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Primate phylogenetic relationships and divergence dates inferred from complete mitochondrial genomes

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Abstract

The origins and the divergence times of the most basal lineages within primates have been difficult to resolve mainly due to the incomplete sampling of early fossil taxa. The main source of contention is related to the discordance between molecular and fossil estimates: while there are no crown primate fossils older than 56 Ma, most molecule-based estimates extend the origins of crown primates into the Cretaceous. Here we present a comprehensive mitogenomic study of primates. We assembled 87 mammalian mitochondrial genomes, including 62 primate species representing all the families of the order. We newly sequenced eleven mitochondrial genomes, including eight Old World monkeys and three strepsirrhines. Phylogenetic analyses support a strong topology, confirming the monophyly for all the major primate clades. In contrast to previous mitogenomic studies, the positions of tarsiers and colugos relative to strepsirrhines and anthropoids are well resolved. In order to improve our understanding of how fossil calibrations affect age estimates within primates, we explore the effect of seventeen fossil calibrations across primates and other mammalian groups and we select a subset of calibrations to date our mitogenomic tree. The divergence date estimates of the Strepsirrhine/Haplorhine split support an origin of crown primates in the Late Cretaceous, at around 74 Ma. This result supports a short fuse model of primate origins, whereby relatively little time passed between the origin of the order and

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the diversification of its major clades. It also suggests that the early primate fossil record is likely poorly sampled.

Keywords

Mitogenomics; Molecular clock; Divergence date; Fossil calibration; Strepsirrhini; Haplorhini

1. Introduction

The order Primates, including 420 species and 73 genera (IUCN 2013.2 IUCN Red List of Threatened Species), is a major mammalian radiation with a rich diversity of morphological, behavioral, and ecological adaptations. Today the non-human members of this order are mainly restricted to tropical or subtropical regions of Asia, Central and South America, and Africa. The fossil record shows that primates were more widely distributed in the past, including in North America and Europe (Covert, 2002). Despite the great interest of evolutionary biologists in this radiation, numerous issues about their origin and diversification remain unclear.

The traditional view of primate origins is that crown primates or euprimates (i.e., all descendants of the last common ancestor of the living species) originated and diversified soon after the Cretaceous-Paleogene (K-Pg) boundary (*explosive model*). According to this model, the abrupt extinction of dinosaurs and the radiation of flowering plants (angiosperms) opened a new set of ecological niches that placental mammals (Eutheria) and birds (Neoaves) could exploit (Conroy, 1990; Rose, 2006). As a consequence, those groups of animals that remained fairly small and cryptic during the Cretaceous underwent a rapid adaptive radiation soon after the mass extinction 65 millions year ago (Rose, 2006; Wible et al., 2007; O'Leary et al., 2013).

The main line of evidence supporting the explosive model is the absence of euprimate fossils prior to the K-Pg boundary. The earliest unambiguous fossil euprimates date from the beginning of the Eocene epoch and are no older than 56 Ma (Miller et al. 2005; Smith et al. 2006; Williams et al. 2010). The best-known representatives of these early euprimates are the two groups Omomyoidea (e.g., *Teilhardina* and *Melanerimia*) and Adapoidea (e.g., *Cantius* and *Donrussellia*), possibly related to the living haplorhines and strepsirrhines, respectively (Gingerich, 1986; Martin, 1993; Ni et al., 2004; Miller et al., 2005; Smith et al., 2006; Rasmussen, 2007). The North African *Altiatlasius koulchii* (Sigé et al., 1990) is slightly earlier, dated to approximately 57 Ma in the late Paleocene, but is not unambiguously a euprimate (Rasmussen, 2007; Williams et al., 2010). Finally, the plesiadapiforms, which have in the past been included in the order Primates, are found in the fossil record between the latest Cretaceous and the early Eocene, with most specimens recovered from deposits dating to the Paleocene (56–66 Ma). However, in contemporary taxonomies, none of these fossils are included in the euprimates and are thought to represent either a stem-group that diverged prior to the origin of euprimates (Bloch and Silcox, 2001; Bloch and Boyer, 2002; Bloch et al., 2007; Silcox et al., 2007) or a non-primate mammalian group possibly related to primates or colugos (Martin, 1968; Cartmill, 1972; Wible and

Covert, 1987; Beard and Houde, 1989; Beard, 1990, 1993; Kay and Thorington, 1990; Kay et al., 1992).

Analyses of molecular data have provided a quite different scenario for the origin and diversification of primates. Molecular studies have yielded divergence dates older than any known euprimate fossil, extending the origins of crown primates before the K-Pg boundary (63.7–95.0 Ma; Springer et al., 2003, 2012; Chatterjee et al., 2009; Fabre et al., 2009; Meredith et al., 2011; Perelman et al., 2011; Jameson et al., 2011; Wilkinson et al., 2011). This view of primate origins agrees with a number of recent molecular studies on mammal diversification suggesting a deep Cretaceous origin for most of the modern placental lineages (Madsen et al., 2001; Murphy et al., 2001; Springer et al., 2003; Bininda-Emonds et al., 2007; Meredith et al., 2011), despite the lack of fossils showing clear morphological traits of the crown group Placentalia (Wible et al., 2007; O'Leary et al., 2013). In contrast to the *explosive model*, these studies place the origin of eutherian orders in the Cretaceous, supporting either a *long-fuse model* (the origin of the orders extend deep into the Cretaceous, with the origin of the main intraordinal lineages near the K-Pg boundary) or a *short-fuse model* (both interordinal and intraordinal diversity originating deep in the Cretaceous) (Springer et al., 2003; Bininda-Emonds et al., 2007; Meredith et al., 2011).

While it is expected that molecules would overestimate and fossils underestimate divergence times (Steiper and Young, 2008), the early lineage origin dates inferred from molecular studies suggest that we are missing a substantial proportion of primate evolutionary history (~20–25 million years). A possible source of the discrepancy between paleontological and molecular models of primate origins lies in the calibration of molecular clocks. In order to convert molecular evolutionary distance estimates to chronological time, a set of well-dated fossils with well-supported phylogenetic placement is required. These calibration points are critical in molecular dating since they are the only source of information about absolute time (Steiper and Young, 2008; Ho and Phillips, 2009; Parham et al., 2012). Three main problems can affect the use of fossil information in estimating divergence times using molecular sequences: 1) the selection of inappropriate fossils; 2) the incompleteness of the fossil record in some areas of the tree; and 3) the phylogenetic misplacement of a fossil (e.g., placing a stem form in a crown group). Though these problems have long been recognized as critical in dating a molecular tree (Raaum et al. 2005; Rutschmann et al., 2007; Steiper and Young, 2008; Ho and Phillips, 2009), the difficulty of accurately choosing proper calibration points is still underestimated (Pyron, 2010; Dornburg et al., 2011; Parham et al., 2012). Many molecular studies suffer from a non-rigorous use of the paleontological record that may easily include considerable error in fossil calibration and provide misleading divergence estimates (Graur and Martin, 2004).

Here, we present one of the largest time-calibrated phylogenies of primates estimated using complete mitochondrial genome sequences. Mitogenomic analyses have been shown to be useful for phylogenetic reconstruction and divergence time estimation at different taxonomic levels and in different groups, including amphibians (Zardoya et al., 2003; Zhang et al., 2005; Zhang and Wake, 2009), birds (Pereira and Baker, 2006; Slack et al., 2007; Pacheco et al., 2011), fish (Inoue et al., 2003, 2010; Miya et al., 2003), and mammals (Arnason and Janke, 2002; Arnason et al., 2002, 2004, 2008). Mitochondrial genomes have traditionally

been used for phylogenetic and phylogeographic analyses of animal taxa. The use of mitochondrial DNA (mtDNA) has several advantages including: the lack of recombination, a rapid coalescence time, relatively high substitution rates, high copy number, and haploidy. For these reasons, it has been suggested that mitochondrial gene trees are more likely to approximate the history of divergence among species than other loci (Moore, 1995). Today, multilocus phylogenies are very common (Jameson et al., 2011; Perelman et al., 2011; Springer et al., 2012), but the nuclear loci used in these studies are usually quite short (<1500 bp) compared to the large size of mitochondrial genomes (>16,000 bp). Mitochondrial genomes also show higher information content per base than nuclear DNA (Anderson et al., 1982; Cummings et al., 1995). Finally, the high number of copies of mitochondria in each cell makes mitogenomic data easy to obtain and sequence, especially in low quality samples such as museum and fossil specimens (Briggs et al., 2009; Krause et al., 2010; Rowe et al., 2011; Mason et al., 2011; Guschanski et al., 2013). Therefore, a good knowledge of the mitochondrial evolutionary history is not only valuable per se, but it can also provide an important comparative dataset in studies for which mtDNA is the only genetic marker available.

Within primates, mitochondrial genomes have been commonly used in phylogenetic studies at the family or genus level (Raaum et al., 2005; Sterner et al., 2006; Hodgson et al., 2009; Chan et al., 2010; Chiou et al., 2011; Guschanski et al., 2013; Zinner et al., 2013), but to date only two comprehensive mitogenomic studies including all main primate lineages have been performed (Matsui et al., 2009; Finstermeier et al., 2013). Here we assembled whole mitochondrial genome sequences from sixty-two primate species (including eleven new sequences) representing all sixteen living families within the order Primates. The goals of this study are (i) to infer the evolutionary relationships of primates using whole mitochondrial genome sequences, (ii) to investigate how different fossil calibrations selected within and outside primates affect estimates of times of divergence within the order, and (iii) to develop a suite of congruent fossil calibrations for use in future studies.

2. Material and methods

2.1 Taxon sampling

We sequenced complete mitochondrial genomes for eleven species representing three strepsirrhines (*Daubentonia madagascarensis*, *Mirza coquereli*, and *Otolemur crassicaudatus*) and eight Old World monkeys (*Allenopithecus nigroviridis*, *Cercopithecus diana*, *C. lhoesti*, *C. mitis*, *Erythrocebus patas*, *Cercocebus torquatus*, *Lophocebus aterrimus*, and *Mandrillus sphynx*) (Table 1). Complete genome sequences from these specimens are deposited in GenBank (http://www.ncbi.nlm.nih.gov/) under accession numbers KJ158462-KJ158463 and KJ434955-KJ434963 (Table 1).

These genomes were added to a dataset including mitochondrial genomes of 51 other primates, in total representing all major taxonomic lineages within the order, as well as mitogenomes of 23 other species representing major lineages within Boreoeutheria. Rooting was done using two Afrotheria species, *Elephas maximum* and *Loxodonta africanus*, commonly accepted as external to Boreoeutheria in mammal phylogenies (Murphy et al., 2001; Arnason and Janke, 2002; Springer et al., 2003; Bininda-Emonds et al., 2007;

Arnason et al., 2008; Meredith et al., 2011; Song et al., 2012). Overall 87 mammalian genomes were included in the phylogenetic analyses.

2.2 Sequencing

DNA was extracted and isolated from either blood or tissue samples using the QIAamp DNA Micro Kit (Qiagen, Inc.) following the protocol provided by the manufacturer. List of the samples with location, sample type, and GenBank accession number is reported in Supplementary Table A1. All the samples are currently stored at the Molecular Anthropology lab at New York University. Mitochondrial genomes were sequenced by long-template PCR, which minimizes the chance of amplifying mitochondrial pseudogenes in the nuclear genome (*numts*) (Thalmann et al., 2004; Raaum et al., 2005). We employed the same approach previously used in Raaum et al. (2005), Sterner et al. (2006), Hodgson et al. (2009), and Chiou et al. (2011). Briefly, independent sets of amplification primers that amplify the entire mtDNA genome in two or more overlapping long fragments were designed for each species. PCR reactions were performed employing a long-range PCR kit (Expand Long Template PCR System, Roche). The sequencing products were analyzed on a 3730 DNA Analysis System (Applied Biosystems, Foster City, CA). Sequences were then assembled and edited using Sequencher 4.1 (Gene Codes, Corp., Ann Arbor, MI). In addition to the eleven newly sequenced genomes, fifteen of the primate genomes used in this study were previously sequenced in the NYU Molecular Anthropology lab applying the technique described above (see Raaum et al. 2005; Sterner et al. 2006; Hodgson et al. 2009). All the remaining genomes (61) obtained from GenBank were analyzed to minimize the presence of nuclear copies of mitochondrial genes (*numts*) in the dataset. First, we translated all of the protein-coding mitochondrial genes into amino acids to check for the presence of premature stop codons and frame shifting insertions or deletions. Then, for each protein-coding gene and RNA sequence independently, we constructed a neighbor-joining tree to identify any sequence that displayed unusual phylogenetic placement.

2.3 Alignment

Heavy-strand protein-coding genes and RNA sequences (2 rRNAs and 22 tRNAs) were automatically extracted from complete genome sequences according to GenBank annotations using a Perl script written by JAH for this purpose. The D-loop was excluded from the dataset because of alignment difficulties due to its high variability. Also the ND6 gene was excluded from the final alignment because it is encoded on the mitochondrial L-strand which has a different nucleotide composition from the H-strand, and has been shown to have poor phylogenetic signal (Gissi et al., 2000; Miya and Nishida, 2000). Protein-coding genes were then aligned based on their corresponding amino acid translations using the software TranslatorX (Abascal et al., 2010). Since homology is best identified at the amino acid level due to the evolution of coding DNA as triplets of nucleotides and the redundancy of the genetic code, this software translates the DNA sequences into amino acids, aligns the amino acid sequences, and then back-translates the alignment to the nucleotide sequences. We allowed the software to automatically identify the most likely reading frame (minimizing the number of stop codons) and we used MUSCLE (Edgar, 2004) to perform the protein alignment. RNA sequences (rRNAs and tRNAs) were aligned with reference to their secondary structure. Due to their stem-and-loop structure, the

identification of homologous regions is best identified when the secondary structure of the RNA sequences is taken into account. This approach has been advocated to improve not only the alignment itself but also the phylogenetic tree reconstruction (Kjer and Honeycutt, 2007; Stocsits et al., 2009; Letsch et al., 2010). We employed two different structure informed alignment methods for RNA sequences: RNAsalsa (Stocsits et al., 2009) and RAF (Do et al., 2006, 2008). RNAsalsa, specifically designed to align ribosomal RNAs, uses preexisting knowledge of RNA structure to simultaneously predict secondary structure in the RNA sequences and to align them based on the secondary structure information. We used secondary structures of *Bos taurus* as reported in the literature (Springer and Douzery, 1996; Burk and Douzery, 2002; Stocsits et al., 2009) to align the remaining 86 sequences in our dataset. RAF (RNA Alignment and Folding) uses an algorithm for simultaneous alignment and consensus folding of unaligned RNA sequences. In contrast to RNAsalsa, RAF does not require preexisting information about secondary structure but identifies sets of likely pairing and alignment candidates for each nucleotide to effectively obtain a simultaneous pairwise alignment and folding (Do et al., 2006, 2008) and then compares the results of the two candidate alignments to identify possible discrepancies.

The resulting alignments (12 protein coding genes, 12S and 16S rRNAs, and 22 tRNAs) were concatenated after removing problematic regions using Gblocks 0.91 (Talavera and Castresana, 2007) under a relaxed approach. Gblocks removes all poorly aligned regions in a dataset and it has been shown to be particularly effective in phylogenetic studies including very divergent sequences (Castresana, 2000; Talavera and Castresana, 2007). Gblocks selects blocks to be deleted following a reproducible set of requirements, including the lack of large segments of contiguous non-conserved positions, lack of gap positions and high conservation of flanking positions. Gblocks was run with the options “Minimum Length Of A Block” = 10 (5 for RNAs), and “Allowed Gap Positions” = “With Half”.

A total of 11,022 bp from the 12 protein-coding genes, 1,313 bp from 22 tRNA genes, and 1,708 bp from two rRNA genes were unambiguously aligned. The individual alignments were then concatenated using the software SequenceMatrix v1.7.6 (Vaidya et al., 2011) to create a master alignment of 14,043 bp total, equivalent to approximately 83% of the mitochondrial genome.

2.4 Data partitions

For both Bayesian and maximum likelihood (ML) analyses, we evaluated six different partition schemes of the mitochondrial genome: 1) no partition (MT0); 2) four partitions (protein-coding genes, 12S rRNA, 16rRNAs, and 22 tRNAs) (MT4); 3) five partitions, identical to the previous one but with the protein coding genes separated in two different partitions (i) codon position 1 and 2 and (ii) codon position 3 (MT5); 4) six partitions, consisting of three partitions for the two rRNAs (12S and 16S) and the 22 tRNAs, and three partitions for the three codon positions of the protein-coding genes (MT6); 5) fifteen partitions, where the 12 protein-coding genes were treated as independent partitions (MT15); and 6) 39 partitions, where the different codon positions of each protein-coding gene were treated as separate partition, plus the three RNA partitions (MT39). The nucleotide substitution model for each partition was selected among 24 models using the

Akaike Information Criterion (AIC) (Posada and Buckley, 2004) as implemented in MrModeltest v2.3 (Nylander, 2004). For each of the data partitions, we evaluated the variable sites and the parsimony-informative sites across all taxa and within the ingroup (primates) only (Table 2).

2.5 Phylogenetic Analysis

Maximum likelihood (ML)—ML analyses were run using a Randomized Accelerated Maximum Likelihood in RAxML version 7.2.6 (Stamatakis et al., 2005; Stamatakis, 2006). For each partition scheme, we ran 50 independent ML inferences (using 50 distinct randomized MP trees) with a GTRGAMMA model to estimate the best topology. We then performed a rapid (–f a -x option) with 1000 replications to assess support on different nodes (Stamatakis, 2006; Stamatakis et al., 2008). Maximum-likelihood bootstrap proportions (MLBS) ≥70% were considered strong support (Hillis and Bull, 1993; Wilcox et al., 2002).

Bayesian analyses (MB)—Bayesian phylogenetic analyses were conducted with MrBayes 3.2.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) using the Metropolis coupled Markov Chain Monte Carlo (Metropolis coupled MCMC or MC^3) algorithm. The most appropriate model of nucleotide evolution was estimated independently for each partition using the AIC test as implemented in MrModelTest 2.3 (Nylander, 2004) as reported in Table 2. Although the model selected in most partitions was GTR+G+I, we also run our analyses using a simplified model GTR+G. This was done because Bayesian analyses may run into problems of convergence of the MCMC when one tries to accommodate among-site substitution rate variation using a combination of proportion of invariant sites model (+I) and a gamma distributed rates model (+G). This conflict may induce two regions of high posterior probability creating clear problems of convergence in the Bayesian analysis (Brian Moore, pers. comm.; Stamatakis 2006). Four independent runs, each including four incrementally heated chains, were run for at least 20 million generations. Different partition schemes required different number of generations to properly reach convergence as reported in Table 3. The sample frequency was adjusted according to the number of generations (Table 3). We assessed convergence visually using Tracer v.1.5 (Rambaut and Drummond, 2007) to plot the likelihood versus generation number and estimate the effective sample size (ESS>200) of all parameters and to compare the performance of the four independent analyses. In addition, we used AWTY (Are We There Yet?, Nylander et al., 2008) to plot pairwise split frequencies for the four independent MCMC runs and to check the posterior probabilities of clades for non-overlapping samples of trees in the sample using the compare and slide commands, respectively. After checking for convergence we summarized the posterior distribution of trees removing the first 10% of generations as burn-in. Posterior probability (PP) support values higher than 0.95 were considered strong support for individual clades (e.g., Alfaro et al., 2003; Erixon et al., 2003; Huelsenbeck and Rannala, 2004).

2.6 Partition models comparison

In order to evaluate the relative support for competing partition models, we compared the marginal likelihood scores for both maximum likelihood and Bayesian analyses (Table 3). We also used Bayes factors (Kass and Raftery, 1995) to select the partition model better

supported by Bayesian analyses. We calculated ln (Bayes factor) as the difference in estimated marginal likelihoods for pairs of models, as described in (Nylander et al., 2004). For Bayesian analyses, marginal likelihoods were estimated from the harmonic means using Tracer v1.5 (Rambaut and Drummond, 2007) by employing the weighted likelihood bootstrap estimator with standard error estimated using 1000 bootstrap pseudoreplicates (Newton, 1994; Suchard et al., 2001). The results for the partition model comparison reported here are based on combining the post–burn-in data from the two runs that achieved the greatest marginal likelihoods, as summarized in Table 3. The similarity of tree topologies resulting from the analyses using different partitions was assessed using the program TOPD-FMTS version 3.3 (Puigbò et al., 2007). Unrooted maximum likelihood and Bayesian trees (without branch lengths) for each of the six partitioning schemes were compared. The TOPD-FMTS analysis was performed under default conditions, with 100 simulated trees as a null model. Analyses were run using the *nodal*, the *split*, and the *disagree* options. The *nodal method* compares the number of nodes that separate each taxon from the other taxa in the tree and calculates pairwise distance matrices from two input trees using only those taxa that are common to both trees (Puigbò et al., 2007). The *split* option uses a crossover method that takes into account the minimum number of operations required to convert one tree into the other, following the algorithm proposed by Robinson and Foulds (1981). Finally, the *disagree* option compares two trees and returns the taxa that make the disagreement between those trees. It first removes one taxon and calculates the gain (reduction in the split distance) between two trees. The taxon that produces most gain is removed for the following iterations. Then this procedure is repeated until the split distance is zero (Puigbò et al., 2007).

2.7 Test for compositional biases

Research over the last decade has shown that phylogenetic inference can be affected by systematic error, such as non-phylogenetic signals that can drive phylogenetic results (Phillips et al., 2004; Phillips, 2009). Even complex models of nucleotide substitution may not be able to capture the substitution process well enough to reliably provide an accurate phylogenetic reconstruction (Phillips et al., 2004; Phillips, 2009). This problem might be even more relevant in large concatenated datasets in which “long sequences exacerbate the potential for biases (systematic error) to be positively misleading” (Phillips et al., 2004: pp. 1455).

The encoding of nucleotides as purines and pyrimidines (RY-coding) has been suggested as an effective approach to assess the effects of compositional biases and non-phylogenetic signal (multiple substitutions) (Phillips et al., 2004; Phillips, 2009). Previous studies on mitochondrial genomes have shown that RY-coding can improve the level of signal relative to compositional heterogeneity, by giving more weight to the slowest evolving sites in the mitochondrial genomes (Brinkmann and Philippe 1999; Phillips and Penny 2003; Delsuc, Phillips, and Penny 2003; Phillips et al., 2004). This approach can therefore increase the ratio between historical and nonhistorical signal, reducing the weight of systematic biases. In order to control for this issue, we compiled and analyzed three additional datasets: 1) third codon position in protein coding regions coded as RY [3RY], 2) positions with Cs and Ts replaced with Y [AGY], and 3) all nucleotides coded as purines (R) and pyrimidines (Y)

[allRY]. For each dataset, relative support of internal nodes was assessed by bootstrap analyses using the bootstopping criteria autoMRE (Majority Rule Criterion) as implemented in RAxML 7.2.6 (Pattengale et al., 2009).

2.8 Taxon sampling effects on phylogenetic reconstruction

Incomplete taxon sampling has been demonstrated to be a major problem in phylogenetic reconstructions (Zwickl and Hillis, 2002; Hillis et al., 2003; Plazzi et al., 2010; Townsend and Leuenberger, 2011; Nabhan and Sarkar, 2012). Here, we explicitly test the importance of taxon representation in resolving the relationships between the major lineages within primates and colugos. We assembled four additional datasets, including different numbers of primate taxa in each: 1) only six primate taxa (one for each main lineage within primates: Lemuriformes, Chyromyiformes, Lorisiformes, Tarsiiformes, Platyrrhini, Catarrhini), 2) ten taxa as used by Schmitz et al. (2002a), 3) 14 taxa as used by Arnason et al. (2008), and 4) 26 taxa as in Matsui et al. (2009). We then compared the results obtained by these four datasets with our original dataset with 62 primate species. Phylogenetic analyses were run using RAxML 7.2.6 as fully described above.

2.9 Divergence date estimation and cross-validation analyses

Fossil constraints were carefully selected based on criteria for choosing appropriate calibration points reviewed elsewhere (Raaum et al., 2005; Ho and Phillips, 2009; Parham et al., 2012). We employed seventeen fossil constraints identified from the literature and previous studies performed on primate and mammal phylogeny. Details about these fossil calibrations, including age, phylogenetic position, minimum and (putative) maximum bounds, and references are reported in Table 4. The detailed procedure of fossil selection, with paleontological details, and assignment to alternative nodes for calibration of the molecular clock is fully reported in Supplementary Material B1.

We evaluated the congruence of these 17 minimum-age constraints using fossil cross-calibration (Near and Sanderson, 2004; Near et al., 2005; Hugall et al., 2007; Rutschmann et al., 2007). Fossil cross-validation involves the use of one fossil at a time to generate age estimates for the nodes to which the other fossils are assigned; then it compares the inferred age with the fossil age at that node. This technique assesses the consistency of date estimates generated from different putative calibration points (Near and Sanderson, 2004; Near et al., 2005). We calculated two different statistics to evaluate the congruence of each calibration point. First, following the method proposed by Near et al., we calculate the mean deviation (*D*) between molecular (MA) and fossil age (FA) estimates for all nodes using a single fossil-dated node as a calibration point, and the SS values ($SS_x = \sum_{i \neq x} D^2$) for a given fossil calibration node when it was used as the single calibration point. Second, we applied the statistics described by Hugall et al. (2007). We first calculate the mean deviation D as described above. Then, for each calibration point, we obtained the sum of the absolute values of differences between the estimated and proposed ages for each calibration node. This value ($\sum|D|$) is an indication of how congruent a specific fossil calibration is with the remainder: the smaller the value the more consistent. For these analyses, we used the topology and branch lengths of the MT39 model tree obtained in our Bayesian analyses.

This topology was strongly favored in the Bayes factor comparison analysis explained above (see Table 3).

For all the analyses described above, we used the rate-autocorrelated Bayesian relaxed molecular clock method implemented in the MULTIDIVTIME program package (Thorne and Kishino, 2002). First, maximum likelihood parameters for the F84 + G model of evolution were estimated with *baseml* from the PAML package (Yang, 1997, 2007). Next, branch lengths and their variance-covariance matrix were calculated with *estbranches*. Finally divergence times were calculated with *multidivtime* using conservative priors and the fossil calibrations. Bayesian priors for *multidivtime* were chosen according to J. Thorne's recommendations in the *multidivtime* manual. Root-to-tip mean was set at 100.0 Ma, with a 100.0 Ma standard deviation. The evolutionary rate at the root was set at 0.009 substitutions per nucleotide site per Myr, with a standard deviation of 0.009. This was calculated as the median root-to-tip branch length divided by the root-to-tip mean prior. The *brownmean* and its standard deviation were set to 0.020, such that the *brownmean* multiplied by the root-to-tip mean prior (100Ma) equals 2. After a burn-in period of 100,000 generations, MCMC chains were sampled every 100 generations until 10,000 samples were taken. To test the effect of the priors on the posteriors, several runs were performed with various prior choices. The results were extremely robust to changes in priors.

During the preliminary analyses fossil constraints were treated as point calibrations (minimum equal to maximum bounds). Based on the results of the preliminary analyses in which all 17 fossil calibrations were tested for congruence, a set of 16 constraints was used to obtain the final chronogram presented in the results. In this final analysis, for each fossil calibration we defined a minimum and a maximum constraint (if available) as reported in Table 4.

3. Results

3.1 Phylogeny

A comparison of the tree topologies that resulted from the analyses showed that varying partition scheme had little effect on the overall tree topology. For ML trees, between zero and six out of 168 possible conflicts were identified across partitions, representing a difference of only 0.00–3.57% (nodal distance: 0–0.474; split distance: 0–0.036). The differences were mainly represented by the position of two taxa: the genus *Cavia* within the rodents (either sister group of *Cricetulus*/(*Mus*/*Rattus*) or sister taxon of the dormouse, *Glis glis*) and the position of *Cercopithecus diana* in relation to the other cercopithecins. Within the MB trees, only 2 possible conflicts were found across different partitioning scheme (1.07%; nodal distance: 0–0.331; split distance: 0–0.0119). The position of *C. diana* was the only taxon with conflicting placements among different partitions.

Bayes factor comparisons strongly supported the model with 39 partitions (MT39), consisting of two partitions for the rRNAs (12S and 16S), one for the 22 tRNAs, and 36 partitions for the three codon positions of each protein-coding gene. Partition scheme MT39 was strongly favored over MT6 (lnBF = 5531.809), MT6 was favored over MT5 (lnBF = 1287.011), MT5 strongly favored over MT15 (lnBF = 5068.416), MT15 favored over MT4

(lnBF = 996.090), and MT4 favored over MT0 (lnBF = 1440.723) (Table 3). The same results were obtained using maximum likelihood (MT39<MT6<MT5<MT15<MT4<MT0). We therefore treat the MT39 ML and Bayesian trees as our "preferred tree". Tree topology comparison analysis using TOPD-FMTS version 3.3 showed identical topologies between ML and Bayesian trees (0/168 nodes; Split Distance random [differents/possibles]: 0.997 +/− 0.007 [167.46 +/− 1.195/168 +/− 0.000]).

The Bayesian clade credibility values (PP) across the entire tree were high, with only two nodes below 0.95: the sister group relationship between *C. diana* and the clade including *Erythrocebus patas* and *C.mitis/C.lhoesti* (PP=0.91), and the sister group relationship between *Erythrocebus patas* and *C.mitis/C.lhoesti* (PP=0.80) (Figure 1). In contrast to the Bayesian results, the maximum likelihood bootstrap (BP) values were quite low in several nodes across the tree (Figure 1). Within primates several nodes were weakly supported in the ML analyses. For instance, within the platyrrhines, the sister relationship between *Ateles* and the clade including *Aotus*, *Saimiri*, *Cebus*, and *Saguinus* was below 70% bootstrap support Also, several nodes within cercopithecins (particularly, the position of *E. patas* and *C. diana*, and the sister group relationship between *Nasalis* and *Pygathrix*) showed bootstrap support below 70% (Figure 1). BP and PP values for all the nodes within primates are reported in Supplementary Material (Table C1).

In order to evaluate the influence of RNA sequences on the topology and support values, we also ran RAxML and MrBayes using only the 12 protein coding genes (11,022 bp). The topology of the reduced dataset did not differ from the one using all data. Similar to the ML results described above, BP values were generally lower than PP in Bayesian analyses (see Supplementary Figure D1–2).

All the analyses supported the same topology and the monophyly of the order Primates (Figure 1). Specifically, mitochondrial genome analyses supported the subdivision of primates into infraorders Strepsirrhini and Haplorhini, with tarsiers as sister group of Anthropoidea (BP>70% and PP>0.95). Moreover, the dermopterans (colugos, here represented by the genus *Galeopterus*) fell outside the primates (BP>70% and PP>0.95), and not the sister group of anthropoids as in many previous studies using mitochondrial DNA (Arnason and Janke, 2002; Arnason et al., 2002, 2008; Schmitz et al., 2002a).

We evaluated the effect of compositional biases and the level of non-phylogenetic signal using three additional datasets (3RY, AGY, and allRY). All analyses strongly supported (BP>70%) the monophyly of primates (with colugos outside primates), and two datasets (3RY and AGY) also strongly supported the sister group relationship between tarsiers and anthropoids. When all nucleotides were coded as RY (allRY), the monophyly of haplorhines (tarsiers+anthropoids) was only weakly supported (BP= 57%) (Supplementary Figures E1–3).

Relationships among the major lineages within primates were well supported by all the analyses, with lemuriformes and lorisoids within strepsirrhines, platyrrhines and catarrhines within anthropoids, and cercopithecoids (Cercopithecinae and Colobinae) and hominoids within catarrhines (Figure 1). Two major discrepancies between our results and widely

accepted relationships within primates are found in the phylogenetic relationships within the lorisoids and the papionins. In the former clade, the division between galagids and lorisids was not supported by our mitogenomic data. The African lorisids were found to be the sister group to the clade composed of Asian lorisids and African galagids in all the analyses performed here. However, while Bayesian analyses strongly supported this topology (PP>0.95), bootstrap values were relatively low across all the analyses (BP<70%). Within the tribe Papionini, our results did not support the presence of an African clade (Theropithecus/Papio/Lophocebus/Mandrillus/Cercocebus). Instead, the group *Mandrillus/Cercocebus* clustered together with the genus *Macaca* (BP>70% and PP>0.95). Within these two primate groups (lorisoids and papionins), the same relationships were found when RY-coded datasets (3RY, AGY, and allRY) were analyzed.

3.2 Taxon sampling effects on phylogenetic reconstruction

In order to investigate the influence of taxon sampling in our dataset we analyzed four additional datasets including a subset of the primate taxa. Interestingly, all the datasets (6-taxa, 10-taxa, 14-taxa, and 26-taxa) showed no support for the monophyly of primates and the sister relationship between tarsiers and anthropoids. All reduced datasets supported instead the sister relationship between colugos and anthropoids and between strepsirrhines and tarsiers. However, the bootstrap values for both nodes were low (BP<70%) across all the analyses: 32–60% for the clade colugos-anthropoids and 45–68% for the clade tarsiers-strepsirrhines (Supplementary Figures F1–4).

3.3 Fossil congruence analyses

The cross-validation results (Table 5) reveal that calibration point 13 (C13: the split between Mysticeti and Odontoceti) is highly inconsistent with the other fossil calibrations. Specifically, when used as the only calibration point, C13 tended to overestimate all the other calibration points used in this study (D_x=23.52). Both statistics used in the study (SS and ΣD) supported this conclusion (Table 5). All the other fossil calibrations used in this study provide plausible age estimates that fall within the range of the other calibrations. We thus used 16 calibration points listed in Table 4 (excepting C13) for all dating analyses.

Because the relative position of fossil calibrations in the tree might influence the precision of the dating estimates for the node of interest, we tested whether the absolute values of the deviation between the estimated and proposed ages for each calibration node were correlated with the branch lengths (nodal distance) between the calibration point used in each run and the remaining 16 nodes. We performed a Mantel test to check the correlation between the matrix of the deviations |D| (index of the estimation error) and the matrix of nodal distances in term of branch length (R Development Core Team, 2009). The Mantel test based on 10,000 replicates produced a positive correlation (p-value: 0.038) with r=0.265. In order to evaluate the effect size of this correlation, we calculated the slope estimate from a linear regression. The analyses showed a positive relationship with β=4.7.

3.4 Divergence dates

Dating analyses were run with *multidivtime* using the MT39 partition strategy, as selected by the preliminary analyses described above. Estimates for the major nodes within primates are

reported in Table 6, along with 95% HPD intervals. The chronogram estimated the origin of crown primates at 74.1 Ma (95% HPD=68.2–81.2; Figure 2 and Table 6). Both crown strepsirrhines and crown haplorhines are inferred to have originated in the Late Cretaceous, 66.3 Ma (95% HPD=61.1–72.8) and 70.0 Ma (95% HPD=64.3–76.7), respectively. The most recent common ancestor of the living platyrrhines was estimated to have lived 20.9 Ma (95% HPD=17.9–24.4), while the origin of crown catarrhines occurred considerably earlier, approximately 32.1 Ma (95% HPD=29.4–33.8). *Homo sapiens* and Neanderthals diverged at around 680,000 years ago (95% HPD=490–926,000), while the date of the most recent common mitochondrial ancestor between the Denisovans, Neanderthals and modern humans was estimated to be approximately 1.39 Ma (95% HPD=1.06–1.83). The estimates for all the nodes within primates are reported in the Supplementary Material (Table C1).

4. Discussion

In this study, we provided an assessment of primate phylogenetic relationships and divergence dates using complete mitochondrial genome data. We compiled one of the largest mitogenomic datasets for primates, and we applied a fossil cross-validation procedure to identify and select the most appropriate set of fossils to calibrate the molecular primate tree.

4.1 Phylogenetic conclusion

All major lineages within the order Primates were included in our analyses and were inferred to be monophyletic. In contrast to previous mitogenomic studies, our analyses posit a monophyletic Primates with colugos as their sister group within Euarchontoglires. Previous mitogenomic studies hypothesized a sister relationship between anthropoid primates and colugos (Dermoptera), making the order Primates paraphyletic (e.g., Arnason and Janke, 2002; Arnason et al., 2002; Schmitz et al., 2002a; Kjer and Honeycutt, 2007). Arnason et al. (2002) proposed the name Dermosimii for this assembly (Arnason and Janke, 2002; Arnason et al., 2002, 2008). However, this group has been suggested to be an artifact of similar nucleotide composition of mitochondrial genomes rather than reflecting the true evolutionary history (Schmitz et al., 2002a; Schmitz and Zischler, 2003; Raina et al., 2005; Zischler, 2007). In contrast to mitochondrial results, the monophyly of primates is in fact strongly supported not only by morphological studies (Silcox et al., 2007) but also by nuclear sequences (Jameson et al., 2011; Meredith et al., 2011; Perelman et al., 2011) and transposable elements (Schmitz et al., 2002a). In this study we suggest that this artifact might also be a consequence of taxon sampling and the small internode distance between Dermoptera and Primates. Incomplete or biased taxon sampling is a major concern in phylogenetic studies and can provide misleading results in reconstructing phylogenetic relationships (Zwickl and Hillis, 2002; Hillis et al., 2003; Plazzi et al., 2010; Townsend and Leuenberger, 2011; Nabhan and Sarkar, 2012), and increased sampling of taxa has been shown to be an effective way to improve overall phylogenetic accuracy (Zwickl and Hillis, 2002). Here, we show that a large taxon sampling within primates can recover phylogenetic relationships consistent with morphological and nuclear data, and supports the monophyly of primates and the invalidity of the taxon Dermosimii. When only a subset of primate taxa was included in the analyses, the position of colugos and tarsiers became poorly supported

and all the analyses recovered a sister relationship between colugos and anthropoids similar to previous studies (e.g., Arnason and Janke, 2002; Arnason et al., 2002; Schmitz et al., 2002a; Kjer and Honeycutt, 2007), thus indicating that insufficient taxon sampling was likely the cause of the discrepancy found between mitochondria DNA and other lines of evidence (e.g., Schmitz et al., 2002a; Silcox et al., 2007; Jameson et al., 2011; Meredith et al., 2011; Perelman et al., 2011)

The difficulty of recovering primate monophyly using mitochondrial sequences is somewhat confirmed by lower support in the ML bootstrap analyses, compared to Bayeasian posterior probabilities. Theoretical and empirical studies showed that Bayesian posterior probability tends to be substantially higher than corresponding ML bootstrap frequencies. In particular, bootstrap analyses have been shown to be particularly sensitive to small numbers of characters and might underestimate confidence in tree topologies compared to Bayesian posterior probability in case of short internodes (Alfaro, 2003; Erixon et al., 2003; Rothfels et al., 2012). The low amount of phylogenetic signal in the mitochondrial genome for these nodes is demonstrated by the relatively short branch length of the internode between Dermoptera and Primates, and between Strepsirrhini and Haplorhini (Figure 1).

Within primates, we found a well-supported division between strepsirrhines (including lorises, galagids, and Malagasy lemurs) and haplorhines (including tarsiers and anthropoids). Overall, support values across the entire primate tree were high with only a few exceptions. Within cercopithecoids, the sister group relationship between *C. diana* and the clade including *Erythrocebus patas* and *C.mitis/C.lhoesti* and the sister group relationship between *Erythrocebus patas* and *C.mitis/C.lhoesti* were poorly supported in both Bayesian and ML analyses. Within the platyrrhines, most of the nodes showed low bootstrap support (but high Bayesian PP support), in agreement with previous studies that employed complete mitochondrial genomes (Hodgson et al., 2009; Chiou et al., 2011).

The phylogenetic position of the newly sequenced mitochondrial genomes was consistent with previous studies. The aye-aye (*Daubentonia madagascariensis*) was inferred as the most basal divergence within lemurs, while *Mirza* (member of the family Cheirogaleidae) was recovered as sister group of sportive lemurs (*Lepilemur*) (Perelman et al., 2011; Springer et al., 2012). Within Cercopithecoidea, the phylogenetic positions of the five guenon genomes (*Allenopithecus nigroviridis*, *Cercopithecus diana*, *C. lhoesti*, *C. mitis*, and *Erythrocebus patas*) were identical to the ones suggested in a larger mitogenomic study for guenons (Guschanski et al., 2013), but in contrast with nuclear data (Perelman et al., 2011; but see Guschanski et al., 2013).

Despite the large taxon representation in this study, we could not overcome some well-known problems affecting mitochondrial-based reconstructions of primate phylogenetic relationships. For example, two well-supported taxonomic groups, the African papionins and the lorisids, were not inferred to be monophyletic in our study. In the former group, our mitogenomic tree clusters *Mandrillus* and *Cercocebus* with *Macaca* instead of with the clade including *Papio*, *Theropithecus*, and *Lophocebus*. Within lorisoids, our mitogenomic tree places Asian lorisids as the sister group to the African galagids rather than to the African lorisid *Perodicticus*. Both the lorisids and the papionins are well supported by

morphological data and nuclear sequences, and previous studies employing mitochondrial sequences have shown similar difficulty in recovering such clades (Papionini: Disotell et al., 1992; Harris, 2000; Finstermeier et al., 2013; Lorisidae: Roos et al., 2004; Masters et al., 2007; Matsui et al., 2009; Finstermeier et al., 2013; Pozzi et al., *accepted*). The mitochondrial genome represents a single locus and its phylogenetic history can differ from the species as a consequence of several events, including gene flow, hybridization, or incomplete lineage sorting (Maddison, 1997; Degnan and Rosenberg, 2006; Maddison and Knowles, 2006; Edwards, 2009). Although mitochondrial genomes are still a powerful resource to estimate phylogeny and phylogeography (Moore, 1995), conclusions based on a single locus should always be made with caution (Edwards, 2009; Ting and Sterner, 2013; Pozzi et al., 2014).

4.2 Divergence dates

To our knowledge, this is the first study that explores the application of fossil cross-validation within primates. The primate fossil record is extremely fragmented and incomplete, and some studies estimate that only 4–7% of all primate species are known from the fossil record (Tavaré et al., 2002; Soligo and Martin, 2006; Martin et al., 2007). The degree of incompleteness of the fossil record is particularly extreme in some lineages, most notably within the Malagasy lemurs in which no fossils are known with the possible – but controversial – exception of *Bugtilemur mathesoni* in Pakistan (see Marivaux et al., 2001, 2006). As a consequence, most of the molecular studies to date employed a small set of calibration points, representing only a few nodes within the primate tree (e.g., Fabre et al., 2009; Matsui et al., 2009). More recently, Wilkinson et al. (2011) tried to better integrate the current knowledge of the fossil record into molecular date estimates. They developed a new methodology to better account for the rates of fossil preservation and discovery when calibrating a molecule-based tree. This study dated the origin for the crown primates at ~84 Ma, confirming a discrepancy between molecular estimates and the known fossil record.

In this study, we present a cross validation analysis of the fossil record to select the most appropriate calibration points to be used with our data set. Our approach consisted of two different steps: 1) we selected *a priori* putative fossils suitable to be used as calibration points for our dataset, following the criteria proposed by Parham et al. (2012); 2) we performed a cross validation analyses aimed to detect possible outliers among our fossil selection. Since one of the main goals of this paper was to estimate the timing of primate origins we also included several calibration points within Boreoeutheria (Euarchontoglires and Laurasiatheria). Using only calibration points within primates might lead to problems in extrapolating dates, especially at the root of the tree (Raaum et al., 2005). In this study we selected sixteen fossils as calibration points, including eleven within and five outside primates. The original cross validation method proposed by Near et al. (2005) has been criticized by several authors because it could lead to several problems in selecting calibration points, including the possibility that the method will select an internally consistent set of fossils, excluding the most informative fossils because they are inconsistent with the majority of the remaining calibrations (Marshall, 2008; Ho and Phillips, 2009). Here, we used a conservative approach aimed to identify only possible outliers in the sets of fossils selected during the *a priori* phase. Although we acknowledge that the approach used

here is not sufficient to address the complex challenges of calibration (Magallón, 2004; Near and Sanderson, 2004; Near et al., 2005; Hugall et al., 2007; Rutschmann et al., 2007; Pyron, 2010; Wilkinson et al., 2011), we believe that our approach allows researchers to better select fossils to be used in molecular phylogenetic analyses. For example, this approach might be beneficial in inferring the correct placement of fossils to specific nodes in a tree and excluding fossils that might be otherwise misused in calibrating molecular phylogenies. Moreover, new fossils can be tested against a good set of calibration points and alternative placements can be evaluated using the approach described here (see also Pyron, 2010 and Ward et al., 2010). This technique also allows researchers to include more fossils in their analyses, providing a better - and much needed - integration between molecular phylogenies and the paleontological record.

In order to identify how nodal distance can affect molecular estimates across the tree, we investigated the relationship between the deviation |D| and the nodal distance for each node selected as putative calibration point. Due to the incomplete nature of the fossil record, many areas of the primate tree have no available calibration point (e.g., the Malagasy lemurs). If the evolutionary distance from calibration points were a limiting factor in our ability to accurately estimate divergence times, then we would expect a strong correlation between nodal distance and the estimation error. The relationship that we found in our data set, although statistically significant, is particularly low. The Mantel test showed a weak positive correlation (r=0.265), suggesting that evolutionary distance from calibration points plays a very small role in the associated error. For instance, we found that the difference in estimated error of the crown Catarrhini divergence date between a near calibration point (*Homo/Pan*, branch length distance = 0.2588) and a more distant calibration point (Lorisidae/Galagidae, branch length = 0.7788) was only ±2.44 million years. For this reason, our confidence in divergences distant from calibrations will be similar to those closer to calibration points. This result is particularly important for some taxonomic groups in which the fossil record is particularly scarce or absent. Therefore, despite the negative relationship between nodal distance and accuracy, modern molecular dating techniques can successfully provide reliable estimates even in areas where no calibration points are available.

The molecular chronogram obtained in this study estimated the crown-group origin of the order Primates in the Late Cretaceous (around 74 Ma), in agreement with most recent molecular studies (Arnason et al., 2008; Fabre et al., 2009; Matsui et al., 2009; Jameson et al., 2011; Perelman et al., 2011; Wilkinson et al., 2011). Nuclear estimates seem to provide slightly older estimates than the ones obtained by mitogenomic data (Perelman et al., 2011; Wilkinson et al., 2011), however all molecular data to date seem to strongly suggest an origin of primates before the K-Pg boundary. This result agrees with estimates obtained for other group of mammals, where the majority of recent molecular studies on mammalian diversification place the origin for most orders in the Cretaceous (Springer et al., 2003; Bininda-Emonds et al., 2007; Meredith et al., 2011).

A recent study on mitochondrial genomes including over 80 species of primates, recovered more recent origins for the crown group, dated around 66Ma (59–73Ma) (Finstermeier et al., 2013). This discordance with our study is particularly interesting given the large overlap in genome sequences used in both studies. Two main differences may have contributed to the

discrepant conclusions between the two studies. First, Finstermeier et al. (2013) only used calibration points within primates, making the age estimates at the root of the tree problematic due to extrapolation biases (see Raaum et al., 2005). The main differences in age estimates between Finstermeier et al.'s study and ours are limited to deeper nodes in the phylogeny. An additional source of discordance might be due to the use of controversial calibration points by Finstermeier et al. (2013). Out of the nine calibration points employed in that study, at least four were either secondary calibrations (i.e., molecular date estimates obtained from other molecular analyses) or based on misinterpretation of the fossil record (i.e., the use of stem fossils to calibrate a crown groups) (Graur and Martin 2004; Steiper and Young, 2008; Ho and Phillips, 2009; Pozzi et al., 2011). For instance, Finstermeier et al. (2013) calibrated the origins of crown catarrhines using the fossil *Saadanius hijazensis* (Zalmout et al., 2010). However, *Saadanius* is a stem catarrhine and cannot possibly inform the divergence between hominoids and catarrhines (see Pozzi et al., 2011).

Similarly to Finstermeier et al. (2013), a recent study conducted by Steiper and Seiffert (2012) also suggested a more recent origin for crown primates, closer to the K-Pg boundary or possibly even in the Paleocene. In their study, the authors showed a negative correlation between three different life history variables (body size, absolute endocranial volume and relative endocranial volume) and molecular rates in primates. While almost all divergence date estimates for placental mammals based on molecular data are broadly consistent with our results, it is possible that there are life history factors that all these studies have failed to take into account (Bromham, 2009, 2011; Lanfear et al., 2010; Steiper and Seiffert, 2012).

Finally, O'Leary et al. (2013) recently integrated molecular and morphological evidence to support an explosive model of evolution for mammals, with most the interordinal diversification dated just after the K-Pg boundary. Based on their analyses, the crown primates originated in the late Paleocene, around 55–56 Ma (O'Leary et al., 2013). The reconstruction proposed by O'Leary et al. (2013) is however based on phylogenetic analyses that fail to discriminate between homology and homoplasy and also implies extremely high acceleration in nucleotide substitution rate in early Paleocene mammals (Springer et al., 2013). One of the major shortcomings of O'Leary et al.'s study is the use of the earliest known fossil representative to date the origin of each group ('ghost lineage' analysis). As a consequence the origin of crown primates coincides with the earliest members of the group found in the fossil record at around 55–56 Ma (e.g., *Teilhardina brandti*) (O'Leary et al., 2013). However, the absence of evidence in the fossil record is not necessarily evidence of absence (Ho and Phillips, 2009) and the estimates obtained by O'Leary et al. (2013) can be considered only minimum divergence times based on ghost-lineage minimization rather than actual divergence times (Slater, 2013; Springer et al., 2013; Yoder, 2013; dos Reis et al., 2014).

The ancestor of the living catarrhines (apes and Old World monkeys) was estimated to have lived approximately 32 Ma. This result is in contrast to Zalmout et al. (2012) who suggested that the divergence for crown catarrhines dates after 29 Ma, based on the stem catarrhine *Saadanius hijazensis*. Inferring a date older than 29 Ma is not surprising because stem fossils do not provide any information about the divergence time of a crown group (Steiper and

Young, 2008; Ho and Phillips, 2009); therefore, *Saadanius* does not inform the time of origin of crown catarrhines (Pozzi et al., 2011).

A recent study conducted by Langergraber et al. (2012) introduced a novel method to date divergence times within great apes without employing fossils as calibration points. This method relies on recent advances in the direct measurement of the mutation rate in humans (Awadalla et al., 2010; Roach et al., 2010; Sanders et al., 2012; Scally and Durban, 2012) and in the generation times within wild populations of chimpanzees and gorillas (Langergraber et al., 2012). Interestingly, the estimates obtained by Langergraber et al. are in line with the ones reported in this study. The only contrast with the above study is the age estimate for the divergence between bonobos (*Pan paniscus*) and common chimpanzee (*Pan troglodytes*) dated between 1.5 and 2.6 Ma by Langergraber et al. and 2.4–3.8 Ma in our study.

Finally, our estimates of 680 ka for Neanderthal and modern humans, and ~1.4 Ma for Denisovans and *Homo sapiens* are older - roughly 1.4x - than the ones obtained in the original paper by Krause et al. (2010) also using mitochondrial genomes. We identified the origin of this discrepancy in the different use of calibration points employed in the two studies. Krause et al. in fact assumed an average divergence of human and chimpanzee mtDNAs of 6 million years, while in our study we allowed this node to date between 5 and 10 Ma. We inferred this divergence to be at approximately 7.6 Ma (95% HPD=6.7–8.8), which is substantially older than the age used by Krause et al. (2010). When the split between *Homo* and *Pan* was set at 6 Ma, we obtained age estimates that agree with Krause et al. (*H. sapiens*-Neanderthal: 530 ka [397–693]; *H. sapiens*-Denisovans: 1.08 Ma [0.87–1.35]). We contend that the mitochondrial divergences obtained in our study are more realistic than the one presented by Krause at al. (2010), because the common ancestor of modern humans and chimpanzees is likely to be older than 6 Ma, based on both molecular estimates (Jameson et al., 2011; Wilkinson et al., 2011; Springer et al., 2012) and the fossil record (Haile-Selassie et al., 2001; Senut et al., 2001; Brunet et al., 2002; Vignaud et al., 2002).

4.3 Conclusion

Our study applied a fossil cross-validation procedure to identify a set of fossil calibrations to be used in dating a molecular phylogeny within primates. We used a two-step approach that includes an *a priori* selection of putative fossils and *a posteriori* cross validation analysis to exclude possible outliers. The set of calibrations obtained by such an analysis can then be used to estimate the ages of the nodes of interest. We therefore recommend the use of these fossil calibrations for future studies aimed to estimate divergence times within primates. Also, our study showed a weak correlation between nodal distance and accuracy, suggesting that distant calibration points can be reliably used for dating areas of the trees where the fossil record is particularly scant.

The results obtained in this study agree with recent studies of primate divergence dates, supporting an origin of the order in the Late Cretaceous. This suggests that the early primate fossil record is likely poorly sampled. Although primate evolution may involve a slowdown in molecular rates, most molecular studies to date suggest that it is highly unlikely that

primates – together with most mammalian orders – originated after the K-Pg boundary. Based on the age estimated obtained in our study we suggest a short fuse model of primate origins, whereby relatively little time (<8Myr) passed between the origin of the order Primates and the diversification of its major clades, Strepsirrhini, Tarsiiformes, and Anthropoidea.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- We present one of the largest phylogenies of primates using complete mitochondrial genomes.
- We included 62 primate species (representing all families) and 25 mammal species.
- We recovered all the major clades within primates.
- A cross-validation procedure was used to identify a set of fossil calibrations.
- Crown primates diversified in the late Cretaceous supporting a short fuse model.

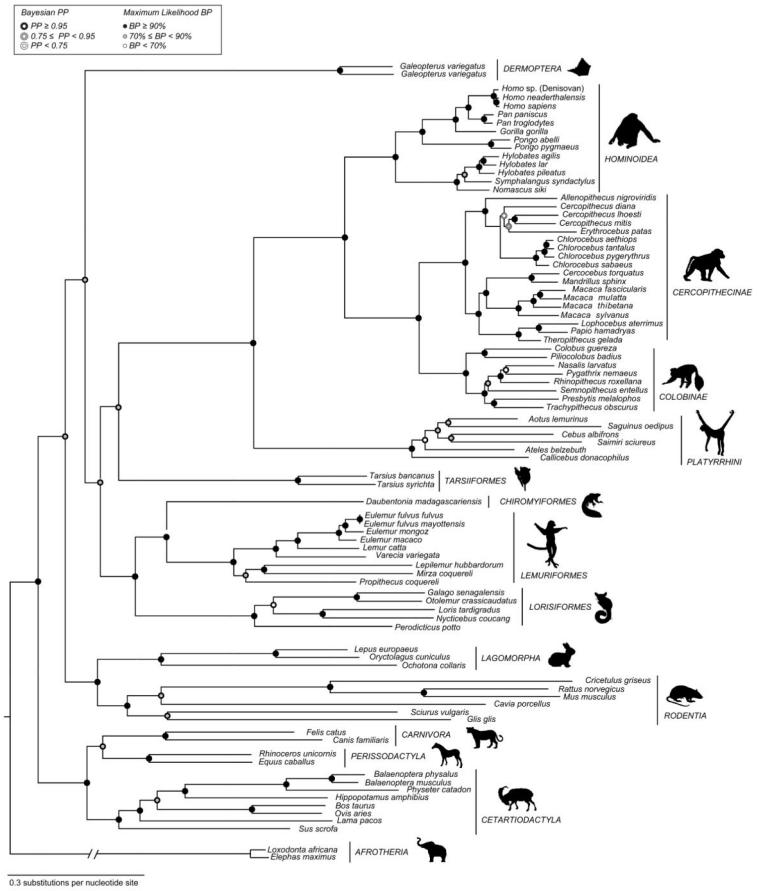

Figure 1.

Single phylogenetic tree inferred from complete mitochondrial genome sequences based on maximum likelihood and Bayesian analyses. The tree topology remained constant across all analyses and branch lengths presented here follow the Bayesian analysis. The tree was rooted using *Loxodonta africana* and *Elephas maximus*. Maximum likelihood bootstrap values (BP) are shown with color coded inner circles (black: BP≥90%, grey 70%≤BP<90%; white: BP<70%); Bayesian posterior probability value (PP) are indicated by the outer ring (black: PP≥0.95, grey 0.75≤PP<0.95; white: PP<0.75).

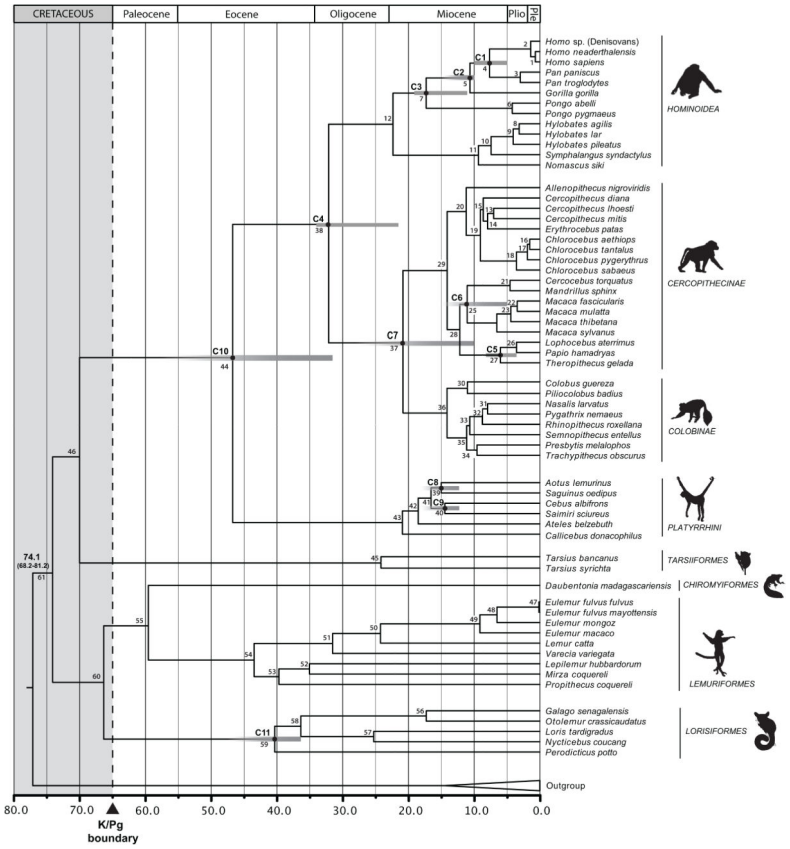

Figure 2.

Single chronogram with divergence date estimates from complete mitochondrial genome sequences. Only age estimates within primates are shown. Calibration points are depicted with filled circles; gray bars indicate constraint ranges. Mean node ages and 95% HPD intervals for major nodes within primates are presented in Table 6. Age estimates for all the nodes in the chronogram are reported in Supplementary Material (Table C1).

Table 1

Sequences used in this study.

Scientific name	Common name	NCBI Accession No	Reference
Primates			
Strepsirrhini			
Lorisiformes			
Lorisidae			
Loris tardigradus	Slender loris	NC_012763	Matsui et al., 2009
Nycticebus coucang	Slow loris	NC_002765	Arnason et al., 2000
Perodicticus potto	Potto	NC_012764	Matsui et al., 2009
Galagidae			
Galago senegalensis	Senegal galago	NC_012761	Matsui et al., 2009
Otolemur crassicaudatus[a]	**Thick-tailed galago**	**KJ434961**	**This study**
Chiromyiformes			
Daubentoniidae			
Daubentonia madagascariensis[a]	**Aye-aye**	**KJ158462**	**This study**
Lemuriformes			
Lemuridae			
Eulemur mongoz	Mongoose lemur	NC_010300	Arnason et al., 2008
Eulemur fulvus fulvus	Brown lemur	NC_012766	Matsui et al., 2009
Eulemur fulvus mayottensis	Brown lemur	NC_012769	Matsui et al., 2009
Eulemur macaco macaco	Black lemur	NC_012771	Matsui et al., 2009
Lemur catta	Ring-tailed lemur	NC_004025	Arnason et al., 2002
Varecia variegata	Black and white ruffled lemur	NC_012773	Matsui et al., 2009
Cheirogaleidae			
Mirza coquereli[a]	**Coquereli's giant mouse lemur**	**KJ158463**	**This study**
Lepilemuridae			
Lepilemur hubbardorum	Hubbard's sportive lemur	NC_014453	Lei at al., 2010
Indridae			
Propithecus coquereli	Coquerel's sifaka	AB286049	Matsui et al., 2007
Haplorrhini			
Tarsiformes			
Tarsiidae			
Tarsius bancanus	Horsfield's tarsier	NC_002811	Schmitz et al., 2002b
Tarsius syrichta	Philippine tarsier	NC_012774	Matsui et al., 2009
Anthropoidea			
Platyrrhini			
Cebidae			
Aotus lemurinus	Lemurine night monkey	FJ785421	Hodgson et al., 2009
Cebus albifrons	White-fronted capuchin	AJ309866	Arnason et al., 2000
Saimiri sciureus	Common squirrel monkey	FJ785425	Hodgson et al., 2009
Saguinus oedipus	Cotton-top tamarin	FJ785424	Hodgson et al., 2009

Scientific name	Common name	NCBI Accession No	Reference
Pithecidae			
Callicebus donacophilus	Bolivian titi	FJ785423	Hodgson et al., 2009
Atelidae			
Ateles belzebuth	White-bellied spider monkey	FJ785422	Hodgson et al., 2009
Catarrhini			
Cercopithecoidea			
Colobinae			
Colobus guereza	Guereza	AY863427	Raaum et al., 2005
Nasalis larvatus	Proboscis monkey	DQ355298	Sterner et al., 2006
Pilocolobus badius	Western red colobus	DQ355301	Sterner et al., 2006
Presbytis melalophos	Mitred leaf monkey	DQ355299	Sterner et al., 2006
Pygathrix nemaeus	Douc langur	DQ355302	Sterner et al., 2006
Rhinopithecus roxellana	Golden snub-nosed monkey	DQ355300	Sterner et al., 2006
Semnopithecus entellus	Hanuman langur	DQ355297	Sterner et al., 2006
Trachypithecus obscurus	Dusky leaf monkey	NC_006900	Raaum et al., 2005
Cercopithecinae			
Allenopithecus nigroviridis[a]	**Allen's swamp monkey**	**KJ434962**	**This study**
Cercocebus torquatus[a]	**Collared mangabey**	**KJ434959**	**This study**
Cercopithecus diana[a]	**Diana monkey**	**KJ434958**	**This study**
Cercopithecus lhoesti[a]	**L'Hoest's monkey**	**KJ434957**	**This study**
Cercopithecus mitis[a]	**Blue monkey**	**KJ434956**	**This study**
Chlorocebus aethiops	Grivet	NC_007009	Raaum et al., 2005
Chlorocebus pygerythrus	Vervet monkey	NC_009747	Wertheim & Worobey, 2007
Chlorocebus sabaeus	Green monkey	EF597503	Wertheim & Worobey, 2007
Chlorocebus tantalus	Tantalus monkey	NC_009748	Wertheim & Worobey, 2007
Erythrocebus patas[a]	**Patas monkey**	**KJ434955**	**This study**
Lophocebus aterrimus[a]	**Black crested mangabey**	**KJ434960**	**This study**
Macaca sylvanus	Barbary ape	NC 002764	Arnason et al., 2000
Macaca mulatta	Rhesus monkey	NC_005943	Gokey et al., 2004
Macaca fascicularis	Crab-eating macaque	NC_012670	Yi et al., unpub.
Macaca thibetana	Pere David's macaque	NC_011519	Li et al., 2009
Mandrillus sphinx[a]	**Mandrill**	**KJ434963**	**This study**
Papio hamadryas	Hamadryas baboon	NC_001992	Arnason et al., 1998
Theropithecus gelada	Gelada	FJ785426	Hodgson et al., 2009
Hominoidea			
Gorilla gorilla	Western gorilla	NC_001645	Horai et al., 1995
Homo neanderthalensis	Neanderthal	AM948965	Green et al., 2008
Homo sapiens	Human	EF061150	Friedlaender et al., 2007
Homo sp. (Denisovan)	Denisova hominin	FR695060	Krause et al., 2010
Hylobates agilis	Agile gibbon	NC_014042	Matsudaira & Ishida, 2010
Hylobates lar	White-handed gibbon	NC_002082	Arnason et al., 1996

Scientific name	Common name	NCBI Accession No	Reference
Hylobates pileatus	Pileated gibbon	NC_014045	Matsudaira & Ishida, 2010
Nomascus siki	Southern white-cheeked gibbon	NC_014051	Matsudaira & Ishida, 2010
Pan paniscus	Pygmy chimpanzee	D38116	Horai et al., 1995
Pan troglodytes	Common chimpanzee	EU095335	Flynn & Carr, 2007
Pongo abelii	Sumatran orangutan	X97707	Xu & Arnason, 1996
Pongo pygmaeus	Bornean orangutan	NC_001646	Horai et al., 1995
Symphalangus syndactylus	Siamang	NC_014047	Matsudaira & Ishida, 2010
Non-primate mammals			
Euarchontoglires			
Dermoptera			
Galeopterus variegatus	Sunda flying lemur	NC_004031	Arnason et al., 2002
Galeopterus variegatus	Sunda flying lemur	AF460846	Schmitz et al., 2002a
Rodentia			
Cavia porcellus	Guinea pig	AJ222767	D'Erchia et al., 1996
Cricetulus griseus	Chinese hamster	NC_007936	Partridge et al., 2007
Glis glis	Fat dormouse	NC_001892	Reyes et al., 1998
Mus musculus	Mouse	NC_005089	Bayona-Bafaluy et al., 2003
Rattus norvegicus	Brown rat	AJ428514	Nilsson et al., 2003
Sciurus vulgaris	Squirrel	AJ238588	Reyes et al., 2000
Lagomorpha			
Lepus europaeus	European hare	AJ421471	Arnason et al., 2002
Ochotona collaris	Collared pika	NC_003033	Lin et al., 2002
Oryctolagus cuniculus	Rabbit	NC_001913	Gissi et al., 1998
Laurasiatheria			
Carnivora			
Canis familiaris	Dog	NC_002008	Kim et al., 1998
Felis catus	Domestic cat	U20753	Lopez et al., 1996
Perissodactyla			
Equus caballus	Horse	NC_001640	Xu & Arnason, 1994
Rhinoceros unicornis	Greater Indian rhinoceros	NC_001779	Xu et al., 1996
Cetartiodactyla			
Balaenoptera musculus	Blue whale	NC_001601	Arnason et al., 1993
Balaenoptera physalus	Finback whale	X61145	Arnason et al., 1991
Bos taurus	Cow	NC_006853	Chung,and Ha, unpub.
Hippopotamus amphibius	Hippopotamus	AJ010957	Ursing & Arnason, 1998b
Lama pacos	Alpaca	NC_002504	Ursing et al., 2000
Ovis aries	Sheep	AF010406	Hiendleder et al., 1998
Physeter catodon	Sperm whale	NC_002503	Arnason et al., 2000
Sus scrofa	Pig	AJ002189	Ursing & Arnason, 1998a
Afrotheria			
Proboscidea			
Elephas maximus	Asiatic elephant	NC_005129	Rogaev et al., 2006

Scientific name	Common name	NCBI Accession No	Reference
Loxodonta africana	African elephant	NC_000934	Hauf et al., 2000

[a] in bold new sequences generated for this study

Table 2

Sequence characteristics and models chosen for each data partition

Partition	No. sites	All taxa		Only primates		Model
		VS	PIS	VS	PIS	
All genes	14043	9080	8244	8396	7532	GTR+I+G
12S rRNA	691	349	308	286	244	GTR+I+G
16S rRNA	1017	489	389	419	313	GTR+I+G
all tRNAs	1313	777	643	652	514	GTR+I+G
All protein-coding genes	11022	7465	6904	7039	6461	GTR+I+G
pos1	3674	2277	2033	2092	1827	GTR+I+G
pos2	3674	1568	1270	1341	1075	GTR+I+G
pos1+2	7348	3845	3303	3433	2902	GTR+I+G
pos3	3674	3620	3601	3606	3559	GTR+I+G
Individual protein-coding genes						
ATP6	*681*	*499*	*465*	*477*	*434*	*GTR+I+G*
pos1	227	163	151	156	134	GTR+I+G
pos2	227	112	92	98	80	GTR+I+G
pos3	227	224	222	223	220	GTR+I+G
ATP8	*225*	*198*	*190*	*193*	*176*	*GTR+G*
pos1	75	64	59	62	55	GTR+G
pos2	75	64	62	61	54	GTR+G
pos3	75	70	69	70	67	GTR+G
COI	*1569*	*807*	*727*	*752*	*694*	*GTR+I+G*
pos1	523	198	153	164	135	GTR+I+G
pos2	523	96	62	76	50	GTR+I+G
pos3	523	513	512	512	509	GTR+I+G
COII	*696*	*443*	*408*	*417*	*385*	*GTR+I+G*
pos1	232	134	121	124	111	GTR+I+G
pos2	232	81	60	66	49	HKY+I+G
pos3	232	228	227	227	225	GTR+I+G
COIII	*804*	*463*	*426*	*443*	*406*	*GTR+I+G*

Partition	No. sites	All taxa		Only primates		Model
		VS	PIS	VS	PIS	
pos1	268	131	110	122	101	GTR+I+G
pos2	268	72	58	62	50	GTR+I+G
pos3	268	260	258	259	255	GTR+I+G
CYTB	*1143*	*747*	*961*	*714*	*648*	*GTR+I+G*
pos1	381	221	200	209	182	GTR+I+G
pos2	381	147	114	126	102	HKY+I+G
pos3	381	379	377	379	364	GTR+I+G
NADH1	*963*	*640*	*599*	*601*	*556*	*GTR+I+G*
pos1	321	203	180	176	160	GTR+I+G
pos2	321	118	101	106	81	GTR+I+G
pos3	321	319	318	319	315	GTR+G
NADH2	*1050*	*848*	*478*	*788*	*729*	*GTR+I+G*
pos1	350	274	252	258	230	GTR+I+G
pos2	350	226	192	185	159	GTR+I+G
pos3	350	348	34	345	340	GTR+G
NADH3	*360*	*248*	*231*	*234*	*219*	*GTR+I+G*
pos1	120	78	71	69	64	GTR+I+G
pos2	120	54	45	50	42	GTR+I+G
pos3	120	116	115	115	113	GTR+G
NADH4	*1386*	*1002*	*911*	*951*	*864*	*GTR+I+G*
pos1	462	319	287	297	258	GTR+I+G
pos2	462	225	179	197	157	GTR+I+G
pos3	462	458	445	457	449	GTR+I+G
NADH4L	*297*	*222*	*205*	*204*	*186*	*GTR+I+G*
pos1	99	74	68	68	60	GTR+I+G
pos2	99	51	40	40	30	GTR+G
pos3	99	97	97	96	96	GTR+I+G
NADH5	*1848*	*1348*	*1253*	*1265*	*1154*	*GTR+I+G*
pos1	616	418	381	387	337	GTR+I+G
pos2	616	322	265	274	221	GTR+I+G

Partition	No. sites	All taxa		Only primates		Model
		VS	PIS	VS	PIS	
pos3	616	608	607	604	596	GTR+I+G

Note: VS = variable sites; PIS = parsimony-informative sites.

Table 3

Summary of model partition comparison (Maximum Likelihood and Bayesian analyses)

Model	Maximum Likelihood	Bayesian				
		Run	Generations	Burn-in	Marginal Likelihood	SD
MT0	−421145.742	1	20,000,000	2,000,000	−417245.466	0.239
		2	20,000,000	2,000,000	−417245.834	0.287
MT4	−420115.955	1	20,000,000	2,000,000	−415805.630	0.329
		2	20,000,000	2,000,000	−415804.224	0.280
MT5	−409906.041	1	20,000,000	2,000,000	−409740.282	0.295
		2	20,000,000	2,000,000	−409740.561	0.329
MT6	−408934.350	1	20,000,000	2,000,000	−408453.105	0.300
		2	20,000,000	2,000,000	−408453.716	0.297
MT15	−419192.485	1	30,000,000	3,000,000	−414803.673	0.311
		2	30,000,000	3,000,000	−414814.001	0.381
MT39	−407068.508	1	40,000,000	4,000,000	−402921.612	0.318
		2	40,000,000	4,000,000	−402921.590	0.364

Table 4

Evolutionary rate calibration constraints (in millions of years).

Divergence	Min.	Max.	Fossil	Reference	Age
Primate fossils					
1. *Homo*	5.0	10.0	*Ardipithecus*	Haile-Selassie et al., 2001	5.2
			Orrorin	Senut et al., 2001	6.0
			Sahelanthropus	Vignaud et al., 2002; Brunet et al., 2002	6.0–7.0
2. *Gorilla*	10.0	NA	*Chororapithecus abyssinicus*	Suwa et al., 2007	≈10.0
3. *Pongo*	12.5	18.0	*Sivapithecus*	Kelley, 2002	≈12.5
4. Crown Catarrhini	21.0	33.9	*Morotopithecus*	Gebo et al., 1997	>20.6
			Victoriapithecus	Pilbeam and Walker, 1968 Benefit and McCrossin, 2002	≈19.0
5. *Theropithecus*	3.5	6.5	*Theropithecus*	Leakey, 1993 Frost, 2007	≈3.5
6. *Macaca*	5.5	NA	*Macaca libyca*	Delson 1980 Jablonski, 2002	≈5.5
7. *Colobinae*	10	NA	*Microcolobus tugenensis*	Benefit and Pickford, 1986; Jablonski, 2002	9.0–11.0
8. *Aotus*	12.1	NA	*Aotus dindensis*	Setoguchi and Rosenberger, 1987	≈12.1
9. *Saimiri*	12.1	NA	*Neosaimiri*	Hartwig and Meldrum, 2002	≈12.1
10. Crown Anthropoidea	31.5	NA	Fayum catarrhines	Rasmussen, 2002; Seiffert, 2005	31.5
11. *Lorisoidea*	36.9	NA	*Saharagalago* *Karanisia*	Seiffert et al., 2003 Seiffert, 2007	≈36.9
Non-primate fossils					
12. Cetartyodactyla	48.6	NA	*Himalayacetus*	Bajpai And Gingerich, 1998	53.5
			Pakicetus	Gingerich and Russel 1981 Thewissen at al, 2001	>48.6
13. Mysticeti-Odontoceti	33.5	53.5	*Llanocetus denticrenatus*	Mitchell, 1989	≈3.5
14. Feliniforma-Caniformia	43.0	63.8	*Tapocyon*	Benton and Donoghue, 2007	43.0–46.0
15. *Bovidae*	18.3	28.55	*Eotragus noyi*	Solounias et al., 1995 Benton and Donoghue, 2007	≈18.3
16. *Mus/Rattus*	7.3	16.0	*Progonomys hussaini*	Jacobs & Downs, 1994 Benton and Donoghue, 2007	11.1–11.5
17. *Equus/Rhinoceros*	54.0	58.0	*Lambdotherium*	Rose, 2006	>50.3

* see Supplementary Material B1 for the detailed procedure of fossil selection, with paleontological details, and assignment to alternative nodes for calibration of the molecular.

Table 5

Cross-validation deviance of candidate calibration points.(Dx: average mean deviation between molecular and fossil age estimates for all available nodes based on the fossil calibration at node x; SS: sum of the squared differences between the molecular and fossil age estimates at all other fossil-dated nodes; ΣD: sum of the absolute values of differences between the estimated and proposed ages for each calibration node)

CP	divergence	age	node																D_x	SS	ΣD
			C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17			
C1	*Homo*	**5.0**	-	7.0	11.9	21.1	4.5	8.1	14.5	10.0	9.6	28.1	21.7	25.7	11.8	27.1	13.7	11.1	27.5	−5.60	2277.0	130.5
C2	*Gorilla*	**10.0**	7.2	-	17.0	30.4	6.5	11.6	20.9	14.4	13.8	40.6	31.4	37.1	17.0	39.2	19.8	16.0	39.7	−0.93	1099.6	110.94
C3	*Pongo*	**12.5**	5.4	7.5	-	21.9	4.8	8.5	15.2	10.4	10.0	29.0	22.3	26.2	11.9	27.7	13.9	11.3	28.1	−6.38	2181.2	127.77
C4	Crown Catarrhini	**21.0**	5.3	7.4	12.2	-	4.6	8.1	14.6	10.0	9.6	27.7	21.3	25.1	11.4	26.5	13.3	10.8	26.9	−5.21	2387.5	133.29
C5	*Theropithecus*	**3.5**	4.2	5.8	9.7	16.8	-	6.3	11.5	8.0	7.7	22.3	17.2	20.4	9.3	21.5	10.9	8.8	21.8	−9.75	3489.7	166.78
C6	*Macaca*	**5.5**	3.7	5.1	8.5	15.0	3.1	-	10.2	7.1	6.8	20.1	15.6	18.5	8.4	19.5	9.8	8.0	19.8	−11.56	4051.0	182.05
C7	*Colobinae*	**10.0**	3.6	5.0	8.4	14.5	3.1	5.6	-	6.9	6.6	19.2	14.8	17.5	7.9	18.5	9.3	7.5	18.7	−11.32	4336.2	188.3
C8	*Aotus*	**12.1**	6.6	9.2	15.2	26.3	5.7	10.2	18.3	-	11.7	35.0	27.1	32.2	14.8	33.9	17.2	13.9	34.4	−2.71	1373.5	110.84
C9	*Saimiri*	**12.1**	6.9	9.6	15.9	27.4	5.9	10.6	19.0	12.6	-	36.2	28.1	33.1	15.1	34.9	17.6	14.2	35.4	−1.88	1306.1	110.76
C10	Crown Anthropoidea	**31.5**	6.0	8.4	13.9	23.9	5.2	9.3	16.7	11.3	10.9	-	24.3	28.9	13.2	30.4	15.3	12.5	30.8	−4.92	1772.1	117.83
C11	*Lorisoidea*	**36.9**	9.6	13.4	22.1	37.9	8.3	14.8	26.5	18.0	17.4	49.7	-	44.8	20.5	47.3	23.8	19.3	48.1	4.81	1599.1	139.06
C12	Cetartyodactyla	**48.6**	10.8	15.0	24.7	42.4	9.3	16.5	29.7	20.3	19.5	55.4	42.2	-	22.1	52.0	25.7	21.4	52.7	8.05	2393.3	168.76
C13	Mysticeti-Odontoceti	**33.5**	15.5	21.5	35.6	61.5	13.4	23.8	42.7	29.2	28.1	81.1	62.3	73.5	-	77.6	39.1	31.9	78.7	23.52	11026.8	383.95
C14	*Felis/Catus*	**43.0**	8.8	12.3	20.3	35.0	7.6	13.6	24.4	16.7	16.0	45.9	35.1	41.1	18.8	-	21.9	17.9	43.8	8.06	1313.0	125.72
C15	*Ovis/Bos*	**18.3**	7.3	10.2	16.8	29.0	6.3	11.2	20.2	13.8	13.3	38.2	29.3	34.4	15.7	36.3	-	14.8	36.8	−0.58	1251.1	113.95
C16	*Mus/Rattus*	**7.3**	4.0	5.6	9.3	16.0	3.5	6.2	11.1	7.6	7.3	21.0	16.1	19.1	8.8	20.2	10.2	-	20.5	−10.29	3836.8	174.83
C17	*Equus/Rhinoceros*	**54.0**	11.1	15.4	25.4	43.6	9.6	17.0	30.5	20.8	20.0	57.1	43.5	51.1	23.4	53.5	27.2	22.3	-	9.21	2694.5	180.89

Table 6

Comparison of divergence time estimates (posterior mean and 95% credible interval in in millions of years) between this study and recent studies on primates.

Divergence (node)	This study	Arnason et al., 2008[c, d]	Matsui et al., 2009[d]	Fabre et al., 2009[e]	Chatterjee et al., 2009 [e]	Perelman et al., 2011[f]	Wilkinson et al., 2011[f]	Jameson et al., 2011[f]	Springer et al., 2012[e]	Finstermeier et al., 2013[d]
Crown Primates (61)	**74.1 (68.2–81.2)**	80.0	76.0 (69.3–82.5)	78.8 (69.9–88.4)	63.7 (58.3–68.7)	87.2 (75.9–98.6)	84.5 (69.2–103.5)	72.6 (69.6–76.9)	67.8 (61.0–76.7)	66.2 (59.6–73.6)
Crown Strepsirrhini (60)	**66.3 (61.1–72.8)**	70.0	64.5 (57.2–71.7)	67.1 (60.2–74.5)	51.6 (47.7–55.7)	68.7 (58.8–76.6)	49.8 (35.9–72.0)	52.4 (47.0–57.2)	54.2 (48.8–57.2)	56.9 (50.5–64.1)
Crown Lorisiformes (59)	**40.3[a] (37.1–46.3)**	N/A	35.4 (28.5–43.1)	39.5 (38.0–41.8)	37.5 (36.9–38.7)	40.3 (35.2–45.6)	N/A	N/A	34.7 (27.9–38.2)	34.5 (30.2–39.0)
Lemuriformes-Chiromyiformes (55)	**59.6 (54.3–65.9)**	64.0	55.3 (47.7–63.0)	59.6 (53.3–66.7)	46.2 (41.3–50.8)	58.6 (38.6–76.8)	N/A	N/A	50.0 (45.2–53.8)	47.1 (40.9–53.6)
Crown Lemuriformes (54)	**43.5 (37.5–50.1)**	N/A	N/A	47.3 (37.9–50.8)	32.4 (28.6–33.6)	38.6 (26.4–50.0)	34.4 (23.5–49.1)	N/A	31.8 (26.7–36.6)	35.5 (30.4–40.4)
Crown Haplorrhini (46)	**70.0 (64.3–76.7)**	N/A	70.1 (63.2–76.7)	N/A	N/A	81.3 (69.5–95.8)	N/A	68.6 (65.6–72.7)	61.2 (57.6–69.6)	63.1 (56.0–69.7)
Crown Anthropoidea (44)	**46.7[a] (42.4–50.8)**	55.0	45.3 (39.4–51.3)	37.7 (33.3–42.7)	42.8 (40.1–45.0)	43.5 (38.6–48.4)	47.2 (38.9–56.5)	40.0 (37.3–43.1)	40.6 (33.6–49.5)	45.3 (40.7–50.1)
Crown Platirrhini (43)	**20.9 (17.9–24.4)**	N/A	N/A	14.5 (9.7–19.9)	26.6 (23.5–30.0)	24.8 (20.6–29.3)	25.1 (20.1–31.0)	N/A	23.3 (19.2–27.5)	22.0 (19.2–24.4)
Crown Catarrhini (38)	**32.1[b] (29.4–33.8)**	39.0	30.5 (28.8–35.3)	23.9 (23.1–25.9)	29.3 (28.0–30.0)	31.6 (25.7–37.9)	31.0 (25.1–37.7)	25.4 (23.7–27.6)	25.1 (19.7–32.8)	31.9 (28.3–35.7)
Crown Cercopithecoidea (37)	**20.8[a] (18.6–22.9)**	N/A	N/A	13.3 (11.6–14.7)	23.4 (21.9–24.9)	17.6 (13.9–21.5)	14.1 (11.0–17.7)	N/A	13.2 (8.9–18.3)	22.8 (20.0–25.6)
Crown Colobinae (36)	**14.1 (12.0–16.3)**	N/A	N/A	8.7 (7.3–10.4)	18.4 (16.8–20.1)	12.3 (9.4–15.1)	N/A	N/A	8.9 (6.3–12.0)	15.5 (13.4–17.8)
Crown Cercopithecinae (29)	**14.1 (12.2–15.8)**	N/A	N/A	9.1 (7.7–10.0)	18.6 (16.5–20.5)	11.5 (9.2–13.9)	10.3 (8.1–13.0)	N/A	8.4 (5.4–11.6)	14.9 (12.9–16.8)
Crown Hominoidea (12)	**22.3 (20.5–23.9)**	N/A	19.9 (16.7–23.0)	18.6 (17.1–20.5)	21.5 (18.9–24.3)	20.3 (16.6–24.2)	19.2 (15.1–24.1)	N/A	17.4 (12.4–23.9)	20.3 (17.4–23.5)

[a] Lower bound defined as calibration point (see Table 4)

[b] Lower and upper bound defined as calibration point (see Table 4)

[c] Confidence intervals not provided

[d] The study used entire mitochondrial genomes

[e] The study used a supermatrix approach (mitochondrial and nuclear genes together)

[f] The study used multiple nuclear loci (Perelman et al.: 54 loci ~35kb - Wilkinson et al.: 2 genomic region ~83kb – Jameson et al.: 1078 genes ~1.26 Mbp)