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Higher Level Relationships of the Arctoid Carnivora Based on Sequence Data and "Total Evidence"

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The relationships of the lesser or red panda, *Ailurus*, have remained elusive even as any doubts about the identity of the giant panda as a bear have been erased. While usually classified as a member of the Procyonidae (raccoons), recent anatomical studies have suggested that the red panda may not fall in any of the arctoid carnivore families but instead may reflect an early offshoot of the lineage leading to ursids (bears) and pinnipeds (seals, sea lions, and walruses). Sequence data from the cytochrome *b* and 12S genes for multiple representatives of all relevant families support this hypothesis. Such a systematic position makes this threatened species particularly worthy of conservation. Sequence data alone, as well as a combined analysis of the sequence and anatomical data, strongly support a single origin of pinnipeds and their aquatic adaptations, lending some resolution to the general disagreement about familial relationships in this group. These molecular data also support canids as the basal members of this caniform clade, but are unresolved with respect to whether mustelids or procyonids constitute the sister group to the (ursid, pinniped, *Ailurus*) clade. There is support for the notion that skunks are a genetically divergent and possibly nonmustelid lineage.

INTRODUCTION

The identity of and relationships among mammalian carnivore families have been hotly debated over the last two centuries (for accounts of the history see Flynn and Galliano, 1982; Wozencraft, 1989a,b). While the order was originally conceived as an order comprising all carnivorous mammals, some members are secondarily omnivorous or even herbivorous. Carnivora is now defined as a natural (monophyletic) group comprising those animals (and their descendants) which have, among prominent features, modified the upper fourth premolar and the lower first molar to shear flesh (Flynn and Galiano, 1982). The order is now accepted to be composed of two major monophyletic lineages, the Caniformia (=Arctoidea+Canidae) and the Feloidea (=Feliformia). The former includes the raccoons and allies (Procyonidae), skunks, otters, weasels, and relatives (Mustelidae), dogs and foxes (Canidae), bears (Ursidae), seals (Phocidae), sea lions or "eared seals" (Otariidae), and the walrus (Obobenidae). The latter group comprises the cats proper (Felidae), hyenas (Hyaenidae), mongooses (Herpestidae), and civets (Viverridae). The arctoid clade is more speciose and morphologically diverse (given its aquatic members), and hence has had the greater number of controversial issues surrounding its internal phylogeny.

Among arctoid groups, placement of the two "panda" species, the giant panda, *Ailuropoda melanoleuca*, and the Red or Lesser panda, *Ailurus fulgens*, has caused considerable controversy in recent years. While placement of the giant panda as a bear is well-established (Davis, 1964; O'Brien *et al.*, 1985; and note that recent morphological studies of arctoid phylogeny find no discrepancy between *Ailuropoda* and other bears in terms of characters which define that family), support for a definitive placement of *Ailurus* has proved to be more equivocal: O'Brien *et al.* (1985) and Wayne *et al.* (1989) grouped *Ailurus* with the Procyonidae, as have many traditional workers (e.g., Nowak and Paradiso, 1991). *Ailurus* has a superficial resemblance to the American raccoon (*Procyon*) and shares several dental similarities. The basicranial anatomy, however, appears quite different: Flynn *et al.* (1988) found *Ailurus* to be an arctoid of uncertain affinities, while Wozencraft (1989a) grouped it with the Ursidae. Still others (e.g., Schaller *et al.* 1985) have considered the two pandas to constitute a separate arctoid family. While there do not appear to be any characteristics that link *Ailurus* to *Ailuropoda* in particular (other than habitat and

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diet), others have found evidence of ursid (and otariid) affinities (Ginsburg, 1982). Wyss and Flynn's recent treatise (1993) on carnivore phylogeny, which incorporated all known anatomical characters (including those of Wozencraft, 1989a), placed *Ailurus* in a peculiar phylogenetic position—not as sister to any one family but to a group that includes pinnipeds and bears (termed Ursida). If so, this would mark a rare familial reassessment of a mammalian species. Molecular support for such an idea is somewhat limited; a cladistic study of hemoglobin amino acid sequences supported placing the red panda as sister to bears relative to two other procyonids, but included only one pinniped sample (Czelusniak *et al.,* 1990).

The inclusion of the pinnipeds (Phocidae, Otariidae, Odobenidae) in the Carnivora generally, and Arctoidea specifically, has gained wide acceptance over the past two decades. However, their exact placement, questions of their monophyly, and relationships to one another have been less firmly established. Numerous authors have espoused independent origins for the phocids and otariids (e.g., Tedford, 1976; Wozencraft, 1989a), most often grouping odobenids as sister to the latter. Seals were thought to have arisen from an early otter/mustelid branch, while sealions have been grouped as sister to the bears on the basis of a host of anatomical similarities. This view has been challenged in recent years by Wyss, Flynn, and co-workers (Wyss, 1987; Wyss, 1988a; Wyss, 1989; Flynn *et al.,* 1988; Berta *et al.,* 1989; Wyss and Flynn, 1993), who have suggested that pinniped monophyly is the more compelling argument. In particular, they have noted that (among other adaptive features) the similarities in flipper structure cannot be dismissed as characters in a phylogenetic analysis. Several lines of molecular data (albeit largely phenetic–gross similarity data) including immunological distance, karyology, degrees of DNA probe (of a repetitive sequence), and hybridization have also made a case for a single-pinniped origin, though with no consensus on their placement within Caniformia (Sarich, 1969; Fay *et al.,* 1967; Arnason, 1974; Arnason and Widegren, 1986). Parsimony analyses of amino acid sequences of single or multiple polypeptides have supported pinniped monophyly (e.g., de Jong, 1982, 1993; Miyamoto and Goodman, 1986; McKenna, 1987), but have also disagreed on placement.

Other disputed issues in arctoid phylogeny include the affinities of the Odobenidae within the Pinnipedia, the possible sister-taxon relationship between the Procyonidae and Mustelidae, and the monophyly of the latter, the most speciose carnivore family. Additionally, there are several fossil taxa (e.g., the Amphicyonidae or "bear-dogs"—the putative ursid sister group, the Desmatophocidae—an extinct pinniped group, and the Nimravidae—a particularly enigmatic group which may be basal feliforms or caniforms) which may play key roles in understanding arctoid phylogeny (Flynn and Galiano, 1982; Wyss and Flynn, 1993). While we have included these three groups in our analysis, we have not included several other groups, the Miacidae, the Viverravidae, and the "order" Creodonta, as these groups (the latter two in particular) are thought to fall outside the modern carnivore radiation. Miacidae, however, may contain basal members of both Caniformia and Feliformia, but has not been adequately dissected (Flynn and Galiano, 1982; Wyss and Flynn, 1993).

Herein, we present sequence data from two mitochondrial genes, small ribosomal subunit (12S) and cytochrome *b* (cyt b), which address these questions—particularly placement of the red panda and the issue of pinniped monophyly. Both 12S and cytochrome *b* have been shown to be useful for elucidating some mammalian intra-ordinal relationships (Irwin *et al.,* 1991; Kraus and Miyamoto, 1991; Milinkovitch *et al.,* 1993). Use of sequences from multiple genes also seems to us preferable to more data from any one locus, so as to avoid any gene-specific peculiarities. In other words, a signal found through an analysis of multiple genes would seem less likely to be an artifact of convergent molecular evolution. We have also utilized a "total evidence" (Kluge, 1989) approach wherein the sequence data were analyzed in conjunction with the anatomical data of Wyss and Flynn (1993), the most comprehensive carnivore data set to date. Such an approach not only takes advantage of all pertinent information, but also lets one include taxa for which molecular data are difficult to obtain (e.g., fossils).

MATERIALS AND METHODS

DNA Isolation

Total genomic DNA was isolated by conventional methods. Tissue samples of various types (Table 1) were ground with a mortar and pestle under liquid nitrogen. The resulting powder was placed in a homogenization buffer containing 10 mM Tris, 25 mM EDTA, 0.5% SDS, 100 mM NaCl, and 0.1 mg/ml proteinase K. After 3+ h incubation with agitation at 55°C, the standard series of phenol/chloroform extractions, ethanol precipitation, and resuspension in TE buffer (10 mM Tris, 1 mM EDTA) followed. Frozen blood samples were treated identically, with the exception that they were thawed and deposited directly into the above buffer.

Amplification

A single-stranded template suitable for sequencing was prepared for cytochrome *b* samples using the methods described by Allard *et al.* (1991). Briefly, this entails performing a double-stranded (ds) reaction for a limited number (i.e., 25–30) of PCR cycles, using that product as a template for a PCR involving only one of the original primers and a higher annealing

TABLE 1

Carnivore Taxa Used in This Study

Family	English	Latin	Tissue	Source
Soricidae	Shortail shrew	*Blarina brevicauda*	Organ	Authors
Felidae	Domestic cat1	*Felis domesticus*	Testis	Authors
	Domestic cat2	*Felis domesticus*	—	Lit.3
	Snow leopard	*Panthera uncia*	Blood	NYZS
	Lion	*Panthera leo*	—	Lit.3
Herpestidae	Meerkat	*Suricata suricatta*	Liver	NYZS
Canidae	Coyote	*Canis latrans*	Muscle	Authors
	Red fox	*Vulpes vulpes*	Muscle	Authors
	Domestic dog	*Canis familiaris*	—	Lit.3
	Blackback jackal	*Canis mesomelas*	—	Lit.3
Mustelidae	Mink1	*Mustela vison*	Organ	Authors
	Mink2	*Mustela vison*	Muscle	Authors
	Longtailed weasel	*Mustela frenata*	Muscle	Authors
	American badger	*Taxidea taxus*	Muscle	Authors
	Skunk	*Mephitis mephitis*	Liver	Authors
	River otter	*Lutra canadensis*	Blood	NYZS
	Smallclaw otter	*Aonyx cinerea*	Blood	NYZS
Procyonidae	Raccoon	*Procyon lotor*	Muscle	Authors
	Ringtail	*Bassariscus astutus*	Muscle	TX A&M U.
	Kinkajou	*Potos flavus*	Blood	NYZS
	Red panda	*Ailurus fulgens* ×2	Blood	NYZS
Ursidae	Black bear1	*Ursus americanus*	Muscle	Authors
	Black bear2	*Ursus americanus*	—	Lit.1
	Brown bear	*Ursus arctos*	—	Lit.1
	Polar bear	*Ursus maritimus*	—	Lit.1
	Sloth bear	*Ursus ursinus*	—	Lit.3
	Giant panda	*Ailuropoda melanoleuca*	Plasma	NYZS
Otariidae	Calif. sea lion	*Zalophus californianus*	Lung	NYZS
Odobenidae	Walrus	*Odobenus rosmarus*	Muscle	U. Alaska
Phocidae	Harbor seal	*Phoca vitulina* ×2	Blood	NYZS;Lit.2
	Gray seal	*Halichoerus grypus*	Liver	NYZS

Note. Abbreviations: organ, whole organ mass; NYZS, New York Zoological Society; ×2, two samples of same species with identical sequences; Lit.1, sequence from Shields and Kocher, 1991; Lit.2, sequence from Arnason and Johnsson, 1992; Lit.3, sequence from Janczewski, 1992.

temperature, and subsequent purification of that single-stranded (ss) product. The primers used were those described by Kocher *et al.* (1989), which amplify a fragment 307 bp in length (i.e., 357 bp including primers). This section of the cytochrome *b* gene contains sequence coding for organelle internal, organelle external, and membrane-spanning domains, and hence is a good representative of the gene as a whole, containing a phylogenetic signal at several levels (Kocher *et al.*, 1989).

Several approaches were taken in amplifying the approximately 394-bp, 12S mtrDNA region delineated by the "universal" primers: some samples were amplified with these primers and cloned into the Invitrogen vector "PCR II." Other samples were amplified with 12S coding and the 16S ribosomal noncoding primers. These samples were either cloned into the same vector or directly sequenced. When the former approach was used, multiple clones were sequenced.

Sequencing

Cyt *b* sequencing was carried out using [^{35}S]dATP, the primers used for PCR amplification, the modified T7 DNA polymerase Sequenase (ver 2.0, U.S. Biochemical Corp.), and the accompanying reagents. Two internal sequencing primers for the cytochrome *b* fragment were also utilized to facilitate reading both strands along the entire fragment: Cytb1a, 5′ GTTACCCATATCTGCCGAG; Cytb2a, 5′ TCAGCCGTAGTTCACGTCTC. The protocol used for ss products is the same as that used for double-stranded templates: product is denatured at 100°C and kept on an ice slurry until the reactions are performed. A similar protocol was used for 12S clones except that the template was denatured at 85°C with NaOH and EDTA. This was followed by a precipitation and resuspension, annealing at 37°C for 15 min, and standard sequencing reactions. No variation was observed among "same frag-

ment" clones. For other 12S samples, the sequencing was carried out using the PRISM cycle sequencing kit (ABI) and run on the ABI 373A automated sequencer. As noted, both strands were sequenced to ensure accurate results.

Selection of Taxa

Multiple representatives of all arctoid families were obtained (Table 1), with the exception of the Otariidae and the Odobenidae (there is only one species of extant walrus). An effort was made to obtain cladistically divergent family representatives (based on prior systematic analyses) so as to better estimate the "molecular groundplan" of the group. Regardless of the practicality of this suggestion, we advocate, in general, increasing the number of taxa examined rather than the bases sequenced (when the two propositions are in conflict), as this appears to be important in accurate phylogeny reconstruction (Wheeler, 1992). Several members of the Feloidea were included as primary outgroups, and a shrew was included as a secondary outgroup as several studies have suggested insectivores may be the carnivores' nearest extant relatives (Novacek, 1986; Czelusniak *et al.*, 1990).

Data Analysis

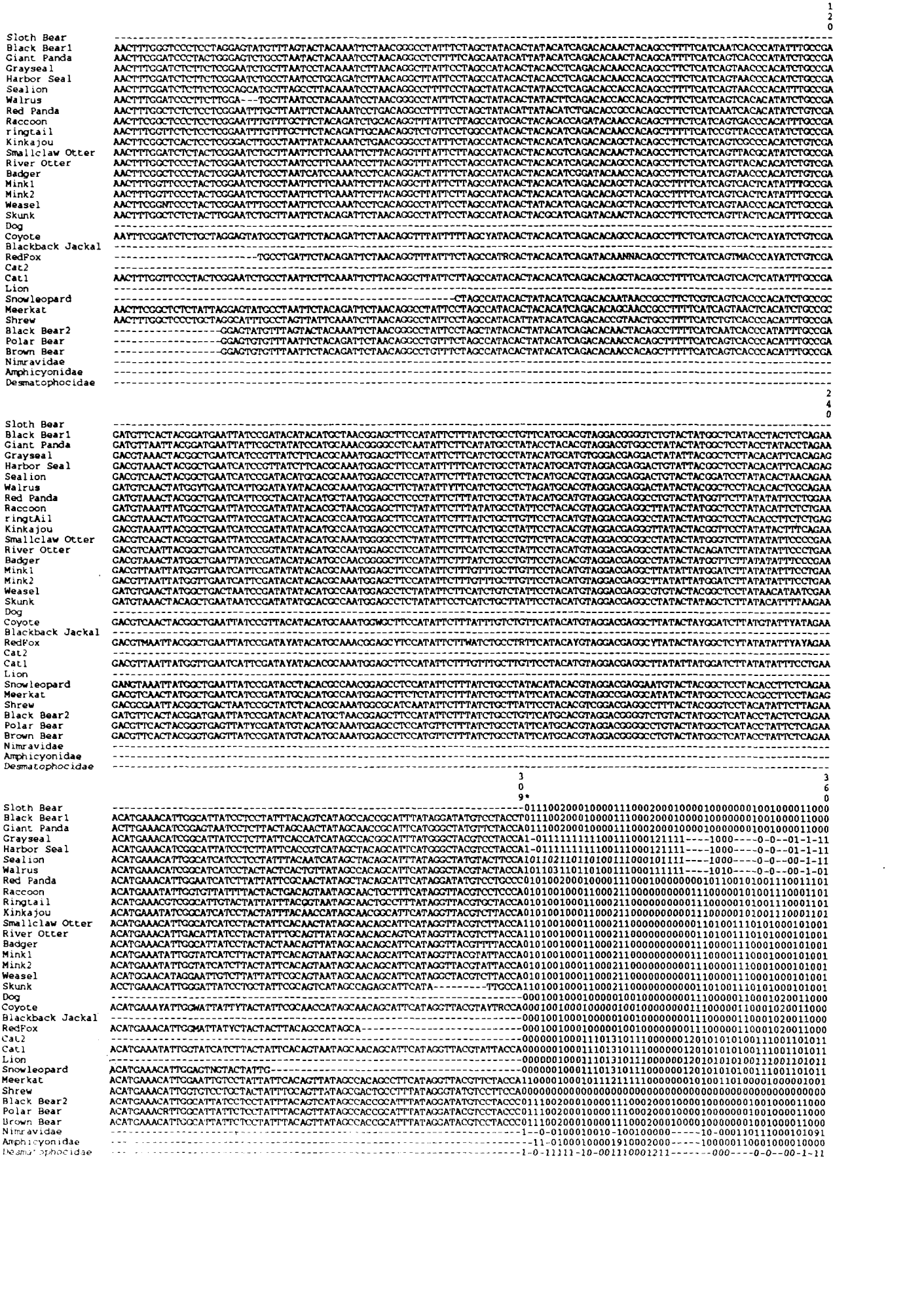
Alignment of the 12S data was done using the parsimony-based multiple alignment program MALIGN (ver. 1.85, Wheeler and Gladstein, 1993). The gap to change cost ratio used was 10:6. Parsimony analysis was performed using the program PAUP ver. 3.1.1 (Swofford, 1993) and Hennig86 ver. 1.85 (Farris, 1988). Heuristic algorithms included TBR branch swapping and mh*bb*. The cytochrome *b* and 12S sequence data set was analyzed both separately and in the same matrix as Wyss and Flynn's (1993) data set. Gaps in the aligned 12S sequences were coded as additional presence/absence characters and added to the molecular data set (interfamilial relationships were identical without these characters, but both RI—retention index—and CI—consistency index—were lower). All characters were weighted equally; however, separate runs were done for the molecular data alone with transversions weighted 2, 3, 4, 5, 9, and 10 times as much as transitions. Gap characters were treated as transversions in the weighted runs. Morphological characters were scored for each taxon used as their coding appeared (for their family) in Wyss and Flynn's matrix (fossil taxa were not included). This matrix contains only characters pertaining to higher level relationships; familial and intrafamilial synapomorphies are not included, with the exception of the Mustelidae (see Fig. 3). A separate total evidence run was performed including the three fossil groups previously mentioned. "Successive approximations" were performed on the molecular data set using mean, maximum, and minimum values of CI and RI. Sequences from either cyt *b* or 12S of pertinent taxa from other studies were scored as missing for the other gene (Fig. 1, Table 1); these taxa always grouped with other members of their respective families. Even incomplete taxa may have a major effect on phylogenetic reconstruction by showing novel character suites (e.g., Gauthier *et al.*, 1988).

RESULTS

The complete data matrix used, including aligned sequences, morphological matrix, and gaps coded as characters, is shown in Fig. 1. The cytochrome *b* data yielded 123 potentially informative (i.e., variable non-autapomorphic) sites, and the 12S sequence 125. Heteroplasmy was observed in the cytochrome *b* sequences of several taxa including the coyote (11 sites), red fox (16 sites), and walrus (3 sites). Each of these specimens was amplified and sequenced several times. This phenomenon may be due to multiple varieties of mitochondrial DNA within the organism, or alternatively to a nuclear copy, as suggested by Smith *et al.* (1992). The absence of intra-organismal variation in the 12S sequences would tend to support the latter hypothesis. Additionally, the odobenid sample shows an apparent deletion at amino position 39, noted as a "hypervariable residue" by Irwin *et al.* (1991). This translated product of this position would be predicted to fall within the inner mitochondrial membrane, as does the only amino acid insertion known in mammals, in the African elephant *Loxodonta* (Irwin *et al.*, 1991). This study also noted that the majority of putative amino replacements found in cytochrome *b* are between hydrophobic residues that fall within the membrane.

A strict consensus of all most parsimonious trees for each of the data sets, separate and combined, is shown in Figs. 2, 3, and 4. Statistics for each are shown in Table 2, including the extra steps required to place *Ailurus* with "other" procyonids, Phocids with mustelids, and several other alternative hypotheses of arctoid phylogeny. As shown (Fig. 2), the molecular data alone are highly congruent with those of the anatomy (Fig. 3; although less resolved when equal weighting is employed) and an exact match with respect to pinniped and red panda placement. Monophyly of all included families is preserved, with two notable excep-

FIG. 1. The data matrix used to construct the phylogenetic trees presented here. Characters 1–309 are the section of the cytochrome *b* gene sequenced, beginning with amino acid residue number 32, asparagine. Characters 310–373 represent the morphological characters compiled by Wyss and Flynn (1993). The 12S sequence alignment constitutes characters 374–781, and the gap characters derived from that alignment are coded as characters 782–801. Asterisks denote the start of each data set.



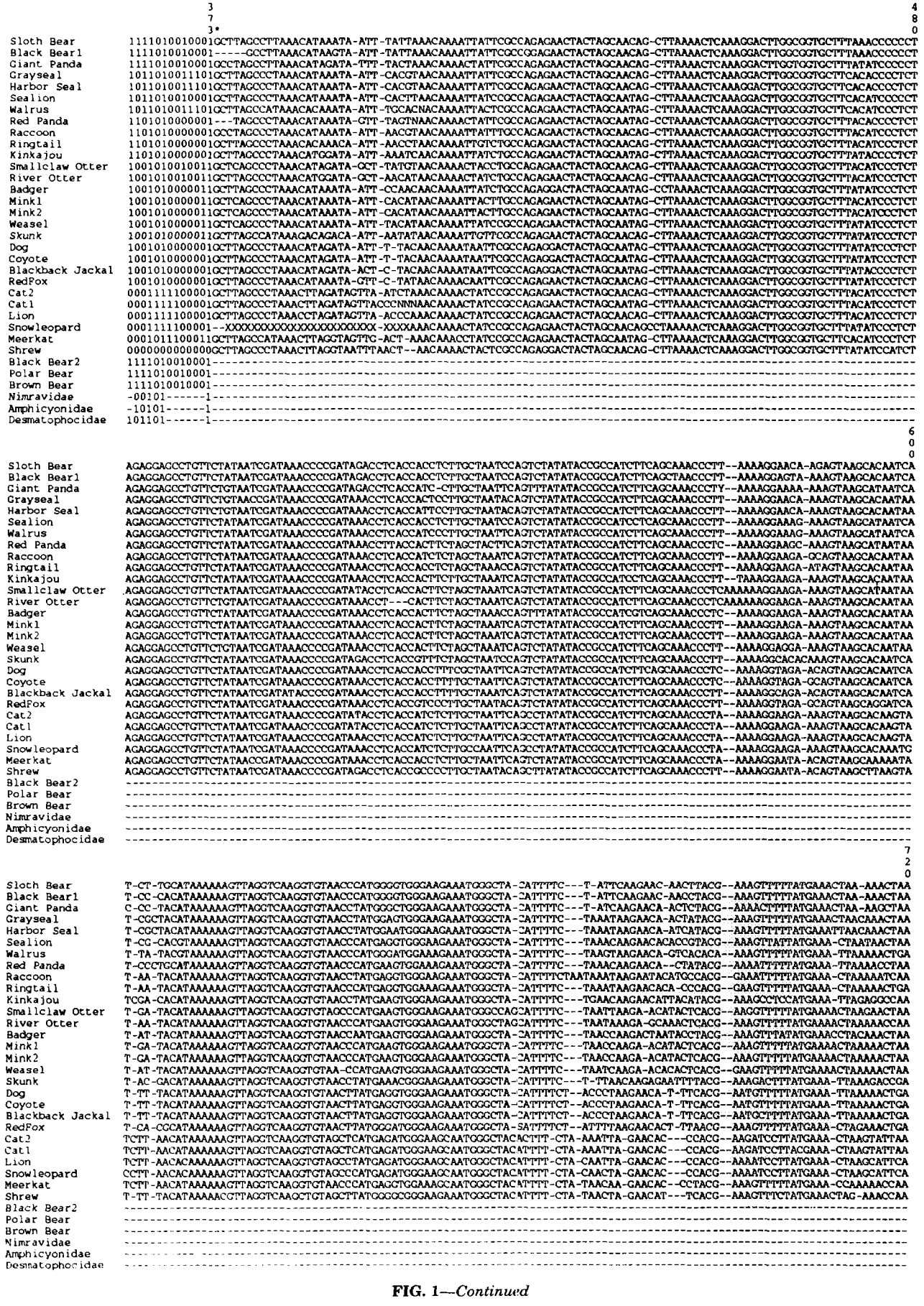


FIG. 1—*Continued*

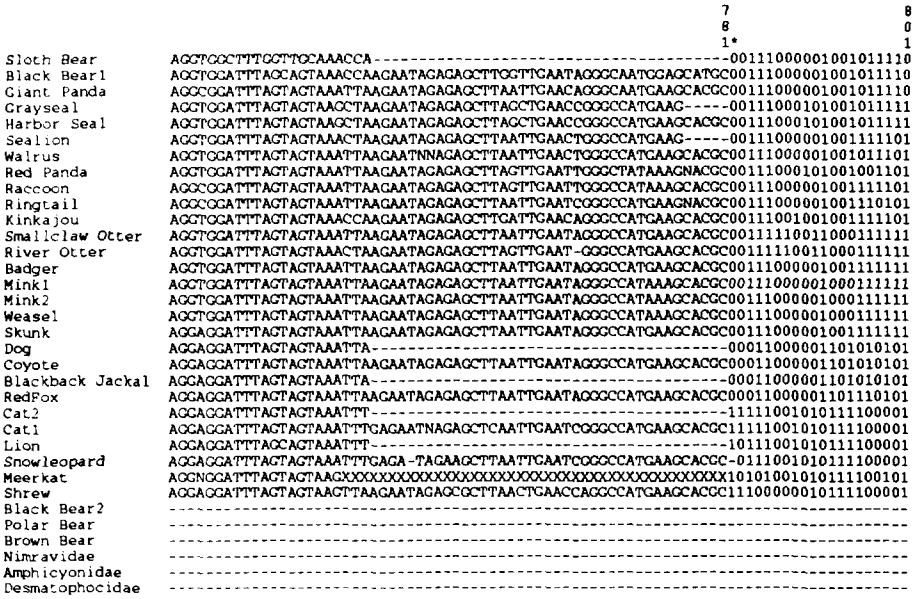


FIG. 1—*Continued*

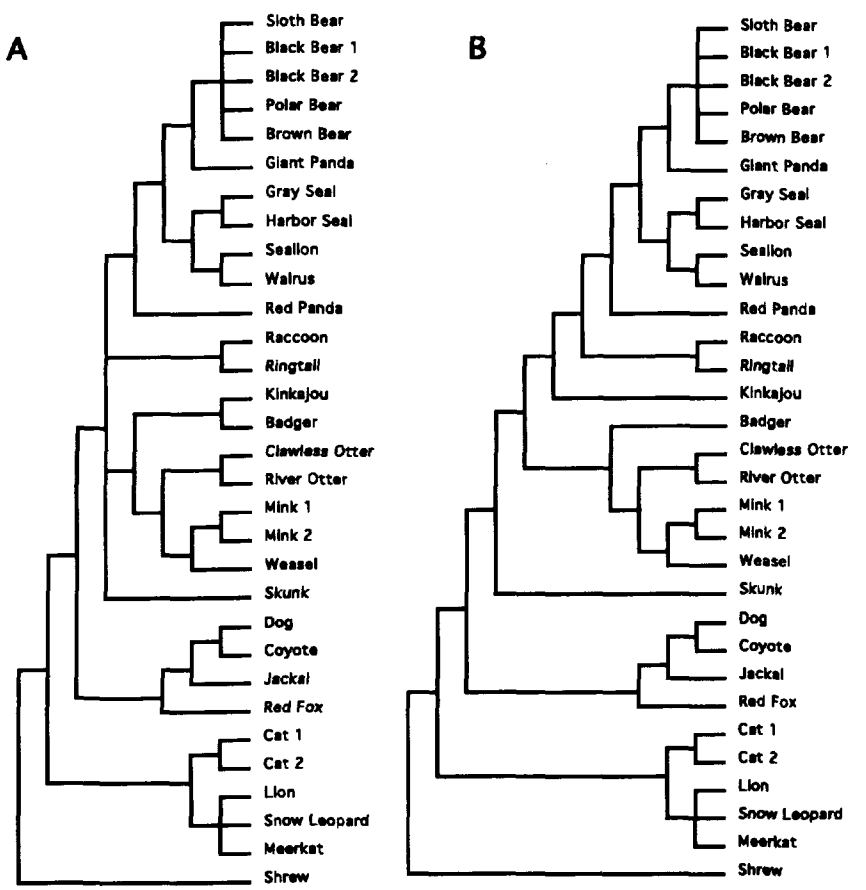


FIG. 2. Trees based on the sequence data obtained for this study. Tree "A" is based on all positions being equally weighted, while tree "B" is yielded when transversions are weighted two, three, and four times as much as transitions. Cladograms shown are strict consensus of all most parsimonious trees found (see Table 2 for details). The weighted trees are particularly congruent with the morphology-based cladogram (Fig. 3). Comparing the two, note that most areas with different branch structures are due to unresolved nodes (rather than disagreement) with the exception of the placement of the walrus (Odobenidae).

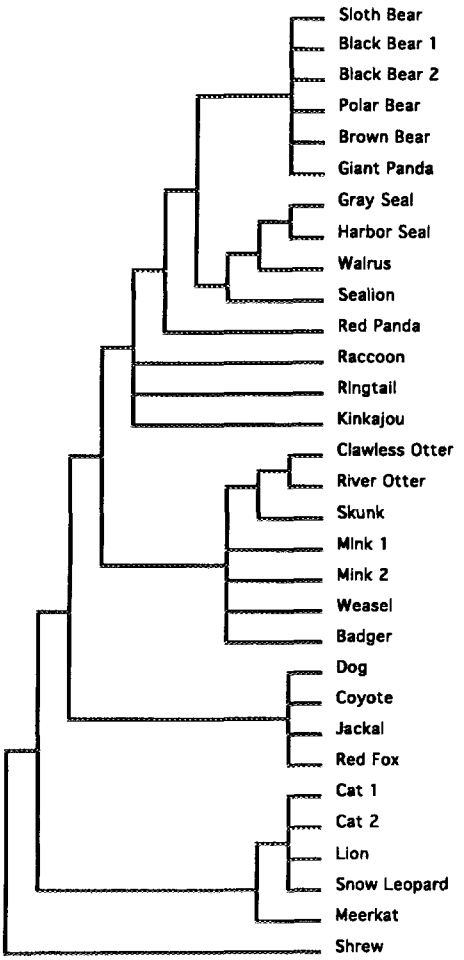


FIG. 3. Strict consensus tree obtained by scoring terminal taxa (i.e., for which sequence data was obtained) for the morphological characters compiled by Wyss and Flynn (1993). Neither characters supporting intrafamilial relationships nor family synapomorphies (though some characters act in the latter respect due to exclusion of fossil taxa) were included, with the exception of the Mustelidae. The mustelid characters were included since the monophyly of this speciose family has been doubted, particularly given the history of including small primitive arctoids in this group.

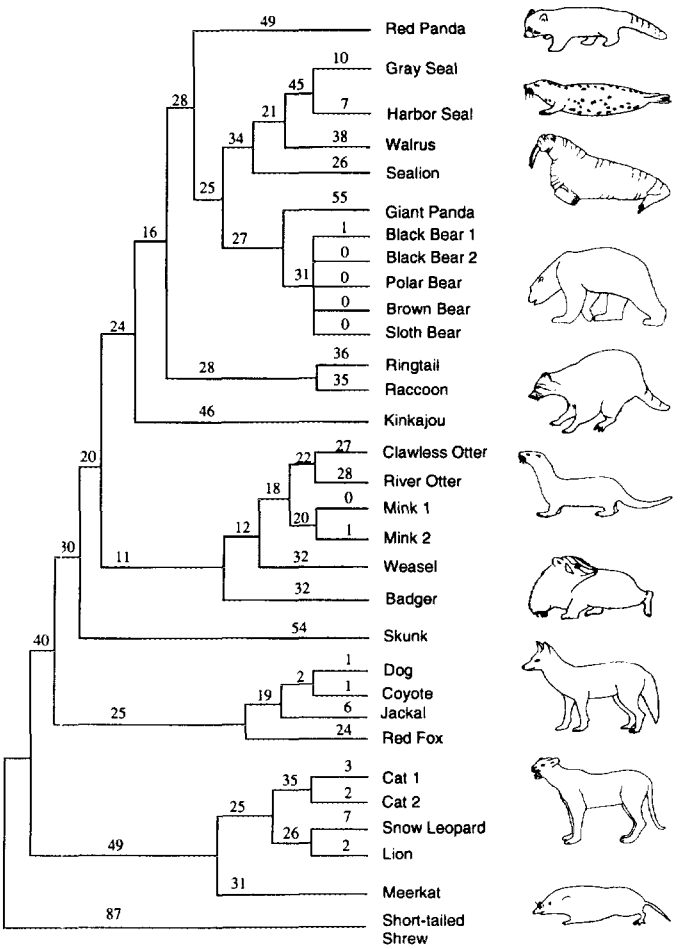


FIG. 4. Carnivore relationships based on a total evidence cladogram consisting of two data sets used to construct the trees in Figs. 1 and 2. Cladogram shown is a strict consensus of all most parsimonious trees. Branch lengths are minima for all most parsimonious trees. When the fossil groups Desmatophocidae, Amphicyonidae, and Nimravidae (see text) were added to the analysis, the topology was unchanged and the former two groups were placed as in Wyss and Flynn (1993). The Nimravidae fell at the base of the Carnivora.

tions: the procyonids and mustelids each have one member which falls adjacent to the others rendering these groups para- or polyphyletic (Fig. 2). In the case of the procyonids, this result may stem from the large divergence between the highly derived *Potos* and *Procyon/Bassariscus* within the family (Decker and Wozencraft, 1991). Perhaps inclusion of its putative sister genus *Bassaricyon* (the Central/South American olingos) would yield a monophyletic Procyonidae by eliminating a "long-branch effect." The other molecular/morphological discrepancy in this clade concerns the affinities of the walrus: with the molecular data alone it groups with the sea lion, while the anatomy supports a seal–walrus clade.

The total evidence analysis including the three fossil taxa mentioned above (not shown) supported the sister group relationship of Amphicyonidae and Ursidae, as well as Desmatophocidae and Phocidae. The Nimravidae, an enigmatic sabre-toothed group, fell at the base of the Carnivora outside feliforms plus caniforms. Clearly this group, as well as miacids, requires greater character study for our understanding of the modern carnivore radiation. In terms of systematic importance, fossil DNA efforts on these taxa could prove worthwhile.

Table 3 shows the statistics for molecular character subsets; these were derived from one of the most parsimonious unweighted (molecular data alone) trees. Both RI and CI are shown since autapomorphies, which contribute no phylogenetic information, have the highest possible CIs (1.0). These statistics may aid in decisions of future data gathering and give sugges-

TABLE 2

Tree Statistics for the Unweighted Molecular (Molec), Morphological (Morph), and Total Evidence (Total) Most Parsimonious Trees

	Molec	Morph	Total
No. of trees	42	2	7
No. characters	737	64	801
Length	1191	110	1316
CI	0.423	0.664	0.438
RI	0.521	0.890	0.582
Rp-Pro steps[a]	5	6	16
Pho-Must steps	10	13	26
Od-Pho steps	4	0	0[b]
Meph-Lutr steps	7	0	4
Gp-Pro steps	13[c]	12	39
Gp-Rp steps	4	6	20
Rp-Urs steps	1	3	6

Note. No. of trees, number of most parsimonious trees; No. characters, number of characters in data set; length, total number of steps; CI, consistency index; RI, retention index; Rp-Pro steps, number of steps required to place *Ailurus* with the procyonids; Pho-Must steps, phocids as sister to mustelids; Od-Pho steps, *Odobenus* as sister to the phocids; Meph-Lutr, skunks as sister to otters; Gp-Pro, Giant Panda-procyonids; Gp-Rp, Giant Panda as sister to Red Panda or vice-versa; Rp-Urs, Red Panda as sister to bears. As implied, the group being moved "to" is the group remaining in the position shown on that particular tree.

[a] As sister to kinkajou, raccoon plus ringtail, or a monophyletic Procyonidae with kinkajou as next branch.

[b] One extra step to form a walrus/sealion clade.

[c] As sister to the kinkajou, placement with the other procyonids required 7 additional steps.

TABLE 3

Statistics and Ranges for Molecular Character Subsets on Tree 1 of Most Parsimonious Trees for All Molecular Data

	Cytb(o)	1st	2nd	3rd	12S(o)	12S(-gaps)	gaps
CI(m)	.37	.37	.51	.36	.49	.48	.61
Lo CI	.11	.14	.2	.11	.14	.14	.25
Hi CI	1.0	1.0	.667	1.0	1.0	1.0	1.0
RI(m)	.42	.43	.37	.42	.62	.59	.86
Lo RI	0	0	0	0	0	0	0
Hi RI	1.0	1.0	.667	1.0	1.0	1.0	1.0
No. con.char.	7	1	0	6	33	25	8

Note. Mean is given (m), with range below (Lo, lowest value; Hi, highest value). Autapomorphies were not included in range. Cytb, cytochrome *b*; (o), overall; 1st, first codon positions, Cytb; 2nd, second codon position, Cytb; 3rd, third codon position, Cytb; 12S(-gaps), 12S without gap characters; gaps, gap characters from 12S mtrDNA alone. No. con.char., number of completely consistent phylogenetically informative characters.

TABLE 4

Bremer Support (aka Decay Index) for Carnivore Clades

Node	Molec	Morph	Total
Pinnepedia	2	11	14
Pinnipeds + bears	1	3	3
Ailurus, pinnipeds, bears	2	2	4
Arctoidea	2	2	9
Caniformia	10	3	14
Skunk outside Mustelidae	1	N.A.	2
Potos outside Procyonidae	1	N.A.	2
Respective placement of walrus within pinnipeds	2	1	1

Note. Number given is the number of extra steps required to break up indicated group. Tree abbreviations as in Table 2.

tions as to character evolution. Clearly the data may be broken into many other subsets (e.g., 12S stems vs loops, silent vs amino acid-altering changes in cyt *b*); those given are not intended to be exhaustive.

Table 4 gives the Bremer support (Bremer, 1988; aka "decay index") for certain nodes in the unweighted molecular, morphological, and total evidence trees. This involves looking at less parsimonious trees for a given data set to see how many additional "steps" are required in order to find trees incompatible with the indicated clade. This test may suggest which phylogenetic questions one would like to test further through gathering additional data.

Application of three rounds of "successive weightings" (Farris, 1969; Carpenter, 1988) to the sequence data, in which more consistent characters are *a posteriori* weighted more heavily, yielded complete congruence (i.e., the positions of mustelids and procyonids were resolved) with the anatomy, again with the exception of odobenid placement and the two taxa mentioned above. Topologies were stable after the first round. Characters reweighted on the basis of maximum, minimum, and mean CI, RI, and rescaled consistency index all yielded identical topologies (CI may be preferable for intradata set character comparison; Goloboff, 1993). The advantage of this approach is that the method is data-set dependent and does not rely on *a priori* models of character evolution which may not be met.

Transversion weighting two, three, and four times more heavily than transitions yielded trees more congruent with the total evidence tree (Fig. 2). Higher ratios all yielded a substantially different topology, with *Ailurus* sister to the pinnipeds, a procyonid/mustelid clade sister to this, followed by ursids, then canids, and finally the feloid representatives. Given the higher congruence of the lower ratios, we would argue for their credence.

DISCUSSION

The data subset statistics (Table 3) suggest that the 12S data are much less "noisy" for these taxa than the cyt *b* sequences. However, the cyt *b* data set clearly contributes phylogenetic information since it contains many characters with little homoplasy, and seven with none (also the combined gene tree is more congruent with morphology than either subset alone). Since homoplasy in different data sets would not be expected to be coincident, combining one "noisier" data set (provided it is not entirely devoid of signal) with fewer homoplastic data need not ruin the signal in the combined data set. While this overall data set will certainly have overall higher levels of homoplasy, most nodes may in fact be strengthened by having more characters supporting them. Table 2 strongly suggests this interaction of data sets; note that many alternative hypotheses require more steps than the sum of those in the individual data sets.

If one were to follow the logic of eliminating data subsets with lower CIs and RIs (i.e., higher levels of homoplasy), one would be left with the gap characters, since they have substantially higher statistics than even the remaining 12S data. The prevailing wisdom that insertion/deletion events are rarer than substitutions may explain this phenomenon. Certainly these statistics argue in favor of including these characters; indeed, it has been argued that it is illogical not to (Wheeler, 1993), given that investigators must weigh them heavily when constructing alignments.

One trend in these data that defies current assumptions is that the level of homoplasy in third codon positions is no lower than that of other positions. In fact, the majority of completely consistent characters are third base positions (though this class also contains a large number of completely homoplastic characters—RI = 0.0). This may be due to certain third codon positions essentially having more degrees of freedom of change than first, and particularly second, positions if there is selection to code for the same amino acid. These data suggest that every position may have different pressures; lumping them into classes may not always be appropriate.

As noted above, the total evidence tree may be by definition the best supported since it contains more of the pertinent evidence than any subset of the data (Kluge, 1989; Jones *et al.*, 1993; Kluge and Wolf, 1993). This tree (Fig. 4) supports the same general conclusions, yielding a walrus–seal clade due to the input of the anatomical characters. Given the use of only one otariid, and two closely related phocids (Wyss, 1988b) in this study, this hypothesis merits further work. Both families may require further systematic work to estimate ancestral conditions in both molecules and morphology (Wyss, 1988b; Berta *et al.*, 1989). While it is easy to imagine that some of the anatomical characters linking phocids and odobenids are homoplastic (e.g., absence of external ears, given the great reduction in otariids), others are harder to ignore (e.g., intraabdominal testes).

Both the molecular and the total evidence trees place the *Mephitis* specimen outside all other mustelids. This is suggestive, as several other lines of molecular data have proposed that skunks may not fall solidly within the family (Arnason and Widegren, 1986), and this question also deserves further study (the putative mustelid synapomorphies—enlarged anal gland and tooth loss—are both homoplastic; likewise, loss of both upper and lower first premolars supports the skunk/otter clade specifically). Similar to the situation seen in the procyonids, more definitive statements on skunk placement will require the sampling of other mephitine species to ensure that this finding is not a "long-branch" artifact. The placement of the Mustelidae relative to the Procyonidae also seems worth further systematic study. While the latter group is resolved in our total evidence tree, transversion-weighted trees, and the morphology alone as the sister to the red panda/Ursida clade, few steps are required to reverse their positions or to place the two as sister taxa.

The sum of available data supports the idea that the red panda is not a procyonid but an early offshoot of a pinniped–ursid lineage. This hypothesis is supported by total evidence, molecular data, and morphology. Ironically, this suggests that the red panda is more systematically important than its more famous namesake, the giant panda. Not only does the red panda retain many primitive arctoid anatomical characteristics (Hunt, 1974), but it is one of the few mammals lacking a sister group relationship to any single family. Thus, by traditional nomenclature, *Ailurus* should be afforded familial status. Indeed, if conservation priority is to be based at all on systematic position, as numerous authors have suggested (e.g., Vane-Wright *et al.*, 1991; Vrana and Wheeler, 1992), then the red panda may be among the most important carnivores. Interestingly, the total evidence tree requires considerably more steps to move *Ailurus* as sister to the procyonids than the sum of the individual data sets (Table 2). Even if further evidence should place *Ailurus* as a basal procyonid, the long branch lengths noted here suggest an early origin for its lineage. An early radiation within the procyonids is suggested regardless, given the similarly long branch length of the kinkajou. Given the controversy over pinniped origins, it is little wonder that until recently no one would have postulated that *Ailurus* would share a closer relationship with these aquatic arctoids than with any of the other small terrestrial carnivores.

With multiple data sets and combined evidence supporting a single origin of pinnipeds, an interesting scenario of convergent evolution is dispelled. The diphyletic hypothesis required independent origins of (quite

similar) flippers and reduction of ears among other aquatic adaptations from both bearlike and otterlike ancestors. That such a similar form should arise twice within the same general carnivore group seemed quite a plausible and attractive hypothesis; however, it is one that now has the burden of evidence against it. Finally, we note that in order to generate this particular phylogenetic arrangement one must include all the pertinent taxa. Since prior molecular studies have failed to incorporate all arctoids, it is little wonder that a link between *Ailurus,* ursids, and the pinnipeds has not been appreciated.

Note added in proof. Zhang and Ryder (1993) have also presented 12S and Cyt *b* data bearing on arctoid relationships and reached different conclusions than ours. However, these authors did not include a number of pertinent families including Mustelidae, Phocidae, Otariidae, Odobenidae, and Canidae, rendering their analysis incomparable to ours.

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