

RESEARCH ARTICLE SUMMARY

ZONOMIA

A genomic timescale for placental mammal evolution

Nicole M. Foley et al.

INTRODUCTION: Resolving the role that different environmental forces may have played in the apparent explosive diversification of modern placental mammals is crucial to understanding the evolutionary context of their living and extinct morphological and genomic diversity.

RATIONALE: Limited access to whole-genome sequence alignments that sample living mammalian biodiversity has hampered phylogenomic inference, which until now has been limited to relatively small, highly constrained sequence matrices often representing <2% of a typical mammalian genome. To eliminate this sampling bias, we used an alignment of 241 whole genomes to comprehensively identify and rigorously analyze noncoding, neutrally evolving sequence variation in coalescent and concatenation-based phylogenetic frameworks. These analyses were followed by validation with multiple classes of phylogenetically informative structural variation. This approach enabled the generation of a robust time tree for placental mammals that evaluated age variation across hundreds of genomic loci that are not restricted by protein coding annotations.

RESULTS: Coalescent and concatenation phylogenies inferred from multiple treatments of the

data were highly congruent, including support for higher-level taxonomic groupings that unite primates+colugos with treeshrews (Euarchonta), bats+cetartiodactyls+perissodactyls+carnivorans+pangolins (Scrotifera), all scrotiferans excluding bats (Fereungulata), and carnivorans+pangolins with perissodactyls (Zooamata). However, because these approaches infer a single best tree, they mask signatures of phylogenetic conflict that result from incomplete lineage sorting and historical hybridization. Accordingly, we also inferred phylogenies from thousands of non-coding loci distributed across chromosomes with historically contrasting recombination rates. Throughout the radiation of modern orders (such as rodents, primates, bats, and carnivores), we observed notable differences between locus trees inferred from the autosomes and the X chromosome, a pattern typical of speciation with gene flow. We show that in many cases, previously controversial phylogenetic relationships can be reconciled by examining the distribution of conflicting phylogenetic signals along chromosomes with variable historical recombination rates.

Lineage divergence time estimates were notably uniform across genomic loci and robust to extensive sensitivity analyses in which the underlying data, fossil constraints, and clock models

were varied. The earliest branching events in placental phylogeny coincide with the breakup of continental landmasses and rising sea levels in the Late Cretaceous. This signature of allopatric speciation is congruent with the low genomic conflict inferred for most superordinal relationships. By contrast, we observed a second pulse of diversification immediately after the Cretaceous-Paleogene (K-Pg) extinction event superimposed on an episode of rapid land emergence. Greater geographic continuity coupled with tumultuous climatic changes and increased ecological landscape at this time provided enhanced opportunities for mammalian diversification, as depicted in the fossil record. These observations dovetail with increased phylogenetic conflict observed within clades that diversified in the Cenozoic.

CONCLUSION: Our genome-wide analysis of multiple classes of sequence variation provides the most comprehensive assessment of placental mammal phylogeny, resolves controversial relationships, and clarifies the timing of mammalian diversification. We propose that the combination of Cretaceous continental fragmentation and lineage isolation, followed by the direct and indirect effects of the K-Pg extinction at a time of rapid land emergence, synergistically contributed to the accelerated diversification rate of placental mammals during the early Cenozoic. ■

The list of author affiliations is available in the full article online.

*Corresponding author. Email: wmurphy@cvm.tamu.edu

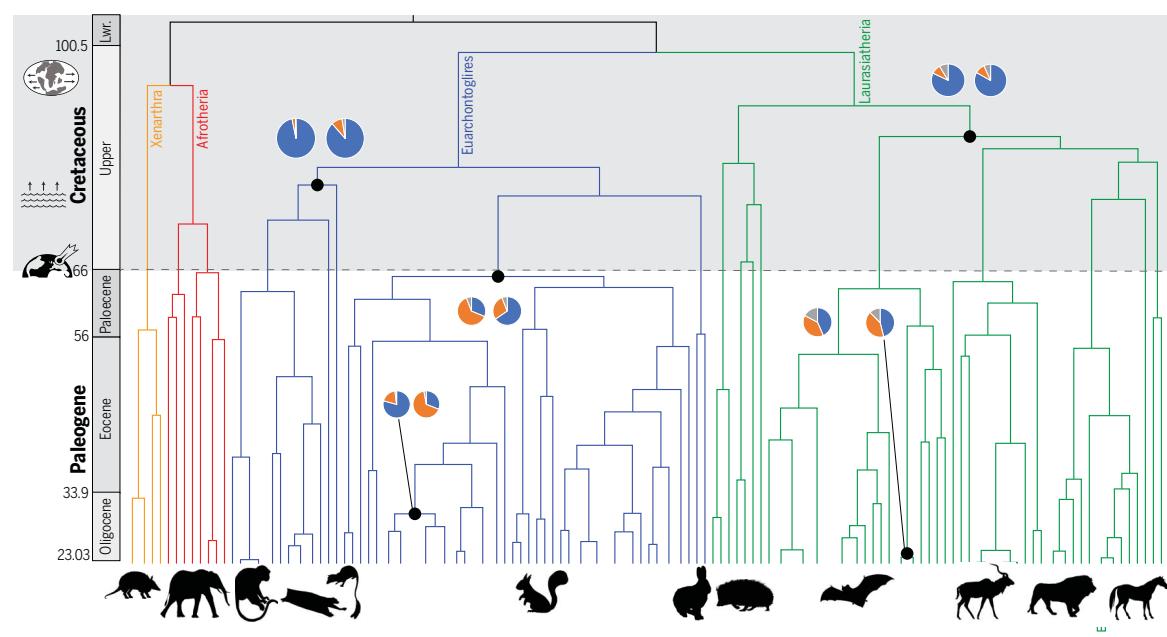
†These authors contributed equally to this work.

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The timing of placental mammal evolution.

Superordinal mammalian diversification took place in the Cretaceous during periods of continental fragmentation and sea level rise with little phylogenomic discordance (pie charts: left, autosomes; right, X chromosome), which is consistent with allopatric speciation. By contrast, the Paleogene hosted intraordinal diversification in the aftermath of the K-Pg mass extinction event, when clades exhibited higher phylogenomic discordance consistent with speciation with gene flow and incomplete lineage sorting.



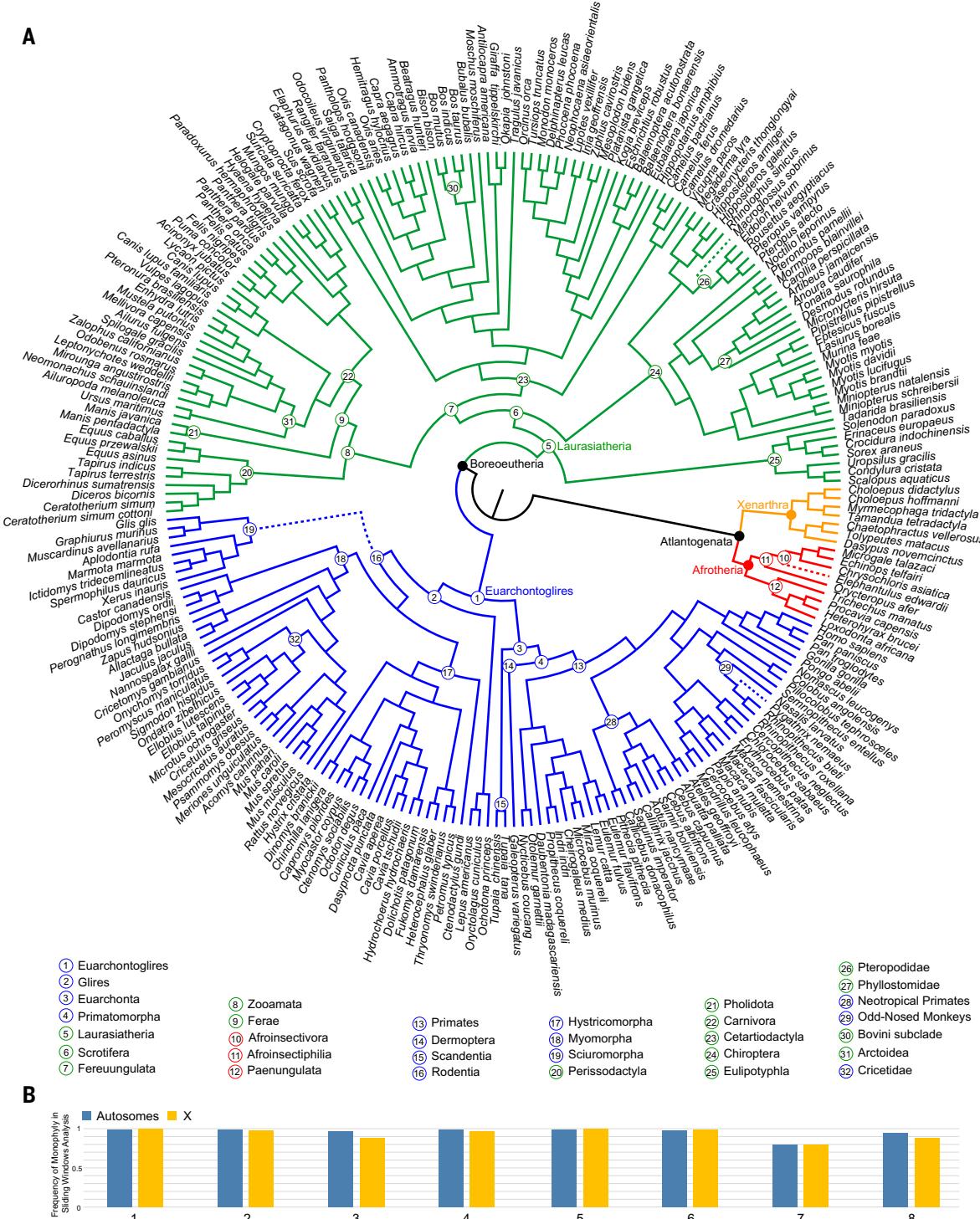


Fig. 1. Placental mammal phylogeny based on coalescent analysis of nearly neutral sites. (A) Fifty-percent Majority-rule consensus tree from a SVDquartets analysis of 411,110 genome-wide, nearly neutral sites from the human-referenced alignment of 241 species. Bootstrap support is 100% for all nodes. Superordinal clades are labeled and identified in four colors. Nodes corresponding to Boreoeutheria and Atlantogenata are indicated with black circles. (B) The frequency at which eight superordinal clades [numbered 1 to 8 in (A)] were recovered as monophyletic in 2164 window-based maximum likelihood trees from representative autosomes (Chr1, Chr21 and Chr22) and ChrX. Dotted lines indicate relationships that differ from the concatenated maximum likelihood analysis.

Genomic distribution of superordinal phylogenomic signal

Coalescent-based approaches such as SVDquartets assume incomplete lineage sorting (ILS) but no

interspecific gene flow. Concatenation methods assume that the most common phylogenetic signal represents the species tree. Both approaches typically mask signatures of ancestral

hybridization or admixture (15–17). To address this problem, we generated 2164 maximum likelihood trees for 228 species from 100-kb alignment windows (locus trees) sampled across

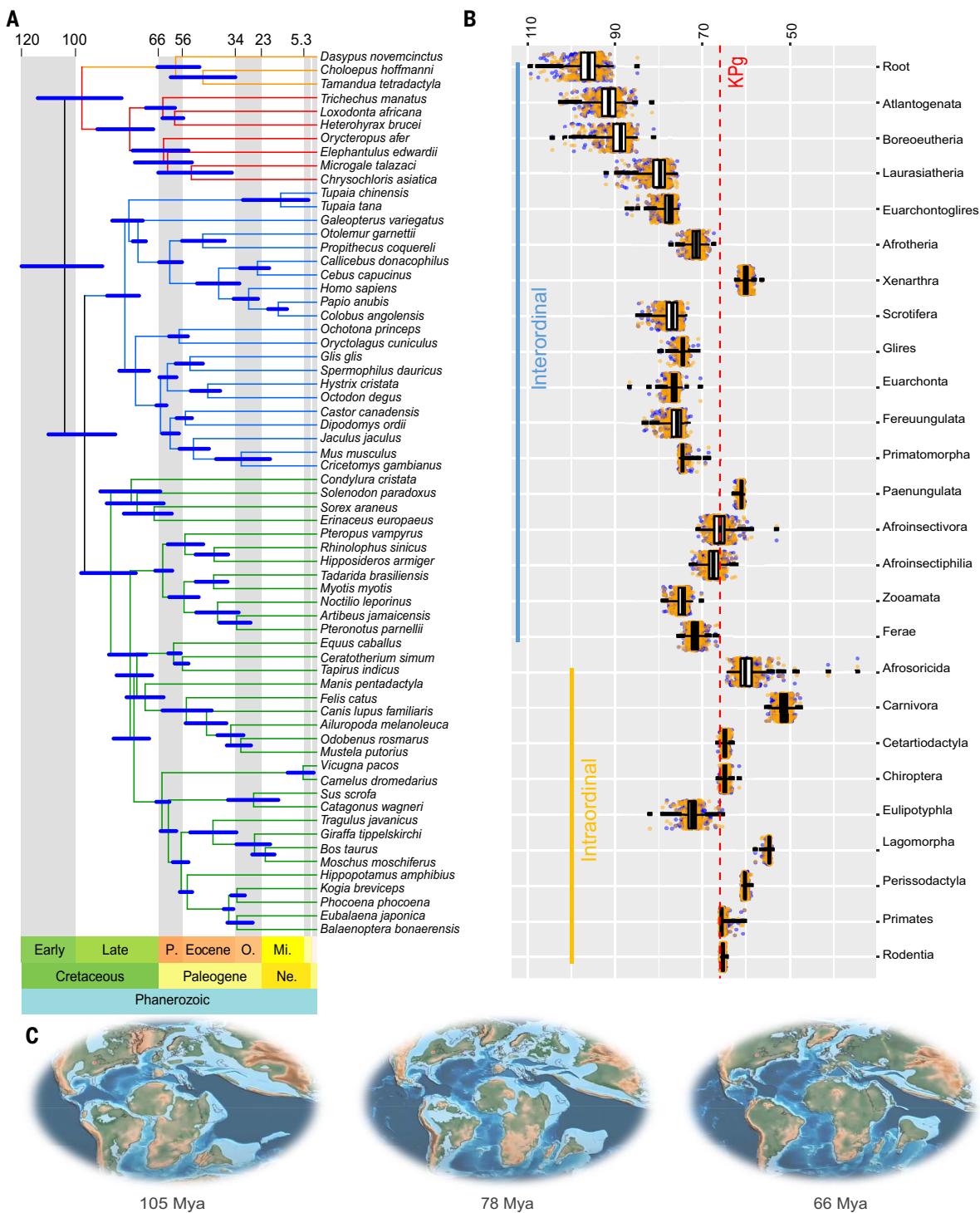


Fig. 4. Genomic timescale for placental mammal diversification. Divergence times estimated with 37 fossil calibrations for interordinal and intraordinal diversification events in mammals. (A) A representative topology from ChrX showing divergence times and CIs for 65 species, estimated by using the Benton2009 root constraint and the independent rate model (IRM) clock model. (B) Genomic estimates for major placental mammal clades based on 316 100-kb windows by using the

Benton2009 + IRM analysis, distributed across Chr1, Chr21, Chr22, and ChrX. The box plots summarize the mean and variation around the mean. The corresponding upper 95% CI and lower 95% CI are displayed as blue and orange circles, respectively, for each of the 316 estimates. The related minimum, maximum, mean, and median 95% CIs are listed in table S10. (C) Paleomaps (38) illustrate the extent of continental fragmentation and sea level rise at a series of time points during the Cretaceous.

of the ancestral placental mammal karyotype that are predicted to have historically lower rates of recombination. However, this topology is depleted on the small autosomes and the

telomeric ends of Chr1, where ancestral reconstructions predict historically higher rates of recombination (Table 1 and table S5) that lead to locus tree conflict.

A basal position for ursids is supported across most locus trees within arctoid carnivorans. However, there is strong enrichment for an ursid+musteloid clade found within

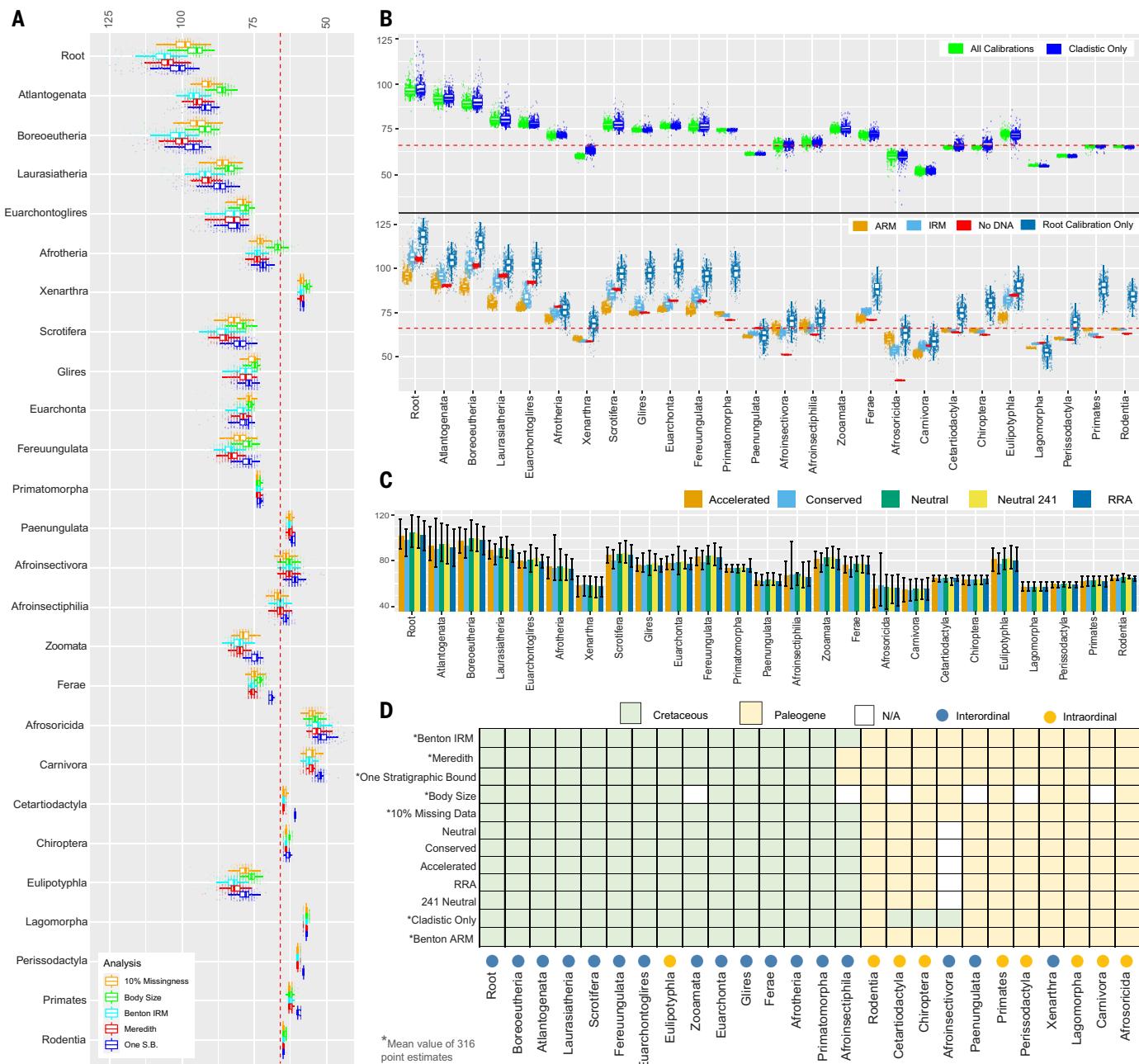


Fig. 5. Divergence time sensitivity analyses. For analyses in which 316 trees were used, point divergence time estimates for all 316 time trees are displayed. The overlaid box plots show the mean of 316 point estimates. The corresponding minimum, maximum, mean, and median 95% CIs are listed in table S10. **(A)** Variation in node ages when the root constraint, stratigraphic bounds (correcting for body size), and missingness are varied. **(B)** Comparison of point estimates when the tree is fully calibrated by using a combination of “cladistic” (fossils assigned to a node based on a formal cladistic analysis) and “opinion” fossil constraints relative to point estimates calibrated only with cladistic fossils

(table S9). (Bottom) Comparison of divergence time estimates using the IRM or autocorrelated rate model (ARM). The effective joint prior (No DNA) is compared with divergence times estimated when only the root of Placentalia is calibrated by using the Benton 2009 soft bound upper constraint.

(C) Comparison of point estimates and 95% CIs for single-tree datasets in which selective pressure, genome alignment reference species, and the number of species are varied (table S10). **(D)** The inferred ages of select interordinal (x axis, blue dots) and intraordinal divergences (x axis, yellow dots) across the range of sensitivity analyses are listed in table S10.

two ChrX recombination coldspots that are enriched for the species tree in other carnivoran families (16, 44). We hypothesize that gene flow between the ancestors of musteloids and pinnipeds may have erased the species tree history across the autosomes, which

was retained in the center of the low recombining region of ChrX, mirroring observations in other animal clades (15, 17). Locus trees for cricetid rodents also reveal a very high disparity in ChrX versus autosomal signal, with ChrX enriched for a *Cricetuslus*+*Ondatra* clade

as the most probable species tree, which echoes findings from phylogenomic studies of other muroid rodents (45). Profiles with low GC content similarly track the inferred species trees in each Cenozoic clade (Fig. 2) (21, 46). Our findings highlight phylogenetically dispersed