

## Molecular phylogenetic inference from saber-toothed cat fossils of Rancho La Brea

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**ABSTRACT** A method for the successful extraction of sequestered cellular DNA from 14,000-year-old fossil bones was developed and applied to asphalt-preserved specimens of the extinct saber-toothed cat, *Smilodon fatalis*. Two distinct gene segments, the mitochondrial gene for 12S rRNA and nuclear *FLA-I* (the feline class I major histocompatibility complex gene), from three different individual fossil specimens were cloned and sequenced after PCR amplification. Comparison of fossil-derived DNA sequences to homologous regions in 15 living carnivorous species, including 9 species of Felidae and 6 nonfelids, affirmed the phylogenetic placement of *Smilodon* within the modern radiation of Felidae distinct from the Miocene paleofelid (Nimravidae) saber-toothed "cat" species. These results raise the prospect of obtaining genetically informative DNA from preserved bones of extinct fossil species, particularly among the 2 million specimens excavated from the asphaltic sediments at Rancho La Brea in metropolitan Los Angeles.

The asphalt deposits of Rancho La Brea in Los Angeles have provided one of the world's richest assemblages of late Pleistocene fossils (1, 2). Collections from over 100 local excavations since the early 1900s have yielded nearly 2 million fossils representing some 465 animal and 159 plant species. Particularly notable is the recovery of over 1 million mammal fossils representing 59 different species, 23 of which are extinct. The cache of large Pleistocene mammals at Rancho La Brea ranges in age from 10,000 to 38,000 years and includes such extinct species as the giant ground sloths (3 spp), dire wolf, tapir, camel, peccary, Colombian mammoth, mastodon, American lion, and the California saber-toothed cat. Anatomical studies and reconstruction of the extinct fossils from the tar pits have provided extensive insight into the faunal ecology of what is now termed the "Rancholabrean Land Mammal Age" and refers to the interval from 10,000 to about 400,000 years before the present in North America (1–7).

Among the large mammalian species in the tar pits, the saber-toothed cat, *Smilodon fatalis* (formerly *Smilodon californicus*) has been the most provocative. The second most common mammal recovered from the asphalt (dire wolf is first), *Smilodon* was the size of the modern African lion (5, 6). Its muscular build and powerful limbs suggest that this saber-toothed cat used cunning and ambush rather than speed to capture relatively large prey (7). The function of the spectacular upper canine teeth still remains an issue of debate. Their knife-like shape combined with powerful jaw muscles suggest the canines could have been used to stab or slash prey animals (7) or even to slice open the soft underbelly of its prey (5, 6). The phylogenetic placement of the saber-

toothed cats has been controversial, largely because of the occurrence of blade-like canine teeth in a variety of extinct fossil carnivore taxa (3, 8–16). It is now generally agreed that the saber-toothed adaptation evolved independently at least twice in carnivorans (members of Carnivora order): first in the extinct Nimravidae (paleofelid) family that was prevalent during the Oligocene and again in the Felidae (neofelid or true cats) radiation that was antecedent to the 37 modern cat species (3, 8–11). Several synapomorphic (shared-derived) cranial characters support the alignment of *Smilodon* with the neofelids but do not resolve whether *Smilodon* represents an early neofelid divergence or a more recent derivative of evolving lineage of the Felidae (8–16).

Recent successes in extracting ancient DNA from museum skin specimens (17–21), from bones (22) and preserved tissues in human burial sites (23–27), and from Miocene *Magnolia* specimens (28, 29) have raised the prospect of reading DNA sequences from extinct species where the organic remains have not yet been replaced. Although such DNAs are generally degraded to small fragments and are subject to chemical decay and gross microbial contamination, ancient DNA sequences have been obtained by cloning or by PCR amplification (17–29). In these cases the derived sequences can be compared to extant species for estimation of phylogenetic affiliation. Material from the Rancho La Brea tar pits was particularly alluring for DNA analysis not only because of the richness of the fossil deposits but also from the manner of fossilization. As bones grow, osteocytes become sequestered with bone layer deposition (30). Entrapment of animals in asphaltic sediments results in biodegradation of soft parts, while the bones are apparently resilient and protective of included cellular DNA even though portions of the bones do become infiltrated with tar (natural petroleum). The combination of empirical methods that extract DNA from tar and amplification of short DNA fragments with the PCR affirmed the persistence of host sequences in these materials that can be used for phylogenetic inference of the extinct *Smilodon* fossils.

### MATERIALS AND METHODS

**Smilodon Fossils.** *S. fatalis* specimens Sfa1–Sfa5 are identified by their Los Angeles County Museum numbers (and date of extraction): HC23223 and HC23224 adult proximal right humeri [Sfa1 (February 7 and May 1, 1989) and Sfa4 (February 7, 1989), respectively]; HCA854 and HCA957 adult proximal right femora [Sfa5 (June 15, 1990) and Sfa2 (May 1, 1989), respectively]; HCA1482 and HCA1486 juvenile proximal right femurs [Sfa3 (April 13, 1989) and Sfa6

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Abbreviations: EtdBr, ethidium bromide; dH<sub>2</sub>O, distilled water; MHC, major histocompatibility complex.

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(June 15, 1990, respectively]. All bones were recovered from Pit 3, grids D, E, and F, from depths of 12–17 ft in 1913 and 1914. Successful extractions were made of specimens HC23223 and HC23224, February 1989; HC23223 (second extraction), May 1989; and HCA854, May 1990.

**DNA Isolation, Amplification, and Sequence.** Methods for successful extraction, cloning, and sequencing of DNA from La Brea materials were developed empirically (31–33, 58). Forty cycles of PCR (31, 32) were performed: each cycle involved incubating at 92°C for 1 min, cooling to 50°C over 50 sec, annealing at 50°C for 1 min, heating to 72°C over 1 min, and extension at 72°C for 1 min in a programmable heat block (Perkin–Elmer/Cetus DNA Thermal Cycler). The prominent band was excised from the gel and resuspended in 1 ml of dH<sub>2</sub>O. Ten microliters of gel-purified PCR product was used in the single-strand amplification reaction mixture containing all of the reagents for double-strand amplification except that the same primers were used in a 100:1 ratio (25 pmol:0.25 pmol). Single-strand products were concentrated with Centricon 30 microconcentrators, dried in a vacuum centrifuge, and resuspended in 12  $\mu$ l of sterile dH<sub>2</sub>O. DNA samples from living species (Table 1) were extracted as described (33).

The mitochondrial DNA (mtDNA) region encoding 12S rRNA was amplified with PCR by using synthetic oligonucleotide primers whose sequence was conserved in feline, human, bovine, and mouse sequences (34, 35). Map positions (35) of the mitochondrial 12S RNA gene primers and their homologous human mtDNA are: 12S-1 (positions 1067–1092; 5'-AAACTGGGATTAGATACCCACTAT-3') and 12S-4 (positions 1253–1279; 5'-GGGTTTGCTGAAGATGGCGG-TATATAG-3'). Amplification and direct sequencing of a 132-base-pair (bp) segment of a 12S-RNA-encoding mtDNA sequence was obtained in DNA from modern felids and carnivores as well as from the fossil samples. PCR amplification of a 397-bp fragment from genomic DNA extracts was achieved by using the straight single-strand amplification method (42) with primers 12S-1 and 12S-2 (positions 1477–1497; 5'-GAGGGTGACGGCGGTGTCT-3') at a 100:1 ra-

tio. Amplification of the larger 12S RNA-encoding mtDNA segment (397 bp) that was successful in the modern cat samples was unsuccessful in *Smilodon* samples. For the dire wolf material, PCR amplification with primer 12S-3 (positions 1253–1279; 5'-CTATATACCGCCATCTTCAGCAAACC-3') was attempted unsuccessfully with 12S-2. Sequences of the major histocompatibility complex (MHC) class I primers are 5'-AATTGGATCCGACACGCAGTTCGTGCGGTT-3' and 5'-AATTGAATTCGTCTCGCTCTGGTTGTAGT-3' (36, 37).

## RESULTS

DNA extractions were attempted from six bone specimens from *S. fatalis*; three of these produced genomic sequences that we judged to be authentic based on criteria presented below. A special difficulty with Rancho La Brea samples was the removal of tar and polymerase inhibitors from the DNA prior to PCR amplification. Successful starting material was derived from inside 5-cm middyaphysis sections of long bone prepared with a hacksaw; the medullar portion of the bone was harvested with a circular file that crushed internal marrow. Tar was effectively removed from bone material without significant effect on DNA by multiple extractions with kerosine. This step produced a "yield" of 10  $\mu$ g of DNA per gram of crushed "marrow." A potent and ubiquitous inhibitor of PCR that coextracted with cellular DNA was removed by separation on acrylamide gels. These three steps combined with standard methods (31, 32, 38–40) produced genomic DNA that was successfully amplified in three of six *Smilodon* specimens (Fig. 1).

Because of the well-known ability of PCR to amplify contaminating DNA and the presumption that the majority of DNA in our sample was microbial and not mammalian, we adopted stringent protocols for preparation and evaluation of PCR products. First, all reagents were handled under sterile conditions in a separate room dedicated to fossil DNA extractions; PCR reagents were treated with UV light prior to

Table 1. Carnivorous species whose mtDNA sequence encoding 12S RNA was determined for comparison to *Smilodon*

Species	Common name	Specimens, no.	Sequences, no.	Sequence divergence from <i>Smilodon</i> , %	
Fossil					
Sfa	<i>Smilodon fatalis</i>	Saber-toothed cat	3	7	0.0–2.3
Felidae					
Ppa	<i>Panthera pardus</i>	Leopard	3	4	2.3–3.0
Pon	<i>P. onca</i>	Jaguar	2	2	1.5–3.8
Pti	<i>P. tigris</i>	Tiger	4	5	1.5–3.8
Ple	<i>P. leo</i>	Lion	11	11	1.5–2.3
Pma	<i>Pardofelis marmorata</i>	Marbled cat	1	2	0.8–2.3
Nne	<i>Neofelis nebulosa</i>	Clouded leopard	3	5	3.0–4.5
Aju	<i>Acinonyx jubatus</i>	Cheetah	6	14	0.8–2.3
Fca	<i>Felis catus</i>	Domestic cat	3	5	1.5–3.8
Lpa	<i>Leopardus pardolus</i>	Ocelot	1	2	3.8–4.5
Hyaenidae					
Ccr	<i>Crocuta crocuta</i>	Spotted hyaena	1	4	4.5–6.1
Mustelidae					
Mev	<i>Mustela eversmanni</i>	Steppe polecat	1	2	6.1–6.8
Canidae					
Cfa	<i>Canis familiaris</i>	Domestic dog	1	3	9.9–10.7
Cme	<i>Canis mesomelas</i>	Black-backed jackal	1	3	10.7–11.5
Ursidae					
Uar	<i>Ursus ursinus</i>	Sloth bear	1	3	8.3–9.8
Uth	<i>Ursus thibetanus</i>	Asiatic black bear	1	2	10.7–12.2
Primate					
Hsa	<i>Homo sapiens</i>	Human*	7	1	15.2

\*Sequence obtained from GenBank.

	1	2	4	6	8	0	1	1
	0	0	0	0	0	0	2	3
	0	0	0	0	0	0	0	2
SfaPCR	TTTATCGATTATAGAACAGG	CTCCTCTAGAGGGATGTAAA	GCACCCGCAAGTCTCTTGAG	TTTAAAGCTGTTGCTAGTAG	TTCTCTGCGGATAGTTTTG	TTTAGGGTAACATCTAAGT	TTAGGCTAAGC	
Sfa1C1;C4	.....	.....	.....	.....	.....	.....	.....	.....
Sfa1C3	.....	.....	.....	.....	.....	.....	.....	.....
Sfa1C2; Sfa4C1	.....	.....	.....	.....	.....	.....	.....	.....
Ppa	.....	.....	.....	.....	.....	.....	.....	.....
Pon	.....	.....	.....	.....	.....	.....	.....	.....
Pti	.....	.....	.....	.....	.....	.....	.....	.....
Ple	.....	.....	.....	.....	.....	.....	.....	.....
Pma	.....	.....	.....	.....	.....	.....	.....	.....
Nne	.....	.....	.....	.....	.....	.....	.....	.....
Aju	.....	.....	.....	.....	.....	.....	.....	.....
Fca	.....	.....	.....	.....	.....	.....	.....	.....
Lpa	.....	.....	.....	.....	.....	.....	.....	.....
Ccr	.....	.....	.....	.....	.....	.....	.....	.....
Mev	.....	.....	.....	.....	.....	.....	.....	.....
Cfa	.....	.....	.....	.....	.....	.....	.....	.....
Cme	.....	.....	.....	.....	.....	.....	.....	.....
Uth	.....	.....	.....	.....	.....	.....	.....	.....
Uar	.....	.....	.....	.....	.....	.....	.....	.....

FIG. 1. Nucleotide sequence of mitochondrial 12S rRNA gene region from 15 species. The complete sequence is shown for *S. fatalis*, SfaPCR, derived from asymmetric direct sequencing of PCR products amplified from three bones, Sfa-1, Sfa-2, and Sfa-4. Dashes and single letters represent identical and distinct nucleotides, respectively, from *Smilodon*. Asterisks show positions of deletions relative to *Smilodon*. Additional Sfa sequences are M13 clones derived from PCR products (Sfa1c1, Sfa1c2, etc.). Consensus sequences were obtained for four species, which are represented by several subspecies (lion, tiger, cheetah, and leopard; see Table 1 for identification of designations). In cases where subspecies variants occurred, the ancestral form was used, determined by the presence in other members of the same genus or family.

DNA amplification with a Stratagene UV-linker to crosslink any potential DNA contaminants (38). Second, two negative controls were routinely included: an H<sub>2</sub>O blank and a sample of tar from the tar pits processed identically to bone samples from the extraction stage to PCR amplification. In four experiments the tar did yield a double-stranded PCR product indicated by EtdBr staining; however, in none of these did asymmetric PCR sequencing produce a homogeneous and readable sequence. Third, DNA was successfully extracted from multiple bones at different times over a 15-month period and one bone, Sfa1, was extracted twice. Multiple PCR amplifications were carried out, and sequences were determined from both direct asymmetric sequencing and by sequencing of multiple M13 clones. Fourth, to control for contamination by DNA from other cat species, we predicted and found nonidentity of *Smilodon*-derived bone sequences with sequences from living species, including those of 29 feline species whose DNA is in this laboratory. As an added caution, we extracted DNA from the internal marrow of six asphalt-embedded fossil bones from dire wolf, *Canis dirus*; in no case were sequences recovered that were recognizable as *Smilodon*. Fifth, short (<200 bp) segments of two different genes, the mitochondrial gene for 12S RNA and nuclear *FLA-I* (the feline MHC class I gene), were amplified from *Smilodon*; longer pieces could not be amplified despite success with the same primers in all DNA of extant species (see *Materials and Methods*). Sixth, the sequences were tested and conformed to phylogenetic placement (see below). All of these measures gave results consistent with the conclusion that the derived sequences were representative of a fossil felid species' mitochondrial and nuclear genomes.

By using mitochondrial 12S RNA gene primers, 30 of 60 attempted PCR amplifications from five of six *Smilodon* bones produced a double-stranded DNA product of the expected size (132 bp) visualized by EtdBr. Of these 30, 7 taken from three bones (designated Sfa1, Sfa4, and Sfa5) produced identical DNA sequences (designated SfaPCR in Fig. 1) in the unbalanced primer method for DNA sequencing of the amplified product (41, 42); the rest produced unreadable sequences that indicated mixtures of sequences or nonspecific priming. PCR products from amplifications of two bone samples (Sfa1 and Sfa4) were cloned in M13, and five clones were sequenced (43, 44). Each was recognizable as carnivore mtDNA encoding 12S RNA. The PCR sequence from asymmetric sequencing and three unique *Smilodon*-derived sequences (from five M13 clones) are presented in

Fig. 1. The five *Smilodon* sequences derived from Sfa1 and Sfa4 differed from each other by 1- to 3-bp substitutions and were different from the homologous sequences of 14 extant carnivore species (including 29 cats) similarly amplified and sequenced (Table 1 and Fig. 1). The *Smilodon* mtDNA sequence for 12S RNA showed a high degree of sequence similarity to corresponding sequences from 14 carnivore species with divergences ranging from 0.8% to 12.2% (Table 1). The repeated verification of nearly identical mtDNA sequences for 12S RNA derived from multiple extractions of *Smilodon* bones over an 18-month period (Fig. 1), the phy-

		37		83		Sfa1-2a		Sfa1-2b	
<i>Smilodon</i>	Sfa1-2a	DSDAPNPREE	PRAPMEEQEG	PEYWDNRTRI	YLDTAQISRVS	NLMTLLR	0.0	19.1	
	Sfa1-2b	.....	.....	.....	EA-K VKN	---F- D	19.1	0.0	
Cat	FLA24	.....	.....	.....	.....	---FR-	1.4	10.5	
	FLA28	.....	.....	.....	.....	---F-L -Q-A-	6.3	14.0	
	FLA10	.....	.....	.....	.....	---F- D--M-	2.8	9.1	
	FLA10	.....	.....	.....	.....	---E-K VKN	---F- D	8.4	0.7
	FLA1	.....	M	V	.....	E-N MKN	---F- D-M-	12.6	6.3
	FLA89	.....	V-Y	.....	.....	F-S-ENTRG	9.1	16.8	
Dog	D1a29	---ATG-M	---V	---Q-T	IKE--RTF-	D-D--RG	20.3	18.2	
Bear	Uar1	---GAS--A-	L--L-L	---F--MK-QN	TRTCTH-L-G	R--EVRG	25.2	24.5	
Mouse	H2Kb	---E--Y	---R	---E-E-QK	AKGNE-SF-	D-R--G	23.1	19.6	
	H2Dp	---E--MK	---VR	---EQE-QN	AK--HE-SF-	S-RN--G	25.2	23.1	
Human	HLA2	---ASQ-M	---I	---GE-K	VKAHQSTH-	D-G--RG	25.2	20.2	
	HLA86B	---ASQ-M	---I	---N	VKAQS-TD-	D-G--RG	22.4	21.0	
	HLA27K	---AS--	---I	---E-Q	CKAK--TDRE	---RIA-	19.6	21.0	
Chimpanzee	Ch1a126	---ASQ-M	---I	---EE--S	VKAS--TD-	D-G--RG	23.8	20.3	

FIG. 2. Deduced amino acid sequence of positions 37-83 of the  $\alpha 1$  coding domain of *FLA-I*. Index sequence is from Sfa1-2a for which five M13 clones gave identical sequence. The second sequence was obtained from two identical clones. Both were derived from a single sample, Sfa1. The *FLA* sequences are domestic cat allele transcripts (36, 37). Dog and noncarnivore sequences are from GenBank. We attempted PCR reactions to produce longer  $\alpha 1$  domain coding sequences from the fossil DNA using the 5' primer 5'-CTCCCACTCCCTGAGGTATT-3' and the same 3' primer as the first successful PCR reaction. All DNA samples from the cheetah, lion, and domestic cat yielded 228-bp products, but no PCR products were observed with the fossil DNA. The large sequence variation of *FLA-I* transcripts within domestic cat species is typical of the  $\alpha 1$  domain, which codes for a region of the MHC class I molecule that interacts with antigen in presentation to T cells (36, 37). As in primates (45-47), a large majority of sequence motifs in this region in cats is conserved from historic polymorphisms that predate species development (N.Y. and S.J.O'B., unpublished data). Recognition of the polymorphic motifs that are present in both cat and *Smilodon* *FLA-I* sequences (as compared with dog or bear) reinforce the felid origin of the *Smilodon* sequences.

logenetic similarity but nonidentity to DNAs from extant felid species (Table 1), and our inability to amplify longer 12S-RNA-encoding segments in fossil DNA support the conclusion that the amplified sequences were derived from *Smilodon* genetic material.

To extend the evidence that the *Smilodon* mtDNA sequences were authentic, we attempted to amplify fossil DNA with oligonucleotide primers homologous to the constant portion of the  $\alpha 1$  domain of the *FLA-I* gene, the feline MHC (36, 37). *FLA* class I genes are present in approximately 20 copies per haploid genome of domestic cats (36). Twenty of 25 M13 clones produced distinct MHC 143-bp class I sequences that were identifiable as feline-specific (Fig. 2). One *Smilodon*-derived *FLA-I* sequence was very similar to the homologous sequence in two allelic transcripts of domestic cat, *FLA-A10* and *FLA-A24* (2.8 and 1.4% DNA sequence divergence respectively, Fig. 2) (37). The second sequence showed 19.1% divergence from the first but closer similarity (0.7%) to *FLA-X10*. Both *Smilodon*-derived sequences were distinct from other felid class I sequences present in our laboratory but were highly divergent from other carnivore, mouse, and primate homologous sequences (Fig. 2). The

recognition of feline-specific MHC sequences from fossil DNA extracts supports the authenticity of the sequence and the phylogenetic placement of *Smilodon* within or near the modern Felidae radiation.

The extent and patterns of mtDNA 12S RNA sequence divergence between feline and other carnivore sequences allowed the construction of evolutionary trees. Based upon the derived topologies both phenetic and phylogenetic methods, trees (Figs. 3 and 4) demonstrated that the mtDNA sequence encoding 12S RNA recapitulates Carnivora and Felidae evolutionary relationships among living species. The molecular results clearly distinguish the two Aeluroidea (cat-like) carnivore families (Hyaenidae and Felidae) from the Arctoidea (bear-like) carnivores (Canidae, Ursidae). In addition, the three principal evolutionary lineages of Felidae (viz. pantherine lineage, domestic cat lineage, and ocelot lineage) (53, 54) are reinforced by the derived topologies. The phenetic analyses clearly aligned all *Smilodon* mtDNA sequences for 12S RNA within the Felidae radiation and separated them from the Arctoid carnivores. The close similarity of *Smilodon* sequences to the great cat sequences support the placement of *Smilodon* on the pantherine lineage, although the parsimony analysis places the *Smilodon* sequence as primitive relative to the consensus minimum-length topology. Taken together, the phylogenetic analyses strongly affirm the placement of *Smilodon* within the modern Felidae and suggest a proximity with the pantherine lineage, although the lineage association is tentative at this stage.

## DISCUSSION

Several factors appear to have contributed to the recovery of fossil DNA from the Rancho La Brea specimens. First, the

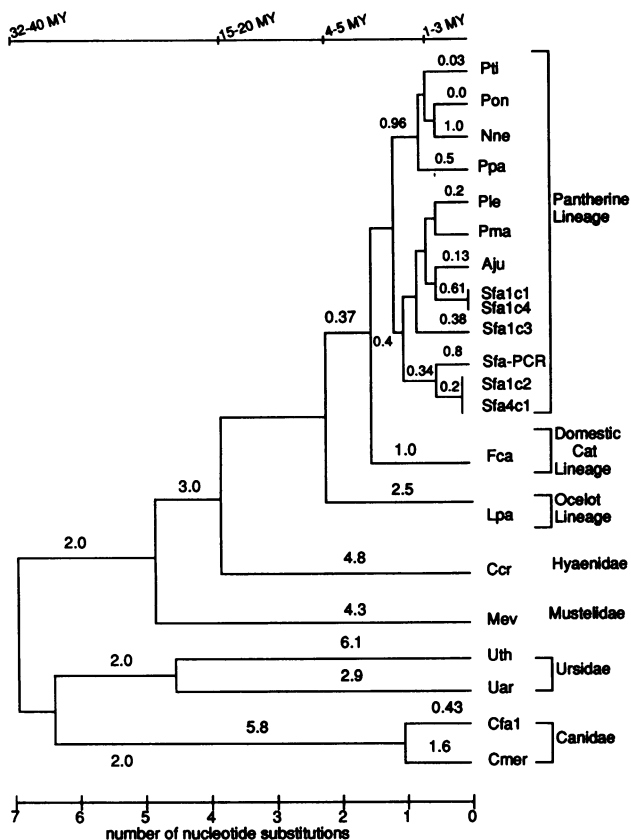


FIG. 3. Phenetic trees based on mitochondrial 12s RNA gene sequences derived from the Fitch-Margoliash algorithm (48) using the KITSCH subroutine of the PHYLIP program (49). This program computes a midpoint-rooted topology based upon the assumption of an evolutionary clock rendering all terminal species as contemporaneous. The numbers are the leg length of an unrooted tree generated by the FITCH algorithm in the absence of these assumptions. The top scale shows fossil dates for the divergent nodes of Felidae and Carnivora specimens (50). The topology was affirmed by sequence analysis of 358-bp segments of the mtDNA encoding 12s RNA from 3-5 individuals from each of 17 living felid species and 5 nonfelid carnivores; 98 residues were variable. The derived topology with both segments were very similar, with differences primarily in limb lengths, supporting the efficacy of using the homologous 132-bp region for tree construction. The UPGMA tree derived from PILEUP of the Genetics Computer Group program of the University of Wisconsin (51) was topologically equivalent.

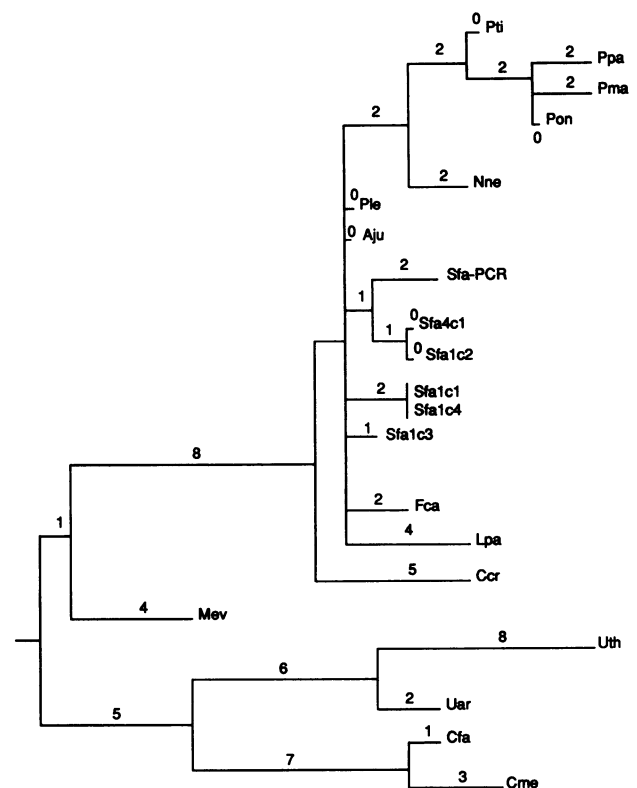


FIG. 4. Phylogenetic tree derived from mitochondrial 12s-RNA gene data using PAUP version 2.3 (52). A strict consensus tree is presented based upon midpoint rooting. Scale and leg lengths are in nucleotide substitutions. Topologically equivalent trees were derived when any or all arctoid carnivores are designated as outgroups. The presented tree has a length of 80 changes and an overall consistence index of 0.637, indicating a 36% convergence level.

preservation of bones in an anaerobic asphalt environment for millennia apparently has provided a chemically inert medium that effectively prevented consumptive degradation of DNA deposited during bone growth (1, 30). Second, the success of empirical trials to remove the ubiquitous tar and potent inhibitors of PCR were critical developments. Third, the relative abundance of mtDNA and the nuclear *FLA-I* genes relative to single-copy sequences likely contributed to our detection. It is not clear, however, that single-copy nuclear DNA segments may not be amenable to detection and amplification as well, particularly if DNA is extracted directly at the excavation site (28).

The results presented here illustrate both the strengths and limitations of sequence analysis in phylogenetic inference of fossil materials. Although the mitochondrial 12S RNA gene segments clearly placed *Smilodon* within the Felidae radiation, they could not resolve it further because of the slowness of evolutionary divergence of the region. The *FLA* sequences affirm the phylogenetic placement of *Smilodon* in the Felidae, but in the absence of comparative data from other Felidae species sequences, they do not increase the precision of this conclusion. Clearly, the selection of DNA sequences that diverge at a rate that is diagnostic in the period of species divergence is very important for precise phylogenetic placement in a multi-typic species radiation (50).

Of six fossil *Smilodon* bones processed with the present protocol, DNA from five yielded double-stranded PCR products; three were sequenced, and two were cloned in M13. This rate of success held promise in analysis of 59 fossil mammals plus the plethora of other nonmammal fossils represented in the Rancho La Brea collections. The materials, particularly the large species likely to have protected DNA, should now be amenable not only to phylogenetic analysis but also to population genetic approaches that may increase our understanding of the incipient extinction of modern species (21, 55). Finally, the molecular resolution of extinct species' genomes raises the hope of discovering infectious agents, endogenous viruses, and pathogens that might have played a regulatory role in historic ecosystems (56, 57).

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