J Mol Evol (1997) 44(Suppl 1):S98–S116

JOURNAL OF MOLECULAR EVOLUTION

Phylogenetic Reconstruction of the Felidae Using 16S rRNA and NADH-5 Mitochondrial Genes

Warren E. Johnson, Stephen J. O'Brien*

Laboratory of Genomic Diversity, National Cancer Institute, Building 560, Room 21-105, Frederick, MD 21702-1201, USA

Received: 28 June 1996 / Accepted: 29 August 1996

Abstract. The Felidae family represents a challenge for molecular phylogenetic reconstruction because it consists of 38 living species that evolved from a relatively recent common ancestor (10–15 million years ago). We have determined mitochondrial DNA sequences from two genes that evolve at relatively rapid evolutionary rates, 16S rRNA (379 bp) and NADH dehydrogenase subunit 5 (NADH-5, 318 bp), from multiple individuals of 35 species. Based on separate and combined gene analyses using minimum evolution, maximum parsimony, and maximum likelihood phylogenetic methods, we recognized eight significant clusters or species clades that likely reflect separate monophyletic evolutionary radiations in the history of this family. The clusters include (1) ocelot lineage, (2) domestic cat lineage, (3) *Panthera* genus, (4) puma group, (5) *Lynx* genus, (6) Asian leopard cat group, (7) caracal group, and (8) bay cat group. The results confirm and extend previously hypothesized associations in most cases, but in others, e.g., the bay cat group, suggest novel phylogenetic relationships. The results are compared and evaluated with molecular, cytogenetic, and morphological data to derive a phylogenetic synthesis of field evolutionary history.

Key words: Phylogeny — 16S rRNA — NADH dehydrogenase subunit 5 (NADH-5) — Mitochondrial sequence data — Felidae

Introduction

The evolutionary history of the Felidae has been marked by rapid speciation and extinction rates. The felid fossil record suggests the occurrence of numerous radiations of apparently now-extinct groups during the last 40 million years (Martin 1989). Modern fields continue to reflect this evolutionary pattern. Although extant felids last shared a common ancestor 10–15 million years ago (MYA) (Martin 1980; Collier and O'Brien 1985; Savage and Russell 1983; Werdelin 1985; Hunt 1996), fossil and molecular data indicate that modern-day cat species evolved more recently. The oldest fossil records of modern cat species are only 3–5 million years old and some first appear <100,000 years ago (Clutton-Brock 1987; Kurten 1967; Savage and Russell 1983; Turner 1985, 1987; Van Valkenburgh et al. 1990). As has been found between human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), and gorilla (*Gorilla gorilla*) (e.g., Sibley and Ahlquist 1987), felid evolution has occurred too recently for large numbers of ancestral polymorphisms to sort the 38 living cat species into reciprocally monophyletic lineages (Avise 1994; Neigel and Avise 1986; Wu 1991).

The phylogenetic relationships among the Felidae have been addressed by several studies that employed both morphological and molecular techniques. Early efforts included comparative karyology (Modi and O'Brien 1988; Wurster-Hill and Centerwall 1982), the genomic occurrence of two felid endogenous retroviruses (Benveniste and Todaro 1974; Benveniste et al. 1975; Reeves and O'Brien 1984), albumin immunological distance (Collier and O'Brien 1985), comparative morphology (Salles 1992), allozyme electrophoresis

*With assistance of collaborators listed in Table 1.

Correspondence to: W.E. Johnson; e-mail johnsonw@fcrfv2.ncifcrf.gov

(O'Brien et al. 1987; Pecon Slattery et al. 1994), and two-dimensional protein electrophoresis (Pecon Slattery et al. 1994). More recently, efforts to resolve phylogenetic relationships have focused on the mitochondrial genome (Janczweski et al. 1995; Johnson et al. 1996; Lopez et al. 1994; Masuda et al. in press).

These molecular genetic studies support the hypothesis that the 38 field species can be divided into three major phylogenetic lineages (Ewer 1973; Hemmer 1978; Leyhausen 1979; Neff 1982; Collier and O'Brien 1985; Nowak 1991). The first group, the ocelot lineage, diverged 10–12 MYA and led to seven species of small spotted cats of Central and South America. The second group, the domestic cat lineage, diverged 8–10 MYA and resulted in six species of small cats which are related to domestic cat and originated from the Mediterranean region. Somewhat more recently, the felid species of the Pantherine lineage emerged, including the great cats of the *Panthera* genus and many medium-sized species such as puma (*Puma concolor*), cheetah (*Acinonyx jubatus*), and caracal (*Caracal caracal*). The molecular data have suggested certain associations below the three primary lineages that require confirmation but also have left a variety of unresolved or conflicting associations among species of each felid lineage (Janczweski et al. 1995; Johnson et al. 1996; Masuda et al. in press).

In the present work, we examine mitochondrial DNA sequence divergence from multiple individuals from each of 35 felid species. Samples from the three missing cat species, Andean mountain cat (*Oreailurus jacobita*), Chinese desert cat (*Felis bieti*), and Iriomote cat (*Felis iriomotensis*), were unavailable because they are extremely rare and have very reduced distributions. We expand on earlier efforts to resolve cat phylogeny by analyzing sequences from segments of 16S rRNA and NADH dehydrogenase subunit 5 (NADH-5) mitochondrial genes. These genes exhibit relatively high rates of mutation in carnivores (Lopez et al. submitted) and had not previously been used in studies of felid evolution. We also build on earlier efforts by analyzing samples from a larger number of species and individuals.

Materials and Methods

Sample Collection and Preparation. Samples were obtained from one to five individuals of 35 felid species (Table 1). When possible, individuals were chosen from different parts of the geographic distribution of each species. Spotted hyena (*Crocuta crocuta*), a hyaenid, and ringtailed mongoose (*Galidia elegans*), a viverrid, were used as outgroups. Total genomic DNA was extracted from either frozen leukocytes, primary fibroblast cultures from skin biopsies, or from frozen organs (liver, kidney, or ovary) following standard methods described in Modi et al. (1987) and Sambrook et al. (1989).

PCR Amplification. Nucleotide sequences were obtained by PCR amplification of genomic DNA (Engelke et al. 1988; Saiki et al. 1985) using 16S rRNA universal primers (Hoezel and Green 1992) and NADH-5 primers developed by Melanie Culver (Johnson et al. submitted), each attached with universal-tailed sequencing primers (Applied Biosystems, Inc.). For all primer sets 30 cycles of PCR were performed in a programmable heat block (Perkin-Elmer Cetus DNA Thermal Cycle); each cycle with 1-min denaturation at 92°C, 1-min annealing at 48°C, and 1-min extension at 72°C. Reactions (75 μl) were prepared using 7.5 ng genomic DNA in 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTP, 2.5 units *Thermus aquaticus* DNA polymerase, and an upper layer of mineral oil. NADH-5 sequences for lion (*Panthera leo*), tiger (*P. tigris*), jaguar (*P. onca*), leopard (*P. pardus*), clouded leopard (*Neofelis nebulosa*), and hyena were obtained by adjusting the annealing temperature to 45°C and using 2.0 mM $MgCl_2$. PCR products were checked by agarose gel electrophoresis.

Sequence and Phylogenetic Analysis. Reaction products were resuspended in 2 ml dH_2O and concentrated with Centricon 100 microconcentrators. Products were sequenced in both forward and reverse directions with a prism dye primer kit and an ABI 373A automated DNA sequencer (Applied Biosystems, Inc.) and consensus sequences (of forward and reverse sequences) were determined using Gene Sequencher computer program (Gene Codes Corporation). Alignments of the consensus sequences were obtained using the algorithm of Needleman and Wunsch (1970) with the PILEUP program of the Genetics Computer Group (University of Wisconsin) computer package and were visually confirmed. Percentage base composition, percentage codon usage, and transition-transversion ratios were estimated using the computer program MEGA (version 1.01; Kumar et al. 1993).

Genetic differences among all pairs of taxa were estimated by the number of base-pair differences between sequences and by Kimura's two-parameter model (Kimura 1980) using the DNADIST program of Phylip (version 3.5; Felsenstein 1993). NADH-5 sequences were translated to amino acids using the TRANSLATE program of the Genetics Computer Group (University of Wisconsin) computer package.

Phylogenetic trees were constructed using three methods: minimum evolution estimated by the neighbor-joining algorithm (Saitou and Nei 1987) using a distance matrix of Kimura distances, maximum parsimony (Swofford 1993), and maximum likelihood (Felsenstein 1981). These methods are based on different evolutionary assumptions and may produce different phylogenetic trees (Huelsenbeck and Hillis 1993; Felsenstein 1993; Sourdis and Nei 1988). We interpret concordance among these methods as strong support for the resulting phylogenetic patterns. Neighbor-joining and maximum likelihood analyses were conducted using the PHYLIP 3.5 computer package (Felsenstein 1993). Maximum parsimony analyses were conducted with PAUP, version 3.1.1 (Swofford 1993) with the heuristic search, accelerated transformation options. Bootstrap resampling analyses using the neighbor-joining and maximum parsimony algorithms (100 iterations) were performed to test the reliability of the data to derive consistent topologies. Bootstrap proportions >70% were considered to reflect reliable support for each node (Hillis and Bull 1993; Hillis 1995).

Because of the large range of variation in transition-transversion ratios between gene segments and among felid groups, we tested the effects of different ratios (2:1, 6:1, 10:1, and 14:1) with one individual of each species (as in Fig. 1) with both neighbor-joining and maximum parsimony analyses. Phylogenetic analyses based on NADH-5 amino acid sequences also provided less resolution than the combined sequence data. We used combined sequence analyses because results from the two mitochondrial genes were largely similar. This total evidence approach combines the phylogenetic information from both genes (697 bp) and provides additional resolution (Kluge 1989; Huelsenbeck et al. 1996).

With the species of each of the phylogenetic groups that we identified through these analyses, we estimated the maximum length of time since they last shared a common ancestor. For these calculations we used the previous calibration of feline mitochondrial gene rates which sets the rate of base-pair divergence to be 0.56% and 0.74% per million

Table 1. Common name, scientific name, geographic location, source, and personal contact for each specimen analyzed in the study

Common name	Scientific name	ID	Origin	Contact	Source
Ocelot lineage					
Ocelot	*Leopardus pardalis*	Lpa 6		Dirk van Damm	Blijdorp Zoo, Rotterdam, The Netherlands
		Lpa 10	Brazil	Peter Crawshaw	Manaos, Brazil
		Lpa 20	Costa Rica	Lilly & Werner Hagnauer	Las Pumas, Cañas, Costa Rica
		Lpa 30	Guatemala	Nini N. de Berger	Auto Safari Chapin, Guatemala
Tigrina	*Leopardus tigrina*	Lti 6		J.M. Lernould	Parc Zool. Et Bot. de Mulhouse, France
		Lti 8	Colombia	Pat Quillen	SOS Care, CA
		Lti 11	Brazil	Pat Quillen	SOS Care, CA
		Lti 13	Costa Rica	Lilly & Werner Hagnauer	Las Pumas, Cañas, Costa Rica
		Lti 14		Juan Romero	Parque Zool. de Buenos Aires, Argentina
Margay	*Leopardus wiedii*	Lwi 22	Costa Rica	Lilly & Werner Hagnauer	Las Pumas, Cañas, Costa Rica
		Lwi 35	Guatemala	Nini N. de Berger	Auto Safari Chapin, Guatemala
Pampas cat	*Lynchailurus colocolo*	Lco 4	Chile	Victor Riveros	Zoológico Nacional de Chile
		Lco 7	Argentina	Sonia Alcazar	Cordoba Zoo, Argentina
		Lco 9	Uruguay	Jose Olazarri	Parque Zoológico de Mercedes, Uruguay
		Lco 10	Chile	Juan Baschelil	Zoológico de Quilpue, Chile
Geoffroy's cat	*Oncifelis geoffrovi*	Oge 14	Argentina	Juan Romero	Parque Zool. de Buenos Aires, Argentina
		Oge 40	Uruguay	Tabare Gonzalez	Pan de Azucar Reprod. Cent., Uruguay
Kodkod	*Oncifelis guigna*	Ogu 2	Chile	Victor Riveros	Zoológico Nacional de Chile
		Ogu 3	Chile	Victor Riveros	Zoológico Nacional de Chile
Domestic cat lineage					
Domestic cat	*Felis catus*	Fca 117	US	Martin Kriete	NIH Animal Center, MD
		Fca 143	Russia	Ivan Korneev	Leningrad Zoo Park, Russia
		Fca 152	Argentina	Juan Romero	Parque Zool. de Buenos Aires, Argentina
Jungle cat	*Felis chaus*	Fch 2		Dirk van Damm	Blijdorp Zoo, Netherlands
		Fch 5		Ivan Korneev	Leningrad Zoo Park, Russia
		Fch 8	Russia	Vladimir Fainstein	Tallinn Zoo Park, Russia
African wild cat	*Felis libyca*	Fli 2	South Africa	Mitchell Bush	Kruger Park, South Africa
Sand cat	*Felis margarita*	Fma 8	Israel	Janis Ott-Joslin	Woodland Park Zoo, Seattle, WA
		Fma 10		Karen Sousman	Living Desert, Palm Desert, CA
		Fma 11		Jill Mellen	Washington Park Zoo, Portland, OR
Flack-footed cat	*Felis nigripes*	Fni 6	S. Africa	Don Janssen	San Diego Zoo, CA
		Fni 11	S. Africa	Don Janssen	San Diego Zoo, CA
		Fni 14	S. Africa	Don Janssen	San Diego Zoo, CA
European wild cat	*Felis silvestris*	Fsi 6	Dubai	Gayle Forman	ISEC, OH
		Fsi 10	East Africa	Louis diSabato	San Antonio Zoological Gardens, TX
		Fsi 14	Saudi Arabia	Don Janssen	San Diego Zoo, CA
		Fsi 25	Kazakhstan	Ivan Korneev	Leningrad Zoo Park, Russia

Table 1. Continued

Common name	Scientific name	ID	Origin	Contact	Source
Pantherine lineage					
Panthera genus					
Lion	*Panthera leo*	Ple 131	Kenya	Pieter Kat	Masai Mara, Kenya
Jaguar	*Panthera onca*	Pon 20	Panama	Anabell Herrera	Zoológico Summit, Panama
		Pon 24	Nicaragua	Victoriano Corazon	Zoológico Nacional de Nicaragua
Leopard	*Panthera pardus*	Ppa 69		R. Schildgen	Zoologischer Garten Frankfurt, Germany
		Ppa 80		Don Janssen	San Diego Zoo, CA
Tiger	*Panthera tigris*	Pti 111	Russia	Howard Quigley	Hornocker Carnivore Research Center, ID
		Pti 115	Russia	Howard Quigley	Hornocker Carnivore Research Center, ID
Snow leopard	*Panthera uncia*	Pun 11		Dan Wharton	New York Zoological Park, NY
		Pun 81		Vladimir Spitzen	Moscow Zoological Park, Russia
		Pun 83		Vladimir Fainstein	Tallin Zoological Park, Russia
Clouded leopard	*Neofelis neofelis*	Nne 27	China	Albert Lewandoski	Cleveland Metroparks Zoological Park, OH
Puma group					
Puma	*Puma concooor*	Pco 14	Florida	Melody Roelke-Parker	Florida Game and Fresh Water Fish Comm.
		Pco 333	Yellowstone	Kerry Murphy	Kornocker Wildlife Research Center, MT
Cheetah	*Acinoyx jubatus*	Aju 861	Namibia	Laurie and Dan Kraus	Cheetah Conservation Fund
		Aju 865	Namibia	Laurie and Dan Kraus	Cheetah Conservation Fund
		Aju 866	Namibia	Laurie and Dan Kraus	Cheetah Conservation Fund
Jaguarundi	*Herpailurus yagouaroundi*	Hya 1	Mexico	Dirk van Damm	Blijdorp Zoo, Netherlands
		Hya 8		Don Janssen	San Diego Zoo, CA
		Hya 12	Guatemala	Nini N. de Berger	Auto Safari Chapin, Guatemala
		Hya 16	Argentina	Juan Romero	Parque Zool. de Buenos Aires, Argentina
Lynx genus					
Bobcat	*Lynx rufus*	Lru 4		Janis Ott-Joslin	Brookfield Zoo, IL
		Lru 31	Florida	Melody Roelke-Parker	Florida Game and Fresh Water Fish Comm.
		Lru 37		Joe Maynard	Rare Feline Breeding Colony
		Lru 66	Florida	Melody Roelke-Parker	Florida Game and Fresh Water Fish Comm.
Canadian lynx	*Lynx canadensis*	Lcu 1		Mike Bleyman	CERI, NC
		Lca 2		Janis Ott-Joslin	Brookfield Zoo, IL
Siberian Lynx	*Lynx lynx*	Lly 1		Mike Bleyman	CERI, NC
		Lly 3		Mike Bleyman	CERI, NC
		Lly 6		Mike Bleyman	CERI, NC
		Lly 8		Mike Bleyman	CERI, NC
Asian leopard cat group					
Asian leopard cat	*Prionailurus bengalensis*	Phe 10		Stephen O'Brien	National Zoological Park, DC
		Pbe 12		Stephen O'Brien	National Zoological Park, DC
Flat-headed cat	*Ictailurus planiceps*	Ipl 4		Tom Meehan	Lincoln Park Zoo, IL
		Ipl 6	Malaysia	JoGayle Howard	States of Sabah and Sarawak, Malaysia
		Ipl 8	Malaysia	Leslie Johnston	Melaka Zoo, Malaysia

Table 1. Continued

Common name	Scientific name	ID	Origin	Contact	Source
Fishing cat	*Prionailurus viverrinus*	Pvi 3		Dirk van Damm	Blijdorp Zoo, Netherlands
		Pvi 10		Don Janssen	San Diego Zoo, CA
Caracal group					
Caracal	*Caracal caracal*	Cca 12		Mike Bleyman	CERI, NC
		Cca 21		Melody Roelke-Parker	Central Florida Zoo, FL
African golden	*Profelis aurata*	Pau 1		Dirk van Damm	Blijdorp Zoo, The Netherlands
Bay cat group					
Bay cat	*Pardofelis badia*	Pba 2	Sarawak	Charles Leh	Sarawak Museum, Kushing, Malaysia
Asian golden cat	*Profelis temmincki*	Pte A	(was Pbal)	Mike Bleyman	CERI, NC
		Pte 8		Joe Maynard	Rare Feline Breeding Colony, CA
		Pte 9		Joe Maynard	Rare Feline Breeding Colony, CA
		Pte 10	Malaysia	Leslie Johnston	Melaka Zoo, Malaysia
Unaligned species					
Serval	*Leptailurus serval*	Lse 17		Vivian Wilson	Chipangali Wildlife Trust, Zimbabwe
		Lse 18		Melody Roelke-Parker	Octagon Wildlife Park, Octagon, FL
		Lse 19		Vladimir Fainstein	Tallin Zoological Park, Russia
Rusty-spotted cat	*Prionailurus rubiginosa*	Pru 2		Ed Maruska	Cincinnati Zoo, OH
Pallas cat	*Otocolobus manul*	Oma 10		Lyndsay Phillips	Brookfield Zoo, IL
		Oma 13		Vladimir Spitzen	Moscow Zoological Park, Russia
		Oma 16	Russia	Vladimir Spitzen	Moscow Zoological Park, Russia
Marbled cat	*Pardofelis marmorata*	Pma 2		Tom Meehan	Lincoln Park Zoo, IL
		Pma 3	Thailand	JoGayle Howard	Khao Kheow Open Zoo, Thailand
Outgroups					
Spotted hyena	*Crocuta crocuta*	Cer 2		Lyndsay Phillips	Henry Doorly Zoo, NE
Ring-tailed mongoose	*Galidia elegans*	Gel 1		Mitchell Bush	National Zoological Park, DC

years for 16S rRNA and NADH-5 mitochondrial genes, respectively (Lopez et al. submitted). The calibration is based upon empirical measures of sequence divergence of mutational rates of mitochondrial genes compared to homologous counterpart genes included in *Numt* (such as 16S rRNA). *Numt* is a nuclear genomic fossil sequence that represents an ancient transfer and tandem amplification of 7.9 kb of mitochondrial DNA to chromosome D1 of an ancestral species of the domestic cat (*Felis catus*) (Johnson et al. 1996; Lopez et al. 1994). When the 16S and NADH-5 divergence rates are weighted by the number of base pairs used in these analyses, we estimate the composite divergence rate for 16S rRNA and NADH-5 mitochondrial genes to be 0.63% bp/MY.

Results and Discussion

The nucleotide sequence of 379 base pairs of 16S rRNA and 318 base pairs of NADH-5 genes was determined from genomic DNA of 92 individuals of 35 feline species. Homologous DNA sequences were aligned and the pattern and phylogenetic relationships of variation among species were determined.

Sequence Characteristics

For 16S rRNA sequences, 100 of 379 nucleotide sites (26.8%) were variable within the Felidae. The overall ratio of transitions to transversions among all pairs of species was 5.0 but varied widely among comparisons. Base-pair composition was 33.5% A, 23.5% T, 23.0% C, and 20.0% G. Kimura genetic distance values ranged from 0.55% between domestic cat (*Felis catus*) and African wild cat (*Felis libyca*) to 10.42% between cheetah (*Acinonyx jubatus*) and snow leopard (*Panthera uncia*). Among the Felidae there were five insertions/deletions in the alignment. Of these, one was a simple adenine repeat

which ranged in size from two (in ocelot *Leopardus pardalis*) to five (in all *Felis* species except *F. nigripes*).

For NADH-5 sequences, 164 of 318 base pairs (51.6%) were polymorphic sites within the Felidae. Forty of these were two-fold redundant and 29 were four-fold redundant. Of 105 codons, 50 (47.6%) were variable. The ratio of transitions to transversions among all pairs of species was 12.4 but varied widely among comparisons. Mean base-pair composition at all three positions was 33.8% A, 31.4% T, 27.1% C, and 7.7% G. These percentages varied depending on codon position (position 1: 40.6% A, 29.0% T, 18.7% C, 11.7% G; position 2: 20.3% A, 46.1% T, 27.1% C, 35.4% G; position 3: 40.8% A, 19.1% T, 35.4% C, 4.7% G). Similar biases against guanine in portions of the mitochondria have been described in domestic cat (Lopez et al. submitted) and other nonfelid species (Ikemura 1985; Sharp et al. 1988). Kimura genetic differences ranged from 0.1% between domestic cat and African wild cat to 21.8% between jaguar (*Panthera onca*) and caracal (*Caracal caracal*).

Initially we examined alignments of a single individual for each gene in 35 cat species plus two outgroup species (Fig. 1). A genetic distance matrix which lists the actual number of nucleotide substitutions between species for the combined NADH-5 and 16S rRNA sequences plus the Kimura distance between species (assuming a transversion-transition weight of 2.0; see Materials and methods) is presented in Table 2. The range of combined sequence divergence was from 1.02% Kimura distance (6 bp out of 697 residues) between European wild cat and domestic cat to 13.76% Kimura distance (88 bp out of 697 residues) between jaguarundi (*Herpailurus yagouaroundi*) and jaguar (*Panthera onca*) (Table 2).

Overall Felid Phylogenetic Patterns

Phylogenetic trees constructed from sequence analyses of combined fragments of 16S rRNA and NADH-5 mitochondrial genes from single individuals using three phylogenetic approaches (neighbor-joining method of minimum evolution, maximum parsimony, and maximum likelihood) are presented in Fig. 1. In general, species' sequences that grouped together in one analysis tended to cluster in the others as well, although hierarchical relationships among these groups were not well resolved. Based upon highly significant ($P < 0.01$) node resolution with maximum likelihood analysis plus high bootstrap values in both neighbor-joining and maximum parsimony analyses (see below) we recognize eight groupings of cats that will be described individually below. Two species, pallas cat (*Otocolobus manul*) and marbled cat (*Pardofelis marmorata*), did not consistently align with other species. Rusty-spotted cat (*Prionailurus rubiginosa*) and serval (*Leptailurus serval*) were weakly associated in several of the analyses, but support for aligning them was considered to be poor.

Although definition of the eight groups was comparable among the three phylogenetic methods with the combined 16S rRNA and NADH-5 sequence data, the relative relationships among the eight groups was not consistently resolved (see asterisks in Fig. 1A,B and collapsed polytomies in Fig. 1C). The lack of hierarchical resolution among the eight clades was reflected by low bootstrap values for nodes which connected the eight groups. Bootstrap percentages for these ancient nodes ranged from 0 to 41% with the minimum evolution and maximum parsimony methods and were indicated by an unresolved polytomy with the maximum likelihood analysis (Fig. 1). Previous results suggest that the ocelot lineage was the first felid lineage to emerge, followed by the domestic cat and Pantherine lineages (Collier and O'Brien 1985; Janczewski et al. 1995).

The quantity of sequence divergence within felid lineages was used to estimate their divergence date, under the assumption of a constant stochastic divergence rate. Using the maximum divergence among sequences from species within a lineage (Table 3) and the previously estimated composite rate for feline 16S rRNA and NADH-5 gene mitochondrial substitution (see Materials and Methods), we estimated the time required to accumulate the observed level of sequence divergence for each lineage and compared it to the fossil record for species within these lineages (Table 3). We discuss the findings for individual lineages below.

Ocelot Lineage

Analyses of mtDNA sequence divergence among multiple individuals of the ocelot lineage species defined three distinct subgroups, each with bootstrap support of >95% with both neighbor-joining and maximum parsimony analyses (Figs. 1, 2). The first subgroup to diverge was composed of ocelot (*Leopardus pardalis*) and margay (*L. wiedii*). Sequence variation among ocelots was relatively high. Ocelots from Central America and Mexico differed from ocelots from Brazil by 12 bp (2.65% difference). The second subgroup within the ocelot lineage consisted of Geoffroy's cat (*Oncifelis geoffroyi*) and kodkod (*O. guigna*). Finally, tigrinas (*Leopardus tigrina*) formed two distinct groups. First, tigrinas from southern Brazil were closely associated with pampas cats from Argentina and Chile. The second group consisted of a divergent group of tigrinas from Colombia and Costa Rica which formed a distinct and genetically polymorphic group. Brazilian tigrinas differed from those from Costa Rica and Colombia by 49 bp (5.68%) compared with only 7–9 bp (0.6–1.3%) difference between Brazilian tigrinas and pampas cat (Table 2). A similarly large degree of intraspecific variation was apparent among tigrina using mtDNA RFLP, although

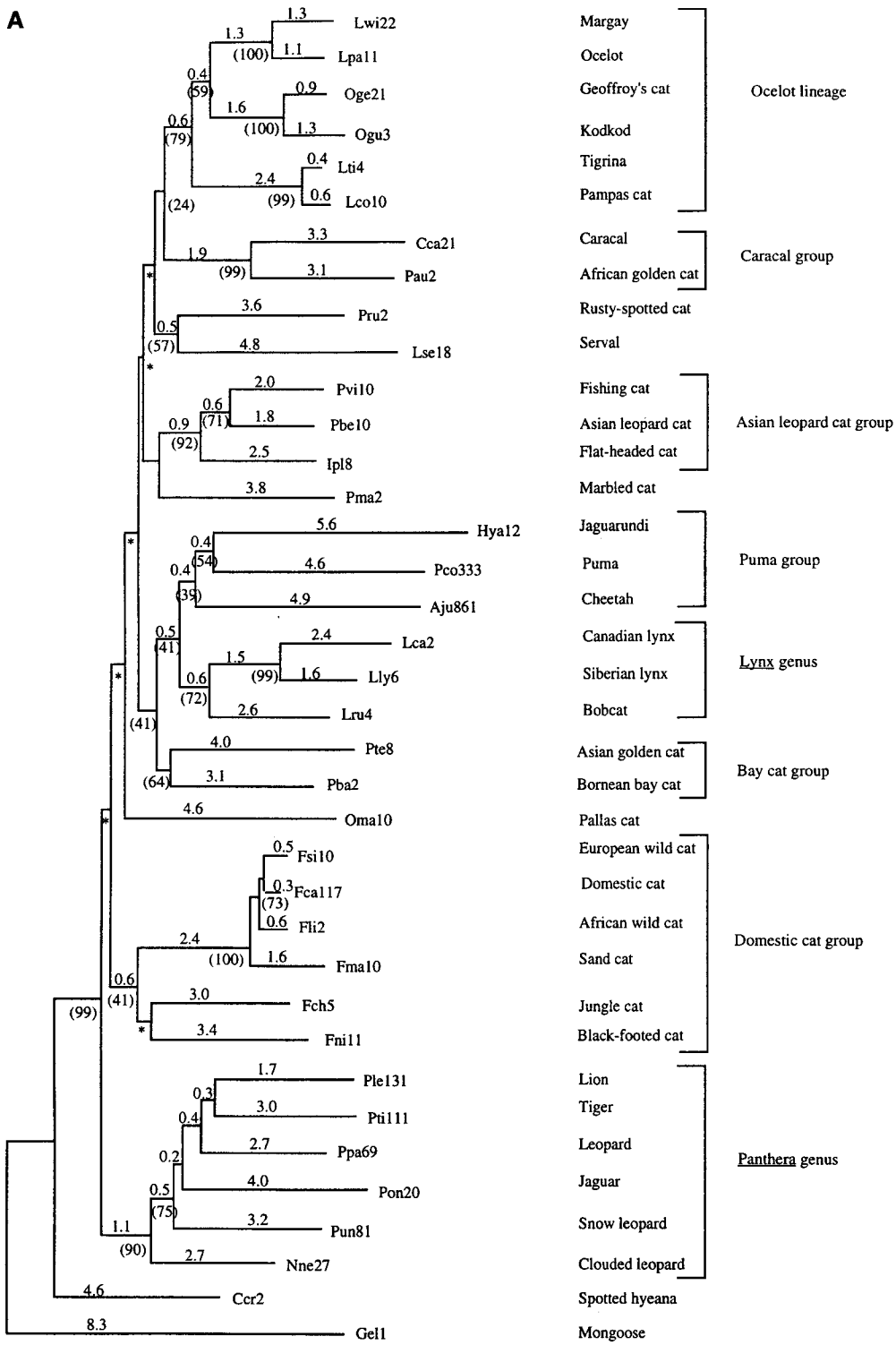

Fig. 1. Phylogenetic relationships among 35 single individuals of felid species based on analyses of combined 16S rRNA (379 bp) and NADH-5 (318 bp) mtDNA gene segments with spotted hyena and mongoose as outgroup species. Clusters represent proposed phylogenetic clades. Phylogenetic trees were constructed with **(A)** minimum evolution method estimated by neighbor-joining method with Kimura distances (*branch lengths* are above the lines and *values* from 100 bootstrap iterations are below the lines in *parentheses; asterisks* indicate topologies not supported in the bootstrap consensus tree); **(B)** maximum parsimony method (*value* above the branch corresponds to number of steps/*number* of homoplasies; percentage of 100 bootstrap iterations is below the branch in *parentheses;* One of two most-parsimonious trees, tree length = 969, CI = 0.382; *asterisks* indicate topologies not supported in the bootstrap consensus tree); and **(C)** maximum likelihood method using a subset of the species (ln = −4,853; 5,905 trees examined); nodes with confidence intervals overlapping zero were reduced to polytomies.

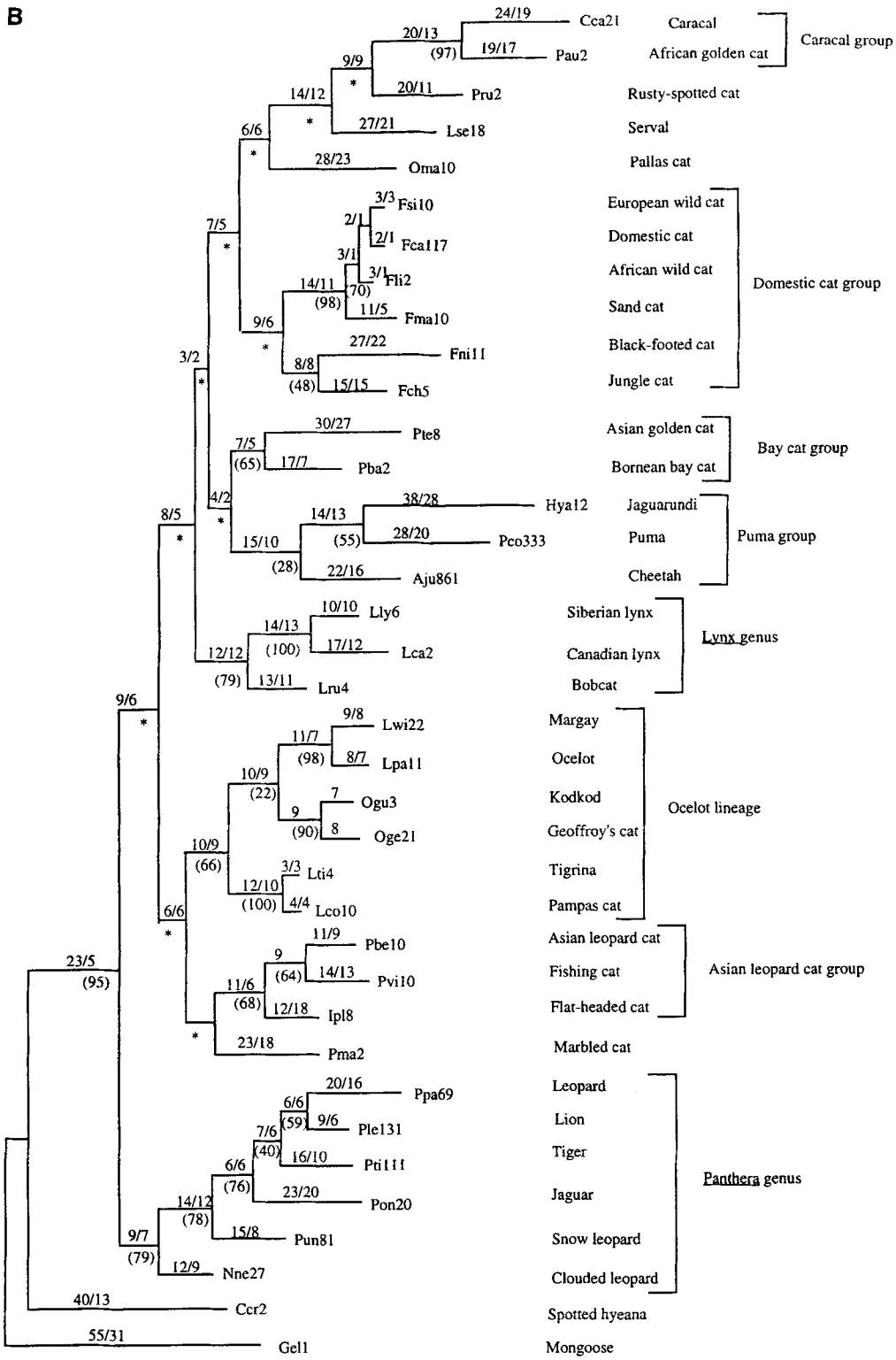

Fig. 1. Continued.

these earlier samples had imprecise geographic origins (Johnson et al. 1996). Pampas cat and Brazilian tigrina currently are distributed in southern South America and are potentially separated from the other tigrina group by the Amazon River Basin.

The observed monophyly of the ocelot lineage is consistent with analyses of cytogenetic, molecular genetic, and morphological characters (Collier and O'Brien 1985; Glass and Martin 1978; Modi and O'Brien 1988; O'Brien et al. 1987; Salles 1992). Pairings of ocelot with margay, pampas cat with tigrina, and Geoffroy's cat with kodkod were indicated by analyses of 12S rRNA and

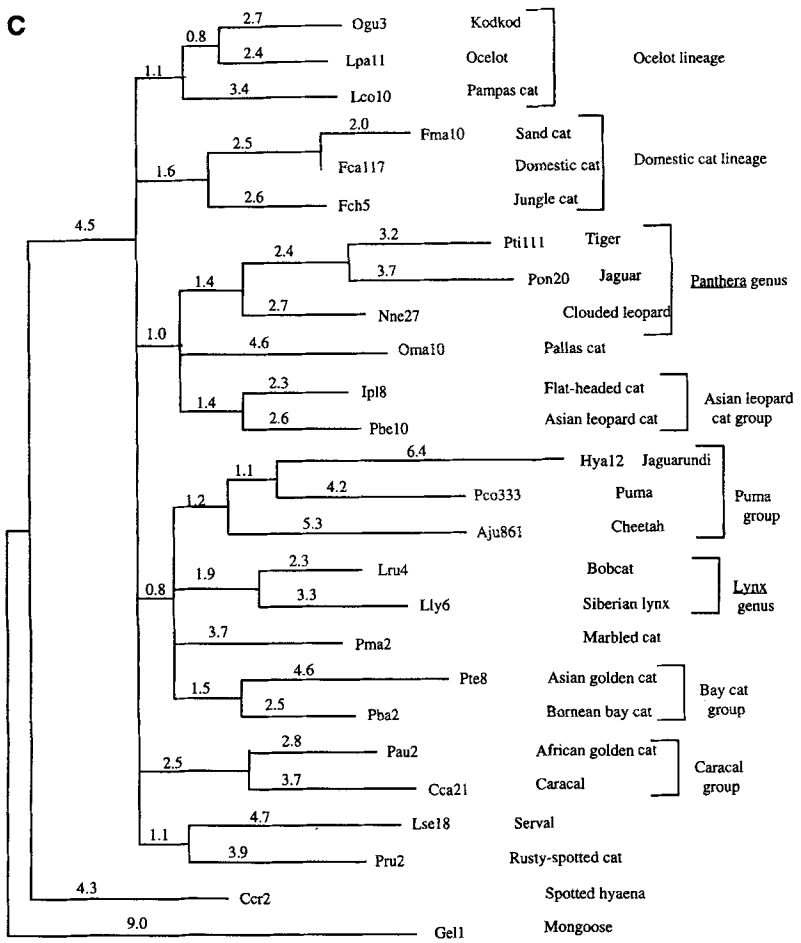

Fig. 1. Continued.

cytochrome b mtDNA sequences (Masuda et al. in press), mitochondrial RFLP variation (Johnson et al. 1996), and to a more limited extent by protein electrophoretic data (Pecon Slattery et al. 1994). However, the relative branching order and relationships among these ocelot-lineage subgroups varies with both the genetic marker examined and the phylogenetic algorithm employed.

Biogeographic evidence is relevant to the interpretation of molecular genetic relationships observed among ocelot lineage species. These felids are restricted in distribution to Central and South America (Nowak 1991), which had been devoid of placental mammals until around the formation of the Panama land bridge 3–5 MYA (Martin 1989; Patterson and Pascual 1972). Although fossils for ocelot lineage species are rare, they are also restricted to South America and southern North America (Berta 1983; Kurten and Anderson 1980). A probable ancestor of South American small cats first appears in the fossil record in North America from 4 to 5 MYA (Werdelin 1985). Our estimate that mitochondrial genes of the ocelot lineage last shared a common ancestor 5.10 MYA (Table 3) supports the supposition that these species radiated during and after the migration through the Panama land bridge.

Domestic Cat Lineage

Analyses of sequence divergence among several individuals from each species of the domestic cat lineage revealed a recently diverged group consisting of domestic cat (*Felis catus*), European wild cat (*F. silvestris*), African wild cat (*F. libyca*), and sand cat (*F. margarita*), and a more ancient association of these species with black-footed cat (*F. nigripes*) and jungle cat (*F. chaus*) (Figs. 1, 3). Our data suggest that black-footed cat was the first species of the lineage to diverge, followed by jungle cat. Inclusion of black-footed cat within the domestic cat lineage was affected by the choice of nonfelid outgroups. Black-footed cat sequences had a strong similarity to mongoose (*Galidia elegans*) and thus tended to be less closely allied with the other domestic cat species when mongoose was an outgroup species (data not shown). Bootstrap support for the clade composed of sand cat, domestic cat, and European and African wild cats was strong (100% for both neighbor-joining and maximum parsimony methods). The sand cat consistently diverged first, while domestic cat, European wild cat, and African wild cat formed a polytomy, with 1–11 bp separating the three species and only 1–6 bp separating domestic cat and European wild cat individuals

Table 2. Number of base-pair differences among 35 felid species and two outgroup species (above diagonal) and Kimura's percentage divergence (below diagonal) in combined segments of 16S rRNA (379 bp) and NADH-5 mtDNA (318 bp) genes

	Ocelot lineage						Domestic cat lineage						Panthera genus						Puma group			Lynx genus			Asian leopard cat group			Caracal group		Bay cat group		Unaligned species				Outgroup species	
	Lpa	Lwi	Oge	Ogu	Lco	Lti	Fca	Fsi	Fli	Fma	Fch	Fni	Ple	Pon	Ppa	Pti	Pun	Nne	Pco	Hya	Aju	Lca	Lly	Lru	Pbe	Pbi	Ipl	Cca	Pau	Pba	Pte	Lse	Pru	Pma	Oma	Gel	Ccr
Lpa11	-	19	40	36	45	44	52	52	54	60	51	55	55	62	58	65	61	54	72	79	67	65	58	60	50	54	54	66	62	57	64	65	57	56	54	100	75
Lwi22	2.50	-	37	35	45	43	53	53	54	59	52	54	56	63	61	62	64	52	72	77	66	66	59	61	49	53	53	64	62	55	67	60	46	57	59	100	75
Oge21	5.41	5.24	-	17	37	35	57	55	58	63	64	63	59	68	67	60	62	54	75	81	64	66	59	56	50	51	51	68	65	62	64	63	52	54	63	99	81
Ogu3	5.08	5.22	2.21	-	39	37	56	56	58	63	63	64	61	68	68	64	66	56	73	82	63	64	57	57	50	54	56	63	65	62	65	62	53	55	60	103	78
Lco10	6.48	6.62	5.09	5.69	-	8	54	54	55	61	58	65	62	71	67	68	67	54	74	77	68	61	59	57	52	55	50	58	53	55	58	60	62	57	58	107	79
Lti4	6.33	6.47	4.94	5.54	1.03	-	54	54	55	61	56	64	58	69	65	66	61	52	72	75	64	55	55	51	49	54	48	58	54	51	52	58	58	53	54	109	79
Fca117	7.45	7.74	8.09	8.22	8.06	7.92	-	6	8	16	35	52	60	74	62	70	53	48	72	71	69	66	61	53	57	56	57	64	68	54	67	66	55	62	59	98	81
Fsi10	7.61	7.90	7.92	8.38	8.22	8.07	1.02	-	8	16	41	52	60	74	62	68	51	48	74	73	71	62	61	55	59	56	59	64	66	56	67	68	53	62	61	98	81
Fli2	7.95	8.08	8.43	8.72	8.40	8.26	1.04	1.17	-	17	41	52	60	73	62	69	52	47	73	73	69	67	62	54	56	53	55	62	69	56	67	68	53	60	60	96	81
Fma10	8.91	8.88	9.22	9.53	9.37	9.22	2.21	2.35	2.51	-	48	53	65	77	67	72	54	52	73	74	74	75	70	59	60	54	61	71	75	60	69	72	55	67	63	106	85
Fch5	7.30	7.59	9.21	9.34	8.71	8.24	5.22	6.01	6.01	7.12	-	45	63	72	65	71	61	61	73	75	73	65	58	58	59	59	61	65	63	53	67	62	58	57	59	106	85
Fni11	7.94	7.92	9.04	9.52	9.66	9.52	7.42	7.56	7.60	7.74	6.32	-	60	75	53	68	62	57	75	79	80	68	63	61	56	57	63	71	65	57	66	67	57	63	62	90	80
Ple131	7.93	8.38	8.58	9.19	9.19	8.70	8.73	8.89	8.92	9.73	9.22	9.04	-	40	29	31	37	41	78	83	71	67	59	55	55	59	62	75	74	60	58	67	64	65	55	100	81
Pon20	9.05	9.51	10.03	10.32	10.64	10.48	11.00	11.17	11.04	11.70	10.69	11.48	5.98	-	51	45	50	53	85	88	77	69	69	61	66	68	63	90	81	73	77	69	71	66	69	114	99
Ppa69	8.41	9.19	9.86	10.32	9.99	9.83	9.05	9.21	9.24	10.05	9.54	7.92	4.30	7.71	-	42	45	46	85	87	82	70	58	55	60	62	67	76	71	65	64	74	67	70	66	94	89
Pti111	9.57	9.38	8.76	9.71	10.22	10.05	10.39	10.20	10.42	10.89	10.56	10.37	4.64	6.81	6.34	-	43	51	84	87	78	73	68	64	67	64	69	83	79	70	71	72	69	70	63	110	92
Pun81	8.75	9.53	8.90	9.85	9.87	9.06	7.45	7.28	7.48	7.78	8.74	9.20	5.40	7.43	6.65	6.35	-	40	86	86	79	71	71	62	61	61	61	78	81	67	70	73	69	66	62	102	89
Nne27	7.74	7.72	7.75	8.35	7.89	7.73	6.78	6.94	6.80	7.59	8.85	8.52	6.16	8.04	6.94	7.75	5.85	-	79	76	69	66	60	65	51	53	52	76	82	62	64	67	63	62	58	102	72
Pco333	10.43	10.72	10.93	10.89	10.91	10.74	10.40	10.90	10.77	10.77	10.60	10.94	11.60	12.77	12.77	12.65	12.80	11.72	-	69	65	75	69	64	72	67	71	78	79	62	73	77	71	59	74	121	98
Hya12	11.75	11.71	12.07	12.54	11.57	11.40	10.40	10.89	10.93	11.09	11.09	12.08	12.91	13.76	13.59	13.62	13.28	11.69	10.20	-	73	73	70	58	74	74	70	85	90	71	76	79	79	69	81	122	96
Aju861	9.92	10.05	9.42	9.56	10.24	9.74	10.23	10.72	10.42	11.24	10.91	12.09	10.73	11.72	12.56	11.93	11.91	10.36	9.71	11.15	-	61	58	56	65	70	67	77	76	58	67	77	74	64	74	118	91
Lca2	9.37	9.83	9.54	9.50	8.86	8.06	9.53	9.05	9.88	11.20	9.38	10.16	10.15	10.48	10.64	11.20	10.69	9.97	10.88	11.03	8.91	-	29	47	62	61	65	74	73	63	71	67	66	64	69	122	92
Lly6	8.23	8.68	8.39	8.36	8.52	8.04	8.70	8.86	9.05	10.35	8.23	9.33	8.84	10.45	8.68	10.35	10.66	8.98	10.19	10.83	8.72	4.00	-	38	57	58	62	70	65	58	65	65	57	52	68	111	81
Lru4	8.70	9.16	8.07	8.52	8.38	7.58	7.59	8.07	7.93	8.73	8.39	8.88	8.07	9.52	9.19	9.72	9.37	8.52	8.26	10.19	8.56	6.62	5.52	-	53	53	56	72	66	54	53	59	53	54	60	107	85
Pbe10	7.27	7.40	7.28	7.56	7.73	7.42	8.38	8.86	8.40	9.04	8.70	8.23	8.22	9.99	9.02	10.19	9.04	7.56	10.72	11.21	9.89	9.18	8.36	7.88	-	25	32	62	59	58	62	54	48	51	60	104	80
Pvl10	7.90	8.03	7.43	8.19	8.21	8.21	8.22	8.38	7.92	8.08	8.70	8.39	8.86	10.32	9.34	9.71	9.04	7.88	9.90	11.21	10.71	9.02	8.52	7.88	3.70	-	35	62	64	61	59	62	50	49	58	107	86
Ipl8	7.88	8.02	7.42	8.49	7.40	7.25	8.36	8.84	8.22	9.19	9.01	9.34	9.33	9.49	10.13	10.50	9.02	7.70	10.54	10.53	10.19	9.65	9.14	8.34	4.76	5.22	-	64	62	57	58	55	50	47	54	102	84
Cca21	9.53	9.34	9.70	9.18	8.38	8.40	9.19	9.36	9.06	10.52	9.36	10.51	11.00	13.49	11.16	12.37	11.35	11.13	11.57	12.91	11.39	10.68	10.32	10.81	9.02	9.02	9.32	-	43	64	75	71	65	71	71	107	86
Pau2	8.89	9.02	9.20	9.50	7.59	7.76	9.84	9.66	10.19	11.16	9.04	9.51	10.83	11.99	10.34	11.68	11.82	12.12	11.74	13.75	11.55	10.51	9.51	9.83	8.54	9.34	9.00	6.45	-	63	74	68	62	67	76	105	88
Pba2	8.11	8.09	8.90	9.21	7.93	7.45	7.63	8.11	8.14	8.78	7.47	8.40	9.05	11.17	9.86	10.74	10.07	9.36	9.10	11.02	8.75	9.34	8.83	8.06	8.55	9.05	8.39	9.39	9.23	-	48	69	62	53	61	109	89
Pte8	9.38	10.16	9.36	9.84	8.55	7.76	9.87	10.01	10.23	10.37	9.88	9.69	8.57	11.66	9.54	10.72	10.37	9.52	11.05	11.68	10.35	10.46	9.79	8.05	9.36	8.87	8.69	11.33	11.15	7.09	-	71	65	61	63	107	88
Lse18	9.55	9.03	9.23	9.36	8.89	8.73	9.71	10.19	9.73	10.88	9.07	9.89	10.02	10.35	11.17	10.88	10.86	10.00	11.74	12.21	12.06	9.87	9.83	9.03	8.07	9.36	8.22	10.68	10.18	10.56	11.03	-	57	66	70	114	91
Pru2	8.55	7.10	7.75	8.21	9.51	9.02	7.92	7.74	7.78	8.08	8.40	8.23	9.83	10.97	10.32	10.66	10.48	9.65	10.42	11.90	11.22	9.68	8.22	7.74	7.42	7.42	7.72	9.35	8.85	9.07	9.68	8.39	-	57	60	106	84
Pma2	8.07	8.52	7.76	8.20	8.38	7.90	9.02	9.19	8.89	10.02	8.23	9.20	9.68	9.84	10.49	10.53	9.70	9.16	8.73	10.51	9.84	9.31	7.70	8.17	7.57	7.25	6.93	10.63	9.98	7.89	9.31	10.17	8.69	-	60	106	81
Oma10	7.75	8.84	9.20	9.00	8.54	8.06	8.55	9.03	8.90	9.38	8.56	9.05	8.08	10.33	9.84	9.40	9.05	8.52	10.91	12.23	11.22	10.17	9.98	8.85	8.98	8.68	8.03	10.33	11.15	8.89	9.36	10.51	9.18	8.85	-	111	91
Gel1	15.51	15.64	15.15	16.17	17.02	17.23	15.23	15.41	15.11	16.90	16.70	13.72	15.43	17.90	14.39	17.30	15.98	15.75	19.24	19.56	18.90	19.24	17.39	16.89	16.31	16.87	15.96	16.70	16.34	17.03	16.89	18.22	16.91	16.71	17.40	-	83
Ccr2	11.12	11.27	12.00	11.78	12.10	11.95	12.24	12.41	12.45	13.68	12.97	11.94	12.13	15.24	13.50	14.08	13.69	10.57	14.99	14.76	14.11	13.82	12.08	12.96	12.09	13.11	12.77	13.12	13.46	13.53	13.50	14.05	12.98	12.27	13.85	12.88	-

Table 3. Maximum length of time (in million years) since species of each group last shared a common ancestor calculated using a rate of base-pair divergence of 0.63% base-pair divergence per million years for 16S rRNA and NADH-5 mitochondrial genes (see Materials and Methods section), followed by estimated minimum date derived from interpretation of available fossil evidence (NA = no date available)

Group/lineage	Number species	Number individuals	Maximum % bp difference	Maximum time	Minimum fossil date
Puma	3	9	10.40	8.25	3.0–5.0[a]
Panthera	6	11	7.55	6.00	>2.0–3.0[b]
Domestic cat	6	17	7.55	6.00	>2.0–3.0[c]
Bay cat	2	5	6.84	5.43	NA
Lynx	3	10	6.70	5.32	3.0–5.0[d]
Ocelot	6	19	6.41	5.10	4.0–5.0[d]
Caracal	2	3	6.13	4.86	>3.0–5.0[e]
Leopard cat	3	7	4.98	3.95	NA

[a] Adams (1979); Ficcarelli (1984); Turner (1987); Van Valkenburgh et al. (1990)
[b] Turner (1987)
[c] Kurten (1968)
[d] Werdelin (1985)
[e] Savage and Russell (1983); Turner (1987)

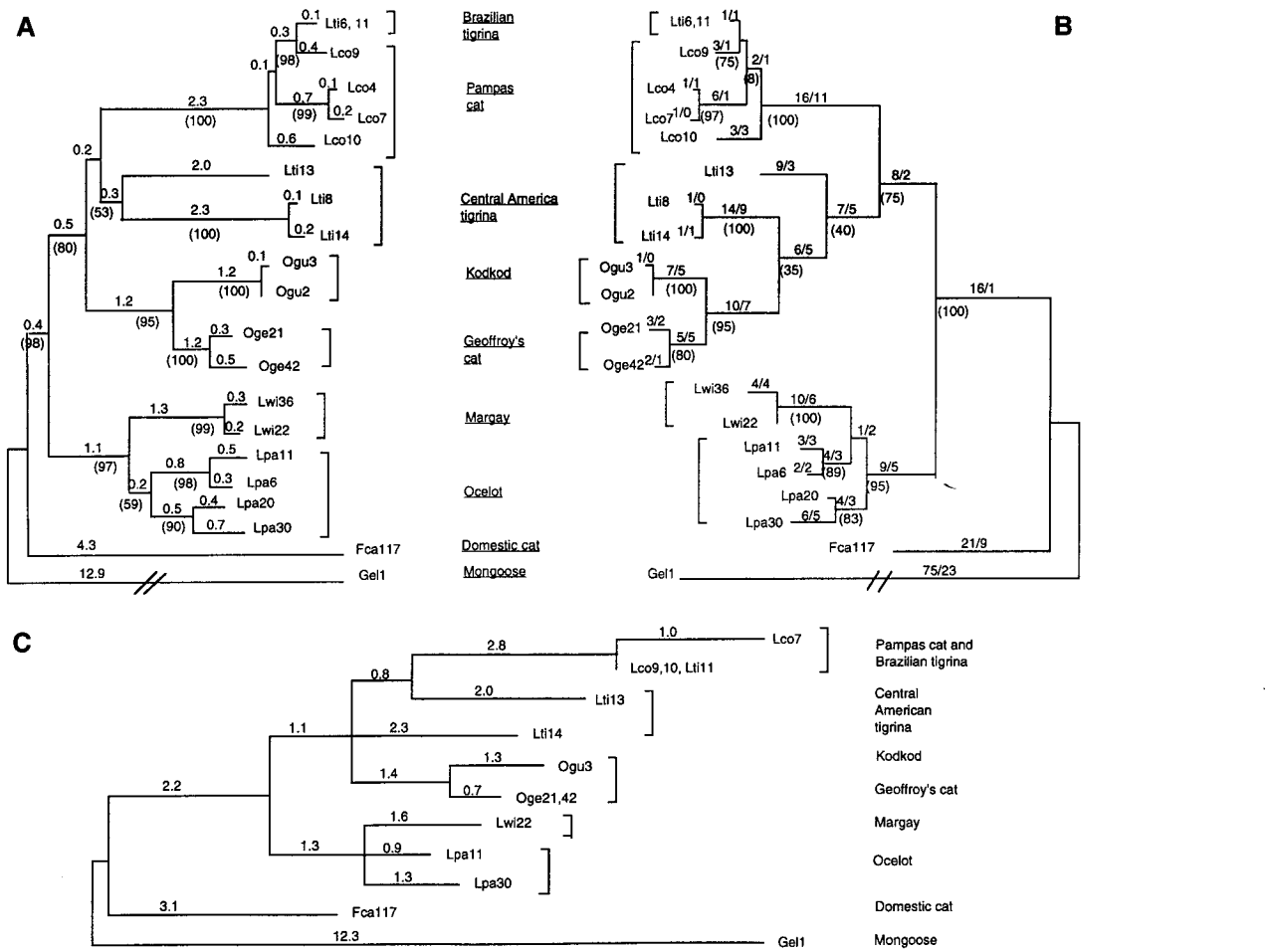

Fig. 2. Phylogenetic relationships among multiple individuals of ocelot lineage species based on analyses of combined 16S rRNA (379 bp) and NADH-5 (318 bp) mtDNA gene segments. Phylogenetic trees constructed with **(A)** minimum evolution method estimated by neighbor-joining method with Kimura distances (*branch lengths* are above the lines and *values* from 100 bootstrap iterations are below the lines in *parentheses*); **(B)** maximum parsimony method (*value* above the branch is number of steps/number of homoplasies; *percentage* of bootstrap iterations is below the branch in *parentheses;* two most-parsimonious trees, tree length = 265, CI = 0.702); and **(C)** maximum likelihood method (ln = −2,249, 1,018 trees examined).

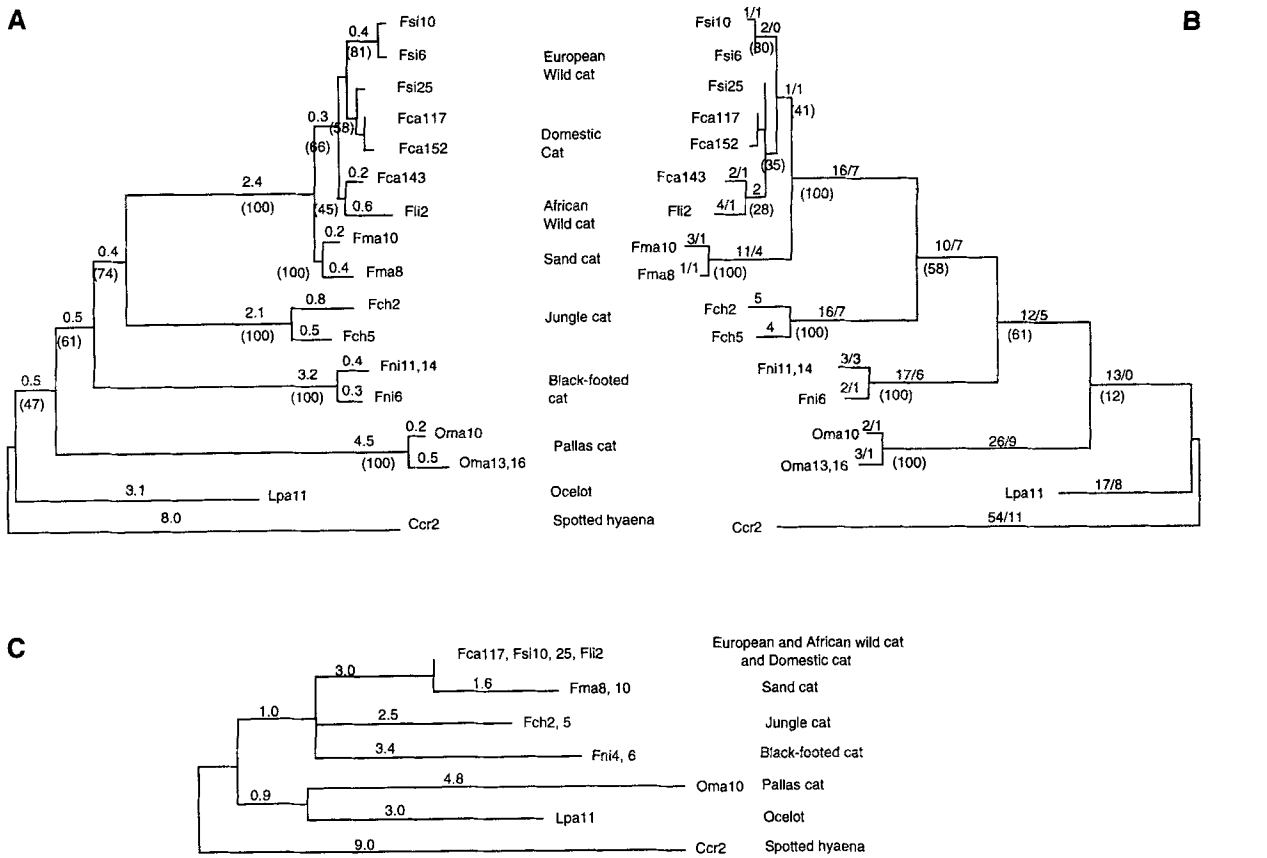

Fig. 3. Phylogenetic relationships among multiple individuals of domestic cat lineage species based on analyses of combined 16S rRNA (379 bp) and NADH-5 (318 bp) mtDNA gene segments. Phylogenetic trees constructed with (**A**) minimum evolution method estimated by neighbor-joining method with Kimura distances (branch *lengths* are above the lines and *value* from 100 bootstrap iterations is below the lines in *parentheses*); (**B**) maximum parsimony method (value above the branch corresponds to number of steps/number of homoplasies; *percentage* of 100 bootstrap iterations is below the branch in *parentheses;* one of two most-parsimonious trees, tree length = 231, CI = 0.749); and (**C**) maximum likelihood method (ln = −2,107, 597 trees examined); nodes with confidence intervals overlapping zero were reduced to polytomies.

(Table 2). The lack of resolution among the domestic cat and samples of the two wild cat species indicates very recent species radiation and may also reflect periodic hybridization and/or the domestication of cats in several places during different time periods. These results support recent suggestions to classify African wild cat and European wild cat within a single species, *Felis silvestris* (Nowak 1991; Wozencraft 1993), as well as the recommendation to subsume domestic cat into *Felis silvestris,* because domestication from *F. silvestris* occurred within the last 4,000 years (Randi and Ragni 1991).

These proposed phylogenetic patterns (Fig. 3) generally corroborate findings from other genetic studies (Collier and O'Brien 1985; Masuda et al. in press; O'Brien et al. 1987) including the presence of two endogenous retroviruses, RD114 and FeLV, in the genomes of domestic cat lineage species: *F. silvestris, F. catus, F. libyca, F. margarita, F. chaus,* and *F. nigripes* (Benveniste et al. 1975, Benveniste 1985). Another supportive cladistic genomic character is the incorporation of a tandem amplification of mtDNA on nuclear chromosome D2 in each domestic cat lineage species except black-footed cat (Johnson et al. 1996; Lopez et al. 1994, 1996).

Biogeographic evidence also supports this taxonomic hierarchy. The recent evolution of species from the domestic cat lineage is centered around the Mediterranean Basin, with black-footed cat being confined to arid parts of southern Africa and jungle cat ranging from Egypt to Thailand (Clutton-Brock 1987; Kurten 1968). Between these regions, African and European wild cats and sand cat are distributed throughout most of Africa (except in tropical rainforests), in southern Europe west to Pakistan, and in some of India and Afghanistan. We estimate that the mitochondrial genes of the domestic cat lineage last converged to a common ancestor 6.0 MYA (Table 3).

Panthera *Genus*

Bootstrap support joining combined mtDNA sequences from the five *Panthera* species and clouded leopard (*Neofelis neofelis*) was high (Figs. 1, 4). Clouded leopard was consistently the most ancient divergence of this group, followed by snow leopard (*Panthera uncia*). There was poor resolution among the other four *Panthera* species, however, as reflected by bootstrap values

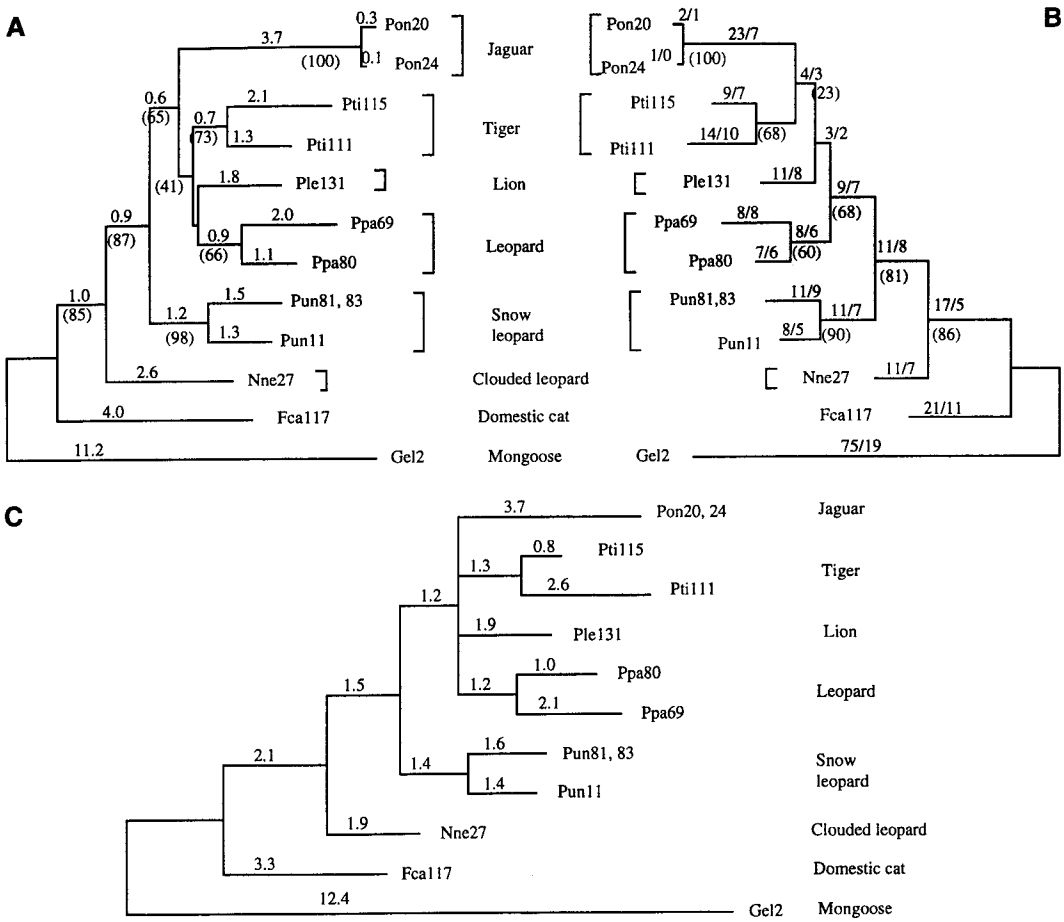

Fig. 4. Phylogenetic relationships with multiple individuals of species of the *Panthera* genus based on analyses of combined 16S rRNA (379 bp) and NADH-5 (318 bp) mtDNA gene segments. Phylogenetic trees constructed with **(A)** minimum evolution method estimated by neighbor-joining method with Kimura distances (branch *lengths* are above the lines and *percentage* of 100 bootstrap iterations is below the lines in *parentheses;* **(B)** maximum parsimony method (*value* above the branch corresponds to number of steps/number of homoplasies; *percentage* of 100 bootstrap iterations is below the branch in *parentheses;* tree length = 278, CI = 0.734); and (C) maximum likelihood method (ln = −2362, 519 trees examined).

of <70% for all internal nodes and by lack of consistency between the three phylogenetic methods. Further, associations previously suggested by mtDNA RFLP and cytochrome *b* and 12S rRNA sequence data (Janczewski et al. 1995; Johnson et al. 1996), such as between lion (*P. leo*) and leopard (*P. pardus*), were not supported with the present data.

Support for a monophyletic clade composed of *Panthera* species is abundant from morphological (Neff 1982; Peters and Hast 1994; Salles 1992) and genetic studies, but genetic support for specific relationships among *Panthera* species has been equivocal (Janczewski et al. 1995; Johnson et al. 1996; O'Brien et al. 1987). Lack of resolution among these large cats is not unexpected due to the relative recency of the *Panthera* genus divergence, whereby large portions of the observed variation results from shard ancestral polymorphisms or synplesiomorphies which are inherently uninformative. We estimate that mitochondrial gene sequences of the *Panthera* group (including clouded leopard) last shared a common ancestor 6.0 MYA (Table 3). If the fossil estimates of *Panthera* origin at 2–3 MYA are accurate (Turner 1987), the radiation was likely polyphyletic, perhaps explaining our failure to resolve the internal branch nodes with mitochondrial sequences.

Puma Group

Mitochondrial DNA sequences combined from 16S rRNA and NADH-5 identified a monophyletic grouping of puma (*Puma concolor*) with jaguarundi (*Herpailurus yagouaroundi*) and a more ancestral association with cheetah (*Acinonyx jubatus*) (Figs. 1, 5). There was a suggestion of a common ancestor between the puma group and the *Lynx* genus with the combined data sets (Figs. 1, 5, Table 4). The relatedness of cheetah and puma was strongly suggested from cytochrome *b* and 12S rRNA sequence data (Janczewski et al. 1995), although protein electrophoretic (O'Brien et al. 1987) and albumin immunological distance data (Collier and O'Brien 1985) did not reveal such an association. Al-

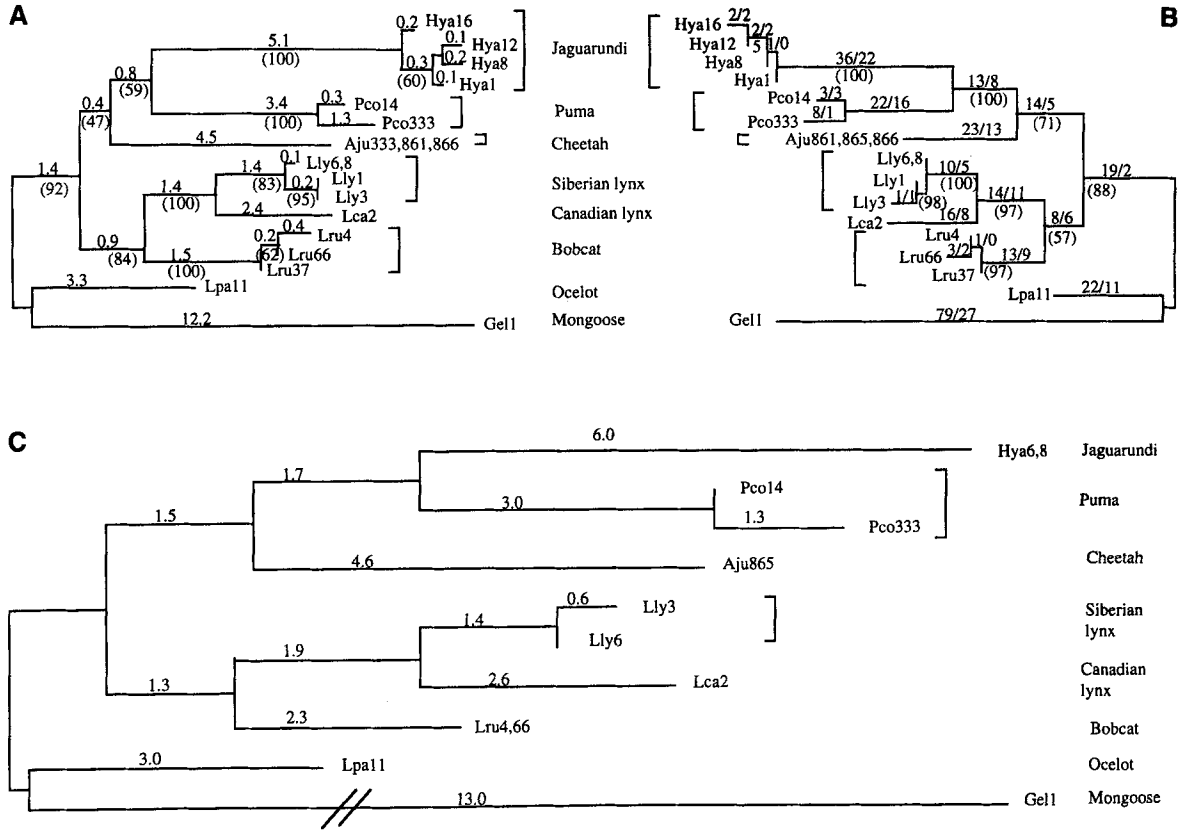

Fig. 5. Phylogenetic relationships among multiple individuals of puma group and *Lynx* group species based on analyses of combined 16S rRNA (379 bp) and NADH-5 (318 bp) mtDNA gene segments. Phylogenetic trees constructed with **(A)** minimum evolution method estimated by neighbor-joining algorithm with Kimura distances (branch *lengths* are above the lines and *percentage* of 100 bootstrap iterations is below the lines in *parentheses*); **(B)** maximum parsimony method (value above the branch corresponds to number of steps/number of homoplasies; *percentage* of 100 bootstrap iterations is below the branch in *parentheses;* two most-parsimonious *trees,* tree length = 312, CI = 0.721); and **(C)** maximum likelihood method (ln = −2,446, 370 trees examined).

though cheetahs are currently restricted to Africa and Iran, well separated from puma and jaguarundi in the Americas, North American fossil specimens (2–3 MYA) have been suggested to link these species (Adams 1979; Orr 1969; Van Valkenburgh et al. 1990). We estimate that this is a relatively ancient lineage, whose three member species last shared a common ancestor 8.25 MYA based upon mtDNA gene divergence (Table 3).

Lynx *Genus*

The *Lynx* genus analyses of 16S rRNA and NADH-5 sequences indicated that Siberian lynx (*Lynx lynx*) and Canadian lynx (L. canadensis) were sister taxa, with a more ancestral association with bobcat (*L. rufus*) (Figs. 1, 5). This genus has been widely recognized by taxonomists and morphologists (Nowak 1991). Association between bobcat and Canadian lynx was also apparent from phylogenetic analyses of cytochrome *b* and 12S rRNA sequence data (Janczewski et al. 1995). Fossil evidence suggests that *Lynx* species originated in North America (MacFadden and Galiano 1981; Martin 1989). We estimate that the *Lynx* mtDNA gene sequences coalesced to a common ancestor 6.70 MYA (Table 3).

Leopard Cat Group

Mitochondrial sequences from Asian leopard cat (*Prionailurus bengalensis*), fishing cat (*Ictailurus planiceps*), and flat-headed cat (*Ictailurus planiceps*) were closely related and formed a strongly supported clade with each of the phylogenetic methods (68–92% bootstrap support) (Figs. 1, 6). Flat-headed cat apparently diverged from a common ancestor of both leopard cat and fishing cat. The association of these three species was also corroborated by albumin immunological distance data (Collier and O'Brien 1985) and by 12S rRNA and cytochrome *b* sequence data (Masuda et al. in press), although these data suggested fishing cat diverged earlier from a common ancestor with the other two species.

There is little fossil data for Asian leopard cat, fishing cat, or flat-headed cat, but their close affinity is not surprising based on their current distributions. They are sympatric throughout much of Southeast Asia, with leopard cat having the widest distribution and flat-headed cat the most restricted. We estimated that mtDNA sequences of these three species diverged from a common ancestor only 3.95 MYA, making it one of the most recent felid lineages (Table 3).

Table 4. Summary of support for the phylogenetic groupings of felid species in Table 1 based on separate and combined analyses of sequences of 16S rRNA (379 bp) and NADH-5 (318 bp) mtDNA gene segments[a]

	16S			NADH-5			Combined		
	NJ	MP	ML	NJ	MP	ML	NJ	MP	ML
Ocelot lineage									
[Lpa, Lwi, Oge, Ogu, Lti, Lco]	12	No	No	50	61	Yes	79	66	Yes
[Lpa, Lwi][1]	86	79	Yes	95	96	Yes	97	95	Yes
[Oge, Ogu][1]	67	62	Yes	90	81	Yes	95	95	Yes
[Lco, Lti-Brazil][1]	91	89	Yes	96	100	Yes	100	100	Yes
[Lti-C.A., Lco, Lti-Brazil][1]	No	No	No	No	No	No	No	No	No
Domestic cat lineage									
[Fsi, Fca, Fli, Fma, Fch, Fni]	41	No	No	No	No	No	41	10	Yes
[Fsi, Fca, Fli, Fma, Fch][2]	No	No	No	40	No	Yes	74	58	Yes
[Fsi, Fca, Fli, Fma][2]	66	60	Yes	100	100	Yes	100	100	Yes
Panthera genus									
[Pti, Ppa, Pon, Ple, Pun, Nne]	No	No	No	56	90	Yes	90	79	Yes
[Pti, Ppa, Pon, Ple, Pun]	65	55	Yes	No	87	Yes	75	78	Yes
Puma/Lynx group									
[Lru, Lca, Lly, Pco, Hya, Aju]	14	No	No	12	No	No	41	No	Yes
Puma subgroup									
[Pco, Hya, Aju]	73	69	Yes	No	No	No	39	28	Yes
[Pco, Hya][3]	No	No	No	56	70	Yes	59	100	Yes
Lynx genus									
[Lru, Lca, Lly]	33	No	Yes	54	52	Yes	72	79	Yes
[Lca, Lly][5]	78	22	No	99	48	Yes	100	97	Yes
Caracal group									
[Pau, Cca]	36	No	No	99	99	Yes	99	97	Yes
Asian leopard cat group									
[Pvi, Pbe, Ipl]	85	71	Yes	No	30	No	92	68	Yes
Bay cat group									
[Pba, Pte]	No	No	No	77	75	Yes	64	65	Yes
Serval group									
[Lse, Pru]	25	No	No	No	No	No	57	No	Yes

[a] Phylogenetic trees were derived from three methods: (NJ) the minimum evolution method estimated by the neighbor-joining algorithm from Kimura distances (value from 100 bootstrap iterations), (MP) the maximum parsimony method estimated from a heuristic search (value from 100 bootstrap iterations), and (ML) the maximum likelihood method. Unless otherwise noted, one individual was used from each species and spotted hyena and mongoose were used as outgroups (as in Fig. 1). Groups labeled "no" were paraphyletic for that analysis. Marbled cat and pallas cat are not included in this list. Three-letter species codes are defined in Table 1. *Numbered notes:* [1]Determined with the subset of individuals used in Fig. 2. [2]Determined with the subset of individuals used in Fig. 3. [3]Determined with the subset of individuals used in Fig. 4

Caracal Group

Phylogenetic analyses of sequences from caracal (*Caracal caracal*) and African golden cat (*Profelis aurata*) formed a well-supported clade with each of the methods (86–99% bootstrap support) (Figs. 1, 6), but the species sequences were distantly related (43 bp and 6.45% difference) (Table 3). Phylogenetic association of caracal and African golden cat was also suggested from albumin immunological distance, allozyme genetic distance, and 12S rRNA and cytochrome *b* sequence data (Collier and O'Brien 1985; Janczewski et al. 1995; O'Brien et al. 1987). However, these analyses also associated golden cat with several, an association not reproduced in this study (see below).

Geographically, caracal and African golden cat have adjacent, but nonoverlapping ranges, with caracal occupying drier woodlands and steppe of Africa and parts of the Middle East, west to northwest India, and African golden cat inhabiting mostly the moist forests of West and Central Africa (Nowak 1991). The ancient roots of this clade are supported by 3–5 MYA caracal fossils (Savage and Russell 1983; Turner 1987). We estimate that caracal and African golden cat mtDNA genes last shared a common ancestor 4.85 MYA (Table 3).

Bay Cat Group

Sequences from bay cat (*Pardofelis badia*) and Asian golden cat (*Profelis temmincki*) showed a distant relationship (63 bp and 9.23% difference) (Table 2), but formed a moderately well supported monophyletic clade with 65% and 94% bootstrap support in neighbor-joining and maximum parsimony analyses, respectively, and good support in the maximum likelihood analyses (Figs. 1, 6). Although Asian golden cat has traditionally been suggested to be closely related to African golden cat based on morphological traits, phylogenetic analyses of 12S rRNA and cytochrome *b* sequence data also revealed

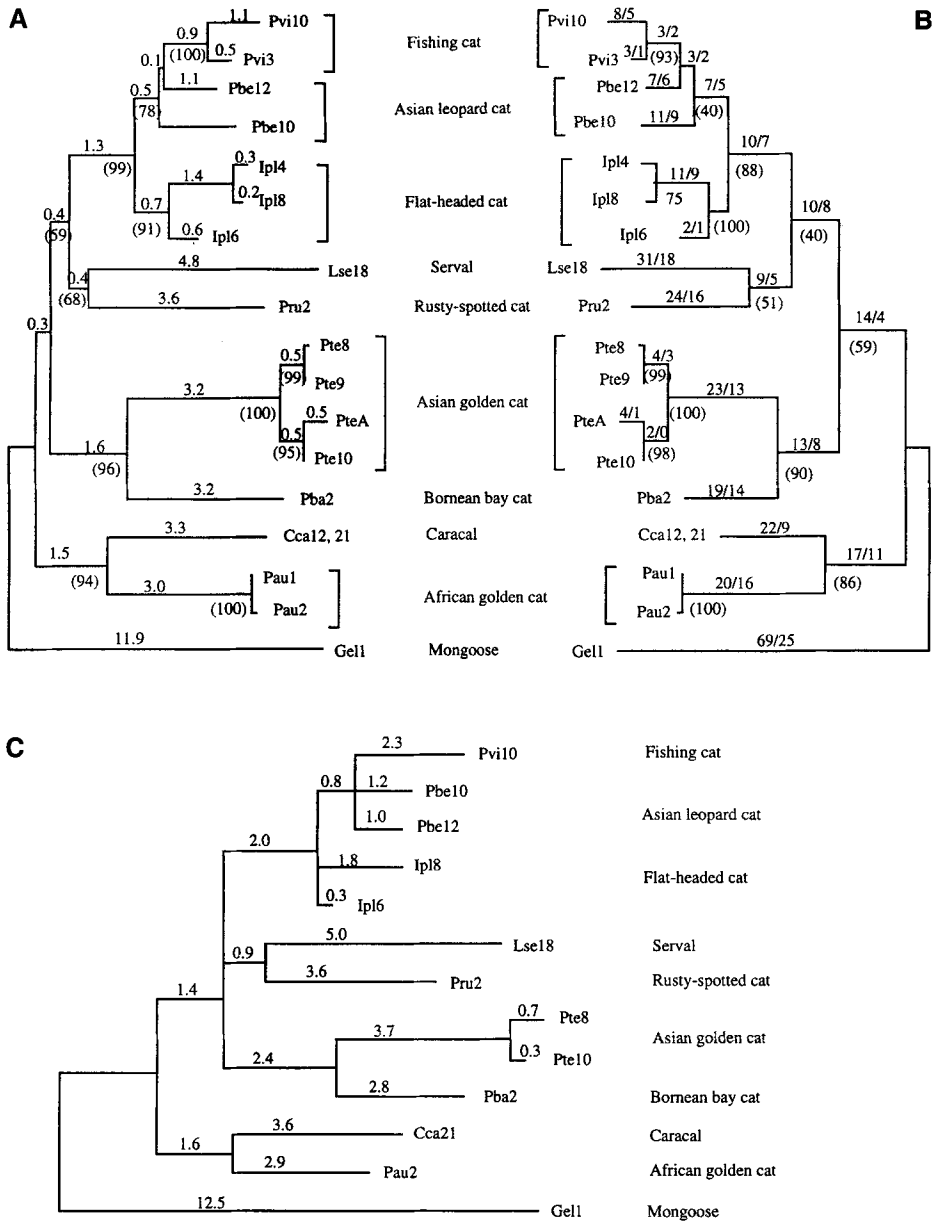

Fig. 6. Phylogenetic relationships among multiple individuals of pantherine lineage Asian/African smaller cats based on analyses of combined 16S rRNA (379 bp) and NADH-5 (318 bp) mtDNA gene segments. Phylogenetic trees constructed with **(A)** minimum evolution method estimated by neighbor-joining algorithm with Kimura distances (branch *lengths* are above the lines and *percentage* of 100 bootstrap iterations is below the line in *parentheses*); **(B)** maximum parsimony method (values above the branch correspond to number of steps/number of homoplasies; *percentage* of 100 bootstrap iterations is below the branch in *parentheses;* two most-parsimonious trees, tree length = 358, CI = 0.654); and **(C)** maximum likelihood method (ln = 2,640, 569 trees examined).

that these two species were distantly related and that African golden cat had a clearer affinity with caracal (Janczewski et al. 1995). The bay cat and Asian golden cat association has not previously been examined with molecular genetic methods, although Hemmer (1978) allied the two species in the same genus *Catopuma* based on morphological traits. Pocock (1932) also suggested a close relationship between bay cat and Asian golden cat based on coat characteristics. The affinity of bay cat and Asian golden cat is not contradicted by geographic evidence. Bay cats are found only on the island of Borneo and Asian golden cat is distributed through Southeast Asia from Sumatra and Malaysia north to Nepal, Burma, and China (Nowak 1991). We estimate that bay cat and Asian golden cat mtDNA sequences last shared a common ancestor 5.43 MYA (Table 3), well before Borneo was last separated from the other islands of the Sunda Shelf 10,000–15,000 years ago (Umbgrove 1949).

Unaligned Species

Analyses of 16S rRNA and NADH-5 sequences demonstrated a weak association between rusty-spotted cat (*Prionailurus rubiginosa*) and serval (*Leptailurus ser-*

val) with neighbor-joining (68% bootstrap support) and maximum parsimony analyses (51% bootstrap support) (Fig. 6). These two species are not closely related, however (57 bp and 8.39% difference) (Table 2), and their association has not been confirmed by other molecular studies. Considering the two gene segments separately, the alignment of the two species is weakly supported by the 16S rRNA gene segment but not by the separate NADH-5 analysis. Finally, there are two chromosome differences between serval and rusty-spotted cat (Modi and O'Brien, 1988). For these reasons, their weak association reported here was considered tentative. Classifications of these two species based on morphological data have been inconsistent. Salles (1992) proposed rusty-spotted cat formed a clade with Andean mountain cat and pampas cat and Hemmer (1978) allied rusty-spotted cat to fishing cat, Asian leopard cat, and flat-headed cat.

Pallas cat (*Otocolobus manul*) and marbled cat (*Pardofelis marmorata*) sequences did not consistently associate with any other field species in the global (Fig. 1) or local (Fig. 3) phylogenetic analyses. With the combined and 16S rRNA data, pallas cat aligned weakly with members of the *Panthera* genus while NADH-5 data suggested it was an early divergence of the domestic cat lineage. Masuda et al. (in press), using cytochrome *b* data, suggested that pallas cat was an early divergence within the domestic cat lineage; however, 12S rRNA analysis did not affirm this association. Pallas cat has also been placed within the domestic cat lineage based on albumin immunological distances (Collier and O'Brien 1985) had similar karyological traits (Wurster-Hill and Centerwall 1982). Mitochondrial RFLP data suggested pallas cat was a distantly related member of the domestic cat lineage (Johnson et al. 1996). From this evidence, we included pallas cat sequences in our analyses of the domestic cat lineage (Fig. 3). These results suggest that if pallas cat falls in this lineage, it is clearly the most ancient divergence. When pallas cat was constrained to combine with the other species of the domestic cat lineage (as in Fig. 1 analysis), the maximum parsimony tree was only four steps longer than the unconstrained tree of 969 steps.

Marbled cat has previously been proposed to align with *Lynx* species based on albumin immunological distance (Collier and O'Brien 1985), allozyme genetic distance (O'Brien et al. 1987), karyology (Wurster-Hill and Centerwall 1982), and, to a limited extent, by 12S rRNA and cytochrome *b* gene sequences (Janczewski et al. 1995). With 16S rRNA and NADH-5 combined analyses, marbled cat did not align with any other species (Fig. 1). When marbled cat was constrained to combine with the *Lynx* group, the maximum parsimony tree was 17 steps longer than the unconstrained shortest tree of 696 steps. When it was constrained to the puma group, however, the maximum parsimony tree was only four steps longer.

Separate Analyses with 16S rRNA and NADH-5 Mitochondrial Genes

The utility of the 16S rRNA and NADH-5 gene segments for resolving the relationships among the Felidae varied depending upon the phylogenetic method and the group of species being examined (Table 4). 16S rRNA sequences provided better support than NADH-5 for several clades including the puma subgroup and the Asian leopard cat group. NADH-5 provided more support for other clades such as the association of Asian golden cat with bay cat and the pairing of caracal with African golden cat. Combining the sequences of 16S and NADH-5 gene segments consistently increased support for the eight proposed groups (Table 4).

Compared with other mitochondrial genes, 16S rRNA and NADH-5 have been estimated to be evolving at 0.56% base-pair divergence/MY and 0.71% base-pair divergence/MY, respectively (Lopez et al. submitted). The phylogenetic patterns resulting from our analyses are generally consistent with those derived from other mitochondrial genes, as discussed above. The improved resolution (based on higher bootstrap values) from our sequence data compared with 12S and cytochrome *b* sequences (Janczewski et al. 1995; Masuda et al. in press) resulted from the more rapid evolution of 16S rRNA and NADH-5 genes and the longer sequence analyzed. From our results, 16S rRNA and NADH-5 genes appear to be most useful at resolving recent (2–5 MY) radiations and were unable to resolve more ancient relationships (6–12 MY), such as the hierarchy of phylogenetic divergence among the eight felid groups plus the four unpaired species.

Several relationships among the Felidae remain unresolved. These include the hierarchical relationships among the eight groups of cats presented in this paper and the placement of the pallas cat, marbled cat, Andean mountain cat, Chinese desert cat, Iriomote cat, rusty-spotted cat, and several relative to these groups. Within the proposed clades, the relationships among big cats of the *Panthera* genus remain unresolved and the large divergence among tigrina from different regions of South America needs further scrutiny.

Acknowledgments. Sequence data was deposited in GenBank. We appreciate the assistance of M. Culver, J. Martenson, M. Thompson, and S. Cevario in data collection and the advice of J. Pecon Slattery in the analysis and in the preparation of earlier drafts. We are grateful to the numerous people who helped obtain blood and tissue samples for this study and to the institutions and individuals listed in Table 1 who provided access to their animals. All tissue samples were collected with full compliance with specific Federal Fish and Wildlife permits (Convention on International Trade in Endangered Species of Wild Fauna and Flora [CITES]; Endangered and Threatened Species, Captive Bred) issued to the National Cancer Institute, National Institutes of Health (principal officer S.J. O'Brien) by the U.S. Fish and Wildlife Service of the Department of the Interior.

References

Adams DB (1979) The cheetah: native American. Science 205:1155–1158

Avise JC (1994) Molecular markers, natural history and evolution. Chapman and Hall, New York

Benveniste RE, Todaro GJ (1974) Homology between type-C viruses of various species as determined by molecular hybridization. Proc Natl Acad Sci USA 70:3316–3320

Benveniste RE, Sherr CJ, Todaro GJ (1975) Evolution of type C viral genes: origin of feline leukemia virus. Science 190:886–888

Benveniste RE (1985) The contributions of retroviruses to the study of mammalian evolution. In: MacIntyre RJ (ed) Molecular evolutionary genetics. Plenum Press, New York, pp 359–417

Berta A (1983) A new species of small cat (Felidae) from the late Pliocene-early Pleistocene Uquian of Argentinian. J Mammal 64: 720–725

Clutton-Brock J (1987) A natural history of domesticated animals. Cambridge University Press, Cambridge, England

Collier GE, O'Brien SJ (1985) A molecular phylogeny of the Felidae: immunological distance. Evolution 39:437–487

Engelke DR, Hoener PA, Collins FS (1988) Direct sequencing of enzymatically amplified human genomic DNA. Proc Natl Acad Sci USA 85:544–548

Ewer RF (ed) (1973) The carnivores. Cornell University Press, Ithaca, NY 409 pp.

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1993) Phylogeny inference package (PHYLIP). Version 3.5. University of Washington, Seattle

Ficcarelli G (1984). The Villafranchian cheetahs from Tuscany and remarks on the dispersal and evolution of the genus *Acinonyx*. Palaeontographia Italica 73:94–103

Glass GE, Martin LD (1978) A multivariate comparison of some extant and fossil Felidae, Carnivora. Carnivora 1:80–88

Hemmer H (1978) The evolutionary systematics of living Felidae: present status and current problems. Carnivore 1:71–79

Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst Biol 42:182–192

Hillis DM (1995) Approaches for assessing phylogenetic accuracy. Syst Biol 44:3–16

Hoelzel AR, Green A (1992) Analysis of population-level variation by sequencing PCR-amplified DNA. In: Hoelzel AR (ed) Molecular genetic analysis of populations, a practical approach. IRL Press, Oxford pp. 159–187

Huelsenbeck JP, Hillis DM (1993) Success of phylogenetic methods in the four-taxon case. Syst Biol 42:247–264

Huelsenbeck JP, Bull JJ, Cunningham CW (1996) Combining data in phylogenetic analysis. Trends Ecol Evol 11:152–157

Hunt MH (1996) Biogeography of the order Carnivora. In: Gittleman JL (ed) Carnivore behavior, ecology, and evolution, vol 2. Cornell University Press, Ithaca, NY, pp. 485–541

Ikemura T (1985) Codon usage and tRNA content in unicellular and multicellular organisms. Mol Biol Evol 2:13–34

Janczewski DN, Modi WS, Stephens JC, O'Brien SJ (1995) Molecular evolution of mitochondrial 12S RNA and cytochrome b sequences in the Pantherine lineage of Felidae. Mol Biol Evol 12:690–707

Johnson WE, Dratch PA, Martenson JS, O'Brien SJ (1996) Resolution of recent radiations within three evolutionary lineages of Felidae using mitochondrial restriction fragment length polymorphism variation. J Mammal Evol 3:97–120

Johnson WE, Culver M, Iriarte A, O'Brien SJ (submitted) Tracking the evolution of the elusive Andean Mountain Cat (*Oreailurus jacobita*) from mitochondrial DNA. J Heredity

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kluge AG (1989) A concern for evidence and a phylogenetic hypothesis of relationships among Epiarates (Boidae, Serpentes). Syst Zool 38:7–25

Kumar S, Tamura K, Nei M (1993) MEGA: Molecular evolutionary genetics analysis, version 1.01. The Pennsylvania State University, University Park, PA

Kurten B (1968) Pleistocene mammals of Europe. Aldine Press, Chicago

Kurten B, Anderson E (1980) In: Pleistocene mammals of North America. Columbia University Press, New York, pp. 108–118

Leyhausen P (1979) Cat behavior. Garland Press, New York

Lopez JV, Yuhki N, Masuda R, Modi W, O'Brien SJ (1994) Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. J Mol Evol 39:174–190

Lopez JV, Cevario S, O'Brien SJ (1996) Complete nucleotide sequences of the domestic cat (*Felis catus*) mitochondrial genome and a transposed mtDNA tandem repeat (Numt) in the nuclear genome. Genomics 33:229–246

Lopez JV, Culver M, Stephens JC, Johnson WE, O'Brien SJ (submitted) Rates of nuclear and cytoplasmic mitochondrial DNA sequence divergence in mammals. Mol Biol Evol

MacFadden BJ, Galiano H (1981) Late Hemphillian cat (Mammalia: Felidae) from the Bone Valley Formation of central Florida. J Paleontolo 55:218–226

Martin LD (1980) Functional morphology and the evolution of cats. Trans Neb Acad Sci 13:141–154

Martin LD (1989) Fossil history of the terrestrial Carnivora. In: Gittleman JL (ed) Carnivore behavior, ecology, and evolution. Cornell University Press, Cornell, NY, pp 536–568

Masuda RM, Lopez JV, O'Brien SJ, Pecon Slattery J, Yuhki N, O'Brien SJ (in press) Molecular phylogeny of mitochondrial cytochrome b and 12S rRNA sequences in the Felidae: ocelot and domestic cat lineages. Mol Phyl Evol

Modi WS, Nash WG, Ferrari AC, O'Brien SJ (1987) Cytogenetic methodologies for gene mapping and comparative analyses in mammalian cell culture systems. Gene Anal Tech 4:75–85

Modi WS, O'Brien SJ (1988) Quantitative cladistic analysis of chromosomal banding among species in three orders of mammals: hominoid primates, felids and arvicolid rodents. In: Gustafson JP, Appels R (eds) Chromosome structure and function. Plenum Press, New York

Needleman SB, Wunsch CD (1970) A general method applicable to the search for similarities in the amino acid sequence of two proteins. J Mol Biol 48:443–453

Neff NA (1982) The big cats: the paintings of Guy Coheleach. Abrams Press, New York

Neigel JE, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: Karlin S, Nevo E (eds) Evolutionary processes and theory. Academic Press, Orlando, FL

Nowak RM (1991) Walker's mammals of the world, vol 2. John Hopkins University Press, Baltimore

O'Brien SJ, Collier GE, Benveniste RE, Nash WG, Newman AK, Simonson JM, Eichelberger MA, Seal US, Janssen D, Bush M, Wildt DE (1987) Setting the molecular clock in Felidae: the great cats, *Panthera*. In: Tilson RL, Seal US (eds) Tigers of the world. Noyes, NJ, pp 10–27

Orr PC (1969) *Felis trumani*, a new radiocarbon dated cat skull from Crypt Cave, Nevada. Santa Barbara Mus Nat Hist Bull 2:1–8

Patterson B, Pascual R (1972) The fossil mammal fauna of South American. In: Keast A, Erk C, Glass B (eds) Evolution, mammals and southern continents. State University of New York Press, Albany, NY, pp 247–309

Pecon Slattery J, Johnson WE, Goldman D, O'Brien SJ (1994) Phylogenetic reconstruction of South American felids defined by protein electrophoresis. J Mol Evol 39:296–305

Peters G, Hast MH (1994) Hyoid structure, laryngeal anatomy, and vocalization in fields (Mammalia: Carnivora: Felidae). Z Saugetierkunde 59:87–104

Pocock, RI (1932) The marbled cat (*Pardofelis marmorata*) and some other Oriental species, with the definition of a new genus of the Felidae. Proc Zool Soc London 132:741–766

Randi E, Ragni B (1991) Genetic variability and biochemical systematics of domestic and wild cat populations (*Felis silvestris: Felidae*). J Mamm 72:79–88

Reeves RH, O'Brien SJ (1984) Molecular genetic characterization of the RD-114 gene family of endogenous feline retroviral sequences of the domestic cat. J Virol 52:164–171

Saiki RK, Scharf S, Fallona F, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of B-globin genomic sequences and restriction site analysis for diagnosis of sickle-cell anemia. Science 230:1350–1354

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–426

Salles LO (1992) Felid phylogenetics: extant taxa and skull morphology (Felidae, Aeluroidae). Am Mus Novit 3047:1–67

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, a lab manual. 2nd ed. Cold Spring Harbor Laboratory publications, Cold Spring Harbor, NY

Savage DE, Russell DE (1983) Mammalian paleofaunas of the world. Addison-Wesley, London

Sharp PM, Cowe E, Higgins DG, Shields DC, Wolfe KH, Wright F (1988) Codon usage patterns in *Escherichia coli, Bacillus subtilis, Saccharomyces cerevisae, Schizosaccharomyces pombe, Drosophila melanogaster,* and *Homo sapiens;* a review of the considerable within-species diversity. Nucleic Acids Res 16:8207–8211

Sibley SG, Ahlquist JE (1987) DNA hybridization evidence of hominoid phylogeny: results from an expanded data set. J Mol Evol 26:99–121

Sourdis J, Nei M (1988) Relative efficiencies of the maximum parsimony and distance-matrix methods in obtaining the correct tree. Mol Biol Evol 5:298–311

Swofford DL (1993) Phylogenetic analysis using parsimony (PAUP), version 3.1.1. University of Illinois, Champaign

Turner A (1985) Extinction, speciation and dispersal in African larger carnivores from the late Miocene to Recent. S Afr J Sci 81:256–257

Turner A (1987) New fossil carnivore remains from the Sterkfontein hominid site (Mammalia: Carnivora). Ann Transvall Mus 34:319–347

Umbgrove JHF (1949) Structural history of the East Indies. Cambridge University Press, Cambridge, England

Van Valkenburgh B, Grady F, Kurten B (1990) The Plio-Pleistocene cheetah-like cat *Miracinonyx inexpectatus* of North America. J Vert Paleo 10:434–454

Werdelin L (1985) Small pleistocene felines of North America. J Vert Paleo 5:194–210

Wozencraft C (1993) Order Carnivora, Family Felidae. In: Wilson DE, Reeder DA (eds) Mammal species of the world. Smithonsian Institution Press, 2nd edition. Washington, DC, pp 288–299

Wu CI (1991) Inference of species phylogeny in relation to segregation of ancient polymorphisms. Genetics 127:429–435

Wurster-Hill DH, Centerwall WR (1982) The interrelationships of chromosome banding patterns in Procyonids, Viverrids and Felids. Cytogenet Cell Genet 34:178–192