points; divergences that are older than calibration points are underestimated and younger divergences are overestimated (Arbogast et al. 2002; Zheng et al. 2011). The four node calibrations used previously (Kistler et al. 2015) were based on fairly recent divergences in the Haplorhini (human-chimp ~ 5-8 Ma, baboons ~ 1-3.5 Ma), and two older calibrations (apes-Old World Monkeys ~ 21-34 Ma, Lorisiformes ~ 37 Ma). If divergence times previously derived from mitochondrial DNA sequences were biased towards calibration points, then the inferred divergences of lemurs should be close to those calibrations, which they are (Table 4). We included the mitochondrial sequences from previous studies, and combined them with morphological data that are most likely coded by multiple nuclear loci. In addition, with our calibrations based on 33 fossils actually in the tree, spread across the chronology of early to recent primate diversification, it is expected that lineage divergence times should be earlier and spread more evenly through time than observed in the mitochondrial node-dating divergence time estimates, which is the case in our results.

Joint Inference of Phylogeny and Divergence Times: Ancient Primate Fossils

Support for the phylogenetic placement of possible stem and early crown primate fossil taxa was weak, and this was expected given that many taxa had high proportions of missing data,

few synapomorphic characters linking them with extant lineages, and autapomorphic character states that make their derived morphology difficult to place (e.g., *Altiatlasius, Teilhardina,* some adapiforms). Including them in these analyses allowed us to place the taxa in the tree with empirical data and the temporal occurrence information of the fossils calibrated the speciation and extinction rates of the tree. This is the first study that could use the temporal information of possible stem primates such as plesiadapiforms, particularly the oldest known fossils, in estimates of divergence times. While previous studies have calibrated the root of crown primates based on the oldest known crown fossils (e.g., Wilkinson et al. 2011), placing those fossils on the tree and inferring their stem ages is unique to this study. Some inferred relationships were unexpected and most likely due to the paucity of fossils in this sample compared to previous studies focused on fossils (e.g., Seiffert et al. 2010). For example, some fossil clades which are considered to be crown clades sister to Haplorhini (Omomyiformes such as *Altiatlasius* and *Teilhardina*, Hartwig 2002; Beard 2008, or *Altiatlasius* sister to Anthropoidea, Ni et al. 2013) and Strepsirrhini (Adapiformes sister to Strepsirrhini, Seiffert et al. 2009) were inferred to be outside crown primates (Table 3). The underrepresentation of omomyiform and adapiform species in the present sample precludes conclusions regarding relationships for those taxa. Other fossils were well supported, firmly anchoring the topology and divergence times for catarrhines (*Aegyptopithecus*) and platyrrhines (*Branisella*). The plesiadapiforms, which are accepted to be outside crown primates (Bloch et al. 2007), were well supported as sister to the other lineages. The occurrence of Plesiadapiformes and early primate taxa in the Paleocene/Eocene and their placement at the base of the tree calibrates the total tree depth to ~75 Ma, and key divergences among crown primate lineages occurred after the Cretaceous-Paleogene boundary.

The divergence times we estimated with the FBD model for the deepest nodes are generally more recent than previously suggested using molecular data for only extant taxa. Total evidence dating techniques do not assume that fossil species represent minimum ages for the MRCAs of living taxa, as node dating does. Rather, the extinct taxa share a common ancestor with sister lineages sometime before their appearance in the fossil record (Ronquist et al. 2012a). Fossil taxa represent a minimum bound for a node, but the maximum bound may be much earlier than allowed by most hard prior distributions used to date. Our results suggest the divergence of crown Haplorhini and Strepsirrhini ~54-70 Ma, with a rapid subsequent divergence among lineages during the Paleocene and Eocene. These dates are more concordant with the fossil record than the deep Cretaceous estimates found by some molecular studies (Horvath et al. 2008; Wilkinson et al. 2010; Perelman et al. 2011).

Table 5. Comparison of divergence time estimates at key nodes in the phylogeny, in millions of years ago. The results of this study using the fossilized birth-death process and combined data are compared to those published previously using node dating and molecular data.

Node	**This study**	**Kistler et al. 2015**	**Yoder and Yang 2004**	**Horvath et al. 2008**	**Perelman et al. 2011**	**Springer et al. 2012**	**Chatterjee et al. 2009**
Haplorhini/ Strepsirrhini	64 (48,70)	68 (60,76)	85* (77,90)	-	87 (76, 99)	68 (63,71)	67 (64,73)
Crown Strepsirrhini	61 (56,67)	59 (52,66)	69 (61,75)	75 (67,84)	69 (59,77)	54 (53,55)	52 (48,56)
Lorises + Galagos	38 (32,39)	38* (37,41)	39* (38,42)	39* (37,42)	40* (35,46)	35* (31,37)	38 (37,39)
Lemuriformes	55 (49,61)	50 (42,57)	62 (58,73)	66 (55,75)	59 (39,77)	50 (49,51)	46 (41,51)
Lemurs (*sans Daubentonia*)	42 (34,50)	31 (27,35)	42 (35,50)	39 (33,46)	39 (26,50)	32 (27,37)	32 (29,34)
Archaeolemuridae	28 (21,35)	24 (20,28)	-	-	-	-	-
Palaeopropithecidae	23 (17,29)	21 (17,24)	-	-	-	-	-
Indriidae	23 (17,28)	17 (14,20)	39	36	17 (10,26)	18 (12,26)	21 (17,25)
Lemuridae	26 (19,33)	19 (16,22)	32 (26,39)	23 (19,29)	26 (16,37)	21 (15,26)	21 (18,25)
Lepilemuridae	16 (12,22)	12 (9,15)	37-38	32 (26,38)	12 (6,17)	9 (6,13)	16 (13,19)
Cheirogaleidae	31 (24,39)	25 (21,30)	29 (23,36)	23 (19,28)	25 (15,35)	22 (17,27)	24 (20,27)

* Node used as calibration point in previous studies.

The origin of strepsirrhines is still poorly understood. The djebelemurid clade of northern Africa is the oldest stem strepsirrhine in this analysis at 45-49 Ma (Seiffert 2012; Marivaux et al. 2013). Djebelemurid fossils have not been informative for studies using node dating because they cannot be assigned to any node in extant-only phylogenies. In the FBD analysis with the full morphological dataset, *Djebelemur* was found to be sister to all other strepsirrhines (a stem strepsirrhine), while analyses with the reduced morphological dataset suggested *Djebelemur* was sister to Lorisiformes, and thus a crown strepsirrhine (similar to Pattinson et al. 2015). *Djebelemur* lacks a toothcomb, the synapomorphy that unites crown strepsirrhines (Seiffert 2012). Thus, either the toothcomb evolved once after the divergence of *Djebelemur* from the crown strepsirrhine lineage as suggested by the FBD analysis with the full dataset, or it evolved with crown strepsirrhines and was independently lost in *Djebelemur*. While the toothcomb is a defining crown strepsirrhine synapomorphy, it has been lost, presumably independently, in several lemur lineages including *Daubentonia* and some extinct giant lemurs including *Archaeolemur* and *Hadropithecus*. The hypothesis that *Djebelemur* was a stem strepsirrhine is most likely better supported, given the results of previous studies with greater sampling of stem strepsirrhine fossils (e.g., Seiffert et al. 2003).

One surprising result in this study was the placement of *Plesiopithecus* as sister to *Daubentonia* in the FBD analysis with the reduced morphological dataset and the TD analyses with both full and reduced datasets. *Plesiopithecus* is an African Eocene fossil with a unique suite of derived and plesiomorphic characters that has made inferring its phylogenetic position relative to other primates difficult. *Plesiopithecus* has been hypothesized to be an early anthropoid (Simons 1992), a lorisiform (Rasmussen & Nekaris 1998), or, more commonly, a stem strepsirrhine (Simons & Rasmussen 1994; Seiffert et al. 2003, reviewed in Seiffert 2012).

Recent total evidence analyses using partitioned Bayesian analysis with extensive fossil sampling found *Plesiopithecus* to be unresolved (Pattinson et al. 2015). The *Daubentonia+Plesiopithecus* relationship has been suggested before (Godinot 2005), and many of our analyses support this sister relationship. The synapomorphic characters linking *Plesiopithecus* and *Daubentonia* include simplification of molar features compared to other taxa in the analyses. Simplifications of the molars may be convergent adaptations to food items that are structurally defended but processed with the anterior teeth. The chisel-like incisors of *Daubentonia* are used to bore holes in tree bark and seeds, but the food items obtained require little physical processing by the posterior teeth (insect larvae, soft inner flesh of seeds, Sterling 1994). A similar adaptive function may explain the enlarged canines of *Plesiopithecus*. The lack of other, non-functionally related synapomorphies indicates that the inferred relationship between *Plesiopithecus* and *Daubentonia* may be the result of convergent evolution, rather than common descent. The biogeographic implications of this result are of great importance to understanding lemuriform origins. The presence of a lemuriform primate in Africa after the split of the most basal lineages would suggest either a single origin of lemurs and a back-dispersal of *Plesiopithecus* to Africa, or two independent dispersals to Madagascar. Further, if *Plesiopithecus* is accepted to be a lemuriform, it is the first and only true fossil lemuriform. This result is controversial in view of the strongly supported single-origins hypothesis from molecular and total evidence analyses (e.g., Yoder et al. 1996). Further validation is necessary to confirm the placement of this taxon by including more complete character sampling (both taxa were sampled for ~60% of characters, mostly dental) and more stem strepsirrhines such as anchomomyine taxa.

There were discrepancies among topologies inferred with the full and reduced morphological matrices. The morphological characters were variable in all analyses and included

autapomorphic characters. In the reduced dataset, characters were culled based on an objective approach that tracks co-distributed state changes among characters. Using the Gower dissimilarity of character state changes among taxa, characters were omitted if, for example, character Y always changed from state 0 to state 1 when character X changed from 0 to 1 in all taxa. This situation supports non-independence of characters X and Y, and we removed one character of each pair. If taxa change positions in analyses of the full and reduced matrices, support for those taxa in the analysis of the full matrix may be biased by effectively up-weighting correlated characters. This had the biggest effect on fossils, and one explanation is that these taxa had few synapomorphic characters to link them with strong support to other lineages so removing correlated characters left too few characters to secure their positions. Also, the characters were taken from previous studies that sought to identify those characters which most strongly distinguished major clades, such as Haplorhini / Strepsirrhini, such that many of these characters have only a single transition. Some examples include: (1) allantois development is rudimentary in all haplorhines while all strepsirrhines have large, vesicular structures; (2) primordial amniotic cavity present in haplorhines and absent in strepsirrhines; (3) retinal fovea found in haplorhines and not strepsirrhines. Lastly, this analysis is dataset-dependent; there are taxa not included in the present matrix that may break up the 1:1 state change pattern and future analyses with greater taxonomic sampling of intermediate fossil forms will likely change which characters are considered correlated.

Joint Inference of Phylogeny and Divergence Times: Strepsirrhini and Lemuriformes

Total evidence analyses can test the assumption that the placement of fossil taxa in the phylogeny corresponds to nodes linking extant taxa. An especially important example of fossils representing minimum age bounds in this dataset concerns the MRCA of Lorisiformes, which

was previously calibrated to approximately 37 Ma based on *Saharagalago* and *Karanisia* (Seiffert et al. 2003; Pozzi et al. 2014a). In our analyses, the relationships of these fossils and their MRCAs with crown sister lineages were inferred jointly, and the results showed these fossils shared a common ancestor with crown lorisiforms ~35-56 Ma. We inferred the MRCA of crown Lorisiformes ~31-39 Ma, concordant with estimates from node calibration. By including these fossils in this study, their placement in the tree was inferred empirically and the divergence times for lorisiforms was estimated from the data, rather than calibrated *a priori*. Before the discovery of *Saharagalago* and *Karanisia*, the MRCA of Lorisiformes was inferred to exist ~40 Ma based on calibrations from non-strepsirrhine primates (Yang & Yoder 2003), further validating the Eocene origins of the clade.

This study is the first to infer the position of nearly all subfossil lemurs and their divergence times jointly from empirical analysis of combined data. The strepsirrhine phylogenies were generally well supported, especially at key nodes within Lemuriformes which have been contentious until now. Many extinct species were placed with moderate to strong support, corroborating inferences from both morphological affinities (e.g., Jungers et al. 1991; Jungers et al. 1997) and ancient DNA (Karanth et al. 2005; Orlando et al. 2008), especially Archaeolemuridae, Palaeopropithecidae, *Pachylemur, Daubentonia robustus*. One exception was the paraphyly of Indriidae; while Indriidae was previously hypothesized to be sister to Palaeopropithecidae, here we inferred that *Indri* was sister to a clade consisting of Palaeopropithecidae and *Propithecus* + *Avahi*, but with low posterior probability. The relationships among indriid genera have always been contentious, and new data are needed to resolve this issue. For example, there are no nuclear loci available for *Indri*, and the recent

recovery of ancient nuclear DNA from *Megaladapis* holds promise for acquiring those data for other subfossil taxa as well (Perry et al. 2015).

Another unique finding in this study was the placement of *Megaladapis* as sister to all lemurs other than *Daubentonia*, a hypothesis that conflicts with its morphological similarities to Lepilemuridae (e.g. Tattersall and Schwartz 1974), and the sister relationship to Lemuridae found with ancient mitochondrial DNA. This result was surprising, given that we included the published molecular data that had recovered the *Megaladapis*+Lemuridae relationship (Kistler et al. 2015). These differences may be related to different data partitioning schemes between studies; previous studies had applied a single molecular model to the entire mitochondrial genome or partitioned the genome by codon position (Kistler et al. 2015), instead of partitions based on the best subset of substitution rate categories as in this study. The specification of molecular models in phylogenetic inference is an important yet often overlooked issue, and misspecification of the molecular partitions and models can lead to poor inferences (Brown and Lemmon 2007, Lanfear et al. 2012). In this study, the best partitioning scheme of the multi-gene alignment included a fast-evolving partition (*cytochrome B* third codon position and *NADH dehydrogenase 4* first codon position), and a slow-evolving partition (all other loci together). With this partitioning scheme, the position of *Megaladapis* we inferred was more strongly supported by the data than the alternative *Megaladapis*+Lemuridae relationship (OSM Table S8). These differences in molecular evolution and partitioning scheme between previous studies and this study may account for the discrepancies in fossil placement observed.

Ours are among the most complete phylogenetic inferences for lemurs to date. Accurate and complete dated phylogenies are necessary for testing hypotheses about lineage and character evolution (Felsenstein 1985; Nunn 2011). Our time-tree inferences have important implications

for the diversification dynamics in this biologically diverse and endangered primate group. For example, the tree shape and balance is indicative of the tempo of diversification and possible shifts in diversification rate through time (Pybus and Harvey 2000; Rabosky 2014). Including fossil species in phylogeny-based inferences of lineage diversification rates is at the forefront of macroevolution (Pyron and Burbrink 2012; Silvestro et al. 2014). With increasing availability of molecular and morphological data, paleontological databases, and innovative models of divergence times and character evolution, researchers in phylogenetic systematics and macroevolution are primed to clarify the structure and the ages of the tree of life.

SUPPLEMENTARY MATERIAL

Supplementary material, data files and/or online-only appendices, can be found in the Dryad Digital Repository: http://dx.doi.org/ 10.5061/dryad.10.5061, MorphoBank project P2167, and TreeBASE submission # 17704.

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REFERENCES

Andriaholinirina N., Fausser J.L., Roos C., Zinner D., Thalmann U., Rabarivola C., Ravoarimanana I., Ganzhorn J.U., Meier B., Hilgartner R. 2006. Molecular phylogeny and taxonomic revision of the sportive lemurs (*Lepilemur*, Primates). BMC Evol. Biol. 6:17.

Andriantompohavana R., Runhua L., Zaonarivelo J.R., Engberg S.E., Nalanirina G., McGuire S.M., Shore G.D., Andrianasolo J., Herrington K., Brenneman R.A., Louis E.E. 2007. Molecular phylogeny and taxonomic revision of the woolly lemurs, Genus *Avahi* (Primates: Lemuriformes). Special Publictions of the Museum of Texas Tech University 51:1-64.

Arbogast B.S., Edwards S.V., Wakeley J., Beerli P., Slowinski J.B. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annu. Rev. Ecol. Syst. 33:707-740.

Arcila D., Pyron R.A., Tyler J.C., Ortí G., Betancur-R R. 2015. An evaluation of fossil tip-dating versus node-age calibrations in tetraodontiform fishes (Teleostei: Percomorphaceae). Mol. Phylogen. Evol. 82:131-145.

Bapst D.W. 2012. paleotree: an R package for paleontological and phylogenetic analyses of evolution. Methods in Ecology and Evolution 3:803-807.

Beard K.C. 1990. Gliding behaviour and palaeoecology of the alleged primate family Paromomyidae (Mammalia, Dermoptera). Nature 345:340-341.

Beard K.C. 2008. The oldest North American primate and mammalian biogeography during the Paleocene–Eocene Thermal Maximum. Proc. Natl. Acad. Sci. U.S.A. 105:3815-3818.

Beck R.M., Lee M.S. 2014. Ancient dates or accelerated rates? Morphological clocks and the antiquity of placental mammals. Proc. Royl. Soc. B: Biol. Sci. 281:20141278.

Behrensmeyer A.K., Turner A. accessed 2015. Taxonomic occurrences of Primates recorded in the Paleobiology Database. Fossilworks. http://fossilworks.org.

Bloch J.I., Silcox M.T., Boyer D.M., Sargis E.J. 2007. New Paleocene skeletons and the relationship of plesiadapiforms to crown-clade primates. Proc. Natl. Acad. Sci. U.S.A. 104:1159-1164.

Cartmill M. 1975. Strepsirhine basicranial structures and the affinities of the Cheirogaleidae. In: Luckett W. P. and Szalay F. S. editors. Phylogeny of the Primates: A Multidisciplinary Approach. US, Springer, p. 313-354.

Cartmill M. 1978. The orbital mosaic in prosimians and the use of variable traits in systematics. Folia Primatol. 30:89-114.

Catlett K.K., Schwartz G.T., Godfrey L.R., Jungers W.L. 2010. “Life history space”: a multivariate analysis of life history variation in extant and extinct Malagasy lemurs. Am. J. Phys. Anthropol. 142:391-404.

Chatterjee H., Ho S., Barnes I., Groves C. 2009. Estimating the phylogeny and divergence times of primates using a supermatrix approach. BMC Evolutionary Biology 9:259.

Condamine F.L., Nagalingum N.S., Marshall C.R., Morlon H. 2015. Origin and diversification of living cycads: a cautionary tale on the impact of the branching process prior in Bayesian molecular dating. BMC Evol. Biol. 15:65.

Craul M., Zimmermann E., Rasoloharijaona S., Randrianambinina B., Radespiel U. 2007. Unexpected species diversity of Malagasy primates (*Lepilemur* spp.) in the same biogeographical zone: a morphological and molecular approach with the description of two new species. BMC Evol. Biol. 7:83.

dos Reis M., Donoghue P.C., Yang Z. 2014a. Neither phylogenomic nor palaeontological data support a Palaeogene origin of placental mammals. Biol. Lett. 10:20131003.

dos Reis M., Zhu T., Yang Z. 2014b. The Impact of the rate prior on Bayesian estimation of divergence times with multiple loci. Syst. Biol. 63:555-565.

Drummond A.J., Ho S.Y., Phillips M.J., Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4:e88.

Eernisse D.J., Kluge A.G. 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. Mol. Biol. Evol. 10.

Fabre P.H., Rodrigues A., Douzery E.J.P. 2009. Patterns of macroevolution among Primates inferred from a supermatrix of mitochondrial and nuclear DNA. Mol. Phylogen. Evol. 53:808-825.

Felsenstein J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1-15.

Felsenstein J. 2004. Inferring phylogenies. Sunderland, Massachusetts, Sinauer Associates.

FitzJohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084-1092.

Foote M. 2000. Origination and extinction components of taxonomic diversity: general problems. Paleobiology 26:74-102.

Gebo D.L. 2002. Adapiformes: Phylogeny and adaptation. In: Hartwig WC editor. The Primate Fossil Record. UK, Cambridge University Press, p. 21-43.

Godfrey L.R., Jungers W.L., Burney D.A. 2010. Subfossil lemurs of Madagascar. In: Werdelin L, Sanders W editors. Cenozoic Mammals of Africa. Berkeley, The University of California Press.

Godinot M. 2005. Lemuriform origins as viewed from the fossil record. Folia Primatol. 77:446-464.

Grimm G.W., Kapli P., Bomfleur B., McLoughlin S., Renner S.S. 2014. Using more than the oldest fossils: Dating Osmundaceae with three Bayesian clock approaches. Syst. Biol. 64:396-405.

Groeneveld L.F., Blanco M.B., Raharison J.L., Rahalinarivo V., Rasoloarison R.M., Kappeler P.M., Godfrey L.R., Irwin M.T. 2010. MtDNA and nDNA corroborate existence of sympatric dwarf lemur species at Tsinjoarivo, eastern Madagascar. Mol. Phylogen. Evol. 55:833-845.

Groves C.P., Eaglen R.H. 1988. Systematics of the Lemuridae (primates, Strepsirhini). J. Hum. Evol. 17:513-538.

Heath T.A., Huelsenbeck J.P., Stadler T. 2014. The fossilized birth-death process for coherent calibration of divergence-time estimates. Proc. Natl. Acad. Sci. U.S.A.:E2957-E2966.

Horvath J.E., Weisrock D.W., Embry S.L., Fiorentino I., Balhoff J.P., Kappeler P., Wray G.A., Willard H.F., Yoder A.D. 2008. Development and application of a phylogenomic toolkit: Resolving the evolutionary history of Madagascar's lemurs. Genome Res. 18:489-499.

IUCN 2015. The IUCN Red List of Threatened Species. Version 2015-3. <http://www.iucnredlist.org>. Downloaded February 28 2015.

Johnson S.E., Lei R., Martin S.K., Irwin M.T., Louis E.E. 2008. Does *Eulemur cinereiceps* exist? Preliminary evidence from genetics and ground surveys in southeastern Madagascar. Am. J. Primatol. 70:372-385.

Jungers W.L., Godfrey L.R., Simons E.L., Chatrath P.S. 1997. Phalangeal curvature and positional behavior in extinct sloth lemurs (Primates, Palaeopropithecidae). Proc. Natl. Acad. Sci. U.S.A. 94:11998.

Jungers W.L., Godfrey L.R., Simons E.L., Chatrath P.S., Rakotosamimanana B. 1991. Phylogenetic and functional affinities of *Babakotia* (Primates), a fossil lemur from northern Madagascar. Proc. Natl. Acad. Sci. U.S.A. 88:9082.

Karanth K.P., Delefosse T., Rakotosamimanana B., Parsons T.J., Yoder A.D. 2005. Ancient DNA from giant extinct lemurs confirms single origin of Malagasy primates. Proc. Natl. Acad. Sci. U.S.A. 102:5090-5095.

Kass R.E., Raftery A.E. 1995. Bayes factors. J. Amer. Stat. Assoc. 90:773-795.

Katoh K., Kuma K.-i., Toh H., Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33:511-518.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649.

Kistler L., Ratan A., Godfrey L.R., Crowley B.E., Hughes C.E., Lei R., Cui Y., Wood M.L., Muldoon K.M., Andriamialison H. 2015. Comparative and population mitogenomic analyses of Madagascar's extinct, giant ‘subfossil’ lemurs. J. Hum. Evol. 79:45-54.

Ksepka D.T., Parham J.F., Allman J.F., Benton M.J., Carrano M.T., Cranston K.A., Donoghue P.C., Head J.J., Hermsen E.J., Irmis R.B. 2015. The Fossil Calibration Database, a new resource for divergence dating. Syst. Biol.: 10.1093/sysbio/syv025.

Lambert S.M., Reeder T.W., Wiens J.J. 2015. When do species-tree and concatenated estimates disagree? An empirical analysis with higher-level scincid lizard phylogeny. Mol. Phylogen. Evol. 82:146-155.

Lanfear R., Calcott B., Ho S.Y.W., Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29:1695-1701.

Larget B.R., Kotha S.K., Dewey C.N., Ané C. 2010. BUCKy: gene tree/species tree reconciliation with Bayesian concordance analysis. Bioinformatics 26:2910-2911.

Lei R., Engberg S.E., Andriantompohavana R., McGuire S.M., Mittermeier R.A., Zaonarivelo J.R., Brehheman R.A., Louis E.E. 2008. Nocturnal lemur diversity at Masoala National Park. Special Publications of Texas Tech University 53:1-48.

Lewis P.O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50:913.

Lihoreau F., Boisserie J.-R., Manthi F.K., Ducrocq S. 2015. Hippos stem from the longest sequence of terrestrial cetartiodactyl evolution in Africa. Nature Communications 6.

Louis E.E. 2006. Molecular and morphological analyses of the sportive lemurs (Family Megaladapidae: Genus *Lepilemur*) reveals 11 previously unrecognized species. Special Publictions of the Museum of Texas Tech University.

Louis E.E., Coles M.S., Andriantompohavana R., Sommer J.A., Engberg S.E., Zaonarivelo J.R., Mayor M.I., Brenneman R.A. 2006. Revision of the mouse lemurs (*Microcebus*) of eastern Madagascar. Int. J. Primatol. 27:347-389.

Maechler M., Rousseeuw P., Struyf A., Hubert M., Hornik K., Studer M., Roudier P. 2015. cluster: Cluster Analysis Basics and Extensions, R package version 2.0.1, CRAN.

Markolf M., Rakotonirina H., Fichtel C., von Grumbkow P., Brameier M., Kappeler P.M. 2013. True lemurs... true species - species delimitation using multiple data sources in the brown lemur complex. BMC Evol. Biol. 13:233.

Marivaux L., Ramdarshan A., Essid E.M., Marzougui W., Ammar H.K., Lebrun R., Marandat B., Merzeraud G., Tabuce R., Vianey-Liaud M. 2013. *Djebelemur*, a tiny pre-tooth-combed primate from the Eocene of Tunisia: a glimpse into the origin of crown strepsirhines. PLoS ONE 8:e80778.

Masters J., Anthony N., De Wit M., Mitchell A. 2005. Reconstructing the evolutionary history of the Lorisidae using morphological, molecular, and geological data. Am. J. Phys. Anthropol. 127:465-480.

Meredith R.W., Janečka J.E., Gatesy J., Ryder O.A., Fisher C.A., Teeling E.C., Goodbla A., Eizirik E., Simão T.L., Stadler T. 2011. Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. Science 334:521-524.

Miller M.A., Pfeiffer W., Schwartz T. 2010. Creating the CIPRESS Science Gateway for inference of large phylogenetic trees. Proc. Gateway Computing Environments Workshop. New Orleans, LA, GCE, p. 1-8.

Murphy W.J., Eizirik E., Johnson W.E., Zhang Y.P., Ryder O.A., O'Brien S.J. 2001. Molecular phylogenetics and the origins of placental mammals. Nature 409:614-618.

Ni X., Gebo D.L., Dagosto M., Meng J., Tafforeau P., Flynn J.J., Beard K.C. 2013. The oldest known primate skeleton and early haplorhine evolution. Nature 498:60-64.

Novacek M.J., Wheeler Q. 1992. Extinction and Phylogeny. NY, Columbia University Press.

Nunn C.L. 2011. The comparative approach in evolutionary anthropology and biology. Chicago, University of Chicago Press.

Nylander J.A., Wilgenbusch J.C., Warren D.L., Swofford D.L. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581-583.

Olivieri G., Zimmermann E., Randrianambinina B., Rasoloharijaona S., Rakotondravony D., Guschanski K., Radespiel U. 2007. The ever-increasing diversity in mouse lemurs: three new species in north and northwestern Madagascar. Mol. Phylogen. Evol. 43:309-327.

Orlando L., Calvignac S., Schnebelen C., Douady C.J., Godfrey L.R., Hänni C. 2008. DNA from extinct giant lemurs links archaeolemurids to extant indriids. BMC Evol. Biol. 8:121.

Parham J.F., Donoghue P.C., Bell C.J., Calway T.D., Head J.J., Holroyd P.A., Inoue J.G., Irmis R.B., Joyce W.G., Ksepka D.T. 2011. Best practices for justifying fossil calibrations. Syst. Biol. 61:346-359.

Pastorini J., Forstner M.R.J., Martin R.D. 2001a. Phylogenetic history of sifakas (*Propithecus*: Lemuriformes) derived from mtDNA sequences. Am. J. Primatol. 53:1-17.

Pastorini J., Martin R.D., Ehresmann P., Zimmermann E., Forstner M.R.J. 2001b. Molecular phylogeny of the lemur family Cheirogaleidae (Primates) based on mitochondrial DNA sequences. Mol. Phylogen. Evol. 19:45-56.

Pastorini J., Forstner M.R., Martin R.D. 2002. Phylogenetic relationships of gentle lemurs (*Hapalemur*). Evol. Anthrop. 11:150-154.

Pastorini J., Thalmann U., Martin R.D. 2003. A molecular approach to comparative phylogeography of extant Malagasy lemurs. Proc. Natl. Acad. Sci. U.S.A. 100:5879-5884.

Pattinson D.J., Thompson R.S., Piotrowski A.K., Asher R.J. 2015. Phylogeny, paleontology, and primates: do incomplete fossils bias the tree of life? Syst. Biol. 64:169-186.

Patzkowsky M.E., Holland S.M. 2012. Stratigraphic paleobiology: understanding the distribution of fossil taxa in time and space. Chicago, University of Chicago Press.

Perelman P., Johnson W.E., Roos C., Seuánez H.N., Horvath J.E., Moreira M.A., Kessing B., Pontius J., Roelke M., Rumpler Y., Schneider M., Silva A., O'Brien S.J., Pecon-Slattery J. 2011. A molecular phylogeny of living primates. PLoS Genet. 7:e1001342.

Perry G., Kistler L., Godfrey L.R., Crowley B.E., Muldoon K.M., Malhi R., Schuster S., Miller W., Yoder A.D., Louis E.E. 2015. Nuclear genome sequences from the extinct subfossil lemurs *Palaeopropithecus ingens* and *Megaladapis edwardsi*. Am. J. Phys. Anthropol. Volume 156 (S60): 251.

Pozzi L., Disotell T.R., Masters J.C. 2014a. A multilocus phylogeny reveals deep lineages within African galagids (Primates: Galagidae). BMC Evol. Biol. 14:72.

Pozzi L., Hodgson J.A., Burrell A.S., Sterner K.N., Raaum R.L., Disotell T.R. 2014b. Primate phylogenetic relationships and divergence dates inferred from complete mitochondrial genomes. Mol. Phylogen. Evol. 75:165-183.

Pybus O.G., Harvey P.H. 2000. Testing macro–evolutionary models using incomplete molecular phylogenies. Proc. Royl. Soc. London B: Biol. Sci. 267:2267-2272.

Pyron R., Burbrink F. 2012. Trait-dependent diversification and the impact of palaeontological data on evolutionary hypothesis testing in New World ratsnakes (tribe Lampropeltini). J. Evol. Biol. 25:497-508.

Pyron R.A. 2011. Divergence time estimation using fossils as terminal taxa and the origins of Lissamphibia. Syst. Biol. 60:466-481.

Pyron, R. A. 2015. Post-molecular systematics and the future of phylogenetics. Trends Ecol. Evol.: doi:10.1016/j.tree.2015.1004.1016.

R Core Team. 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Rabosky D.L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PloS One 9:e89543.

Rambaut A., Suchard M., Xie D., Drummond A. 2014. Tracer v1. 6. Available at: http://beast. bio. ed. ac. uk/Tracer.

Rasmussen T., Nekaris K. 1998. Evolutionary history of lorisiform primates. Folia Primatol. 69:250-285.

Rasoloarison R.M., Goodman S.M., Ganzhorn J.U. 2000. Taxonomic revision of mouse lemurs (*Microcebus*) in the western portions of Madagascar. Int. J. Primatol. 21:963-1019.

Rasoloarison R.M., Weisrock D.W., Yoder A.D., Rakotondravony D., Kappeler P.M. 2013. Two new species of mouse lemurs (Cheirogaleidae: *Microcebus*) from eastern Madagascar. Int. J. Primatol. 34:455-469.

Reeder T.W., Townsend T.M., Mulcahy D.G., Noonan B.P., Wood Jr P.L., Sites Jr J.W., Wiens J.J. 2015. Integrated analyses resolve conflicts over squamate reptile phylogeny and reveal unexpected placements for fossil taxa. PloS one 10:e0118199.

Revell L.J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217-223.

Robinson D., Foulds L.R. 1981. Comparison of phylogenetic trees. Math. Biosci. 53:131-147.

Ronquist F., Klopfstein S., Vilhelmsen L., Schulmeister S., Murray D.L., Rasnitsyn A.P. 2012a. A total-evidence approach to dating with fossils, applied to the early radiation of the Hymenoptera. Syst. Biol. 61:973-999.

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A., Huelsenbeck J.P. 2012b. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61:539-542.

Rose K.D. 2006. The beginning of the age of mammals. Baltimore, MD, Johns Hopkins University Press.

Rumpler Y., Hauwy M., Fausser J.-L., Roos C., Zaramody A., Andriaholinirina N., Zinner D. 2011. Comparing chromosomal and mitochondrial phylogenies of the Indriidae (Primates, Lemuriformes). Chrom. Res. 19:209-224.

Sansom R.S. 2015. Bias and sensitivity in the placement of fossil taxa resulting from interpretations of missing data. Syst. Biol. 64:256-266.

Schwitzer C., Mittermeier R., Davies N., Johnson S.E., Ratsimbazafy J., Razafindramanana J., Louis Jr E.E., Rajaobelina S. 2013. Lemurs of Madagascar: a stragey for their conservation 2013-2016. Bristol, UK, IUCN SSC Primate Specialist Group, Bristol Conservation and Science Foundation, Conservation International.

Seiffert E.R. 2007. Evolution and extinction of Afro-Arabian primates near the Eocene-Oligocene boundary. Folia Primatol. 78:314-327.

Seiffert E.R. 2012. Early primate evolution in Afro-Arabia. Evol. Anthropol. 21:239-253.

Seiffert ER, Costeur L, and Boyer DM. 2015. Primate tarsal bones from Egerkingen, Switzerland, attributable to the middle Eocene adapiform Caenopithecus lemuroides. PeerJ 3:e1036.

Seiffert E.R., Perry J.M.G., Simons E.L., Boyer D.M. 2009. Convergent evolution of anthropoid-like adaptations in Eocene adapiform primates. Nature 461:1118-1121.

Seiffert E.R., Simons E.L., Attia Y. 2003. Fossil evidence for an ancient divergence of lorises and galagos. Nature 422:421-424.

Seiffert E.R., Simons E.L., Boyer D.M., Perry J.M.G., Ryan T.M., Sallam H.M. 2010. A fossil primate of uncertain affinities from the earliest late Eocene of Egypt. Proc. Natl. Acad. Sci. U.S.A. 107:9712.

Seiffert E.R., Simons E.L., Ryan T.M., Attia Y. 2005. Additional remains of *Wadilemur elegans*, a primitive stem galagid from the late Eocene of Egypt. Proc. Natl. Acad. Sci. U.S.A. 102:11396.

Silvestro D., Schnitzler J., Liow L.H., Antonelli A., Salamin N. 2014. Bayesian estimation of speciation and extinction from incomplete fossil occurrence data. Syst. Biol. 63:349-367.

Simons E.L. 1992. Diversity in the early Tertiary anthropoidean radiation in Africa. Proc. Natl. Acad. Sci. U.S.A. 89:10743-10747.

Simons E.L., Rasmussen D.T. 1994. A remarkable cranium of *Plesiopithecus teras* (Primates, Prosimii) from the Eocene of Egypt. Proc. Natl. Acad. Sci. U.S.A. 91:9946-9950.

Slater G.J. 2013. Phylogenetic evidence for a shift in the mode of mammalian body size evolution at the Cretaceous-Palaeogene boundary. Meth. Ecol. Evol. 4:734-744.

Slater G.J., Harmon L.J. 2013. Unifying fossils and phylogenies for comparative analyses of diversification and trait evolution. Meth. Ecol. Evol. 4:699-702.

Slater G.J., Harmon L.J., Alfaro M.E. 2012. Integrating fossils with molecular phylogenies improves inference of trait evolution. Evolution 66:3931-3944.

Spaulding M., O'Leary M.A., Gatesy J. 2009. Relationships of Cetacea (Artiodactyla) among mammals: increased taxon sampling alters interpretations of key fossils and character evolution. PLoS ONE 4:e7062.

Springer M.S., Meredith R.W., Gatesy J., Emerling C.A., Park J., Rabosky D.L., Stadler T., Steiner C., Ryder O.A., Janečka J.E. 2012. Macroevolutionary dynamics and historical biogeography of primate diversification inferred from a species supermatrix. PLoS ONE 7:e49521.

Steiper M.E., Seiffert E.R. 2012. Evidence for a convergent slowdown in primate molecular rates and its implications for the timing of early primate evolution. Proc. Natl. Acad. Sci. U.S.A. 109:6006.

Sterling E.J. 1994. Aye-Ayes: specialists on structurally defended resources. Folia Primatol. 62:142-154.

Tattersall I., Schwartz J. 1974. Craniodental morphology and the systematics of the Malagasy lemurs (Primates, Prosimii). Anthropol. Papers Amer. Mus. Nat. Hist. 52:1-60.

Tattersall I., Schwartz J. 1991. Phylogeny and nomenclature in the Lemur-group of Malagasy strepsirhine primates. Anthropol. Papers Amer. Mus. Nat. Hist. 69:1-24.

Thiele D., Razafimahatratra E., Hapke A. 2013. Discrepant partitioning of genetic diversity in mouse lemurs and dwarf lemurs–Biological reality or taxonomic bias? Mol. Phylogen. Evol. 69:593-609.

Thiele K. 1993. The holy grail of the perfect character: the cladistic treatment of morphometry data. Cladistics 9:275–304.

Thorne J.L., Kishino H. 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51:689-702.

Wagner P.J. 2000. Exhaustion of morphologic character states among fossil taxa. Evolution 54:365-386.

Weisrock D.W., Rasoloarison R.M., Fiorentino I., Ralison J.M., Goodman S.M., Kappeler P.M., Yoder A.D. 2010. Delimiting species without nuclear monophyly in Madagascar's mouse lemurs. PLoS ONE 5:e9883.

Wiens J.J. 2000. Phylogenetic analysis of morphological data. Washington, DC.: Smithsonian Institution Press 1:233-235.

Wiens J.J., Kuczynski C.A., Townsend T., Reeder T.W., Mulcahy D.G., Sites Jr J.W. 2010. Combining phylogenomics and fossils in higher-level squamate reptile phylogeny: molecular data change the placement of fossil taxa. Syst. Biol. 59:674-688.

Wiens J.J., Morrill M.C. 2011. Missing data in phylogenetic analysis: reconciling results from simulations and empirical data. Syst. Biol. 60:719-731.

Wiens J.J., Tiu J. 2012. Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. PLoS One 7:e42925.

Wilkinson R.D., Steiper M.E., Soligo C., Martin R.D., Yang Z., Tavaré S. 2010. Dating primate divergences through an integrated analysis of palaeontological and molecular data. Syst. Biol. 60(1):16-31.

Wood H.M., Matzke N.J., Gillespie R.G., Griswold C.E. 2012. Treating fossils as terminal taxa in divergence time estimation reveals ancient vicariance patterns in the palpimanoid spiders. Syst. Biol. 62:264-284.

Xie W., Lewis P.O., Fan Y., Kuo L., Chen M.-H. 2010. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. Syst. Biol. 60:150-160.

Yang Z. 2008. Empirical evaluation of a prior for Bayesian phylogenetic inference. Phil. Trans. Royl. Soc. B: Biol. Sci. 363:4031.

Yang Z., Rannala B. 2005. Branch-length prior influences Bayesian posterior probability of phylogeny. Syst. Biol. 54:455-470.

Yoder A.D. 1994. Relative position of the Cheirogaleidae in strepsirhine phylogeny: a comparison of morphological and molecular methods and results. Am. J. Phys. Anthropol. 94:25-46.

Yoder A.D., Cartmill M., Ruvolo M., Smith K., Vilgalys R. 1996. Ancient single origin for Malagasy primates. Proc. Natl. Acad. Sci. U.S.A. 93:5122-5126.

Yoder A.D., Irwin J.A. 1999. Phylogeny of the Lemuridae: effects of character and taxon sampling on resolution of species relationships within Eulemur. Cladistics 15:351-361.

Yoder A.D., Irwin J.A., Payseur B.A. 2001. Failure of the ILD to determine data combinability for slow loris phylogeny. Syst. Biol. 50:408-424.

Yoder A., Yang Z. 2000. Estimation of Primate Speciation Dates Using Local Molecular Clocks. Mol. Biol. Evol. 17:1081 - 1090.

Yoder A.D., Yang Z. 2004. Divergence dates for Malagasy lemurs estimated from multiple gene loci: geological and evolutionary context. Mol. Ecol. 13:757-773.

Zaramody A., Fausser J.-L., Roos C., Zinner D., Andriaholinirina N., Rabarivola C., Norscia I., Tattersall I., Rumpler Y. 2006. Molecular phylogeny and taxonomic revision of the eastern woolly lemurs (Avahi laniger). Primate Reports 74:9-23.

Zhang J., Mi X., Pei N. 2010. Phylotools: Phylogenetic tools for ecologists. R package version 0.0 7:201019.

Zhang C., Stadler T., Klopfstein S., Heath T.A., Ronquist F. 2015. Total-Evidence Dating under the Fossilized Birth-Death Process. Syst. Biol. doi:10.1093/sysbio/syv080.

Zheng Y., Peng R., Kuro-o M., Zeng X. 2011. Exploring patterns and extent of bias in estimating divergence time from mitochondrial DNA sequence data in a particular lineage: a case study of salamanders (Order Caudata). Mol. Biol. Evol. 28:2521-2535.

Figure legends

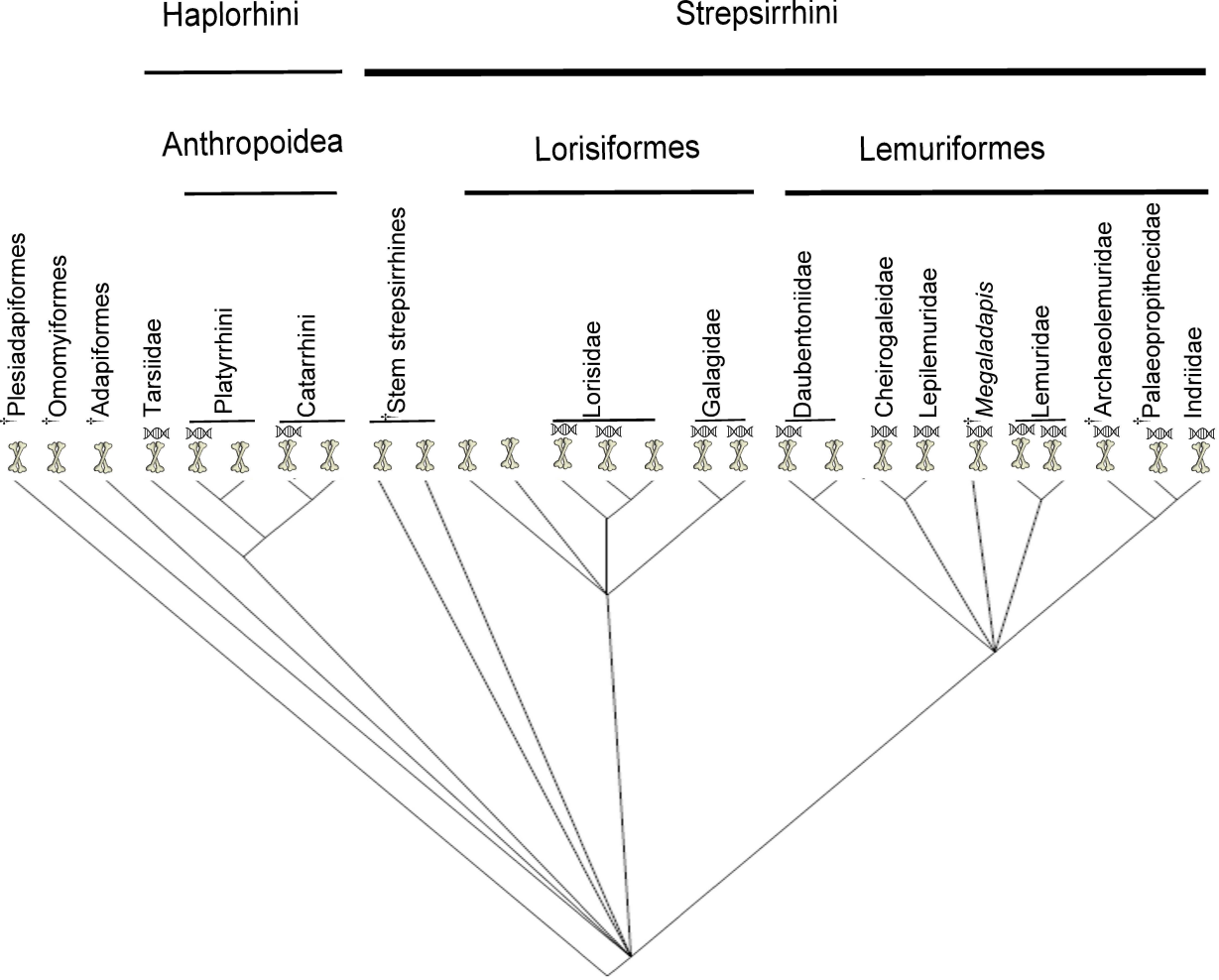
Figure 1. Simplified phylogeny of study taxa illustrating the systematics referred to in the text and the relationships of fossil taxa (indicated with crosses). Taxa for which morphological data were available are depicted with crossbones, and taxa for which molecular data were available are depicted with a double helix.

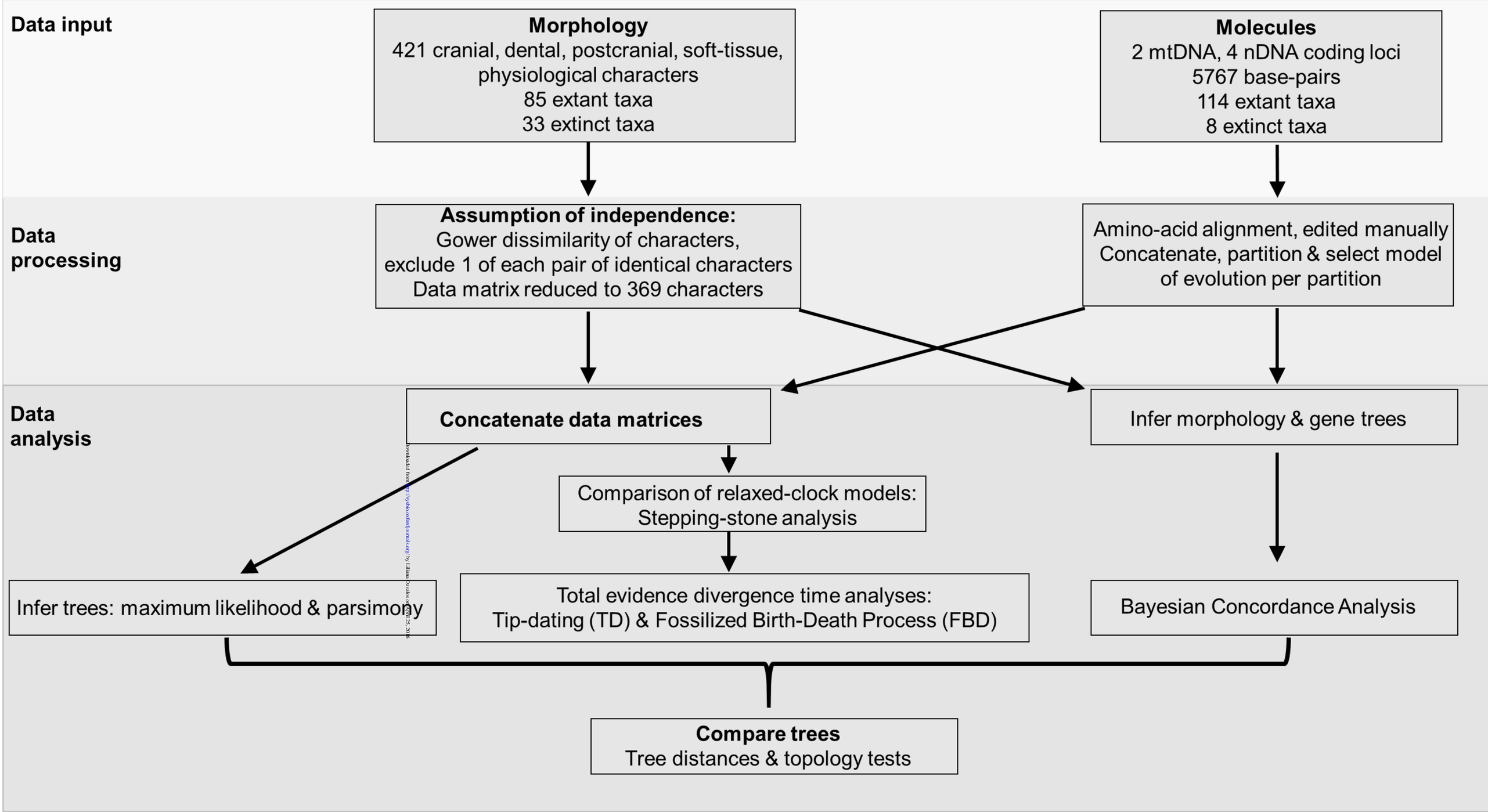
Figure 2. Schematic of the study workflow illustrating data used, data processing procedures, and analytical techniques.

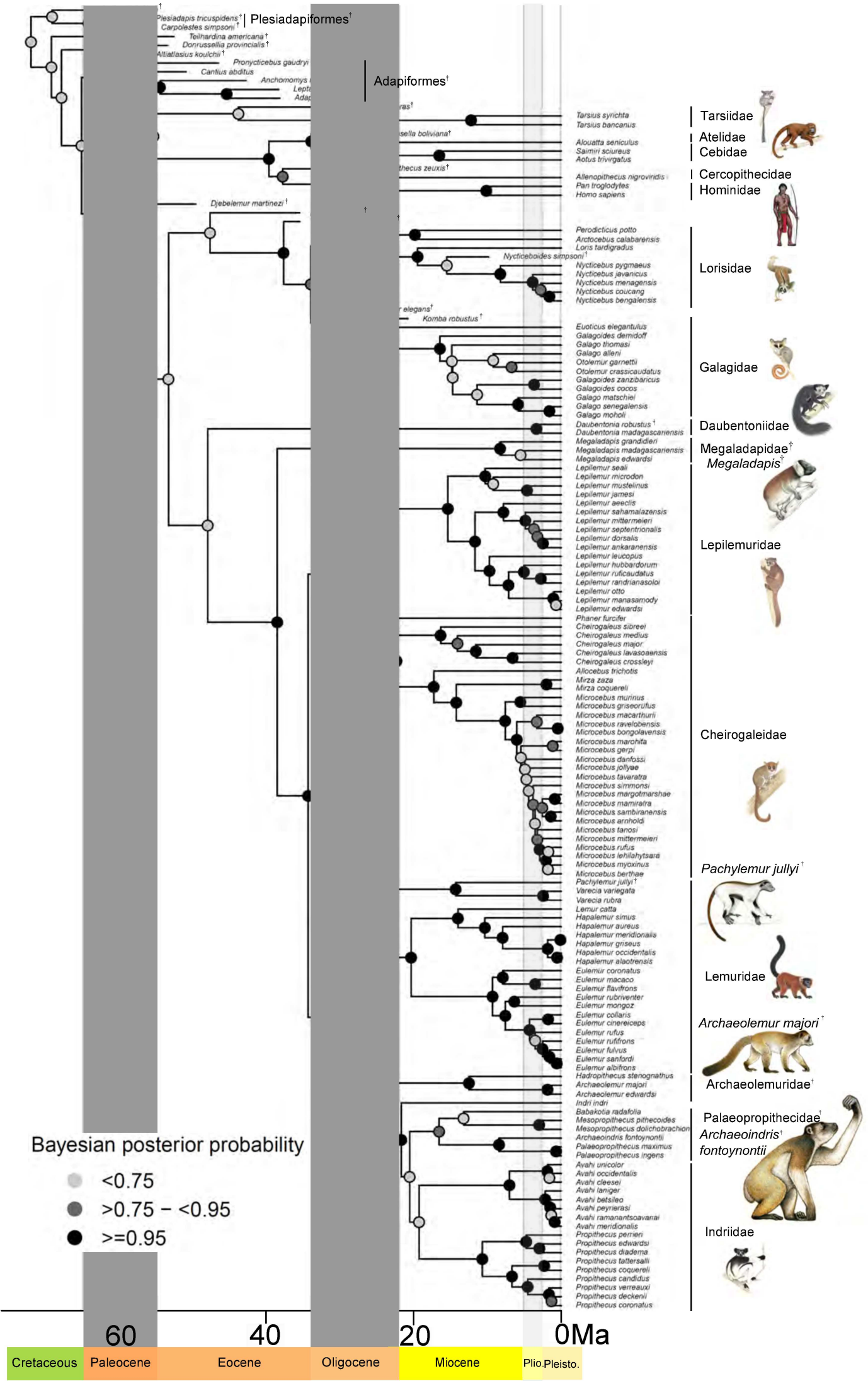
Figure 3. Time-calibrated maximum clade credibility phylogeny inferred from a total evidence dataset (421 morphological, 5767 protein-coding molecular characters) using the fossilized birth-death process model. Node supports are illustrated with color coding. The time scale is in millions of years ago. The family names are given with illustrations of representative taxa. Representatives of the extinct subfossils are shown for each family. Illustrations of extant taxa by S. Nash in Schwitzer et al. 2013, extinct subfossils are by S. Nash in Mittermeier et al. 2010.

Figure 4. Comparison of divergence-time estimates from two techniques used in this study, the Tip-Dating (TD) and the Fossilized Birth-Death Process (FBD) methods, and the full and reduced morphological data matrix for each technique. Circles indicate the median age estimate and bars encompass the 95% highest probability distribution (HPD), in millions of years ago.

Nodes are referred to by taxonomic names as in Figures 1 and 3. The two techniques differ in the divergence time estimates for key nodes in the phylogeny, with the TD method estimating ages that are ~9-12 million years older than the FBD method, on average.


Haplorhini
Strepsirrhini
Anthropoidea
Lorisiformes
Lemuriformes
†Plesiadapiformes
†Omomyiformes
†Adapiformes
Tarsiidae
Platyrrhini
Catarrhini
†Stem strepsirrhines
Lorisidae
Galagidae
Daubentoniidae
Cheirogaleidae
Lepilemuridae
†Megaladapis
Lemuridae
†Archaeolemuridae
†Palaeopropithecidae
Indriidae




Plesiadapiformes†
Adapiformes†
Plesiadapis tricuspidens†
Carpolestes simpsoni†
Teilhardina americana†
Donrussellia provincialis†
Altiatlasius koulchii†
Pronycticebus gaudryi
Cantius abditus
Djebelemur martinezi†
Komba robustus†
Nycticeboides simpsoni†
Tarsius syrichta
Tarsius bancanus
Alouatta seniculus
Saimiri sciureus
Aotus trivirgatus
Allenopithecus nigroviridis
Pan troglodytes
Homo sapiens
Perodicticus potto
Arctocebus calabarensis
Loris tardigradus
Nycticebus pygmaeus
Nycticebus javanicus
Nycticebus menagensis
Nycticebus coucang
Nycticebus bengalensis
Euoticus elegantulus
Galagoides demidoff
Galago thomasi
Galago alleni
Otolemur garnettii
Otolemur crassicaudatus
Galagoides zanzibaricus
Galagoides cocos
Galago matschiei
Galago senegalensis
Galago moholi
Daubentonia robustus†
Daubentonia madagascariensis
Megaladapis grandidieri
Megaladapis madagascariensis
Megaladapis edwardsi
Lepilemur seali
Lepilemur microdon
Lepilemur mustelinus
Lepilemur jamesi
Lepilemur aeeclis
Lepilemur sahamalazensis
Lepilemur mittermeieri
Lepilemur septentrionalis
Lepilemur dorsalis
Lepilemur ankaranensis
Lepilemur leucopus
Lepilemur hubbardorum
Lepilemur ruficaudatus
Lepilemur randrianasoloi
Lepilemur otto
Lepilemur manasamody
Lepilemur edwardsi
Phaner furcifer
Cheirogaleus sibreei
Cheirogaleus medius
Cheirogaleus major
Cheirogaleus lavasoaensis
Cheirogaleus crossleyi
Allocebus trichotis
Mirza zaza
Mirza coquereli
Microcebus murinus
Microcebus griseorufus
Microcebus macarthurii
Microcebus ravelobensis
Microcebus bongolavensis
Microcebus marohita
Microcebus gerpi
Microcebus danfossi
Microcebus jollyae
Microcebus tavaratra
Microcebus simmonsi
Microcebus margotmarshae
Microcebus mamiratra
Microcebus sambiranensis
Microcebus arnholdi
Microcebus tanosi
Microcebus mittermeieri
Microcebus rufus
Microcebus lehilahytsara
Microcebus myoxinus
Microcebus berthae
Pachylemur jullyi†
Varecia variegata
Varecia rubra
Lemur catta
Hapalemur simus
Hapalemur aureus
Hapalemur meridionalis
Hapalemur griseus
Hapalemur occidentalis
Hapalemur alaotrensis
Eulemur coronatus
Eulemur macaco
Eulemur flavifrons
Eulemur rubriventer
Eulemur mongoz
Eulemur collaris
Eulemur cinereiceps
Eulemur rufus
Eulemur rufifrons
Eulemur fulvus
Eulemur sanfordi
Eulemur albifrons
Hadropithecus stenognathus
Archaeolemur majori
Archaeolemur edwardsi
Indri indri
Babakotia radafolia
Mesopropithecus pithecoides
Mesopropithecus dolichobrachion
Archaeoindris fontoynontii
Palaeopropithecus maximus
Palaeopropithecus ingens
Avahi unicolor
Avahi occidentalis
Avahi cleesei
Avahi laniger
Avahi betsileo
Avahi peyrierasi
Avahi ramanantsoavanai
Avahi meridionalis
Propithecus perrieri
Propithecus edwardsi
Propithecus diadema
Propithecus tattersalli
Propithecus coquereli
Propithecus candidus
Propithecus verreauxi
Propithecus deckenii
Propithecus coronatus
Tarsiidae
Atelidae
Cebidae
Cercopithecidae
Hominidae
Lorisidae
Galagidae
Daubentoniidae
Megaladapidae†
Megaladapis†
Lepilemuridae
Cheirogaleidae
Pachylemur jullyi†
Lemuridae
Archaeolemur majori†
Archaeolemuridae†
Palaeopropithecidae†
Archaeoindris†
fontoynontii
Indriidae
Bayesian posterior probability
<0.75
>0.75 − <0.95
>=0.95
60
40
20
0Ma
Cretaceous
Paleocene
Eocene
Oligocene
Miocene
Plio.
Pleisto.