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The ‘‘*Phoca* Standard’’: An External Molecular Reference for Calibrating Recent Evolutionary Divergences

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Abstract. Comparison of the complete mitochondrial DNA (mtDNA) of the high-Arctic ringed seal (*Phoca hispida*) and the sub-Arctic harbour (*P. vitulina*) and grey (*Halichoerus grypus*) seals shows that they are genetically equidistant from one another. We relate the evolutionary divergence of the three species to expanding glaciation in the Arctic Basin and establish, in conjunction with mtDNA data, a standard reference for calibration of recent divergence events among mammalian taxa. In the present study, we apply the ‘‘*Phoca* standard’’ to the dating of divergences within the hominid phylogenetic tree. After determining the relative rates of substitution over all mitochondrial protein-coding genes in the different evolutionary lineages, we estimate that humans and chimpanzees diverged from each other 6.1 Mya (95% confidence limits: 5.2–6.9 Mya). The corresponding lower-limit divergence between common chimpanzee, *Pan troglodytes*, and pygmy chimpanzee, *P. paniscus*, occurred 3 (2.4–3.6) Mya, and the primary split within the *P. troglodytes* complex 1.6 (1.3–2.0) Mya. The analyses suggest that the split between *Gorilla* and *Pan*/*Homo* occurred 8.4 (7.3–9.4) Mya. They also suggest that *Pongo* (orangutan) and the lineage leading to gorillas, chimpanzees, and humans diverged 18.1 (16.5–19.6) Mya. The present analysis is independent of the hominid paleontological record and inferential morphological interpretations and thus is a novel approach to the lower-limit dating of recent divergences.

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Introduction

Molecular dating of evolutionary divergence depends on the assignment of reliable dates to at least one of the nodes of the phylogenetic tree and on the calibration of the molecular clocks for the branches involved. In mammals there are very few paleontological estimates that allow unequivocal dating of either distant or recent evolutionary divergencies. The primary reasons for this are the fragmentary nature of the fossil record and the potential incongruity between evolutionary divergence and morphological distinction.

The evolutionary history of the Hominoidea (gibbons, great apes, and human) has attracted a great deal of attention. There are only a dozen or so unambiguous hominid (great ape and human) specimens dated to between 14 and 4 Mya, but none of them preserves much anatomical detail. Moreover, no fossil evidence of ancestral chimpanzee (*Pan*) has yet been recognized, and therefore the paleontological record does not permit conclusive dating of the evolutionary separation between humans and their closest relatives (Pilbeam 1984; Andrews 1987; Andrews and Martin 1987; Hill and Ward 1988; Pilbeam et al. 1990; White et al. 1994).

The problems with the molecular dating of the hominid divergence events are the same as for mammals in general—namely, the absence of data on divergence times that can be associated with the various nodes

within or outside the phylogenetic tree. For example, estimates for the divergence time between Hominoidea and Cercopithecoidea (Old World monkeys) as well as that for Catarrhini (Old World monkeys and apes) and Platyrrhini (New World monkeys) are still contentious and have very wide ranges (Gingerich 1984, 1986; Godinot and Mahboubi 1992). It is evident that an extrapolation of inconclusive ancestral dates to different divergence events within the hominid tree cannot provide reliable estimates of the more recent divergences. Putative dates of divergence between *Pongo* (orangutan) and the lineage leading to *Gorilla, Pan,* and *Homo,* ranging from 10 to 15 Mya, have been frequently used in the past to calibrate the hominid tree. Recently it has been suggested that 12 Mya should be taken as the *minimum* estimate of the divergence between the African apes and the orangutan (Kappelman et al. 1991). However, the use of *Pongo* as a reference for dating the evolutionary separation of the African hominids has its limitations, because a common ancestor of *Pongo* and the lineage ancestral to *Gorilla*/*Pan*/*Homo* has not yet been identified in the fossil record, and therefore the paleontological estimate has no well-defined upper limit.

Here we use newly sequenced mtDNAs of *Homo* and *Pan troglodytes verus,* the West African common chimpanzee subspecies (Arnason et al. 1996), together with the mtDNAs of *Gorilla gorilla, Pan paniscus,* and *Pongo pygmaeus* (Horai et al. 1995), in a phylogenetic study that includes all the living great apes and *Homo.* In order to date the various hominid divergences we use as an external standard the mtDNA sequences from the harbour (Arnason and Johnsson 1992) and grey (Arnason et al. 1993) seals. The mtDNA from the opossum *Didelphis virginiana* (Janke et al. 1994) was used as an outgroup. The divergence of the seals has been set at 2.7 Mya. This figure is in accord with newly calibrated datings for expanding glaciation in the Arctic Basin (H. Kleiven and E. Jansen in preparation). Cooling of the Arctic Basin, however, was a gradual process and the figure 2.7 Mya should therefore be taken as a lower-limit dating for the seal divergence.

Materials

In the present study we use a newly determined human sequence. The reason is that the original sequence (Anderson et al. 1981) was based partly on placental tissue and partly on cultured HeLa cells. The mtDNA molecules of human and the common chimpanzee used are each derived from a single source and are, thus, not chimeric (Arnason et al. 1996). Both sequences were determined in their entirety on the basis of sequence data of natural (not PCR) clones.

Results and Discussion

Between the human and chimpanzee sequences the ratio for total difference according to codon positions 1,2, and 3, is 2.7:1:12 (Arnason et al. 1996). The values are very similar to those, 2.7:1:16, of a corresponding comparison between the harbour and grey seals (Arnason et al. 1993a). The ratio for conservative nucleotide changes (Irwin et al. 1991) between chimpanzee and human is 2:1.2:1 (Arnason et al. 1996), virtually the same as that found in the two seal species, 2:1.2:1.1 (Arnason et al. 1993a). The pronounced conformity between the values for *Pan*/*Homo* and the harbour/grey seals suggests that the mode of evolution of mtDNA is basically similar in the two lineages.

The mtDNA data of the *Phoca* complex of the family Phocidae (true seals) are extensive. The complete mtDNAs of the harbour and grey seals, as well sequence data of the complete cytochrome *b* gene of many members of the family Phocidae, have been reported (Arnason et al. 1995). The mtDNAs of the ringed seal and an additional harbour seal specimen have also been completely sequenced (unpublished sequences). The latter study was undertaken in order to investigate whether within-species variation might affect comparisons between species. Between the two harbour seal specimens, one from the Baltic Sea and one from Iceland, the total nucleotide difference outside the control region was only 0.17%, negligible for comparisons between species. The ringed, harbour, and grey seals are approximately equidistant from one another. Outside the control region, the mtDNA difference between the harbour and grey seals is 3.48%. The corresponding figure for the harbour and ringed seals is 3.46% and that for the ringed and grey seals is 3.15%.

The commonly accepted understanding of the genesis of a new species is that an ancestral lineage splits into two lineages which thereafter gradually diverge. The tripartite phocid evolutionary split giving rise to the high-Arctic ringed seal and the sub-Arctic harbour and grey seals has been connected with a major geological event, the acceleration of the Northern Hemisphere glaciation (Arnason et al. 1995). The cooling of the Arctic Basin and the expansion of Northern Hemisphere glaciation was a progressive process. The dramatic events of very extensive glaciation are, however, marked geologically by the first major peaks of ice-rafted detritus in the subpolar Nordic seas and the North Atlantic (Shackleton et al. 1984; Jansen and Sjøholm 1991; H. Kleiven and E. Jansen in preparation). Renewed age calibration of this event in Ocean Drilling Program Site 644 in the Norwegian Sea and in Deep Sea Drilling Program Site 610 in the NE Atlantic, using new time scales (Cande and Kent 1992; Shackleton et al. 1995), places it in the upper part of the Gauss magnetic Chron at 2.7 Mya in isotope stage G6 (H. Kleiven and E. Jansen in preparation). We use this dating for defining the lower limits of the seal divergence.

In order to determine the relative rates of evolution in the seal and the hominid lineages, we used as outgroup the mtDNA sequence from the opossum *Didelphis virginiana* (Janke et al. 1994). We did not use any of the completely sequenced eutherian mtDNAs in the literature (e.g., rat, mouse, blue whale, cow, horse) because

Table 1. Total number of substitutions in 13 mitochondrial protein-coding genes (above diagonal) and rRNA-specifying genes (below diagonal)[a]

	Pan troglodytes	*Pan paniscus*	*Homo sapiens*	*Gorilla gorilla*	*Pongo pygmeaeus*	*Halichoerus grypus*	*Phoca vitulina*	*Didelphis virginiana*
Pan troglodytes	—	0.1482 (0.0078)	0.4192 (0.0153)	0.4984 (0.0175)	0.6751 (0.0228)	1.4436 (0.0617)	1.3854 (0.0573)	1.8250 (0.1003)
		0.0106 (0.0011)	0.0212 (0.0016)	0.0305 (0.0019)	0.0651 (0.0029)	0.1768 (0.0050)	0.1759 (0.0050)	0.2318 (0.0059)
Pan paniscus	0.0271 (0.0034)	—	0.4079 (0.0150)	0.4833 (0.0171)	0.6604 (0.0223)	1.4370 (0.0612)	1.3937 (0.0580)	1.8467 (0.1031)
			0.0219 (0.0016)	0.0298 (0.0019)	0.0645 (0.0029)	0.1761 (0.0050)	0.1753 (0.0050)	0.2325 (0.0059)
Homo sapiens	0.0486 (0.0046)	0.0609 (0.0051)	—	0.5358 (0.0186)	0.6593 (0.0223)	1.4643 (0.0633)	1.4500 (0.0622)	2.0405 (0.1318)
				0.0295 (0.0019)	(0.0659 (0.0029)	(0.1772 (0.0050)	0.1767 (0.0050)	0.2338 (0.0059)
Gorilla gorilla	0.0618 (0.0052)	0.0574 (0.0050)	0.0743 (0.0057)	—	0.6689 (0.0226)	1.4546 (0.0626)	1.4620 (0.0631)	2.0378 (0.1314)
					0.0663 (0.0029)	0.1786 (0.0051)	0.1791 (0.0051)	0.2358 (0.0060)
Pongo pygmeaeus	0.1132 (0.0072)	0.1057 (0.0069)	0.1190 (0.0074)	0.1128 (0.0072)	—	1.3632 (0.0557)	1.3168 (0.0526)	1.9791 (0.1216)
						0.1838 (0.0051)	0.1844 (0.0052)	0.2399 (0.0060)
Halichoerus grypus	0.2298 (0.0109)	0.2399 (0.0112)	0.2427 (0.0113)	0.2456 (0.0114)	0.2692 (0.0121)	—	0.1542 (0.0080)	1.7679 (0.0937)
							0.0072 (0.0009)	0.2077 (0.0055)
Phoca vitulina	0.2309 (0.0110)	0.2416 (0.0113)	0.2399 (0.0112)	0.2461 (0.0114)	0.2628 (0.0119)	0.0203 (0.0029)	—	1.7941 (0.0968)
								0.2062 (0.0055)
Didelphis virginiana	0.3276 (0.0138)	0.3257 (0.0138)	0.3378 (0.0141)	0.3372 (0.0141)	0.3619 (0.0148)	0.2798 (0.0124)	0.2768 (0.0123)	—

[a] Above diagonal, upper values: numbers of synonymous substitutions per synonymous site; lower values: number of nonsynonymous substitutions per nonsynonymous site. Standard errors for all comparisons are shown in parenthesis

none of them is regarded unequivocally as an outgroup of both primates and carnivores (seals). The data analyses were based on all protein-coding genes and the two rRNA-specifying genes. The two sets of data were analyzed separately.

For the 13 protein-coding genes, we estimated the number of synonymous and nonsynonymous substitutions for each of the 28 possible pairs of taxa (Table 1) according to the method of Nei and Gojobori (1986). Branch lengths were estimated by the neighbor joining method (Saitou and Nei 1987). The JC (Jukes and Cantor 1969) method was used to correct for multiple hits. The numbers of synonymous and nonsynonymous substitutions were calculated on a combined stretch of 11,397 nucleotides (3,799 codons). For the subsequent calculations, we used only nonsynonymous substitutions, because synonymous sites are saturated in many of the 28 pairwise comparisons. Dates of divergence are listed as 95% confidence limits around the mean.

Our results suggest that on average hominid mtDNAs have evolved about 1.4 times faster than those of the seals. By taking 2.7 Mya as the lower-limit divergence time between the harbour and grey seals, and by correcting for the inequalities in evolutionary rates, we infer that the lower limit for the *Pan*/*Homo* split is 6.1 Mya (95% confidence limits: 5.2–6.9 Mya). Also in the analysis of the rRNA-specifying genes we used JC correction for multiple hits and estimated the branch lengths by the neighbor-joining method. The estimates for the *Pan*/*Homo* divergence derived from this set of data are about the same as those derived from the protein-coding genes, ≈5.5 (5.2–5.7) Mya; essentially the same results are obtained by using other methods of branch-length inference. Estimates of dates of divergence for the other lineages within the hominid tree are given in the legend of Fig. 1.

In order to date the divergence between *P. t. troglodytes*, Central African common chimpanzee, and *P. t. verus*, West African common chimpanze, we compared the complete cytochrome *b* gene (1,140 nt) and a portion of the NADH4 (457 nt) and NADH5 (294 nt) genes of the two subspecies (Arnason et al. 1996). The analysis suggested that the *verus* and *troglodytes* varieties diverged from each other ≈1.6 (1.3–2.0) Mya. This dating is about the same as that obtained in a recent population study of the common chimpanzee that was based on different sets of molecular data (Morin et al. 1994).

Previous molecular estimates of hominid divergence

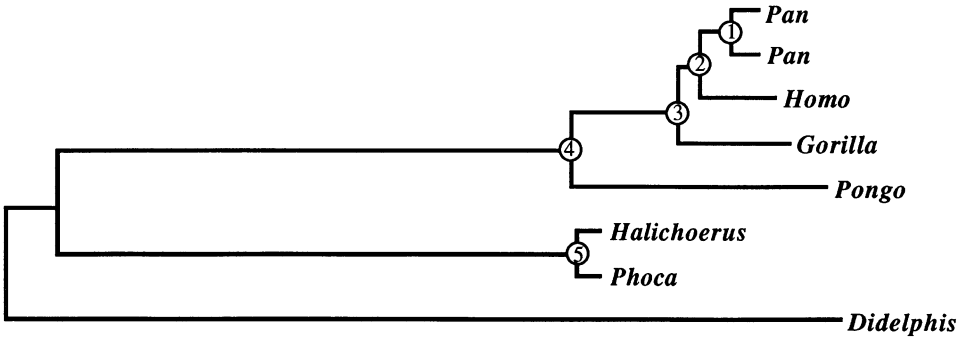

Fig. 1. Phylogeny of five hominids, two phocids (true seals) and an outgroup (opossum) based on a concatenated sequence of all the 13 protein-coding mitochondrial genes. The inferred mean divergence times (and 95% confidence limits) for the marked nodes are: (1) 3.0 Mya (2.3–3.6), (2) 6.1 Mya (5.2–6.9), (3) 8.4 Mya (7.3–9.4), and (4) 18.1 Mya (16.5–19.6). Preliminary analyses using the gibbon as outgroup (Arnason et al. in preparation) suggest that the evolutionary rate of *Pongo* has accelerated relative to other hominids. Based on these data, recalibration of the split between *Pongo* and *Gorilla*/*Pan*/*Homo* places it at 15 Mya. Node (5) set at 2.7 Mya has been used as reference for the calculations. At this date extensive glaciation had taken place in the Arctic Basin and this figure is therefore proposed as the under limit for the above divergences. An earlier split cannot be excluded, however.

times were based on internal or external calibrations of the primate tree on the basis of fossil data. However, dating of evolutionary separations on the basis of paleontological data and morphological comparisons is difficult to accomplish. There are several reasons for the difficulties: (1) temporal incompleteness of the paleontological record, (2) morphological incompleteness of the fossil remains, (3) difficulties in the assignment of particular fossils to internal branches of the phylogenetic tree, (4) problematic temporal calibrations, and (5) incongruity between morphological distinction and time of evolutionary divergence. Among the hominids the latter problem was recently highlighted in studies of gorillas (Ruvolo et al. 1994) and the common chimpanzee (Morin et al. 1994), which showed that populations within each species have diverged from each other $\geqslant$1.5 Mya without this pronounced genetic and evolutionary divergence being accompanied by any significant morphological distinction. Thus in *Pan troglodytes* no osteological difference has become established that permits conclusive identification of two subspecies (*P. t. troglodytes* and *P. t. verus*) that have existed for about half the age of the species (i.e., the divergence between *P. troglodytes* and *P. paniscus*).

Similar cases of incongruity between morphological and evolutionary distinctions have also been demonstrated among marine mammals. The most notable case is probably that of the morphologically distinct grey whale of the monotypic family Eschrichtiidae, which molecular analyses place within genus *Balaenoptera* of the family Balaenopteridae, rorquals (Arnason et al. 1993b; Arnason and Gullberg 1994, 1996). The evolutionary separation of the gray whale and the balaenopterids took place ≈5–6 Mya, but the paleontological record goes back to only 100,000 years. Also within genus *Balaenoptera* the same molecular analyses have shown that there is a greater distinction between the morphologically very similar Antarctic and North Atlantic minke whales than that between the two distinct species the sei and the Bryde's whales. Similarly among the true seals it has been shown in the *Phoca* complex that evolutionary divergencies of 5–6 million years have produced very limited morphological distinction, whereas in the case of the Weddell and leopard seals a pronounced morphological distinction coincides with a relatively short evolutionary separation (Arnason et al. 1995).

Five divergence events are commonly used in the literature to calibrate the hominoid tree. These are: the divergence between primates and other eutherian orders (65–100 Mya), the divergence between Prosimii and Anthropoidea (45–65 Mya), the divergence between the New World Plathyrrhini and the Old World Catarrhini (35–55 Mya), the divergence between Cercopithecoidea and Hominoidea (20–37 Mya), and the divergence between *Pongo* and the ancestor of *Homo*/*Pan*/*Gorilla* (>12 Mya). Unfortunately, all the five events are defined quite broadly and the use of different estimates within the ranges given for any particular speciation event may therefore produce widely divergent conclusions. In general, while lower limits of times of divergence are based on paleontological data pertaining to the existence of representatives from both descending branches, upper limits are frequently based on absence of the taxonomic grades in question. Thus the earliest estimates of a divergence event can be pushed back quite substantially. The above-mentioned examples of hominids and marine mammals demonstrate in quite a dramatic way how difficult it may be, even among recent mammals, to determine evolutionary affinities purely on morphological grounds. Problems of this kind are much more difficult to solve when evolutionary affinities can be judged solely on the basis of a fossil record that may be fragmentary both with respect to continuity and completeness of specimens.

It may be argued that the mean difference in the rates of evolution that we have registered between hominids and seals are an artifact of fluctuating evolutionary rates within each of the lineages. In the case of the seals, we have data of the complete cytochrome *b* gene from about 40 different species of carnivores, all of which are approximately equally distant from external references. The relative rate of evolution in the primate and carnivore lineages were determined by using the opossum as outgroup. The reason for this is that other complete mtDNAs described do not represent an unequivocally accepted outgroup to both Primates and Carnivora. It is likely, however, that within a few years it will be possible to reassess the relative rate of evolution among both Primates and Carnivora by using a less-distant outgroup than the opossum. Preliminary analysis using a closely related outgroup (white-handed gibbon, *Hylobates lar*) suggests that the rate of nonsynonymous nucleotide substitution in *Pongo* has accelerated relative to *Gorilla*/*Pan*/*Homo* (Arnason et al. in preparation). At this time we do not detail datings of divergence within the hominid phylogenetic tree that have been obtained by different approaches. Rather, we wish to present the dating of the expansion of the Northern Hemisphere glaciation as a useful lower-limit standard for calibrating recent divergencies in mammalian phylogenetic trees. Although we have addressed only one specific tree, it is clear that the approach has general applicability for dating other recent mammalian divergence events.

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References

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organisation of the human mitochondrial genome. Nature 290:475–465

Andrews P (1987) Aspects of hominoid phylogeny. In: Patterson C (ed) Molecules and morphology in evolution: conflict or compromise. Cambridge University Press, Cambridge, GB, pp 23–53

Andrews P, Martin L (1987) Cladistic relationships of extant and fossil hominoids. J Hum Evol 16:101–118

Arnason U, Gullberg A (1994) Relationship of baleen whales established by cytochrome *b* gene sequence comparison. Nature 367: 726–728

Arnason U, Gullberg A (1996) Cytochrome *b* nucleotide sequences and the identification of five primary lineages or extant cetaceans. Mol Biol Evol 13:407–417

Arnason U, Johnsson E (1992) The complete mitochondrial sequence of the harbor seal, *Phoca vitulina.* J Mol Evol 34:493–505

Arnason U, Gullberg A, Johnsson E, Ledje C (1993a) The nucleotide sequence of the mitochondrial DNA molecule of the grey seal, *Halichoerus grypus,* and a comparison with mitochondrial sequences of other true seals. J Mol Evol 37:323–330

Arnason U, Gullberg A, Widegren B (1993b) Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. Mol Biol Evol 10:960–970

Arnason U, Gullberg A, Ledje C, Mouchaty S (1995) A molecular view of pinniped relationships with particular emphasis on the true seals. J Mol Evol 40:78–85

Arnason U, Xu X, Gullberg A (1996) Comparison between the complete mitochondrial DNA sequences of human and the common chimpanzee. J Mol Evol 42:145–152

Cande S, Kent DVJ (1992) A new geomagnetic polarity time scale for the late Cretaceous and Cenozoic. J Geophys Res 97:13917–13951

Gingerich PD (1984) Primate evolution: evidence from the fossil record. Yearbook Phys Anthropol 27:57–72

Gingerich PD (1986) Temporal scaling of molecular evolution in primates and other mammals. Mol Biol Evol 3:205–221

Godinot M, Mahboubi M (1992) Earliest known Simian primate found in Algeria. Nature 357:324–326

Hill A, Ward S (1988) Origin of the Hominidae, the record of African large hominoid evolution between 14 My and 4 My. Yearbook Phys Anthropol 31:49–83

Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N (1995) Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci 92:532–536

Irwin DM, Kocher TD, Wilson AC (1991) Evolution of the cytochrome *b* gene of mammals. J Mol Evol 32:128–144

Janke A, Feldmaier-Fuchs G, Thomas WK, von Haeseler A, Pääbo S (1994) The marsupial mitochondrial genome and the evolution of placental mammals. Genetics 137:243–256

Jansen E, Sjøholm J (1991) Reconstruction of glaciation over the past 6 million years from ice-borne deposits in the Norwegian Sea. Nature 349:600–604

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic Press, New York, pp 21–132

Kappelman J, Kelley J, Pilbeam D, Sheikh KA, Ward S, Anwar M, Barry JC, Brown B, Hake P, Johnson NM, Raza SM, Shah SMI (1991) The earliest occurrence of *Sivapithecus* from the middle Miocene Chinji Formation of Pakistan. J Hum Evol 21:61–73

Morin PA, Moore JJ, Chakraborty R, Jin L, Goodall J, Woodruff DS (1994) Kin selection, social structure, gene flow and the evolution of chimpanzees. Science 265:1193–1201

Nei M, Gojobori T (1986) Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol 3:418–426

Pilbeam D (1984) The descent of hominoids and hominids. Sci Am 250(3):60–69

Pilbeam D, Rose MD, Barry JC, Shah SMI (1990) New *Sivapithecus* humeri from Pakistan and the relationship of *Sivapithecus* and *Pongo.* Nature 348:237–239

Ruvolo M, Pan D, Zehr S, Golberg T, Disotell TR, von Dornum M (1994) Gene trees and hominoid phylogeny. Proc Natl Acad Sci USA 91:8900–8904

Saitou N, Nei M (1987) The neighbor-joining method—a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Shackleton NJ, Backman J, Zimmerman H, Kent DV, Hall MA, Roberts DG, Schnitker D, Baldauf JG, Desprairies A, Homrighausen R, Huddlestun P, Keene JB, Kaltenback AJ, Krumsiek KAO, Morton AC, Murray JW, Westberg-Smith J (1984) Oxygene isotope calibration of the onset of ice-rafting and history of glaciation in the North Atlantic region. Nature 307:620–623

Shackleton NJ, Crowhurst S, Hagelberg T, Pisias NG, Schneider DA (1995) A new late Neogene time scale: application to Leg 138 Sites. In: Mayer LA, Pisias NG, Janecek T (eds) Ocean drilling program—scientific results 138:73–101

White TD, Suwa G, Asfaw B (1994) *Australopithecus ramidus,* a new species of early hominid from Aramis, Ethiopia. Nature 371:306–312