

*Phylogenetics series*

Molecules consolidate the placental mammal tree

Mark S. Springer¹, Michael J. Stanhope², Ole Madsen³ and Wilfried W. de Jong³¹Department of Biology, University of California, Riverside, CA 92521, USA²Bioinformatics, GlaxoSmithKline, Collegeville, PA 19426, USA³Department of Biochemistry, University of Nijmegen, 6500 HB Nijmegen, the Netherlands

Deciphering relationships among the orders of placental mammals remains an important problem in evolutionary biology and has implications for understanding patterns of morphological character evolution, reconstructing the ancestral placental genome, and evaluating the role of plate tectonics and dispersal in the biogeographic history of this group. Until recently, both molecular and morphological studies provided only a limited and questionable resolution of placental relationships. Studies based on larger and more diverse molecular datasets, and using an array of methodological approaches, are now converging on a stable tree topology with four major groups of placental mammals. The emerging tree has revealed numerous instances of convergent evolution and suggests a role for plate tectonics in the early evolutionary history of placental mammals. The reconstruction of mammalian phylogeny illustrates both the pitfalls and the powers of molecular systematics.

Are we, humans, more closely related to mice or to cows and dogs? A long history of debate surrounds this and other questions pertaining to relationships among the orders of placental mammals. Difficulties in reconstructing relationships among the orders have been attributed to a rapid radiation following the Cretaceous–Tertiary boundary [1]. Even if we consult the recent literature, we find that the relationship of primates to other placental orders is the subject of fierce debate. There are many contradictory hypotheses about placental mammal relationships, both between and among molecules and morphology. Yet, it is clear that knowing the actual pattern of mammalian phylogeny is very important, not only because it reveals our own genealogy, but also because this family tree provides the framework to interpret the evolution of morphological, physiological, behavioral, and genomic features that characterize different mammalian taxa. Understanding placental mammal phylogeny is also a crucial prerequisite for unraveling the biogeographical history of this group. Mammals are better known from morphological and molecular data than are all other

groups. Thus, reconstructing their phylogeny can serve as a model for research on other organisms.

Here, we highlight that, in spite of the ongoing debate, the congruence of most recent molecular evidence is striking and consensus is approaching rapidly. Progress has been achieved by using larger and more diverse molecular datasets, increasing taxon sampling to subdivide long branches, and using LIKELIHOOD (see Glossary) methods of phylogeny reconstruction that explicitly model the nucleotide substitution process and are less susceptible to problems of STATISTICAL INCONSISTENCY than are methods such as MAXIMUM PARSIMONY [2]. In addition, results of phylogenetic analyses that rely on nucleotide or amino acid substitutions are now complemented by rare genomic changes (RGCs; Box 1) that constitute genetic markers of common descent. The major molecular finding is that the 18 placental orders are divided into four clades, of which three were never suspected based on morphology. Here, we discuss the reliability of the new tree, discuss reasons for earlier discrepancies, highlight the remaining problems and offer a prospectus on future studies.

The growth of molecular consensus

Until the advent of molecular approaches, mammalian phylogeny was necessarily the domain of morphology and paleontology. Since Darwin, the study of placental mammal relationships has seen episodic development and has culminated in a morphological tree that remains prominent in the current literature ([3–5]; Figure 1a). Variations of this tree largely conform to the topology of ordinal relationships proposed by Novacek [6], which evolved from the mammalian classifications of Gregory in 1910, Simpson in 1945, and McKenna in 1975. The major characteristics of this tree are that Xenarthra (e.g. armadillos, anteaters) are the most basal placental group, and that most of the remaining orders are grouped into three generally accepted clades: (i) UNGULATA, (ii) ARCHONTA, and (iii) ANAGALIDA. This topology deviates in essential aspects from the currently emerging molecular tree, which recognizes three novel superordinal clades: AFROTHERIA, LAURASIATHERIA and EUARCHONTOGLIRES, the latter two of which are SISTER GROUPS [i.e. BOREOEUTHERIA; Figure 1b].

Corresponding author: Mark S. Springer (mark.springer@ucr.edu).

Available online 17 June 2004

Glossary

Afrotheria: the molecular superordinal hypothesis that includes the orders Proboscidea (elephants), Sirenia (manatees and dugongs), Hyracoidea (hyraxes), Tubulidentata (aardvarks), Afrosoricida (golden moles and tenrecs) and Macroscelidea (elephant shrews).

Anagalida: the morphology-based superordinal hypothesis that includes Rodentia (e.g. rats, mice and guinea pigs), Lagomorpha (rabbits, hares and pikas) and Macroscelidea (elephant shrews).

Archonta: the morphology-based superordinal hypothesis that includes Chiroptera (bats), Dermoptera (flying lemurs), Primates (e.g. humans, apes and monkeys) and Scandentia (tree shrews).

Analogy: characters that have similar functions, but that evolved independently in different groups and are not descended from a common ancestral precursor character.

Atlantogenata: the molecular superordinal hypothesis that includes the order Xenarthra (sloths, armadillos and anteaters) and the superordinal group Afrotheria.

Boreoeutheria: the molecular superordinal hypothesis that includes the superordinal groups Euarchontoglires and Laurasiatheria.

Condylarth: an extinct group of primitive hoofed mammals.

Diphyletic: a group with two separate origins. For example, Edentata is diphyletic on the molecular tree because xenarthrans and pangolins have separate origins and do not share a common ancestor with each other to the exclusion of other placental mammals.

Euarchontoglires: the molecular superordinal hypothesis that includes the orders Rodentia (e.g. rats, mice and guinea pigs), Lagomorpha (rabbits, hares and pikas), Scandentia (tree shrews), Dermoptera (flying lemurs) and Primates (e.g. humans, apes and monkeys).

Eutheria: a stem group that includes Placentalia plus extinct mammalian taxa that are outside of Placentalia but more closely related to placentals than to marsupials.

Fossorial: a term that is used to describe animals that are adapted to digging, such as moles and golden moles.

Glires: the morphology-based superordinal hypothesis that includes Rodentia (e.g. rats, mice, guinea pigs) and Lagomorpha (rabbits, hares, pikas).

Homology: characters are homologous if they trace back to a common ancestral precursor character.

Homoplasy: molecular or morphological similarities that evolved independently in different lineages and were not inherited from a common ancestor.

Laurasiatheria: the molecular superordinal hypothesis that includes the orders Eulipotyphla (hedgehogs, moles and shrews), Chiroptera (bats), Perissodactyla (horses, tapirs, and rhinos), Cetartiodactyla (e.g. camels, pigs, cows, hippos, whales and porpoises), Carnivora (e.g. dogs, bears and cats) and Pholidota (pangolins).

Maximum likelihood: in phylogenetics, the maximum likelihood estimate of the phylogeny is the hypothesis (e.g. evolutionary tree) that gives the highest probability of observing the data (e.g. nucleotide sequences).

Maximum parsimony: a phylogeny reconstruction method that searches for one or more trees that minimize the number of evolutionary changes that are required to explain the observed differences among taxa included in the study.

Monophyletic: a group that includes a common ancestor and all its descendants.

Paenungulata: the morphology-based superordinal hypothesis that includes the orders Hyracoidea (hyraxes), Sirenia (manatees and dugongs) and Proboscidea (elephants).

Paraphyletic: a group that includes a common ancestor but only a fraction of its descendants.

Placentalia: a crown group that includes the most recent common ancestor of all placental mammal and all the descendants, living and extinct, of this common ancestor.

Sister groups: taxa that are each other's closest relatives.

Statistical inconsistency: in phylogenetics, methods are consistent when they converge on the correct answer given enough data. Conversely, inconsistent methods will converge on an incorrect answer given enough data.

Ungulata: the morphology-based superordinal hypothesis that includes the orders Hyracoidea (hyraxes), Sirenia (manatees and dugongs), Proboscidea (elephants), Perissodactyla (horses, tapirs and rhinos), Artiodactyla (e.g. camels, pigs, cows, pigs), Cetacea (e.g. whales and porpoises) and, variably, Tubulidentata (aardvarks).

Some conspicuous features of the present molecular tree emerged during the 1980s, when comparative sequencing was performed on proteins such as hemoglobins, myoglobin, α A-crystallin, cytochrome *c* and ribonuclease [7]. In spite of the limited ordinal representation, these protein sequences separated PAENUNGULATES (e.g. elephants, hyraxes, dugongs) from the other

ungulates, and placed them with aardvarks in what later became the Afrotheria.

As sequences for complete mitochondrial genomes became available, molecular studies of interordinal relationships were dominated by these data. This led to various unorthodox proposals, some of which have now been well corroborated, notably the sister-group relationship of whales to hippos [8] and the grouping of bats closer to ungulates rather than to primates [9]. Indeed, all subsequent sequence data [10], as well as SINE insertions [11] and a cladistic analysis of morphological characters [12], support an artiodactyl ancestry for Cetacea, whereas bats became firmly nested within Laurasiatheria (Figure 1b). The proposals that the guinea pig is not a rodent [13], that hedgehog or rodents are the oldest placental offshoots [14], and that the egg-laying monotremes are the sister-group of marsupials [15] were strongly advocated based on mitochondrial DNA (mtDNA) data and still persist. These hypotheses have provoked much discussion about the reliability of deeper phylogenetic inference from mitochondrial data [16], but are now contradicted by both morphological and other molecular evidence supporting rodent monophyly (including guinea pigs), a more nested position for hedgehogs within the placental tree, and a sister group relationship between placentals and marsupials (Figure 1). The use of PCR also made the comparative sequencing of nuclear genes feasible. In general, phylogenetic analyses of nuclear gene segments (i) led to poorly resolved and unstable topologies; and (ii) showed that single genes can give misleading topologies. However, analyses of individual nuclear genes agree with more recent molecular studies in supporting the whale-hippo clade, Paenungulata and Afrotheria, including enlarging the latter clade to also include elephant shrews, golden moles and tenrecs [17].

A shortcoming of most molecular studies from the 1980s and 1990s was incomplete and unbalanced taxon sampling that was also mostly based on relatively short segments of single genes. Nevertheless, by combining evidence from various separate analyses, a division of all placentals into the four currently recognized major clades (Figure 1b) was first proposed by Waddell *et al.* [18]. Solid support for these superordinal groups has come from independent studies that concatenated DNA sequences from many different nuclear genes, including representatives of all extant placental orders [19–25]. Subsequently, additional support for the four major clades has emerged from analyses of the complete set of mitochondrial tRNA and rRNA gene sequences [26]. Analyses of mitochondrial protein-coding sequences have returned mixed results, but reconciliation with nuclear trees is reached when methods that mitigate against known phylogeny reconstruction problems are employed [27,28] and/or taxon sampling is improved [29]. Beyond sequence analyses, the four major clades are forcefully corroborated by RGCs (Box 1).

Considerable resolution within the four major groups has also been achieved. Within Afrotheria, molecular phylogenies support Paenungulata, which also appears in several morphological classifications [30]. A novel molecular result is a sister-group relationship between elephant shrews and golden moles + tenrecs [21,25]. Fetal

Box 1. Rare genomic changes in mammalian phylogenetics

Rare genomic changes (RGCs) include events such as insertions or deletions (indels), retrotransposon integrations, diagnostic amino acid signatures, changes in gene order or genome organization, gene duplications, and genetic code changes [55,56]. RGCs have become increasingly important in systematics and complement phylogenetic analyses of primary sequence data. It has been argued that they constitute excellent markers of common descent (synapomorphies or shared derived characters) because homoplasy and secondary loss are less likely than for single nucleotide substitutions. RGCs can serve as

arbiters in cases where primary sequences generate conflicting or inconclusive results. Table I lists important RGCs that have contributed to our understanding of higher-level placental phylogenetics. Figure 1 illustrates deletions that support Euarchontoglires and Afrotheria. In spite of their usefulness in higher-level systematics, RGCs are not immune to homoplasy and other problems and must be interpreted with caution [57]. Waddell *et al.* [22] provide a statistical framework for testing alternate hypotheses using SINE data that explicitly addresses the gene tree/species tree problem.

Table I. Important rare genomic changes (RGCs) in placental mammal systematics

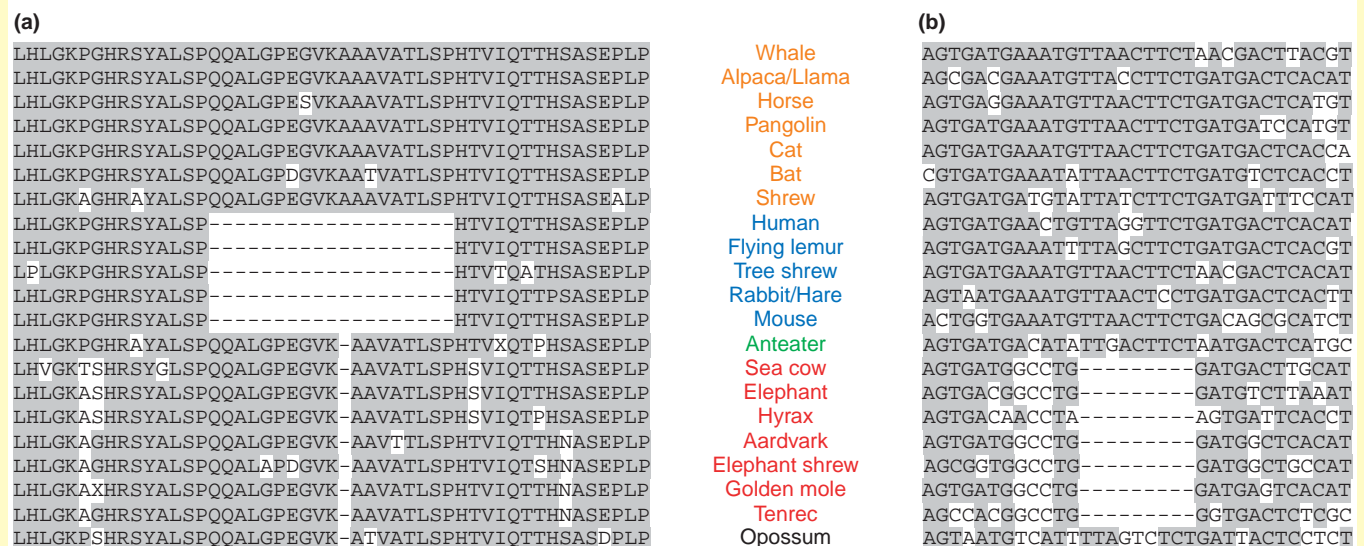
RGC ^a	Clade supported	Refs
79–82 amino-acid deletion in aligned <i>APOB</i> sequences	Afrotheria	[24]
Chromosomal rearrangements ^b	Afrotheria	[58]
AfroSINEs ^c	Paenungulata	[59]
3 amino-acid deletion in α A-crystallin protein	Xenarthra	[60]
6-bp deletion in <i>PRNP</i>	Euarchontoglires	[57,61]
18 amino-acid deletion in <i>SCA1</i> protein alignment	Euarchontoglires	[57,61]
MLT1A0 element insertions ^d	Euarchontoglires	[62]
10-bp deletion in aligned sequences for the 5' untranslated region of the <i>PLCB4</i> gene	Laurasiatheria	[63]
363-bp deletion in aligned <i>APOB</i> sequences	Carnivora + Pholidota	[24]
SINE insertions	Hippopotamidae + Cetacea	[11,22]
LINE1 insertion between exons 40 and 41 of the <i>COLIA2</i> gene	Primates	[33]
FLAM integration between exons 5 and 6 of the <i>HBX2</i> gene	Primates	[33]
Presence of Alu SINEs	Primates	[33]

^aAbbreviations: *APOB*, apolipoprotein B; *COLIA2*, collagen type I α 2; *FLAM*, free left Alu monomer; *HBX2*, homeobox gene 2; *LINE*, long interspersed nuclear element; *PLCB4*, phosphoinositide-specific phospholipase-C β 4; *PRNP*, prion protein; *SCA1*, spinocerebellar ataxia type 1; *SINE*, short interspersed nuclear element.

^bFroncicke *et al.* [58] identified two chromosomal rearrangements that link the representative afrotherians (African elephant and aardvark) that were investigated: first, a syntenic association of human chromosomes 5 and 21, and, second, a syntenic association of human chromosomes 1 and 19.

^cAfroSINEs are a novel family of short interspersed nuclear elements that are distributed exclusively among afrotherian taxa [59]. This distribution supports the monophyly of Afrotheria. The HSP (Hyracoidea, Sirenia, Proboscidea) subfamily of AfroSINEs contains a 45-bp deletion in the middle region of the SINE and is unique to paenungulate taxa.

^dThree LINE insertions have been detected in rodents and primates, but not in carnivores, artiodactyls, or non-mammalian vertebrates that have been examined [62]. These putative RGCs for Euarchontoglires remain to be investigated in additional taxa.

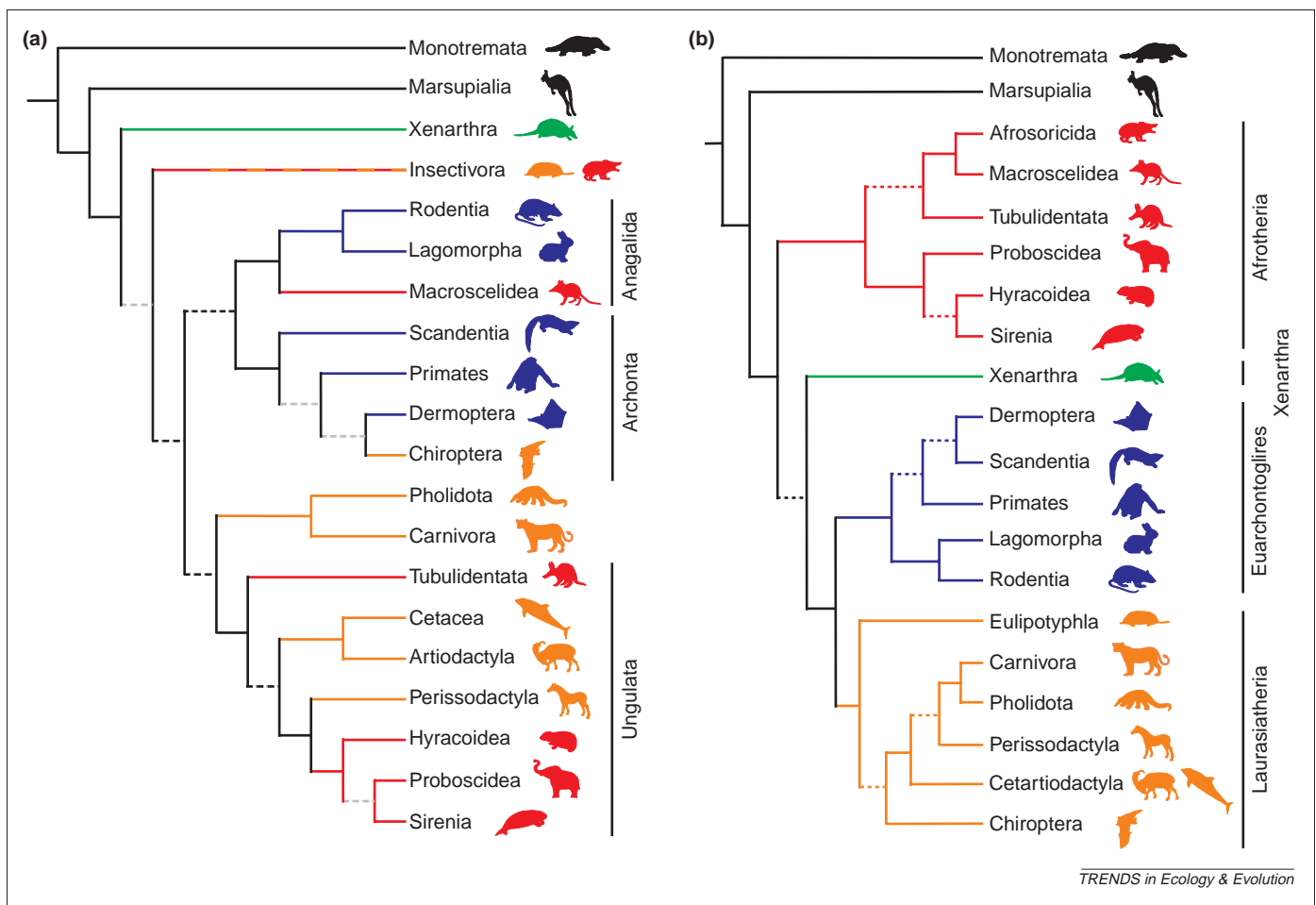


TRENDS in Ecology & Evolution

Figure 1. Examples of rare genomic changes (RGCs) that support the major clades of placental mammals include (a) an 18 amino-acid deletion (relative to outgroup) in the *SCA1* protein for Euarchontoglires [61] and (b) a 9-bp deletion in the *BRCA1* gene (breast and ovarian cancer susceptibility gene 1) for Afrotheria [19]. Color-coding for higher-level taxa is as follows: black, Marsupialia; red, Afrotheria; green, Xenarthra; blue, Euarchontoglires; and orange, Laurasiatheria.

membrane structures provide additional support for this hypothesis [31]. Within Euarchontoglires, there is a fundamental split between GLIRES (rodents + lagomorphs) and Euarchonta (primates + tree shrews + flying

lemurs). Glires is a bastion of morphological trees; Euarchonta differs from the morphological Archonta hypothesis by removing bats from this clade. The molecular exclusion of bats from Archonta requires



TRENDS in Ecology & Evolution

Figure 1. The prevailing morphological tree (a) and the emerging molecular tree (b) of the placental orders. (a) Morphology generally places Xenarthra (sloths, anteaters and armadillos) as basal, and most of the remaining orders into three well-established clades: Ungulata (thought to be derived from CONDYLARTH ancestors, Archonta and Anagalida. The depicted tree is from Shoshani and McKenna [3]. The tree obtained by Liu *et al.* [4] is identical, apart from placing cetaceans as sister group to the perissodactyl-paenungulate clade. The tree of Novacek ([6]; <http://tolweb.org/tree?group=Eutheria&contgroup=Mammalia>) places Pholidota (pangolins) as basal sister to Xenarthra, makes Primates and Scandentia (tree shrews) sister groups, and collapses several clades (black dotted lines). Novacek [5] subsequently collapses some further clades (gray dotted lines), which increases reconciliation with the molecular tree. (b) The molecular tree recognizes four major clades: Afrotheria, Xenarthra, Laurasiatheria and Euarchothoglires, of which the latter two are joined into Boreoeutheria. The presented placental ordinal topology is according to Murphy *et al.* [21]. Placing Marsupialia as sister to Placentalia is based on Phillips and Penny [54] and references therein. Clades indicated by solid lines are, with rare exceptions, supported independently by all other molecular data and analyses [24–29]. Notable exceptions are the strong tendency of mitochondrial protein sequences to place hedgehogs and rodents as basal in the tree [14]. Colors distinguish the four basal placental clades in the molecular tree.

convergent evolution of features related to volancy in bats and flying lemurs, but eliminates the need to postulate the loss of archontan ankle specializations in bats [32]. Complete mtDNA analyses recently placed flying lemurs within primates and render the latter PARAPHYLETIC [14]. However, SINE and LINE insertions [33] and analyses of nuclear genes [21,24] recover traditional primate MONOPHYLY. Within Laurasiatheria, Eulipotyphla (e.g. moles, shrews, hedgehogs) is the probable sister-taxon to the remaining orders. The emerging molecular support for a sister-group relationship between carnivores and pangolins includes concatenated nuclear sequences [21], mitochondrial protein sequences [14] and an RGC (Box 1). Morphologically, carnivores and pangolins are unique among living placental mammals in possessing an osseous tentorium that separates the cerebral and cerebellar compartments of the cranium [3].

Molecular data are also resolving relationships within orders, sometimes with unexpected results. In addition to nesting whales within Artiodactyla, molecular data

separate hippos from other Suiformes (e.g. pigs) [10]. In Eulipotyphla, shrews and hedgehogs group to the exclusion of moles [25,34]. This result contrasts with morphological hypotheses that favor either moles + shrews to the exclusion of hedgehogs or moles + hedgehogs to the exclusion of shrews. In Rodentia, molecular data suggest a novel mouse-related clade that includes murids (mice and rats), dipodids (jerboas), castorids (beavers), geomyids (pocket gophers), heteromyids (pocket mice), anomalurids (scaly-tailed flying squirrels), and pedetids (springhares) [35]. This group had never been proposed based on morphological and paleontological data. Within Chiroptera (bats), both nuclear and mitochondrial sequences favor microbat paraphyly, which has profound implications for understanding the origins of laryngeal echolocation (Box 2).

The deployment of morphological character evolution
Darwin [36] recognized that ANALOGICAL or adaptive characters would be almost valueless to the systematist

Box 2. Bat relationships and the evolution of flight and echolocation

Bats (order Chiroptera) have traditionally been viewed as a monophyletic order and members of the superordinal clade Archonta, which also includes flying lemurs, tree shrews and primates. Bats are the only mammals with the capacity for powered flight. Bat monophyly implies homology of the flight apparatus and a single origin for mammalian flight (Figure 1a).

Chiroptera is divided into the suborders Microchiroptera (microbats) and Megachiroptera (megabats). Microbats are generally smaller than megabats and are characterized by complex laryngeal echolocation systems that transmit, receive and process ultrasonic calls. Megabats, commonly known as Old World fruitbats, have enhanced visual acuity and do not echolocate, with the exception of a few forms that use a different type of echolocation based on tongue-clicks.

In the 1980s, Smith and Madkour [64] suggested that megabats were more closely related to primates than to microbats based on morphological characteristics of the penis. This hypothesis implied that bats were diphyletic and that flight had evolved independently in

microbats and megabats (Figure 1b). Pettigrew and colleagues [65] provided additional support for the 'flying primate' hypothesis by showing that primates and megabats share retino-tectal pathways from the eye to the cortex. Subsequently, both morphological and molecular data falsified the bat diphyly hypothesis and supported traditional bat monophyly [66,67]. Nevertheless, surprising results from molecular studies were the dissociation of bats from Archonta [9] and the nesting of megabats within Microchiroptera (Figure 1c). Microbat paraphyly mandates either dual origins of laryngeal echolocation in rhinolophoid and yangochiropteran microbats or a single origin in the common ancestor of bats followed by loss of this feature in the common ancestor of megabats. In analyses with living taxa only, these possibilities are equally parsimonious. Molecular scaffold analyses based on morphological data for living bat families plus fossil bat genera from the early Eocene favor a single origin of laryngeal echolocation with subsequent loss in the ancestor of megabats [68].

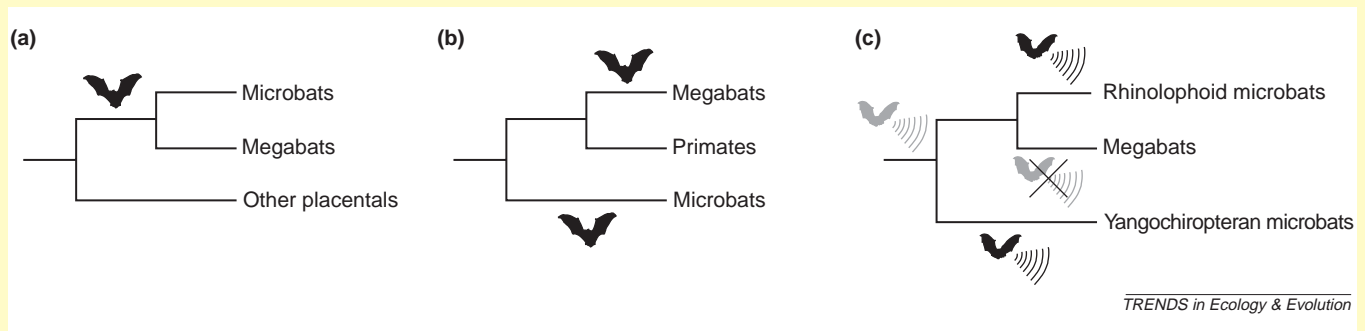


Figure 1. Relationships among the major bat lineages. **(a)** Traditional bat monophyly coupled with a sister-group relationship between the suborders Megachiroptera and Microchiroptera. **(b)** Bat diphyly, with a sister-group relationship between megabats and primates. **(c)** Microbat paraphyly, with a sister-group relationship between megabats and the rhinolophoid microbat families Hipposideridae (Old World leaf-nosed bats), Rhinolophidae (horseshoe bats), Megadermatidae (false vampire bats) and Rhinopomatidae (mouse-tailed bats) [27,69,70]. Silhouettes in (a) and (b) indicate originations of flight. Black and gray silhouettes in (c) indicate alternate scenarios for the evolution of laryngeal echolocation.

and would conceal rather than reveal true blood relationship. Deciphering between HOMOLOGOUS (revealing) characters, which trace back to a common ancestor, and analogous (concealing) characters, which have similar functions but evolved separately in different groups (e.g. bird wings and bat wings), requires independent lines of evidence. Among marsupial and placental mammals, there are numerous examples of taxa that are ecological analogs, including volant forms (sugar gliders versus flying squirrels), FOSSORIAL forms with specializations for digging (marsupial mole versus African golden moles), ant-termite eating forms (Australian numbat versus South American anteater), and carnivores of various sizes (thylacine versus wolf, marsupial sabertooth versus placental sabertooth). In these examples, independent adaptation to similar conditions was revealed through other lines of evidence such as fundamental differences in reproductive anatomy. Unfortunately, deciphering between homologous and analogous characters is less obvious in comparisons of anatomical features among placental mammals.

In light of the molecular tree in Figure 1b, it is clear that both revealing and concealing characters have impacted morphological trees of the orders of placental mammals. Revealing characters include those that support Glires (e.g. loss of upper and lower first incisor) and Paenungulata

(e.g. bones in wrist are dorsoventrally compressed and serially arranged) [37]. Concealing characters have supported clades such as Edentata (xenarthrans + pangolins), Lipotyphla, Ungulata, and Volitantia (bats + flying lemurs). For example, the dissociation of xenarthrans and pangolins on the molecular tree (Figure 1b) suggests that suppression of tooth development and poorly developed (or absent) enamel are features that evolved independently in these two groups. Similarly, flying lemurs share numerous anatomical features with bats including a humeropatagialis muscle extending from the humerus to the patagium (i.e. flight membrane) and extensions of the patagium between the fingers [38]. With the deployment of bats in Laurasiatheria and flying lemurs in Euarchontoglires, shared features of Volitantia must be interpreted as analogous characters that evolved independently in the two orders. Overall, the splintering of numerous morphological groups across the four major clades suggests that there have been extensive parallel adaptive radiations among placental mammals [19,39]. These resemblances are perhaps most striking for taxa in Afrotheria and Laurasiatheria (Figure 2), but also extend to Xenarthra (e.g. anteaters have external features that parallel both pangolins and aardvarks) and Euarchontoglires (e.g. flying lemurs share features with bats). With the identification of HOMOPLASTIC features in different

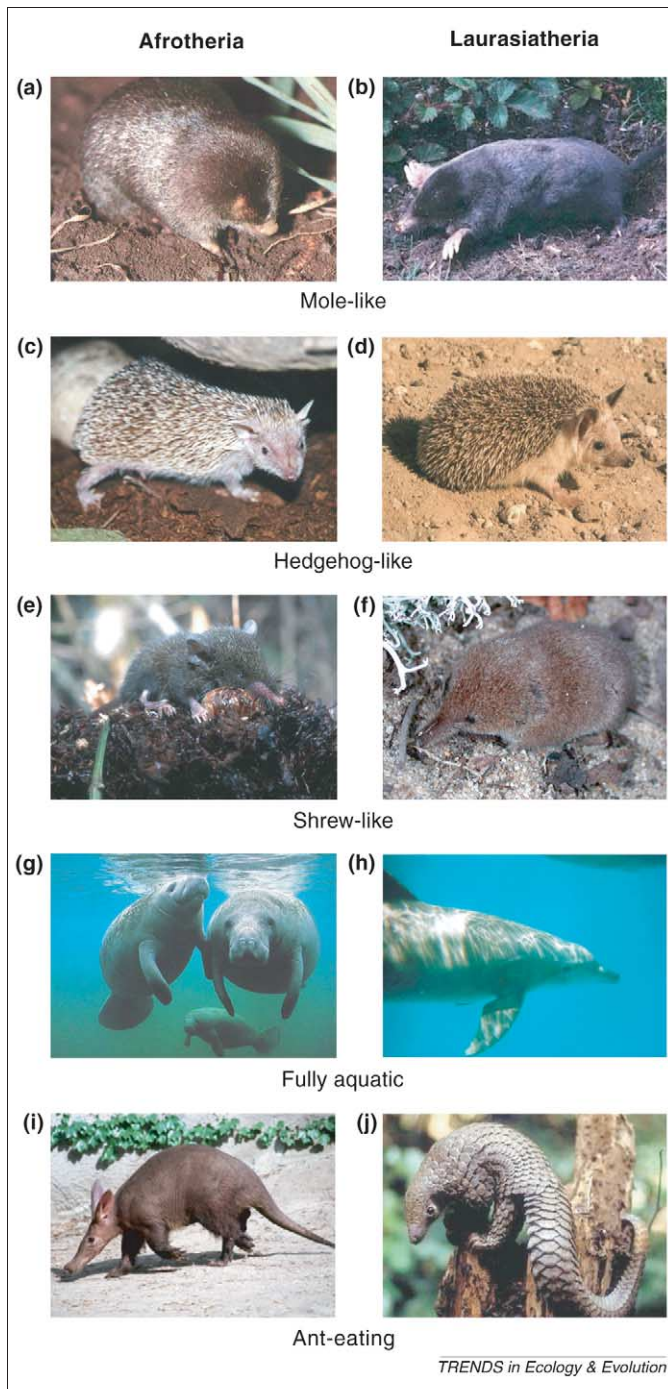


Figure 2. Parallel morphological radiations in Afrotheria and Laurasiatheria illustrate homoplasy in external morphology. (a) African golden mole (*Chrysochlorinae*) and (b) Old World mole (*Talpinae*); (c) Malagasy hedgehog (*Tenrecinae*) and (d) common hedgehog (*Erinaceinae*); (e) shrew tenrec (*Oryzorictinae*; *Microgale thomasi*; Copyright Link Olson) and (f) common shrew (*Soricinae*); (g) manatee (*Trichechidae*) and (h) dolphin (*Delphininae*); (i) armadillo (*Orycteropodidae*) and (j) pangolin (*Maninae*).

mammalian taxa, new questions arise, such as whether the underlying genetic architecture responsible for these changes involves the same or different genes.

The root of the placental tree and other remaining problems

With the proposal of and strong support for the four major clades of placental mammals, as well as Boreoeutheria

(Euarchontoglires + Laurasiatheria), there are only three viable locations for the root of the placental tree [19,21–23]. These are between (i) Afrotheria and other placental orders, (ii) Xenarthra and other placental orders (as favored by morphology), and (iii) ATLANTOGENATA (Xenarthra + Afrotheria) and Boreoeutheria. Numerical simulations [21] reject the latter two hypotheses, but these tests might be too liberal in rejecting alternate hypotheses if real data are not simulated accurately according to current models of sequence evolution [40]. Resolving the placental root remains the most fundamental problem for future studies of placental phylogeny and has implications for understanding early placental biogeography. For all three competing hypotheses, molecular data give the separation of South American xenarthrans and African-origin afrotheres as being ~100 million years ago, which coincides with the vicariant separation of South America and Africa. Whereas some workers have suggested a causal connection between these plate-tectonic dates and molecular dates separating Xenarthra and Afrotheria [18,21], others dismiss this as coincidence [41].

Similar to the placement of the placental root, remaining problems associated with resolving relationships within the major clades involve minor perturbations of the tree shown in Figure 1b. The discovery of further RGCs will be crucial in testing alternate hypotheses that involve short time intervals [22]. Within Laurasiatheria, it is unclear if perissodactyls are more closely related to pangolins + carnivores or to Cetartiodactyla. Within Afrotheria, it has proved difficult to resolve the relationship among the three paenungulate orders (elephants, hyraxes, dugongs–manatees). By contrast, morphology strongly supports a sister-group relationship between Proboscidea and Sirenia (Tethytheria) [3,4,42], which is also supported by complete mitochondrial genomes [43].

Minority views

The emerging consensus for placental ordinal relationships (Figure 1b), with its four major clades that are supported by overwhelming sequence evidence and RGCs, is not without critics [4,14,44]. Arnason *et al.*'s [14] mtDNA analysis suggests that hedgehogs are dissociated from other core insectivores, such as shrews and moles, and were the earliest offshoot of the placental tree. Arnason *et al.* [14] also find that rodents, Glires, Euarchontoglires, and Boreoeutheria are all paraphyletic taxa. However, Lin *et al.* [27] found that mtDNA trees recover the same four clades as nuclear genes when outgroup taxa are removed. Peculiar features of rooted mtDNA trees can result from inadequate models of sequence evolution [27,28] and/or unbalanced taxon sampling [28,29]. In particular, some marsupials have unusual nucleotide compositions and there have been changes in the mutational process in both hedgehogs and murid rodents relative to most other placental mammal mitochondrial genomes [27]. These changes violate the assumptions of most methods of phylogeny reconstruction. For example, general time reversible models of nucleotide substitution assume that base composition remains the same in different lineages. Other analyses suggest that protein-coding regions of the

mitochondrial genome lack sufficient power to resolve the placental radiation [45].

Some analyses of nuclear sequences have also recovered rodents at or near the base of the placental radiation, sometimes as a paraphyletic taxon [42,44]. These studies, which typically have poor taxon sampling among rodents and/or result from maximum parsimony analyses, are reminiscent of the guinea pig is not a rodent hypothesis, which was emphatically rejected by some morphologists [46]. Confronted by the morphologists' challenge to increase taxon sampling in the molecular studies, recent studies with nuclear genes that have subdivided rodent branches provide compelling support for rodent monophyly [20,35] and underscore the importance of adequate taxon sampling [47] in phylogeny reconstruction. Taxon sampling that breaks up long branches becomes especially important with certain phylogenetic methods, such as maximum parsimony, to mitigate against the potential effects of long-branch attraction. Long-branch attraction can occur when parallel/convergent substitutions on long branches outnumber homologous substitutions on short interior branches. The potential pitfalls of long-branch attraction with maximum parsimony were exposed in an analysis of DNA sequences from exon 11 of the breast and ovarian cancer susceptibility gene 1 (BRCA1) [19].

Conclusions and future challenges

After more than a century, we are now in the final stages of resolving the interordinal tree for living placental mammals. Morphology and molecules agree on the monophyly of 16 out of 18 placental orders, whereas molecular analyses nest whales within Artiodactyla (e.g. cows, pigs, hippos) and make Lipotyphla (e.g. hedgehogs, moles, shrews, golden moles) DIPHYLETIC. Above the ordinal level, analyses of molecular data corroborate the morphology-based Glires and Paenungulata hypotheses, as well as a variation of Archonta, dubbed Euarchonta [18], which includes primates, tree shrews and flying lemurs. Other morphological superordinal hypotheses are no longer viable in the face of robust molecular support for Afrotheria, Euarchontoglires and Laurasiatheria. With independent lines of support for Euarchontoglires, we are now confident that humans are more closely related to mice than to cows and dogs. We have learned that improved taxon sampling; the procurement of large, diverse and independent molecular datasets; modern methods of phylogenetic analysis; the discovery of RGCs; and application of the principle of congruence together constitute a powerful approach for resolving difficult phylogenetic problems. Resolving mammalian relationships shows that mitochondrial protein-coding genes can be misleading for deeper phylogenetic relationships, but that this can be improved by increased taxon sampling and/or removing the data that violate the model the most [22,27].

For mammalian systematists, the far more daunting challenge is to now integrate molecular and morphological data for living and fossil EUTHERIANS. This will surely require new fossil discoveries and novel analytical approaches. Numerous extinct taxa from the Cenozoic, often with highly divergent morphological specializations,

are placed within crown-group PLACENTALIA. There are also extinct eutherians from the Mesozoic, some predating the origin of crown-group Placentalia [48], whereas others might be included within Placentalia [42,49]. It is now fundamentally important to re-examine the phylogenetic placement of these extinct taxa in the context of the emerging molecular phylogeny for living taxa, with its division of placental orders into distinct groups with southern (Xenarthra, Afrotheria) and northern (Euarchontoglires, Laurasiatheria) hemisphere origins. Total evidence with maximum parsimony has been the method of choice for combined molecular and morphological datasets [42,50]. New approaches include Bayesian methods, which allow molecular and morphological data to have their own evolutionary models [51,52]. The Lewis [52] model for morphological characters can potentially take advantage of autapomorphies (i.e. uniquely derived characters) that are traditionally omitted from morphological character matrices because they are uninformative under the maximum-parsimony criterion. One potential difficulty with combined analyses is that they fail to address the weighting problem posed by including molecular and morphological data in the same data matrix. Another alternative is to impose molecular scaffolds with morphological data. Molecular scaffolds are topological constraints derived from previous molecular analyses that constrain the topology for living taxa, but not for fossil taxa. Sánchez-Villagra *et al.* [53] recently employed molecular scaffolds with morphological data to investigate the phylogenetic placement of the giant, extinct rodent *Phoberomys*. Given the prevalence of morphological convergence suggested by trees from molecular data, the challenge of placing fossil taxa is sure to lead to reassessments of morphological characters and new methods of phylogenetic analysis.

Acknowledgements

We thank Michael Novacek, Peter Waddell, and an anonymous reviewer for constructive comments about this article. This work was supported by NSF (M.S.S.) and the Training and Mobility of Researchers (TMR) program of the European Commission (M.J.S. and W.W.d.J.).

References

- Miyamoto, M.M. and Goodman, M. (1986) Biomolecular systematics of eutherian mammals: phylogenetic patterns and classification. *Syst. Zool.* 35, 230–240
- Whelan, S. *et al.* (2001) Molecular phylogenetics: state-of-the-art methods for looking into the past. *Trends Genet.* 17, 262–272
- Shoshani, J. and McKenna, M.C. (1998) Higher taxonomic relationships among extant mammals based on morphology, with selected comparisons of results from molecular data. *Mol. Phylog. Evol.* 9, 572–584
- Liu, F.-G.R. *et al.* (2001) Molecular and morphological supertrees for eutherian (placental) mammals. *Science* 291, 1786–1789
- Novacek, M.J. (2001) Mammalian phylogeny: genes and supertrees. *Curr. Biol.* 11, R573–R575
- Novacek, M.J. (1992) Mammalian phylogeny: shaking the tree. *Nature* 356, 121–125
- Czelusniak, J. *et al.* (1990) Perspectives from amino acid and nucleotide sequences on cladistic relationships among higher order taxa of Eutheria. In *Current Mammalogy* (Genoways, H.H., ed.), pp. 545–572, Plenum Press
- Irwin, D.M. and Arnason, U. (1994) Cytochrome *b* gene of marine mammals: phylogeny and evolution. *J. Mammal. Evol.* 2, 37–55
- Pumo, D.E. *et al.* (1998) Complete mitochondrial genome of a

- neotropical fruit bat, *Artibeus jamaicensis*, and a new hypothesis of relationships of bats to other eutherian mammals. *J. Mol. Evol.* 47, 709–717
- 10 Gatesy, J. and O'Leary, M.A. (2001) Deciphering whale origins with molecules and fossils. *Trends Ecol. Evol.* 16, 562–570
- 11 Nikaido, M. *et al.* (1999) Phylogenetic relationships among cetartiodactyls based on insertions of short and long interspersed elements: hippopotamuses are the closest extant relatives of whales. *Proc. Natl. Acad. Sci. U. S. A.* 96, 10261–10266
- 12 Geisler, J.H. and Uhen, M.D. (2003) Morphological support for a close relationship between hippos and whales. *J. Vert. Paleont.* 23, 991–996
- 13 D'Erchia, A.M. *et al.* (1996) The guinea pig is not a rodent. *Nature* 381, 597–600
- 14 Arnason, U. *et al.* (2002) Mammalian mitogenomic relationships and the root of the eutherian tree. *Proc. Natl. Acad. Sci. U. S. A.* 99, 8151–8156
- 15 Janke, A. *et al.* (2002) Phylogenetic analysis of 18S rRNA and the mitochondrial genomes of the wombat, *Vombatus ursinus*, and the spiny anteater, *Tachyglossus aculeatus*: increased support for the Marsupionta hypothesis. *J. Mol. Evol.* 54, 71–80
- 16 Curole, J.P. and Kocher, T.D. (1999) Mitogenomics: digging deeper with complete mitochondrial genomes. *Trends Ecol. Evol.* 14, 394–398
- 17 Stanhope, M.J. *et al.* (1998) Molecular evidence for multiple origins of insectivora and for a new order of endemic African insectivore mammals. *Proc. Natl. Acad. Sci. U. S. A.* 95, 9967–9972
- 18 Waddell, P.J. *et al.* (1999) Towards resolving the interordinal relationships of placental mammals. *Syst. Biol.* 48, 1–5
- 19 Madsen, O. *et al.* (2001) Parallel adaptive radiations in two major clades of placental mammals. *Nature* 409, 610–614
- 20 Murphy, W.J. *et al.* (2001) Molecular phylogenetics and the origins of placental mammals. *Nature* 409, 614–618
- 21 Murphy, W.J. *et al.* (2001) Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* 294, 2348–2351
- 22 Waddell, P.J. *et al.* (2001) A phylogenetic foundation for comparative mammalian genomics. *Genome Inform.* 12, 141–154
- 23 Delsuc, F. *et al.* (2002) Molecular phylogeny of living xenarthrans and the impact of character and taxonomic sampling on the placental tree rooting. *Mol. Biol. Evol.* 19, 1656–1671
- 24 Amrine-Madsen, H. *et al.* (2003) A new phylogenetic marker, apolipoprotein B, provides compelling evidence for eutherian relationships. *Mol. Phylog. Evol.* 28, 225–240
- 25 Waddell, P.J. and Shelley, S. (2003) Evaluating placental inter-ordinal phylogenies with novel sequences including RAG1, g-fibrinogen, ND6, and mt-tRNA, plus MCMC-driven nucleotide, amino acid, and codon models. *Mol. Phylog. Evol.* 28, 197–224
- 26 Hudelot, C. *et al.* (2003) RNA-based phylogenetic methods: application to mammalian mitochondrial RNA sequences. *Mol. Phylog. Evol.* 28, 241–252
- 27 Lin, Y-H. *et al.* (2002) Four new mitochondrial genomes and the increased stability of evolutionary trees of mammals from improved taxon sampling. *Mol. Biol. Evol.* 19, 2060–2070
- 28 Nikaido, M. *et al.* (2003) Mitochondrial phylogeny of hedgehogs and monophyly of Eulipotyphla. *Mol. Phylog. Evol.* 28, 276–284
- 29 Reyes, A. *et al.* (2004) Congruent mammalian trees from mitochondrial and nuclear genes using Bayesian methods. *Mol. Biol. Evol.* 21, 397–403
- 30 McKenna, M.C. and Bell, S.K. (1997) *Classification of Mammals Above the Species Level*, Columbia University Press
- 31 Carter, A.M. (2001) Evolution of the placenta and fetal membranes seen in the light of molecular phylogenetics. *Placenta* 22, 800–807
- 32 Szalay, F.S. and Drawhorn, G. (1980) Evolution and diversification of the Archonta in an arboreal milieu. In *Comparative Biology and Evolutionary Relationships of Tree Shrews* (Lockett, W.P., ed.), pp. 133–169, Plenum Press
- 33 Schmitz, J. and Zischler, H. (2003) A novel family of tRNA-derived SINEs in the colugo and two new retrotransposable markers separating dermopterans from primates. *Mol. Phylog. Evol.* 28, 341–349
- 34 Douady, C.J. *et al.* (2002) Molecular phylogenetic evidence confirming the Eulipotyphla concept and in support of hedgehogs as the sister group to shrews. *Mol. Phylog. Evol.* 25, 200–209
- 35 Huchon, D. *et al.* (2002) Rodent phylogeny and a timescale for the evolution of Glires: evidence from an extensive taxon sampling using three nuclear genes. *Mol. Biol. Evol.* 19, 1053–1065
- 36 Darwin, C. (1859) *The Origin of Species*, John Murray
- 37 Novacek, M. (1990) Morphology, paleontology, and the higher clades of mammals. In *Current Mammalogy* (Vol. 2) (Genoways, H.H., ed.), pp. 507–543, Plenum Press
- 38 Simmons, N.B. (1995) Bat relationships and the origin of flight. *Symp. Zool. Soc. Lond.* 67, 27–43
- 39 Helgen, K.M. (2003) Major mammalian clades: a review under consideration of molecular and paleontological evidence. *Mammal. Biol.* 68, 1–15
- 40 Buckley, T.R. (2002) Model misspecification and probabilistic tests of topology: evidence from empirical data sets. *Syst. Biol.* 51, 509–523
- 41 Archibald, J.D. (2003) Timing and biogeography of the eutherian radiation: fossils and molecular compared. *Mol. Phylog. Evol.* 28, 350–359
- 42 Asher, R.J. *et al.* (2003) Relationships of endemic African mammals and their fossil relatives based on morphological and molecular evidence. *J. Mammal. Evol.* 10, 131–194
- 43 Murata, Y. *et al.* (2003) Afrotherian phylogeny as inferred from complete mitochondrial genomes. *Mol. Phylog. Evol.* 28, 253–260
- 44 Misawa, K. and Janke, A. (2003) Revisiting the Glires concept – phylogenetic analysis of nuclear sequences. *Mol. Phylog. Evol.* 28, 320–327
- 45 Corneli, P.S. (2002) Complete mitochondrial genomes and eutherian evolution. *J. Mammal. Evol.* 9, 281–305
- 46 Lockett, W.P. and Hartenberger, J-L. (1993) Monophyly or polyphyly of the order Rodentia: possible conflict between morphological and molecular interpretations. *J. Mammal. Evol.* 1, 127–147
- 47 Hillis, D.M. *et al.* (2003) Is sparse taxon sampling a problem for phylogenetic inference? *Syst. Biol.* 52, 124–126
- 48 Ji, Q. *et al.* (2002) The earliest known eutherian mammal. *Nature* 416, 816–822
- 49 Archibald, J.D. *et al.* (2001) Late Cretaceous relatives of rabbits, rodents, and other extant eutherian mammals. *Nature* 414, 62–65
- 50 Gatesy, J. *et al.* (1999) Stability of cladistic relationships between Cetacea and higher-level artiodactyl taxa. *Syst. Biol.* 48, 6–20
- 51 Ronquist, F. and Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574
- 52 Lewis, P. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* 50, 913–925
- 53 Sánchez-Villagra, M.R. *et al.* (2003) The anatomy of the world's largest extinct rodent. *Science* 301, 1708–1710
- 54 Phillips, M.J. and Penny, D. (2003) The root of the mammalian tree inferred from whole mitochondrial genomes. *Mol. Phylog. Evol.* 28, 171–185
- 55 Rokas, A. and Holland, P.W. (2000) Rare genomic changes as a tool for phylogenetics. *Trends Ecol. Evol.* 15, 454–459
- 56 Ragg, H. *et al.* (2001) Vertebrate serpins: construction of a conflict-free phylogeny by combining exon-intron and diagnostic site analyses. *Mol. Biol. Evol.* 18, 577–584
- 57 de Jong, W.W. *et al.* (2003) Indels in protein-coding sequences of Euarchontoglires constrain the rooting of the eutherian tree. *Mol. Phylog. Evol.* 28, 328–340
- 58 Fronicke, L. *et al.* (2003) Towards the delineation of the ancestral eutherian genome organization: comparative genome maps of human and the African elephant (*Loxodonta africana*) generated by chromosome painting. *Proc. R. Soc. Lond. Ser. B* 270, 1331–1340
- 59 Nikaido, M. *et al.* (2003) Ancient SINEs from African endemic mammals. *Mol. Biol. Evol.* 20, 522–527
- 60 van Dijk, M.A.M. *et al.* (1999) The virtues of gaps: Xenarthran (edentate) monophyly supported by a unique deletion in α A-crystallin. *Syst. Biol.* 48, 94–106
- 61 Poux, C. *et al.* (2002) Sequence gaps join mice and men: phylogenetic evidence from deletions in two proteins. *Mol. Biol. Evol.* 19, 2035–2037
- 62 Thomas, J.W. *et al.* (2003) Comparative analyses of multi-species sequences from targeted genomic regions. *Nature* 424, 788–793
- 63 Springer, M.S. *et al.* A molecular view on relationships among the extant orders of placental mammals. In *Origin, Timing, and Relationships Among the Major Clades of Extant Placental Mammals*

- (Rose, K.D. and Archibald, J.D., eds), Johns Hopkins University Press (in press)
- 64 Smith, J.D. and Madkour, G. (1980) Penial morphology and the question of chiropteran phylogeny. In *Proceedings Fifth International Bat Research Conference* (Wilson, D.E. and Gardner, A.L., eds), pp. 347–365, Texas Tech Press
- 65 Pettigrew, J.D. *et al.* (1989) Phylogenetic relations between microbats, megabats and primates (Mammalia: Chiroptera, Primates). *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 325, 489–559
- 66 Bailey, W.J. *et al.* (1992) Rejection of the flying primate hypothesis by phylogenetic evidence from the ϵ -globin gene. *Science* 256, 86–89
- 67 Simmons, N.B. (1994) The case for chiropteran monophyly. *Am. Mus. Novitates* 3103, 1–54
- 68 Springer, M.S. *et al.* (2001) Integrated fossil and molecular data reconstruct bat echolocation. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6241–6246
- 69 Hutcheon, J.M. *et al.* (1998) Base-compositional biases and the bat problem. III. The question of microchiropteran monophyly. *Philos. Trans. R. Soc. Lond. Ser. B* 353, 607–617
- 70 Teeling, E.C. *et al.* (2002) Microbat paraphyly and the convergent evolution of a key innovation in Old World rhinolophoid microbats. *Proc. Natl. Acad. Sci. U. S. A.* 99, 1431–1436

Have you contributed to an Elsevier publication?

Did you know that you are entitled to a 30% discount on books?

A 30% discount is available to ALL Elsevier book and journal contributors when ordering books or stand-alone CD-ROMs directly from us.

To take advantage of your discount:

1. Choose your book(s) from www.elsevier.com or www.books.elsevier.com

2. Place your order

Americas:

TEL: +1 800 782 4927 for US customers

TEL: +1 800 460 3110 for Canada, South & Central America customers

FAX: +1 314 453 4898

E-MAIL: author.contributor@elsevier.com

All other countries:

TEL: +44 1865 474 010

FAX: +44 1865 474 011

E-MAIL: directorders@elsevier.com

You'll need to provide the name of the Elsevier book or journal to which you have contributed. Shipping is FREE on pre-paid orders within the US, Canada, and the UK.

If you are faxing your order, please enclose a copy of this page.

3. Make your payment

This discount is only available on prepaid orders. Please note that this offer does not apply to multi-volume reference works or Elsevier Health Sciences products.

www.books.elsevier.com