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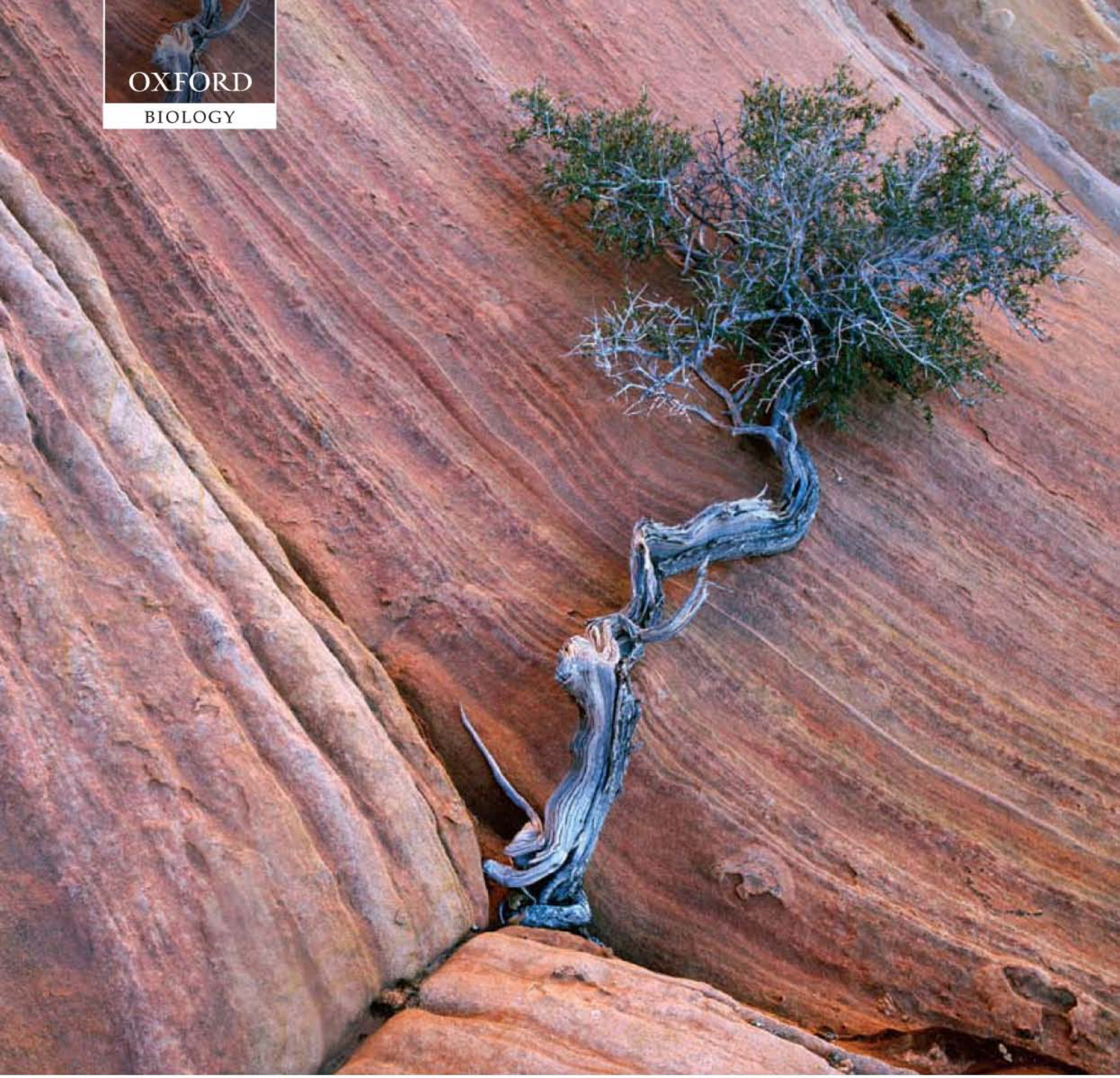

# the TIMETREE of LIFE

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# Armadillos, anteaters, and sloths (Xenarthra)

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## Abstract

**Armadillos, anteaters, and sloths (31 sp.) are grouped into five families within the mammalian Order Xenarthra. The xenarthran timetree shows that armadillos diverged from anteaters + sloths ~70 million years ago (Ma), which was followed by the divergence of anteaters and sloths ~60 Ma. Molecular dating also reveals that the pygmy anteater diverged from other anteaters ~40 Ma, and that the two living genera of sloths diverged ~20 Ma. Molecular dates suggest that the paleoenvironmental changes that occurred during the Cenozoic (66–0 Ma) in South America have influenced the evolutionary history of Xenarthra.**

Despite their heterogeneous morphologies, armadillos (Cingulata), anteaters (Vermilingua), and sloths (Folivora) form a monophyletic group of curious placental mammals, the Order Xenarthra. Indeed, all living and extinct members share the presence of supplementary intervertebral articulations termed "xenarthrales" in the posterior dorsal vertebrae (*1*). Extant xenarthrans are currently represented by 31 species of South American origin (*2*). This reduced taxonomic diversity is the result of the last mass extinction event just 10,000 years ago that wiped out most of the product of an evolutionary radiation triggered by the isolation of South America during most of the Cenozoic era, 65–3 Ma (*3*). Five families are generally recognized: Dasypodidae (armadillos; Fig. 1), Cyclopedidae and Myrmecophagidae (anteaters), and Bradypodidae and Megalonychidae (sloths) (*4*). Here, we review the phylogenetic relationships and divergence times among the five families of extant xenarthrans.

Few studies have been dedicated to reconstructing the phylogenetic relationships of xenarthrans. The first attempts based on morphological and anatomical characters have conflicted regarding the interrelationships of the three xenarthran lineages: some grouped armadillos and sloths together (*5, 6*), whereas others suggested that anteaters are the closest relatives of sloths (*7, 8*). Early molecular studies based on evolutionary comparisons of protein sequences of *alpha crystallin-A* (*9*) and immunological distances derived from serum albumins (*10*) did not contribute much to this debate. The most recent classification (*4*) nevertheless groups anteaters (Vermilingua) and sloths (Folivora) into a clade called Pilosa, referring to their coat which constitutes a derived character in Xenarthra since it is interpreted as a reversal to the ancestral condition of mammals.

Xenarthran molecular systematics has been conducted using a variety of molecular markers and has resulted in a well-resolved interfamilial phylogeny (Fig. 2). The earliest study analyzed a combination of the mitochondrial *12S* and *16S ribosomal RNA* (rRNA) genes and the nuclear exon 28 of the *von Willebrand Factor* gene (*VWF*) for eight of the 13 living xenarthran genera (*11*). Subsequently, taxon sampling was increased to 12 genera in analyses of three protein-coding nuclear genes, adding the complete *ά-2B adrenergic receptor* gene (*ADRA2B*) and exon 11 of the *breast cancer susceptibility* gene (*BRCA1*) to the exon 28 *VWF* data (*12*). This nuclear data set

**Fig. 1** A Southern Three-banded Armadillo (*Tolypeutes matacus*), Family Dasypodidae, from Argentina. Credit: F. Delsuc.

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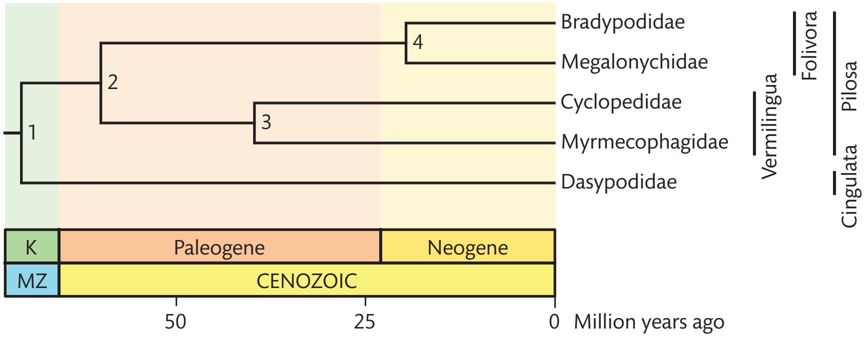

**Fig. 2** A timetree of armadillos, anteaters, and sloths (Xenarthra). Divergence times are shown in Table 1. *Abbreviations*: MZ (Mesozoic) and K (Cretaceous).

was later enlarged by the addition of two mitochondrial genes (*12S rRNA* and *NADH dehydrogenase 1* [*ND1*]) (*13*). Finally, retroposed elements and their flanking regions have been studied for representatives of all 13 extant genera (*14*).

The monophyly of Xenarthra is morphologically well supported by characters generally thought to reflect adaptations toward fossoriality and myrmecophagy (*1*). The common ancestry of the three xenarthran lineages was evidenced in early molecular studies (*9, 10*). Subsequent sequence-based phylogenetic studies focused on xenarthrans have also found strong statistical support for their monophyly (*11, 12*), with the notable occurrence of a rare diagnostic three amino acid deletion in their *α-crystallin A* protein (*15*).

The four molecular studies sampling all anteater and sloth genera provided unambiguous support for Pilosa (*11–14*), confirming the results of large-scale analyses including fewer taxa (*16–18*). These results contradicted studies of the ear region (*5*) and cephalic arterial patterns (*6*) that supported a basal position of anteaters within Xenarthra. The extreme specialization of the skull toward myrmecophagy in anteaters might have confounded these early morphological studies. Subsequent cladistic studies of morphological and anatomical features (*7*) including the structure of the ear region (*8*) favored Pilosa and provided shared-derived characters such as the interruption of the zygomatic arch and the intra-pelvic location of the testes.

The two living genera of three-toed (Genus *Bradypus*) and two-toed (Genus *Choloepus*) sloths (Folivora) are strictly arboreal and virtually unknown in the fossil record (*3*). On the basis of numerous morphological differences and a presumably diphyletic origin suggested by fossils, they have been classified into distinct families (Bradypodidae and Megalonychidae, respectively) (*19*). This taxonomic distinction is supported by immunological data revealing considerable evolutionary distance between their albumins (*10*). Ancient DNA studies based on mitochondrial *12S* and *16S rRNA* fragments from fossil ground sloths also provide some support for the diphyly hypothesis (*20, 21*). These studies suggest that modern three-toed sloths (Bradypodidae) are closely related to the Shasta Ground Sloth *Nothrotheriops shastensis* (Megatheriidae), whereas two-toed sloths (Megalonychidae) appear closer to the Giant Ground Sloth *Mylodon darwinii* (Mylodontidae).

Within anteaters (Vermilingua), the Pygmy Anteater (*Cyclopes didactylus*) is considered morphologically divergent from the closely related Giant Anteater (*Myrmecophaga tridactyla*) and tamanduas (genus *Tamandua*) (*7, 22, 23*). Based on this morphological divergence, *C. didactylus* is classified in its own distinct family Cyclopedidae (*4*). All molecular results confirm this arrangement by supporting a close relationship between *Myrmecophaga* and *Tamandua* (Myrmecophagidae) (*11–14*).

The newly established phylogenetic framework has been used to derive a molecular timescale for xenarthran evolutionary history. The earliest molecular dating study used a maximum likelihood local molecular clock approach with three calibration points to calculate divergence dates among eight xenarthran species from amino acid sequences of the *VWF* exon 28 (*11*). A Bayesian relaxed molecular clock method approach (*24*) was subsequently used to analyze a large combination of both mitochondrial and nuclear genes but including only one representative per xenarthran lineage (*25*). Finally, the

**Table 1.** Divergence times (Ma) and their confidence/credibility intervals (CI) among armadillos, anteaters, and sloths (Xenarthra).

| Timetree | | Estimates | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Node | Time | Ref. (*11*) | | Ref. (*25*)(a) | | Ref. (*25*)(b) | | Ref. (*26*) | |
| | | Time | CI | Time | CI | Time | CI | Time | CI |
| 1 | 70.5 | 66.0 | 83–49 | 69.9 | 78–62 | 81.4 | 96–68 | 64.7 | 75–55 |
| 2 | 60.0 | 56.7 | 71–42 | 61.4 | 70–52 | 66.8 | 83–52 | 55.2 | 65–46 |
| 3 | 39.7 | 39.3 | 49–29 | – | – | – | – | 40.0 | 49–32 |
| 4 | 19.6 | 18.3 | 23–13 | – | – | – | – | 20.8 | 28–15 |

*Note*: Node times in the timetree represent the mean of time estimates from different studies. Divergence time from ref. (*11*) is the mean estimates from three calibration points. Times estimates from ref. (*25*) are based on the analysis of (a) nuclear and (b) mitochondrial genes. The 95% Bayesian credibility intervals are reported for two studies (*25, 26*).

same dating method has also been applied to a combined data set of three nuclear genes (*ADRA2B*, *BRCA1*, and *VWF*) encompassing 12 of the 13 living genera (*26*). The age of the xenarthran last common ancestor, corresponding to the separation between Cingulata and Pilosa, was estimated at 70.5 Ma (Table 1). This estimate, preceding the Cretaceous–Paleogene boundary (Fig. 2), is slightly more recent than previous ones suggesting a date ~80 Ma (*10, 20*). It is also more compatible with the first occurrence of fossil xenarthrans in the late Paleocene of Brazil (~58 Ma), in the form of the earliest armadillo scutes (*27*).

This initial split within Xenarthra was followed, only about 10 Ma later, by the divergence of Vermilingua and Folivora at ~60 Ma (Table 1). A relatively early split between anteaters and sloths may have occurred in the middle of the Paleocene epoch about ~60 Ma (Fig. 2). Within anteaters, molecular dating revealed a deep split between Cyclopedidae and Myrmecophagidae with the pygmy anteater lineage emerging in the middle Eocene ~40 Ma (Table 1). The first appearance of undoubted anteater fossils dates from the early Miocene of Patagonia (~20 Ma) (*28*). Our fairly ancient estimates for the age of their last common ancestor suggest a ghost lineage of ~20 Ma in the early fossil record of anteaters, which supports the contention that the fossil record is fairly incomplete for this group. These molecular estimates reveal the evolutionary antiquity of the Pygmy Anteater (*C. didactylus*) and support its classification in the distinct Family Cyclopedidae (*4*).

The molecular dating estimated the separation between the two modern sloth genera at 19.6 Ma. The oldest undisputed sloth remains come from the middle Eocene of Patagonia and Antarctica (~40 Ma) but they cannot be precisely assigned to any of the recognized lineages (*29*). The deep molecular estimate confirms the considerable divergence between the two modern sloth genera and supports their taxonomic distinction at the family level.

Finally, the molecular estimates obtained for xenarthran divergence dates suggested a potential role played by paleoenvironmental changes in the diversification of living xenarthrans (*26*). The paleoenvironmental changes that occurred during much of the Cenozoic era in South America and their interaction with the mammalian fauna have been well documented (*3, 30*). Actually, some diversification events in Xenarthra appear to follow periods of important environmental changes, possibly triggered by major phases of Andean uplift defined as "tectonic crises" (*30*).

First, the separation between Cyclopedidae and Myrmecophagidae seems to correspond with a well-dated deformation pulse of the large Incaic uplift episode in the Andes of Peru estimated at ~43 Ma in the middle Eocene (*30*). Second, the diversification of modern sloth lineages in the middle of the early Miocene also correlates well with the end of the first major Bolivian crisis. This diastrophic event was an intense deformational and magmatic episode widespread along the Andes (*30*). It is seen as a turning point in Andean tectonics which significantly influenced South American climates and also marked a major shift in South American mammalian fossil communities. The evolutionary history of living xenarthrans, therefore, seems to have been influenced by the environmental changes that occurred in South America over the last 65 million years (*26*).

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