

consideration of primary and secondary structures. Sequence positions for which putative homology could not be asserted were excluded. Phylogenetic relationships are inferred by the neighbour-joining method with the PHYLIP package, version 3.5c (ref. 30). Evolutionary distances were calculated with the Kimura two-parameter model (transition:transversion ratio = 2.0). Bootstrap methods provided confidence estimates for tree topology. To avoid potential bias, taxon addition order was randomized.

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Parallel adaptive radiations in two major clades of placental mammals

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Higher level relationships among placental mammals, as well as the historical biogeography and morphological diversification of this group, remain unclear^{1–3}. Here we analyse independent molecular data sets, having aligned lengths of DNA of 5,708 and 2,947 base pairs, respectively, for all orders of placental mammals. Phylogenetic analyses resolve placental orders into four groups: Xenarthra, Afrotheria, Laurasiatheria, and Euarchonta plus Glires. The first three groups are consistently monophyletic with different methods of analysis. Euarchonta plus Glires is monophyletic or paraphyletic depending on the phylogenetic method. A unique nine-base-pair deletion in exon 11 of the *BRCA1* gene provides additional support for the monophyly of Afrotheria, which includes proboscideans, sirenians, hyracoids, tubulidentates, macroscelideans, chrysochlorids and tenrecids. Laurasiatheria contains cetartiodactyls, perissodactyls, carnivores, pangolins, bats and eulipotyphlan insectivores. Parallel adaptive radiations have occurred within Laurasiatheria and Afrotheria. In each group, there are aquatic, ungulate and insectivore-like forms.

The combination of fossil and anatomical data has suggested moderately well-resolved phylogenetic trees for the 18 extant orders of placental mammals^{1,2}. DNA sequences have provided consistent support for only a few of the proposed superordinal groups, notably for Paenungulata (elephants, sea cows and hyraxes) and Cetartiodactyla (artiodactyls and whales)³, and have rejected some traditional clades such as Archonta (primates, tree shrews, flying lemurs and bats)⁴. Molecular data have also suggested new sets of relationships; Afrotheria, a group that includes paenungulates, aardvarks, elephant shrews, golden moles and tenrecs, is supported by both mitochondrial ribosomal RNA and nuclear protein-coding genes^{5,6}. These same sequences suggest that lipotyphlan insectivores are paraphyletic or polyphyletic.

Increased resolving power may result from concatenations of individual genes⁷. We concatenated DNA sequences for mitochondrial RNA genes and three nuclear genes (A2AB, IRBP, vWF), including 16 new sequences (see Methods). This data set includes 26 placental taxa, representative of all eutherian orders, and two marsupial outgroups (see Fig. 1A). In addition, golden mole and tenrec, belonging to the insectivore families Chrysochloridae and Tenrecidae, respectively, are represented by sequences for all genes

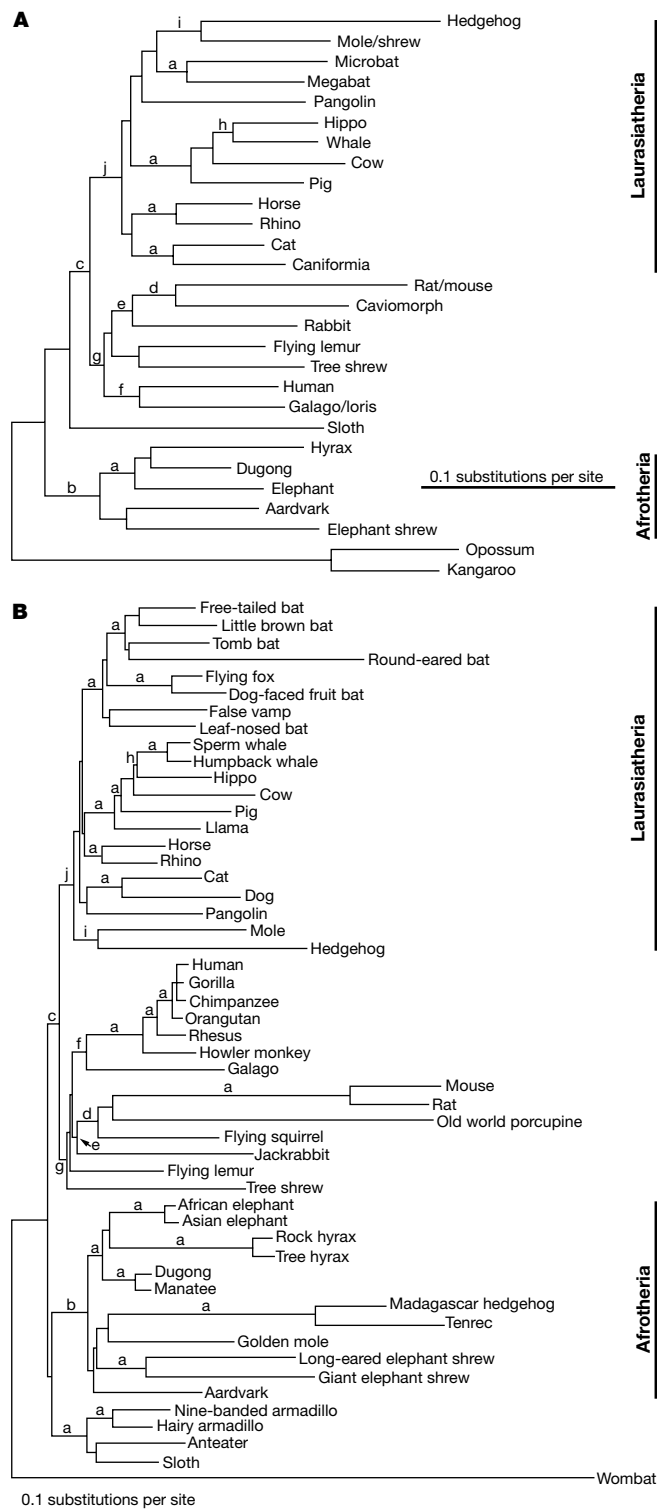


Figure 1 Rooted maximum-likelihood trees. **A**, The 5,708-bp data set. **B**, The 2,947-bp data set. For chimaeric sequences in **A**, the combined species or the higher taxonomic unit are indicated. Branch lengths are proportional to the amount of sequence change (scale bar, 10% sequence divergence). Bootstrap support values for nodes b–j with different phylogenetic methods are shown in Table 1; nodes labelled a are supported at or above 90% by all methods. Afrotheria also includes golden moles and tenrecs, which are not represented by IRBP sequences, and therefore not shown in **A**; in analyses with the 5,708-bp data set that included golden mole and tenrec, bootstrap support for Afrotheria ranged from 88% to 100%. For the 5,708-bp data set, the 12 statistically acceptable

branches for the location of the root, in decreasing order of likelihood score, are branch b (as shown), sloth, rabbit, branch leading to rat/mouse, branch c, branch d, branch e, caviomorph, branch j, tree shrew, branch leading to flying lemur + tree shrew, and galago/oris; other branches were rejected. For the 2,947-bp data set, the statistically acceptable options, in decreasing order of likelihood score, were branch c (as shown), branch b, and the base of Xenarthra. The name Laurasiatheria (“from the area of Laurasia or Europe + Asia + North America”)¹⁸ was suggested for this clade, but no support in the form of bootstrap analyses and/or statistical tests was provided.

except IRBP; inclusion of these taxa in a subset of analyses was necessary to investigate the proposed diphyly of the insectivores and to confirm the naturalness of Afrotheria. In all, 5,708 aligned nucleotide positions were used in phylogenetic analyses.

Hypothesis testing with independent data sets is fundamental in phylogenetics. To establish a data set that was independent of the 5,708-bp concatenation, we sequenced roughly 3 kilobases (kb) of exon 11 of the single-copy breast and ovarian cancer susceptibility gene 1 (*BRCA1*) for 33 taxa and combined these with 19 GenBank sequences (see Methods). The aligned *BRCA1* data set includes representatives of all placental orders and one marsupial outgroup (for names, see Fig. 1B). Finally, we combined the 5,708-bp and 2,947-bp data sets (total 8,655 bp) for 26 overlapping placentals and one marsupial.

Phylogenetic trees (maximum likelihood) for the 5,708-, 2,947- and 8,655-bp data sets are shown in Fig. 1A and B, and Supplementary Information, respectively. Bootstrap support values for different phylogenetic methods are given in Table 1 for the 5,708- and 2,947-bp data sets, and in Supplementary Information for the 8,655-bp data set. In spite of the differences in taxonomic sampling, as well as some conspicuously long branches on the *BRCA1* tree, placental orders are resolved into the following four groups on all three maximum-likelihood trees: Xenarthra, Afrotheria, Laurasiatheria, and Glires (rodents and lagomorphs) + Euarchonta (flying lemurs, tree shrews and primates). Xenarthra, Afrotheria, and Laurasiatheria are robust with different phylogenetic methods (Fig. 1B; Table 1; Supplementary Information). Afrotheria is also supported by a 9-bp deletion that is shared by all 12 afrotherians that were included in our study (Fig. 2). The absence of this deletion in other placentals, as well as in marsupials, indicates that this feature may be a shared derived character of Afrotheria. Despite the absence of any morphological evidence for Afrotheria^{2,8}, it now becomes increasingly difficult to explain this hypothesis except in the context of shared common ancestry. Within Afrotheria, there is strong support for Paenungulata. Our analyses provide the first robust support for the Laurasiatheria clade. Within this clade, there is strong support for Cetartiodactyla and the monophyly of eulipotyphlan insectivores (hedgehogs, shrews and moles).

Support for Euarchonta + Glires was inconsistent, largely because the position of the root was sensitive to the phylogenetic method that was used (Table 1; Supplementary Information). Maximum-likelihood analyses with the *BRCA1* data set provided 100% bootstrap support for the monophyly of Glires + Euarchonta, whereas parsimony provided only 18% support. The shortest parsimony trees (two at 10,867 steps; Supplementary Information) for *BRCA1* root on Old World porcupine, rendering Euarchonta + Glires, Glires and Rodentia paraphyletic; 35 additional steps are required to root in the same location as the likelihood tree shown in Fig. 1B. Among the individual bootstrap replicate trees for parsimony, rootings occurred on long, topologically separated branches as

follows: Old World porcupine (69%); rat-mouse (11%); Madagascar hedgehog-tenrec (11%); tree shrew (4%); hedgehog (3%); and round-eared bat (2%). Maximum likelihood did not root on these long branches. Instead, there was 100% bootstrap support for the monophyly of Laurasiatheria + Euarchonta + Glires (branch c), and individual bootstrap replicate trees, with or without an allowance for rate-heterogeneity, rooted on branch b (base of Afrotheria), branch c or at the base of Xenarthra; these three branches are topologically adjacent, which suggests a strong affinity of the root for this general region of the tree. Furthermore, statistical tests with likelihood reject rooting the *BRCA1* tree on all branches except for these three. Finally, Monte Carlo simulations mirrored our results for the actual *BRCA1* data set and showed that parsimony will root on long branches and fail to recover the correct root given the topology depicted in Fig. 1B (Supplementary Information).

Twelve roots are statistically acceptable on the likelihood tree for the 5,708-bp data set (Fig. 1A). The three possible *BRCA1* roots coincide with three of the five best roots on the 5,708-bp data set tree. These are also the three best roots for the 8,655-bp data set. Although several roots in the Euarchonta + Glires group could not be rejected with the 5,708- and 8655-bp data sets, we do not favour these roots. First, there are numerous morphological synapomorphies for both rodent monophyly⁹ and Glires². Second, other molecular results also favour rodent monophyly and even the monophyly of Glires, when phylogenetic methods are used that address problems such as nonstationarity and long-branch attraction^{10,11}.

Afrotheria and Xenarthra have observed first occurrences in the fossil record on Southern Hemisphere landmasses—Africa in the case of Afrotheria^{3,6} and South America in the case of Xenarthra¹². First occurrences in the fossil record do not permit unequivocal assignment of continental origin. Nevertheless, there is a strong possibility that Afrotheria and Xenarthra have Gondwanan origins. Of the three roots (base of Afrotheria, branch b; base of Laurasiatheria + Euarchonta + Glires, branch c; base of Xenarthra) that are statistically acceptable on all three trees, rooting on the xenarthran branch is the only hypothesis that is consistent with the morphology-based Epitheria hypothesis². At the same time, rooting on the Xenarthra branch results in paraphyly of a possible Gondwanan group (i.e., Xenarthra + Afrotheria) at the base of the placental tree. Rooting on branch b also results in paraphyly of this Southern Hemisphere group. The suggestion that a Gondwanan assemblage is paraphyletic at the base of the tree contrasts with traditional views on the evolution of placental mammals, which place their most recent common ancestor in the Northern Hemisphere¹³. Considering the debate about the timing of the principal placental divergences^{14,15}, the paucity of Cretaceous mammal fossils from the Southern Hemisphere¹⁵, and the discoveries of tribosphenic fossils in the Southern Hemisphere, including a possible placental¹⁶, a Gondwanan origin for extant placentals should not be excluded. A Southern Hemisphere ancestry for

Table 1 Bootstrap support percentages for branches b–j in Fig. 1 with different phylogenetic methods

Node	Parsimony				Distance methods						Maximum likelihood			
	Unweighted		Transversion		NJ-WAVE		ML-GTR		Logdet		No rate heterogeneity		Rate heterogeneity	
	5,708	2,947	5,708	2,947	5,708	2,947	5,708	2,947	5,708	2,947	5,708	2,947	5,708	2,947
b	100	89	100	83	100	96	100	76	100	94	100	100	100	100
c	53	11	62	29	51	56	19	40	30	39	45	100	64	100
d	79	22	74	30	41	69	99	48	100	61	80	99	94	99
e	53	3	4	26	5	34	42	17	54	15	41	67	59	68
f	97	88	71	83	98	89	99	84	99	85	99	99	96	98
g	48	18	58	30	6	49	5	35	10	34	44	100	66	100
h	93	13	72	42	100	85	100	88	100	88	98	36	96	44
i	95	95	94	100	100	100	76	99	74	99	100	100	96	100
j	96	93	87	97	76	97	81	89	78	98	99	100	99	100

In the first row, 5,708 and 2,947 refer to the 5,708-bp and 2974-bp data sets, respectively. In maximum-likelihood analyses that did not allow for rate heterogeneity, transition/transversion ratios were set of 1.81 and 2.22 for the 5,708- and 2947-bp data sets, respectively. In maximum-likelihood analyses that allowed for rate heterogeneity, transition/transversion ratios were set of 1.89 and 2.24 for the 5,708- and 2947-bp data sets, respectively. ML-GTR, maximum likelihood with general time reversible model; WAVE, weighted average distances (see Methods).

acid repeat region of the A2AB gene and a 21-bp region of the *BRCA1* gene that is repeated up to four times. The aligned data sets were the following lengths: A2AB (1,164 bp); IRBP (1,292 bp); vWF (1,251 bp); 12S rRNA/tRNA valine-16S rRNA (2,001 bp); and *BRCA1* (2,947 bp). Alignments for the concatenated (A2AB + IRBP + vWF + rRNA) and *BRCA1* data sets are available in the Supplementary Information. Phylogenetic analyses included unweighted and transversion parsimony, minimum evolution (5,708-bp data set) or neighbour joining (*BRCA1* data set) with logdet and maximum likelihood-GTR²⁴ distances, neighbour joining with weighted average (WAVE) maximum likelihood distances²⁵, and maximum likelihood under the HKY85 (ref. 24) model of sequence evolution. Gaps were coded as missing in parsimony analyses. Maximum-likelihood estimates of relative rates and transition to transversion ratios were obtained from maximum parsimony trees and used in subsequent maximum-likelihood analyses and in calculating weighted-average maximum-likelihood distances. Bootstrap support values are based on 500 replications except for maximum likelihood (100 replications). Maximum-likelihood bootstrap analyses with the *BRCA1* data set used the following backbone constraint, where taxon numbers correspond to the ordering of taxa (top to bottom) in Fig. 1B: (((1–4), (5, 6), (7, 8)), ((9, 10), 11–14), (15, 16), (17, 18), 19–21, (((22–24), 25), 26), 27), 28, (29, 30), 31–35, ((36, 37), (38, 39), (40, 41)), ((42, 43), 44), (45, 46), 47, ((48, 49), 50, 51)). Kishino and Hasegawa tests²⁴ were used to examine *a priori* hypotheses and to examine statistically acceptable root locations. In the latter case, we obtained the best unrooted likelihood tree for each data set and then evaluated all possible root positions. All phylogenetic analyses and statistical tests were performed with PAUP 4.0b2 (ref. 26), except for neighbour-joining with weighted average distances, where analyses were performed with PHYLIP 3.572 (J. Felsenstein) and WAVEBOOT (D. King and C. Krajewski). Maximum-likelihood analyses with rate partitions allowed the following eight rate partitions with the 5,708-bp data set: third positions of each nuclear gene; first + second positions of each nuclear gene; RNA stems; and RNA loops. Two rate partitions, corresponding to first + second and third codon positions, respectively, were used with the *BRCA1* data set. NJ-WAVE analyses used a weighted-average distance approach²⁵ with the eight partitions indicated above for the 5,708-bp data set and two partitions (first + second codon positions; third codon positions) for the 2,947-bp data set; each partition was allowed its own rate, base composition, and transition to transversion ratio. Monte Carlo simulations were performed with Seq-Gen 1.1 (ref. 27) (Supplementary Information). Molecular dates were estimated using QDATE²⁸ (Supplementary Information).

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Molecular phylogenetics and the origins of placental mammals

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The precise hierarchy of ancient divergence events that led to the present assemblage of modern placental mammals has been an area of controversy among morphologists, palaeontologists and molecular evolutionists. Here we address the potential weaknesses of limited character and taxon sampling in a comprehensive molecular phylogenetic analysis of 64 species sampled across all extant orders of placental mammals. We examined sequence variation in 18 homologous gene segments (including nearly 10,000 base pairs) that were selected for maximal phylogenetic informativeness in resolving the hierarchy of early mammalian divergence. Phylogenetic analyses identify four primary superordinal clades: (I) Afrotheria (elephants, manatees, hyraxes, tenrecs, armadillo and elephant shrews); (II) Xenarthra (sloths, anteaters and armadillos); (III) Glires (rodents and lagomorphs), as a sister taxon to primates, flying lemurs and tree shrews; and (IV) the remaining orders of placental mammals (cetaceans, artiodactyls, perissodactyls, carnivores, pangolins, bats and core insectivores). Our results provide new insight into the pattern of the early placental mammal radiation.

The panoply of morphological, ecological and genomic diversity among extant mammals offers considerable potential for studies of speciation, adaptation, molecular evolution, genome organization and biogeography^{1–3}. Studies on morphology and both mitochondrial and nuclear genes have revealed several higher level phylogenetic associations^{2,4–7}, but a full resolution of the earliest placental