

Molecular Dating and Biogeography of the Early Placental Mammal Radiation

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The timing and phylogenetic hierarchy of early placental mammal divergences was determined based on combined DNA sequence analysis of 18 gene segments (9779 bp) from 64 species. Using rooted and unrooted phylogenies derived from distinct theoretical approaches, strong support for the divergence of four principal clades of eutherian mammals was achieved. Minimum divergence dates of the earliest nodes in the placental mammal phylogeny were estimated with a quartet-based maximum-likelihood method that accommodates rate variation among lineages using conservative fossil calibrations from nine different nodes in the eutherian tree. These minimum estimates resolve the earliest placental mammal divergence nodes at periods between 64 and 104 million years ago, in essentially every case predating the Cretaceous-Tertiary (K-T) boundary. The pattern and timing of these divergences allow a geographic interpretation of the primary branching events in eutherian history, likely originating in the southern supercontinent Gondwanaland coincident with its breakup into Africa and South America 95–105 million years ago. We propose an integrated genomic, paleontological, and biogeographic hypothesis to account for these earliest splits on the placental mammal family tree and address current discrepancies between fossil and molecular evidence.

The phylogenetic pattern and timing of the radiation of eutherian (placental) mammals has been the subject of considerable debate over several decades. Classical and modern analyses based on paleontological data have suggested a rapid adaptive radiation following, and perhaps facilitated by, the extinction of dinosaurs at the Cretaceous-Tertiary (K-T) boundary about 65 million years ago (MYA) (Benton 1999; Bromham et al. 1999; Foote et al. 1999). Recent molecular studies reported genetic divergence estimates that question this view and suggest much earlier dates (74–130 MYA) for the interordinal divergences among placental mammals (Easteal 1999; Hedges et al. 1996; Kumar and Hedges 1998). A pre-K-T boundary differentiation scenario led to the hypothesis that the initial radiation of placental mammals was triggered by vicariant events derived from continental breakup during the Cretaceous (Bromham et al. 1999; Hedges et al. 1996), which may have been enhanced much later by the sudden opening of numerous ecological niches previously occupied by dinosaurs. To distinguish between these two scenarios it is important to consider the variance of molecular dating estimates for early eu-

therian divergences (e.g., Hedges and Kumar 1999) and to compare results from different approaches and independent data sets. A potentially confounding aspect of molecular dating methods would be rate heterogeneity among lineages, which has been apparent in some studies of placental mammals including rodents (e.g., Gu and Li 1992; Li et al. 1996). A confident interpretation of deep divergence nodes such as those of placental mammals would benefit from explicit tests and adjustment for rate heterogeneity among lineages (Li 1997; Kumar and Hedges 1998; Bromham et al. 2000).

A critical requirement for dating divergence nodes among the earliest placental mammals would be a robust resolution of the phylogenetic topology of the 18 living orders. This information is of direct relevance to designing comprehensive and representative taxon comparisons for molecular dating estimates that span the deepest nodes of Eutheria. Studies using morphological and molecular characters have contributed to the resolution of certain parts of the eutherian tree (e.g., McKenna and Bell 1997; Miyamoto and Goodman 1986; Novacek 1992; Springer et al. 1997), but the structure of the earliest di-

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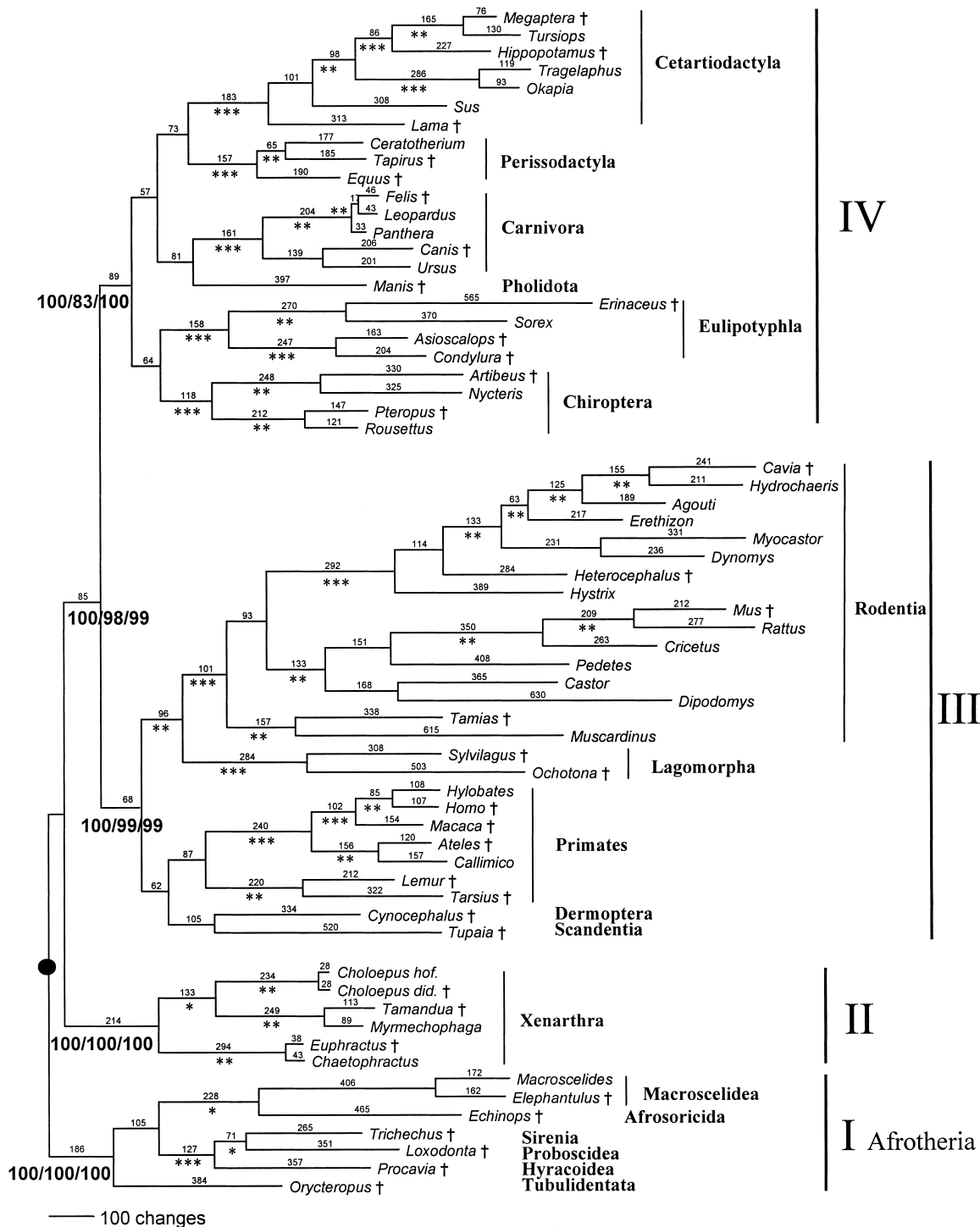
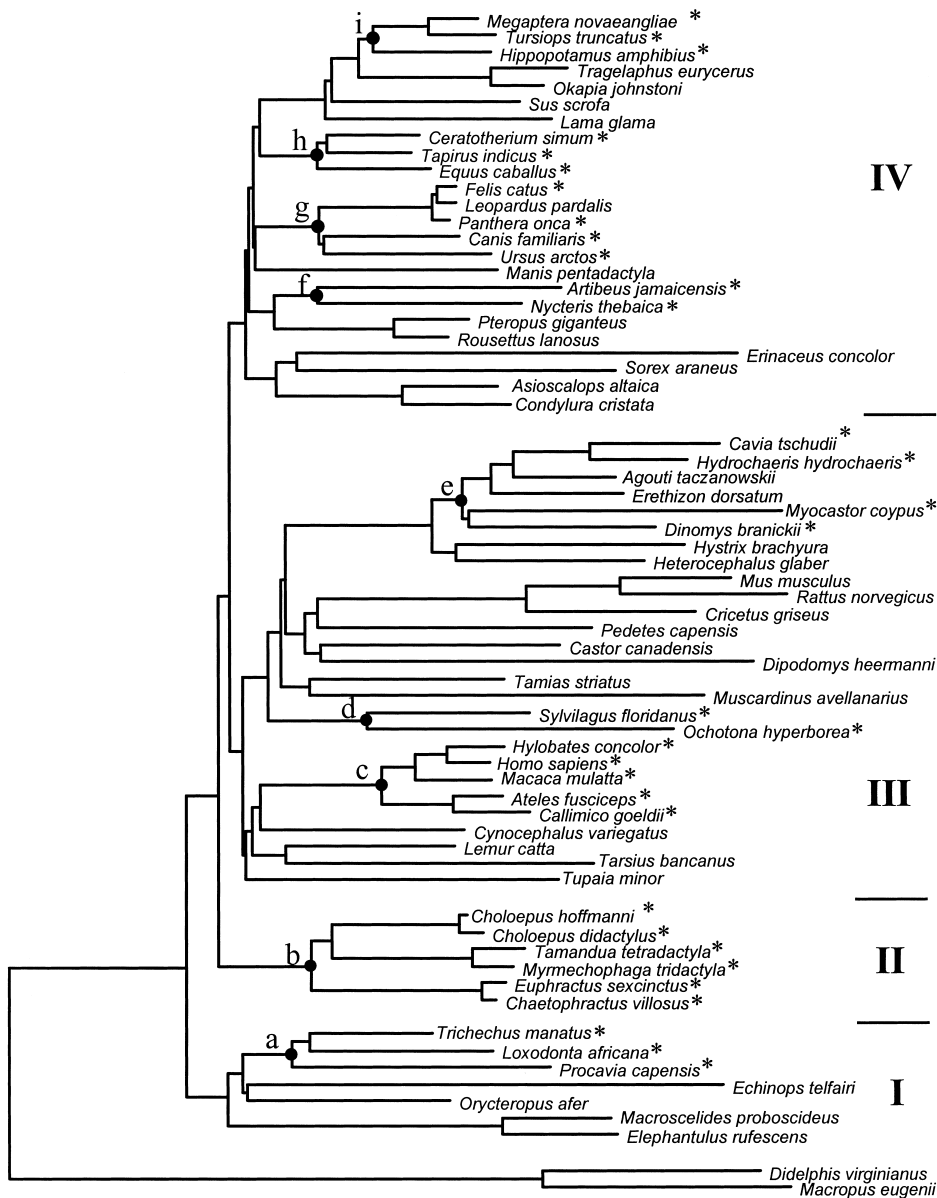


Figure 1. Unrooted maximum parsimony (MP) phylogeny of placental mammals (TL = 25,106, CI = 0.346, RI = 0.479). A concordant topology was obtained with maximum-likelihood (ML) and minimum evolution (neighbor joining, NJ) analyses (see Methods). Numbers above branches are the number of steps in the MP analysis. Bootstrap values are shown under basal branches (MP/NJ/ML). Bootstrap support (bs) for other nodes is indicated by asterisks: * = bs > 90% in one of the three methods; ** = bs > 90% in two methods; *** = bs > 90% in all three methods. See Table 3 for a complete list of bootstrap values for all major eutherian groups. † Species included in the pruned dataset for ML analyses. Our current estimate of the placement of the root (Murphy et al. 2001; see also Madsen et al. 2001) is shown by a black circle, and the tree is presented here with this arrangement for clarity. Eutherian orders are identified on the right (boldface type) and the four principal clades of placental mammals are indicated by roman numerals (I–IV).

vergences among placental mammals has remained unclear. Recent molecular data have contributed to resolving these basal nodes (Madsen et al. 2001; Murphy et al. 2001), supporting the existence of four principal

clades of placental mammals. Although these studies provide evidence for the placement of the root of the eutherian tree (between clade I [Afrotheria] and the other three clades), we could not exclude two

alternative roots (at the base of Xenarthra or between [Afrotheria, Xenarthra] and the other two clades). Moreover, it is possible that the bootstrap support for the basal relationships among the four major



— 0.01

Figure 2. Neighbor-joining phylogram of the placental radiation (derived from ML, gamma-corrected distances, see Methods) displaying the rapid basal divergences, the extreme rate heterogeneity among lineages, and conservative estimates for the first fossil occurrence of representative modern groups (a–i). These labeled nodes were used as fossil-based calibration points in the quartet dating analyses (asterisks indicate taxa used in examined pairs): (a) Paenungulata (hyrax, elephant, manatee), 55 MYA (Gheerbrant et al. 1996); (b) Xenarthra (2 armadillos, 2 sloths, 2 anteaters), 61 MYA (Benton 1993); (c) anthropoid primates (spider monkey, Goeldi’s monkey, macaque, gibbon, human), 35 MYA (Kay et al. 1997); (d) Lagomorpha (rabbit, pika), 50 MYA (Benton 1993); (e) caviomorph rodents (capybara, coypu, guinea pig, pacarana), 32 MYA (Wyss et al. 1993); (f) Microchiroptera (Jamaican fruit-eating bat, slit-faced bat), 50 MYA (Novacek 1985); (g) Carnivora (cat, jaguar, dog, bear), 50 MYA (Benton 1993); (h) Perissodactyla (rhino, tapir, horse), 55 MYA (Benton 1993); (i) Cetartiodactyla (whale, dolphin, hippo), 49 MYA (Gingerich et al. 1994).

clades of placentals was actually decreased by inclusion of the marsupial outgroup, due to its unstable placement (Swofford et al. 1996).

In this article we reexamine the phylogeny, dating, and biogeography of early placental mammals using three approaches: (1) a comparison of the bootstrap support for basal eutherian nodes in extensive rooted versus unrooted phylogenetic anal-

yses of nearly 10,000 aligned nucleotides examined in 64 placental mammal species; (2) a maximum-likelihood-based rate constancy test (quartet dating), which allows for rate heterogeneity among lineages, aiming to specifically test whether the supraordinal divergences within Eutheria preceded the K-T boundary; and (3) an estimation of the dates and 95% confidence intervals for the deep nodes which define

the four major clades of eutherian mammals. Using conservative fossil calibrations (which are almost certainly underestimates of true divergence times at those internal nodes) our results support the hypothesis that most, if not all, of the early supraordinal eutherian diversification did precede the K-T boundary (Hedges et al. 1996; Cooper and Penny 1997; Kumar and Hedges 1998). These results, along with current estimates of the position of the eutherian root (Madsen et al. 2001; Murphy et al. 2001), suggest that this early separation of placental lineages was likely the result of vicariant events associated with the breakup of Gondwanaland in the late Cretaceous period.

Materials and Methods

The analyses performed here are based on the dataset presented by Murphy et al. (2001), which consists of 18 gene segments (15 nuclear: *ADORA3*, *ADRB2*, *APP*, *ATP7A*, *BDNF*, *BMII*, *CNR1*, *CREM*, *EDG1*, *PLCB4*, *PNOC*, *RAG1*, *RAG2*, *TYR*, *ZFX*; and three mitochondrial: 12S rRNA, tRNA^{Val}, partial 16S rRNA; total alignment of 9779 bp) examined in 64 placental mammals, broadly representing all extant orders (Table 1), as well as two marsupial outgroups. The primers used to amplify these segments are listed in Table 2. The use of a long, concatenated data set as opposed to several separate short segments has been suggested to improve the reliability of phylogenetic and dating estimates (Bromhan et al. 2000; Huelsenbeck et al. 1996; Nei et al. 2001).

Phylogenetic analyses were performed with PAUP*4.0b4a (Swofford 1998) using three optimality criteria: maximum parsimony (MP), minimum evolution with the neighbor-joining algorithm (NJ), and maximum likelihood (ML) using a gamma-corrected HKY85 model with parameters estimated from the dataset (Ts/Tv = 2.0; α = 0.45). Parsimony methods employed heuristic searches (50 replicates, random addition of taxa, TBR branch swapping), and were subdivided into three separate analyses: (1) all sites given equal weight, (2) third codon position transitions removed (CS, conservative substitution parsimony), and (3) transversions weighted two times greater than transitions (2:1 parsimony). Distance analyses employed different distance corrections (Kimura two-parameter, Logdet paraligner, maximum likelihood with an HKY85 model and parameters estimated from the dataset) to examine effects on topological stability.

Table 1. List of taxa analyzed in this study

Order	Scientific name	Code ^a	Common name	Source ^b
Clade I				
Sirenia	<i>Trichechus manatus</i>	Tmn*	West Indian manatee	USFWS
Hyracoidea	<i>Procavia capensis</i>	Pca*	Rock hyrax	LGD
Proboscidea	<i>Loxodonta africana</i>	Laf*	African elephant	LGD
Macroscelidea	<i>Macroscelides proboscideus</i>	Mpr	Short-eared elephant shrew	SDZ
Macroscelidea	<i>Elephantulus rufescens</i>	Eru	Rufous elephant shrew	SDZ
Afrosoricida	<i>Echinops telfairi</i>	Ete	Lesser hedgehog tenrec	NZP
Tubulidentata	<i>Orycteropus afer</i>	Oaf	Aardvark	SDZ
Clade II				
Xenarthra	<i>Choloepus hoffmanni</i>	Cho*	Hoffmann's two-toed sloth	SDZ
Xenarthra	<i>Choloepus didactylus</i>	Cdi*	Linne's two-toed sloth	SDZ
Xenarthra	<i>Euphractus sexinctus</i>	Ese*	Six-banded armadillo	LGD
Xenarthra	<i>Chaetophractus villosus</i>	Cvi*	Hairy armadillo	NZP
Xenarthra	<i>Tamandua tetradactyla</i>	Ttt*	Tamandua	SDZ
Xenarthra	<i>Myrmecophaga tridactyla</i>	Mtr*	Giant anteater	SDZ
Clade III				
Rodentia	<i>Tamias striatus</i>	Tsr	Eastern chipmunk	LGD
Rodentia	<i>Castor canadensis</i>	Ccn	North American beaver	USFWS
Rodentia	<i>Muscardinus avellanarius</i>	Mav	Dormouse	T. Oleksyk
Rodentia	<i>Pedetes capensis</i>	Pcp	Springhare	USFWS
Rodentia	<i>Mus musculus</i>	Mmu	Mouse	LGD
Rodentia	<i>Rattus norvegicus</i>	Rno	Rat	LGD
Rodentia	<i>Cricetus griseus</i>	A23	Hamster	LGD
Rodentia	<i>Dipodomys heermanni</i>	Dhe	Kangaroo rat	NZP
Rodentia	<i>Heterocephalus glaber</i>	Hgl	Naked mole rat	USFWS
Rodentia	<i>Hystrix brachyurus</i>	Hba	Malayan porcupine	SDZ
Rodentia	<i>Erethizon dorsatum</i>	Edr	North American porcupine	USFWS
Rodentia	<i>Cavia tschudii</i>	Cts*	Guinea pig	SDZ
Rodentia	<i>Hydrochaeris hydrochaeris</i>	Hhd*	Capybara	SDZ
Rodentia	<i>Myocastor coypus</i>	Mco*	Coypu	USFWS
Rodentia	<i>Dinomys branicki</i>	Dbr*	Pacarana	SDZ
Rodentia	<i>Agouti taczanowski</i>	Ata	Mountain paca	SDZ
Lagomorpha	<i>Ochotona hyperborea</i>	Ohy*	Northern pika	KIZ
Lagomorpha	<i>Sylvilagus floridanus</i>	Sfl*	Eastern cottontail	LGD
Dermoptera	<i>Cynocephalus variegatus</i>	Cva	Malayan flying lemur	UTSW
Scandentia	<i>Tupaia minor</i>	Tmi	Lesser tree shrew	SDZ
Primates	<i>Lemur catta</i>	Lct	Ring-tailed lemur	LGD
Primates	<i>Tarsius bancanus</i>	Tsp	Western tarsier	NZP
Primates	<i>Ateles fusciceps</i>	Afc*	Brown-headed spider monkey	LGD
Primates	<i>Callimico goeldi</i>	Cgo*	Goeldi's monkey	LGD
Primates	<i>Macaca mulatta</i>	Mma*	Rhesus macaque	LGD
Primates	<i>Hylobates concolor</i>	Hco*	Gibbon	LGD
Primates	<i>Homo sapiens</i>	Hsa*	Human	LGD
Clade IV				
"Eulipotyphla"	<i>Erinaceus concolor</i>	Eco	Eastern European hedgehog	A. Grafodatsky
"Eulipotyphla"	<i>Asioscalops altaica</i>	Aal	Siberian mole	A. Grafodatsky
"Eulipotyphla"	<i>Condylura cristata</i>	Cct	Star-nosed mole	NZP
"Eulipotyphla"	<i>Sorex araneus</i>	Sar	European common shrew	O. Serov
Chiroptera	<i>Artibeus jamaicensis</i>	Aja*	Neotropical fruit bat	SDZ
Chiroptera	<i>Nycteris thebaica</i>	Nth*	Slit-faced bat	LGD
Chiroptera	<i>Pteropus giganteus</i>	Pgi	Indian flying fox	SDZ
Chiroptera	<i>Rousettus lanosus</i>	Rla	Ruwenzori long-haired rousette	SDZ
Cetartiodactyla	<i>Megaptera novaeangliae</i>	Mno*	Humpback whale	LGD
Cetartiodactyla	<i>Tursiops truncatus</i>	Ttr*	Bottlenose dolphin	LGD
Cetartiodactyla	<i>Hippopotamus amphibius</i>	Ham*	River hippopotamus	SDZ
Cetartiodactyla	<i>Lama glama</i>	Lgl	Llama	SDZ
Cetartiodactyla	<i>Tragelaphus eurycerus</i>	Teu	Bongo	LGD
Cetartiodactyla	<i>Sus scrofa</i>	Ssr	Domestic pig	LGD
Cetartiodactyla	<i>Okapia johnstoni</i>	Ojo	Okapi	LGD
Perissodactyla	<i>Equus caballus</i>	Eca*	Domestic horse	LGD
Perissodactyla	<i>Ceratotherium simum</i>	Csi*	White rhinoceros	LGD
Perissodactyla	<i>Tapirus indicus</i>	Tin*	Malayan tapir	SDZ
Carnivora	<i>Canis familiaris</i>	Cfa*	Domestic dog	LGD
Carnivora	<i>Ursus arctos</i>	Uar*	Brown bear	LGD
Carnivora	<i>Felis catus</i>	Fca*	Domestic cat	LGD
Carnivora	<i>Leopardus pardalis</i>	Lpa	Ocelot	LGD
Carnivora	<i>Panthera onca</i>	Pon*	Jaguar	LGD
Pholidota	<i>Manis pentadactyla</i>	Mpe	Chinese pangolin	KIZ
Marsupials				
Didelphimorphia	<i>Didelphis virginianus</i>	Dvi	North American opossum	LGD
Diprotodontia	<i>Macropus eugenii</i>	Meu	Tammar wallaby	LGD

^a Three-letter codes are referred to in Table 4; asterisks indicate taxa used for the quartet dating analysis.

^b LGD (Stephen J. O'Brien, Laboratory of Genomic Diversity, USA), SDZ (Oliver Ryder, Center for Reproduction of Endangered Species, San Diego Zoo, USA), NZP (Richard Montali, National Zoological Park, USA), USFWS (Gina Harris, U.S. Fish and Wildlife Service), KIZ (Ya Ping Zhang, Kunming Institute of Zoology, China), UTSW (Robert Baker, University of Texas, Southwestern Museum, USA).

The maximum-likelihood analysis was based on a pruned dataset containing 37 taxa representing all major eutherian lineages. Rooted analyses were performed using two marsupials (representing the neotropical and Australasian lineages, see Table 1) as outgroups, whereas unrooted trees included only eutherian taxa. Bootstrap support was assessed using 1000 replicates for MP and NJ analyses, and 100 iterations for the ML phylogeny.

We calculated divergence estimates among placental mammal groups using the quartet dating method (with the program QDate, version 1.11; Cooper and Penny 1997; Rambaut and Bromham 1998) applied to the concatenated dataset of 18 gene segments. This approach involves maximum-likelihood tests of rate constancy in multiple four-taxon trees (quartets), with internal calibration points used for each pair of taxa. In this case we incorporated a two-rate model in which a different rate is allowed for each pair of taxa. Only quartets that did not depart significantly (using a chi-square test) from the expectations of the two-rate model were considered (Rambaut and Bromham 1998). To specifically test whether basal placental lineages diverged prior to the K-T boundary, we calibrated our molecular divergences with conservative dates for the first appearance of nine different mammalian groups in the fossil record. These dates can be considered to be underestimates of true divergence times for these calibration nodes, and therefore our calculations are aimed to be minimum, rather than absolute estimates of basal eutherian divergences.

Results

Our analyses provided consistently high bootstrap support for the four major phylogenetic clades of placental mammals identified by Madsen et al. (2001) and Murphy et al. (2001), as well as for the branch separating Afrotheria + Xenarthra from all other placentals. These results strongly suggest that these basal relationships among eutherians are stable (Figure 2, Table 3), although the ultimate resolution of the early branching order will only be achieved with the firm establishment of the root. We observed that in nearly all cases the bootstrap values for basal branches did increase in the unrooted analyses relative to the rooted trees (Table 3), supporting the prediction that the unstable root was affecting the confidence

Table 2. Primers used for generation of nucleotide dataset (see Murphy et al. 2001)

Locus	Forward primer (5'→3')	Reverse primer (5'→3')
ADORA3	ACCCCATGTTGGCTGGAA	GATAGGGTTCATCATGGAGTT
ADRB2	ATCGTCTGGCCATCGTGT	CTCCTGGAAGCAATCCTGA
APP	TCCAAGATGCAGCAGAACG	CTAATGTGTGCACATAAAACAGG
ATP7A	TCCCTGGACAATCAAGAACG	AAGGTAGCATCAAATCCCATGT
BDNF	CATCCTTTCTTACTACTGGTT	TTCCAGTGCCTTTTGTCTATG
BMI1	CATTTGGCCATAGTTTGTAACTCAA	CCAATATGGCATTGTACAACAAGC
CNR1	CCAAATAGGTTACTTCCCACA	CATAGATGATGGGGTTCACG
CREM	AGGAACTCAAGGCCCTCAA	GGGAGGACAAATGCTTTTCAA
EDG1	CGTCCGGCATTACAATACTACA	GACGTTTCCAGAAGACATAATGG
PLCB4	GTGAAATTTGGAAGCCGAGAT	CACCAAGCTCATTACTTGTGA
PNOC	GCATCCTTGAGTGTGAAGAGAA	TGCCTCATAAAGTCACTGAACC
RAG1	AAGACATCTTGAAGGCATGA	AAAGTTGCCATTTCATCTCA
RAG2	TCTGGAGGAAACACCAAA	TGCCTGAGACAGAGATTC
TYR	TGTGGCCAGCTTTCAGGCAG	CTTCATGGGCAAAATCAATGT
ZFX	TGGCCAGATGGACATCC	AATGCTTTCGGACTCATCG

12S + tRNAval Springer MS, Holler L J, and Burk A. Mol Biol Evol 1995;12:1138–1150.

16S Springer et al. 1995 (above); Palumbi S. In: Molecular systematics (Hillis DM, et al., eds.), 1997.

of ingroup relationships (Swofford et al. 1996).

The branching pattern observed in Figure 1 shows that the early eutherian radiation was rather rapid (note the short basal internodes coupled with long terminal branches). Furthermore, the observed starlike topology within the supraordinal clades III and IV clearly suggests rapid bursts of diversification within these lineages. Also apparent is the considerable

degree of rate variation among placental lineages (Figure 1). Several taxa are clearly accelerated relative to the others, such as the caviomorph rodents (e.g., *Cavia*, *Myocastor*), muroid rodents (e.g., *Mus*, *Rattus*), hedgehog (*Erinaceus*), and tenrec (*Echinops*). These features of the eutherian tree pose a challenge to its complete resolution, but also provide insights into the causative processes and time interval of these events.

Table 3. Summary of topological and bootstrap support for major eutherian clades in different phylogenetic analyses

Clade	ML	NJ-ML dist.	NJ-K2P	NJ-LogDet	MPEqual	MP-3posTs ^a	MP-2:1 ^b
Afrotheria (clade I)	99/100	94/100	90/100	91/100	NS/100	57/100	NS/100
Paenungulata ^c	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Tethytheria ^d	55/NS	97/97	100/100	100/100	63/79	*/53	55/72
Xenarthra (clade II)	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Clades III + IV	79/100	65/98	72/98	69/99	64/100	71/100	71/100
Clade III	85/100	93/99	84/98	84/99	64/99	67/91	73/100
Primates	82/83	NS/NS	NS/NS	NS/NS	66/70	66/74	78/82
Anthropoids	100/100	100/100	100/100	100/100	100/100	100/100	100/100
<i>Tarsius</i> + <i>Lemur</i>	99/99	98/99	98/99	98/99	80/82	78/84	91/94
Dermoptera + Scandentia	59/55	NS/NS	NS/NS	NS/NS	55/61	60/71	60/71
Primates + Dermoptera + Scandentia	72/68	*/*	55/*	54/54	*/52	*/*	*/*
Glires ^e	95/100	99/99	99/100	99/99	52/67	52/64	51/71
Lagomorpha	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Rodentia	100/100	97/97	94/95	94/96	87/100	92/100	85/100
Clade IV	99/100	74/66	94/83	94/81	99/100	90/94	98/100
Cetartiodactyla	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Cetacea + Hippo	100/100	100/100	100/100	100/100	96/97	82/86	98/100
Perissodactyla	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Cetartiodactyla + Perissodactyla	67/64	63/64	65/65	67/63	72/76	71/66	50/58
Carnivora	100/100	100/100	100/100	100/100	100/100	100/100	100/100
Carnivora + Pholidota	63/60	*/*	NS/NS	NS/NS	*/*	NS/NS	51/52
Chiroptera	100/100	90/100	98/100	97/99	100/100	100/100	100/100
“Eulipotyphla”	98/99	100/99	99/100	99/100	97/98	94/94	99/100

Bootstrap support is depicted as [rooted/unrooted] value.

^a MP-3posTs = maximum parsimony excluding third codon position transitions.

^b MP-2:1 = maximum parsimony weighting transversions two times more than transitions.

^c Paenungulata = Proboscidea + Sirenia + Hyracoidea.

^d Tethytheria = Proboscidea + Sirenia.

^e Glires = Rodentia + Lagomorpha.

* Monophyletic in optimal tree, <50% bootstrap support.

NS = not monophyletic in optimal tree, <50% bootstrap support.

Three hundred seventy-seven quartets of taxa covering all major eutherian lineages were tested for rate constancy under a two-rate model (Rambaut and Bromham 1998). We list in Table 4 the 205 of these taxon quartets (54%) that conformed to this model. These quartets that passed this rate constancy test were used to estimate minimum ages of divergence for five basal nodes in the eutherian tree (Table 4). The estimated dates for the early placental mammal divergences range from 64 to 104 MYA (Table 5), consistently older (with one exception, see below) than the K-T boundary. Considerable variance was observed around estimates of the same phylogenetic node, in large part due to the occurrence of outliers associated with particular calibration points (see Table 4). For example, estimates using the caviomorph rodent calibration at 32 MYA consistently resulted in divergence dates much younger than those obtained using other pairs of taxa, perhaps suggesting a more ancient origin for this group. Conversely, estimates using the Cetartiodactyla calibration point tended to produce outlier values that were considerably older than those obtained with other pairs. In spite of these caveats, all of our results strongly support a rapid diversification of the four principal eutherian lineages (I–IV) prior to the K-T boundary, likely 70–110 MYA (Table 5).

Discussion

The controversy over the timing of early eutherian radiation centers around the discrepancy so far observed between early (Cretaceous) and widely dispersed molecular divergence estimates for extant placental groups, and the abrupt, more recent (post K-T boundary) first fossil appearance of all diagnosable modern lineages of eutherian mammals (Benton 1999; Bromham et al. 1999; Eastale 1999; Foote et al. 1999; Kumar and Hedges 1998). The present results may offer a plausible explanation for this perceived discrepancy and provide evidence for an integrated interpretation of the early steps in the diversification of placental mammals.

The structure of our phylogenetic trees suggests a rather rapid early radiation of eutherians and an extremely fast diversification inside at least two of the major clades (III and IV), which appears to conflict with the more distinctive branching pattern inferred from previous molecular clock estimates (Eastale 1999; Kumar and Hedges 1998). The more spaced pattern

Table 4. Minimum divergence estimates (with 95% confidence intervals) for the 205 quartets of eutherian taxa that conformed to a two-rate model of nucleotide evolution

Taxa ^a	Date	Low	High	Taxa ^a	Date	Low	High	Taxa ^a	Date	Low	High
Tmn,Laf:Aja,Nth	83	79	88	Ttt,Ese:Hsa,Afc	84	79	89	Mno,Ham:Fca,Cfa	89	84	96
Tmn,Laf:Hco,Afc	92	87	98	Ttt,Ese:Mma,Afc	84	79	89	Mno,Ham:Pon,Cfa	88	83	94
Pca,Laf:Cho,Ese	85	80	90	Ttt,Ese:Hco,Cgo	82	77	87	Mno,Ham:Fca,Uar	86	81	92
Pca,Laf:Cdi,Ese	84	80	89	Cho,Cvi:Aja,Nth	79	75	83	Mno,Ham:Pon,Uar	86	81	92
Pca,Laf:Mtr,Ese	85	81	89	Cho,Cvi:Mno,Ham	103	97	110	Mno,Ham:Hco,Afc	93	86	100
Pca,Laf:Ttt,Ese	85	81	89	Cho,Cvi:Ttr,Ham	94	89	101	Mno,Ham:Hsa,Afc	95	89	102
Pca,Laf:Cho,Cvi	84	80	89	Cho,Cvi:Fca,Cfa	88	83	94	Ttr,Ham:Csi,Eca	90	85	96
Pca,Laf:Cdi,Cvi	84	79	89	Cho,Cvi:Pon,Cfa	87	82	93	Ttr,Ham:Tin,Eca	89	83	95
Pca,Laf:Mtr,Cvi	86	82	91	Cho,Cvi:Fca,Uar	88	82	93	Ttr,Ham:Fca,Cfa	84	79	89
Pca,Laf:Ttt,Cvi	85	81	90	Cho,Cvi:Pon,Uar	87	82	93	Ttr,Ham:Pon,Cfa	83	78	88
Pca,Laf:Aja,Nth	76	72	81	Cho,Cvi:Cts,Dbr	73	68	77	Ttr,Ham:Fca,Uar	81	76	86
Pca,Laf:Mno,Ham	102	96	109	Cho,Cvi:Hhd,Dbr	75	70	80	Ttr,Ham:Pon,Uar	81	76	86
Pca,Laf:Ttr,Ham	98	93	104	Cho,Cvi:Hco,Afc	82	77	88	Ttr,Ham:Hco,Afc	87	81	93
Pca,Laf:Csi,Eca	94	89	100	Cho,Cvi:Hsa,Afc	83	78	89	Ttr,Ham:Hsa,Afc	90	84	96
Pca,Laf:Tin,Eca	94	88	99	Cho,Cvi:Mma,Afc	84	79	89	Ttr,Ham:Mma,Afc	89	83	95
Pca,Laf:Fca,Cfa	87	82	92	Cdi,Cvi:Aja,Nth	79	75	83	Ttr,Ham:Hco,Cgo	84	79	90
Pca,Laf:Pon,Cfa	86	82	91	Cdi,Cvi:Mno,Ham	102	96	110	Ttr,Ham:Hsa,Cgo	86	81	92
Pca,Laf:Fca,Uar	85	80	90	Cdi,Cvi:Ttr,Ham	94	88	100	Csi,Eca:Fca,Cfa	82	77	88
Pca,Laf:Pon,Uar	85	80	90	Cdi,Cvi:Fca,Cfa	89	83	95	Csi,Eca:Pon,Cfa	82	77	87
Pca,Laf:Hco,Afc	83	78	88	Cdi,Cvi:Pon,Cfa	88	82	94	Csi,Eca:Fca,Uar	81	76	86
Pca,Laf:Hsa,Afc	83	79	89	Cdi,Cvi:Fca,Uar	88	83	94	Csi,Eca:Pon,Uar	81	76	86
Pca,Laf:Mma,Afc	83	78	88	Cdi,Cvi:Pon,Uar	87	82	93	Csi,Eca:Hco,Afc	85	80	91
Cho,Ese:Aja,Nth	79	75	84	Cdi,Cvi:Cts,Dbr	73	68	78	Csi,Eca:Mma,Afc	87	81	93
Cho,Ese:Mno,Ham	104	98	111	Cdi,Cvi:Hhd,Dbr	75	71	80	Tin,Eca:Fca,Cfa	81	76	86
Cho,Ese:Ttr,Ham	96	90	102	Cdi,Cvi:Hco,Afc	82	76	88	Tin,Eca:Pon,Cfa	81	76	86
Cho,Ese:Fca,Cfa	89	84	95	Cdi,Cvi:Mma,Afc	83	78	89	Tin,Eca:Fca,Uar	80	76	85
Cho,Ese:Pon,Cfa	88	83	94	Cdi,Cvi:Mma,Cgo	81	76	87	Tin,Eca:Pon,Uar	81	76	86
Cho,Ese:Fca,Uar	88	83	94	Mtr,Cvi:Aja,Nth	80	76	85	Tin,Eca:Hco,Afc	85	79	91
Cho,Ese:Pon,Uar	88	83	93	Mtr,Cvi:Mno,Ham	102	96	108	Tin,Eca:Hsa,Afc	87	81	93
Cho,Ese:Cts,Dbr	73	68	78	Mtr,Cvi:Ttr,Ham	94	89	100	Tin,Eca:Mma,Afc	87	81	93
Cho,Ese:Hhd,Dbr	75	71	80	Mtr,Cvi:Tin,Eca	95	90	101	Tin,Eca:Hco,Cgo	83	78	89
Cho,Ese:Hco,Afc	83	78	89	Mtr,Cvi:Fca,Cfa	89	84	95	Tin,Eca:Mma,Cgo	83	78	89
Cho,Ese:Hsa,Afc	84	79	90	Mtr,Cvi:Pon,Cfa	89	84	94	Fca,Cfa:Cts,Mco	64	60	68
Cho,Ese:Mma,Afc	84	79	90	Mtr,Cvi:Fca,Uar	89	84	94	Fca,Cfa:Hco,Afc	80	75	86
Cdi,Ese:Aja,Nth	80	75	84	Mtr,Cvi:Pon,Uar	89	84	94	Fca,Cfa:Hsa,Afc	81	76	87
Cdi,Ese:Mno,Ham	104	98	112	Mtr,Cvi:Hhd,Dbr	74	70	79	Fca,Cfa:Mma,Afc	80	75	86
Cdi,Ese:Ttr,Ham	95	89	102	Mtr,Cvi:Hco,Afc	84	79	89	Fca,Cfa:Hco,Cgo	78	73	83
Cdi,Ese:Fca,Cfa	90	84	96	Mtr,Cvi:Hsa,Afc	85	80	90	Fca,Cfa:Hsa,Cgo	79	74	84
Cdi,Ese:Pon,Cfa	89	83	95	Mtr,Cvi:Mma,Afc	85	80	90	Fca,Cfa:Mma,Cgo	77	72	82
Cdi,Ese:Fca,Uar	89	83	94	Mtr,Cvi:Hco,Cgo	82	78	87	Pon,Cfa:Cts,Mco	64	59	68
Cdi,Ese:Pon,Uar	88	83	93	Mtr,Cvi:Mma,Cgo	82	78	87	Pon,Cfa:Hco,Afc	80	75	85
Cdi,Ese:Cts,Dbr	73	69	78	Ttt,Cvi:Aja,Nth	79	75	83	Pon,Cfa:Hsa,Afc	81	76	86
Cdi,Ese:Hhd,Dbr	76	71	81	Ttt,Cvi:Mno,Ham	100	94	107	Pon,Cfa:Mma,Afc	80	75	86
Cdi,Ese:Hco,Afc	83	77	89	Ttt,Cvi:Ttr,Ham	93	88	99	Pon,Cfa:Hco,Cgo	77	73	83
Cdi,Ese:Mma,Afc	84	79	90	Ttt,Cvi:Csi,Eca	94	89	99	Pon,Cfa:Hsa,Cgo	78	73	83
Mtr,Ese:Aja,Nth	79	75	84	Ttt,Cvi:Tin,Eca	94	89	99	Pon,Cfa:Mma,Cgo	77	73	82
Mtr,Ese:Mno,Ham	101	95	107	Ttt,Cvi:Fca,Cfa	88	84	94	Fca,Uar:Hhd,Dbr	74	70	79
Mtr,Ese:Ttr,Ham	93	88	99	Ttt,Cvi:Pon,Cfa	88	83	93	Fca,Uar:Hco,Afc	83	78	89
Mtr,Ese:Csi,Eca	93	88	99	Ttt,Cvi:Fca,Uar	88	83	93	Fca,Uar:Hsa,Afc	84	78	90
Mtr,Ese:Tin,Eca	93	88	98	Ttt,Cvi:Pon,Uar	88	83	93	Fca,Uar:Mma,Afc	83	78	88
Mtr,Ese:Fca,Cfa	88	84	94	Ttt,Cvi:Hco,Afc	83	78	88	Fca,Uar:Hco,Cgo	81	76	86
Mtr,Ese:Pon,Cfa	88	83	93	Ttt,Cvi:Hsa,Afc	84	79	89	Fca,Uar:Hsa,Cgo	81	76	86
Mtr,Ese:Fca,Uar	88	83	93	Ttt,Cvi:Mma,Afc	84	80	89	Fca,Uar:Mma,Cgo	80	75	85
Mtr,Ese:Pon,Uar	87	83	92	Aja,Nth:Mno,Ham	79	75	84	Pon,Uar:Hhd,Dbr	74	70	79
Mtr,Ese:Hco,Afc	83	78	88	Aja,Nth:Ttr,Ham	76	72	81	Pon,Uar:Hco,Afc	83	78	89
Mtr,Ese:Hsa,Afc	83	79	88	Aja,Nth:Csi,Eca	72	69	77	Pon,Uar:Hsa,Afc	83	78	89
Mtr,Ese:Mma,Afc	84	79	89	Aja,Nth:Tin,Eca	73	69	77	Pon,Uar:Mma,Afc	83	78	89
Mtr,Ese:Hco,Cgo	82	77	86	Aja,Nth:Fca,Cfa	72	68	76	Pon,Uar:Hco,Cgo	80	75	86
Mtr,Ese:Mma,Cgo	82	77	87	Aja,Nth:Pon,Cfa	71	67	76	Pon,Uar:Mma,Cgo	80	75	86
Ttt,Ese:Aja,Nth	78	74	82	Aja,Nth:Fca,Uar	70	66	74	Cts,Dbr:Hco,Afc	69	65	74
Ttt,Ese:Mno,Ham	100	94	106	Aja,Nth:Pon,Uar	69	66	73	Cts,Dbr:Hsa,Afc	69	65	73
Ttt,Ese:Ttr,Ham	93	88	98	Aja,Nth:Cts,Mco	64	60	69	Cts,Dbr:Mma,Afc	68	64	72
Ttt,Ese:Csi,Eca	93	88	98	Aja,Nth:Hhd,Mco	66	62	71	Cts,Dbr:Hco,Cgo	68	64	73
Ttt,Ese:Tin,Eca	92	87	98	Aja,Nth:Hco,Afc	72	68	77	Hhd,Dbr:Hco,Afc	69	65	74
Ttt,Ese:Fca,Cfa	88	83	93	Aja,Nth:Hsa,Afc	74	70	78	Hhd,Dbr:Hsa,Afc	69	65	74
Ttt,Ese:Pon,Cfa	87	83	93	Aja,Nth:Mma,Afc	73	69	78	Hhd,Dbr:Mma,Afc	68	64	73
Ttt,Ese:Fca,Uar	88	83	93	Aja,Nth:Hco,Cgo	72	68	76	Hhd,Dbr:Hco,Cgo	68	64	73
Ttt,Ese:Pon,Uar	87	82	92	Mno,Ham:Csi,Eca	98	91	104				
Ttt,Ese:Hco,Afc	83	78	88	Mno,Ham:Tin,Eca	96	90	102				

^a Taxa are indicated by three-letter codes; see Table 1 for key to full species names.

Table 5. Summary of minimum divergence estimates for placental superordinal lineages based on fossil calibrations

Clade comparison	Taxon comparison	Range of divergence estimates (MYA)	Range of 95% confidence intervals (MYA)	No. of quartets compared	Calibration points employed ^a
I versus II+III+IV	Afrotheria—other placentals	76.1–102.1	72.1–108.8	22	a–i
II versus III+IV	Xenarthra—clades III and IV	72.6–104.4	68.2–111.8	100	b–i
III	Rodentia—Primates	68.0–69.4	64.0–73.9	8	c, e
III versus IV	Rodentia—Clade IV	63.5–74.5	59.5–79.3	6	e, f–i
III versus IV	Primates—Clade IV	71.7–95.3	67.5–102.3	41	c, f–i
IV	Carnivora—Cetartiodactyla	80.7–89.5	76.0–95.8	8	g, i
IV	Carnivora—Perissodactyla	80.3–82.2	75.8–87.6	8	g, h
IV	Carnivora—Chiroptera	69.5–71.5	66.0–76.0	4	f, g
IV	Cetartiodactyla—Perissodactyla	88.8–97.5	83.4–104.3	4	h, i
IV	Cetartiodactyla—Chiroptera	76.1–79.4	71.8–84.4	2	f, i
IV	Chiroptera—Perissodactyla	72.5–73.2	68.8–77.3	2	f, h

^a See Methods for details about calibration points. Includes only those quartets that fit the constrained two-rate model. All quartets using the lagomorph pair (calibration point d) were rejected. MYA = million years ago.

observed in previous studies could have been influenced by undetected and therefore uncorrected differences in gene divergence rates among lineages. Bromham et al. (2000) pointed out that currently available rate constancy tests often fail to detect moderate levels of rate heterogeneity, potentially leading to overestimates of divergence dates. Rate heterogeneity may have introduced a bias in the previously reported very old divergence estimates between primates and rodents (Easteal 1990; Kumar and Hedges 1998), and also in the phylogenetic position of rodents in several molecular studies (e.g., Reyes et al. 2000). More recent and extensive molecular analyses (Huchon et al. 2000; Madsen et al. 2001; Murphy et al. 2001) have observed that, with increased taxon sampling and larger nucleotide datasets, rodents are not basal among eutherians, rather they comprise an internal group allied to lagomorphs (cohort Glires), a view consistent with previous inference developed from strong morphological evidence (Novacek 1992). The only other major point of discrepancy derives from Kumar and Hedges' (1998) estimate of the Xenarthra-Primates divergence at about 129 MYA, however, this calculation was based on only three genes and exhibited a large variance (Hedges and Kumar 1999). The remaining estimates provided by Kumar and Hedges (1998) are actually compatible with a rapid diversification of placental mammals and are largely consistent with our results (Table 5). For example, Kumar and Hedges' (1998) estimate of the divergences between Afrotheria versus Primates (105 ± 6.6 MYA), Ferungulata (part of our clade IV) versus Primates (92 ± 1.3 MYA), and within Ferungulata (74 ± 5.7 MYA to 83 ± 4 MYA) clearly overlap with our 95% confidence intervals (Table 5).

Regarding the age of these divergences, the quartet dating analyses indicate minimum dates for the basal eutherian nodes that are almost exclusively pre-K-T boundary (Table 5), thus supporting previous independent molecular studies (Cooper and Penny 1997; Hedges et al. 1996; Kumar and Hedges 1998). Given the observed variance around these estimates, we conclude that even larger datasets will be needed to produce narrower confidence intervals. The accuracy of such estimations will also be improved by the availability of progressively more reliable fossil calibrations. In spite of these limitations, our results strongly indicate that the basal eutherian radiation did occur prior to the K-T boundary (perhaps preceding it by tens of millions of years), although not dispersed over the long period suggested by previous molecular studies.

In the absence of compelling molecular evidence linking the age of the primary placental mammal divergences with the K-T boundary, some authors noted that the timing of placental diversification coincides with that of Cretaceous continental breakup (Hedges et al. 1996; Kumar and Hedges 1998). However, this provocative hypothesis had no phylogenetic pattern of diversification that might correlate with specific geological events during the Cretaceous (but see Springer et al. 1997). The topology and timing reported here provide support for a role of vicariant events in the early diversification of placental mammals and implicate the breakup of the southern supercontinent Gondwanaland in the earliest eutherian divergences. Similar patterns of vicariance (the historical separation of formerly continuous faunas as a result of geographic barriers) have been implicated in the diversification of Gondwanan taxa within several other ver-

tebrate groups, including fishes (Murphy and Collier 1997; Farias et al. 1999), amphibians (Feller and Hedges 1998), and birds (Cooper and Penny 1997; van Tuinen et al. 1998).

If one interprets the known geographic distribution of fossils from the four major groups in the context of the basal positions of the two southern hemisphere clades [Afrotheria (Africa) and Xenarthra (South America)] (Figure 1; see also Madsen et al. 2001; Murphy et al. 2001) and the 70–110 MYA estimated date of the basal divergences (Table 5), the results suggest a southern origin for placental mammals prior to the complete breakup of the southern supercontinent Gondwanaland. Considering our molecular data in light of geological and paleontological evidence, the most probable scenario depicts an ancestral placental radiation occurring in Gondwanaland (possibly Africa) around 95–110 MYA, with some biogeographic event isolating the ancestor of Afrotheria in Africa prior to the isolation of Xenarthra in South America. This must have occurred no later than 95 MYA (possibly as early as 105 MYA), since this is the youngest estimated date for the breakup of Africa and South America (Smith et al. 1994). The progenitors of clades III and IV (Figures 1 and 2) emerged shortly thereafter, and their fossil distribution suggests a general Laurasian (northern continents) origin (Benton 1993; Carrol 1988; Dawson and Krishtalka 1984). Paleogeographic reconstructions indicate that Eurasian continental fragments were in close proximity to northern Africa in the Late Cretaceous (Scotese 2000; Smith et al. 1994), providing a potential corridor for migration to and dispersal through northern continents by the ancestors of clades III and IV.

Two alternative scenarios cannot be statistically rejected by our data (Murphy et al. 2001) given the uncertainty of the exact position of the root. The first is a sister group relationship between Afrotheria and Xenarthra, implying a strict drift-vicariance hypothesis relating to the separation of Africa and South America. Second is a hypothesis depicting Xenarthra as the most basal eutherian lineage, which would be in agreement with current morphological views (McKenna and Bell 1997), and would suggest that the early placental radiation took place in an interval constrained between 105 MYA and 65 MYA.

Two other points can be made that further bridge the discrepancy between fossil and molecular views of the early eutherian radiation. The phylogenetic resolution of

four primary clades of placental mammals (Madsen et al. 2001; Murphy et al. 2001; this study) indicates that each clade retains insectivorous species that bear features some believe are primitive for all placentals. This is in agreement with the hypothesis that the earliest lineage-splitting events in eutherian history were decoupled from the subsequent morphological diversification that culminated in extant groups, most likely after the K-T boundary (Easteal 1999; Foote et al. 1999). If those primitive insectivorous lineages became initially isolated by continental breakup, the impressive adaptive radiation leading to modern orders seems to have occurred in parallel in different geographic locations (as suggested by Madsen et al. 2001), and may have been constrained in each of them until diverse ecological niches became available after the abrupt demise of the dinosaurs.

A final consideration is the inferred Gondwanan origin for the major eutherian lineages. This hypothesis may help explain the current lack of recognizable fossils of modern placentals prior to the K-T boundary, since the Late Cretaceous mammalian fossil record of the southern hemisphere continents (particularly Africa) is relatively poorly known (Foote et al. 1999). These findings, inferences, and their biogeographic implications may help resolve some of the previous discrepancies between paleontological and molecular approaches, and will hopefully stimulate further investigation into the plausible origin of all major extant mammal lineages (monotremes, marsupials, and eutherians) in the southern continents.

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