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The evolution of armadillos, anteaters, and sloths depicted by nuclear and mitochondrial phylogenies: implications for the status of the enigmatic fossil **Eurotamandua**.

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Running title: Evolution of Xenarthra

ABSTRACT

The mammalian order Xenarthra (armadillos, anteaters and sloths) is one of the four major clades of placentals, but it remains poorly studied from the molecular phylogenetics perspective. We here present a study encompassing most of the order's diversity, to establish xenarthrans intra-ordinal relationships, discuss the evolution of their morphological characters, search for their extant sister-group, and specify the timing of their radiation with special emphasis on the status of the controversial fossil Eurotamandua. Sequences of three genes (nuclear exon 28 of the von Willebrand Factor [vWF]; mitochondrial 12S and 16S rRNAs) are compared for eight of the 13 living genera. Phylogenetic analyses confirm the order's monophyly and that of its three major lineages: armadillos (Cingulata), anteaters (Vermilingua), and sloths ("Tardigrada", renamed in "Folivora") and our results strongly support the grouping of hairy xenarthrans (anteaters and sloths) into Pilosa. Within placentals, Afrotheria might be the first lineage to branch off, followed by Xenarthra. The morphological adaptative convergence between New World xenarthrans and Old World pangolins is confirmed. Molecular datings place the early emergence of armadillos around the Cretaceous/Tertiary boundary, followed by the divergence between anteaters and sloths in the Early Eocene. These Tertiary dates contradict the concept of a very ancient origin of modern xenarthran lineages. They also question the placement of the purported fossil anteater (Eurotamandua) from the Middle Eocene of Europe, with the Vermilingua, and instead suggest the independent and convergent evolution of this enigmatic taxon.

Keywords: Molecular phylogeny — Xenarthra — Pilosa — 12S rRNA — 16S rRNA — von Willebrand Factor (vWF) — local molecular clocks — Eurotamandua.

INTRODUCTION

The mammalian order Xenarthra (ex Edentata; see Glass 1985) is composed of three major lineages corresponding to three distinct living morphotypes: armadillos (Cingulata: Dasypodidae), anteaters (Vermilingua: Myrmecophagidae) and sloths (Bradypodidae and Megalonychidae). Xenarthrans include 30 living species classified in 13 genera, almost all endemic of South and Central Americas, the nine-banded armadillo (Dasypus novemcinctus) being the only one to occur in North America. Extant species provide only a glimpse of xenarthran past diversity since 218 fossil genera are recognised (McKenna & Bell 1997). This order was still greatly diversified no later than 10000 years ago, when the majority of genera became extinct possibly because of human impact (Patterson & Pascual 1972; Fariña 1996).

The radiation of Xenarthra occurred between Palaeocene and Eocene at an epoch where South America was isolated from other continental masses (Patterson & Pascual 1972). Their fossil record is almost restricted to the American continent (Carrol 1988) with three striking exceptions: a "primitive edentate" (Ernanodon antelios) from the Upper Palaeocene of China (Ding 1979), a purported anteater (Eurotamandua joresi) discovered in the Middle Eocene of Europe (Storch 1981), and an unidentified Eocene sloth from Antarctica (Vizcaíno & Scillato-Yané 1995). These unexpected reports complicated the reconstruction of xenarthran biogeographical history and raised doubts on their confinement to the New-World. More specifically, the affinities of Eurotamandua with Xenarthra are still highly debated (Gaudin 1999; Rose 1999).

Despite highly specialised and distinct morphologies of the three lineages, the monophyly of the order Xenarthra is classically recognised (McKenna & Bell 1997). All living and fossil xenarthrans exhibit a dental reduction by the loss of enamel, reaching its paroxysm in anteaters which totally lack teeth (Carrol 1988). The name "Edentata" has been used to recall this evolutionary trend, but it leads to confusion because it originally referred to

the grouping of New-World xenarthrans and Old-World pholidotans (pangolins) which are also lacking teeth (Glass 1985). One exclusive morphological synapomorphy of Xenarthra is the presence of "xenarthry" constituted by additional atypical articulations between vertebrae (Engelmann 1985; Patterson et al. 1992; Rose & Emry 1993; Gaudin 1999). From the molecular point of view, xenarthran monophyly is strongly supported by a three consecutive amino acids deletion in the eye lens αA-crystallin protein, that is unique among studied eutherian mammals (de Jong et al. 1985; van Dijk et al. 1999).

The order Xenarthra is of crucial importance in understanding mammalian phylogenetics. Following McKenna's (1975) assumption that Xenarthra represents the sister-group to all other living eutherians, thereby named Epitheria, numerous molecular workers have used its representatives to root eutherian phylogenies (see e.g. Allard et al. 1996). Gaudin et al. (1996), however, emphasised the weakness of the morphological synapomorphies defining epitherians. The comparison of complete mitochondrial genomes suggested a close relationship between Xenarthra and either Ferungulata (Cetartiodactyla, Perissodactyla and Carnivora: Arnason et al. 1997) or Afrotheria (Proboscidea, Hyracoidea, Sirenia, Macroscelidea, Chrysochloridae and Tenrecidae; Waddell et al. 1999a,b). Recently, nuclear DNA sequences showed that Xenarthra represents one of the four major clades of placentals (Madsen et al. 2001; Murphy et al. 2001).

Within Xenarthra, the intra-ordinal relationships and their timing remain unclear. Morphological data suggest the grouping of anteaters and sloths into Pilosa, a clade defined by the replacement of the carapace by a coat (Engelmann 1985; Patterson et al. 1992; McKenna & Bell 1997). However, the possibility of an early emergence of anteaters from the ancestral xenarthran stock is suggested by studies on fossils (Carrol 1988), ear region (Guth 1961), and cephalic arterial pattern (Bugge 1979). From the molecular perspective, the first immunological (Sarich 1985) and protein (de Jong et al. 1985) studies, as well as the analysis

of partial mitochondrial 12S and 16S rRNA genes (Höss et al. 1996), did not clarify intra-xenarthran relationships. Data from the mitochondrial ND1 favoured the grouping of armadillos and sloths to the exclusion of anteaters (Cao et al. 1998), contra the eye lens αA-crystallin which provided support for a monophyletic Pilosa, but suggested the paraphyly of armadillos (van Dijk et al. 1999). Most recently, Murphy et al. (2001), in an analysis of a data set including five xenarthran genera, present a tree with strong support for Pilosa, but with no statistical analysis of xenarthran issues or estimates of dates for the various splitting events within the order.

To better understand xenarthran evolution, we here present a study encompassing most of the order's diversity, based on three genes already used to explore mammalian phylogenetics: the exon 28 of the von Willebrand Factor (vWF) — a single copy nuclear marker —, and the mitochondrial 12S and 16S rRNAs (e.g. Huchon et al. 1999; Montgelard et al. 1997; Höss et al. 1996). The aims of this study are (1) to investigate the intra-ordinal relationships of Xenarthra using a larger taxonomic diversity than in previous molecular studies; (2) to discuss the evolution of morphological characters in light of the phylogeny obtained; and (3) to specify the chronology of their radiation in relation to South American biogeography, with special reference to the occurrence of the purported fossil anteater Eurotamandua in Europe.

MATERIALS AND METHODS

Taxonomy

Throughout the text, we introduced "Folivora" for the clade containing the sloths, because the usual terms of "Phyllophaga" (e.g. Engelmann 1985) or "Tardigrada" (e.g. Patterson et al. 1992) both referred to different groups of protostomians. Etymologically, "Folivora" means "leaf eater" and is the Latin correspondence for "Phyllophaga".

Data acquisition

Xenarthran samples preserved in 95% ethanol were stored in the Collection of Mammalian Tissues from the Institut des Sciences de l'Evolution de Montpellier (Catzeflis 1991). Total DNAs were extracted for Chaetophractus villosus (larger hairy armadillo), Cabassous unicinctus (southern naked-tailed armadillo), Dasypus novemcinctus (nine-banded armadillo), Cyclopes didactylus (pygmy anteater), Tamandua tetradactyla (collared anteater), Myrmecophaga tridactyla (giant anteater), Bradypus tridactylus (pale-throated three-toed sloth) and Choloepus didactylus (southern two-toed sloth).

PCR amplifications of the complete 12S rRNA gene were conducted following Hassanin & Douzery (1999). The 16S rRNA fragment (722 bp) for Myrmecophaga was amplified with primers R9 = 5'-CGCCTGTTTACCAAAAACATC-3' (direct) and S1 = 5'-TTATGCAATTACCGAGATCTGCCA-3' (reverse), and cloned using the pCR™ 2.1 plasmid vector and Escherichia coli strain INVαF' (Original TA cloning kit, Invitrogen). For the vWF exon 28 of sloths and armadillos, two overlapping fragments (V1/W2: 907 bp, and V2/W1: 962 bp) were amplified according to Huchon et al. (1999). Three overlapping fragments (V1/W11: 398 bp, V8/W10: 504 bp, and V9/W12: 523 bp) were amplified for anteaters, with the following direct (V) and reverse (W) primers: V8 = 5'-GGAGCTGCGGCGCRTCGCCAGCCAGGTGAA-3', V9 = 5'-ACGAGATCATCAGCTACCTCTGYGRCCTG-3', W10 = 5'-TGTTGAAGCCGGCCTCGCCGATCCTGTC-3', W11 = 5'-CCGAAGACCTGGAACAGCGTGTAC-3', and W12 = 5'-TGAGGGCCCACRCCRATGGG-3'. PCR products were purified from 1% agarose gels using Amicon Ultrafree-DA columns (Millipore), and sequenced on both strands using manual (Thermo Sequenase cycle kit, Amersham) and automatic sequencing (Big Dye Terminator cycle kit) on an ABI 310 (PE Applied Biosystems). Three distinct clones were sequenced for the partial 16S rRNA of Myrmecophaga. Sequences have been deposited in EMBL under accession numbers

AJ278151-AJ278161, and AJ297939 (Electronic Appendix I). Two already published vWF sequences have been updated (Chaetophractus villosus: AF076480 and Bradypus tridactylus: U31603).

Taxonomic sampling

Since species sampling has been shown to have a major impact on phylogenetic reconstruction (Philippe & Douzery 1994), we chose a data set encompassing a wide range of ordinal mammalian diversity. To reduce potential long branch attraction artefacts, we analysed, whenever possible, at least two species per placental order. The sequences of two marsupials from Australia (Macropus giganteus) and America (Didelphis virginiana) were used to unambiguously root eutherian trees. Thirty five mammalian genera were considered for the three genes. All sequences accession numbers are listed in Electronic Appendix I.

Sequence alignment and phylogenetic analyses

Sequences were manually aligned with the ED editor of the MUST package (Philippe 1993). The alignment of vWF exon 28 sequences started at position 38 of the human sequence, was 1233 bp long, and non sequenced positions were coded as missing data. Indels and hypervariable zones of the 12S and 16S rRNAs were removed from subsequent analyses. Alignments are available upon request to FD.

Phylogenetic reconstructions were conducted with PAUP* 4.0b4a (Swofford 1998), TREE-PUZZLE 4.02 (Strimmer & von Haeseler 1996), and PAML 3.0b (Yang 2000). Distance analyses used the Minimum Evolution (ME) criterion with TBR branch swapping on a Neighbor Joining (NJ) tree based on paralinear Log-Det distances with constant site removal. Maximum Parsimony (MP) analyses consisted of heuristic searches using 100 random addition sequence replicates with TBR branch swapping. Maximum Likelihood (ML) analyses with PAUP* were made in two steps in order to speed up computation times. First, ML parameters were estimated using a heuristic search with nearest neighbour interchange

(NNI) branch swapping on a NJ starting tree. Second, a new search was conducted with random addition of sequences and tree bisection-reconnection (TBR) branch swapping, using the previously estimated parameters. Substitution rates were described by the GTR (under PAUP* and PAML) and JTT (under TREE-PUZZLE and PAML) models of sequence evolution for nucleotides and amino acids respectively. Among sites substitution rate heterogeneity for DNA and vWF protein was described by a gamma distribution with eight categories (Γ_8) (Yang 1996).

Robustness of the different nodes was estimated by Bootstrap Percentages (BP) after 1000 replications for ME and MP with TBR branch swapping, and after 300 replications using previously estimated parameters for ML, with NJ starting trees and 1000 TBR rearrangements per resampling. Reliability Percentages (RP) for the protein dataset were obtained after 10000 quartet-puzzling (QP) steps with TREE-PUZZLE. Bremer Support Indices, i.e. the number of extra steps required to break the corresponding node under the MP criterion, were computed with PAUP*. Log-likelihoods of alternative topologies were compared by the ML test of Kishino & Hasegawa (1989) implemented in PAML 3.0b. Three different GTR+Γ_8 models were assumed for the combination of the three genes.

Molecular datings

The presence of local molecular clocks (Yoder & Yang 2000) was tested for different data sets, and molecular dates were calculated by PAML 3.0b using maximum likelihood branch lengths in a locally clocklike tree. Taxa were removed one by one until acceptance by the likelihood ratio test (LRT) of the hypothesis of three local clocks: one for paenungulates, one for perissodactyls, and one for the remaining species. Three independent paleontological calibration points were taken into account to check for molecular datings with cross-calibrations (Huchon et al. 2000): (1) 55 million years (Myr) for the split between Ceratotherium and Equus (Waddell et al. 1999b) ; (2) 60 Myr for the split between Procavia

and Elephas (Gheerbrant et al. 1996); and (3) 63 Myr for the Arctocyonia – Mesonychia divergence (Gingerich & Uhen 1998), represented here by the Sus (domestic pig) – Physeter (sperm whale) split. To discuss our molecular dates in the light of geochronology, we have used the geological timescale compiled for the Tertiary by McKenna & Bell (1997).

RESULTS

Phylogenetic results

Nuclear exon 28 of vWF

Due to significant base composition heterogeneity (evaluated by a χ^2 test in TREE-PUZZLE) for numerous taxa (including Dasypus, Chaetophractus, Choloepus, Tamandua and Myrmecophaga within Xenarthra), third codon positions were excluded from all subsequent analyses. All phylogenetic reconstructions provided strong support for most nodes involving xenarthrans (Table 1): (i) monophyly of Xenarthra; (ii) monophyly of each of its three lineages: armadillos, anteaters and sloths; (iii) grouping of anteaters and sloths into a clade called Pilosa; and (iv) close relationship within anteaters between Myrmecophaga and Tamandua — the subfamily Myrmecophaginae — to the exclusion of Cyclopes. Within armadillos, the grouping of Cabassous with Chaetophractus to the exclusion of Dasypus received mixed support (Table 1). Phylogenetic analyses on vWF amino acids yielded high support for all nodes within Xenarthra, and provided several exclusive amino acid substitutions supporting them (Table 1 and Electronic Appendix II).

Mitochondrial 12S and 16S rRNAs

All nodes involving xenarthrans were retrieved by phylogenetic analyses using 12S and 16S rRNAs (Table 1), with high support (monophyly of Cingulata, Vermilingua, Folivora, and Myrmecophaginae), moderate support (grouping of anteaters and sloths), or low support (monophyly of the order). One conflict arose from 12S rRNA relationships within armadillos

(Table 1): ME favoured the grouping of Cabassous with Chaetophractus, contra MP and ML which suggested a sister-group relationship between Cabassous and Dasypus.

Combination of the three genes

The ML phylogram obtained with the combined nuclear and mitochondrial data sets (2107 bp) is presented in Figure 1. Concatenation resulted in a general increase of robustness indices (Table 1). Xenarthra, Pilosa, Cingulata, Folivora, Vermilingua and Myrmecophaginae were clearly monophyletic ($BP_{ML} > 97$), and the grouping of the armadillos Cabassous and Chaetophractus to the exclusion of Dasypus was favoured ($BP_{ML} = 60$).

Tests of alternative hypotheses

The three alternative branching patterns connecting armadillos, anteaters, and sloths were compared by Kishino & Hasegawa (1989) likelihood tests. Pilosa appeared to be the most likely hypothesis in all cases. Alternative hypotheses placing either anteaters or sloths as sister-group to the remaining xenarthrans involved a drop in log-likelihoods which is significant when the three markers are combined (Table 2). With regard to Xenarthra sister-group relationships, their emergence second amongst placentals — after Afrotheria — was the most likely hypothesis in the vWF and combination cases. Alternative hypotheses, i.e. Xenarthra sister-group of either remaining placentals (Epitheria), or Afrotheria, or Ferungulata, all appeared less likely when the three markers were combined as they involved a drop in log-likelihood by ca. 1 standard error (Table 2). The morphological hypothesis of "Edentata" (Xenarthra + Pholidota) was significantly rejected in all cases (Table 2).

Molecular timings

Local clocks have been used in the ML datings because they represent a compromise between setting a single substitution rate among all lineages (global clock constrained) and independent rates for each branch (no clock constrained). Amino acid substitutions in vWF

behaved locally clocklike for a reduced data set of 18 taxa, including Xenarthra, Paenungulata, Perissodactyla, and Cetartiodactyla (Lama excluded due to a fast rate of evolution) with the two marsupials as outgroup (LRT between non-clock and locally clocklike tree: $2\Delta\ln L = 27.2$ with 19 degrees of freedom; P = 0.10). Using respectively the perissodactyl, cetartiodactyl, and paenungulate calibration points, we obtained average substitution rates of 0.160, 0.194, and 0.205 % amino acid / Myr / lineage in the vWF protein (Figure 2). Cross-calibration comparisons between these three points showed that the cetartiodactyl and paenungulate points were compatible, with the latter yielding the youngest ages, whereas the perissodactyl point provided the oldest dates.

Within placentals, the separation between Afrotheria (here represented by two paenungulates: Figure 2) and the remaining groups was estimated to have occurred 122 Myr (range of datings depending on the choice of calibration points: 115-148 Myr), whereas Xenarthra diverged from Cetartiodactyla + Perissodactyla some 106 Myr (range: 100-128 Myr). Our estimates then placed the xenarthran radiation — corresponding to the split between Cingulata and Pilosa — at ca. 63 Myr (Figure 2), around the Cretaceous/Tertiary (K/T) boundary (range: 59-76 Myr). Within Xenarthra, the following splits have been dated: (i) Vermilingua vs. Folivora: 54 Myr in Early Eocene (range: 51-65 Myr) ; (ii) Dasypus vs. other armadillos: 33 Myr in Early Oligocene (31-40 Myr), followed by Chaetophractus vs. Cabassous: 21 Myr in Early Miocene (20-25 Myr) ; (iii) pygmy anteater vs. other anteaters: 38 Myr in Late Eocene (35-45 Myr), followed by giant vs. collared anteaters: 13 Myr in Middle Miocene (12-15 Myr) ; (iv) Bradypus vs. Choloepus: 18 Myr in Early Miocene (16-21 Myr).

Datings were also conducted with the three concatenated markers at the DNA level, assuming three independent ML models, and using the same set of 18 species (LRT: $2\Delta\ln L = 28.8$; P = 0.07). The paenungulate and the cetartiodactyl points yielded similar dating estimates between them (data not shown), and also similar relative to vWF amino acids. For

example, within Xenarthra, the first (armadillos vs. others) and second (anteaters vs. sloths) splits are estimated to be respectively 57-69 Myr and 50-60 Myr old at the DNA level, against 59-63 Myr and 51-54 Myr for vWF amino acids (Figure 2). The perissodactyl calibration point provided the oldest dating estimates, and was not compatible with the two other points: it estimated the Hyracoidea / Proboscidea split to have occurred 118 Myr, against 60 Myr for palaeontology.

DISCUSSION

Molecular systematics and character evolution in Xenarthra

Xenarthra monophyly

The Xenarthra monophyly is unambiguously supported in all reconstructions by the vWF alone or in combination with the 12S and 16S rRNAs (Table 1). The single common ancestry of the eight xenarthrans here studied is also defined by three exclusive vWF amino acid replacements (Table1 and Electronic Appendix II), which should be added to the unique derived deletion in the αA-crystallin known for seven xenarthrans (van Dijk et al. 1999). The monophyly of Xenarthra is also supported by morphological characters like accessory articulations between vertebrae ("xenarthry"), ischiosacral fusion — with a secondary reversal in Cyclopes likely related to its strictly arboreal life style (Gaudin & Branham 1998) — and dental simplification by the loss of enamel (Engelmann 1985). These characters are generally thought to reflect adaptation towards fossoriality and myrmecophagy (Carroll 1988; Gaudin 1999).

Xenarthra intra-ordinal relationships

The monophyly of Cingulata, Vermilingua, and Folivora finds strong support from vWF and mitochondrial rRNAs sequence comparisons. It is also corroborated by numerous morphological characters like, for example, the modification of dermal ossicles into articulate

plates for Cingulata, the total absence of teeth for Vermilingua, and the presence of paired perforations of the centra in lumbar vertebrae for Folivora (Engelmann 1985).

Our data, including all anteater and sloth genera, provide strong support for the clade Pilosa (Tables 1, 2), extending the results of Madsen et al. (2001) and Murphy et al. (2001). This arrangement is in agreement with studies of the ear region (Patterson et al. 1992) and other morphological characters which provided remarkable synapomorphies for Pilosa like, e.g. the interruption of the zygomatic arch or the intra-pelvian location of the testes (Engelmann 1985). These results contrast with those of Guth (1961), Bugge (1979), Carrol (1988), and Cao et al. (1998) who claimed that anteaters are the sister-group of the remaining xenarthrans. In the light of our results, it is likely that earlier studies of the ear region (Guth 1961) and cephalic arterial pattern (Bugge 1979) may have been misled by the extreme specialisation of the skull towards myrmecophagy in anteaters. Reanalysis of ear characters (Patterson et al. 1992) supported the Pilosa hypothesis, and the character pointed by Bugge (1979) for the grouping of armadillos and sloths (i.e. the median course of the internal carotid artery) is likely to be symplesiomorphic. Carrol (1988) suggested an early emergence of anteaters by emphasising the fact that no fossil anteaters were found to have retained dermal ossicles, unlike some mylodontid sloths which possess these relicts of the cingulate-like xenarthrans' ancestor. The fossil record of anteaters is particularly scarce (Gaudin & Branham 1998), and our results showing that anteaters are the sister-group of sloths suggest that fossil anteaters with armour relicts remain to be found. Thus, if fossils are taken under consideration, the replacement of the ancestral carapace by a coat could not be considered as a synapomorphy defining Pilosa. However, this evolutionary trend has led to a discrete pattern in living xenarthrans where armoured xenarthrans (armadillos) have retained the ancestral carapace and hairy xenarthrans (anteaters and sloths) share the possession of a true coat. Our conclusions also contradict those of Cao et al. (1998) based on the mitochondrial NADH

dehydrogenase 1 gene (ND1) which moderately supports a close relationship between the armadillo Cabassous and the sloth Bradypus to the exclusion of the anteater Tamandua. This result may be due to the reduced taxonomic sampling and/or to the particular behaviour of this fast evolving molecule which seems to be subject to convergence (Cao et al. 1998).

Our results confirm the classical arrangement within Vermilingua by favouring the grouping of the strictly terrestrial Myrmecophaga and semi-arboreal Tamandua into the subfamily Myrmecophaginae, to the exclusion of the strictly arboreal Cyclopes. Such a relationship is corroborated by numerous myological (Reiss 1997) and morphological (Gaudin & Branham 1998) characters.

The phylogeny of Cingulata is still poorly understood from a morphological point of view (Patterson et al. 1989). Our results suggest the grouping of Cabassous (Priodontini) and Chaetophractus (Euphractini) to the exclusion of Dasypus (Dasypodini), contra Engelmann (1985) who claims a close relationship between Dasypus and Cabassous. Our results are in agreement with a recent study of sperm morphology and morphometry (Cetica et al. 1998) showing that Cabassous and Chaetophractus have rather similar spermatozoa. However, the discrepancies existing between vWF, 12S, and 16S rRNA suggest that additional genes for a greater taxonomic diversity are needed to further resolve relationships within armadillos.

Searching for the Xenarthra sister-group

The vWF and mitochondrial rRNA genes do not provide a clear response to the difficult question of Xenarthra affinities within Eutheria. However, our analyses including eight armadillos, anteaters and sloths genera suggest that Xenarthra may constitute the second offshoot of the placental tree, Afrotheria being the first one to branch off (Figure 1 and Table 2). This is in agreement with the results obtained by Madsen et al. (2001) and Murphy et al. (2001) on respectively four and five genera. Alternative affinities of Xenarthra with either epitherians, afrotherians, or ferungulates appear less likely (Table 2),

but cannot be significantly rejected. Sequencing of additional molecular markers to provide an increased number of phylogenetically informative positions concomitant with adequate taxonomic diversity of Xenarthra is needed to further resolve their position within the eutherian tree. Interesting to note, the classical hypothesis of "Edentata" (Novacek 1992) is significantly rejected in all cases (Table 2). Together with other molecular results (de Jong 1998; Madsen et al. 2001; Murphy et al. 2001), this confirms that morphological similarities between xenarthrans and pangolins are a spectacular example of convergence in relation to myrmecophagy (Bugge 1979; Rose & Emry 1993).

Molecular timing of the xenarthran radiation

With vWF amino acids, the three calibration points — cetartiodactyls, perissodactyls, paenungulates — are reciprocally compatible in the local molecular clocks analysis (Figure 2). One should note that setting 63 Myr for the split between Bos (rather than Sus) and Physeter would involve 85 Myr for the split between Procavia and Elephas, i.e. a divergence time 25 Myr older than the paleontological one. This is the reason why our molecular datings tend to yield younger dates than previously published for the Hippopotamus / Physeter split: 41-53 Myr (Figure 2) vs. 53-54 Myr (Montgelard et al. 1997; Ursing & Arnason 1998). However, our datings of deeper splits become more compatible with other proposed dates because of increasing intervals between the dates obtained from the three different calibration points. For example, our estimates of the xenarthran separation from other placentals (Figure 2: 100-128 Myr) are in the range of those of Springer (1997) and Waddell et al. (1999b).

The xenarthran radiation, corresponding to the emergence of armadillos, is here estimated to have occurred close to the K/T boundary (59-76 Myr) and the separation between anteaters and sloths during the Palaeocene (51-65 Myr). These results are younger than other molecular estimations yielding about 80 Myr for the radiation of xenarthran families (review in Bromham et al. 1999). Indeed, immunological comparisons suggest ca. 80 Myr (Sarich

1985), partial 12S and 16S rRNA gave the same result (Höss et al. 1996), and Cao et al. (1998) even suggest an interval of 65 to 130 Myr based on the deep divergences they observed in ND1. These different molecular dates suggest that breaking long isolated branches through increased taxonomic sampling within Xenarthra might have modified the divergence time estimates.

Our estimates obtained after extensive taxonomic sampling within Xenarthra are compatible with paleontological data. The first armadillo scutes are from the late Palaeocene (ca. 58 Myr) of Brazil (Scillato-Yané 1976), the first sloth remains are from the Middle Eocene of Antarctica (Vizcaíno & Scillatto-Yané 1995), and the first apparition of undoubted myrmecophagid anteaters is from the Early Miocene of Patagonia (Carlini et al. 1992). Long gaps in the fossil record are clearly not unusual, and our dating of the origin of anteaters and sloths (around the Palaeocene/Eocene limit) suggests a gap of about 30 million years in the South American fossil record of anteaters, and emphasises its incompleteness. Regarding Myrmecophagidae, the very ancient origin of the pygmy anteater (Cyclopes) explains its very divergent morphology shaped by ca. 30 Myr of arboreal life-style, since arboreality is considered ancestral for anteaters (Gaudin & Branham 1998). The separation between the two modern sloths, unknown as fossils (Patterson & Pascual 1972), is dated by our data at ca. 18 Myr (end of Early Miocene). Two-toed (Choloepus) and three-toed sloths (Bradypus) have been placed into two distinct families (respectively Megalonychidae and Bradypodidae) on the basis of their numerous morphological differences and a presumably diphyletic origin (Webb 1985). Our estimation is slightly younger than the 25 Myr of Sarich (1985) but confirms their considerable divergence. It suggests that their apparent external similarities are the result of paralellism and that arboreality may have evolved at least twice within Folivora (Höss et al. 1996; Greenwood et al. 2001). The quite ancient dates obtained for the armadillos radiation strengthen the fact that Cingulata contains strikingly divergent taxa, a result coherent with

marked differences observed in the structure of their spermatozoa (Cetica et al. 1998). Finally, it is noteworthy that numerous cladogenic events within Xenarthra seem to occur close to transitions between Epochs of the chronostratigraphic scale (Figure 2). Since those transitions are defined by dramatic environmental and climatic changes (Pascual & Ortiz Jaureguizar 1990), such a synchrony suggests a major role of paleobiogeographic changes in the diversification of xenarthrans.

Eurotamandua: helping to solve the enigma?

Our molecular estimates of divergence dates for armadillos, anteaters and sloths raise the question of the occurrence of purported fossil xenarthrans outside of South America. Indeed, if the presence of a sloth in the Eocene of Seymour Island is compatible with land connections between Antarctica and the Patagonian province until the Oligocene (Vizcaíno & Scillatto-Yané 1995), the occurrence of Ernanodon in the Palaeocene of China (Ding 1979) and Eurotamandua in the Middle Eocene (45 Myr) of Europe (Storch 1981) are more difficult to explain since South America was an island at these epochs. The true belonging of Ernanodon and Eurotamandua to Xenarthra have been widely debated (Rose & Emry 1993; Gaudin 1999). Actually, Ernanodon antelios seems likely to be assigned to a group of endemic Chinese mammals whose morphological similarities with Xenarthra would reflect convergent adaptations to fossorial habits (Rose & Emry 1993; Gaudin 1999). However, there is no consensus about the status of Eurotamandua joresi (Storch 1981). Vermilinguan affinities of Eurotamandua have been suggested (Storch 1981; Storch & Habersetzer 1991), but several of the most crucial features for assessing its phylogenetic position remain equivocal (Gaudin & Branham 1998; Gaudin 1999; Rose 1999). If Eurotamandua belongs to Vermilingua, our results dating the origin of Vermilingua well nested in the Tertiary (between 54 and 38 Myr: Figure 2) raise again the question of how an anteater reached Europe at a time where South America was isolated from all other continental masses? The only possible response is

dispersal which seems to be very unlikely in the present state of our knowledge of paleobiogeography based on plate tectonic models. Thus, our results, contra Höss et al. (1996), cast doubt on the true belonging of Eurotamandua to Vermilingua. They suggest that the striking morphological resemblances between this taxon and Myrmecophagidae might be the result of adaptative convergence towards fossoriality and ant-feeding. This example underlines, once again, how morphological adaptation to similar ecological niches could be positively misleading in terms of phylogenetic signal.

The xenarthran status of Eurotamandua was also widely questioned and this fossil was interpreted as either an endemic Old World xenarthran sister-group of New World xenarthrans (Szalay & Schrenk 1994), or a Pholidota (Shoshani et al. 1997), or a sister-group to Pilosa (Gaudin & Branham 1998). However, the evidence for the presence of true xenarthry in Eurotamandua is far from convincing (Rose & Emry 1993; Gaudin & Branham 1998; Rose 1999). Based on an exhaustive and detailed study of the evolution of xenarthrous vertebrae, Gaudin (1999) concluded that there is at present little evidence of a close phylogenetic relationship between true xenarthrans and Eurotamandua. Moreover, on the study of forelimbs from a second specimen, Rose (1999) argued for a close relationship of Eurotamandua and Palaeanodonta (a group of extinct fossorial mammals with reduced dentitions). Our results placing the origin of Pilosa in the Early Tertiary contradict those of Gaudin & Branham (1998), but our dating of the xenarthrans origin into Cretaceous (between 106 and 63 Myr: Figure 2) leave opened the possibility that Eurotamandua could be a basal member of Xenarthra. Only the discovering of additional specimens will provide us with a better understanding on the evolutionary affinities of this enigmatic fossil.

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FIGURE CAPTIONS

Figure 1: Maximum Likelihood phylogram obtained using the combination of complete mitochondrial 12S rRNA, partial 16S rRNA and positions 1 and 2 of the nuclear vWF exon 28 (2107 sites whose 1150 variable). The GTR model was used (rate matrix: A-C: 5.28, A-G: 11.33, A-T: 4.86, C-G: 3.05, C-T: 23.03 and G-T: 1.00) and among site variation following a gamma distribution (eight categories) of shape $\alpha = 0.44$ with $\theta = 16\%$ of invariable sites. Branch lengths are proportional to the estimated number of substitution per site, and that leading to the outgroup has been reduced by one-half. Xenarthrans are connected by bold lines. Node labels are bootstrap percentages obtained after 300 ML replications. Note that the combination representing Elephantidae is a chimera between Elephas maximus (asian elephant) for 12S rRNA and vWF, and Loxondonta africana (african elephant) for 16SrRNA. Parameters for 12S rRNA: 838 sites with 485 variable, rate matrix: A-C: 3.80, A-G: 10.32, A-T: 4.67, C-G: 0.41, C-T: 28.00, G-T: 1.00 ; $\theta = 5\%$; $\alpha = 0.30$. Parameters for 16S rRNA: 447 sites with 194 variable, rate matrix: 31.84, 33.33, 20.59, 0.64, 123.70 ; $\theta = 34\%$; $\alpha = 0.41$. Parameters for vWF (codon positions 1 + 2): 822 sites with 494 variable, rate matrix: 2.27, 6.66, 1.48, 2.64, 5.90, 1.00 ; $\theta = 12\%$; $\alpha = 0.77$.

Figure 2: Molecular estimates of divergence dates for the xenarthran radiation based on a reduced dataset of vWF amino acids (415 sites of which 319 were variable). The JTT model of protein evolution and a gamma distribution (eight categories ; $\alpha = 0.94$) were used as likelihood assumptions. The maximum likelihood phylogram constrained to be clocklike was obtained with three local clocks: one for the branch leading to perissodactyls (Ceratotherium and Equus ; rate: 0.09), one for the branch leading to paenungulates (Procavia and Elephas ; rate: 0.45), and one for the remaining branches (default rate: 1.00). Divergence dates (in

million years; Myr) are indicated, and were deduced from three calibration points: C_{PER} (55 Myr: split between Ceratotherium and Equus), C_{PAE} (60 Myr: split between Procavia and Elephas), and C_{CET} (63 Myr: split between Sus and Bos + Hippopotamus + Physeter). Rectangles at nodes represent the range of dates estimated using the three calibration points. Youngest and oldest estimates are respectively provided by the paenungulate and perissodactyl points. Intermediate values are given by the cetartiodactyl point and are indicated near the nodes. Cross-calibration results: (i) the perissodactyl split is estimated to have occurred either 43 [calibration by C_{PAE}] or 46 [C_{CET}] Myr, (ii) paenungulate split: 64 [C_{CET}] or 77 [C_{PER}] Myr, and (iii) cetartiodactyl split: 59 [C_{PAE}] or 76 [C_{PER}] Myr. The split between Macropus and Didelphis has not been dated, because arbitrary dates can be obtained by assuming different relative rates on the two sides of the root (Yoder & Yang 2000). The time scale is given above the tree. The main geological epochs are indicated and delimited by dashed lines: U C = Upper Cretaceous ; Tertiary: Pa = Palaeocene, E = Eocene, O = Oligocene, M = Miocene, and Pl = Plio-Pleistocene. The Cretaceous/Tertiary boundary (K/T) is represented by the vertical striped line. Fossil records of Xenarthra (Eurotamandua excluded) are figured by continuous lines below the tree. Broken lines indicate gaps in the fossil record suggested by our molecular estimates.

TABLES

Table 1. Indices of robustness for the nodes of the Xenarthra subtree, obtained for 12S rRNA, partial 16S rRNA, vWF exon 28 (codon positions 1 + 2), and combination of the three genes, with three methods of phylogenetic reconstruction (ME: distance, MP: maximum parsimony, ML: maximum likelihood). Reliability percentages (RP) after Quartet Puzzling and number of Exclusive (non homoplasic) Synapomorphies (ExS) observed for the vWF exon 28 amino acid data set are also indicated for each node. Position and nature of these exclusive synapomorphies are indicated in Electronic Appendix II.

	12S rRNA				**Partial 16S rRNA**				**vWF (1 + 2)**				**Combination**				**vWF a.a.**	
	BSI	**ME**	**MP**	**ML**	**BSI**	**ME**	**MP**	**ML**	**BSI**	**ME**	**MP**	**ML**	**BSI**	**ME**	**MP**	**ML**	**RP**	**ExS**
Cingulata (armadillos)	+ 25	100	100	100	+ 4	100	94	96	+ 10	100	99	99	+ 36	100	100	100	99	2
Cabassous + Chaetophractus	0	75	-	-	+ 1	85	61	48	+ 2	92	67	50	+ 1	83	61	60	90	2
Vermilingua (anteaters)	+ 3	88	73	96	+ 4	99	80	84	+ 11	100	99	100	+ 16	97	100	100	98	3
Tamandua + Myrmecophaga	+ 22	100	100	100	+ 3	100	92	87	+ 32	100	100	100	+ 44	100	100	100	99	5
Folivora (sloths)	+ 15	100	99	100	+ 5	100	95	95	+ 34	100	100	100	+ 33	100	100	100	99	6
Pilosa (anteaters + sloths)	+ 3	78	53	75	+ 2	73	43	59	+ 4	97	89	93	+ 6	87	88	97	93	2
Xenarthra	+ 3	43	29	48	0	50	24	62	+ 17	100	100	100	+ 27	100	100	100	93	3

Notes: BSI = Bremer Support Indices. ME = bootstrap percentages obtained after 1000 replications of minimum evolution on paralinear Log-Det distances. MP = bootstrap percentages obtained after 1000 replications of standard parsimony. ML = bootstrap percentages obtained after 100

replications of maximum likelihood. RP = reliability percentages obtained with TREE-PUZZLE after 10000 QP steps. A dash indicates that the node does not appear in the corresponding majority-rule consensus bootstrap tree.

Table 2. Results of Kishino & Hasegawa (1989) likelihood tests of alternative topologies computed for the nuclear vWF (codon positions 1 + 2), the mitochondrial 12S and 16S rRNAs, and their combination. δ = ΔlnL / S. E. refers to the ratio between the difference in log-likelihood (ΔlnL) of two phylogenetic hypotheses and its standard error (S. E.). P refers to the one-tailed significance level of the Kishino & Hasegawa test as performed in PAML (*: significantly worse at the 5% level).

	12S rRNA			Partial 16S rRNA			vWF (1+2)			Combination		
Phylogenetic a priori hypotheses	**- lnL**	**δ**	**P**	**- lnL**	**δ**	**P**	**- lnL**	**δ**	**P**	**- lnL**	**δ**	**P**
INTRA XENARTHRA RELATIONSHIPS												
Vermilingua + Folivora (= Pilosa)	11216.44	Best	—	4602.55	Best	—	8569.98	Best	—	24587.14	Best	—
Vermilingua + Cingulata	11223.96	1.26	0.10	4605.04	0.93	0.18	8576.64	1.35	0.09	24603.76	2.06	0.02 *
Folivora + Cingulata	11223.93	1.25	0.11	4604.51	0.58	0.28	8576.42	1.26	0.10	24603.14	1.92	0.03 *
XENARTHRA SISTER-GROUP RELATIONSHIPS												
Xenarthra + Pholidota ("Edentata")	11225.16	1.84	0.03 *	4619.23	2.54	< 0.01 *	8585.99	1.86	0.03 *	24620.34	3.04	< 0.01 *
Xenarthra + Epitheria	11216.94	0.44	0.33	4602.55	Best	—	8574.29	1.09	0.14	24591.23	1.07	0.14
Xenarthra 2nd offshoot	11216.46	0.08	0.47	4604.30	0.75	0.23	8569.98	Best	—	24587.14	Best	—
Xenarthra + Afrotheria	11216.94	0.44	0.33	4604.13	0.62	0.27	8574.29	1.09	0.14	24591.31	1.11	0.13
Xenarthra + Ferungulata	11216.44	Best	—	4609.68	0.99	0.16	8572.31	0.41	0.34	24594.88	0.95	0.17

Electronic Appendix I: Taxonomic sampling used for this study. Accession numbers and corresponding references are given for each sequence used. Tissue numbers refer to the collection of mammalian tissues of the "Institut des Sciences de l'Evolution" de Montpellier (Catzeflis 1991).

Species	Common name	12S rRNA	Partial 16S rRNA	vWF exon 28
MARSUPIALIA				
DIDELPHIMORPHIA				
Didelphis virginiana	Virginia opossum	Z29573, Janke *et al.* (1994)	Z29573, Janke *et al.* (1994)	AF226848, Madsen *et al.* (2001)
DIPROTODONTIA				
Macropus giganteus	Eastern grey kangaroo	X86941, Douzery & Catzeflis (1995)	—	AJ224670, Huchon *et al.*(1999)
Macropus robustus	Wallaroo	—	Y10524, Janke *et al.* (1997)	—
PLACENTALIA				
CHRYSOCHLORIDAE				
Amblysomus hottentotus	Hottentot golden mole	M95108, Allard & Miyamoto (1992)	U97336, Springer *et al.* (1997)	U97534, Springer *et al.* (1997)
TENRECIDAE				
Echinops telfairi	Lesser hedgehog-tenrec	AF069540, Stanhope *et al.* (1998)	AF069540, Stanhope *et al.* (1998)	AF076478, Stanhope *et al.* (1998)
EULIPOTYPHLA				
Erinaceus europaeus	West European hedgehog	X88898, Krettek *et al.* (1995)	X88898, Krettek *et al.* (1995)	U97536, Springer *et al.* (1997)
Scalopus aquaticus	Eastern mole	AF069539, Stanhope *et al.* (1998)	AF069539, Stanhope *et al.* (1998)	AF076479, Stanhope *et al.* (1998)
MACROSCELIDEA				
Elephantulus rufescens	Rufous elephant shrew	U97339, Springer *et al.* (1997)	U97339, Springer *et al.* (1997)	U31612, Porter *et al.* (1996)
SIRENIA				
Dugong dugon	Dugong	U60185, Lavergne *et al.* (1996)	AF179291, Springer *et al.* (in press)	U31608, Porter *et al.* (1996)
PROBOSCIDEA				
Elephas maximus	Asian elephant	X93602, Lavergne *et al.* (1996)	—	U31611, Porter *et al.* (1996)
Loxodonta africana	African elephant	—	AJ224821, Hauf *et al.* (2000)	—
HYRACOIDEA				
Procavia capensis	Common rock hyrax	U60184, Lavergne *et al.* (1996)	U97335, Springer *et al.* (1997)	U31619, Porter *et al.* (1996)
TUBULIDENTATA				
Orycteropus afer	Aardvark	U97338, Springer *et al.* (1997)	U97338, Springer *et al.* (1997)	U31617, Porter *et al.* (1996)
CARNIVORA				
Canis familiaris	Domestic dog	Y08507, Ledje & Arnason (1996)	U96639, Kim *et al.* (1998)	L16903, Mancuso *et al.* (unp.)
Felis catus	Domestic cat	U20753, Lopez *et al.* (1996)	U20753, Lopez *et al.* (1996)	U31613, Porter *et al.* (1996)
PRIMATES				

Homo sapiens	Human	J01415, Anderson *et al.* (1981)	J01415, Anderson *et al.* (1981)	X06828, Bonthron & Orkin (1988)
Ateles sp. †	Spider monkey	AF069978, Horovitz *et al.* (1998)	U39011, Horovitz & Meyer (1995)	—
Ateles belzebuth	White-bellied spider monkey	—	—	AF061059, Stanhope *et al.* (1998)
CETARTIODACTYLA				
Bos Taurus	Domestic cow	J01394, Anderson *et al.* (1982)	J01394, Anderson *et al.* (1982)	X63820, Bakhshi *et al.* (1992)
Sus scrofa	Domestic pig	AJ002189, Ursing & Arnason (1998a)	AJ002189, Ursing & Arnason (1998a)	S78431, Bahnak *et al.* (1992)
Physeter catodon	Sperm whale	Arnason *et al.* (1993)*	AJ277029, Arnason *et al.* (2000)	AF108834, Gatesy *et al.* (1999)
Hippopotamus amphibius	Common hippopotamus	AJ010957, Ursing & Arnason (1998b)	AJ010957, Ursing & Arnason (1998b)	AF108832, Gatesy *et al.* (1999)
Lama guanicoe	Guanaco	Y08809, Montgelard *et al.* (1997)	AJ010815, Douzery *et al.* (unp.)	—
Lama glama	Lama	—	—	AF108835, Gatesy *et al.* (1999)
PHOLIDOTA				
Manis sp. †	Pangolin	U61079, Springer & Douzery (1996)	U97340, Springer *et al.* (1997)	U97535, Springer *et al.* (1997)
RODENTIA				
Mus musculus	Domestic mouse	X84382, Hänni *et al.* (1995)	V00711, Bibb *et al.* (1981)	AJ238390, Huchon *et al.* (1999)
Cavia porcellus	Domestic Guinea pig	AJ222767, D'Erchia *et al.* (1996)	AJ222767, D'Erchia *et al.* (1996)	AJ224663, Huchon *et al.* (1999)
PERISSODACTYLA				
Equus asinus	Donkey	X97337, Xu *et al.* (1996)	X97337, Xu *et al.* (1996)	U31610, Porter *et al.* (1996)
Ceratotherium simum	White rhinoceros	X86942, Douzery & Catzeflis (1995)	Y07726, Xu & Arnason (1997)	U31604, Porter *et al.* (1996)
CHIROPTERA				
Megaderma lyra	False vampire bat	AF069538, Stanhope *et al.* (1998)	AF069538, Stanhope *et al.* (1998)	U31616, Porter *et al.* (1996)
Dobsonia moluccensis	Bar-backed fruit bat	U93065, Hollar & Springer (1997)	AF179290, Teeling *et al.* (2000)	U31609, Porter *et al.* (1996)
XENARTHRA				
Dasypus novemcinctus	Nine-banded armadillo	Y11832, Arnason *et al.* (1997)	Y11832, Arnason *et al.* (1997)	AJ278158, tissue T-1830, this study
Chaetophractus villosus	Larger hairy armadillo	U61080, Springer & Douzery (1996)	AF069534, Stanhope *et al.* (1998)	AF076480, Stanhope *et al.* (1998)
Cabassous unicinctus	Southern naked-tailed armadillo	AJ278151, tissue T-1641, this study	Z48940, Höss *et al.* (1996)	AJ278159, tissue T-1641, this study
Myrmecophaga tridactyla	Giant anteater	AJ278153, tissue T-2080, this study	AJ297939, tissue T-2080, this study	AJ278157, tissue T-2080, this study
Tamandua tetradactyla	Collared anteater	AJ278154, tissue T-1640, this study	Z48946, Höss *et al.* (1996)	AJ278161, tissue T-2038, this study
Cyclopes didactylus	Pygmy anteater	AJ278155, tissue T-1724, this study	Mark Springer (unpublished)	AJ278156, tissue T-1724, this study
Bradypus tridactylus	Pale-throated three-toed sloth	AF038022, Springer *et al.* (unp.)	AF069535, Stanhope *et al.* (1998)	U31603, Porter *et al.* (1996)
Choloepus didactylus	Southern two-toed sloth	AJ278152, tissue T-1722, this study	Z48942, Höss *et al.* (1996)	AJ278160, tissue T-1722, this study

unp. : unpublished.

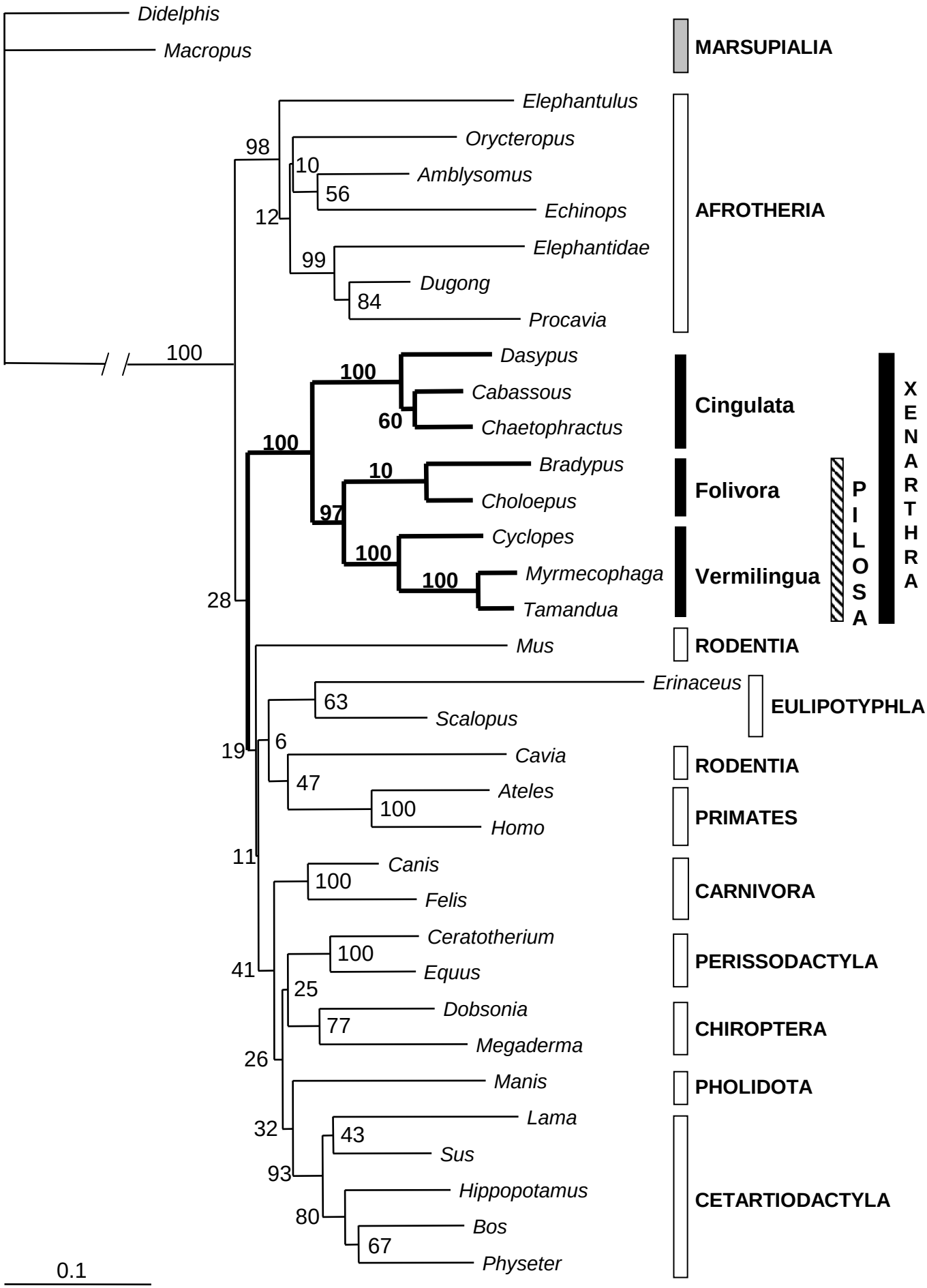
* : This sequence was manually copy from the reference cited because it has not been yet deposited in EMBL.

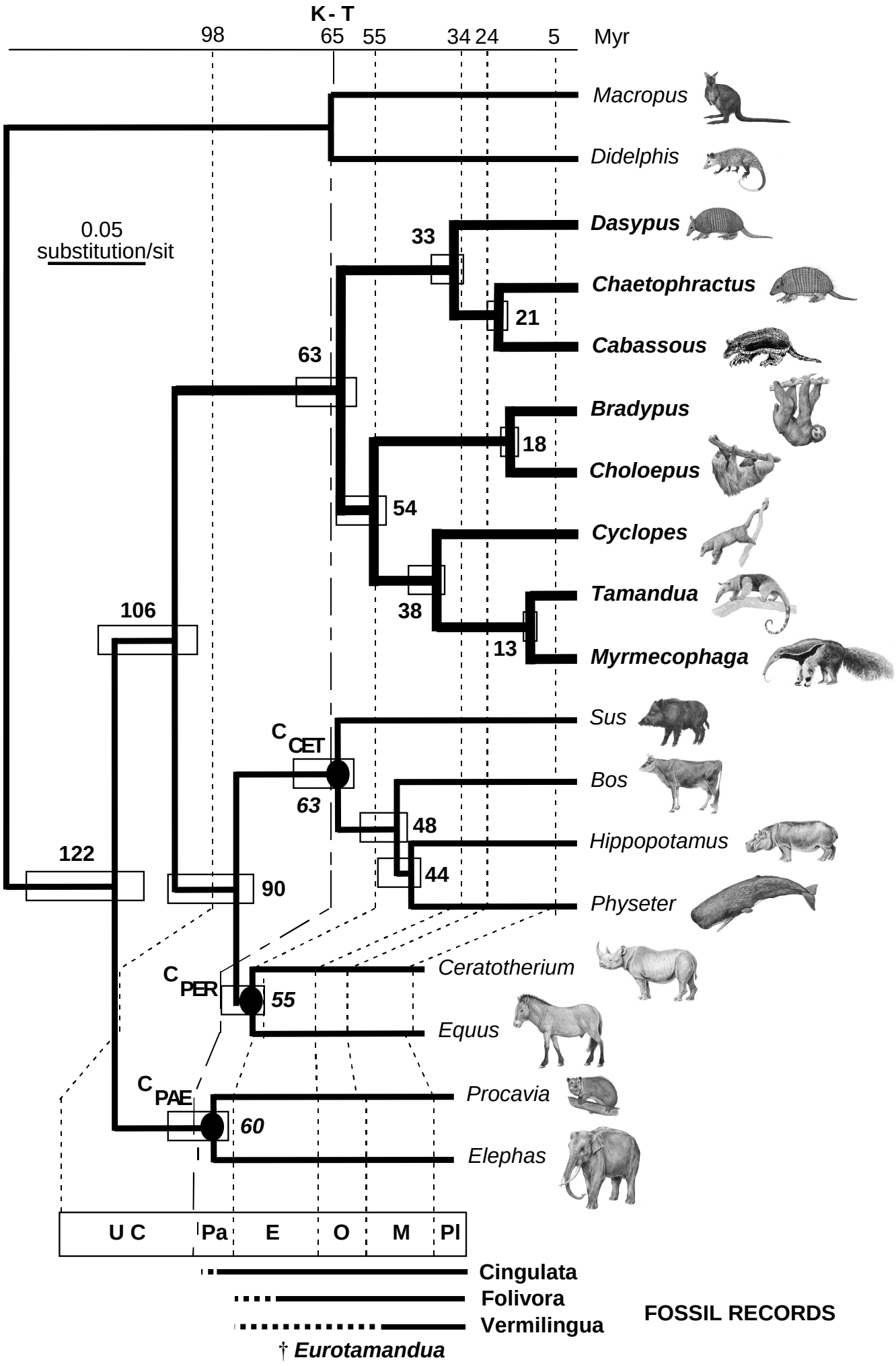
† : The species name of the specimens are unknown.

Abridged references: Allard & Miyamoto (1992) Mol. Biol. Evol. 9: 778-786; **Anderson *et al.* (1981)** Nature 290: 457-465; **Anderson *et al.* (1982)** J. Mol. Evol. 156: 683-717; **Arnason *et al.* (1993)** Biochem. Syst. Ecol. 21: 115-122; **Arnason *et al.* (1997)** Mol. Biol. Evol. 14: 762-768; **Arnason *et al.* (2000)** J. Mol. Evol. 50: 569-578; **Bahnak *et al.* (1992)** Biochem. Biophys. Res. Commun. 182: 561-568; **Bakhshi *et al.* (1992)** Biochim. Biophys. Acta 1132: 325-328; **Bibb *et al.* (1981)** Cell 26: 167-180; **Bonthron & Orkin (1988)** Eur. J. Biochem. 171: 51-57; **D'Erchia *et al.* (1996)** Nature 381: 597-600; **Douzery (1993)** C. R. Acad. Sci. Paris, Sciences de la vie 316: 1511-1518; **Douzery & Catzeflis (1995)** J. Mol. Evol. 41: 622-636; **Höss *et al.* (1996)** Proc. Natl. Acad. Sci. U.S.A. 93: 181-185; **Gatesy *et al.* (1999)** Syst. Biol. 48: 16-20; **Hänni *et al.* (1995)** Isr. J. Zool. 41: 131-146; **Hauf *et al.* (2000)** Zoology 102: 184-195; **Hollar & Springer (1997)** Proc. Natl. Acad. Sci. U.S.A. 94: 5716-5721; **Horovitz & Meyer (1995)** Mol. Phylogenet. Evol. 4: 448-456; **Horovitz *et al.* (1998)** Am. J. Phys. Anthropol. 106: 261-281; **Huchon *et al.* (1999)** Mol. Biol. Evol.16: 577-589; **Huchon *et al.* (2000)** Proc. R. Soc. Lond. B 267: 393-402; **Janke *et al.* (1994)** Genetics 137: 243-256; **Janke *et al.* (1997)** Proc. Natl. Acad. Sci. U.S.A. 94: 1276-1281; **Kim *et al.* (1998)** Mol. Phylogenet. Evol. 10: 210-220; **Krettek *et al.* (1995)** J. Mol. Evol. 41: 952-957; **Lavergne *et al.* (1996)** Mol. Phylogenet. Evol. 6: 245-258; **Ledje & Arnason (1996).** J. Mol. Evol. 43: 641-649; **Lopez *et al.* (1996)** Genomics 33: 229-246; **Madsen *et al.* (2001)** Nature 409: 610-614; **Mancuso *et al.* (1989)** J. Biol. Chem. 264: 19514-19527; **Montgelard *et al.* (1997)** Mol. Biol. Evol. 14: 550-559; **Porter *et al.* (1996)** Mol. Phylogenet. Evol. 5: 89-101; **Springer & Douzery (1996)** J. Mol. Evol. 43: 357-373; **Springer *et al.* (1997)** Nature 388: 61-64; **Stanhope *et al.* (1998)** Proc. Natl. Acad. Sci. U.S.A. 95: 9967-9972; **Teeling *et al.* (2000)** Nature 403: 188-192; **Ursing & Arnason (1998a)** J. Mol. Evol. 47: 302-306; **Ursing & Arnason (1998b)** Proc. R. Soc. Lond. B. 265: 2251-2255; **Xu *et al.* (1996)** Mol. Biol. Evol. 13: 1167-1173; **Xu & Arnason (1997)** Mol. Phylogenet. Evol. 7: 189-194.

Electronic Appendix II. Position and nature of the exclusive Amino-Acids synapomorphies observed in the vWF exon 28 protein sequences alignment supporting nodes of the Xenarthra subtree.

Clades	**Positions**	**Substitutions**
Cingulata (armadillos)	25	Asp, Glu => Gly
	162	Phe, Val, Leu, Ala, Gly => Pro
Cabassous* + *Chaetophractus	254	Gly, Ala, Glu, Leu, Ile, Thr, Arg, Asn => Ser
	294	Asp, Ala => His
Vermilingua (anteaters)	35	Phe, His, Tyr, Leu => Glu
	360	Gly, Asp => His
	375	Asn, Ser => Gly
Tamandua* + *Myrmecophaga	2	Ala, Glu, Lys, Arg, Gly => Ser
	212	Gln, Arg, His, Pro => Ala
	223	Asp => Gly
	303	Thr, Ala, Ser, Met => Glu
	320	Ala, Thr, Pro, Val => Arg
Folivora (sloths)	168	Leu => Met
	233	Gln, Arg, Pro, Val, Met, Ile, Leu => Ala
	234	Pro, Gln, Arg, His, Phe => Ala
	235	Pro, Ser, Thr, His => Ala
	307	Tyr => Phe
	375	Asn, Ser => Thr
Pilosa (anteaters + sloths)	121	Thr, Ile => Ala
	154	Pro => Ser
Xenarthra	173	Val, Ala => Ile
	331	Arg, Gln, Glu, His, Asp => Gly
	371	Met => Leu


Didelphis
Macropus
MARSUPIALIA
Elephantulus
Orycteropus
Amblysomus
Echinops
Elephantidae
Dugong
Procavia
AFROTHERIA
98
10
56
12
99
84
100
Dasypus
Cabassous
Chaetophractus
Cingulata
100
60
100
Bradypus
Choloepus
Folivora
10
97
Cyclopes
Myrmecophaga
Tamandua
Vermilingua
100
100
28
PILOSA
XENARTHRA
Mus
RODENTIA
Erinaceus
Scalopus
EULIPOTYPHLA
63
6
19
Cavia
RODENTIA
47
Ateles
Homo
PRIMATES
100
11
Canis
Felis
CARNIVORA
100
Ceratotherium
Equus
PERISSODACTYLA
100
41
25
Dobsonia
Megaderma
CHIROPTERA
77
26
Manis
PHOLIDOTA
Lama
Sus
Hippopotamus
Bos
Physeter
CETARTIODACTYLA
32
43
93
80
67
0.1


K - T
98
65
55
34
24
5
Myr
Macropus
Didelphis
0.05
substitution/sit
Dasypus
33
Chaetophractus
21
Cabassous
63
Bradypus
18
Choloepus
54
Cyclopes
Tamandua
38
106
13
Myrmecophaga
Sus
C CET
63
Bos
48
Hippopotamus
122
44
90
Physeter
Ceratotherium
C PER
55
Equus
C PAE
Procavia
60
Elephas
U C
Pa
E
O
M
Pl
Cingulata
Folivora
Vermilingua
† Eurotamandua
FOSSIL RECORDS