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Molecular Phylogenetics and Evolution 33 (2004) 694–705
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Phylogenetic relationships within mammalian order Carnivora indicated by sequences of two nuclear DNA genes

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Received 11 April 2004; revised 13 July 2004
Available online 1 October 2004

Abstract

Phylogenetic relationships among 37 living species of order Carnivora spanning a relatively broad range of divergence times and taxonomic levels were examined using nuclear sequence data from exon 1 of the IRBP gene (≈1.3 kb) and first intron of the TTR gene (≈1 kb). These data were used to analyze carnivoran phylogeny at the family and generic level as well as the interspecific relationships within recently derived Felidae. Phylogenetic results using a combined IRBP + TTR dataset strongly supported within the superfamily California, the red panda as the closest lineage to procyonid-mustelid (i.e., Musteloidea) clade followed by pinnipeds (Otariidae and Phocidae), Ursidae (including the giant panda), and Canidae. Four feliform families, namely the monophyletic Herpestidae, Hyaenidae, and Felidae, as well as the paraphyletic Viverridae were consistently recovered convincingly. The utilities of these two gene segments for the phylogenetic analyses were extensively explored and both were found to be fairly informative for higher-group associations within the order Carnivora, but not for those of low level divergence at the species level. Therefore, there is a need to find additional genetic markers with more rapid mutation rates that would be diagnostic at deciphering relatively recent relationships within the Carnivora.

Keywords: Interphotoreceptor retinoid binding protein; Transthyretin; Molecular evolution; Phylogenetic analysis

1. Introduction

The mammalian order Carnivora includes 11 families and classically has been divided into two monophyletic superfamilies, Caniformia and Feliformia (Eisenberg, 1989; Wozencraft, 1989; Wyss and Flynn, 1993). Caniformia was usually organized into the families Canidae, Ursidae, Procyonidae, Mustelidae, Otariidae, Odobenidae, and Phocidae while Feliformia was partitioned into the families Viverridae, Felidae, Herpestidae, and Hyaenidae (Eisenberg, 1989; Flynn and Nedbal, 1998). Despite numerous efforts, however, evolutionary relationships within and among the diverse families of living carnivorans remain controversial.

Within the superfamily Caniformia, the phylogenetic positions of two pandas have been focal points of controversies. Though growing evidence supports the giant panda, *Ailuropoda melanoleuca* as a basal branch of the bear family (Ursidae), the precise relationship of the red panda, *Ailurus fulgens*, to the other carnivorans was still unresolved (Flynn et al., 2000; Pecon Slattery and O'Brien, 1995; Vrana et al., 1994; Zhang and Ryder, 1993). Another hotly disputed area comes from the aquatic carnivorans involving otariids, odobenids, and phocids. Sharp disagreement exists concerning their true

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1055-7903/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2004.08.001

non-aquatic closest relatives within the caniform carnivorans (Arnason and Ledje, 1993; Flynn and Nedbal, 1998; Vrana et al., 1994; Wyss and Flynn, 1993). Among the four families comprising Feliformia, the cat family Felidae, which was further grouped into pantherine, domestic cat, and ocelot lineages, has been the center of interest in recent analyses. Phylogenetic affinities among the 38 species in this family have remained problematic, especially within the pantherine lineage, the most recently evolved and largest group of Felidae.

During the past decades, substantive work on the phylogenetic reconstruction of the carnivorans has produced a diversity of conclusions, but few analyses have included information from nuclear sequences. Flynn and Nedbal (1998) provided a family-level analysis of 22 carnivorans, in which the sequences data from the first intron of nuclear transthyretin (TTR) gene was used. Unfortunately, many of the proposed relationships were weakly supported, thereby highlighting the need for independent estimates of phylogeny from additional nuclear genes for comparison. In more recent studies, sequences from two other nuclear genes, interphotoreceptor retinoid-binding protein gene (IRBP) and recombination-activating gene 1 (RAG1), have also been applied in phylogenetic analyses (Sato et al., 2003, 2004; Yoder et al., 2003; Yu et al., 2004). However, rather than analyzing an inclusive sampling, these studies only considered certain constituent subgroups of carnivoran taxa. Moreover, the internal relationships within family Felidae have never been addressed using nuclear-encoded sequence data.

The present study extends sequences from the IRBP exon 1 and TTR intron 1 genes by covering a wider spectrum of carnivoran species and reanalyzes existing phylogenetic hypotheses. The resulting dataset includes both closely and distantly related taxa, thus enabling us to (1) make a full-scale investigation of overall carnivoran phylogenetic patterns, (2) evaluate evolutionary relationships among cat species within family Felidae and compare results with phylogenies based solely on mitochondrial (mt) markers, and (3) explore the usefulness of IRBP exon 1 and TTR intron 1 gene regions to resolve questions concerning different levels of carnivoran phylogeny.

2. Materials and methods

2.1. DNA samples and PCR amplifications

Thirty-seven species (17 belonging to Caniformia and 20 belonging to Feliformia) were examined in this study (Table 1). At least one species from all currently recognized carnivoran families except Odobenidae is represented, i.e., six families of Caniformia (Canidae, Ursidae, Procyonidae, Mustelidae, Otariidae, and Phocidae) and four families of Feliformia (Viverridae, Felidae, Herpestidae, and Hyaenidae). For each sample, total genomic DNA was isolated from blood or frozen tissues following the method of Sambrook et al. (1989) and prepared for subsequent polymerase chain reaction (PCR).

Target gene segments corresponding to a portion of exon 1 of IRBP gene (≈1.3 kb) and first intron of TTR gene (≈1 kb) were amplified by PCR from the same species using previously reported primers (I217 and I1531 for IRBP gene, Stanhope et al., 1992; T635 and T1628 for TTR gene, Flynn and Nedbal, 1998) and additional internal primers (Yu et al., 2004; Table 2). The optimal thermal cycling conditions for the PCR amplifications were as follows: 95 °C for 5 min, 35 cycles of 94 °C denaturation for 1 min, 50–63 °C annealing for 1 min and 72 °C extension for 1 min. In almost all cases, both target segments were successfully amplified from each template except the TTR intron 1 from *Nasua nasua* (coatimundi), *Martes flavigula* (marten), *Arctonyx collaris* (hog badger), and *Panthera tigris* (Bengal tiger). Redesigned primer pairs, TFnew1/TRnew1 and Tfnew2/Trnew2, were used to amplify this segment from the first three and the last species, respectively, as listed in Table 2.

2.2. Sequencing and alignments

PCR products were purified and sequenced directly from both strands with an ABI automated sequencer using standard protocols provided by the manufacturer. Acquired sequences were analyzed by using BLAST searching algorithm in Genbank (Altschul et al., 1997) to validate the data. Among the sequences used in the present paper, 36 are newly determined (21 IRBP exon gene and 15 TTR intron gene), five have been previously published by us (four IRBP exon gene and one TTR intron gene, Yu et al., 2004) and the remaining 33 are directly extracted from the GenBank. The sequences extracted from GenBank mostly originated from Flynn et al. (1998) (19 TTR intron gene) and Yoder et al. (2003) (nine IRBP exon and two TTR intron gene) (Table 1). Three non-carnivoran outgroup species representing insectivores, primates, and cetaceans in this study were utilized as that in Flynn and Nedbal (1998) (Table 1).

Separate alignments of IRBP exon and TTR intron sequences for 37 ingroup and three outgroup taxa were carried out by use of CLUSTAL X program (Thompson et al., 1997) and manually refined by eye. The alignment for IRBP exon 1 sequence was straightforward. In contrast, TTR intron 1 sequences varied greatly in length from 784 bp (*Felis temminckii*) to 1063 bp (*Ailuropoda melanoleuca*). A long insertion of approximately 250bp in length exclusive to Caniformia at the 3′ end of TTR gene fragment, in which many short repeats disturbed

Table 1
List of taxonomic samples and sequences used in this study

Taxon[a]					Gene			
Order	Family	Scientific name	Common name	Sample source	IRBP		TTR	
					References	Accession numbers	References	Accession numbers
Cetacea	Monodontidae	*Delphinapterus leucas*	Beluga whale		Waddell et al. (2000)	AF231341(1079 bp)	Flynn and Nedbal (1998)	AF039722 (953 bp)
Insectivora	Talpidae	*Uropsilus* sp.[b]			Murphy et al. (2001)	AY057831(1232 bp)		
		Scalopus aquaticus[b]	Eastern mole				Flynn and Nedbal (1998)	AF039723 (831 bp)
Primates	Hominidae	*Homo sapiens*	Human		Fong et al. (1990)	JO5253(1282 bp)	Sasaki et al. (1985)	M11518 (977 bp)
Carnivora	Canidae	*Canis lupus*	Wolf	China	This study	AY525044 (1274 bp)	Flynn and Nedbal (1998)	AF039732 (1060 bp)
		Vulpes velox[b]/	Swift fox		Springer et al. (2001)	AF179293 (1239 bp)		
		Vulpes vulpes[b]	Red fox				Flynn and Nedbal (1998)	AF039733 (1061 bp)
	Procyonidae	*Procyon lotor*	Raccoon	San Diego Zoo, USA	This study	AY525029 (1274 bp)	Flynn and Nedbal (1998)	AF039736 (1054 bp)
		Potos flavus	Kinkajou	San Diego Zoo, USA	This study	AY525030 (1274 bp)	Flynn and Nedbal (1998)	AF039737 (1039 bp)
		Nasua nasua	Coatimundi	San Diego Zoo, USA	This study	AY525031 (1274 bp)	This study	AY525054 (1003 bp)
	Mustelidae	*Martes flavigula*	Marten	Kunming Zoo, China	This study	AY525048 (1274 bp)	This study	AY525050 (991 bp)
		Martes zibellina	Sable	Haerbin Zoo, China	This study	AY525047 (1274 bp)	This study	AY525051 (989 bp)
		Mustela kathia[b]/	Yellow-bellied weasel	Yunnan Province, China	This study	AY525046 (1271 bp)		
		Mustela frenata[b]	Long-tailed weasel				Flynn and Nedbal (1998)	AF039735 (1034 bp)
		Enhydra lutris[b]/	Sea otter		Sato et al. (2003)	AB082978 (1188 bp)		
		Lontra longicaudis[b]	River otter				Flynn and Nedbal (1998)	AF039734 (1051 bp)
		Arctonyx collaris	Hog badger	Yunnan Province, China	This study		This study	
	Ursidae (+two pandas)	*Ursus arctos*	Brown bear	Heilongjiang Province, China	Previous study	AY303842 (1274 bp)	Flynn and Nedbal (1998)	AF039741 (1020 bp)
		Ursus thibetanus	Asiatic black bear	Yunnan Province, China	Previous study	AY303841 (1274 bp)	Previous study	AY303847 (1007 bp)
		Tremarctos ornatus	Spectacled bear	San Diego Zoo, USA	Previous study	AY303840 (1280 bp)	Flynn and Nedbal (1998)	AF039740 (1040 bp)
		Ailuropoda melanoleuca	Giant panda	Sichuan Province, China	Previous study	AY303836 (1274 bp)	Flynn and Nedbal (1998)	AF039738 (1066 bp)
		Ailurus fulgens	Red panda	Near Burma	This study		Flynn and Nedbal (1998)	AF039739 (1043 bp)

Otaeiidae	*Zalophus californianus*	Sea lion	San Diego Zoo, USA	This study		Flynn and Nedbal (1998)	AF039745 (1034 bp)
Phocidae	*Erignathus barbatus*	Bearded seal		Yoder et al. (2003)	AY170077 (1034 bp)	Flynn and Nedbal (1998)	AF039742 (1044 bp)
Felidae	*Panthera pardus*	Panther	South of Yunnan Province, China	This study	AY525041 (1274 bp)	This study	AY525064 (839 bp)
	Panthera leo	Lion	Kunming Zoo, China	This study	AY525036 (1274 bp)	Flynn and Nedbal (1998)	AF039725 (839 bp)
	Panthera tigris	Bengal tiger	Yunnan Province, China	This study	AY525037 (1274 bp)	This study	AY525061 (785 bp)
	Panthera (Uncia) uncia	Snow leopard	China	This study	AY525042 (1274 bp)	This study	AY525065 (839 bp)
	Neofelis nebulosa	Clouded leopard	Yunnan Province, China	This study	AY525032 (1274 bp)	This study	AY525056 (839 bp)
	Felis (Otocolobus) manul	Pallas' cat	Xining Zoo, China	This study	AY525039 (1274 bp)	This study	AY525063 (839 bp)
	Felis (Profelis) temminckii	Asiatic golden cat	South of Yunnan Province, China	This study	AY525034 (1243 bp)	This study	AY525059 (785 bp)
	Felis (Prionailurus) bengalensis	Asiatic leopard cat	Yunnan Province, China	This study	AY525035 (1274 bp)	This study	AY525060 (839 bp)
	Felis (Lynx) lynx	Lynx	Xinjiang Province, China	This study	AY525038 (1274 bp)	This study	AY525062 (837 bp)
	Felis catus	Domestic cat	Guangxi Province, China	Stanhope et al. (1992)	Z11811 (1150 bp)	This study	AY525058 (839 bp)
	Felis silvestris	Wild cat		Yoder et al. (2003)	AY170072 (1034 bp)	Flynn and Nedbal (1998)	AF039724 (839 bp)
	Felis bieti	Chinese desert cat	Qinghai Province, China	This study	AY525033 (1274 bp)	This study	AY525057 (838 bp)
Viverridae	*Nandinia binotata*	African palm civet		Yoder et al. (2003)	AY170083 (1034 bp)	Flynn and Nedbal (1998)	AF039729 (837 bp)
	Viverra tangalunga	Oriental civet		Yoder et al. (2003)	AY170078 (1034 bp)	Flynn and Nedbal (1998)	AF039731 (826 bp)
	Paguma larvata	Masked palm civet	Yunnan Province, China	This study		This study	
	Paradoxurus hermaphroditus	Common palm civet		Yoder et al. (2003)	AY170086 (1034 bp)	Flynn and Nedbal (1998)	AF039730 (796 bp)
Herpestidae	*Crossarchus obscurus*	Long-nosed mongoose		Yoder et al. (2003)	AY170071 (1034 bp)	Flynn and Nedbal (1998)	AF039726 (836 bp)
	Cynictis penicillata	Yellow mongoose		Yoder et al. (2003)	AY170079 (1034 bp)	Yoder et al. (2003)	AY170024 (837 bp)
	Mungos mungo	Banded mongoose		Yoder et al. (2003)	AY170065 (1034 bp)	Yoder et al. (2003)	AY170017 (836 bp)
Hyaenidae	*Crocuta crocuta*	Spotted hyaena		Yoder et al. (2003)	AY170087 (1034 bp)	Flynn and Nedbal (1998)	AF039728 (856 bp)

[a] Taxonomic denomination followed classification of Nowak (1999).
[b] In a few cases, taxa belonging to different genus of the same family or different species of the same genus, rather than the same specimen, were sequenced for both genes.

Table 2
List of PCR primers for amplification and sequencing of carnivoran IRBP exon 1 and TTR intron 1 genes

Target gene	Primer name[a]		Sequence (5′–3′)
IRBP exon 1	External primers	IF217	ATGGCCAAGGTCCTCTTGGATAACTACTGCTT
		IR1531	CGCAGGTCCATATAGGTGCTCCGTGTCCTG
	Internal primers	IF494	ACGAGGTTCTGGAGGGCAATGTGG
		IR1275	ACGGCCCGCACCAGGAGCCTG
		IF835	GGCGTGGCTGAGGACATCACTTAC
		IR954	GGACACGGGCACGGTGAGGAAG
		IF1160	ACCGTGTGCCCACCCTGCTGC
		IR546	CCAGCTTGCTCACCACCTCCTG
TTR intron 1	External primers	TF635	TGCCTCGCTGGACTGGTATT
		TR1628	GACAGCATCTAGAACTTTGACCAT
		TFnew1	TAGAAGTGAATTCTTTCAGCTCTGC
		TRnew1	GCCAGGGAGAGGTCAGCAAAAC
		TFnew2	TGTACACCTGATGAAGTAGAAGGG
		TRnew2	CAACAAAACTGGT TAAGAGTGAAA
	Internal primers[b]	TF633	TACAACTAGTAAGTGGGAATGAC
		TR853	TTCTGCCTCCGGACATGCTGCG

[a] Numbers in the primer names refer to the position of the 5′ end of the primer in the published human sequence.
[b] Numbers in the primer names refer to the position of the 5′ end of the primer from alignment of TTR sequence.

optimum matching, was removed from original alignment as suggested by Flynn and Nedbal (1998). A new 1058 bp alignment with sequence lengths ranging from 781 bp (*Martes zibellian*) to 856 bp (*Crocuta crocuta*) across ingroup taxa was produced and used for subsequent analyses.

2.3. Data analyses and phylogenetic reconstructions

Pairwise divergences based on the method of Tamura and Nei (1993) (TN93) for IRBP and TTR data were calculated with the program MEGA (Kumar et al., 2001). Stability of nucleotide composition across taxa in each gene region was examined using the χ^2test executed in PAUP*4.0b8 (Swofford, 2001). To test for the uniformity of IRBP exon and TTR intron substitutions through the course of carnivoran evolution, we adopted relative-rate test using the program PHYLTEST (Kumar, 1996). Outgroup rooting is one of the most influential factors causing unstable tree topologies in the phylogenetic reconstruction (Tarrio et al., 2000; Wheeler, 1990). Thus, various rooting strategies were considered with all possible combinations of three outgroups. In addition, we also simultaneously analyzed both data sets.

Maximum parsimony (MP) and maximum likelihood (ML) analyses were conducted using PAUP*4.0b8 (Swofford, 2001) for both separate and concatenated datasets. In MP analyses, a heuristic search strategy was employed with the TBR branch swapping algorithm, random addition of taxa and 1000 replicates per search. For the IRBP exon analysis, in addition to weighting all of the characters equally, we also applied a successive weighting procedure (Farris, 1969, 1989; Goloboff, 1991), in which characters were reweighted according to the rescaled consistency index. For the ML analyses, the best-fitting models of sequence evolution were estimated using program MODELTEST 3.06 (Posada and Crandall, 1998), in which parameters of base frequencies, transition/transversion ratio, the proportion of invariable sites and rate heterogeneity among sites were considered.

The reliability of the tree topologies from MP and ML methods were evaluated using bootstrap analysis (Felsenstein, 1985) (1000 and 100 replicates for MP and ML, respectively). Groups that appeared in 50% or more of the trees were retained. For nodes from the combined dataset, we also conducted a partitioned Bremer support analysis (PBS; Bremer, 1988, 1994) with the program TreeRot.v2 (Sorenson, 1999) to measure the respective contribution of each gene towards the total Bremer support.

The Bayesian method (Larget and Simon, 1999) was also applied using MrBayes2.01 (Huelsenbeck and Rpnquist, 2001). The same model for nucleotide evolution used in the ML method, was incorporated in the Bayesian analyses. Posterior probabilities were estimated and used to assess branch support in the inferred phylogeny, with probabilities $\geqslant 95\%$ indicating significant support (Reeder, 2003).

3. Results

In base composition analyses, IRBP sequences were obviously G + C-rich (64.1%) across the entire dataset. The third codon positions displayed the greatest deviation (81%). Conversely, the TTR sequences were composed of a slightly high proportion of A + T (56.9%). For both data sets, no evidence of composition heterogeneity across the studied taxa was observed, whether using all positions (IRBP, $\chi^2 = 50.318$, $P = 0.999$;

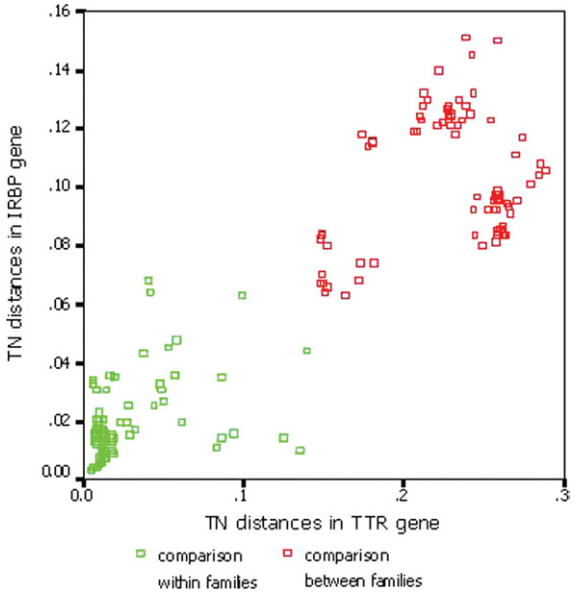

Fig. 1. Plot of IRBP TN distances vs. TTR TN distances.

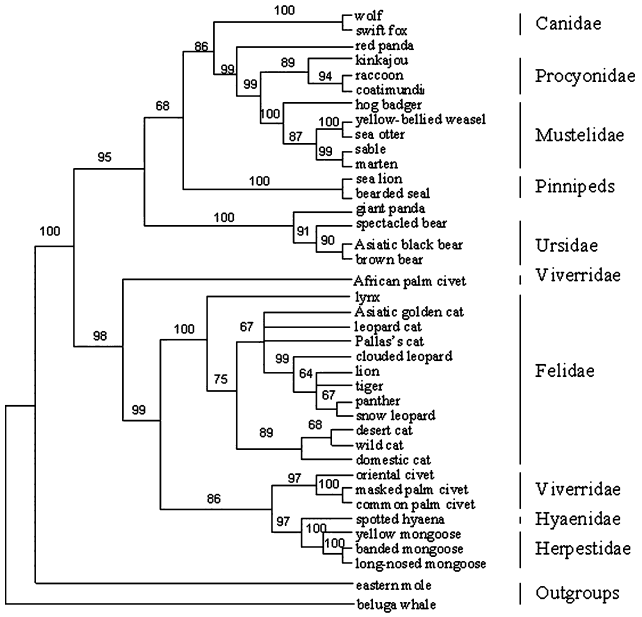

Fig. 2. MP tree found for nuclear IRBP gene datasets, rooted with whale + mole outgroups and analyzed by successive-weighting scheme (consensus of two MP tree with length of 551.998; CI = 0.858, RI = 0.905). Bootstrap percentages greater than 50% are shown above the branches.

TTR, $\chi^2 = 115.423$, $P = 0.524$) or the third codon position alone (IRBP, $\chi^2 = 132.881$, $P = 0.149$). TN93 distances between ingroup species ranged from 0.2 to 15.1% and 7.5% on average for the IRBP data set while TTR TN93 distances ranged from 0.0 to 29.6% and 17.30% on average. The mean ratio of transition to transversion was 3.290 and 1.960 from the IRBP and TTR gene regions, respectively. We compare the substitution rate between IRBP and TTR genes (Fig. 1) and observed a noticeably higher divergence (about 2.3-fold greater) in TTR intron than in IRBP exon region.

3.1. Separate analyses of IRBP exon data set

Taking weighting strategies and outgroup combinations into account, numerous parsimony analyses (14 cases) were performed for the IRBP exon dataset. The constructed phylogenies were vulnerable to outgroup choice and weighting schemes, producing a total of six different tree topologies. However, those based on successive weighting procedures always displayed a remarkable increase of tree resolution and branch support compared with their respective equally weighted parsimony analyses.

Our results indicated that the best pair of outgroups to use for tree construction was the mole + whale because this pairing successfully identified the monophyly of Caniformia and Feliformia and resulted in a relatively high rescaled consistency index. The MP tree from this dataset analyzed by the successive-weighting scheme is presented in Fig. 2. Of the 1280 aligned nucleotides, 526 (41.09%) were variable and 302 (23.59%) were phylogenetically informative. The trees obtained under other outgroup combinations, regardless of weighting scheme, had less desirable topologies. Either several major caniform families collapsed into large basal polytomies or the family Canidae was positioned as the earliest branching lineage within the order Carnivora, disbanding the monophyly of superfamily Caniformia. Using the mole + whale as outgroups (Fig. 2), a close relationship of the canids to the Musteloidea (Mustelidae plus Procyonidae) and the red panda was strongly supported. The ursid lineage was the first to branch off within Caniformia clade. The pinniped forms represented by the sea lion and the bearded seal, diverged prior to the canids, though the placement of pinnipeds was uncertain in the other parsimony analyses. In all cases, the red panda represented a sister taxon nearest to the Musteloidea clade (bootstrap values > 85%), in which procyonids and mustelids were monophyletic sister groups (bootstrap values > 91%). The giant panda was always closest to the monophyletic family Ursidae with high bootstrap values of 100%.

All IRBP MP trees supported the monophyly of the superfamily Feliformia (bootstrap support >85%) and moreover, the branching patterns within this clade were relatively stable. Monophyly for the Herpestidae, Hyaenidae, and Felidae, as well as paraphyly for the Viverridae were consistently recovered. As revealed in Fig. 2, the analyses of the family Felidae, including nine pantherine and three domestic cat species examined in this paper, suggest that the lynx was the earliest species to branch from the cat family (bootstrap value = 75%). This was followed by a distinct separation between the

lineage leading to the domestic cat (89%) and the remaining cat species of the pantherine lineage (67%). Therefore, the monophyly of the pantherine lineage was not supported due to the distant relationship of the lynx with other pantherine species. However, the monophyletic *Panthera* genus within the pantherine lineage (composed of lion, panther, tiger, and snow leopard) was consistently recovered. The snow leopard and panther were more closely related to each other than either was to lion or tiger (bootstrap values = 67%). A close sister-group relationship of this genus with the clouded leopard was strongly supported (bootstrap values = 99%) while the other members of the pantherine lineage including the Asiatic golden cat, leopard cat, and Pallas's cat, excluding the lynx, collapsed into an unresolvable polytomy.

ML and Bayesian trees based on the IRBP dataset were similar to each other and despite various outgroup combinations for rooting, displayed almost identical branching patterns to that in Fig. 2 rather than to the other parsimony analyses.

3.2. Separate analyses of TTR intron data set

Independent of outgroup species designation and optimization criteria, our TTR intron dataset always yielded topologically congruent trees and those utilizing successive-weighting parsimony (e.g., the tree in Fig. 3) were always slightly better resolved and supported than those obtained under equal weighting scheme. Of the 1058 aligned nucleotides, 668 (63.14%) were variable and 442 (41.78%) were phylogenetically informative.

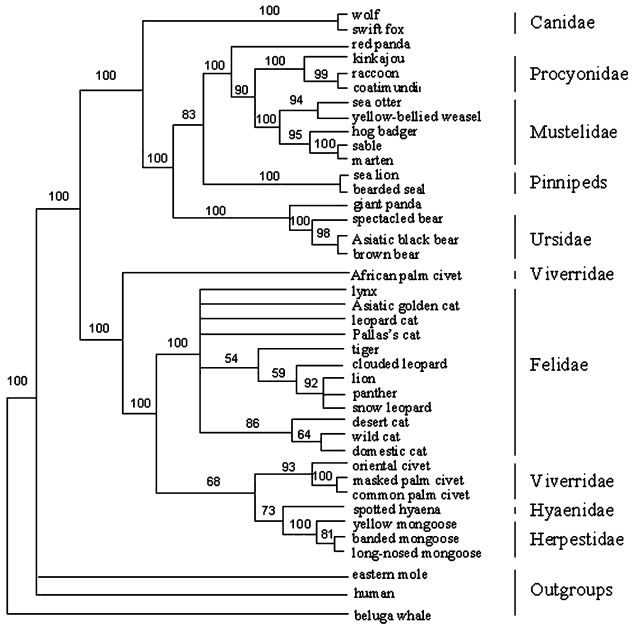

Fig. 3. MP tree found for nuclear TTR gene dataset rooted with three outgroups and analyzed by successive-weighting scheme (consensus of two MP tree with length of 751.23635; CI = 0.869, RI = 0.943). Bootstrap percentages greater than 50% are shown above the branches.

The basal split separating two superfamilies (Caniformia and Feliformia) within the order Carnivora was readily identified (bootstrap values = 100%). In the superfamily Caniformia, the red panda was depicted as the closest lineage to the Musteloidea clade, followed by the pinnipeds, Ursidae (including the giant panda), and then the Canidae. Our TTR intron analyses are consistent with the conventional notion that the canids are basal to the other caniform carnivorans (bootstrap values = 100%). This result is in agreement with previous studies but was not found in any of our IRBP exon gene trees. The TTR intron trees also lent strong support to the closer grouping of pinnipeds with Musteloidea and the red panda than with Ursidae or Canidae (bootstrap values = 83%). Among the Feliformia, the TTR intron gene demonstrated less resolving power in recovering shallow-level feline relationships than did the IRBP exon analyses (Fig. 3) The genus *Panthera* and the clouded leopard, the domestic cat lineage, Pallas's cat, the Asiatic leopard cat, Asiatic golden cat, and the lynx formed an unresolved polytomy in the TTR intron data set. Within the genus *Panthera*, the lion, panther, and snow leopard were more closely related to each other than to tiger, the latter representing an early offshoot within this group, even prior to the divergence of the clouded leopard (bootstrap value = 59%). The ML and Bayesian analyses of TTR dataset produced similar tree topologies as that in Fig. 3.

Comparison of our TTR results and those from Flynn and Nedbal (1998) that focused on higher-level groupings in the order Carnivora using the same TTR gene region, reveals that our inclusion of additional taxa within each family not only has corroborated their overall topology of the TTR intron tree, but also reinforced some of the familial-level affinities with considerably increased confidence. For instance, support for a hyaenas (Hyaenidae) + mongooses (Herpestidae) clade increases from 78–81 to 100%. Support for a raccoon (Procyonidae) + otters/weasels (Mustelidae) clade increases from 53–85 to 77–100% and support for a pinniped + Musteloidea plus the red panda clade increases from 66–88 to 80–100%.

3.3. Concatenated analyses of data sets

We utilized a "total evidence approach", which involves combining all available characters unconditionally, to conduct a simultaneous analysis of IRBP and TTR gene data sets. The combined aligned character matrix comprised 2341 nucleotides, of which 1244 (53.14%) were variable and 761 (32.51%) were parsimony informative.

Compared with either individual gene tree, pooling two genes demonstrated a clear increase in level of sup-

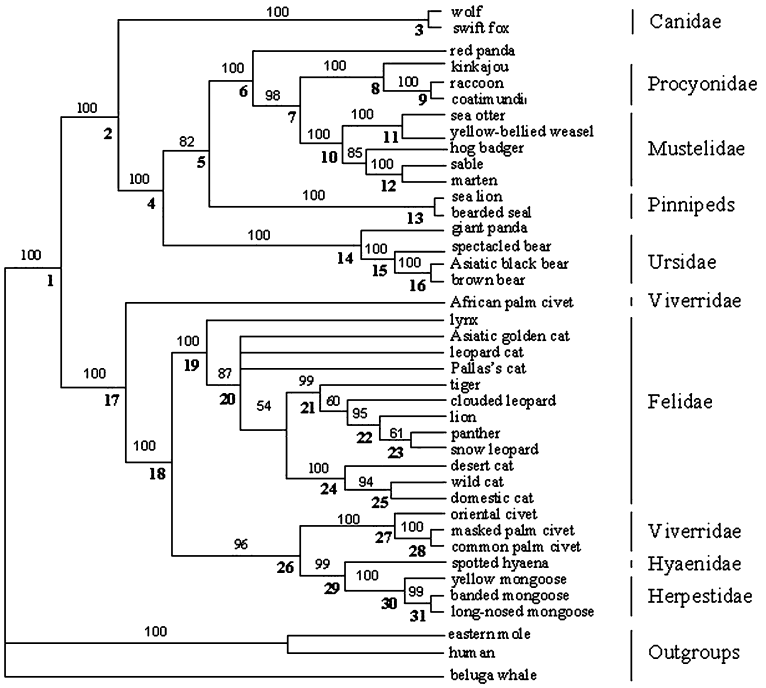

Fig. 4. MP tree found for concatenated IRBP and TTR dataset rooted with three outgroups and analyzed by successive-weighting scheme (consensus of seven MP tree with length of 1350.6686; CI = 0.862, RI = 0.926). Bootstrap percentages greater than 50% are shown above the branches. Partitioned Bremer support analyses for numbered nodes are given in Table 3.

port for most nodes, with a few exceptions. The concatenated MP tree topology using three outgroups under successive-weighting analysis is presented in Fig. 4. We note that the combined analysis results in a topology very similar to our TTR single-gene results. Identical relationships among caniform carnivorans are obtained but with greater support. However, the branching orders for taxa within family Felidae are still enigmatic. The lynx lineage was the earliest diverged from the feline radiation as was found with the IRBP analysis alone (bootstrap values = 87%). The remaining five closely related clades consisting of the domestic cat lineage, the genus *Panthera* plus the clouded leopard, Pallas's cat, the Asiatic leopard cat, and the Asiatic golden cat remained unresolved. However, internal relationships among five *Panthera* species (including the clouded leopard) appear resolved in analysis of the combined dataset. The lineage leading to the tiger diverged first, followed by the clouded leopard, lion, and finally a clade consisting of the panther and snow leopard. The ML and Bayesian methods produced similar tree topologies as presented in Fig. 4.

Partitioned Bremer support analysis indicated that, of the 31 resolved nodes on the concatenated parsimony tree (Fig. 4), 19 received significant support attributable to the TTR gene partition while nine received significant support attributable to the IRBP gene partition (ignoring three showing conflicting PBS values) (Table 3). In sum, the IRBP partition contributed 39.18% and the TTR partition 60.82% in PBS values (Table 3). Fundamentally, many aspects of the trees obtained from the analyses of these two nuclear genes, separately or in combination, showed no significant incongruence. In areas where the two genes disagree, mostly in recent feline relationships, the trees were characterized by short internodes and low bootstrap support, reflecting a lack of data instead of genuine conflict. Phylogenetic inconsistencies, such as the varying placement of Canidae, could also be effectively overcome by simultaneous analyses of both datasets. Therefore, the hypothesis based on combined IRBP and TTR data sets (Fig. 4) may represent the best current estimate of carnivoran phylogeny.

4. Discussion

4.1. Phylogenetic position of family Canidae and long-branch attraction

A significant conflict among the individual IRBP, TTR, and combined gene trees stems from the family Canidae within Caniformia. In analyses of both TTR by itself and the combined data sets, the results strongly confirmed the traditional view that the family Canidae was imbedded within Caniformia as the earliest diverging lineage (Flynn and Nedbal, 1998; Vrana et al., 1994; Wyss and Flynn, 1993), regardless of the analytical approaches. In numerous IRBP parsimony analyses, with the exception of the one using mole + whale for

Table 3
Results of partitioned Bremer support (PBS) analyses for each node on the combined MP tree topology

Node	IRBP	TTR	Combined tree[a]
1	15	20	35
2	−7	24	17
3	68	66	134
4	−2	18	16
5	0	2	2
6	11	14	25
7	5	1	6
8	4	11	15
9	2	5	7
10	15	25	40
11	6	3	9
12	3	6	9
13	14	27	41
14	16	20	36
15	3	5	8
16	2	4	6
17	3	16	19
18	3	11	14
19	16	28	44
20	2	−1	1
21	5	1	6
22	0	3	3
23	1	0	1
24	6	1	7
25	0	1	1
26	3	2	5
27	3	5	8
28	16	26	42
29	4	3	7
30	15	24	39
31	7	0	7
Total	239 (39.18)	371 (60.82%)	610

[a] See Fig. 4. Unnumbered nodes were not supported by equally weighting parsimony analysis greater than 50%.

outgroup rooting (Fig. 2) that strongly supported a closer relationship of the canids to Musteloidea and the red panda, the family Canidae was resolved as basal to the Caniformia and Feliformia. This result leads to the unorthodox paraphyly of caniform carnivorans. Detailed examination of these trees revealed that long-branch attraction between sequences from the family Canidae and the selected outgroups was causing an inaccurate representation of phylogeny by MP estimation.

This interpretation was assessed by the statistical examination of branch lengths variation across carnivoran families. The relative rate test rejected the constancy of the molecular clock for IRBP analysis and revealed several instances of lineage-specific rate differences. The longest branch leading to the canids suggests that they evolved at an elevated rate, approximately 1.5 (canids vs. felids; $z = 2.66415$, $P < 0.05$) ~2.5 (canids vs. ursids; $z = 5.30364$, $P < 0.05$) times faster than many as those of the other families except as observed between canids and herpestids ($z = 1.4292$, $P > 0.05$). Three outgroup species were all identified to be long-branch taxa and moreover, rate homogeneity of the canids with one of the outgroup species, i.e., human, was also unexpectedly detected ($z = 1.33411$, $P > 0.05$). If the human data are excluded and mole + whale are considered jointly as the outgroup taxa we did find evidence for the inclusion of Canidae within Caniformia (Fig. 2). This is consistent with the long-branch error and partially explains the results of the IRBP parsimony analyses. The application of successive weighting schemes failed to circumvent the long-branch problem, however, IRBP analyses based on ML and Bayesian methods demonstrated general superiority to that of MP in this respect, irrespective of outgroup sampling.

Our IRBP studies, excluding those biased by long-branch artifacts, favor a novel view supporting a closer relationship of canids to Musteloidea and the red panda (Fig. 2). However, we prefer to regard the family Canidae to be the most basal group within the superfamily Caniformia as suggested by the consistent resolution and robust support afforded by our TTR and combined analyses.

4.2. Phylogenetic positions of two pandas and pinniped forms

Both IRBP and TTR nuclear genes, analyzed individually or together, not only reinforced the sister-group relationships between the giant panda and Ursidae, but also between the red panda and Musteloidea. The latter contrasted with previously accepted views that either placed the red panda within the procyonid radiation on the basis of mtDNA, protein electrophoresis, DNA hybridization, immunological distance and karyological evidence (Goldman et al., 1989; O'Brien et al., 1985; Pecon Slattery and O'Brien, 1995; Wayne et al., 1989; Zhang and Ryder, 1993) or associated it with the bear family (sometimes plus pinnipeds) on the basis of mtDNA and anatomic characters (Vrana et al., 1994; Wozencraft, 1989; Wyss and Flynn, 1993). Our IPBP and TTR data do agree with the view advanced by Flynn and Nedbal (1998) and Flynn et al. (2000) who analyzed combined mtDNA and nuclear sequences. Bininda-Emonds et al. (1999) also reached the same conclusion from their supertree construction, in which multi- sourced information reported since 1970 was integrated and analyzed.

Based on less complete taxon sampling, the TTR gene analysis by Flynn and Nedbal (1998) placed pinnipeds within the Caniformia as a sister group to Musteloidea and the red panda. However, this conclusion was not convincingly supported by bootstrapping (not greater than 70%). In the current study, this branching pattern was well established in both the TTR and the combined phylogenies with strong nodal support (>80%) and high posterior probability (=100%). Thus, it seems that the placement of pinnipeds within caniform carnivorans

has been strengthened by the addition of more carnivoran sequence data. The traditional view of the bears (Hunt and Barnes, 1994; Vrana et al., 1994; Wyss and Flynn, 1993) or the mustelids (Arnason and Widegren, 1986; Arnason and Ledje, 1993) as the sister group of these aquatic carnivorans was not supported by our study. Dragoo and Honeycutt (1997), using mtDNA and morphological data, positioned pinnipeds with the procyonid-mustelid clade, but they did not include the red panda in their analysis. Therefore, adding data from the red panda to their analysis might further corroborate our conclusions.

4.3. Phylogenetic relationships among the cat species of family Felidae

This is the first study to utilize nuclear genes (on the autosomes) for the phylogenetic investigation within the cat family Felidae. The largest pantherine lineage of the Felidae consisting of the genus *Panthera* and several medium-size felids diverged most recently (within 1–8 MYA; Janczewski et al., 1995) but taxa within this lineage are not phylogenetically resolved. The IRBP and TTR genes from nine pantherine and three domestic cat species were sequenced in the present study. A comparison of these cat species with other carnivorans resulted in extraordinarily short branch lengths for both genes due to a lack of fixation of mutations during their brief evolutionary history. Inspection of mean genetic distances of each carnivoran family revealed the lowest divergence within the Felidae (1.2% vs. 1.3–5.1% for IRBP and 1.1% vs. 1.9–9.8% for TTR sequences). Low levels of divergence were also observed in numbers of informative sites supplied by both genes among felids. Just 21 of 55 variable sites were informative in 1280 bp aligned IRBP sequences and 12 of 34 variable sites in 1058 bp aligned TTR sequences.

Due to the low divergence, exact affinities among these cat species are difficult to define from our data. Nevertheless, useful findings were still derived from our IRBP and TTR data analyses. Notably, the monophyly of the genus *Panthera* that diverged within 1–2 MYA (Turner, 1987; Wayne et al., 1989) was confirmed. Of the five recognized species of *Panthera*, we included four representatives: the leopard, lion, tiger, and snow leopard. The assemblage of these four species was consistently recovered in our analyses with confidence and moreover, internal relationships among them were resolved in the combined dataset (Fig. 4). Secondly, the *Panthera* species grouped with the clouded leopard. In our study, the clouded leopard was joined with *Panthera* species as either the basal lineage (in IRBP analysis) or the next branch after *P. tigris* (in TTR and combined analyses). The close relationships of the clouded leopard to *Panthera* sp. was also observed in prior studies. The clouded leopard has been placed within the genus *Panthera* as the sixth species diverging after the tiger or as a sister taxon of the tiger on the basis of mt12S rRNA and mt cytochrome *b* (cytb) gene results (Janczewski et al., 1995) or as a sister taxon to *Panthera* based on mt 16S rRNA and NADH5 gene results (Johnson and O'Brien, 1997; see also of Bininda-Emonds et al., 1999 and present IRBP results). Additionally, the Pallas's cat was included in the pantherine lineage. There are two conflicting hypotheses with regard to the systematic placement of Pallas's cat based upon previous analyses of mt genes. One supports its position as a pantherine species with phylogenetic uncertainty, as derived from mt cytb (Masuda et al., 1996), 16SrRNA (Johnson and O'Brien, 1997) and sex chromosomes gene data analyses (Pecon Slattery and O'Brien, 1998). The other considers it as a basal species arising from the domestic cat lineage based on 12SrRNA (Masuda et al., 1996) and NADH-5 (Johnson and O'Brien, 1997) analyses. Our results, from non sex-linked nuclear gene data, positioned this enigmatic species inside the pantherine clade rather than with the domestic cat lineage. This was especially clear in the IRBP and combined gene analyses, though the explicit affinity of the Pallas's cat relative to the other felids remains undetermined. Finally, the monophyly of the domestic cat lineage was confirmed. The three domestic cat species, i.e., domestic cat, Chinese desert cat, and wild cat, always grouped together to the exclusion of other felids. However, the position of the domestic cat clade relative to the pantherine species varied with the genetic markers and analytical approaches used.

4.4. Phylogenetic performance of IRBP exon and TTR intron genes

Neither the IRBP exon nor the TTR intron region alone has resolved all of the phylogenetic questions concerning carnivorans. However, our results indicate that these two nuclear genes provided useful and informative information at some levels. IRBP data allows for better resolution in the Feliformia part of the carnivoran tree, especially at the familial and generic levels, but provides few and sometimes misleading signals for more ancient divergences within the caniform groups. In contrast, the TTR analyses consistently gave superior resolution at all levels of Caniformia phylogeny. Examination of consistency indices and phylogenetic behavior of successive weighting analyses for the Caniformia suggest a lower level of homoplasy in the TTR intron data relative to IRBP exon data. The topologies derived from the combined data set compared to each data set analyzed separately, show improved nodal support for most internal branches.

Although the TTR intron and IRBP exon gene regions appear phylogenetically complementary for the higher level analyses (i.e., interfamilial and intergeneric

relationships), in our analyses species-level associations, particularly among 12 felids were left largely unresolved. The IRBP and TTR gene regions may have accumulated sufficient informative variation for phylogenetic reconstruction among higher groups of the order Carnivora, but insufficient variations to resolve low level divergences. This suggests the need to study additional nuclear genetic markers with more rapid nucleotide substitution rates that could resolve terminal nodes in the phylogeny.

We have also obtained some unforeseen results that are noteworthy. Although the TTR intron demonstrates an overall higher degree of sequence divergence in comparison to the IRBP exon, the intron is, nonetheless, highly similar among closely related cat species. The leopard and the snow leopard, sequenced as part of this study, and the lion from GenBank were found to have identical TTR intron sequences. This unanticipated situation is paralleled in another pair of feline species, the domestic cat (reported here first) and the wild cat (from GenBank). Although the extremely low divergence in TTR noncoding sequences among the 12 felids is not unexpected in view of the relatively recent diversification of this family and in particular, the pantherine lineage, however, it is notable that IRBP exon 1, a protein-coding exon, exhibited a few mutations between the felid species, thus implying that the similarity in TTR sequences should not be simply attributed to close evolutionary relatedness at the species level. Rather, selective constraints may exist for these TTR noncoding sequences and could account for the extreme degree of sequence conservation identified here. Elucidating the cause of these sequence would provide insight into the nature of TTR sequence evolution. In addition, the observed accelerated rate of IRBP evolution in canids needs to be further expanded in future investigations.

Appendix A. Supplementary material

The sequences reported in the paper have been deposited in the GenBank database. Accession Nos.: AY525029–AY525051 and AY525053–AY525065.

Acknowledgments

We thank Dr. F. Allendorf for providing samples. This work was supported by National Natural Science Foundation of China (NSFC) and Chinese Academy of Sciences. Additionally, we thank Ms. Vera Tai, Center for Reproduction of Endangered Species, Zoological Society of San Diego for helpful comments on the manuscript.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Arnason, U., Widegren, B., 1986. Pinniped phylogeny enlightened by molecular hybridizations using highly repetitive DNA. Mol. Biol. Evol. 3, 356–365.

Arnason, U., Ledje, C., 1993. The use of highly repetitive DNA for resolving cetacean and pinniped phylogenies. In: Szalay, F.S., Novacek, M., McKenna, M. (Eds.), Mammal Phylogeny. Springer, NY.

Bininda-Emonds, O.R.P., Gittleman, J.L., Purvis, A., 1999. Building large trees by combining phylogenetic information: A complete phylogeny of the extant Carnivora (Mammalia). Biol. Rev. 74, 143–175.

Bremer, K., 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42, 795–803.

Bremer, K., 1994. Branch support and tree stability. Cladistics 10, 295–304.

Dragoo, J.W., Honeycutt, R.L., 1997. Systematics of mustelid-like carnivores. J. Mamm. 78, 426–443.

Eisenberg, J.F., 1989. An introduction to the Carnivora. In: Gittleman, J.L. (Ed.), Carnivore Behavior, Ecology, and Evolution. Cornell University Press, Ithaca, NY, pp. 1–9.

Farris, J.S., 1969. A successive approximation approach to character weighting. Syst. Zool. 18, 374–385.

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

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

Flynn, J.J., Nedbal, M.A., 1998. Phylogeny of the Carnivora (Mammalia): congruence vs incompatibility among multiple data sets. Mol. Phylogenet. Evol. 9, 414–426.

Flynn, J.J., Nedbal, M.A., Dragoo, J.W., Honeycutt, R.L., 2000. Whence the red panda?. Mol. Phylogenet. Evol. 17, 190–199.

Fong, S.L., Fong, W.B., Morris, T.A., Kedzie, K.M., Bridges, C.D., 1990. Characterization and comparative structural features of the gene for human interstitial retinol-binding protein. J. Biol. Chem. 265, 3648–3653.

Goldman, D., Giri, P.R., O'Brien, S.J., 1989. Molecular genetic-distance estimates among the Ursidae as indicated by one- and two-dimensional protein electrophoresis. Evolution 43, 282–295.

Goloboff, P.A., 1991. Homoplasy and the choice among cladograms. Cladistics 7, 215–232.

Huelsenbeck, J.P., Rpnquist, F.R., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Hunt, R.M., Barnes, L.G., 1994. Basicranial evidence for ursid affinity of the oldest pinnipeds. Proc. San Diego Soc. Nat. Hist. 29, 57–67.

Janczewski, D.N., Modi, W.S., Stephens, J.C., O'Brien, S.J., 1995. Molecular evolution of mitochondrial 12S RNA and cytochrome *b* sequences in the pantherine lineage of Felidae. Mol. Biol. Evol. 12, 690–707.

Johnson, W.E., O'Brien, S.J., 1997. Phylogenetic reconstruction of the Felidae using 16SrRNA and NADH-5 mitochondrial genes. J. Mol. Evol. 44, 98–116.

Kumar, S., 1996. Phyltest: A Program For Testing Phylogenetic Hypothesis. Version 2.0. The Pennsylvania Statee University, University Park.

Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Analysis Software. Version 2.1. Arizona State University, Tempe, AZ, USA.

Larget, B., Simon, D.L., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Mol. Biol. Evol. 16, 750–759.

Masuda, R., Lopez, J.V., Pecon Slattery, J., Yuhki, N., O'Brien, S.J., 1996. Molecular phylogeny of mitochondrial cytochrome *b* and 12S rRNA sequences in the Felidae: ocelot and domestic cat lineages. Mol. Phylogenet. Evol. 3, 351–365.

Murphy, W.J., Eizirik, E., O'Brien, S.J., Madsen, O., Scally, M., Douady, C.J., Teeling, E., Ryder, O.A., Stanhope, M.J., de Jong, W.W., Springer, M.S., 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294, 2348–2351.

Nowak, R.M., 1999, sixth ed.Walker's Mammals of the World, vol. 2 Johns Hopkins University Press, Baltimore MD.

O'Brien, S.J., Nash, W.G., Wildt, D.E., Bush, M.E., Benveniste, R.E., 1985. A molecular solution to the riddle of the giant panda's phylogeny. Nature 317, 140–144.

Pecon Slattery, J., O'Brien, S.J., 1995. Molecular phylogeny of the red panda *Ailurus fulgens*. J. Hered. 86, 413–422.

Pecon Slattery, J., O'Brien, S.J., 1998. Patterns of Y and X chromosome DNA sequence divergence during the felidae radiation. Genetics 148, 1245–1255.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Reeder, T.W., 2003. A phylogeny of the Australian *Sphenomorphus* group (Scincidae: Squamata) and the phylogenetic placement of the crocodile skinks (*Tribolonotus*): Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood inferred relationships. Mol. Phylogenet. Evol. 27, 384–397.

Sambrook, E., Fritsch, F., Maniatis, T., 1989. Molecular Cloning. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Sasaki, H., Yoshioka, N., Takagi, Y., Sakaki, Y., 1985. Structure of the chromosomal gene for human serum prealbumin. Gene 37, 191–197.

Sato, J.J., Hosoda, T., Wolsan, M., Tsuchiya, K., Yamamoto, M., Suzuki, H., 2003. Phylogenetic relationships and divergence times among Mustelids (Mammalia: Carnivora) based on nucleotide sequences of the nuclear interphotoreceptor retinoid binding protein and mitochondrial cytochrome *b* genes. Zool. Sci. 20, 243–264.

Sato, J.J., Hosoda, T., Wolsan, M., Suzuki, H., 2004. Molecular phylogeny of Arctoids (Mammalia: Carnivora) with emphasis on phylogenetic and taxonomic positions of the Ferret-badgers and skunks. Zool. Sci. 21, 111–118.

Sorenson, M.D., 1999. TreeRot, version 2. Boston University, Boston, MA.

Springer, M.S., Debry, R.W., Douady, C., Amrine, H.M., Madsen, O., de Jong, W.W., Stanhope, M.J., 2001. Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. Mol. Biol. Evol. 18, 132–143.

Stanhope, M.J., Czelusniak, J., Si, J.S., Nickerson, J., Goodman, M., 1992. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for bat monophyly. Mol. Phylogenet. Evol. 1, 148–160.

Swofford, D.L., 2001. PAUP*: Phylogenetic Analysis using Parsimony (* and Other Methods). Version 4.0b8. Sinauer Associates, Sunderland, MA.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Tarrio, R., Rodriguez-Trelles, F., Ayala, F.J., 2000. Tree rooting with outgroups when they differ in their nucleotide composition from the ingroup: the *Drosophila saltans* and *Willistoni* groups, a case study. Mol. Phylogenet. Evol. 16, 344–349.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The clustalx windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

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

Vrana, P.B., Milinkovitch, M.C., Powell, J.R., Wheeler, W.C., 1994. Higher level relationships of the arctoid Carnivora based on sequence data and total evidence. Mol. Phylogenet. Evol. 3, 47–58.

Waddell, V.G., Milinkovitch, M.C., Berube, M., Stanhope, M.J., 2000. Molecular phylogenetic examination of the delphinoidea trichotomy: congruent evidence from three nuclear loci indicates that porpoises (Phocoenidae) share a more recent common ancestry with white whales (Monodontidae) than they do with true dolphins (Delphinidae). Mol. Phylogenet. Evol. 15, 314–318.

Wayne, R.K., Benveniste, R.E., Janczewski, D.N., O'Brien, S.J., 1989. Molecular and Biochemical Evolution of the Carnivora. In: Gittleman, J.L. (Ed.), Carnivore Behavior, Ecology, and Evolution. Cornell University Press, Ithaca, NY, pp. 465–495.

Wheeler, W.C., 1990. Nucleic acid sequence phylogeny and random outgroups. Cladistics 6, 363–367.

Wozencraft, W.C., 1989. The phylogeny of the recent carnivora. In: Gittleman, J.L. (Ed.), Carnivore Behavior, Ecology, and Evolution. Cornell University Press, Ithaca, NY, pp. 495–535.

Wyss, A.R., Flynn, J.J., 1993. A phylogenetic anaysis and definition of the carnivore. In: Szalay, F.S., Novacek, M., McKenna, M. (Eds.), Mammal Phylogeny. Springer, NY.

Yoder, A.D., Burns, M.M., Zehr, S., Delefosse, T., Veron, G., Goodman, S.M., Flynn, J.J., 2003. Single origin of Malagasy carnivore from an African ancestor. Nature 421, 734–737.

Yu, L., Li, Q.W., Ryder, O.A., Zhang, Y.P., 2004. Phylogeny of the bears (Ursidae) based on nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 32, 480–494.

Zhang, Y.P., Ryder, O.A., 1993. Mitochondrial DNA sequence evolution in the Arctoidae. Proc. Natl. Acad. Sci. USA 90, 9557–9561.