SYSTEMATICS OF MUSTELID-LIKE CARNIVORES

JERRY W. DRAGOO AND RODNEY L. HONEYCUTT

Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843
Present address of JWD: Museum of Southwestern Biology, Department of Biology, University of New Mexico, Albuquerque, NM 87131

The phylogenetic relationships of the skunks to the Mustelidae and other caniform carnivores were examined using mitochondrial-DNA (mtDNA) sequence data from portions of the 12S and 16S ribosomal RNA (rRNA) genes. Data were combined with partial sequences of the cytochrome *b* gene and morphological data obtained from the literature, and used in a total-evidence analysis. The Mustelidae represented a paraphyletic group, with the skunks (*Conepatus, Mephitis,* and *Spilogale*) and the Oriental stink badger (*Mydaus*) forming a monophyletic clade separate from a clade containing the rest of the Mustelidae and the monophyletic Procyonidae. Within the Mustelidae, minus the skunks and stink badger, only one currently recognized subfamily, the Lutrinae, represented a monophyletic group. The families Phocidae, Otariidae, and Odobenidae formed a monophyletic group that was the sister group to the clade composed of the skunks, procyonids, and mustelids. The families Ursidae and Canidae occurred at the base of the Caniformia clade. It is proposed that the skunks be elevated to the level of family and be referred to as the Mephitidae. The family Mephitidae includes the genera *Mephitis* (striped and hooded skunks), *Conepatus* (hog-nosed skunks), *Spilogale* (spotted skunks), and *Mydaus* (Oriental stink badgers).

Key words: Mephitidae, Mustelidae, Carnivora, skunks, phylogeny, taxonomy

In F. Gabriel Sagard-Théodat's "Histoire du Canada," skunks were described as "les enfants du diable," children of the devil (Coues, 1877; Seton, 1926), and their unique method of defense and self-preservation has sustained that reputation. The enlarged anal scent glands of skunks have long been one of the characters that unites them with the weasel family, Mustelidae. However, this character alone does not define the relationship of skunks to any of the other mustelids.

Extant members of the Mustelidae are diagnosed as a monophyletic group on the basis of the loss of the carnassial notch on the upper fourth premolar, the loss of the upper second molar, as well as the enlarged scent glands (Bryant et al., 1993; Martin, 1989; Wozencraft, 1989). Four, to as many as seven, subfamilies are recognized currently (Anderson, 1989; Eisenberg, 1989; Wozencraft, 1989, 1993). The four subfamilies commonly accepted are the Mephitinae (skunks), Melinae (badgers), Lutrinae (otters), and Mustelinae (the rest of the mustelids). Classically, the Mellivorinae (honey badger) has been recognized as a fifth subfamily (Anderson, 1989; Simpson, 1945; Wozencraft, 1993). Wozencraft (1989, 1993) placed the American badger (*Taxidea*) as a separate subfamily, the Taxidiinae, and Anderson (1989) recognized the South American genera *Eira, Galictis,* and *Lyncodon* as components of a distinct subfamily, the Galictinae. Anderson (1989) also mentioned the possibility of distinguishing the South American subgenus *Grammogale* (genus *Mustela*) as a distinct subfamily.

Not only has the designation of subfamilies in the Mustelidae been problematic, but the relationships among taxa within subfamilies and among many subfamilies have been difficult to decipher. The monophyly of the North American skunks (Mephitinae) is well supported (Anderson, 1989; Dragoo et al., 1993; Wozencraft, 1989, 1993), with

three extant genera recognized, including *Mephitis* (hooded and striped skunks), *Spilogale* (spotted skunks), and *Conepatus* (hog-nosed skunks). Two Old World taxa, *Ictonyx* (African zorilla) and *Mydaus* (Oriental stink badger), among others, have been included at various times in the subfamily Mephitinae.

The inability to determine monophyletic groups within the Mustelidae has contributed to confusion regarding sister-group relations within the family. The African zorilla (*Ictonyx*) has a color pattern that converges on that of the North American spotted skunks (*Spilogale*). Many of the early naturalists confused the two genera (Nowak, 1991). Coues (1877) recognized the African Zorillinae as the Old World representative nearest to the Mephitinae. O'Brien et al. (1989), in a protein electrophoresis study of black-footed ferrets and other weasels in the genus *Mustela,* used skunks, including *Ictonyx* (which was referred to as the African striped skunk) as an outgroup. They suggested an ancient split between the skunks *sensu lato* and the genus *Mustela.*

Traditionally, *Mydaus* has been placed within the subfamily Melinae, yet some consider the Melinae to be a polyphyletic group, primarily based on its inclusion (Bryant et al., 1993; Petter, 1971; Pocock, 1921; Radinsky, 1973; Simpson, 1945). Earlier researchers (Petter, 1971; Pocock, 1921; Simpson, 1945) suggested a sister-group relationship between the skunks and the badgers based on similar cranial characters shared by the stink badger and the Mephitinae. Radinsky (1973) asked the question: Are stink badgers skunks? His answer was inconclusive because the characters he identified as being shared were symplesiomorphies. Although the shared characters were plesiomorphic, Radinsky (1973) argued that the fossil record provided support for the skunks and stink badgers being a monophyletic group, with fossil skunks dating from the Miocene and Pliocene in the Old World. Bryant et al. (1993) based on a cladistic analysis of the Mustelidae using cranial, post-cranial, and soft anatomy suggested that *Mydaus* is a member of the Mephitinae.

Recent morphological data (several features of the cranium, dentition, and soft anatomy) have indicated a sister-group relationship between the Lutrinae and the Mephitinae (Hunt, 1974; Wozencraft, 1989; Wyss and Flynn, 1993). These studies, however, were interested in higher-level relationships and, thus, did not examine relationships below the familial level, except within the Mustelidae, which was analyzed at the subfamilial level.

One of the primary difficulties in determining the relationships among subfamilies of mustelids is the diagnosis of monophyletic groups on the basis of synapomorphies as opposed to symplesiomorphies. The morphological data uniting the skunks with any particular subfamily of the Mustelidae, or even to the family, have been based on plesiomorphic character states and convergent similarity. Recent examinations of non-morphological characters have revealed a somewhat different picture of relationships of mustelids. For example, Wurster and Benirschke (1968:374) studied chromosomal data of various carnivores and indicated that the "skunks are remarkably different from the rest of the family." These data suggest that, karyologically, skunks are apomorphic relative to other mustelids; however, these characters provide little information about the relationships of skunks to the mustelids. Ledoux and Kenyon (1975) studied serum proteins and suggested that the Mustelinae, Melinae, and Lutrinae shared a common ancestry long after the lineage leading to the modern Mephitinae diverged.

Recent molecular studies of relationships of carnivores based on DNA hybridization (Árnason and Widegren, 1986; Wayne et al., 1989) suggested that the family Mustelidae is paraphyletic. Although nucleotide-sequence data (Vrana et al., 1994) have provided additional support for this observation, these authors did suggest that data for

more taxa of both skunks and mustelids were needed. Finally, Ledje and Árnason (1996) examined sequence data for the entire cytochrome *b* gene and concluded that *Mephitis* and *Spilogale* were not part of the mustelid clade.

Is the family Mustelidae monophyletic? Part of the answer to this question pertains to the placement of the subfamily Mephitinae. The mustelids have been a difficult group to classify, and the Mustelidae is in need of systematic revision. The objective of this research was to address, using a molecular approach, the phylogenetic relationships among taxa of mustelids, especially those pertinent to the problem of monophyly of mustelids. Sequence data from two mitochondrial genes (12S and 16S ribosomal RNA) were used to assess the phylogenetic relationships among lineages of carnivores, with a primary focus on diagnosing the major clades within the Mustelidae and examining the relationship of skunks to the mustelids and other carnivores. The results also were compared with findings in recent publications of molecular and morphological studies investigating relationships of carnivores.

The 12S and 16S ribosomal RNA (rRNA) genes were selected for several reasons. There is a large existing database of these genes for carnivores and other mammals, thus allowing the examination of more taxa. Relative to other genes within the mammalian mitochondrial genome, the rate of sequence divergence for these genes is suitable for the suggested time periods over which these lineages of carnivores diverged (Janczewski et al., 1992; Lento et al., 1995; Miyamoto et al., 1990; Zhang and Ryder, 1993). Finally, recent studies of systematics of carnivores have shown these genes to be robust in terms of their phylogenetic information content and ability to diagnose monophyletic groups (Janczewski et al., 1992; Lento et al., 1995; Vrana et al., 1994; Zhang and Ryder, 1993). We expanded upon existing molecular studies of carnivores by increasing the sampling of taxa, especially within the family Mustelidae, and in so far as possible, taxa were selected in a manner that would allow for the consideration of both morphological and molecular data.

Materials and Methods

Molecular characters were used to examine relationships among 26 species of carnivores (Table 1). Representatives of the Feliformia (Felidae and Herpestidae) were used as the outgroup for analyses of the suborder Caniformia, which includes the skunks and the mustelids. The molecular data consisted of mitochondrial-DNA (mtDNA) sequences obtained during this study and, whenever possible, compared to data in the GenBank database and recent publications (Árnason and Johnsson, 1992; Janczewski et al., 1992; Vrana et al., 1994).

Total genomic DNA was isolated from frozen tissue, blood, and skin samples using the method of Hillis et al. (1990). Specific regions of mtDNA were amplified using the polymerase chain reaction (PCR). The primers used to amplify portions of the 12S and 16S rRNA genes were (LGL Ecological Genetics, Inc., Bryan, TX); LGL284 (5′ TGG GAT TAG ATA CCC CAC TAT 3′) and LGL383 (5′ ATT GGT GGC TGC TTT TAG GCC 3′). These primers correspond to positions 1,432 and 2,554 of the mtDNA of the harbor seal, *Phoca vitulina* (Árnason and Johnsson, 1992). The amplification product for the rRNA genes was ca. 1,110 base pairs, including 518 base pairs of the 12S rRNA gene, 513 base pairs of the 16S rRNA gene, and 79 base pairs of the transfer RNA valine gene. Double-stranded DNA (dsDNA) products were obtained with PCR amplification using *Taq* DNA polymerase (Saiki et al., 1986, 1988), following similar procedures used by Dragoo et al. (1993). All of the taxa were sequenced on an Applied Biosystems 373A DNA Sequencer. Sequences were attained using the *Taq* DyeDeoxy™ Terminator Cycle Sequencing Kit. Two procedures were used for nucleotide-sequence alignment. The Clustal V program (Higgins and Sharp, 1989) was used to align sequences, followed by visual inspection and alignment, taking into account highly conserved areas. Sequences reported in this paper have

TABLE 1.—*Carnivore taxa used for molecular analyses of phylogenetic relationships. Taxonomy follows Wilson and Reeder (1993).*

Family	Subfamily	Scientific name	Common name	Source[a]
Mustelidae	Mephitinae	*Conepatus mesoleucus*	Hog-nosed skunk	JWD423
		Mephitis mephitis	Striped skunk	JWD389
		Spilogale putorius	Spotted skunk	JWD405
	Lutrinae	*Enhydra lutris*	Sea otter	LGL
		Lutra canadensis	River otter	Vrana et al. (1994)
		Aonyx cinerea	Small-clawed otter	IZL2940
	Melinae	*Mydaus marchei*	Stink badger	CMNH
		Meles meles	Eurasian badger	IZL1111
	Taxidiinae	*Taxidea taxus*	American badger	TK26747
	Mustelinae	*Ictonyx striatus*	Zorilla	SP7550
		Gulo gulo	Wolverine	AF0354
		Martes americana	Pine marten	AF0055
		Mustela vison	Mink	AK11208
		Mustela frenata	Long-tailed weasel	JWD421
		Mustela putorius	European ferret	IZL686
Phocidae	Phocinae	*Phoca vitulina*	Harbor Seal	Árnason and Johnson (1992)
Odobenidae		*Odobenus rosmarus*	Walrus	LGL
Otariidae	Otariinae	*Zalophus californicus*	Sea lion	LGL
Ursidae	Ursinae	*Ursus americanus*	Black bear	AMNH
Procyonidae	Procyoninae	*Procyon lotor*	Raccoon	JWD329
		Bassaricus astutus	Ringtail	JWD392
Procyonidae	Potosinae	*Potos flavus*	Kinkajou	NK13928
Canidae		*Canis latrans*	Coyote	H170
		Urocyon cinereoargenteus	Gray fox	JWD516
Herpestidae	Herpestinae	*Galerella pulverulenta*	Cape gray mongoose	TM1495
Felidae	Felinae	*Leopardus pardalis*	Ocelot	TAMU Kingsville

[a] Collections loaning frozen tissues including the University of Alaska, Fairbanks (AF); Texas A&M University, College Station (AK, H, and JWD); Texas A&M University, Kingsville (TAMU Kingsville); American Museum of Natural History, New York (AMNH); Cincinnati Museum of Natural History (CMNH); Institute of Zoology, London (IZL); LGL Ecological Genetics, Bryan, Texas (LGL); Museum of Southwestern Biology, University of New Mexico, Albuquerque (NK); Section of Mammals, Carnegie Museum of Natural History, Pittsburgh, Pennsylvania (SP); The Museum, Texas Tech University, Lubbock (TK); Transvaal Museum, Johannesburg, South Africa (TM).

been deposited in the GenBank database (accessions U78326-U78350).

Maximum parsimony was used to derive a phylogeny from the nucleotide sequence data. All analyses were conducted using PAUP, version 3.1.1 (Swofford, 1993). The characters were treated as unordered, discrete characters with four possible states (A, C, G, and T). Gaps were coded as present or absent, and an interleave matrix was appended to the end of the sequence data. Both equal and unequal weighting schemes were used. In equally weighted parsimony, all substitutions were used regardless of either the type (transition or transversion) or position in the gene. Unequal weighting involved the use of transversions only, and an evaluation of stems and loops where stems were down weighted to 0.6 as suggested by Springer et al. (1995) for the 12S and 16S rRNA genes.

Sequences for the 12S and 16S datasets were analyzed both separately and in combination. Due to the large number of taxa involved, all maximum-parsimony analyses were performed using the heuristic-search option with 100 replications, a random addition of taxa, and tree bisection-reconnection (TBR) branch swapping. Homoplasy was evaluated using the consistency index (CI—Kluge and Farris, 1969) and the retention index (RI—Farris, 1989). Tree length was used to determine the most-parsimonious solution, and support for individual clades was evaluated using both the decay index, the number of extra steps needed to collapse a node (Bremer, 1988), and boot-

strap resampling using 500 replications (Felsenstein, 1985).

Results

Patterns of sequence variation.—Patterns of nucleotide-base composition were examined using a chi-square test. No bias is indicated by equal frequency (50%) of purines and pyrimidines and equal frequency (25%) of each base (A, C, G, and T). Tests were performed on each species to determine if nucleotide composition varied between sequences (Lockhart et al., 1994). The results were nearly identical among taxa and between the two gene fragments. Although the frequency of purines and pyrimidines in the 12S rRNA gene did not differ from that expected by chance alone ($0.5 > \alpha > 0.1$), there was a general tendency for more purines than pyrimidines. The frequencies of purines and pyrimidines also were about equal in the 16S sequence. There were, however, significantly more adenines than guanines in both genes ($\alpha < 0.005$). The percentage composition of adenine ranged from 35.6 to 39.6% for 12S and 35.6 to 41.8% for 16S, cytosine ranged from 20.6 to 24.9% for 12S and 15.8 to 21.7% for 16S, guanine ranged from 16.7 to 19.4% for 12S and 16.1 to 19.8% for 16S, and thymine ranged from 20.7 to 24.6% for 12S and 22.6 to 27.0% for 16S.

The type of nucleotide substitution also was similar in all pairwise comparisons between sequences. Transition changes between thymine and cytosine tended to be more common than changes between adenine and guanine; changes between adenine and thymine were, on average, the more common transversions. Few transversions involving guanine and either cytosine or thymine were observed.

Genetic distances were calculated by the method of Tajima and Nei (1984) using the program MEGA, version 1.01 (Kumar et al., 1993). This distance, which provides a better estimate of the number of nucleotide substitutions when nucleotide frequencies deviate substantially from 0.25%, is useful when a strong transition-transversion bias is absent (Kumar et al., 1993; Nei, 1991). Neither transition nor transversion saturation was apparent in either gene relative to the genetic distances compared. However, there were more transition changes than transversion changes in the 12S gene, especially at lower levels of divergence. In the 16S gene, the transition and transversion changes were about equal, again with a bias toward transitions between less-divergent taxa. Beyond divergences of 10–15%, the ratio of transitions to transversions was relatively low, averaging 1.75:1 and 1.22:1 for the 12S and 16S genes, respectively.

There were 103 insertion-deletion events in the sequences examined. About 65% of the gaps that occurred in the 12S sequences were the result of single insertion-deletions events, whereas 70% of the gaps were single insertion-deletion events in the 16S sequences. The largest insertion-deletion (seven bases) occurred between *Conepatus* and the outgroup in the 12S gene; no gaps were longer than four bases in the 16S gene.

Phylogenetic analyses.—Parsimony analyses of the 12S and 16S sequence data were performed separately. Five equally parsimonious trees (not shown) resulted from the analysis of the 12S gene; analysis of the 16S gene produced nine most-parsimonious solutions (trees not shown). In all 14 trees, the taxa currently comprising the family Mustelidae did not form a monophyletic group. The Mephitinae (skunks) and *Mydaus* (traditionally placed in Melinae) formed a monophyletic clade separate from a larger clade containing the remainder of the Mustelidae (Lutrinae, Melinae, and Mustelinae) and the family Procyonidae. The relationships of taxa within the Mustelidae were unresolved. The genus *Ictonyx* (subfamily Mustelinae) was placed either sister to the Lutrinae or outside a clade containing Lutrinae and the genus *Mustela* (Mustelinae), thereby making the Mustelinae paraphyletic. The placement of *Gulo* and *Martes* outside a clade containing *Ictonyx, Mustela,* and the Lutrinae also resulted in a paraphy-

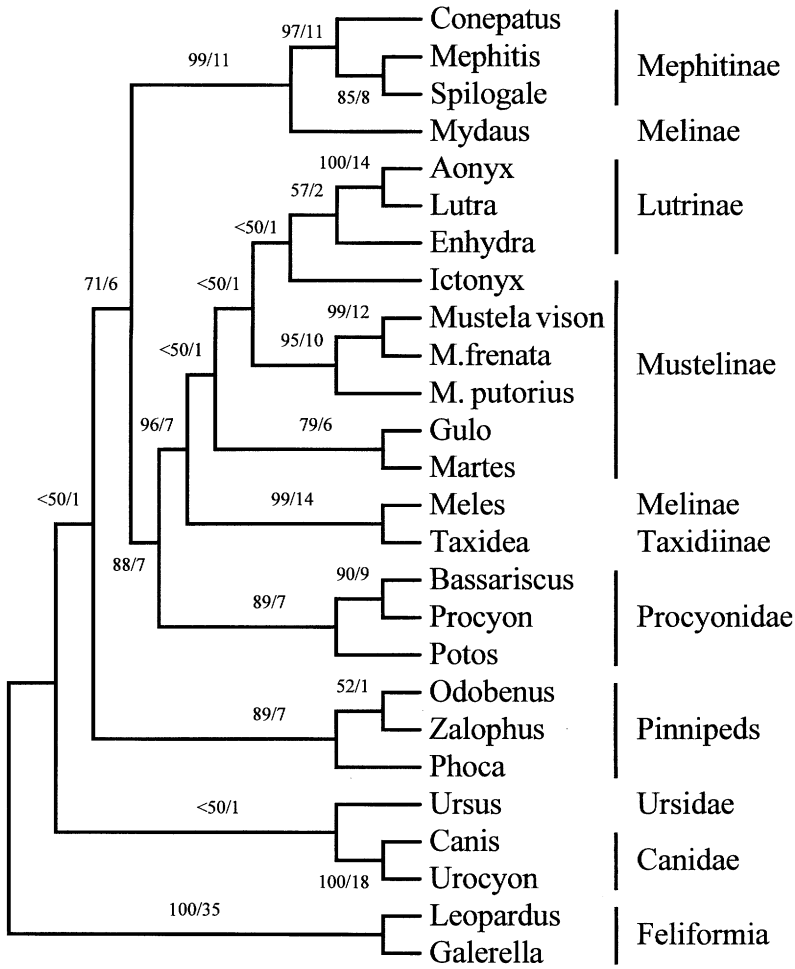

FIG. 1.—Phylogenetic relationships among carnivores based on parsimony analysis of all nucleotide substitutions (tree length = 2,036; CI = 0.365; RI = 0.444) among 12S and 16S rRNA gene sequences. Numbers represent bootstrap values/decay indices.

letic Mustelinae. Other discrepancies among the nine trees obtained from the 16S rRNA sequences involved the placement of the Mephitinae and *Mydaus* clade relative to the pinnipeds, and the placement of the Canidae relative to the Ursidae.

The two datasets were combined because both genes are linked and, thus, share a common evolutionary history (Miyamoto et al., 1994), and demonstrate similar patterns of divergence and base composition. Combining data has the potential of increasing resolution due to the greater number of potentially informative characters that support nodes not well supported by a single gene alone (Miyamoto et al., 1994; Olmstead and Sweere, 1994). Parsimony analysis of the combined 12S and 16S data resulted in a single most-parsimonious tree (Fig. 1). The bootstrap value and decay index for the node uniting *Mydaus* with the Mephitinae were 99% and 11 additional steps, respectively. As was found with the separate analyses of the 12S and 16S data, the mustelids did not form a monophyletic group. The Mephitinae and *Mydaus* grouped outside the clade (bootstrap = 71%, decay index = 6 steps) containing a monophyletic

Procyonidae and the rest of the mustelids, a clade supported by a bootstrap of 88% and decay index of seven steps.

Within the mustelid clade (minus the Mephitinae and *Mydaus*), there was little support for any particular grouping among the three recognized subfamilies. The Mustelinae was paraphyletic based on the placement of *Ictonyx, Gulo,* and *Martes*. There was, however, strong support (bootstrap = 96%, decay index = 7 steps) for the monophyly of the mustelid taxa minus the Mephitinae and *Mydaus*.

An analysis using transversions only produced a tree with a topology similar to that seen in Fig. 1, with the exception of the placement of the Ursidae relative to the Canidae and the pinnipeds. As was found in the equal-weighting analysis, the Mustelidae was paraphyletic when only transversions were used. When stems were down weighted relative to loops, the resulting topology was identical to that seen in Fig. 1.

Discussion

In recent years, several cladistic analyses of morphological characters have addressed relationships among carnivores. Flynn et al. (1988) reviewed morphological characters used in studies of phylogenetics of carnivores and suggested that two major clades were present in the monophyletic Carnivora. One clade, the Caniformia, consisted of Cynoidea (Canidae) and Arctoidea. The Arctoidea was an unresolved trichotomy comprised of Ursidae, the monophyletic Pinnipedia, and Musteloidea (Procyonidae and Mustelidae). Relationships of subfamilies within Mustelidae were not examined.

Wozencraft (1989) examined 100 morphological characters in a cladistic analysis of extant carnivores and proposed a different hypothesis for the phylogeny of Caniformia. He suggested that there were two superfamilies, Ursoidea and Canoidea. The clade containing Otariidae and Odobenidae with Ursidae as the sister taxon represented the Ursoidea. The Canoidea was comprised of Canidae, Procyonidae, and the sister taxa Mustelidae and Phocidae. Within Mustelidae, the subfamilies Mustelinae and Melinae were sister groups, as were Mephitinae and Lutrinae.

To evaluate how well our molecular data conformed to Wozencraft's (1989) hypothesis derived from morphology, we produced a constraint tree for the taxa used in our study, based on the topology presented by Wozencraft (1989). The length of the tree was determined using our 12S and 16S sequence data. Fifty-six additional steps (relative to the most-parsimonious tree shown in Fig. 1) would be needed for the molecular data to support Wozencraft's (1989) hypothesis. This suggests a lack of congruence between Wozencraft's (1989) morphological results and those obtained from the molecular data presented here.

Wyss and Flynn (1993), in an investigation of the relationships among families of carnivores, re-examined Wozencraft's (1989) characters and made the following changes in their interpretation: 1) the scoring of some characters for extant taxa was changed based on the inclusion of fossil taxa; 2) characters not considered independent were combined; 3) ambiguous characters were rejected; 4) series of complex characters with co-varying components were combined as a single character; 5) the polarity of some characters was corrected based on outgroup comparison; 6) poorly justified ordered transformations were dismissed. Their final dataset consisted of 64 morphological characters and both extinct and extant taxa. A phylogenetic analysis of the data produced the following results. Canidae was the sister group to the Arctoidea as defined by Flynn et al. (1988), Mustelidae was at the base of the Arctoidea and the sister group to the remaining taxa, Pinnipedia was the sister group to Ursidae, and Procyonidae was the sister group to the clade comprised of Pinnipedia and Ursidae. In addition, they examined relationships among subfamilies of Mustelidae and found that Mephitinae and Lutrinae were sister

taxa. When the 12S and 16S data were constrained to Wyss and Flynn's (1993) hypothesis, the resultant phylogeny was 29 steps longer than the tree shown in Fig. 1. This result also suggested incongruence between the morphological and molecular data.

In addition to the morphological studies noted above, several molecular studies have examined phylogenetic relationships among carnivores (Ledje and Árnason, 1996; Miyamoto and Goodman, 1986; Vrana et al., 1994; Wayne et al., 1989). Miyamoto and Goodman (1986) studied amino acid sequences and suggested that within the Caniformia, Canidae and Ursidae were sister taxa, pinnipeds were monophyletic, and Procyonidae and Mustelidae were sister taxa. Miyamoto and Goodman (1986) did not examine all the carnivores used in the present study, and did not include any taxa of the subfamily Mephitinae. Nevertheless, the 12S and 16S data constrained on their phylogenetic hypothesis was only eight steps longer than the most parsimonious phylogeny (Fig. 1). The absence of taxa of skunks in their analysis may be the primary difference between the 12S and 16S data and the amino-acid-sequence data. Whereas the 12S and 16S data suggested that the family is paraphyletic, the results of Miyamoto and Goodman (1986) indicated the Mustelidae to be monophyletic.

In a DNA hybridization study by Wayne et al. (1989), the relationships of many of the major clades in the Caniformia were unresolved, with the exception of the Canidae, which was the sister taxon to the arctoid group. Within the arctoid carnivores, the Procyonidae, Ursidae, the pinnipeds, and part of the Mustelidae were part of a polytomy. The subfamily Mephitinae was basal to the arctoid bush. The results of the present study support Wayne et al. (1989) in placing the skunks outside the mustelids and also resolves the arctoid polytomy.

Vrana et al. (1994) examined DNA sequence from partial sequences of the 12S rRNA and cytochrome *b* genes to ascertain relationships of carnivores. They determined that Ursidae and pinnipeds were sister taxa. Procyonidae was paraphyletic; the sister taxa *Procyon* and *Bassariscus* were placed at the base of the ursid-pinniped clade and *Potos* was the sister taxon to this larger clade. Mustelidae was paraphyletic and basal to an arctoid clade containing the ursids, pinnipeds, and procyonids. Mephitinae, represented by *Mephitis,* was placed outside the rest of the arctoid carnivores, and Canidae was the sister group to this entire clade. This hypothesis required 18 additional steps relative to our tree (Fig. 1).

Ledje and Árnason (1996) examined DNA sequences for the entire mitochondrial cytochrome *b* gene. Although they were not able to resolve the major clades of arctoid carnivores, they found that the skunks were not a part of the mustelid clade and suggested that they should be recognized as a separate family. Our results, based on 12S and 16S data, are consistent with theirs, based on cytochrome *b*.

Based on the above mentioned studies, one can conclude that the pinnipeds are part of the arctoid carnivore radiation and are monophyletic, a result supported by other molecular studies (Lento et al., 1995; Sarich, 1969). However, the relationships among the major clades in the Caniformia are unresolved. A strict consensus of the topologies derived from five of the studies discussed above resulted in a polytomy for the Caniformia. The Lutrinae, Melinae (minus *Mydaus*), Procyonidae, Canidae, and Mephitinae (including *Mydaus*) each formed separate branches. The pinnipeds represented three distinct branches, and the Mustelinae also was separated into three different branches. A 50% majority rule consensus for these topologies revealed a polytomy consisting of five lineages, including pinnipeds, Canidae, Procyonidae, Ursidae, and Mustelidae. Within the mustelid clade, Mephitinae (including *Mydaus*) was basal, and Melinae was the sister group

to an unresolved clade containing Lutrinae and Mustelinae.

To examine all of the existing evidence used to describe the families of caniform carnivores, the 12S and 16S rRNA gene sequences from the present study were combined with character-state data from molecular studies (Vrana et al., 1994) and morphological studies (Bryant et al., 1993; Wozencraft, 1989; Wyss and Flynn, 1993) in a maximum-parsimony analysis. The short-tailed shrew (*Blarina*) and the extinct order Creodonta were used as outgroups. A total of 62 specimens representing 44 taxa of carnivores, (including multiple individuals of a species and multiple species in a genus) and 2 outgroup taxa were analyzed. Sequences from the 12S and 16S rRNA genes and gap codes, sequences from the cytochrome *b* gene, and morphological characters from Wyss and Flynn (1993) and Bryant et al. (1993) were combined to produce a dataset of 1,647 characters. A consensus of the 48 most-parsimonious trees was generated (Fig. 2).

Several interesting results were obtained with this combined analysis. The two major groups of carnivores, Caniformia and Feliformia (Wozencraft, 1989; Wyss and Flynn, 1993) formed monophyletic groups. As with the molecular data, the family Mustelidae was paraphyletic, with the subfamily Mephitinae (plus *Mydaus*) being the sister taxon to a clade containing Procyonidae and the remaining Mustelidae. In contrast to the molecular data, except for *Ictonyx,* the Mustelinae formed a monophyletic group that was the sister group to the monophyletic Lutrinae. This clade and *Ictonyx* were the sister group to the American and European badgers (Melinae). The red panda, *Ailurus,* and Procyonidae were sister taxa; the giant panda, *Ailuropoda,* grouped with Ursidae. The pinnipeds formed a monophyletic clade that was the sister group to the clade containing the mustelids, procyonids, and skunks. The basal taxon of the pinniped clade, the extinct Desmatophocidae, was placed as the sister group to the Phocidae as was found by Wyss and Flynn (1993). Finally, Canidae and Ursidae represented more basal caniform families.

Total-evidence and missing data.—The phylogenetic results from the total-evidence analysis (Fig. 2), were similar to the results of the 12S and 16S rRNA gene analysis (Fig. 1). This is surprising given the lack of congruence among independent analyses of morphological and molecular data. Recently, de Queiroz (1993) summarized the two general approaches used to evaluate phylogenetic hypotheses derived from multiple datasets. One approach, which derives a consensus tree from independently derived phylogenies, has the advantage of providing equal weights for each dataset and eliminates any potential swamping of small datasets by larger amounts of data from another source. Nevertheless, this approach may not always provide the most-parsimonious solution for character change (Miyamoto, 1985) and will result in decreased resolution. With regard to the evaluation of relationships among caniform carnivores, a consensus approach results in a total lack of resolution due to low taxonomic congruence among the various existing hypotheses derived using morphological and molecular data.

A second approach, combining all data into a single phylogenetic analysis, has been advocated by several authors (Barrett et al., 1991; Kluge, 1989; Miyamoto, 1985). Phylogenies derived from a broad range of characters may overcome many of the biases associated with a particular set of characters that individually yield erroneous phylogenies (Bull et al., 1993). Arguments for combining data prior to analysis include: 1) different character classes may provide better levels of resolution at different nodes of a tree (Hillis, 1987); 2) individual datasets may contain only a weak phylogenetic signal and adding multiple datasets with low signal should be additive and override the noise; 3) phylogenetic analyses should explain all of the data simultaneously (Kluge, 1989).

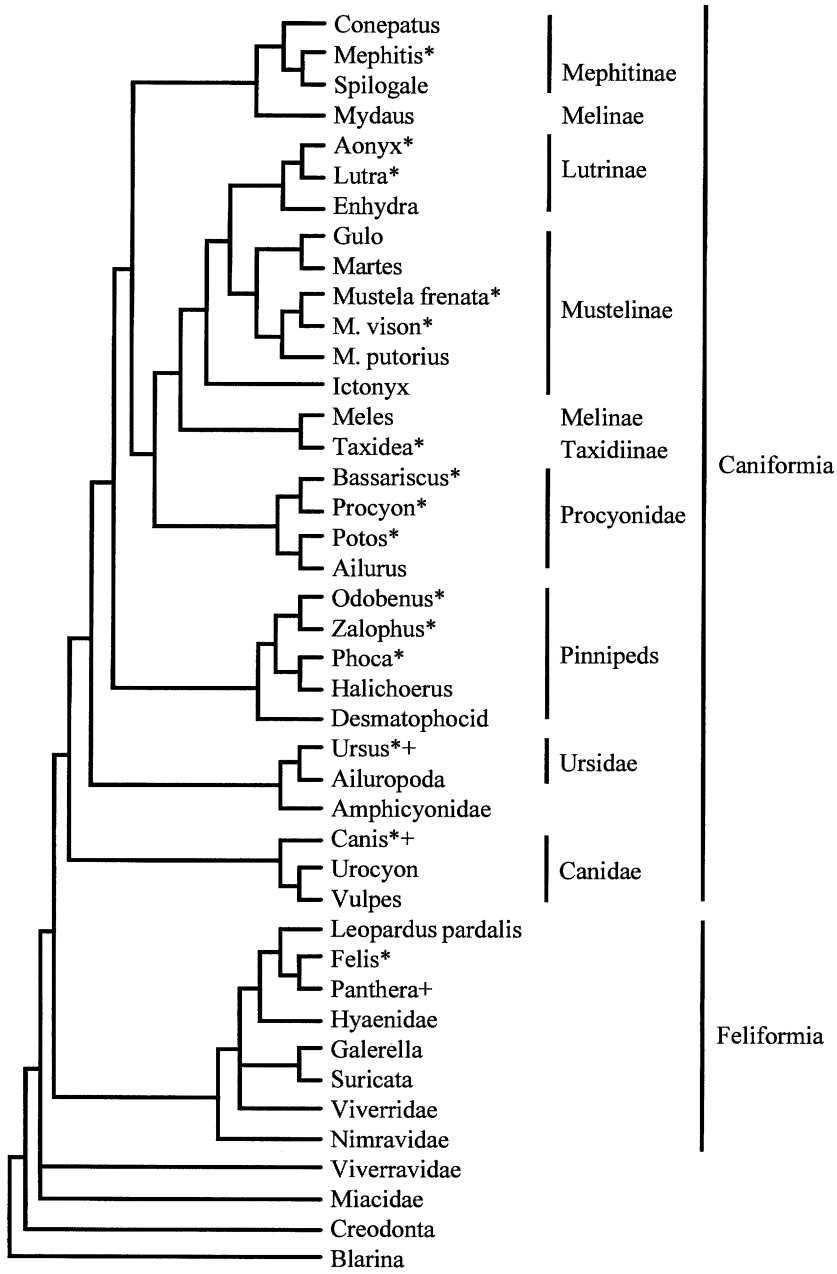

FIG. 2.—Strict consensus tree (tree length 3,534; CI = 0.346; RI = 0.591) of the Carnivora and outgroups derived from 48 equally parsimonious trees based on total evidence including 12S and 16S rRNA sequences, cytochrome *b* sequences, and morphological characters. Taxa followed by (*) are represented by multiple individuals and taxa followed by (+) are represented by multiple species. Terminal taxa denoted by (*) or (+) have been combined into a single branch for ease of presentation.

When different datasets are congruent, it can be assumed that the resultant phylogeny approximates the true species phylogeny (Kluge, 1989). Alternatively, different traits or sets of characters may not share a common evolutionary history in terms of the species phylogeny or differ with respect to the amount of homoplasy associated with each type of data. In such cases, combining highly heterogeneous data may result in an inaccurate representation of relationships among species. Bull et al. (1993) have argued that prior to combining datasets one should test for homogeneity among the different sets of characters. If individual datasets demonstrate considerable heterogeneity and produce highly incongruent phylogenies, then the data should not be combined. They suggest that these character sets may have different evolutionary histories, be evolving at different evolutionary rates, and have different levels of associated homoplasy. For example, individual gene trees, derived from independently evolving loci, may not reflect the phylogeny of species. Such a result is likely when comparing multiple nuclear-gene loci (Moore, 1995). Incongruence among phylogenies derived from different mitochondrial genes may be a result of differences in levels of constraint, amounts of homoplasy, and rates of nucleotide substitution, because the mitochondrial genome is evolving as a single locus (Miyamoto et al., 1994). Morphological data also can show different degrees of homoplasy associated with particular suites of characters as a result of primarily parallel and convergent evolution.

To avoid some of the potential problems with combining the morphological and molecular data, an analysis was conducted using mitochondrial gene sequences (12S, 16S, and cytochrome *b*) to examine relationships among 31 taxa of carnivores obtained from the present study and Vrana et al. (1994). A single most-parsimonious tree was obtained (Fig. 3). The result is congruent with both the analysis of the 12S and 16S genes (Fig. 1) and the total-evidence approach (Fig. 2), except that several nodes received less support as indicated by the low bootstrap values and decay indices. The resultant phylogeny clearly demonstrates more resolution than seen by the consensus analyses performed earlier. As suggested by Kluge (1989) and Eernisse and Kluge (1993), combining all of the characters, regardless of the degree to which they differ in terms of homoplasy, results in a consistent phylogeny that, for the most part, is identical to the molecular analysis.

A potential problem with combining sets of characters is missing data. This is a common occurrence when analyzing both extant and extinct taxa in a combined analysis that includes morphological and molecular data. In such an analysis, the extinct taxa will have missing data associated with all the molecular characters. According to Swofford (1993), only those characters that have non-missing values will affect the location of any taxon on the tree. Nevertheless, there is an indication that missing data can be problematical (Platnick et al., 1991), especially when taxa have large amounts of missing data.

Wyss and Flynn's (1993) analysis of extant and extinct carnivores produced a phylogeny in which the extinct Desmatophocidae was the sister group to Phocidae. In the total-evidence analysis (Fig. 2), the Desmatophocidae was at the base of the pinniped radiation. Only 38 morphological characters were needed to place the desmatophocid on the tree, because of a number of synapomorphies with the Phocidae. However, 1,500 characters were used to place Phocidae on the same tree (Fig. 2). Due to the close relationship among the other pinnipeds, the larger number of synapomorphies in the molecular data swamped the morphological data so that the phocids became the sister group to the otariid and odobenid clade rather than to the desmatophocids.

In contrast to the apparent problems associated with taxa represented only by morphological characters in the total-evidence

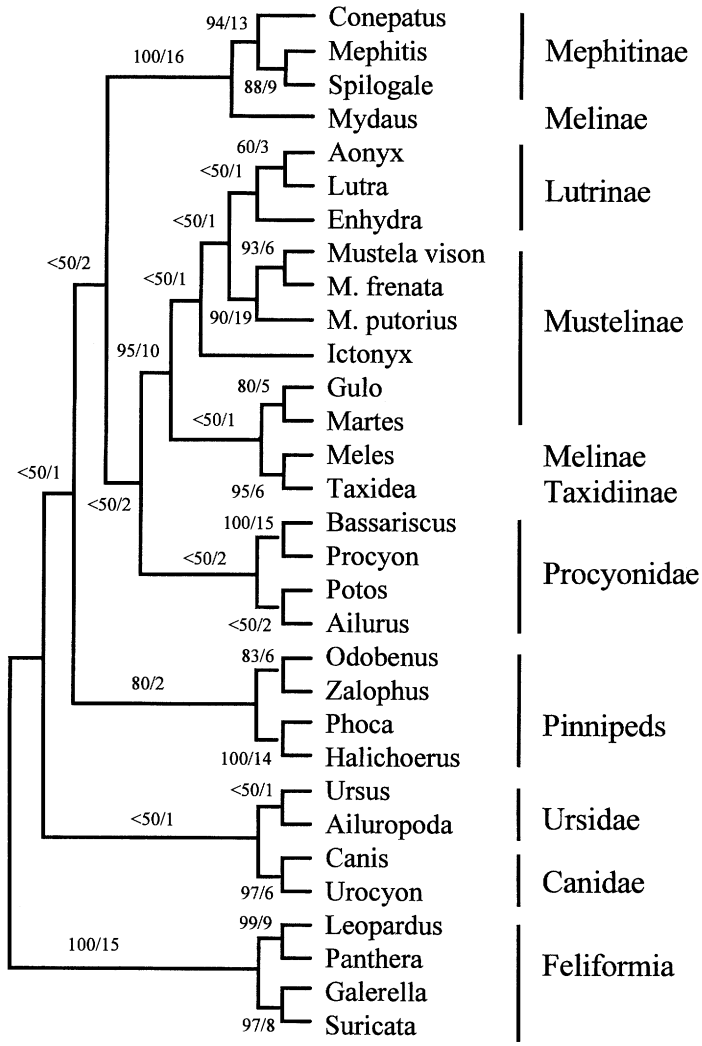

FIG. 3.—Phylogenetic relationships among 31 taxa of carnivores based on parsimony analysis (tree length 2,429; CI = 0.363; RI = 0.437) of the 12S, 16S, and cytochrome *b* gene sequences. Some taxa have partial data. Numbers represent bootstrap values/decay indices.

analysis, there appears to be less sensitivity to missing data associated with the genes examined in this study. To examine the affects of missing data, a parsimony analysis (Fig. 4) was performed using the 12S, 16S, and cytochrome *b* datasets for the three North American genera of skunks, *Mustela frenata* and *M. vison,* the three genera of otters, *Ictonyx,* and Canidae. The tree topology (Fig. 4A) is similar to that of the 12S and 16S analysis (Fig. 1). *Conepatus* was the sister group to the clade formed by *Mephitis* and *Spilogale.* This clade was joined to the clade containing the paraphyletic Mustelinae and the monophyletic Lutrinae. A second analysis (Fig. 4B) was performed on the datasets where the cytochrome *b* data (ca. 25% of the data) for *Mephitis, Mustela vison, Ictonyx,* and *Lutra* were scored as missing. These taxa were chosen because of the varying amounts of support for their placement on the tree shown in Fig. 1. The single most-parsimonious tree had lower bootstrap values and support indices, com-

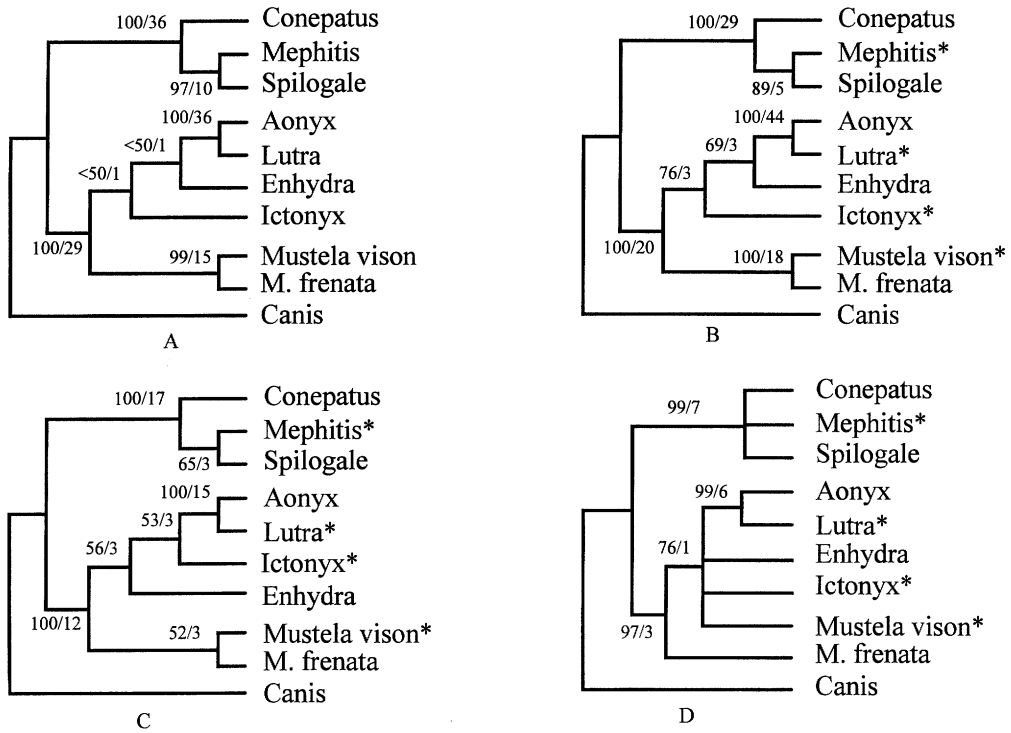

FIG. 4.—The most-parsimonious trees obtained from analyses of datasets containing various amounts of missing data for: A) complete dataset consisting of 12S, 16S, and cytochrome *b* sequences; B) cytochrome *b* sequence scored as missing (*) for *Mephitis, Lutra, Ictonyx,* and *Mustela vison;* C) cytochrome *b* and 16S sequence scored as missing for the same taxa; D) all, but ca. 200 base pairs, of the 12S sequence scored as missing. Numbers represent bootstrap values/decay indices.

pared to the first analysis (Fig. 4A), for the *Mephitis-Spilogale* clade, yet higher support for the placement of *Ictonyx* and *Enhydra.* Next, the 16S data was scored as missing for the same taxa (Fig. 4C). A single most-parsimonious tree was found and the bootstrap values and support indices were lower still, compared to the first two analyses (Figs. 4A and 4B), for the *Mephitis-Spilogale* clade, as well as the *Mustela* clade; the placement of *Ictonyx* and *Enhydra* also changed positions on the tree, resulting in a paraphyletic Lutrinae. When data were scored as missing for all but ca. 200 base pairs of the 12S gene for the four taxa, eight equally parsimonious trees were found and the resulting topology (Fig. 4D) consisted of a polytomy for the skunks and a polytomy for *M. vison, Ictonyx, Enhydra,* and the other two otters.

The above results suggest that the severity of the effects from missing data may relate more to the relationship of the taxa involved and the phylogenetic signal in the data than to the amount of missing data. For example, if several taxa from a strongly supported monophyletic group, such as *Mustela* or the river otters, are included in an analysis, missing data from a single taxon may not affect its placement. This suggests that clades supported by a large number of characters from one dataset can withstand relatively high degrees of missing data and still maintain the same phylogenetic position on the tree. A second example can be seen for members of the Feliformia examined in this study. The monophyly of this group was strongly supported by all analyses as suggested by the morphological characters reviewed by Wyss and

Flynn (1993), despite the fact that some feliform taxa had large amounts of missing data.

Missing data may be more problematical when a taxon has no close relatives, such as monotypic taxa. For example, when more and more data were recorded as missing for *Ictonyx,* the placement of this taxon became obscure. The red panda, *Ailurus,* also was difficult to place, with trees only one or two steps longer resulting in entirely different topologies to that shown in Fig. 3. In general, the addition of *Ailurus* lowered bootstrap values and decay indices for well-supported clades. Vrana et al. (1994) indicated that *Ailurus* may be an interesting taxon to examine in terms of evolution of arctoid carnivores. The red panda has been considered a procyonid (Nowak, 1991), a bear (Wozencraft, 1989), at the base of the radiation of bears and pinnipeds (Vrana et al., 1994), at the base of a clade comprised of ursids and procyonids (Zhang and Ryder, 1993), and as a separate family (Wozencraft, 1989). In an analysis of mitochondrial and nuclear-molecular data, Slattery and O'Brien (1995) found *Ailurus* to be a member of the procyonid clade. Ledje and Árnason (1996) also used mitochondrial molecular data, but suggested that *Ailurus* should be relegated to a separate family. Although the red panda was the sister taxon to *Potos* in the Procyonidae clade, in analysis of the total evidence (Fig. 2) and the analysis of the molecular data (Fig. 3) in the present study the bootstrap values and support indices were low.

Monophyly of the Mustelidae.—Most morphological studies (Bryant et al., 1993; Wozencraft, 1989; Wyss and Flynn, 1993) place skunks as sister to the mustelid subfamily Lutrinae, whereas molecular data (Árnason and Widegren, 1986; Ledje and Árnason, 1996; Vrana et al., 1994; Wayne et al., 1989), including the present study, show the skunks to be outside the mustelids. That the family Mustelidae, as currently recognized, is not monophyletic, as shown by the molecular data, suggests that the interpretation of some morphological character state changes may be compromised (Anderson, 1989; Bryant et al., 1993).

Many of the morphological studies that examined the higher-level relationships of carnivores assumed not only that the family Mustelidae was monophyletic, but that the subfamilies were monophyletic (Wozencraft, 1989; Wyss and Flynn, 1993). There are few synapomorphies to support the monophyly of the Mustelidae, including enlarged scent glands, loss of the carnassial notch on the upper fourth premolar, and the loss of the upper second molar (Bryant et al., 1993; Martin, 1989; Wozencraft, 1989). These characters, however, may be homoplasious. For example, Wozencraft (1989) suggested that the loss of the notch on the carnassial occurred more than once in independent lineages of carnivores. All carnivores have scent glands; they are enlarged in mustelids, and greatly enlarged in skunks. The association of a nipple with the scent gland, rather than a duct as in mustelids, suggests that the scent gland in skunks is apomorphic. Other characteristics shared between skunks and mustelids may represent symplesiomorphies (e.g., type of auditory bulla—Hunt, 1974).

In a recent study of the Mustelidae, Bryant et al. (1993) used 46 morphological characters and 23 extant genera of the presumed monophyletic Mustelidae to test the subfamilial relationships based on Simpson's (1945) classification. They suggested a sister-group relationship between the monophyletic subfamilies Lutrinae and Mephitinae. The subfamily Mustelinae was found to be paraphyletic, which agreed with the findings of earlier studies (Anderson, 1989; Pocock, 1921; Radinsky, 1973). The Melinae was polyphyletic; three of the five genera (*Meles, Arctonyx,* and *Mydaus*) were members of the clade containing Lutrinae and Mephitinae.

Although morphological studies (Bryant et al., 1993; Wyss and Flynn, 1993) have suggested a monophyletic Mustelidae, this is not strongly supported by their data. Only

three additional steps are required to collapse the mustelid clade derived from morphology, whereas the 12S and 16S data in the present study (Fig. 1) require an additional 11 steps to make the Mustelidae monophyletic and 15 steps to place the Mephitinae sister to the Lutrinae. The weak support for a monophyletic Mustelidae based on morphology may be due to plesiomorphic or homoplastic characters uniting the skunks and the mustelids. Bryant et al. (1993) reported that two of the shared character states were plesiomorphic and the polarity of two additional characters was uncertain. Of the five characters shared between Mephitinae and Lutrinae, four were based on tooth morphology, and the fifth (auditory bullae) was plesiomorphic.

Other data also suggest that the skunks may not be a member of the Mustelidae. Based on chromosomal evidence (Wurster and Benirschke, 1968), the Mustelidae is at the base of the Caniformia radiation. Diploid and fundamental numbers are variable within Mustelidae, compared to the rest of the Carnivora, and they noted that the skunks had a unique karyotype relative to the mustelids. Árnason and Widegren (1986) examined the relationship of the pinnipeds using DNA hybridization of highly repetitive DNA components and found that the pinnipeds were monophyletic and more closely related to the Mustelidae (not including the Mephitinae) than to any other carnivore family. The pinnipeds and the procyonids were more genetically similar to the Mustelidae than was the subfamily Mephitinae. These observations are congruent with the 12S and 16S rRNA gene data presented here. Parsimony analyses of the 12S and 16S data in this study suggest that the family Mustelidae is not monophyletic, with the skunks being more divergent from the rest of the mustelids than the family Procyonidae. Our data also provide support for the Melinae being diphyletic with *Mydaus* being the sister group to skunks, and the American and European badgers as the sister group to the other mustelids. Although the remaining mustelid taxa form a monophyletic group, the subfamily Mustelinae is paraphyletic in relation to the Lutrinae.

The results of the present study, based on molecular data and total-evidence data, support the paraphyly of the Mustelidae. How can the patterns of morphological evolution in skunks and mustelids be explained with respect to our results? Part of the answer may be obtained by considering existing information from the fossil record. The earliest Carnivora, which first appeared during the late Paleocene-early Eocene, were small, arboreal, viverrid-like (or weasel-like) forms belonging to the extinct Viverravidae and Miacidae (Martin, 1989). According to Anderson (1989) and Martin (1989), during the late Eocene and early Oligocene these forms gave rise to the Caniformia (Mustelidae, Canidae, Procyonidae, and Ursidae) and the Feliformia (Felidae, Viverridae, and Hyaenidae).

The early mustelid-like forms that appeared in the late Eocene cannot be traced to the modern mustelids (Kurtén and Anderson, 1980), which first appeared in the Old World during the mid-Miocene (Kurtén and Anderson, 1980; Martin, 1989). *Miomephitis,* one of the first recognizable skunks (Anderson, 1989; Kurtén and Anderson, 1980), retained many of the plesiomorphic traits associated with the Eocene mustelid-like carnivores. Radinsky (1973) suggested that most of the earliest known modern mustelids (20–25 $\times$ 10^7 years ago) exhibit several advanced cranial features compared with modern skunks and the stink badger. In addition, Wayne et al. (1989) suggested that the origin of the skunk lineage occurred in the Oligocene (ca. 40 $\times$ 10^7 years ago), which is prior to the appearance in the fossil record of the musteloid stem group that gave rise to the Procyonidae. Because mustelids, in general, and skunks in particular, retain many plesiomorphic traits (Anderson, 1989), it is difficult to find synapomorphies for a monophyletic Mustelidae as well as

other groups of mustelid-like carnivores. As a result, the Mustelidae (especially the Mustelinae) has been a catch-all category for many of the early, undifferentiated taxa as well as divergent genera of doubtful affinity (Anderson, 1989).

Taxonomic considerations.—Although the relationships among some lineages of carnivores are uncertain, the results of the present study, as well as other recent molecular studies (Árnason and Widegren, 1986; Ledje and Árnason, 1996; Vrana et al., 1994; Wayne et al., 1989) suggest a need for some changes in the classification of caniform carnivores. If the Mustelidae are to be treated as a monophyletic group, then the skunks need to be reclassified. The solution is to elevate the subfamily, Mephitinae (Bonaparte, 1845), to a distinct family, the Mephitidae. This family includes the extant genera *Mephitis* (the striped and hooded skunks), *Conepatus* (the hog-nosed skunks), *Spilogale* (the spotted skunks), and *Mydaus* (the stink badger).

ACKNOWLEDGMENTS

We thank the following people who have contributed DNA or tissue samples from various taxa of carnivores; J. Cooke, J. W. Bickham, P. B. Vrana, L. A. Ruedas, R. K. Wayne, J. C. Patton, T. L. Yates, D. Schlitter, R. J. Baker, M. Tewes, and K. Rautenbach. For help in the laboratory, technical assistance, data analyses, and financial support, we thank R. D. Bradley, J. Derr, M. Smolen, T. Lee, P. Lee, L. A. Ruedas, J. Templeton, K. Rittenhouse, R. Adkins, M. Nedbal, A. Walton, J. Rice, C. Walker, J. Patton, and J. Wickliffe. Finally, we thank G. A. Dragoo, T. Taylor, S. Davis, D. Schmidly, J. Bickham, J. McEachran, and two anonymous reviewers for reviewing earlier drafts of this manuscript. This research was supported by grants from the National Science Foundation to J. W. Bickham, R. L. Honeycutt, and J. W. Dragoo (DEB-9310553), and to R. L. Honeycutt (DEB-9208022). Data analyses were performed at the Center for Biosystematics and Biodiversity, a facility funded in part by the National Science Foundation (DIR-8907006). This paper represents contribution 58 of the center.

LITERATURE CITED

ANDERSON, E. 1989. The phylogeny of mustelids and the systematics of ferrets. Pp. 10–20, *in* Conservation biology and the biology of the black-footed ferret (U. S. Seal, E. T. Thorne, M. A. Bogan, and S. H. Anderson, eds.). Yale University Press, New Haven, Connecticut, 209 pp.

ÁRNASON, Ú., AND E. JOHNSSON. 1992. The complete mitochondrial DNA sequence of the harbor seal, *Phoca vitulina*. Journal of Molecular Evolution, 34:493–505.

ÁRNASON, Ú., AND B. WIDEGREN. 1986. Pinniped phylogeny enlightened by molecular hybridizations using highly repetitive DNA. Molecular Biology and Evolution, 3:356–365.

BARRETT, M., M. J. DONOGHUE, AND E. SOBER. 1991. Against consensus. Systematic Zoology, 40:486–493.

BONAPARTE, C.-L. J. L. 1845. Catalogo methodico dei mammiferi Europei. L. di Giacomo Pirola, Milano, Italy, 36 pp.

BREMER, K. 1988. The limits of amino acid sequence data in Angiosperm phylogenetic reconstruction. Evolution, 42:795–803.

BRYANT, H. N., A. P. RUSSELL, AND W. D. FITCH. 1993. Phylogenetic relationships within the extant Mustelidae (Carnivora): appraisal of the cladistic status of the Simpsonian subfamilies. Zoological Journal of the Linnean Society, 108:301–334.

BULL, J. J., P. HUELSENBECK, C. W. CUNNINGHAM, D. L. SWOFFORD, AND P. J. WADDELL. 1993. Partitioning and combining data in phylogenetic analysis. Systematic Biology, 42:384–397.

COUES, E. 1877. Fur-bearing animals: a monograph of North American Mustelidae. Miscellaneous Publications of the Department of the Interior, United States Geological Survey, Washington, D.C., 8:1–348.

DE QUEIROZ, A. 1993. For consensus (sometimes). Systematic Biology, 42:368–372.

DRAGOO, J. W., R. D. BRADLEY, R. L. HONEYCUTT, AND J. W. TEMPLETON. 1993. Phylogenetic relationships among the skunks: a molecular perspective. Journal of Mammalian Evolution, 1:225–267.

EERNISSE, D. J., AND A. G. KLUGE. 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules, and morphology. Molecular Biology and Evolution, 10:1170–1195.

EISENBERG, J. F. 1989. An introduction to the Carnivora. Pp. 1–9, *in* Carnivore behavior, ecology, and evolution (J. L. Gittleman, ed.). Comstock Publishing Associates, Ithaca, New York, 620 pp.

FARRIS, J. S. 1989. The retention index and the rescaled consistency indes. Cladistics, 5:417–419.

FELSENSTIEN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39:783–791.

FLYNN, J. J., N. A. NEFF, AND R. H. TEDFORD. 1988. Phylogeny of the Carnivora. Pp. 73–116, *in* The phylogeny and classification of the tetrapods (M. J. Benton, ed.). Oxford University Press, New York, 2:1–329.

HIGGINS, D. G., AND P. M. SHARP. 1989. Fast and sen-

sitive multiple sequence alignments on a microcomputer. Cagios, 5:151–153.

HILLIS, D. M. 1987. Molecular versus morphological approaches to systematics. Annual Review of Ecology and Systematics, 18:23–42.

HILLIS, D. M., A. LARSON, S. K. DAVIS, AND E. A. ZIMMER. 1990. Nucleic acids III: sequencing. Pp. 318–370, *in* Molecular systematics (D. M. Hillis and C. Moritz, eds.). Sinauer Associates, Inc., Sunderland, Massachusetts, 588 pp.

HUNT, R. M., JR. 1974. The auditory bulla in Carnivora: an anatomical basis for reappraisal of carnivore evolution. Journal of Morphology, 143:21–76.

JANCZEWSKI, D. N., N. YUHKI, D. A. GILBERT, G. T. JEFFERSON, AND S. J. O'BRIEN. 1992. Molecular phylogenetic inference from saber-tooth cat fossils of Rancho La Brea. Proceedings of the National Academy of Sciences, 89:9769–9773.

KLUGE, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Systemic Zoology, 38:7–25.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phylogenetics and the evolution of anurans. Systematic Zoology, 18:1–32.

KUMAR, S., K. TAMURA, AND M. NEI. 1993. MEGA: molecular evolutionary genetics analysis, version 1.01. The Pennsylvania State University, University Park, 130 pp.

KURTÉN, B., AND E. ANDERSON. 1980. Pleisticene mammals of North America. Columbia University Press, Irvington, New York, 442 pp.

LEDJE, C., AND Ú. ÁRNASON. 1996. Phylogenetic analyses of complete cytochrome *b* genes of the order Carnivora with particular emphasis on the Caniformia. Journal of Molecular Evolution, 42:135–144.

LEDOUX, R. G., AND A. J. KENYON. 1975. Protides of the Mustelidae-II. Immunologic relatedness. Comparative Biochemistry and Physiology, A. Comparative Physiology, 51:213–217.

LENTO, G. M., R. E. HICKSON, G. K. CHAMBERS, AND D. PENNY. 1995. Use of spectral analysis to test hypotheses on the origin of pinnipeds. Molecular Biology and Evolution, 12:28–52.

LOCKHART, P. J., M. A. STEEL, M. D. HENDY, AND P. DAVID. 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. Molecular Biology and Evolution, 11:605–612.

MARTIN, L. D. 1989. Fossil history of the terrestrial Carnivora. Pp. 536–568, *in* Carnivore behavior, ecology, and evolution (J. L. Gittleman, ed.). Comstock Publishing Associates, Ithaca, New York, 620 pp.

MIYAMOTO, M. M. 1985. Consensus cladograms and general classifications. Cladistics, 1:186–189.

MIYAMOTO, M. M., AND M. GOODMAN. 1986. Biomolecular systematics of eutherian mammals: phylogenetic patterns and classification. Systematic Zoology, 35:230–240.

MIYAMOTO, M. M., F. KRAUS, AND O. A. RYDER. 1990. Phylogeny and evolution of antlered deer determined from mitochondrial DNA sequences. Proceedings of the National Academy of Sciences, 87:6127–6131.

MIYAMOTO, M. M., M. W. ALLARD, R. M. ADKINS, L. L. JANECEK, AND R. L. HONEYCUTT. 1994. A congruence test of reliability using linked mitochondrial DNA sequences. Systematic Biology, 43:236–249.

MOORE, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution, 49:718–726.

NEI, M. 1991. Relative efficiencies of different tree making methods for molecular data. Pp. 90–128, *in* Phylogenetic analysis of DNA sequences (M. M. Miyamoto and J. L. Cracraft, eds.). Oxford University Press, New York, 358 pp.

NOWAK, R. M. 1991. Walker's mammals of the world. Fifth ed. The Johns Hopkins University Press, Baltimore, 2:643–1629.

O'BRIEN, S. J., J. S. MARTENSON, M. A. EICHELBERGER, E. T. THORNE, AND F. WRIGHT. 1989. Genetic variation and molecular systematics of the black-footed ferret. Pp. 21–33, *in* Conservation biology and the biology of the black-footed ferret (U. S. Seal, E. T. Thorne, M. A. Bogan, and S. H. Anderson, eds.). Yale University Press, New Haven, Connecticut, 209 pp.

OLMSTEAD, R. G., AND J. A. SWEERE. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Systematic Biology, 43:467–481.

PETTER, G. 1971. Origine, phylogenie et systematique des blaireaux. Mammalia, 35:567–597.

PLATNICK, N. I., C. E. GRISWOLD, AND J. A. CODDINGTON. 1991. On missing entries in cladistic analysis. Cladistics, 7:337–343.

POCOCK, R. I. 1921. On the external characters and classification of the Mustelidae. Proceedings of the Zoological Society of London, 1921:803–837.

RADINSKY, L. 1973. Are stink badgers skunks? Implications of neuroanatomy for mustelid phylogeny. Journal of Mammalogy, 54:585–593.

SAIKI, R. K., T. L. BUGAWAN, G. T. HORN, K. B. MULLIS, AND H. A. ERLICH. 1986. Analysis of enzymatically amplified beta-globin and HLA-DQalpha DNA with allele-specific oligonucleotide probes. Nature, 324:163–166.

SAIKI, R. K., ET AL. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239:487–491.

SARICH, V. M. 1969. Pinniped phylogeny. Systematic Zoology, 18:416–422.

SETON, E. T. 1926. Lives of game animals. Doubleday, Doran & Company, Inc., Garden City, New York, 746 pp.

SIMPSON, G. G. 1945. The principles of classification and a classification of mammals. Bulletin of the American Museum of Natural History, 85:1–350.

SLATTERY, J. P., AND S. J. O'BRIEN. 1995. Molecular phylogeny of the red panda (*Ailurus fulgens*). Journal of Heredity, 86:413–422.

SPRINGER, M. S., L. J. HOLLAR, AND A. BURK. 1995. Compensatory substitutions and the evolution of the mitochondrial 12S rRNA gene in mammals. Molecular Biology and Evolution, 12:1138–1150.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1.1. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois, 264 pp.

TAJIMA, F., AND M. NEI. 1984. Estimation of evolutionary distance between nucleotide sequences. Molecular Biology and Evolution, 1:269–285.

VRANA, P. B., M. C. MILINKOVITCH, J. R. POWELL, AND W. C. WHEELER. 1994. Higher level relationships of the arctoid Carnivora based on sequence data and "total evidence." Molecular Phylogenetics and Evolution, 3:47–58.

WAYNE, R. K., R. E. BENVENISTE, D. N. JANCZEWSKI, AND S. J. O'BRIEN. 1989. Molecular and biochemical evolution of the Carnivora. Pp. 465–494, *in* Carnivore behavior, ecology, and evolution (J. L. Gittleman, ed.). Comstock Publishing Associates, Ithaca, New York, 620 pp.

WILSON, D. E., AND D. M. REEDER (EDS). 1993. Mammal species of the world: a taxonomic and geographic reference. Second ed. Smithsonian Institution Press, Washington, D.C., 1206 pp.

WOZENCRAFT, W. C. 1989. The phylogeny of the Recent Carnivora. Pp. 495–535, *in* Carnivore behavior, ecology, and evolution (J. L. Gittleman, ed.). Comstock Publishing Associates, Ithaca, New York, 620 pp.

———. 1993. Carnivora. Pp. 279–348, *in* Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). Smithsonian Institution Press, Washington, D. C., 1206 pp.

WURSTER, D. H., AND K. BENIRSCHKE. 1968. Comparative cytogenetic studies in the order Carnivora. Chromosoma (Berlin), 24:336–382.

WYSS, A. R., AND J. J. FLYNN. 1993. A phylogenetic analysis and definition of the Carnivora. Pp. 32–52, *in* Mammal phylogeny: placentals (F. S. Szalay, M. J. Novacek, and M. C. McKenna, eds.). Springer-Verlag, New York, 321 pp.

ZHANG, Y., AND O. A. RYDER. 1993. Mitochondrial DNA sequence evolution in the Arctoidea. Proceedings of the National Academy of Science, 90:9557–9561.

Submitted 9 December 1995. Accepted 16 July 1996.

Associate Editor was Janet K. Braun.