

## Supplementary Information

### Bayesian estimation of divergence times

The objective of the Bayesian analysis is the estimation of the posterior probability,  $f(\mathbf{t}, \mathbf{r}, \theta | D)$ , of divergence times  $\mathbf{t}$ , molecular evolutionary rates  $\mathbf{r}$ , and model parameters  $\theta$ , given the molecular data  $D$ . This posterior distribution is given by the Bayes theorem[1]

$$f(\mathbf{t}, \mathbf{r}, \theta | D) \propto f(\theta) f(\mathbf{t}) f(\mathbf{r} | \mathbf{t}, \theta) f(D | \mathbf{t}, \mathbf{r}, \theta) \quad (1)$$

where  $f(\theta)$  is the prior of model parameters;  $f(\mathbf{t})$  is the prior of times;  $f(\mathbf{r} | \mathbf{t}, \theta)$  is the prior of rates on the tree; and  $f(D | \mathbf{t}, \mathbf{r}, \theta)$  is the likelihood, or the probability of the molecular data given the times, rates, and model parameters. The posterior is generated by MCMC sampling.

In this study we faced two important challenges in using eq. (1). The first challenge was constructing the time prior,

$$f(\mathbf{t}) = f(\mathbf{t}_C) f_{BD}(\mathbf{t}_C | \mathbf{t}_C), \quad (2)$$

which has two components: the density of ages for nodes in the tree for which fossil calibrations are available,  $f(\mathbf{t}_C)$ ; and the conditional density, based on the birth and death (BD) process, for those nodes where no fossil calibrations are available,  $f_{BD}(\mathbf{t}_C | \mathbf{t}_C)$ . The time prior must comply with the requirement that any node cannot be older than its ancestral nodes. During the MCMC iteration, node ages that violate this requirement are never proposed. Effectively  $f(\mathbf{t}_C)$  is truncated, and the effective prior density used by the program may be very different from the user specified calibration density (e.g. node 40 in Supplementary Fig. 2). For this reason it is always necessary to examine the time prior, which can be easily generated by MCMC iteration without sequence data (that is, by setting  $f(D | \mathbf{t}, \mathbf{r}, \theta) = 1$  in eq. 1).

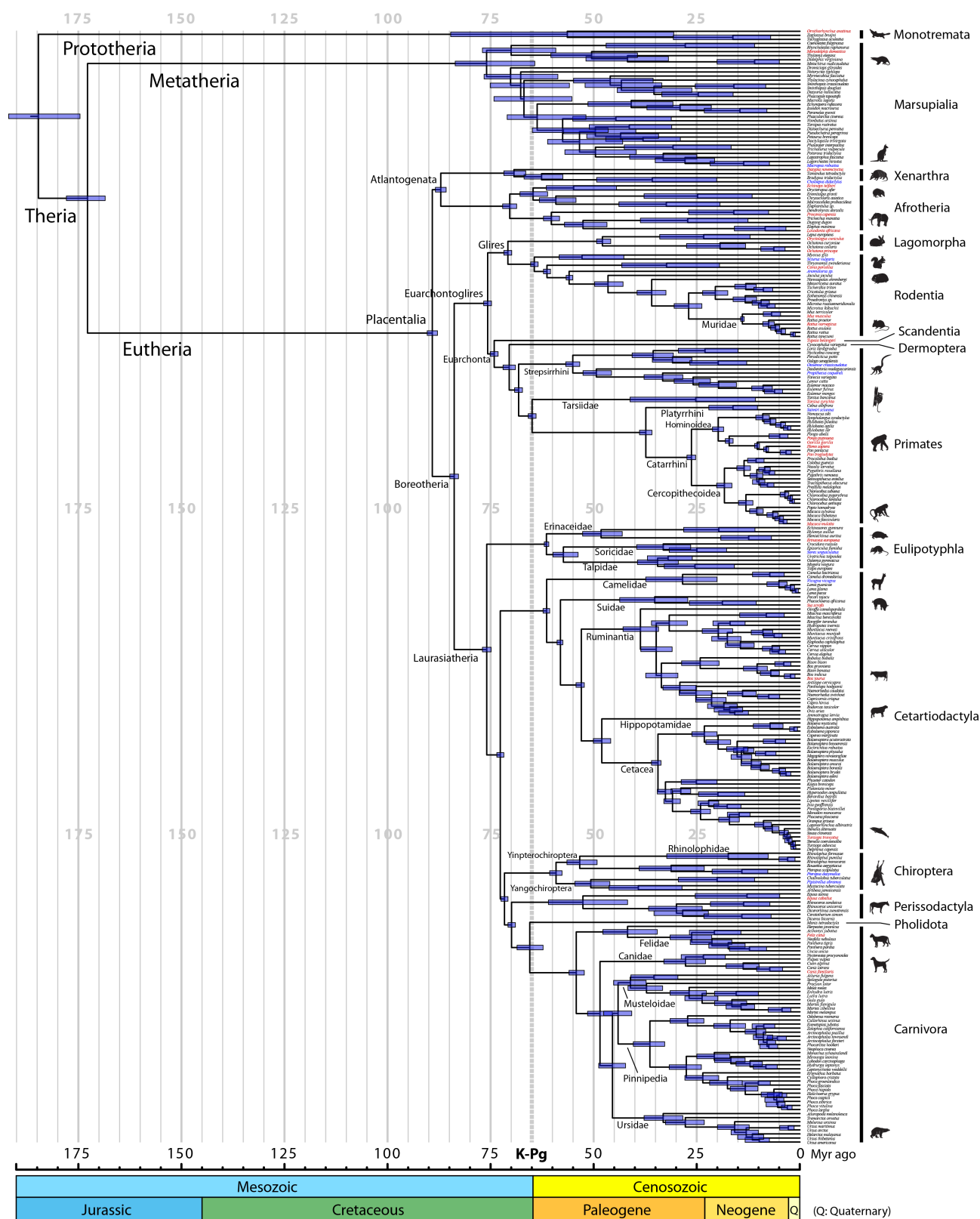
We stress the importance of reporting the time prior in divergence time studies. For some data sets the truncation effect may be quite substantial[2]. Furthermore, not all Bayesian divergence time programs construct the time prior in the same manner, and reporting the time prior is essential for valid comparisons among different studies. In the program Beast[3], the time prior is generated not by a conditional construction as in eq. (2), but instead by a multiplicative construction that does not respect the rules of probability calculus[4]. The resulting effective prior may be in conflict with the user-specified fossil calibrations.

The second challenge was the likelihood function, which has to be calculated many times during the MCMC iteration. In this study we used an approximate method to calculate the likelihood[5]. This method saves computation time, and its accuracy has been extensively tested[6, 7]. However, the large number of partitions and the large number of species in this study still made the analysis computationally demanding. A typical MCMC run for the 14K data set and 20 partitions took ~20 days, and a typical MCMC run on the 274 species tree took 8 days. All the analyses of this study took over 874 processor days. The exact likelihood method, as is used in Beast[3], takes > 100 times more computation and would not have been feasible[7].

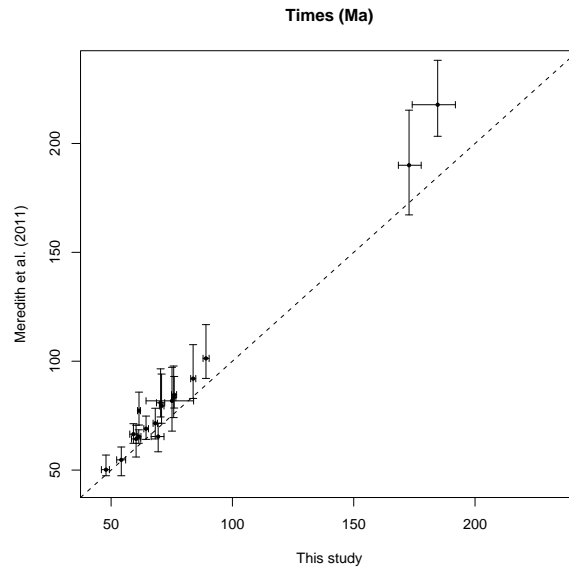
**Supplementary Table 1 | Fossil calibrations used in this study.**

	Node	Minimum soft bound	Maximum soft bound
37	Root	162.9 † <i>Amphitherium</i> (Theriimorpha)[8]	191.1 absence of theriimorphs from early Jurassic[8]
38	Theria	124 † <i>Eomaia</i> (Eutheria)[9]	171.2 absence of therians in mid-Jurassic[8]
39	Marsupialia	48.6 † <i>Djarthia</i> (Australidelphia)[10]	-
40	Placentalia	—	131.5 † <i>Eomaia</i> (Eutherian) and † <i>Sinodelphys</i> (Metatheria)[8]
43	Paenungulata	55.6 † <i>Eritherium</i> (Proboscidea)[11]	-
44	Xenarthra	55.6 † <i>Riostegotherium</i> [12]	-
47	Eulipotyphla	61.5 † <i>Adunator</i> (Erinaceomorpha)[13]	-
49	Cetartiodactyla	—	65.8 absence of crown artiodactyls in Paleocene[14]
	Cetacea	33.8 † <i>Llanocetus</i> (stem mysticete, calibration applied to the 274 species tree only)[15]	-
51	Dolphin/Cow	52.4 † <i>Himalayacetus</i> (Cetacea)[14]	-
53	Horse/ cat	62.5 † <i>Lambdotherium</i> (Perissodactyla)[16]	-
54	Carnivora	39.68 † <i>Daphoenus</i> (Canifomia, Carnivora)[17]	65.8 absence of crown (but not stem, e.g., † <i>Protictis</i> ) carnivorans in early Paleocene[17]
55	Chiroptera	48.6 † <i>Icaronycteris</i> (Chiroptera)[18]	-
57	Glires	61.5 † <i>Heomys</i> (Rodentia)[19]	-
58	Lagomorpha	48.6 Vastan calcanei (Leporidae, Lagomorpha)[20]	65.8 absence of crown lagomorphs in early Paleocene[21]
59	Rodentia	55.6 † <i>Sciuravus</i> (nested within crown Rodentia)[22]	65.8 absence of crown rodents in early Paleocene[21]
60	Guinea pig/ rat	52.5 † <i>Birbalomys</i> (stem Hystricognathi)[23]	58.9 absence of caviomorphs in late Paleocene[23]
61	Kangaroo rat/ rat	40.2 † <i>Ulkenulastomys</i> (Muridae)[24]	56.0 absence of crown rodents in Paleocene[25]
62	Muridae	10.4 † <i>Karnimata</i> (linage leading to <i>Rattus</i> )[26]	14.0 absence of crown murines in early Miocene[26]
63	Euarchonta	61.5 carpolestids and plesiadapids (extinct primate sister taxa)[27]	-
64	Primates	55.6 † <i>Altiasius</i> (Euprimates)[28]	-
65	Strepsirrhini	33.7 † <i>Karanisia</i> (Lorisiformes)[29]	55.6 absence of strepsirrhines in Paleocene[29]
67	Anthropoidea	33.7 † <i>Catopithecus</i> (Catarrhini)[28]	-
68	Catarrhini	23.5 † <i>Proconsul</i> (Hominoidea)[30]	34.0 absence of hominoids in late Eocene[28]
69	Hominidae	11.2 † <i>Sivapithecus</i> (Ponginae)[31]	33.7 absence of pongines in late Eocene[28]
70	Homininae	7.25 † <i>Chororapithecus</i> (stem-gorilla)[32]	-
71	Hominini	5.7 † <i>Orrorin</i> (Hominini)[33]	10 absence of hominines in mid-Miocene[34]

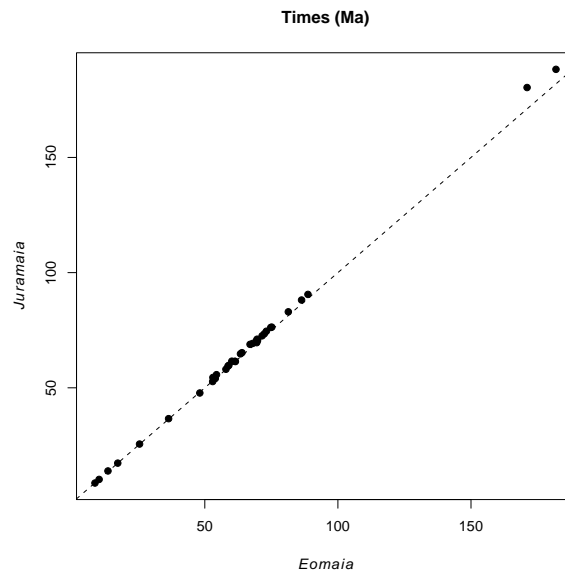
**Note on fossil calibrations:** Choice of fossil calibrations followed Benton et al.[8], to which readers are referred for a more exhaustive discussion of fossil constraints in molecular clock studies. We added a soft minimum for Chiroptera based on early Eocene microchiropterans[18]. Also, contra Benton et al.[8], the oldest stem hystricognaths are early Eocene chapattimyids from Pakistan (e.g., *Birbalomys*[23]). Our calibrations were never used as hard constraints but served as the basis for probability distributions around which clock estimates were made. We assigned a minimum constraint based on reasonable consensus on the oldest occurrence of a member of a specific crown clade, as noted in the literature for each calibration in Supplementary Table 1. Maximum constraints were defined by the youngest occurrence of a well sampled interval which lacks the crown clade in question but yields members of one or more related but less-nested clades. For example, the late Eocene deposits of the Fayum lack any evidence of hominoids (i.e., catarrhines more closely related to apes than monkeys), despite a diverse record of anthropoid and some strepsirrhine primates[28, 29]. For further discussion see Benton et al.[8]



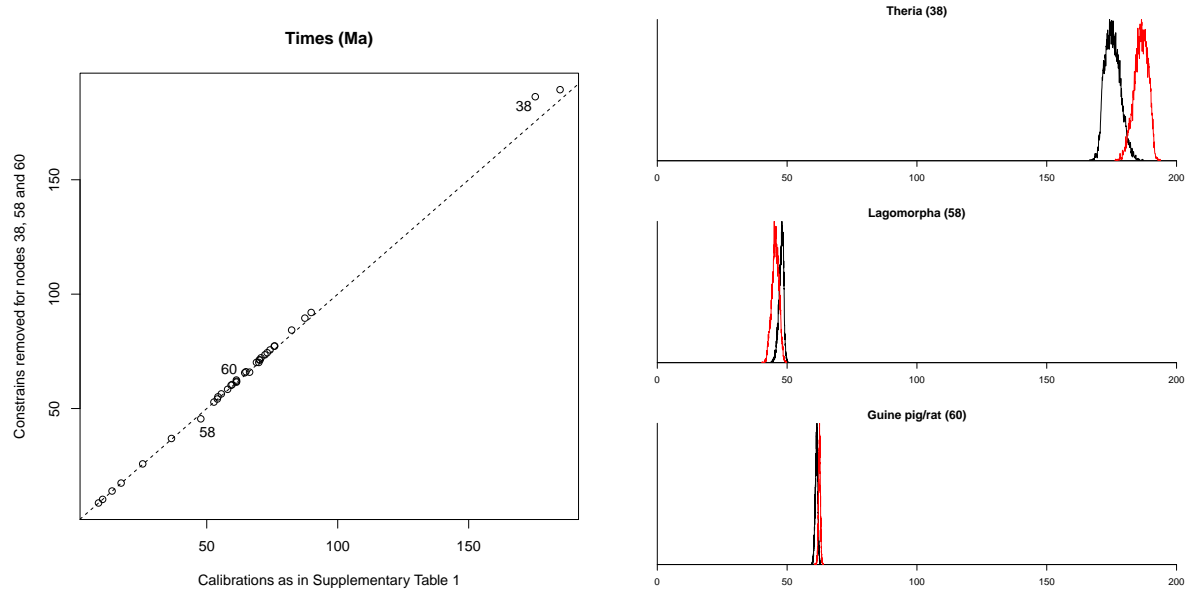
**Supplementary Figure 1. The timetree of 274 mammal species.** Blue bars represent the posterior 95% CI of node ages. Species for which nuclear genome data was analysed are shown in red. Species with nuclear genome data but without sequenced mitochondrial genomes were substituted by closely related species in the 274-species tree (indicated in blue). The tree was estimated by maximum likelihood using a guide tree incorporating well supported clades[35].



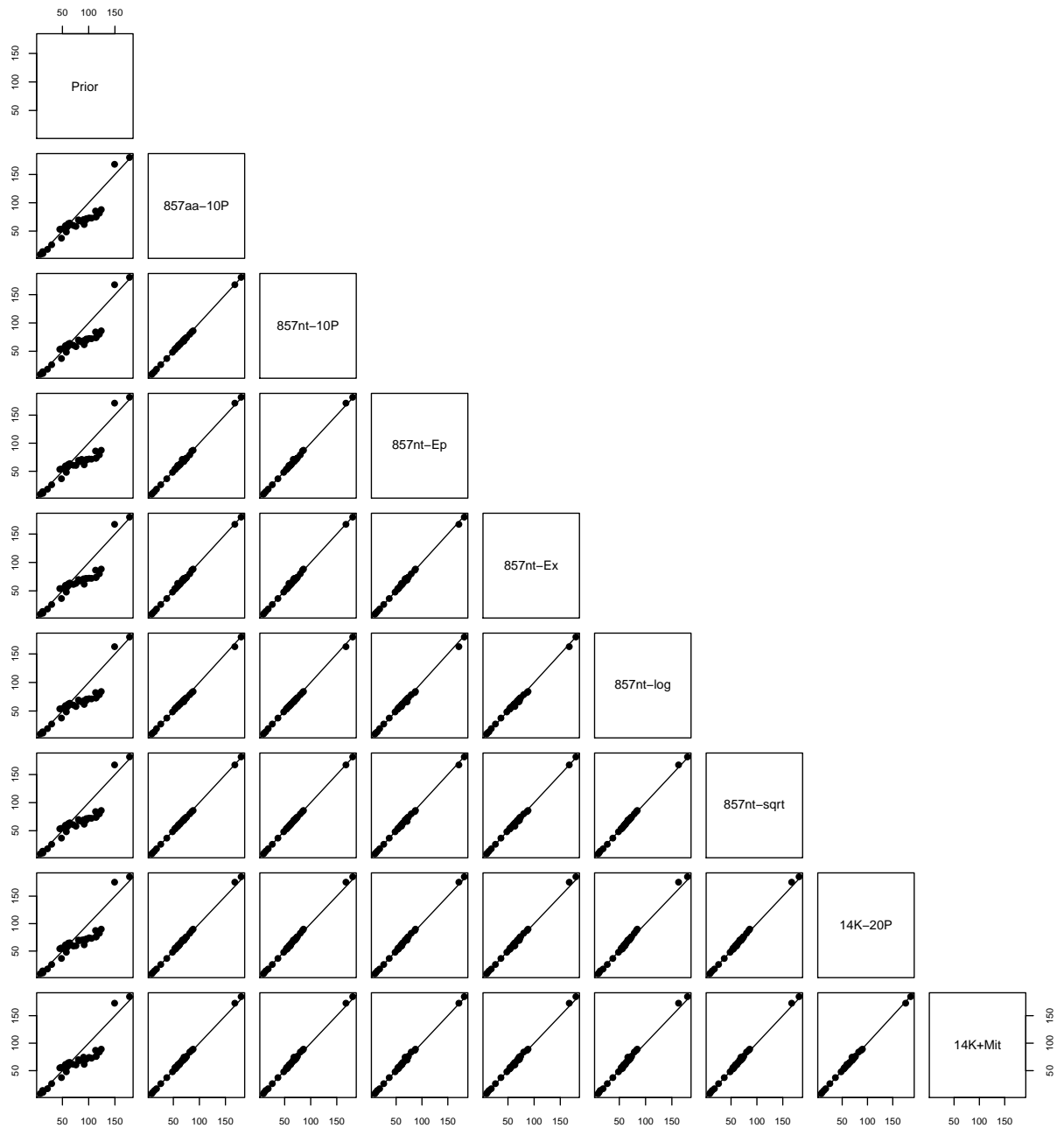
**Supplementary Figure 2. Estimated divergence times in this study vs. the study of Meredith et al.[36]** Vertical bars show the 95% CI in the study of Meredith et al.[36], and horizontal bars show the 95% CI in this study. Note the large uncertainty in Meredith et al.'s estimates.



**Supplementary Figure 3. Estimated divergence times using †*Eomaia* vs. †*Juramaia* calibrations.** Divergence times were re-estimated on the 36 species tree (Atlantogenata topology) using the recently discovered †*Juramaia* fossil[37]. That is, the minimum calibration for node 38 was set to 149 Ma (Supplementary Table 1). The tail probability for the maximum bounds for nodes 37 and 38 was set to 0.1 (instead of the usual 0.025) to assess the effect of a more ancient root on estimated times. The rest of the calibrations were the same as in Supplementary Table 1.



**Supplementary Figure 4. Effect of conflicting fossil calibrations.** Divergence times are estimated on the 36 species tree (Atlantogenata topology). A single analysis was carried out where the maximum age constraints of nodes 38 (Theria), 60 (Guinea pig/rat) and the joint constraints of node 58 (Lagomorpha) were removed because the original calibrations were in conflict with posterior time estimates (Fig. 3). Left panel: scatter plot of mean posterior ages calculated before ( $x$  axis) and after ( $y$  axis) removing the four constraints. Right panel: posterior time densities before (black lines) and after (red lines) removing the four constraints. After removing the constraints, the posterior age of node 38 became substantially older at 186 Ma (181-191), the posterior age of node 60 had little change at 62.5 Ma (61.7-63.2), and the posterior age of node 58 became slightly younger at 45.6 Ma (42.9-48.2). The posterior ages for the remaining nodes were marginally affected by the modified calibrations.



**Supplementary Figure 5. Robustness of mean posterior time estimates.** The molecular data are very informative and estimated times (in Myr) are very similar under various analysis conditions. 857aa-10P: 857 nuclear genes, amino acid substitution model, 10 partitions; 857nt-10P: 857 genes, nucleotide substitution model, 10 partitions; 857nt-Ep: nucleotide analysis, 10 partitions, with Epitheria placental topology; 857nt-Ex: nucleotide analysis, 10 partitions, with Exafroplacentalia placental topology; 857nt-log: 10 partitions generated by PCA of log-transformed branch lengths and k-means clustering of genes (see methods); 857nt-sqrt: 10 partitions generated by PCA of square root-transformed branch lengths and k-means clustering; 14K-20P: 14,632 nuclear genes, 20 partitions; and 14K+Mit: 12 mitochondrial proteins for 274 species, with prior constructed from the 14K-20P posterior times. Note that mean prior times are substantially older than mean posterior times for nodes dated 50 – 100 Ma.

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