Acta Theriologica, Suppl. 5: 89–122, 1998.
PL ISSN 0001-7051

Elephantidae phylogeny: morphological versus molecular results

Jeheskel SHOSHANI*, Edward M. GOLENBERG and Hong YANG**

Shoshani J., Golenberg E. M. and Yang H. 1998. Elephantidae phylogeny: morphological versus molecular results. [In: Ecological genetics in mammals III. G. B. Hartl and J. Markowski, eds]. Acta Theriologica, Suppl. 5: 89–122.

Traditionally, morphologically based phylogenetic relationships within the family Elephantidae (mostly *Loxodonta*, *Elephas*, and *Mammuthus*) depicted *Elephas* and *Mammuthus* as closely related taxa with *Loxodonta* as a sister-group to this clade. Until recently, molecular studies were unable to resolve relationships among the woolly mammoth (*Mammuthus*), Asian (*Elephas*), and African (*Loxodonta*) elephants, or indicated a phenetic pairing of *Loxodonta* and *Mammuthus* with *Elephas* as a sister-group to this grade. In this study we provide further morphological evidence for the traditional hypothesis and data from aligned DNA sequences of the mitochondrial gene cytochrome *b* in support of the monophyletic *Mammuthus-Elephas* clade. In both data sets, morphological and molecular, the extinct American mastodon (*Mammut*) was employed as an outgroup. The molecular results demonstrate the importance of using a closely related taxon as an outgroup for resolving phylogenies of highly derived species. Tests on the importance of the numbers of outgroups and which outgroup may be better for testing phylogenetic relationships reveal that the closer the outgroup to the ingroup, the more corroborative the results will be. Evolutionary rates calculated from the morphological characters indicate that, among the three genera studied, *Loxodonta* is the slowest evolving taxon, followed by *Elephas*, and *Mammuthus*. DNA sequences indicate similar rate differences among the three taxa. Morphological data also corroborate the classical hypothesis that the family Stegodontidae is monophyletic and its members (*Stegolophodon* and *Stegodon*) do not group within Elephantidae. A comparison among mammoths reveals that many of the skull characters are interlinked and may be considered as one, or as a suite of characters, eg antero-posterior compression of skull. An important trend has been observed – the most primitive mammoths had fewer numbers of plates per given tooth and lower lamellar frequencies. A simplified possible mammoth ancestry and radiation is provided.

Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202 USA (JS, EMG, HY); Cranbrook Institute of Science, Bloomfield Hills, Michigan 48304 USA (JS); Department of Human Genetics, Medical Science II M4708, University of Michigan Medical School, Ann Arbor, Michigan 48109-0618 USA (HY)

Key words: Elephantidae, phylogeny, morphology, molecules, outgroups

Introduction

Taxonomists find it tantalizing to resolve questions related to phylogenetic relationships, especially if these are obscured by the fossil record, making such

*Present address: Department of Biology, University of Asmara, P.O. Box 1220, Asmara, Eritrea

** Present address: Department of Science and Technology, Bryant College, Smithfield, Rode Island, 02917 USA

studies scholarly challenges, a paleontological and neontological game. Early workers (eg Blumenbach, 1779) employed the generic name *Elephas* for mammoths and living Asian elephants, a format also followed by some more recent workers (eg Ezra and Cook 1959, Krause 1978). This is not surprising since it is very difficult to distinguish between many closely related taxa the closer one gets to their splitting time in the geological record. For example, earliest elephantid genera are phenotypically extremely similar to one another, and this caused confusion in early stages of research until better diagnostic characters were made available (Maglio 1973, Coppens *et al.* 1978). Dispute over the generic validity of *Loxodonta* and *Elephas* dates back to Cuvier (1825), who regarded them as distinct taxa "differing from each other as much as *Canis* from *Hyaena*, or *Lagomys* from *Lepus*". Based on external ear morphology, however, Lydekker (1907, 1916), considered *Loxodonta* a subgenus of *Elephas* which he designated as *Elephas* (*Loxodonta*) *africana*. If one accepts the earlier hypothesis that the extinct mammoth, *Mammuthus*, should be synonymized with *Elephas* (Blumenbach, 1779), then *Elephas* would span all three genera. Recent workers (eg Aguirre 1969, Maglio 1973, Coppens *et al.* 1978, Jones 1984, Tassy and Shoshani 1988) consider these three genera distinct because of unique characters, especially of the skull and teeth. Shoshani *et al.* (1985b) analyzed 251 characters (a part of Shoshani's 1986 characters) and provided cladistic evidence for the uniqueness of the three separate genera.

Of course, it would be impossible to study phylogenetic relationships with only two living species, the African elephant (*Loxodonta africana*), and the Asian elephant (*Elephas maximus*). Fortunately, well-preserved carcasses of woolly mammoths (*Mammuthus primigenius*), discovered in the Arctic, have provided a unique opportunity to study their soft tissues morphologically and their genes by molecular methods. Together with other extinct proboscideans, *Mammuthus* and the American mastodon (*Mammut americanum*) provide the framework for character evaluation. The richness of proboscidean fossils has facilitated our search for overall evolutionary pathways within the Proboscidea.

Detailed osteological (dentition included) studies of elephantid taxa provided evidence to suggest that among members of the subfamily Elephantinae, *Loxodonta* is the most primitive genus (eg Aguirre 1969, Maglio 1973). These results appeared unchallenged and were accepted as the "traditional hypothesis", even though not all morphological studies confirmed these findings. For example, Valente (1983), employing hair structure, was unable to corroborate these results; he reported on an unresolved trichotomy among *Loxodonta*, *Elephas*, and *Mammuthus*. Shoshani *et al.* (1985a, b) reported on polytomies based on soft tissue or bone proteins and collagen, although Shoshani *et al.* (1985b) reported on additional osteological evidence that supports the traditional hypothesis. A challenge to this hypothesis came from DNA data on modern and extinct taxa. Hagelberg *et al.* (1994) studied DNA sequences of cytochrome *b* gene of *Loxodonta africana*, *Elephas maximus*, and *Mammuthus primigenius* and using dolphin and rhinoceros as outgroups,

suggested "... that *Mammuthus* and *Loxodonta* could be sister-taxa in a monophyletic clade". Höss *et al.* (1994) also studied DNA sequences of these elephantid species (their outgroups were cow and horse) and concluded that the four woolly mammoths they studied "... differ from each other by 0-5 substitutions whereas the Indian and African elephants, representing two genera, differ by only two substitutions". Such an extensive polymorphism among individual mammoths, as opposed to smaller differences between genera, raises a caution flag and requires additional investigation of the same samples and of the data generated by Höss *et al.* (1994). A close examination of Hagelberg *et al.*'s (1994, p. 334) DNA sequences reveals a two codon deletion. As these codons are present in all other mammals, we assume that the sequence differences may be due to a typographical error. Further, their results are in contrast to anatomical (Shoshani *et al.* 1985b) and molecular (Yang *et al.* 1996) data, and are inconsistent with the classical morphologically based hypothesis. We believe these contradicting findings of Hagelberg *et al.*'s (1994) may have resulted from using distant outgroups (a cetacean and a perissodactyl). Indeed, Ulrich Joger (personal communication, 28. June 1996) reported to J. Shoshani "... contrary to our previous data and to the results of Hagelberg, Thomas, and Lister, our extended cytochrome *b* fragment (which is different from yours) has a large number of shared derived characters of both *Mammuthus* and *Elephas*". When a proboscidean taxon such as the American mastodon (*Mammut americanum*) is used to study sequences of cytochrome *b* mitochondrial DNA (mtDNA) sequences within Elephantinae, a corroboration of the traditional hypothesis was achieved (Yang *et al.* 1996). Most recently, Ozawa *et al.* (1997) confirmed the findings of Yang *et al.* (1996) without the inclusion of *Mammut americanum* in their molecular study but with a larger cytochrome *b* mtDNA segment.

A more recent controversy concerns whether the stegodontids, *Stegolophodon* and *Stegodon*, should be grouped within the Elephantidae or should be considered as a separate family, the Stegodontidae. Traditionally, *Stegolophodon* and *Stegodon* have been an inseparable part of the family Stegodontidae (eg Osborn 1942, Coppens *et al.* 1978, Saegusa 1996, Shoshani 1996). Kalb *et al.* (1996), however, reported that Stegodontidae is paraphyletic (or polyphyletic, depending on the relative extent of the group examined) and argued that both *Stegolophodon* and *Stegodon* should be classified within the family Elephantidae, and thus, abolishing the family Stegodontidae.

In this paper we provide expanded data of morphological (mostly osteological, including dentition) characters to test further the relationship among *Loxodonta*, *Elephas*, and *Mammuthus*. In addition, we test the phylogenetic position of *Stegolophodon* and *Stegodon* within Proboscidea. To strengthen the methods of testing, other elephantid genera are included in the analysis. We also increased the number of outgroups to include gomphotheres (eg *Gomphotherium*) in addition to *Mammut*. This expanded morphological data base provides stronger evidence and further support for the traditional hypothesis of relationships within elephantid

genera. This study addresses the following specific questions: (1) which taxa should be included in Elephantidae, (2) do members of the subfamily Elephantinae have a resolved branching arrangement, (3) how significant is DNA isolated from an extinct taxon in testing phylogenetic hypotheses, (4) what are the evolutionary rates among elephantid taxa. An additional related question is the validity of various elephantid genera and species that had to be considered in the early stages of this study because synonymies affect the choice of taxa for analysis. The choice of characters and methods of analysis (see Material and methods) are also important considerations.

Material and methods

Morphological characters examined

Characters studied in this paper include 95 skeletal as well as dental features, taken from the matrices of Shoshani (1986, 1996), Tassy and Darlu (1986), Kalb *et al.* (1996), Saegusa (1996), and Tassy (1996) and summarized in Appendix 1. Of these 95 characters, 72 are binary (only "0" and "1" codes) and 23 are multistate characters (codes 0, 1, 2, ,3 ,4 , and 5 used). Missing data were coded as "?". Note that only 66 of 123 characters of Shoshani (1996) are used; characters that are monomorphic in all taxa within this study were deleted (see details below). In addition, we included 12 of the 40 characters of Tassy and Darlu (1986) and 16 of the 34 characters of Kalb *et al.* (1996) to test their hypotheses (Appendix 1).

Our combined data matrix was constructed after comparing the characters of Shoshani (1996) to those of Tassy and Darlu (1986) and Kalb *et al.* (1996). Duplications of characters were deleted after verifying that there were no conflicts in the coding of these characters. Conflicts were edited based on re-examination of the characters for the specific taxa in question. For example, character 7 of Kalb *et al.* (1996) was not included in our combined set because it is identical to character 9 of Shoshani (1996). Similarly, characters 8 and 9 (also 10, 11, and 12) of Kalb *et al.* (1996) were not included because both (or the three) are part of our character nos. 7 and 94, respectively (note: when analyzing the data with unordered option, splitting one multistate character into two or three binary characters gives them more weight than if they were combined into one). Also, character 24 of Kalb *et al.* (1996) is subsumed by their character nos. 32–34. Character nos. 32–34 of Kalb *et al.* (1996), were better defined than those of Shoshani (1996, eg nos. 43 and 50), and thus we retained those of Kalb *et al.* (1996) instead of nos. 43 and 50. After careful comparison of Kalb *et al.*'s (1996) data matrix to that of Shoshani (1996), we deleted 18 of their characters (12 duplicates, 2 from condensing, 3 not applicable – because we did not employ four of their genera, or because a character is autapomorphic, and 1 because a character was vague). Similarly, 28 of 40 of Tassy and Darlu (1986) were deleted, bringing the total combined set in this analysis to 95 characters (16 of Kalb *et al.*, 12 of Tassy and Darlu, 66 of Shoshani, and 1 new characters as given in Appendix 1, and tabulated in Appendix 2).

Taxa and specimens studied by morphological methods

The choice of these taxa was dictated, in part, by the taxa studied by the authors whose hypotheses we are testing. For this reason, a total of 14 genera/taxa is included in this study. Of these, eight are ingroup or terminal taxa (*Mammuthus*, *Elephas*, *Loxodonta*, *Primelephas*, *Stegodibelodon*, *Stegotetrabelodon*, *Stegodon*, and *Stegolophodon*) and the remaining seven are outgroups (*Paratetralophodon*, *Anancus*, *Tetralophodon*, *Gomphotherium*, *Mammut*, and *Phiomia*) as given in Appendix 2. The most plesiomorphic outgroup employed is *Phiomia*. In earlier studies (eg Shoshani *et al.* 1985b) *Mammut* was used as an outgroup; *Phiomia*, *Gomphotherium* and other gomphotheres are included

here to test a broader scope of hypotheses (relationships within Elephantidae and Stegodontidae), and to test the branching pattern of Kalb *et al.* (1996), who also used *Phiomia* as their outgroup. The character matrix of Kalb *et al.* (1996) for *Phiomia* was coded, however, with "0" (the primitive condition) for all the characters, whereas we used actual data for the characters we analyzed. Also note that the inclusion of *Phiomia* as an outgroup does not negate the function of *Mammut* as an outgroup taxon. On the contrary, it strengthens the testing because, with more than one outgroup, the polarities (direction of evolution) and transformation (changes of character states of one character) of characters for the ingroup – Elephantidae and Stegodontidae – are better defined. For simplicity,

Table 1. A non-ranked simplified classification of 14 proboscidean genera employed in this study (cf Fig. 1A and Appendix 2; partly after Shoshani and Tassy 1996, and after McKenna *et al.* 1997).

Mammalia Linnaeus, 1758
Theria Parker and Haswell, 1897
Placentalia Owen, 1837 (= Eutheria Gill, 1872)
Epitheria McKenna, 1975
Ungulata Linnaeus, 1766
Uranotheria McKenna *et al.*, 1997 (= Paenungulata Simpson, 1945, in part)
Tethytheria McKenna, 1975
Proboscidea Illiger, 1811
Elephantiformes Tassy, 1988 (b)
Unnamed or Phiomiidae Kalandadze and Rautian, 1992 †
Phiomia Andrews and Beadnell, 1902 †
Elephantimorpha Tassy and Shoshani, 1977 (c)
Mammutida Tassy and Shoshani, 1997 † (= Mammutoidea Osborn, 1921) (d)
Mammutidae Hay, 1922 †
Mammut Blumenbach, 1799
Elephantida Tassy and Shoshani, 1997 (e)
Gomphotherioidea Maglio, 1973 (in part) (f)
Gomphotheriidae Hay, 1922 † (g)
Gomphotherium Burmeister, 1837 †
Tetralophodon Falconer, 1857 † (h)
Anancus Aymard, 1855 † (h)
Paratetralophodon Tassy, 1983 † (h)
Elephantoidea Gray, 1821 (= Stegodontoidea Osborn, 1918, in part) (i)
Stegodontidae Osborn, 1918 †
Stegolophodon Schlesinger, 1917 (j)
Stegodon Falconer, 1857 (j)
Elephantidae Gray, 1821 (k)
Stegotetrabelodon Petrocchi, 1941 † (l)
Stegodibelodon Coppens, 1972 † (l)
Elephantinae Gray, 1821 (m)
Primelephas Maglio, 1970 †
Loxodontini Osborn 1918 (n)
Loxodonta Anonymous, 1827 (o)
Elephantini Gray, 1821 (p)
Elephas Linnaeus, 1758
Mammuthus Brookes, 1828 † (q)

† – extinct taxon

→

(a) Arrangements of genera, families, or higher categories in this classification (from top to bottom), correspond to the general sequence of the branching pattern from left to right in Fig. 1A. It is believed that comprehensive classification of proboscidean terminal taxa (about 40 genera) will not alter the general scheme presented here. Most references to authorships are not included in this paper unless they are mentioned elsewhere in this article; they may be found in Osborn (1936, 1942), Shoshani and Tassy (1996), and McKenna *et al.* (1997). (b) This entry (Elephantiformes) corresponds to node A in Fig. 1A. (c) This clade (tentatively named "Elephantimorpha", corresponds to node B in Fig. 1A) may be given a suborder (eg Osborn 1921 p. 2) or infraorder rank. Tassy (1988, p. 46) named members of this clade (not in the same arrangement) "Elephantoidea", a superfamily (as used by Osborn 1942, p. 1582), in accordance with the International Commission for Zoological Nomenclature (ICZN) Recommendation 29a (since the classification is based on extant species; for the same reason we use "subfamily Elephantinae" and "family Elephantidae", and all receive the same authorship "Gray 1821"). P. Tassy and J. Shoshani (in a meeting in Paris, France March 1997) agreed to restrict the suffix of "-oidea" to a superfamily rank. For this reason, the name Mammutida is used instead of Mammutoidea, and Elephantida as a sister taxon. (d) Tentatively called "Mammutida", this clade may be given a suborder (eg Osborn 1921, p. 2, Maglio 1973, p. 15, Coppens *et al.* 1978, p. 339) or infraorder rank. If superfamily rank is given, then the authorship should be Hay 1922, following ICZN Recommendation 29a. Note that the content of this clade as shown here is very different from that of Maglio (1973) and Coppens *et al.* (1978) who grouped Stegodontidae and Mammutidae in Mammutoidea. (e) This clade (tentatively called "Elephantida") corresponds to node C in Fig. 1A and may be of a suborder or infraorder rank. (f) Maglio (1973) and Coppens *et al.* (1978, p. 339) employed the term "Gomphotherioidea" as a suborder of Proboscidea to embrace Gomphotheriidae and Elephantidae. Stegodontidae, according to these authors, was included in the suborder Mammutoidea. In this study, Stegodontidae is a sister group to Elephantidae. (g) This monophyletic family (Gomphotheriidae) may include, in addition to *Gomphotherium*, other trilophodont grade gomphotheres. Positions of the tetralophodont grade gomphotheres are shown in Fig. 1A and noted below, note (h). (h) These three tetralophodont gomphothere taxa (*Tetralophodon*, *Anancus*, *Paratetralophodon* – probably form a grade, or a paraphyletic group; cf Shoshani and Tassy 1996), are included here to accommodate part of the data set of Tassy and Darlu (1986) and Kalb *et al.* (1996). (i) This entry ("Elephantoidea") corresponds to node G in Fig. 1A. (j) Kalb *et al.* (1996) proposed that *Stegolophodon* and *Stegodon* should be classified within the family Elephantidae, and thus abolishing the family Stegodontidae. (k) This entry ("Elephantidae") corresponds to node H in Fig. 1A. According to Maglio (1973) and Coppens *et al.* (1978), Elephantidae includes six genera with 25 species as follows: *Stegotetrabelodon* (with 2 species), *Stegodibelodon* (with 1), *Primelephas* (with 1), *Loxodonta* (with 3), *Elephas* (with 11) and *Mammuthus* (with 7). In the summary of Shoshani and Tassy (1996, incorporating the ideas of Webb and Dudley 1995) the total of elephantid species varies between 39 and 43. Another possible valid species is *Loxodonta cyclotis* (Groves *et al.* 1993). Shoshani (1993), however, considered "*Loxodonta cyclotis*" a plesiomorphic sister group to *Loxodonta africana*, and suggested the use of two subspecies – ie *Loxodonta africana africana* and *L. a. cyclotis* – until additional evidence becomes available. If one accepts Kalb *et al.* (1996) hypothesis that Stegodontidae (*Stegolophodon* and *Stegodon*) be included in Elephantidae, then the total number of species would rise and vary from 42 to 77. (l) Maglio (1973) and Coppens *et al.* (1978) proposed to classify *Stegotetrabelodon* and *Stegodibelodon* in the subfamily Stegotetrabelodontinae. This hypothesis does not seem to be corroborated by recent workers (eg Tassy and Darlu 1986, Tassy 1996, Kalb *et al.* 1996, Shoshani 1996). (m) This entry ("Elephantinae") corresponds to node K in Fig. 1A. (n) Following ICZN, Articles 62–65, the authorship for Loxodontini should be Osborn 1918 (see page 1191 in Osborn 1945), since he gave the name "Loxodontinae", one of the family group names. An error appears in Shoshani (1998, p. 482) – the authorship for Loxodontini should be Osborn 1918, not Kalandadze and Rautian 1992. (o) The author is "Anonymous 1827", not "F. Cuvier 1825"; details in Appendix C of Shoshani and Tassy 1996). (p) This entry (Elephantini) corresponds to node L in Fig. 1A. (q) The author is "Brookes 1828", not "Burnett 1830"; details in Appendix A of Shoshani and Tassy (1996).

Table 2. Hypotheses testing among proboscidean taxa.

Test number	Hypotheses tested*	Tree length	Change from Test 0
0	The most parsimonious tree; Fig. 1A (1)	137	–
1	*Stegodibelodon* joined with *Stegotetrabelodon* (2)	140	3
2	*Stegodon* in front of *Stegotetrabelodon* (3)	139	2
3	*Stegodon* joined with *Stegotetrabelodon* (3)	141	4
4	*Stegodon* in front of *Stegodibelodon* (3)	140	3
5	*Stegodon* joined with *Stegodibelodon* (3)	142	5
6	*Stegodon* in front of *Primelephas* (3)	141	4
7	*Stegodon* joined with *Primelephas* (3)	144	7
8	*Stegodon* in front of *Loxodonta* (4)	143	6
9	*Stegodon* joined with *Loxodonta* (4)	147	10
10	*Elephas* joined with *Loxodonta* (4)	142	5
11	*Mammuthus* joined with *Loxodonta* (4)	142	5
12	As in test no. 6, but also exchange the positions of *Tetralophodon* with *Anancus* (5)	145	8
13	*Tetralophodon* and *Anancus* exchange positions (6)	143	6
14	*Tetralophodon* and *Anancus* joined together (6)	144	7
15	*Tetralophodon* and *Paratetralophodon* exchange positions (6)	146	9
16	*Tetralophodon* and *Paratetralophodon* joined together (6)	145	8
17	*Anancus* and *Paratetralophodon* exchange positions (6)	139	2
18	*Anancus* and *Paratetralophodon* joined together (6)	139	2
19	Stegodontidae in front of *Gomphotherium* (7)	159	22
20	Stegodontidae joined with *Gomphotherium* (7)	159	22
21	Stegodontidae joined with *Mammut* (8)	165	28
22	*Gomphotherium* joined with *Phiomia* (9)	144	9
23	All 4 gomphotheres separately joined with *Phiomia* as outgroup (9)	159	22
24	All 4 gomphotheres together joined with *Phiomia* as outgroup (9)	174	37
25	All 4 gomphotheres joined *Phiomia*, *Mammut* is outgroup (10)	174	37

* The 25 tests presented in this table are a sample of tests conducted "manually" with MacClade program. All test conducted with the characters "unordered", ie, in all characters it costs only one step in the sequence of transformation from one multistate character to another. The numbers in the third column are the length of the cladogram (or the minimum number of evolutionary steps required for a given tree topology) obtained when running PAUP, and the numbers of changes (fourth column) are the differences between this tree topology and one of the most parsimonious trees, Test no. 0. (1) Each hypothesis testing was performed on the original tree; in other words, after one test was completed, the branching arrangement was returned to its original topology (as shown in Fig. 1A) before conducting the next test. Other details are given under Material and methods. (2) Hypothesis after Maglio (1973) and Coppens *et al.* (1978) who proposed to classify *Stegodibelodon* and *Stegotetrabelodon* in the subfamily Stegotetrabelodontinae, family Elephantidae. (3) A part of a hypothesis proposed by Kalb *et al.* (1996), or towards understanding or testing this hypothesis. Kalb *et al.* (1996) proposed to abolish the family Stegodontidae because it is paraphyletic (or polyphyletic, depending on the relative extent of the group examined) and both *Stegolophodon* and *Stegodon* should be classified within the family Elephantidae. In test no. 2, *Stegodon* as a sister-group of Elephantidae (content as in Maglio 1973 and Coppens *et al.* 1978). (4) Test conducted to learn the range in step changes when *Stegodon*, *Elephas*, and *Mammuthus* are joined to *Loxodonta*. (5). This branching pattern is similar to that proposed by Kalb *et al.* (1996, p.

106), except that here we have two additional genera, *Gomphotherium* and *Mammut*. (6) The purpose of these tests is to further examine the relationships among gomphothere taxa and to further elucidate their paraphyletic ("the wastebasket hypothesis") or monophyletic status (cf Shoshani 1996, Tassy 1996). (7) Test conducted to understand better the plasticity of the characters used with reference to notes nos 4–5 above. (8) A hypothesis suggested by Maglio (1973) and Tobien (1988). Note, however, that in this study Mammutidae is represented by *Mammut*, and could be the reason for the high (28 steps) difference, as opposed to 15 steps difference when tested with a denser character matrix (cf. Shoshani 1996, p. 171). (9) Towards understanding or testing the hypothesis that *Phiomia* was on the line of ancestry to gomphothere taxa (eg Tobien 1976, Coppens *et al.* 1978). (10) Exchanging the positions of *Mammut* and *Phiomia* on the most plesiomorphic branch on this cladogram (Fig. 1A) is a rhetorical test.

and to allow us to focus on the relationships between and among Stegodontidae and Elephantidae taxa, we present two sets of results, with and without all the gomphotheres.

This study reports results of analysis of specimens studied in the following museums: American Museum of Natural History (New York, USA); Beijing Natural History Museum (Beijing, Peoples Republic of China); Bristol University, Geology Museum (Bristol, England); Institute of Vertebrate Paleontology and Paleoanthropology (Beijing, Peoples Republic of China); Laboratoire de Paléontologie des Vertébrés et de Paléontologie Humaine, Université P. & M. Curie (Paris, France); Muséum National d'Histoire Naturelle (Paris, France); Natural History Museum (London, England), formerly "British Museum (Natural History)". We also employed data – based on descriptions and illustrations – taken from selected literature reports. The validity of names, on the generic and species levels, used here (cf Table 1) necessitated synonymies of taxa and specimens for analysis (current senior synonyms we use are after Shoshani and Tassy 1996).

Cladistic analysis of morphological data

For the morphological data, computer analysis was conducted with Swofford's (1993) Phylogenetic analysis using parsimony (PAUP) version 3.1.1, and Maddison and Maddison's (1992) MacClade version 3.0. We used the standard specifications for PAUP and MacClade analyses, except for the following (in PAUP): (1) Optimization was conducted using ACCTRAN (accelerated transformation) and DELTRAN (delayed transformation), MAXTREE was set to 100 and to "Automatically increase by 100" intervals; (2) The "Heuristic" and "Exact Method" ("Branch and Bound") search options of PAUP were employed to identify the most parsimonious tree(s). In both searches characters were treated with equal weight and as unordered (for each multistate character, all transformations cost only one step), and the program allows reversals; (3) in the Heuristic search, all three options were tried (General, Stepwise addition, and Branch swapping); the Stepwise addition has four sub-options (As is, Closest, Simple, and Random) – all were performed, with 10 and 100 replications; (4) in all runs, the outgroups and the ingroups were not constrained, ie not specified. Results from PAUP with brief comments are given in Appendix 3.

For the purpose of comparing molecular and morphological studies, we also tried different topologies of cladograms (manual branch swapping with MacClade; Table 2). In addition, we ran PAUP with only one ingroup (Stegodontidae and Elephantidae), but changed the number of the outgroups (Table 3).

Molecular materials

Ten specimens of Proboscidea were used for DNA extraction and analysis (seven extinct, two extant and one hybrid; details in Appendix 4). Fossil specimens ranged from about 46 000 to 10 200 years old. Note that *Mammuthus* (extinct), *Elephas*, and *Loxodonta* are classified in the family Elephantidae and *Mammut* (extinct) is placed in the family Mammutidae (Table 1).

Table 3. Testing the importance of outgroups among proboscidean taxa. (1) The 10 tests* presented in this table are a sample of tests conducted "manually" with PAUP program (specifications in text). The first entry indicates the results as shown in Fig. 1A when all 14 taxa are included in the analysis. Subsequent tests were conducted such that some taxa were deleted from the matrix and the program was executed individually to obtain the results shown in the third and fourth columns. (2) The first number in this column refers either to a single tree/cladogram or two to three equally parsimonious trees as obtained from PAUP analysis, and the second number in parentheses refers to the tree length (TL). (3) Here the ingroups are defined as Stegodontidae and Elephantidae. Elephantidae always remained as a monophyletic taxon, thus the emphasis is on testing whether or not Stegodontidae would remain as a monophyletic taxon (cf Saegusa 1996, Shoshani 1996, Tassy 1996) or a paraphyletic (cf Kalb *et al.* 1996). An entry of "yes" means that the ingroup, specifically the Stegodontidae, is a monophyletic; "no" means that the *Stegodon* is joined with the stem of Elephantidae instead of with *Stegolophodon*. (4) *Mammut* + Stegodontidae (2 taxa) + Elephantidae (6 taxa), a total of 9 taxa. (5) This tree is the same as in Fig. 1A, and in the first entry to this table. (6) Tests from this entry to the end of this table were conducted by deleting individual taxa, rather than adding them as was done in the entries above.

Number of taxa tested	Explanation (1)	Number of trees and (TL) (2)	Monophyly of the ingroups (3)
14	As in the most parsimonious tree; Fig. 1A (1)	1 (137)	yes
9	Only *Mammut*, Stegodontidae and Elephantidae used (4)	1 (93)	yes
10	As above with *Phiomia* added	1 (121)	yes
11	As above with *Gomphotherium* added	3 (128)	no
12	As above with *Tetralophodon* added	2 (132)	no
13	As above with *Anancus* added	1 (136)	yes
14	As above with *Paratetralophodon* added (5)	1 (137)	yes
13	As in Fig. 1A, but after deletion of Anancus (6)	1 (134)	yes
12	As above, but after deletion of *Tetralophodon*	1 (131)	yes
11	As above, but after deletion of *Gomphotherium*	1 (127)	yes
10	As above, but after deletion of *Mammut*	1 (114)	yes
9	As above, but after deletion of *Phiomia*	1 (62)	yes

* Note: there are 12 entries to this table but the first and the seventh are the same as in Fig. 1A, and thus not counted as "tests".

DNA extraction, amplification, and sequencing

Three different DNA isolation and purification approaches were used: traditional proteinase K (Hagelberg and Clegg 1991, Cooper 1994, Hardy *et al.* 1994), 2% CTAB approach (Doyle and Doyle 1987, Golenberg 1994, Yang *et al.* 1997b), and glass bead approaches (Boom *et al.* 1990, Höss and Pääbo 1993, Cano and Poinar 1993). Experiments were conducted in a plant molecular lab where no mammalian DNA (except human) was handled previously by following a blind test design (Yang *et al.* 1997a). Special care was taken to prevent contamination (eg equipment and reagents were dedicated solely for ancient DNA work). Whenever possible, disposable equipment was used, and reusable utensils were soaked in 0.5% sodium hypochlorite and then exposed to UV light for one hour prior to experimentation (for details, see Yang *et al.* 1997b).

Polymerase chain reaction (PCR) was performed using primers designed based on modern elephant cytochrome *b* gene mtDNA sequences (Yang *et al.* 1996). Amplification of specimens *Mammuthus* (EL#2) and *Mammut* (EL#23) DNA were carried out using two-stage nested PCR with newly designed internal primers Elcytb65 (5' CTA CCC CAT CCA ACA TAT CAA CAT GAT 3') and Elcytb320R (5' CGG TAT TTC AAG TTT CCG AGT ATA GGT 3'); other details are given in Yang *et*

al. (1996). The primary PCR product was used as a template without further purification in the second stage of the nested amplification. To monitor contamination, extraction and negative PCR controls from primary amplification were carried through the secondary PCR. DNA sequences were derived by direct dideoxy sequencing of PCR products (Barnard *et al.* 1994), and each sequence was read from both strands. To avoid or reduce bias in our study, the taxonomic identities of the samples were initially known only to the one author (JS) who was not performing the laboratory experiments; the other co-authors (EMG and HY) worked with numbers for the genetic testing, a type of blind testing design (Yang *et al.* 1997a). Correct identifications of Recent species and duplicates from ancient specimens were achieved when sequences determined in the laboratory were compared with previously published data (eg Irwin *et al.* 1991).

Phylogenetic analyses on molecular data

Phylogenetic analyses were performed using maximum parsimony with exhaustive search and equal character weighting (Swofford 1993) and by neighbor-joining analysis using two-parameter sequence distance estimates with a 10 to 1 transition to transversion ratio (Kumar *et al.* 1993, Felsenstein 1993). As explained below, a ratio of expected transitions to transversions is required to generate distance estimates, and functions in estimating the number of multiple hits (mutations) occurring at a particular position or character. Thus, this ratio does not function as a character

Table 4. Evolutionary rates that occurred during geological time span for certain proboscidean taxa. Letters for nodes/clades, number of evolutionary changes (EC) along a branch, and divergence times are given in Fig. 1A. (1) For simplicity (and because of the close "splitting times"), individual rates for the time spans among nodes C through G and nodes H through K were omitted; they can be easily calculated from the data given on the cladogram (Fig. 1A). (2) Numbers before the slash (/) refer to ECs for ACCTRAN option in PAUP, and the numbers following the slash are ECs for DELTRAN option in PAUP (cf caption to Fig. 1A and text for explanations). (3) Rates of evolution (calculated as the number of evolutionary changes (ECs) divided by the time over which these changes took place). Numbers before the slash (/) are calculated rates for ACCTRAN and numbers following the slash are for DELTRAN. (4) The calculation of the rate between nodes K (Elephantinae, ca 6 Ma) and L (Elephantini, ca 4.5 Ma) is subsumed by the next two entries.

Time span between	Duration (time in Ma, approximate)	ECs along this node (2)	Calculated rate (3)
Nodes A and B – ie between Elephantiformes and Elephantimorpha	from 35 to 28, span of 7	15/15	2.14/2.14
Nodes B and C – ie between Elephantimorpha and Elephantida	from 28 to 24, span of 4	13/13	3.25/3.25
Nodes C and G – ie between Elephantida and Elephantoidea	from 24 to 19, span of 5	40/35	8.0/7.0
Nodes G and H – ie between Elephantoidea and Elephantidae	from 19 to 7, span of 12	3/5	0.25/0.42
Nodes H and K – ie between Elephantidae and Elephantinae	from 7 to 6, span of 1	15/16	15/16
Nodes K and *Loxodonta* – ie from the common ancestor of Elephantinae to *Loxodonta*	from 6 to 0, span of 6	3/1	0.50/0.17
Nodes K and *Elephas* – ie from the common ancestor of Elephantinae to *Elephas* (4)	from 6 to 0, span of 6	5/7	0.83/1.17
Nodes K and *Mammuthus* – ie from the common ancestor of Elephantinae to *Mammuthus* (4)	from 6 to 0, span of 6	7/9	1.17/1.50

weighting in parsimony analysis. Additionally, in our data, only one transversion occurred and it falls on the branch leading to the outgroup, *Mammut*. Therefore, a weighted parsimony analysis would not change the maximum parsimony tree topology and so it was not used.

Calculating evolutionary rates

Rates of evolution (calculated as the number of evolutionary changes (ECs) divided by the time over which these changes took place) provide a measure for understanding the evolution of lineages over a given geological time span. The time of divergence of each of the major proboscidean clades along the "spine", or major axis of the cladogram in Fig. 1A from *Phiomia* to Elephantini, was extrapolated from the illustrations and discussion of Maglio (1973), Coppens *et al.* (1978), Tassy and Shoshani (1996). The approximate dates of divergence in million years ago (Ma) are given under

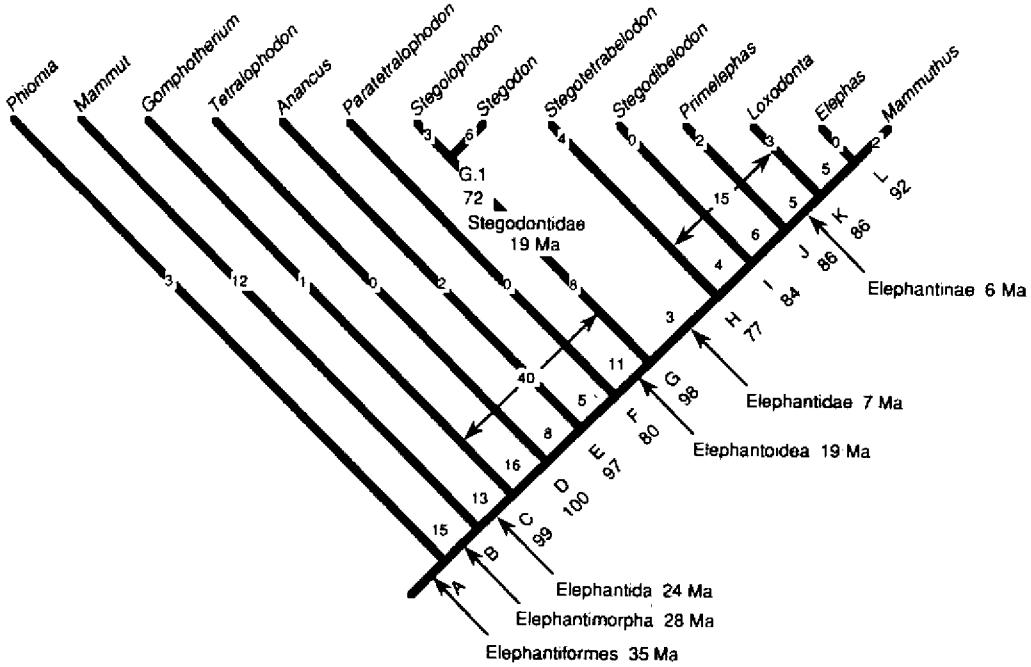

Fig. 1A. A maximum parsimony cladogram obtained with PAUP using 95 unordered characters on 14 taxa; tree length (TL) = 137, Consistency Index (CI) = 0.78, Retention Index (RI) = 0.84. This cladogram was used to test phylogenetic hypotheses explained in Table 2, and testing the importance of outgroups among proboscidean taxa (Table 3). Letters along the main axis of this cladogram represent nodes/clades that are referred in Tables 1 and 4. Numbers inside the cladogram (to the left of the main axis) and along the terminal taxa, represent evolutionary changes (ECs) for these clades or branches as obtained from the ACCTRAN option in PAUP**. Numbers outside the cladogram (to the right of the main axis) are bootstraps values based on 1,000 replications. **Numbers of ECs for these clades as obtained from DELTRAN option in PAUP are: node B–15, node C–13, *node D–13*, node E–8, *node F–3*, node G–11, *node G.1–3*, *node H–5*, *node I–3*, *node J–5*, *node K–8*, *node L–7* (numbers in italics represent differences between ACCTRAN and DELTRAN). Differences in ECs for the terminal taxa, from left (*Phiomia*) to right (*Mammuthus*) are: *Tetralophodon* 2 vs 0, *Paratetralophodon* 1 vs 0, *Stegolophodon* 4 vs 3, *Stegodon* 7 vs. 6, *Stegodibelodon* 1 vs 0, *Primelephas* 3 vs 2, and *Loxodonta* 1 vs 3. Regardless of the differences for specific nodes or terminal taxa between ACCTRAN and DELTRAN, the total length of the tree remains the same–137 steps. The subfamily Elephantinae includes *Primelephas* as shown in Table 1.

Results and discussion in parentheses after each of the subheadings below; cf Table 4 and Fig. 1A for names and geological times of approximate divergence (splitting) of taxa. Relative rates of cytochrome *b* gene evolution among the terminal taxa, *Loxodonta*, *Mammuthus*, and *Elephas*, were determined using a relative rates test (Sarich and Wilson 1967, Wu and Li 1985). Sequence distances from these three taxa to *Mammut americanum* were determined using a Kimura two parameter estimate, using PHYLIP (Felsenstein 1993).

Results and discussion

Morphological analysis

Using the combined set of 95 characters shown in Appendix 1, the topology of the cladogram for the 15 taxa studied is depicted in Fig. 1A. For the purpose of direct comparison with the taxa employed in the molecular analysis (Fig. 2), we also present Fig. 1B which is a condensed version of Fig. 1A; it includes one ingroup (Elephantidae) and one outgroup (*Mammut*). Note that only one tree was obtained with PAUP analysis (no equally parsimonious trees).

Branch swapping was conducted on the cladogram (Fig. 1A); results are shown in Table 2. It is noted that the changes in the number of steps from the most parsimonious to other alternatives within Stegodontidae or within Elephantidae tested are small, ranging from one to five steps. Results confirm that *Mammuthus* and *Elephas* are more closely related to each other than either is to *Loxodonta*. Five additional steps are required when *Mammuthus* and *Loxodonta* or *Elephas* and *Loxodonta* are joined in a common branch (Table 2). It is also noted that, although members of the family Stegodontidae (*Stegolophodon* and *Stegodon*) are

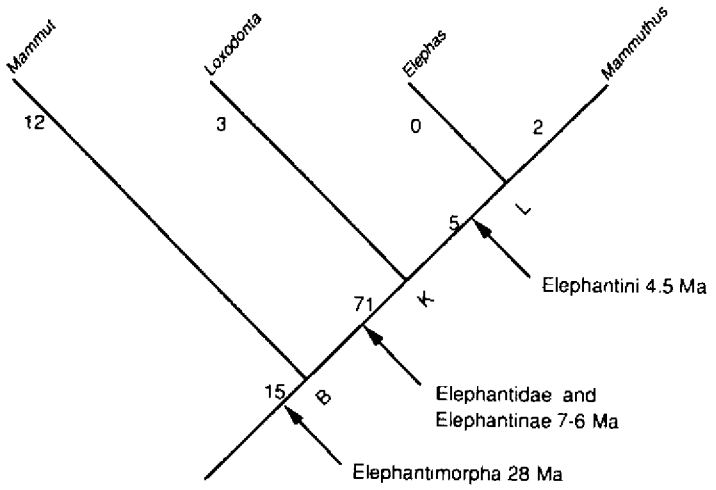

Fig. 1B. This is a condensed version of Fig. 1A which includes one ingroup (Elephantidae) and one outgroup (*Mammut*). Note that only one tree was obtained with PAUP analysis (no equally parsimonious trees; TL = 60, CI = 1.0, RI = 1.0); cf this tree to Fig. 2, obtained from molecular analysis.

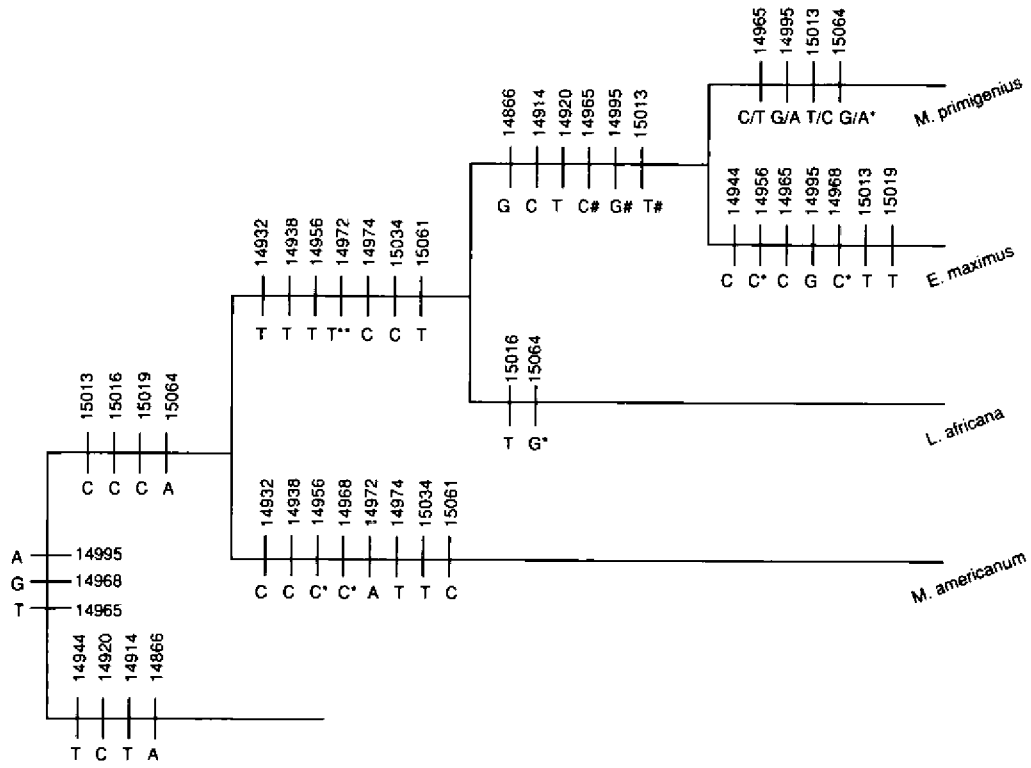

Fig. 2. A cladogram based on Neighbor joining and Maximum Parsimony tree of 228 base pairs mitochondrial gene cytochrome *b* fragment of four proboscidean taxa (two extant and two extinct, after tree in Yang *et al.* (1996). Tree length (TL) = 23, Consistency Index (CI) = 0.87, Retention Index (RI) = 0.70). Vertical bars indicate inferred base composition at variable sites within the sequence along branches. Nucleotide bases (character states) at sites that differ between the Elephantidae and *Mammut* cannot be unarbitrarily distinguished between ancestral and derived. Therefore, to display all character states for all variable sites, we list the nucleotides at these positions on both branches leading to *Mammut* and to Elephantidae. Our listing of these nucleotides does not imply two independent substitution events at each sequence position from an original ancestral state. Numbers are sequence position in reference to the human sequence (Anderson *et al.* 1981). Asterisks (*) indicate inferred homoplasies. Pound signs (#) indicate inferred polymorphism along the branch of the most recent common ancestor. Characters listed on the root branch are inferred shared ancestral characters as opposed to the unique derived characters along terminal branches. Note that throughout this tree, all but one substitution are synonymous (there is no change in the product). The unique exception was observed in codon 75 (nucleic acid position 14972 of human numbering following Anderson *et al.* 1981, indicated by **) where *Mammut* sequence differed in a first position, non synonymous substitution. Also note that *Mammuthus primigenius* was paraphyletic in the original analysis; data on this branch represent two specimens.

monophyletic, breaking this clade costs only two additional steps – weak evidence, considering their long accepted history (since Osborn 1918) as a monophyly. Certainly, additional characters must be examined before a definite conclusion can be reached.

A series of tests was conducted with the present morphological character matrix to evaluate the influence of outgroups on the cohesiveness of the ingroups. In these experiments, we deleted certain outgroup taxa from the matrix and ran PAUP to test whether the family Stegodontidae remained monophyletic as it was suggested by Saegusa (1996), Shoshani (1996), and Tassy (1996), or, if *Stegolophodon* and *Stegodon* would separate as suggested by Kalb *et al.* (1996). In ten of 12 tests, the Stegodontidae is monophyletic, whereas in two of 12 tests, *Stegodon* groups with Elephantidae, making Stegodontidae paraphyletic (Table 3). Outgroups that are phylogenetically closer to the ingroups are more reliable (more accurate) candidates for testing relationships within the ingroups, because the shorter branches (between the ingroup and the closer outgroup) have accumulated fewer mutations than shorter branches between the ingroup and the distantly related outgroup (Adachi and Hasegawa 1995, Yang *et al.* 1996). Less mutation results in fewer possibilities for parallelism and convergence, better polarization of characters, and overall better, and more accurate resolution of relationships among terminal taxa. Our results are corroborated by the vast majorities of similar studies (cf to references given above). This was also demonstrated by molecular data of Yang *et al.* (1996; further discussion below). We also noted that employing more than one outgroup (in our case, two or more) appears to be a more founded character polarity, and possibly a better resolution for taxa within the ingroups.

Mammoths of Europe and Asia

According to Lister (1996) and Lister and Bahn (1994), three lineages or successive stages of mammoths were present in Eurasia: the "ancestral mammoth", *Mammuthus meridionalis* (lived from about 3.0 to 0.7 Ma), the "steppe mammoth", *M. trogontherii* (lived from about 0.7 to 0.5 Ma), and "woolly mammoth", *M. primigenius* (lived from about 0.4 to 0.01 Ma). The evolutionary histories of these mammoths are complex and often intertwined (overlapping geological records and variations in characters). Nevertheless, these overall trends have been observed (Osborn 1942, Maglio 1973, Lister 1996): shift from long and low to short and high skull, including the brevirostry of the mandibular symphysis (antero--posterior compression); shift in the angle of tusk alveoli from projecting forward to projecting almost vertically downward; increase in angle of molar eruption; increase in lamellar frequency; increase in hypsodonty index; decrease in enamel thickness; and decrease in shoulder height. As can be noted, many of the characters are interlinked and may be considered as one, or suites of characters, eg antero--posterior compression of skull. By most accounts (summarized by Lister 1996), the morphology of *M. trogontherii* appears to be more derived than that of *M. meridionalis*. Thus, *M. trogontherii* more resembles *M. primigenius* than *M. meridionalis*. *M. meridionalis* appears to be the most plesiomorphic (among *M. meridionalis*, *M. trogontherii*, and *M. primigenius*) for these reasons: it has

lamellae with median loops and presence of P^4 as observed in one cranium (from Khapry, Russia). Median loops are vestiges of central conules found in gomphothere (more primitive proboscidean than *M. meridionalis*). Similarly, the presence of a permanent upper premolar is more often found in earlier, less advanced proboscideans, and thus is considered a primitive character, although it has been suggested that presence of P^4 in certain crania of advanced proboscideans may be an atavistic character (Dubrovo 1989). Lister (personal communication) noted that the significance of the P^4 in the Khapry *M. meridionalis* is uncertain because it has been observed in only one individual, so it might be considered an individual atavistic character.

Mammoths of North and Central America

A discussion of taxonomy and systematics of the North American mammoth was provided by Agenbroad (1994). According to Agenbroad (1984 – p. 91, 1994 – p. 160), five mammoth species were present in North and Central America: the "Meridional mammoth", *Mammuthus meridionalis* (lived during the early Pleistocene, ca 1.8 Ma – 700 000 years BP), the "Imperial mammoth", *M. imperator* (lived during the middle Pleistocene, ca 700 000–130 000 years BP) the "Columbian mammoth", *M. columbi*, and the "Dwarf mammoth", *M. exilis* (lived during the early part of the late Pleistocene, ca 130 000 – 10 000 years BP), and the "Woolly mammoth", *M. primigenius* (lived during the later part of the late Pleistocene, ca 40 000 – 10 000 years BP). Webb and Dudley (1995) reduced the number of mammoth species in North and Central America to three: *M. hayi*, *M. columbi*, and *M. primigenius*. In this scheme, *M. hayi* is considered the oldest mammoth in the New World (North and Central America; no mammoths are recorded from South America), instead of *M. meridionalis*, a hypothesis first proposed by Madden (1981). In addition, Webb and Dudley's (1995) formula could mean that *M. columbi* is composed of three subspecies, ie *M. columbi columbi*, *M. c. jeffersonii*, and *M. c. exilis*. Examination of characters of New World mammoths reveals that, as with the Eurasian mammoths, their evolutionary histories are complex. Nevertheless, the most important trend has been observed – the most primitive ones had fewer numbers of plates per given tooth and lower lamellar frequencies.

Mammoth ancestry and inter-relationships

Based on the available literature (eg Maglio 1973, Coppens *et al.* 1978, Agenbroad 1984, 1994, Lister and Bahn 1994, Webb and Dudley 1995, Lambert 1995, Lister 1996), the ancestry of mammoths may be simplified as follows (an arrow indicates possible direction of evolution). In Eurasia: *M. meridionalis* → *M. trogontherii* → *M. primigenius*. In North and Central America: *M. hayi* → *M. columbi*. *M. primigenius* is an immigrant to the New World. A possible progenitor

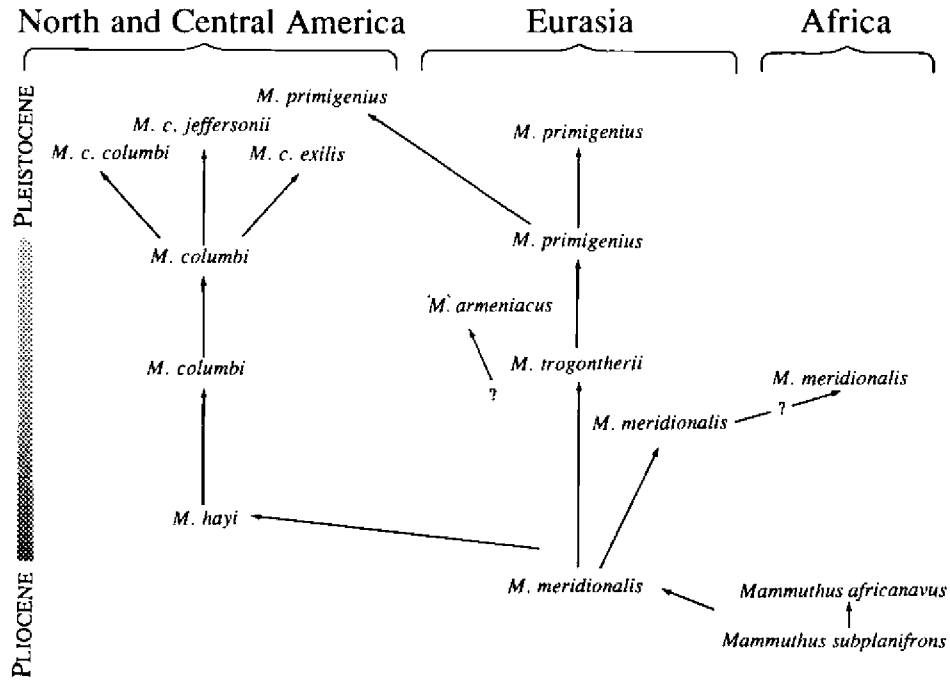

Fig. 3. A simplified possible mammoth ancestry and radiation in Africa, Eurasia and North and Central America. Sources for this chart include Maglio (1973, pp. 77, 79, 116–117), Agenbroad (1984, p. 91), Graham (1986, p. 169), Lister and Bahn (1994, pp. 11–35), Webb and Dudley (1995, pp. 654–657), Lister (1996, p. 203), Shoshani and Tassy 1996, pp. 361–362), Todd and Roth (1996, p. 198), and Webb (1996, p. 71A). The exact phylogenetic position of *Mammuthus armeniacus* is not known; A. M. Lister (pers. comm. 1997) suggested to place it in "Elephantidae *incertae sedis*". *Mammuthus imperator* (Leidy 1858) of North America is considered a junior synonym of *M. columbi* (Falconer, 1857) *fide* S. D. Webb (pers. comm. 1997). Artwork by Jann S. Grimes.

of *M. hayi* is *M. meridionalis* which may have arisen from *M. africanavus* or *M. subplanifrons* (cf Fig. 3).

Molecular analysis

A total of 228 base pairs of cytochrome *b* gene sequences from four proboscidean taxa was used in phylogenetic analysis, and they were aligned with the published *Loxodonta* sequence of Irwin *et al.* (1991). Phylogenetic analysis was performed using domestic pig (*Sus scrofa*), black rhinoceros (*Diceros bicornis*), and human (*Homo sapiens*) sequences as outgroups, or, the fossil American mastodon (*Mammut americanum*) as an alternative outgroup. When modern non-proboscidean taxa (*Sus*, *Diceros*, *Homo*) were used as outgroups, all proboscideans were clustered together forming a monophyletic clade with *Mammut* as the first branching outgroup taxon. This monophyly is supported by 100% bootstrap resampling

analysis. Nonetheless, the relationship among mammoths (*Mammuthus*) and the two modern elephants (*Elephas* and *Loxodonta*) could not be resolved above the 50% consensus level. On the other hand, when *Mammut* was added as the outgroup, both parsimony and neighbor-joining trees suggested that *Elephas* and *Mammuthus* formed a natural clade with *Loxodonta* as the sister-group. The topology is supported by relatively high bootstrap numbers (74%), especially in comparison to a bootstrap support of 80% for monophyly of *Loxodonta*. This analysis demonstrates the effectiveness of using an appropriate outgroup for resolving phylogenies of highly derived elephantine lineages.

Long branches between the outgroups and ingroups could and do affect the relationships among the ingroup taxa. This phenomenon is probably more prevalent in molecular than morphological characters, because there is a bias in the ratio of transition to transversion substitutions (Transition substitutions are defined as a replacement of a purine (either adenine or guanine) with the other purine, or a replacement of a pyrimidine (cytosine or thymine) with the other pyrimidine. A transversion is defined as the substitution of a purine in place of a pyrimidine, or a pyrimidine in place of a purine). There are twice as many possible transversions as there are transitions. Yet, most studies indicate a clear predominance of transitions over transversions. The bias toward transitions is the result of a chemical process of most nucleotide base substitutions. When such a bias occurs on long branches, parallel and convergence mutations accumulate with time, and these mutations cause these long branches to be pulled together in a false clade. The best way to reduce or prevent such a bias is to choose an outgroup as closely related to the ingroup as possible.

Our molecular data also indicate that there is heterogeneity in evolutionary rates between *Elephas-Mammuthus* and *Loxodonta* clades relative to the common outgroup *Mammut* sequence. The *Elephas-Mammuthus* clade seems to have evolved at a more rapid rate relative to *Loxodonta*, in agreement with previous morphological data (Shoshani *et al.* 1985b). Caution should be taken with the interpretation of these results because our sequences are relatively short (228 base pairs).

Evolutionary rates

Table 4 provides approximate divergence times for taxa shown in Fig. 1A, followed by the evolutionary changes that occurred during this period and the calculated evolutionary rates (additional explanations are given under Material and methods). Letters for nodes/clades, number of evolutionary changes (EC) along a branch, and divergence times are included in Fig. 1A.

In summary, evolutionary rates of morphological characters display a relatively slow rate close to the ancestry of Proboscidea, followed by an acceleration along the branch from Elephantida to Elephantoidea (during the early and middle Miocene, from ca 24 to 19 Ma). This accelerated event was followed by a decelerated rate (during the late Miocene), followed by a burst of acceleration rate towards the suggested emergence and diversification of Elephantidae (from about the late

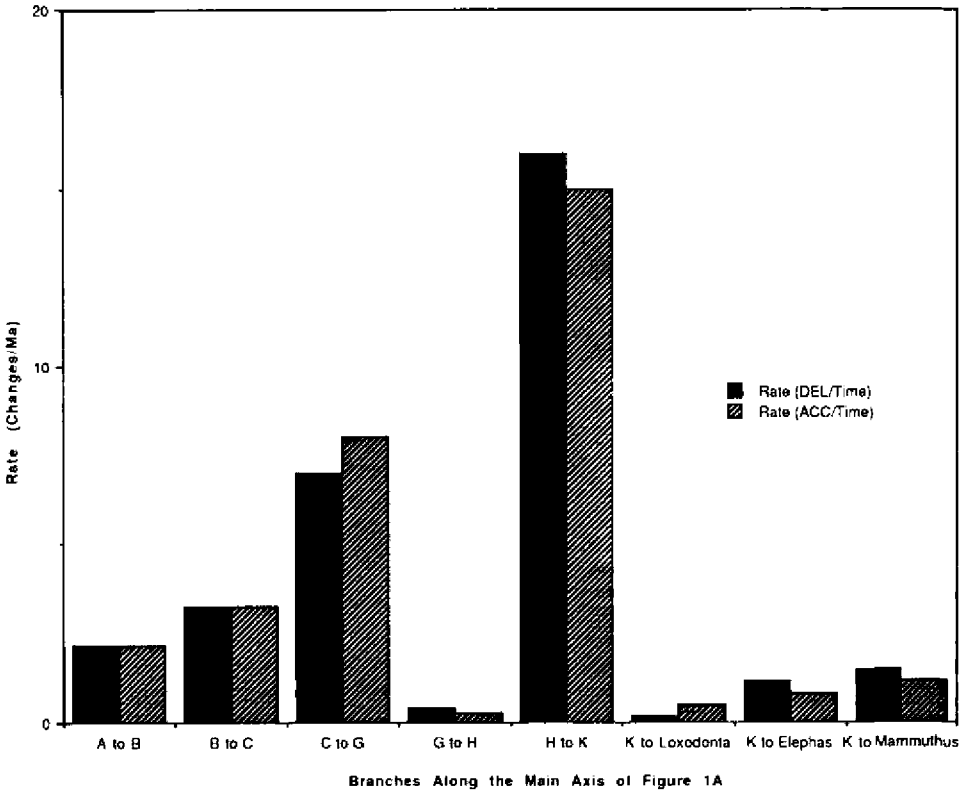

Fig. 4. Histograms depicting generalized patterns of evolutionary rates of morphological characters for the nodes/clades shown in Fig. 1A – main figure. Note the similarity between rates among elephantine taxa (*Loxodonta*, *Elephas*, and *Mammuthus*) shown here, and those of Maglio (1973, pp. 105–106).

Miocene to late Pliocene), and varying rates among Loxodontini and Elephantini taxa (Fig. 4). The observation of very fast acceleration at about the emergence of Elephantidae may be correlated with the fact that "... the Miocene saw major changes in global climate". The early Miocene "was warmer and had less seasonal variation than at any time ...", and by late Miocene, "a new low in average global temperatures" was prevalent (Tattersall *et al.* 1988, pp. 350, 351). A burst of acceleration of morphological characters within Proboscidea may also be correlated with the newly available ecological niches that were utilized by certain developing lineages.

Since the end of the Pliocene, at the time when Loxodontini and Elephantini diverged, through the end of the Pleistocene or Holocene, each lineage took a different adaptive path. Within these approximately six million years, evolutionary

rate values for *Loxodonta*, *Elephas*, and *Mammuthus* were calculated to be 0.50, 0.83, and 1.17, respectively (Table 4, 4th column, numbers on left of "/"). These findings corroborate those of Maglio (1973) and of Shoshani *et al.* (1985b), indicating that *Mammuthus* was the fastest evolving genus, having evolved at about twice the rate of *Loxodonta*. Data from our molecular investigation indicate a 25–36% increase in rates of substitution in *Elephas* and *Mammuthus* compared to *Loxodonta* (Yang *et al.* 1996). It should be noted, however, that these differences do not reach statistical significance, presumably due to the small sequence length studied.

Morphological vs molecular results: a comparison

Focusing on the Elephantinae taxa (*Loxodonta*, *Elephas*, and *Mammuthus*) which were tested with both approaches, and only on the results when *Mammut americanum* was employed as an outgroup, we note the following.

In the morphological portion of this study we used 95 characters, whereas the molecular characters number 228. The maximum number of character states in the morphological data was six (0, 1, 2, 3, 4, 5), whereas there are four possible character states (A – adenine, G – guanine, C – cytosine, T – thymine) for the molecular characters. The number of characters is not significant as long as they are good and chosen objectively. The latter statement holds true for molecular more than for morphological data because of the possible subjectivity involved in choosing morphological characters. The number of character states is perhaps more significant than the number of characters because (when running PAUP with the unordered option), the greater the number of character states the more ambiguous the polarities of these character states will be (see also discussion in Shoshani *et al.* 1996); based on our experience, with such a small difference of six vs four the ambiguity of the polarities is not much different.

Cladistic analyses of morphological and molecular data support the traditional hypothesis that *Mammuthus* and *Elephas* are more closely related to each other than either is to *Loxodonta*. *Mammut americanum* proved to be an excellent choice as an outgroup for both, morphological and molecular studies, because of the recency of common ancestor within Proboscidea and because of the superb preservation of the bones that provided DNA for comparison with another extinct taxon (*Mammuthus*) and the two extant genera (*Loxodonta* and *Elephas*). Results with molecular data demonstrate the importance of using a closely related taxon as an outgroup for resolving phylogenies of highly derived species. The choice of a distantly related outgroup (outside of Proboscidea) would result in inferring evolutionary events over a long period of time (twice the time since the existence of the last common ancestor of the outgroup and ingroup). During this period, we assume that neutral nucleotide substitutions will accrue along both independent lineages (outgroup and ingroup). Because the gene sequence of interest must be evolving rapidly enough to distinguish hierarchical relationships among the

Elephantinae, the gene sequences within the long independent lineages will accumulate a considerable number of substitutions. The result will be that distantly related gene sequences will be equally distinct from any terminal taxa within the ingroups, and will not, therefore, be useful in determining the hierarchical relationships of the inner nodes. This, in turn, results in incorrect rooting of the trees, especially when the branch lengths of the ingroups are heterogeneous. Evolutionary rates for the morphological characters indicate that among the Elephantinae, *Loxodonta* is the slowest evolving genus, followed by *Elephas* and *Mammuthus*. An indication for such rates was also obtained from the molecular data.

Conclusions

Results presented here are based on 95 morphological (osteological and dental) characters and 228 molecular characters (mtDNA base pairs). In the morphological part we studied 14 taxa (2 proboscidean outgroups, 4 gomphotheres, 2 stegodontids, and 6 elephantids), and in the molecular part we analyzed 7 taxa (3 non-proboscidean outgroups, 1 proboscidean outgroup, and 3 elephantids). Two of the proboscidean taxa studied by molecular methods are extinct genera, and were radiometrically dated between 46 000 and 10 220 years ago. Our results are summarized as follows:

(1) It is imperative that morphological characters are clearly defined and illustrated. Taxonomists must make special effort to describe morphological characters as clearly as possible; illustrations/photographs perhaps with arrows indicating exactly the nature of a character are very useful. In some cases, one misunderstanding or misinterpretation in the meaning of one writer by another can produce drastically different results, especially when the coding of a pivotal character produces different polarities by different interpreters. This type of subjectivity in describing characters is non-existent in molecular studies – the sequences (characters) are, in turn, analyzed by a computer (see discussion in Shoshani 1996, and Shoshani *et al.* 1996). One solution is collaborative studies, another is concentration on a few characters, but to study them thoroughly (this statement includes the works of Shoshani!).

(2) Cladistic analysis of morphological and molecular data corroborate the traditional hypothesis that *Mammuthus* and *Elephas* are more closely related to each other than either is to *Loxodonta*. Studying relationships within Elephantidae (using morphology or molecules) must not be conducted in isolation; the outgroup chosen – preferably within the Proboscidea – is an important decision, and two outgroups are better than one.

(3) It is extremely important to employ an outgroup which is closer (at least one which belongs in the same taxonomic order) to the ingroup – the closer the outgroup to the ingroup, the more accurate the results will be. In addition, more

than one outgroup will probably help to define the transformation (morphoclines; ie, changes that occur from one character state to another with one character) of characters and their polarities.

(4) *Stegotetrabelodon* and *Stegodibelodon*, once thought to belong in one subfamily (Stegotetrabelodontinae; Maglio 1973, Coppens *et al.* 1978), do not remain grouped in one clade. *Stegodibelodon* is a sister-group to Elephantinae followed by *Stegotetrabelodon*, a relationship also corroborated by the studies of Kalb *et al.* (1996), and Shoshani (1996). It costs three additional steps to support Maglio's (1973) hypothesis (cf Table 2).

(5) Our findings also suggest that the family Stegodontidae (*Stegolophodon* and *Stegodon*) is a monophyletic taxon although with weaker support than for relationships within the Elephantinae. Kalb *et al.* (1996) hypothesized that Stegodontidae is not a monophyletic taxon.

(6) Recent detailed studies of Eurasian and North American mammoths indicate that their evolutionary histories are complex and often intertwined with overlapping geological records and variations in characters. Despite these complications, overall trends have been observed, and they can be summarized by observing changes in the skull – from low and long in primitive taxa to high and short in more advanced ones – and also by increase of lamellar frequencies through geological ages.

(7) Mammutidae (here represented by *Mammut*) is not a sister-group to Stegodontidae. Mammutidae is the plesiomorphic sister-group to the clade comprising all gomphotheres, stegodontids, and elephantids. The close association of *Tetralophodon*, *Anancus*, and *Paratetralophodon* with Stegodontidae and Elephantidae corroborates the hypothesis that Stegodontidae is more closely related to gomphotheres and elephantids than to mammutids.

(8) The gomphotheres, *sensu lato*, are definitely not a monophyletic assemblage, a conclusion also reached by Tassy (1996) and Shoshani (1996).

(9) Calculated evolutionary rates for the morphological and molecular characters indicate that among the three elephantine genera studied, *Mammuthus* had evolved at the fastest rate, followed by *Elephas*, and then, *Loxodonta*. For the given characters we studied, autapomorphies along the *Mammut* branch are greater in the morphological than in the molecular characters (12 vs 8; cf Fig. 1B to Fig. 2).

(10) The suggested partial classification for the proboscidean taxa studied (Table 1) is based on the works of several authors (eg Shoshani 1986, 1996, Tassy and Darlu 1986, Kalb *et al.* 1996, Tassy 1996), and is not intended to present a "final" branching pattern among these taxa – it is only a tentative working hypothesis based on the available evidence.

Acknowledgements: Staffs of the museums listed above, who allowed us to study specimens in their care and/or collect samples for DNA analyses, are deeply appreciated. Individuals who helped us in various aspects of this study include: J. Alexander, S. K. Bell, D. Domning, M. Goodman, J. S. Grimes, J. E. Kalb, A. M. Lister, M. C. McKenna, E. C. Marsac, J. L. Pierce, H. Saegusa, P. Tassy, R. Tedford,

and S. D. Webb. Tassy's (1990 and 1996) characters were invaluable and discussions of their polarities were pivotal. A. M. Lister's and S. D. Webb's help with simplifying and correcting the mammoth evolutionary chart (Fig. 3, drawn by J. S. Grimes) is much appreciated. Molecular studies of ancient proboscideans were supported, in part, by Alfred P. Sloan Foundation fellowship (93-4-6-ME) to H. Y.

References

Adachi J. and Hasegawa M. 1995. Phylogeny of whales: dependence of inference on species sampling. Molecular Biology and Evolution 12: 177–179.

Agenbroad L. D. 1984. New World mammoth distributions. [In: Quaternary extinctions: a prehistoric revolution. P. S. Martin and R. G. Klein, eds]. University of Arizona Press, Tucson: 90–108.

Agenbroad L. D. 1994. Taxonomy of North American *Mammuthus* and biometrics of the Hot Springs mammoths. [In: The Hot Springs Mammoth Site: a decade of field and laboratory research in paleontology, geology, and paleoecology. L. D. Agenbroad and J. I. Mead, eds]. The Mammoth Site of South Dakota, Hot Springs: 158–207.

Aguirre É. E. 1969. Evolutionary history of the elephant. Science 164 (3886): 1366–1376.

Anderson S., Bankier A. T., Barrell B. G., de Brujin M. H. L., Coulson A. R., Drouin J., Eperon I. C., Nierlich D. P., Roe B. A., Sanger F., Schreier P. H., Smith A. J. H., Staden R. and Young I. G. 1981. Sequence and organization of the human mitochondrial genome. Nature (London) 290: 457–465.

Barnard G. F., Pude R. M., Begum N. A. and Chen L. B. 1994. PCR product sequencing with (a-33P) and (a-32P) dATP. BioTechniques 16: 572–573.

Blumenbach J. F. 1779. Handbuch der Naturgeschichte (1st edn). J. H. Dieterich, Göttingen: 1–559.

Boom R., Sol C. J. A., Salimans M. M. M., Jansen C. L., Wertheim-van Dillen P. M. E. and van der Nordaa J. 1990. Rapid and simple method for purification of nucleic acids. Journal of Clinical Microbiology 28: 495–503.

Cano R. J. and Poinar H. N. 1993. Rapid isolation of DNA from fossil and museum specimens suitable for PCR. BioTechniques 15: 432–435.

Cooper A. 1994. DNA from museum specimens. [In: Ancient DNA. B. Herrmann and S. Hummel, eds]. Springer-Verlag, New York: 149–165.

Coppens Y., Maglio V., Madden C. T. and Beden M. 1978. Proboscidea. [In: Evolution of African mammals. V. J. Maglio and H. B. S. Cooke, eds]. Harvard University Press, Cambridge, Massachusetts: 336–367.

Cuvier F. 1825. Eléphant d'Afrique. [In: Histoire naturelle des Mammifères, avec des figures originales, coloriées, dessinées d'après des animaux vivants: publiée sous l'autorité de l'administration du Museum d'Histoire Naturelle. E. Geoffroy Saint-Hilaire and F. Cuvier, eds, for the years of 1824–1847, 7 volumes]. Belin, Paris: 1–2 + 1plt. Volume 3, A.

Doyle J. J. and Doyle J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Dubrovo I. A. 1989. Systematic position of the Khapry elephants. Paleontologicheskiĭ Zhurnal 1989/1: 78–87.

Ezra H. C. and Cook S. F. 1959. Histology of mammoth bone. Science 129: 465–466.

Felsenstein J. 1993. PHYLIP (Phylogeny Inference Package), Version 3. Department of Genetics, University of Washington, Seattle, Washington: 1–5.

Golenberg E. M. 1994. DNA from plant compression fossils. [In: Ancient DNA. B. Herrmann and S. Hummel, eds]. Springer Verlag, New York: 237–256.

Graham R. W. 1986. Taxonomy of North American mammoths. [In: The Colby mammoth site – taphonomy and archaeology of a Clovis kill in northern Wyoming. G. C. Frison and L. C. Todd, eds]. University of New Mexico Press, Albuquerque: 165–169.

Groves C., Grubb P. and Bricknell S. 1993. Taxonomy of African elephants. Abstracts, Sixth International Theriological Congress, Sydney, Australia, 4–10 July 1993: 120.

Hagelberg E. and Clegg J. B. 1991. Isolation and characterization of DNA from archaeological bone. Proceedings of the Royal Society of London: B, 244: 45–50.

Hagelberg E., Thomas M. G., Cook C. E., Sher A. V., Baryshnikov G. F. and Lister A. M. 1994. DNA from ancient mammoth bones. Nature 370: 333–334.

Hardy C., Casane D., Vigne J. D., Callou C., Dennebouy N., Mounolou J.-C. and Monnerot M. 1994. Ancient DNA from Bronze Age bones of European rabbit (*Oryctolagus cuniculus*). Experientia 50: 564–570.

Höss M. and Pääbo S. 1993. DNA extraction from Pleistocene bones by a silica-based purification method. Nucleic Acids Research 21: 3913–3914.

Höss M., Pääbo S. and Vereschagin N. K. 1994. Mammoth DNA sequences. Nature 370: 333.

Irwin D. M., Kocher T. D. and Wilson A. C. 1991. Evolution of the cytochrome *b* gene of mammals. Journal of Molecular Evolution 32: 128–144.

Jones C. 1984. Tubulidentates, proboscideans, and hyracoideans. [In: Orders and families of Recent mammals of the world. S. Anderson and J. K. Jones, Jr, eds]. John Wiley & Sons, New York: 523–535.

Kalb J. E., Froehlich D. J. and Bell G. L. 1996. Phylogeny of African and Eurasian Elephantoidea of the late Neogene. [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 101–116.

Krause H. 1978. The mammoth – in ice and snow? Cold-adaptation of woolly mammoth: fact or fiction? Self-publisher, Stuttgart: 1–107 + 1 page Epilog.

Kumar S., Tamur K. and Nei M. 1993. MEGA: molecular evolutionary genetics analysis 1.01. The Pennsylvania State University, University Park.

Lambert W. D. 1995. *Mammuthus* from the late Blancan Santa Fe 1B locality: the oldest record of *Mammuthus* in North America. Journal of Vertebrate Paleontology 15 (3, Supplement): 40A.

Lister A. M. 1996. History and taxonomy of Eurasian mammoths. [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 203–213.

Lister A. M. and Bahn P. 1994. Mammoths. MacMillan, New York: 1–168.

Lowenstein J. M. and Shoshani J. 1996. Proboscidean relationships based on immunological data. [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 49–54.

Lydekker R. 1907. The ears as a race-character in the African elephant. Proceedings of the Zoological Society of London 26: 380–403.

Lydekker R. 1916. Catalogue of the ungulate mammals in the British Museum (Natural History) 5: 1–207.

Madden C. T. 1981. Mammoths of North America. Unpublished Ph D thesis. University of Colorado, Boulder. University Microfilms, Ann Arbor: 1–271.

Maddison W. P. and Maddison D. R. 1992. MacClade: analysis of phylogeny and character evolution. Version 3.0. Computer program distributed by Sinauer Associates, Sunderland, Massachusetts.

Maglio V. J. 1973. Origin and evolution of the elephantidae. Transactions of The American Philosophical Society of Philadelphia. New Series 63(3): 1–149.

McKenna M. C., Bell S. K., Simpson G. G., Nichols R. H., Tedford R. H., Koopman K. F., Musser G. G., Neff N. A., Shoshani J. and McKenna D. M. 1997. Classification of mammals above the species level. Columbia University Press, New York: 1–631.

Osborn H. F. 1918. March. A long-jawed mastodon skeleton from South Dakota and phylogeny of the Proboscidea. (Abstract) Bulletin of the Geological Society of America 29: 133–137.

Osborn H. F. 1921. The evolution, phylogeny, and classification of the Proboscidea. American Museum Novitates 1: 1–15.

Osborn H. F. 1936. Proboscidea: a monograph on the discovery, evolution, migration and extinction of the mastodonts and elephants of the world. Vol. I: Moeritherioidea, Deinotherioidea, Mastodontoidea. The American Museum Press, New York: I: xl + 1–802 (page 802 includes errata).

Osborn H. F. 1942. Proboscidea: a monograph of the discovery, evolution, migration and extinction of the mastodonts and elephants of the world. Vol. II: Stegodontoidea, Elephantoidea. The American Museum Press, New York: xxvii + 805–1675 (plus one errata page).

Ozawa T., Hayashi S. and Mikhelson V. M. 1997. Phylogenetic position of mammoth and Steller's sea cow within Tethytheria demonstrated by mitochondrial DNA sequences. Journal of Molecular Evolution 44: 406–413.

Saegusa H. 1996. Stegodontidae: evolutionary relationships. [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 178–190.

Sarich V. M. and Wilson A. C. 1967. Immunological time scale for hominid evolution. Science 158: 1200–1203.

Shoshani J. 1986. On the phylogenetic relationships among Paenungulata and within Elephantidae as demonstrated by molecular and osteological evidence. Unpublished Ph D thesis, Wayne State University, Detroit (Michigan). Michigan University Microfilms, Ann Arbor: 1–649.

Shoshani J. 1993. How many species or subspecies of forest *Loxodonta* exist? Abstracts, Sixth International Theriological Congress, Sydney, Australia, 4–10 July 1993: 278–279.

Shoshani J. 1996. Para- or monophyly of the gomphotheres and their position within the Proboscidea. [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 149–177.

Shoshani J. 1998. Understanding proboscidean evolution: a formidable task. Trends in Ecology and Evolution 13: 480–487.

Shoshani J. and Tassy P. (eds) 1996. The Proboscidea: evolution and paleoecology of elephants and their relatives. Oxford University Press: 1–472.

Shoshani J. *et al.* [76 co-authors] 1982. On the dissection of a female Asian elephant (*Elephas maximus maximus* Linnaeus, 1758) and data from other elephants. Elephant 2: 3–93.

Shoshani J., Fisher D. C., Zawiskie J. M., Thurlow S. J., Shoshani S. L., Benninghoff W. S. and Zoch F. H. 1989. The Shelton Mastodon Site: a multidisciplinary study of a late Pleistocene (Twocreekan) locality in southeastern Michigan. Contributions from the Museum of Paleontology, the University of Michigan 27: 393–436.

Shoshani J., Groves C. and Simons E. 1996. Primate phylogeny: morphological vs. molecular results. Molecular Phylogenetics and Evolution 5: 102–154.

Shoshani J., Lowenstein J. M., Walz D. A. and Goodman M. 1985a. Proboscidean origins of mastodon and woolly mammoth demonstrated immunologically. Paleobiology 11: 429–437.

Shoshani J., Walz D., Goodman M., Lowenstein J. M. and Prychodko W. 1985b. Protein and anatomical evidence of the phylogenetic position of *Mammuthus primigenius* within the Elephantinae. Acta Zoologica Fennica 170: 237–240.

Swofford D. L. 1993. PAUP: phylogenetic analysis using parsimony. Version 3.1.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois: 1–257.

Tassy P. 1988. The classification of Proboscidea: how many cladistic classifications? Cladistics 4: 43–57.

Tassy P. 1990. Phylogénie et classification des Proboscidea (Mammalia): historique et actualité. Annales de Paléontologie (Vert.-Invert.) 76: 159–224.

Tassy P. 1996. Who is who among the Proboscidea? [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 39–48.

Tassy P. and Darlu P. 1986. Analyse cladistique numérique et analyse de parcimonie: l'exemple des Elephantidae. Géobios 19: 587–600.

Tassy P. and Shoshani J. 1988. The Tethytheria: elephants and their relatives. [In: The phylogeny and classification of the tetrapods. Volume 2: Mammals. M. Benton, ed]. The Systematics Association, Special Volume No. 35B. Clarendon Press, Oxford: 283–315.

Tassy P. and Shoshani J. 1996. Historical overview of classification and phylogeny of the Proboscidea. [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 3–8.

Tattersall I., Delson E. and Van Couvering J. 1988. Encyclopedia of human evolution and prehistory. Garland Publishing, New York: 1–603.

Tobien H. 1976. Zur paläontologischen Geschichte der Mastodonten (Proboscidea, Mammalia). Mainzer Geowissenschaftliche Mitteilungen 5: 143–225.

Tobien H. 1988. Some major features in the evolution of mastodonts (Proboscidea, Mammalia). Geological Survey of India, Special Publication No 11: 109–116.

Todd N. E. and Roth V. L. 1996. Origin and radiation of the Elephantidae. [In: The Proboscidea: evolution and palaeoecology of elephants and their relatives. J. Shoshani and P. Tassy, eds]. Oxford University Press: 193–202.

Valente A. 1983. Hair structure of the woolly mammoth, *Mammuthus primigenius* and the modern elephants, *Elephas maximus* and *Loxodonta africana*. Journal of Zoology, London 11: 271–274.

Webb S. D. 1996. Evolution of mammoths in North and Central America. Journal of Vertebrate Paleontology 16 (3, Supplement): 71A.

Webb S. D. and Dudley J. P. 1995. Proboscidea from the Leisey Shell Pits, Hillsborough County, Florida. [In: Paleontology and geology of the Leisey Shell Pit, early Pleistocene of Florida. R. C. Hulbert, Jr, G. S. Morgan and S. D. Webb, eds]. Bulletin of the Florida Museum of Natural History, Biological Sciences 37, Part II(20): 645–660.

Wu C.-I. and Li W.-H. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. Proceedings of the National Academy of Science, USA 82: 1741–1745.

Yang H., Golenberg E. M. and Shoshani J. 1996. Phylogenetic resolution within the Elephantidae using fossil DNA sequence from American mastodon (*Mammut americanum*) as an outgroup. Proceedings of the National Academy of Sciences 93: 1190–1194.

Yang H., Golenberg E. M. and Shoshani J. 1997a. A blind testing design for authenticating ancient DNA sequences. Molecular Phylogenetics and Evolution 7: 261–265.

Yang H., Golenberg E. M. and Shoshani J. 1997b. Proboscidean DNA from museum and fossil specimens: an assessment of ancient DNA extraction and amplification techniques. Biochemical Genetics 35: 165–179.

Received 23 March 1998, accepted 30 May 1998.

Appendix 1. Proboscidean characters examined to test hypotheses discussed in this study.
* Characters in this table are from three main sources: Shoshani (1996, pp. 162–166), Tassy and Darlu (1986, pp. 599–600), and Kalb *et al.* (1996, p. 104). Each set of characters is listed separately for easy reference. Sequential numbers are given first and these are followed (in parentheses) by the original number of the authors to make it easy to trace the sources. This system is also followed in Appendix 2, where the numbers at the top are sequential, and the numbers beneath them are from the original papers.
* Characters from Shoshani (1996) are adapted from, or slightly modified after many sources, details and key to references are given in Shoshani (1996, p. 162). Whenever possible, characters were observed on adult individuals, and at least three specimens were examined for each taxon (other details under Material and methods). Characters are arranged from anterior to posterior of body, beginning with incisors.
Note K18a: In characters K18 through K22, the word "plates" does not define which plates. This is confusing because the configuration of the posterior and anterior face of each lophid or lower plate is different at the anterior, middle, and posterior of a tooth. We have not attempted to correct or modify Kalb *et al.*'s data for this question, but placed a "?" for *Stegolophodon* and *Stegodon* because they are most likely to be incorrectly coded and are the crux of the main disagreement between Kalb *et al.* (1996) results compared to other authors (eg Tassy and Darlu 1986, Saegusa 1996, Shoshani 1996).
Note K 18b: "absent" under character state (1) may be interpreted as if is not "very convex" or not "shallow convex", in which case some taxa, eg, *Stegolophodon* and *Stegodon* might be coded with "?"or "0".

Feature	Primitive (only state "0")	Derived (all states except "0")
1	2	3
	Part I – Characters after Shoshani (1996)	
1(7). UPPER TUSKS: viewed laterally	(0)=curve ventrally	(1)=straight (2)=curve dorsally (3)=curve dorsally, much
2(10). UPPER TUSKS: enamel	(0)=surrounds tooth	(1)=partly surrounds tooth (2)=a longitudinal band of enamel (3)=little or usually absent (4)=enamel absent
3(11). LOWER TUSKS: viewed laterally	(0)=curve dorsally	(1)=straight (2)=curve ventrally
4(12). LOWER TUSKS: viewed anteriorly	(0)=wear at tip, anteriorly	(1)=wear at tip, dorsally and ventrally (2)=wear at tip, ventrally only
5(13). LOWER TUSKS: protruding portion, viewed dorsally	(0)=small (less than 10 cm long)	(1)=longer than approximately 10 cm
6(15). LOWER TUSKS:	(0)=cross section (c.s.) flat	(1)=c.s. pyriform (2)=round or oval
7(16). LOWER TUSKS: medial edge	(0)=round	(1)=straight
8(22). dp^2: parastyle	(0)=not reduced	(1)=reduced
9(23). dp^3: posterior loph (oblique, ie metacone displaced posterior to hypocone)	(0)=not oblique	(1)=oblique
10(24). dp^3: oblique contact between pre- and posttrite central conule	(0)=absent	(1)=present
11(25). dp_3: oblique contact between pre- and posttrite central conule	(0)=absent	(1)=present

Appendix 1 – continued.

1	2	3
12(27). P^2 and P_2 through P^4 and P_4:	(0)=P2/2, P3/3, P4/4 present	(1)=P2/2 absent (2)=P2/2, P3/3 absent (3)=P2/2, P3/3, P4/4 absent
13(31). P3/3 - P4/4: posterior supplementary loph(id)s	(0)=absent	(1)=present
14(32). M^1:	(0)=bilophodont	(1)=third loph outlined (2)=third loph complete (3)=fourth loph complete
15(33). M^2:	(0)=bilophodont	(1)=trilophodont outlined (2)=trilophodont distinct (3)=tetralophodont
16(34). M^3: postentoconule (postentoconule is the third pretrite main cusp of upper molars, and it is the counterpart of postentoconulid (=entoconid II) of lower molars)	(0)=reduced or absent	(1)=enlarged (2)=M^3 with two large cusps posterolabially (3)=M^3 is trilophodont (ie two valleys formed) (4)=M^3 is tetralophodont (5)=M^3 is pentalophodont or more
17(35). M^3:	(0)=large, wide [robustness index (I=100/L) is between 70–100]	(1)=I<70 (2)=I<60
18(37). M^{1-3}: central conules	(0)=not enlarged	(1)=enlarged
19(39). M_1:	(0)=bilophodont	(1)=trilophodont (2)=tetralophodont
20(40). M_2:	(0)=bilophodont	(1)=trilophodont (2)=tetralophodont or more
21(42). M_3:	(0)=bilophodont	(1)=trilophodont (2)=4th lophid with central conule in 3rd interlophid
22(45). M_1 and M_2:	(0)=without central conule in the 2nd interlophid	(1)=with central conules (small or large)
23(46). M_{1-3}: anterolingual cingulum on posttrite side	(0)=not reduced	(1)=reduced
24(48). M_{1-3}:	(0)=2nd and 3rd lophids perpendicular to longitudinal axis of tooth	(1)=2nd and 3rd lophids oriented postero-labially in relation to longitudinal axis
25(49). M1: elephantine plates	(0)=absent	(1)=present
26(55). MOLARS: trefoils	(0)=none	(1)=on pretrite plus quasi posttrite (2)=on pretrite and posttrite
27(56). UPPER MOLARS: cingulum on medial side	(0)=present	(1)=absent
28(57). MOLARS:	(0)=brachyodont non-accentuated	(1)=brachyodont, accentuated
29(58). MOLARS: anterior pretrite central conule and adaxial conelet (mesoconelet)	(0)=not fused	(1)=fused

Appendix 1 – continued.

1	2	3
30(59). MOLARS: abaxial and adaxial cones (and their conelets)	(0)=are of unequal size	(1)=are of about equal size
31(60). MOLARS' PATTERN	(0)=with cusps or lophs	(1)=with laminae (2)=laminae with height/width=1, 1+ or more
32(61). MOLARS: with thinner and more numerous laminae	(0)=absent	(1)=present
33(62). PREMOLARS and MOLARS: Note: "zygodont" refers to the outline of ridges in anterior view; they are 'yoke-shaped' and sharp when worn	(0)=non-zygodont	(1)=zygodont
34(63). PREMOLARS and MOLARS: crowns of	(0)=cement absent	(1)=little cement present (2)=abundant cement present
35(64). PREMOLARS and MOLARS: hypsodonty index	(0)=brachyodonty	(1)=hypsodonty
36(65). PREMOLARS and MOLARS: central conule in a form of a crest and does not block the valley	(0)=absent	(1)=present
37(66). PREMOLARS and MOLARS: zygodont crest on posttrite	(0)=absent	(1)=present
38(67). CHEEK TEETH: horizontal displacement and succession associated with remodeling of adjacent bones	(0)=absent	(1)=present
39(73). CRANIUM:	(0)=swelling absent (saggital crest present)	(1)=swelling present with loss of saggital crest (2)=wide cerebral area
40(74). CRANIUM: large dorsal parietal bulges and frontoparietal concavity	(0)=absent	(1)=present
41(76). PREMAXILLA: with posterodorsal process in the mid-line, at the floor of the nasal fossa	(0)=absent	(1)=present, but does not protrude vertically (2)=protrudes vertically
42(77). MAXILLA: ridges prominent and close to midline in ventral view	(0)=absent	(1)=present
43(79). NAREAL OPENING:	(0)=narrow (lateral limits are vertical)	(1)=wide (lateral limits are diagonal) (2)=deep depressions laterally
44(80). NAREAL OPENING: lateral limits of	(0)=do not extend laterally	(1)=extend laterally up to or beyond width of rostrum
45(82). PALATINE: spina nasalis posterior above choanae	(0)=absent	(1)=present
46(83). ORBIT: anterior border of	(0)=situated above or posterior to M^1	(1)=forward of M^1
47(85). ORBIT: rim with angular corners	(0)=absent	(1)=present
48(88). CANALIS TEMPORALIS (= squamosal-sinus canals)	(0)=present	(1)=absent
49(89). INFRAORBITAL FORAMEN: duplication, on maxilla	(0)=absent	(1)=always present (2)=sometimes present

Appendix 1 – continued.

1	2	3
50(92). FORAMEN OVALE and FORAMEN LACERUM MEDIUM	(0)=not confluent	(1)=confluent
51(93). MANDIBULAR FORAMEN: process at the anterior border	(0)=absent	(1)=present
52(94). MANDIBULAR SYMPHYSIS	(0)=reduced symphysis	(1)=long (2)=short, spout-like
53(96). MANDIBULAR ANGULAR PROCESS:	(0)=distinct	(1)=reduced
54(98). BULLA (ectotympanic area)	(0)=not enlarged	(1)=slightly enlarged posterolaterally
55(99). STYLOHYOID: posterior ramus	(0)=absent	(1)=present
56(100). STYLOHYOID: reduced shelf at the base of the inferior ramus	(0)=absent	(1)=present
57(101). STYLOHYOID: more gracile	(0)=absent	(1)=present
58(102). THORACIC VERTEBRAE: number of	(0)=20–21	(1)=18–19, 20
59(106). HUMERUS: deep bicipital groove and higher trochanter	(0)=absent	(1)=present
60(110). ULNA: olecranon process tilted medially and extends proximally beyond the trochlear notch	(0)=absent	(1)=present
61(111). CARPUS & TARSUS: low and wide, ie less 'cursorial'	(0)=absent	(1)=present
62(112). MAGNUM: reduced lateral bulging of the articular facet of scaphoid	(0)=absent	(1)=present
63(113). PELVIS: well-delineated distal obturator notch	(0)=absent	(1)=present
64(121). ASTRAGALUS: protruding tuberculum mediale	(0)=absent	(1)=present, large (2)=present, reduced
65(131). Humeral condyles	(0)=asymmetrical, ie lateral condyle is larger	(1)=symmetrical
66(134). Femoral head, fovea of (the fovea houses the ligamentum teres)	(0)=deep and centrally placed	(1)=displaced to posterior surface of femoral head (2)=fovea absent
Part II – Characters after Kalb *et al.* (1996)		
67(K1). Cranium	(0)=flattened	(1)=rounded
68(K2). Occipital condyles	(0)=protruding	(1)=recessed
69(K6). Mandible width	(0)=narrow	(1)=wide
70(K15). Curvilinear alignment of apices	(0)=absent	(1)=present
71(K18). Posterior face of lophids or lower plates (see notes K18a and K18b in the caption to this appendix)	(0)=very convex	(1)=shallow convex or absent
72(K19). Posterior face of lophids or lower plates (see note K18a)	(0)=not concave	(1)=shallow concave or very concave
73(K20). Posterior face of lophids or lower plates (see note K18a)	(0)=convex or mixed	(1)=concave

Appendix 1 – concluded.

1	2	3
74(K21). Anterior face of lophids or lower plates (see note K18a)	(0)=convex	(1)=concave or mixed
75(K22). Anterior face of lophids or lower plates (see note K 18a)	(0)=convex or mixed	(1)=concave
76(K25-26). Median sulcus	(0)=complete	(1)=incomplete
77(K27). Four apices (cones, conelets)	(0)=present	(1)=five or more
78(K30). On M_2 and M_3, posterior pretrite conules/columns posteriorly	(0)=present	(1)=reduced/fused
79(K31). On M^3, posterior pretrite conules/columns posteriorly	(0)=absent	(1)=present
80(K32). On M_2 and M^2, minimum of plates	(0)=three	(1)=four (2)=five (3)=six
81(K33). On M_3, minimum of plates	(0)=three	(1)=five (2)=six (3)=seven (3)=eight
82(K34). On M^3, minimum of plates	(0)=three	(1)=five (2)=six (3)=seven (3)=nine
Character after Maglio (1973, pp. 89–90)		
83(V). Base of the transverse valley between molar plates	(0)=V-shaped	(1)=U-shaped
Part III – Characters after Tassy and Darlu's (1986)		
84(T&D3). Alveoli of maxilla	(0)=low	(1)=high
85(T&D4). Occlusal surface of M^3	(0)=horizontal	(1)=convex
86(T&D5). Mesoconelets in upper molars	(0)=of normal height	(1)=increase in height
87(T&D6). Mesoconelets in lower molars	(0)=of normal height	(1)=increase in height
88(T&D9). Postglenoid fossa	(0)=absent	(1)=present
89(T&D10). Mastoid groove	(0)=absent	(1)=present
90(T&D13-4). Mandibular horizontal ramus	(0)=of normal height	(1)=increase in height
91(T&D19). Molars	(0)=wide	(1)=narrow
92(T&D20). Palate	(0)=wide	(1)=narrow
93(T&D30). Pre-posttrite functional dissymmetry	(0)=present	(1)=absent ie erosion of loph(id)s
94(T&D31). I_2	(0)=present	(1)=absent
95(T&D32). Enamel on loph(id)s of premolars and molars	(0)=relatively thick	(1)=thinner

Appendix 2. Character matrix for 14 proboscidean taxa; cf Appendix 1 for list of characters and explanations.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
		7.	10.	11.	12.	13.	15.	16.	22.	23.	24.	25.	27.	31.	32.	33.	34.	35.	37.	39.	40.	42.	45.	46.	48.	49.	55.	56.	57.	58.	59.	60.	61.	62.
1	*Phiomia*	0	2	0	1	0	0	1	0	0	0	0	0	0	2	2	2	0	0	1	1	1	1	0	1	?	0	0	0	0	0	0	?	0
2	*Mammut*	2	3	1	0	0	2	0	1	1	?	?	3	?	2	2	3	1	0	1	1	2	1	1	1	?	0	0	0	0	0	0	?	1
3	*Gomphotherium*	0	2	0	1	1	1	0	1	1	0	0	1	0	2	2	3	2	0	1	1	2	1	1	1	?	0	0	0	0	0	0	?	0
4	*Tetralophodon*	0	4	1	1	1	2	0	1	1	1	1	1	1	3	3	5	2	1	2	2	2	1	1	1	?	1	0	0	0	0	0	?	0
5	*Anancus*	1	4	?	?	?	?	?	1	1	1	1	2	1	3	3	5	2	1	2	2	2	1	1	1	?	1	1	0	0	0	0	?	0
6	*Paratetralophodon*	0	4	?	?	?	?	?	?	?	?	?	?	?	3	3	5	2	1	?	?	2	1	1	?	?	1	1	0	0	0	0	?	0
7	*Stegolophodon*	0	2	0	1	1	1	0	1	1	?	?	1	1	3	3	5	2	1	2	2	2	1	1	1	?	0	1	1	1	1	1	?	0
8	*Stegodon*	2	3	?	?	?	?	?	1	1	?	?	3	?	3	3	5	2	0	2	2	2	1	1	1	?	0	1	1	1	1	1	?	0
9	*Stegotetrabelodon*	1	4	1	1	1	2	0	?	?	?	1	2	1	3	3	5	2	1	2	2	2	1	1	1	0	0	1	0	0	1	1	?	0
10	*Stegodibelodon*	?	?	?	?	?	?	?	?	?	?	?	?	?	3	3	5	2	1	2	2	2	?	1	0	?	0	1	0	0	?	1	?	0
11	*Primelephas*	?	?	1	?	?	2	0	1	?	?	1	2	1	3	3	5	2	0	2	2	2	1	1	0	0	0	?	0	0	1	1	?	0
12	*Loxodonta*	2	4	?	?	?	?	?	1	1	?	?	3	?	3	3	5	2	0	2	2	2	1	1	0	1	0	1	0	0	1	2	?	0
13	*Elephas*	2	4	?	?	?	?	?	1	1	?	?	2	1	3	3	5	2	0	2	2	2	1	1	0	1	0	1	0	0	1	2	1	0
14	*Mammuthus*	3	4	?	?	?	?	?	1	1	?	?	2	1	3	3	5	2	0	2	2	2	0	1	0	1	0	1	0	0	1	2	1	0

		34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
		63.	64.	65.	66.	67.	73.	74.	76.	77.	79.	80.	82.	83.	85.	88.	89.	92.	93.	94.	96.	98.	99.	100.	101.	102.	106.	110.	111.	112.	113.	121.	131.	134.
1	*Phiomia*	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	2	0	0	1	0	0	?	?	?	?	0	?	0	?	?	1	0	1
2	*Mammut*	1	0	1	1	1	1	0	2	0	0	0	1	0	0	1	2	1	0	2	0	1	0	?	0	?	0	?	0	?	?	2	1	2
3	*Gomphotherium*	1	0	0	0	1	2	0	2	0	2	1	0	0	0	1	2	1	0	1	1	1	1	?	0	?	1	?	1	?	?	1	1	2
4	*Tetralophodon*	1	0	0	0	1	2	?	2	?	2	1	0	0	?	1	2	1	?	1	1	1	?	?	?	?	1	?	1	?	?	?	1	2
5	*Anancus*	1	0	0	0	1	2	?	2	?	2	1	0	1	?	1	2	1	?	2	1	1	?	?	?	?	1	?	1	?	?	?	1	2
6	*Paratetralophodon*	2	0	0	0	1	2	?	2	?	2	1	0	1	?	1	1	1	?	?	?	1	?	?	?	?	?	?	?	?	?	?	1	2
7	*Stegolophodon*	1	0	0	0	1	2	0	2	0	2	1	0	1	0	1	2	1	0	?	1	1	?	?	?	?	?	?	?	?	?	?	1	2
8	*Stegodon*	2	0	0	0	1	2	0	2	0	2	1	0	1	?	1	2	1	?	2	1	1	1	?	0	?	1	?	1	?	1	2	1	2
9	*Stegotetrabelodon*	2	1	0	0	1	?	?	?	?	?	?	0	1	?	1	?	1	?	1	1	1	?	?	?	?	1	?	?	?	?	?	1	2
10	*Stegodibelodon*	2	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	2	1	?	?	?	?	?	?	?	?	?	?	?	1	2
11	*Primelephas*	2	1	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	2
12	*Loxodonta*	2	1	0	0	1	2	0	2	0	2	1	0	1	0	1	1	1	0	2	1	1	1	1	0	?	1	1	1	?	1	2	1	2
13	*Elephas*	2	1	0	0	1	2	1	2	1	2	1	0	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	1	2
14	*Mammuthus*	2	1	0	0	1	2	1	2	1	2	1	0	1	1	1	?	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	1	2

		67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
		K1	K2	K6	K15	K18	K19	K20	K21	K22	K25	K27	K30	K31	K32	K33	K34	V	T&D	T&D	T&D	T&D	T&D	T&D	T&D	T&D	T&D	T&D	T&D	T&D
1	*Phiomia*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	*Mammut*	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3	*Gomphotherium*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	?	0	0	0	0	0	0	0	0	1	0	0	0	0
4	*Tetralophodon*	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0
5	*Anancus*	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	0	1	0
6	*Paratetralophodon*	1	0	?	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	?	0	?	?	1	0	0	?	0
7	*Stegolophodon*	?	?	1	1	1	?	0	0	?	0	1	0	1	1	2	1	0	1	1	1	1	1	1	1	0	0	1	?	0
8	*Stegodon*	1	0	1	1	1	?	0	?	?	0	1	0	?	3	3	3	0	1	1	1	1	1	1	1	0	1	1	1	0
9	*Stegotetrabelodon*	1	1	1	1	1	?	0	0	0	0	?	0	1	2	3	1	0	1	0	1	1	1	?	1	1	0	1	0	0
10	*Stegodibelodon*	?	?	1	1	1	?	0	0	0	0	?	1	?	?	3	3	1	?	?	1	1	?	?	1	1	?	1	1	0
11	*Primelephas*	?	?	?	1	1	1	1	1	1	1	1	0	?	2	4	3	1	?	1	1	1	?	?	?	1	?	1	0	0
12	*Loxodonta*	1	1	1	1	1	1	1	0	0	1	1	1	1	3	4	4	1	1	1	1	1	1	1	1	1	0	1	1	1
13	*Elephas*	1	1	1	1	1	1	1	1	1	1	1	1	?	3	4	4	1	1	1	1	1	1	1	1	1	0	1	1	1
14	*Mammuthus*	1	1	1	1	1	1	1	1	1	1	1	1	?	3	4	4	1	1	1	1	1	1	1	1	1	0	1	1	1

Appendix 3. Synapomorphies for clades in Figs. 1A and 1B.
Notes: *Appendix 1 provides a complete list of characters employed in this study, their primitive and derived conditions, and references consulted. Appendix 2 contains the data matrix upon which cladistic analyses were conducted. Sometimes the change of character state for a particular node is given, eg "(12:3 → 4)" means that, for this node, the synapomorphy for multistate character 12 is a change from state 3 to state 4; in binary characters the change is either from 0 → 1, or from 1 → 0 in the case of a reversal. To avoid confusion, in the listing below, sequential numbers of the characters are given first and these are followed by the original numbers. Nodes below are grouped according to those which appear on Figs 1A and 1B, and in selected cases, notes are added. Results below are for ACCTRAN option in PAUP; two asterisks (**) followed by a "/" and a number refer to differences in number of character changes in DELTRAN option (cf caption to Fig. 1A and text for explanations). To simplify this appendix, details for these changes are not given here; they can be obtained from the senior author (some changes are given in Table 4).
"A fat arrow (➜) means that the change occurs in all possible reconstructions (ie is unambiguous). A thin arrow (→) indicates that change occurs under some reconstructions but not others". Further, ➜ indicates that the direction of change is undetermined and it occurs along branches connecting outgroups to ingroups. Whereas, → indicates that the direction of change is unambiguously within the ingroups (Swofford 1993, p. 120).

Node A – Elephantiformes, ie, *Phiomia* and all taxa to the right of it: Since *Phiomia* was employed the most plesiomorphic outgroup, no characters for the node A are available; they have been discussed by Tassy and Shoshani (1988), Tassy (1996) and Shoshani (1996).

Node B – Elephantimorpha, ie, Mammutidae and all taxa to the right of it: fifteen characters – * 8(22; CI=1.0):0 ➜ 1, 9(23; CI=1.0):0 ➜ 1, 16(34; CI=1.0):2 ➜ 3, 21(42; CI=1.0):1 ➜ 2, 23(46; CI=1.0):0 ➜ 1, 34(63; CI=0.667): 0 ➜ 1, 38(67; CI=1.0):0 ➜ 1, 41(76; CI=1.0):1 ➜ 2, 48(88; CI=1.0):0 ➜ 1, 50(92; CI=1.0):0 ➜ 1, 54(98; CI=1.0):0 ➜ 1, 65(131=C40; CI=1.0):0 ➜ 1, 66(134=C55; CI=1.0):1 ➜ 2, 67(K1; CI=1.0):0 ➜ 1, and 82(K34; CI=0.75):0 ➜ 1. Note that the characters assigned for this node were coded as reversals in the output file of PAUP. This situation occurred because the characters for this node are distributed between *Phiomia* (the outgroup of Elephantoidea) and the clade Elephantoidea. We employed these characters as synapomorphies for the Elephantoidea based on the distribution of character states in Appendix 2. *In the expression "8(22; CI=1.0):0 ➜ 1", 8 is the sequential number, and 22 is the original number (see Appendix 1), and CI is the Consistency Index.

Node C – Elephantida ie, *Gomphotherium* and all taxa to the right of it: thirteen characters – 5(13; CI=1.0):0 ➜ 1, 12(27; CI=0.5):0 → 1, 17(35; CI=1.0):0 → 2, 39(73; CI=1.0):0 → 2, 43(79; CI=1.0):0 ➜ 2, 44(80; CI=1.0):0 ➜ 1, 45(82; CI=1.0):1 ➜ 0, 53(96; CI=1.0):0 ➜ 1, 55(99; CI=1.0):0 → 1, 59(106; CI=1.0):0 ➜ 1, 61(111; CI=1.0):0 ➜ 1, 81(K33; CI=1.0):0 ➜ 1, and 91(T&D19, CI=0.5):0 ➜ 1. Except for characters 12(27) and 91(T&D19) which have a consistency index (CI) of 0.5 each, the other 11 have CI of 1.0. Character 45(82) is a reversal.

Node D – Unnamed, ie, *Tetralophodon* and all taxa to the right of it: sixteen **/thirteen characters – 2(10; CI=0.500):2 ➜ 4, 3(11; CI=0.333):0 → 1, 10(24; CI=1.000):0 ➜ 1, 11(25; CI=1.000):0 ➜ 1, 13(31; CI=1.000):0 ➜ 1, 14(32; CI=1.000):2 ➜ 3, 15(33; CI=1.000):2 ➜ 3, 16(34; CI=1.000):3 ➜ 5, 18(37; CI=0.333):0 ➜ 1, 19(39; CI=1.000):1 ➜ 2, 20(40; CI=1.000):1 ➜ 2, 26(55; CI=0.500):0 ➜ 1, 64(121=C64; CI=0.500):1 → 2, 79(K31; CI=0.500):0 → 1, 80(K32; CI=0.750):0 ➜ 1, 86(T&D5; CI=1.000):0 ➜ 1. Nine of these sixteen characters have a CI of 1.0, four with CI of 0.5, one with CI of 0.75, and two with CI of 0.3.
Overall, this clade is based on solid characters (those with CI of 1.0). Similar results were observed by Tassy (1990, 1996) and Kalb *et al.* (1996). These findings corroborate the hypothesis that Stegodontidae is more closely related to gomphotheres and elephantids than to mammutids (Maglio 1973, Tobien 1988).

Node E – Unnamed, ie, *Anancus* and all taxa to the right of it: eight characters – 12(27; CI=0.500):1 → 2, 27(56; CI=1.000):0 → 1, 46(83; CI=1.000):0 ➜ 1, 52(94; CI=0.333):1 ➜ 2, 84(T&D3; CI=1.000):0 ➜ 1, 87(T&D6; CI=1.000):0 ➜ 1, 90(T&D13–4; CI=1.000):0 ➜ 1, 94 (T&D31; CI=0.333):0 ➜ 1. Five characters are with CI of 1.0, one with CI of 0.5, and two with CI of 0.3.

Node F – Unnamed, ie, *Paratetralophodon* and all taxa to the right of it: five **/three characters – 34(63; CI=0.667):1 → 2, 49(89; CI=0.500):2 → 1, 69(K6; CI=0.500):0 → 1, 85(T&D4; CI=0.500):0 → 1, 89(T&D10; CI=1.000):0 → 1.

Node G – Elephantoidea including and all taxa to the right of it (ie, Stegodontidae and Elephantidae): eleven characters – 1(7; CI=0.500):0 → 2, 26(55; CI=0.500):1 → 0, 30(59; CI=1.000):0 → 1, 31(60; CI=1.000):0 → 1, 70(K15; CI=1.000):0 → 1, 71(K18; CI=1.000):0 → 1, 72(K19; CI=1.000):0 → 1, 77(K27; CI=1.000):0 → 1, 81(K33; CI=1.000):1 → 3, 88(T&D9; CI=1.000):0 → 1, 93(T&D30; CI=1.000):0 → 1.
Character state 0 of number 26(55; absence of trefoils in molars) is a reversal of the primitive condition. Characters 30(59; adaxial and abaxial cones and their conelets are of about equal size) and 31(60; molars with laminae) are good characters with CI of 1.0. Seven other characters with CI=1.0 are given, five of Kalb *et al.* (1996) and two of Tassy and Darlu (1986).

Node G.1 – Family Stegodontidae: eight characters – 2(10; CI=0.500):4 → 2, 3(11; CI=0.333):1 → 0, 6(15; CI=0.667):2 → 1, 12(27; CI=0.500):2 → 1, 28(57; CI=1.000):0 → 1, 29(58; CI=1.000):0 → 1, 49(89; CI=0.500):1 → 2, 91(T&D19; CI=0.500):1 → 0.
Of these eight characters, numbers 28(57; accentuated brachyodont molars) and 29(58; fusion of anterior pretrite central conule and adaxial conelets) – appear to be very good synapomorphies for the Stegodontidae.
Grouping of *Stegodon* and *Stegolophodon* in Stegodontidae, although strongly defended by Saegusa (1996), and also by Shoshani (1996) and Tassy (1996), is not strongly supported, because with two evolutionary steps, *Stegodon* and *Stegolophodon* can be separated and *Stegodon* will be a sister taxon to Elephantidae clade (Test no. 2 in Table 2).

Node H – Family Elephantidae and all taxa to the right of it: three **/five characters – 35(64; CI=1.000):0 → 1, 68(K2; CI=0.500):0 → 1, 80(K32; CI=0.750): 1 → 2.
Among these three characters, number 35(64; hypsodont molars) appears to be the best. Characters 68(K2; occipital condyles recessed) and 80(K32; M_2 and M^2 with minimum five plates), although with weaker CI, are relatively good characters. The content of the Elephantidae was discussed by Maglio (1973), Tassy and Shoshani (1988), Kalb *et al.* (1996), Tassy (1996) and Todd and Roth (1996).

Node I – Unnamed, ie, *Stegodibelodon* and all taxa to the right of it: four **/three characters – 24(48; CI=1.000):1 → 0, 78(K30; CI=0.500):0 → 1, 82(K34; CI=0.750):1 → 3, 83(V; CI=1.000):0 → 1.
Character 24(48) is a reversal.

Node J – Unnamed, ie, *Primelephas* and all taxa to the right of it: six **/five characters – 18(37; CI=0.333):1 → 0, 73(K20; CI=1.000):0 → 1, 74(K21; CI=0.500):0 → 1, 75(K22; CI=0.500):0 → 1, 76(K25; CI=1.000):0 → 1, 81(K33; CI=1.000):3 → 4. Character 18(37) is a reversal.

Node K – Subfamily Elephantinae and all taxa to the right of it: five characters – 25(49; CI=1.000):0 → 1, 31(60; CI=1.000):1 → 2, 80(K32; CI=0.750):2 → 3, 82(K34; CI=0.750):3 → 4, 95(T&D32; CI=1.000):0 → 1.

Node L – *Elephas* and *Mammuthus*: five **/eight characters -- 40(74; CI=1.000):0 → 1, 42(77; CI=1.000):0 → 1, 47(85; CI=1.000):0 → 1, 51(93; CI=1.000):0 → 1, 57(101; CI=1.000):0 → 1.

Appendix 4. Proboscidean samples used in this study (after Yang *et al.* 1997b). (1) The nickname or identification of the animal is given in parentheses. (2) Museum abbreviations: WSUMNH – Wayne State University Museum of Natural History (Detroit, Michigan, USA), MNHN – Museum National d'Histoire Naturelle (Paris, France), BMNH – British Museum of Natural History (London, England, New Name: Natural History Museum), AMNH – American Museum of Natural History (New York City, New York, USA), OCC – Oakland Community College, Highland Lake Campus (Union Lake, Michigan, USA). (3) A or a – Proteinase K organic extraction method (Cooper 1994, Hagelberg 1994),

B or b – Glass bead extraction method (Höss and Pääbo 1993, Cano and Poinar 1993), C or c – CTAB extraction method (Doyle and Doyle 1987, Golenberg 1994). Capital letter indicates success in obtaining amplifiable endogeneous DNA; whereas small letter represents failure. (4) Other references are in Yang *et al.* (1997b). (5) The putative intergeneric hybrid between the two modern elephants, *Loxodonta* and *Elephas*, had an Asian elephant mother. The male elephant calf died ten days after his birth on July 11, 1978 at the Chester Zoo in England. The preserved specimen was prepared by a private company for the museum and the chemical preservatives used to treat the specimen were not revealed.

Taxon (1) sample number and name	Nature of samples	Sample storage (2) and label number in the museum	Age (radiocarbon date in years before present) and origin of sample	Extraction methods tested (3)	References (4)
EL#1. *Elephas maximus* (Iki)	Skin preserved in salt	WSUMNH	(1), died in 1980	a, B, C	Shoshani *et al.* 1982
EL#2. *Mammuthus primigenius* (Lyakhovskiy mammoth)	Air dried skin from frozen specimen	MNHN	(>46,000), Lyakhovskiy Island, Siberian Arctic, Russia	a, b, C	J. Shoshani *et al.* unpubl. data
EL#3. A hybrid between the two extant elephant genera (4, 5) (Motty)	Skin preserved in unknown chemical preservatives	BMNH	(16), died in 1978	a, B, C	Lowenstein and Shoshani 1996
EL#4. *Loxodonta africana* (Mtoto)	Skin preserved in salt	Brookfield Zoo Brookfield, Illinois, USA	(2), died in 1992	A, B, C	J. Shoshani, unpubl. data
EL#5. *Elephas maximus* (Iki)	Air dried muscle	WSUMNH	(14), died in 1980	a, B, C	Shoshani *et al.* 1982
EL#6. *Mammut americanum* (Shelton)	Cranial fragment	WSUMNH	(12,320), Oakland County, Michigan, USA	a, b, c	Shoshani *et al.* 1989
EL#19. *Mammuthus primigenius* (Alaska mammoth)	Cranial fragment	AMNH #FAM A 2001-D	(20,000), Cripple Creek, Alaska, USA	A, b, c	J. P. Alexander, pers. comm. 1994
EL#23. *Mammut americanum* (Elmer)	Rib fragment	OCC	(10,200), Oakland County, Michigan, USA	a, b, C	Shoshani *et al.* 1985a
EL#32. *Mammut americanum* (Elmer)	Rib fragment	OCC	(10,200), Oakland County, Michigan, USA	a, b, C	Shoshani *et al.* 1985a
EL#29. *Mammut americanum* (not named)	Cranial fragment	WSUMNH	Late Pleistocene, radiocarbon date is not available	a, b, c	J. Shoshani, unpubl. data