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Phylogenetic studies of pantherine cats (Felidae) based on multiple genes, with novel application of nuclear β-fibrinogen intron 7 to carnivores

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Abstract

The pantherine lineage of the cat family Felidae (order: Carnivora) includes five big cats of genus *Panthera* and a great many mid-sized cats known worldwide. Presumably because of their recent and rapid radiation, the evolutionary relationship among pantherines remains ambiguous. We provide an independent assessment of the evolutionary history of pantherine lineage using two complete mitochondrial (mt) genes (ND2 and ND4) and the nuclear β-fibrinogen intron 7 gene, whose utility in carnivoran phylogeny was first explored. The available four mt (ND5, cytb, 12S, and 16SrRNA) and two nuclear (IRBP and TTR) sequence loci were also combined to reconstruct phylogeny of 14 closely related cat species. Our analyses of combined mt data (six genes; ≈3750 bp) and combined mt and nuclear data (nine genes; ≈6500 bp) obtained identical tree topologies, which were well-resolved and strongly supported for almost all nodes. Monophyly of *Panthera* genus in pantherine lineage was confirmed and interspecific affinities within this genus revealed a novel branching pattern, with *P. tigris* diverging first in *Panthera* genus, followed by *P. onca*, *P. leo*, and last two sister species *P. pardus* and *P. uncia*. In addition, close association of *Neofelis nebulosa* to *Panthera*, the phylogenetic redefinition of *Otocolobus manul* within the domestic cat group, and the relatedness of *Acinonyx jubatus* and *Puma concolor* were all important findings in the resulting phylogenies. The potential utilities of nine different genes for phylogenetic resolution of closely related pantherine species were also evaluated, with special interest in that of the novel nuclear β-fibrinogen intron 7.

Keywords: β-Fibrinogen intron; Pantherine lineage; Felidae; Phylogenetic analysis

1. Introduction

The Felidae, or cat family, is characterized by recent bursts of diversification within the last 10–15 million years (Johnson and O'Brien, 1997; Martin, 1980; Nowak, 1999; Werdelin, 1985). Thirty-eight cat species of this family are generally divided into the pantherine, domestic cat, and ocelot lineages (Ewer, 1973; Janczewski et al., 1995; Leyhausen, 1979; Masuda et al., 1996). The pantherine lineage, as the most recently evolved (within 1–8 MYA; Janczewski et al., 1995; Pecon Slattery et al., 1994) and largest felid group (around 20 cat species; Janczewski et al., 1995) has demonstrated great confusion in their taxonomy and phylogeny. These pantherine cats consist of five big cats of genus *Panthera* and a great many midsized cats species. They had been disputably assigned to 2–13 genera under various classification schemes in past studies (Ewer, 1973; Hemmer, 1978; Leyhausen, 1979; Nowak, 1999) and moreover, phylogenetic relationships among these pantherine species have also been controversial.

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A wealth of molecular characters have been used to decipher feline evolutionary history, including protein electrophoresis, allozyme data, karyology, endogenous retroviruses, mitochondrial (mt) DNA sequences, sex chromosomes-linked genes, and chemical signals (Binda-Emonds, 2001; Collier and O'Brien, 1985; Johnson and O'Brien, 1997; Lopez et al., 1994; O'Brien et al., 1987; Pecon Slattery and O'Brien, 1998; Reeves and O'Brien, 1984). However, little prior research focused nuclear genes at the DNA level. In the present paper, the seventh intron of the single-copy fibrinogen gene (β-chain; β-fibrinogen intron 7) from the nuclear genome was used for phylogenetic resolution among closely related pantherine cats. The utility of this gene segment has been successfully explored at different taxonomic levels in studies of birds (Johnson and Clayton, 2000; Moyle, 2004; Prychitko and Moore, 1997, 2000, 2003; Weibel and Moore, 2002) and reptiles (Creer et al., 2003; Giannasi et al., 2001), however, still lacking in those of mammals. Our work is the first to explore the potential of β-fibrinogen intron 7 as a genetic marker in carnivoran systematics. We also sequenced two large complete NADH dehydrogenase (ND2 and ND4) genes from mt genome, given the general thought that analysis of multiple independently inherited genes is especially effective in testing for congruence and estimating organismal phylogeny and, in addition, our previously reported nuclear interphotoreceptor retinoid-binding protein (IRBP) and transthyretin (TTR) genes (Yu et al., 2004b), together with four other available mtDNA characters (12SrRNA, cytochrome *b*, 16SrRNA, and ND5 genes; Janczewski et al., 1995; Johnson and O'Brien, 1997; Masuda et al., 1996) for the same set of cat species were also added to the present analyses.

Sequence data from 9 genes (three nuclear and six mt) of 12 pantherine cats and 2 domestic cats was analyzed, separately or in a variety of combinations here, with a view of (1) providing a broader understanding of interspecific relationships within the pantherine group, (2) assessing the utility of β-fibrinogen intron 7 as a novel marker in carnivoran phylogenetics, and (3) comparing evolutionary patterns of different genes and their values for resolution of low level feline questions.

2. Materials and methods

2.1. DNA samples and PCR amplifications

Fourteen felids in the family Felidae were examined and listed in Table 1. All the five currently recognized members of genus *Panthera* in the pantherine lineage, including *P. leo* (lion), *P. tigris* (tiger), *P. pardus* (leopard), *P. onca* (jaguar), and *P. uncia* (snow leopard), and seven other pantherine cats, including *Neofelis nebulosa* (clouded leopard), *Otocolobus manul* (Pallas's cat), *Prionailurus temminckii* (Asiatic golden cat), *Prionailurus bengalensis* (Asiatic leopard cat), *Lynx lynx* (lynx), *Puma concolor* (puma), and *Acinonyx jubatus* (cheetah) were included. In addition, two representatives of the domestic cat lineage and one outgroup taxa from family Viverridae or Hyaenidae were also utilized. Taxonomic classification of cat species followed Nowak (1999). Total genomic DNA was isolated from blood, frozen or hair tissues based on the method of Sambrook et al. (1989) and prepared for subsequent polymerase chain reaction (PCR).

Two felid-specific primers FGB-FelF and FGB-FelR (Table 2) used to amplify β-fibrinogen intron 7 (≈650 bp) were designed from conserved regions in flanking exons, based on the homologous comparisons of available β-fibrinogen sequences for birds (Prychitko and Moore, 1997), human (Chung et al., 1983), and rat (Eastman and Gilula, 1989). MtDNA PCR products were obtained using ND2-FelF/ND2-FelR primer pair for ND2 (1038 bp), as well as ND4-FelF/ND4-FelR primer pair for ND4 (1368 bp). Additional internal primers for amplifying these three genes (Table 2) were also synthesized. The optimal conditions adopted in PCRs were 95 °C initial hot start for 5 min, 35 cycles of 94 °C denaturation for 1 min, 50–56 °C annealing for 1 min, and 72 °C extension for 1 min.

With above-mentioned primers, three new target segments for each sample were acquired in almost all cases except an incomplete ND2 sequence (683 bp) from *P. uncia* (snow leopard) due to sequencing difficulty in one end of the gene, as well as unavailable β-fibrinogen intron 7 sequences from *P. onca* (jaguar) and *Puma concolor* (puma) because of insufficient template content in hair tissues as well as from *Acinonyx jubatus* (cheetah) because of the absence of sample. These three taxa were thus only represented in the mtDNA datasets. ND2 and ND4 sequences for *Felis catus* (domestic cat) and *Acinonyx jubatus* (cheetah) were directly retrieved from GenBank. In all, 34 out of 40 ingroup sequences were produced in this study.

2.2. Sequencing and data analysis

The amplified PCR products were purified and sequenced in both directions with an ABI automated sequencer. Acquired sequences were submitted to GenBank for BLAST searching (Altschul et al., 1997) to verify the data. New GenBank Accession Nos. are given in Table 1.

Separate alignments of β-fibrinogen intron 7 (from 11 ingroup) and two mtDNA (ND2 and ND4; from 14 ingroup) sequences data were conducted with CLUSTAL X program (Thompson et al., 1997) and refined by visual inspection. Protein-coding mtDNA alignments were straightforward while the β-fibrinogen intron 7 alignment exhibited obvious sequence length variation.

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

	Taxon			Newly established datasets			Previously available datasets			
	Scientific name[A]	Common name	Sample source	Nuclear gene	Mitochondrial genes		Nuclear gene		Mitochondrial genes[C]	
				β-Fibrinogen intron	ND2	ND4	IRBP exon	TTR intron	16SrRNA	ND5
Pantherine lineage	*Panthera pardus*	Panther	South of Yunnan Province, China	AY634371	AY634383	AY634395	AY525041	AY525064	AF006443	AF006444
	Panthera leo	Lion	Kunming Zoo, China	AY634374	AY170043	AY634398	AY525036	AF039725	AF006547	AF006458
	Panthera tigris	Tiger	Yunnan Province, China	AY634372	AY634384	AY634396	AY525037	AY525061	AF006459	AF006460
	Panthera onca	Jaguar	Xian Zoo, China		AY634391	AY634403			AF006441	AF006442
	Panthera uncia	Snow leopard	China	AY634370	AY634382	AY634394	AY525042	AY525065	AF006449	AF00 6450
	Neofelis nebulosa	Clouded leopard	Yunnan Province, China	AY634373	AY634385	AY634397	AY525032	AY525056	AF006425	AF006426
	Otocolobus manul	Pallas's cat	Xining Zoo, China	AY634375	AY634387	AY634399	AY525039	AY525063	AF006431	AF006432
	Profelis temminckii	Asiatic golden cat	South of Yunnan Province, China	AY634369	AY634381	AY634393	AY525034	AY525059	AF006447	AF006448
	Prionailurus bengalensis	Asiatic leopard cat	Yunnan Province, China	AY634376	AY634388	AY634400	AY525035	AY525060	AF006437	AF006438
	Lynx lynx	Lynx	Xinjiang Province, China	AY634377	AY634389	AY634401	AY525038	AY525062	AF006413	AF006414
	Puma concolor	Puma	Xian Zoo, China		AY634392	AY634404			AF006455	AF006456
	Acinonyx jubatus	Cheetah			AY463959	AY463959			AY463959	AY463959
Domestic cat lineage[B]	*Felis catus*	Domestic cat	Guangxi Province, China	AY634379	NC_001700	NC_001700	Z11811	AY525058	NC_001700	NC_001700
	Felis silvestris[a]	Wild cat	Qinhai Province, China	AY634378	AY170042	AY634402	AY170072	AF039724	AF006401	AF006402
	Felis bieti[a]	Chinese desert cat			AY634390		AY525033	AY525057		
Outgroups[B]	*Paguma larvata*[b]	Masked palm civet	Yunnan Province, China	AY634380		AY634405	AY525040	AY525055		
	Crocuta crocuta[b]	Spotted hyena			AY170057		AY170087	AF039728	AF006391	AF006392

[A] Taxonomic denomination followed classification of Nowak (1999).
[B] In two cases, taxa belonging to different species of the same genus (a) or different families (b) were utilized for the same genes.
[C] Accession numbers of 12SrRNA and cytb genes are unavailable and their sequences were obtained from publications (Janczewski et al., 1995; Masuda et al., 1996).

Table 2
List of PCR primers for amplification and sequencing of nuclear β-fibrinogen intron, mt ND2, and ND4 genes for felids

Target gene	Primer name[a]		Sequence (5′–3′)
β-Fibrinogen intron	External primers	FGB-FelF	CACAACGGCATGTTCTTCAGCACG
		FGB-FelR	TACCACCATCCACCACCATCTTCTT
	Internal primers	FGB-FelF397	ATCACTCTAAGACGTTTCCACTAT
		FGB-FelR867	CCTCAGTGTTAGAGAACCCTGGAA
mtND2	External primers	ND2-FelF	CCATACCCCGAAAATGTTGGTTTAT
		ND2-FelR	AGCTTTGAAGGCTCTTGGTCT
	Internal primers	ND2-FelF337	TTCTGAGTGCCCGAAGTCACACAAGG
		ND2-FelR689	GTTTTATTTCATGTTTGTGATA
mtND4	External primers	ND4-FelF	GGCACTGACTATGTACAAAACCT
		ND4-FelR	GACAGCATCTAGAACTTTGACCAT
	Internal primers	ND4-FelF275	AAAAACTATACATCACAATACT
		ND4-FelR1066	CTGTAGCTCCTATATAGCTTCAGGG
		ND4-FelF362	TATTTGAAGCCACATTAATCCC
		ND4-FelR916	GCTAGTAGTCATCAGGCAGC

[a] Numbers in the primer names refer to the position of the 5′ end of the primer from alignment of sequences.

Besides one single-nucleotide deletion unique for *Profelis temminckii* and two small indels in the outgroup taxa, we found two unusual 239 bp long insertions, with 87.9% sequences similarity, emerged at different positions of *Profelis temminckii* and *Otocolobus manul* β-fibrinogen intron region, respectively (hereafter referred as Gol-ins and Pal-ins). Prior to phylogenetic analyses, Gol-ins and Pal-ins were removed from the original alignment, leaving 657 nucleotide positions in the new nuclear alignment.

Pairwise distances based on the Tamura and Nei (1993) (TN93) method were calculated with program MEGA (Kumar et al., 2001). Stationary of nucleotide composition across taxa were examined using the χ^2 test in PAUP*4.0b8 (Swofford, 2001). We also plotted the number of transitions and transversions against TN93 distance to measure saturation patterns for all sites and each codon position using DAMBE (Xia, 2000). The assumption of molecular clock was tested using the method of relative-rate test with program PHYLTEST (Kumar, 1996).

Phylogenetic analyses were performed under maximum parsimony (MP), maximum likelihood (ML) and newly developed Bayesian (Larget and Simon, 1999) criteria. Analyses were conducted using PAUP*4.0b8 (Swofford, 2001) and MrBayes 3.0B4 (Rqnquist and Huelsenbeck, 2003). ND2 gene trees were rooted using *Crocuta crocuta* (spotted hyena; Yoder et al., 2003) as outgroup while β-fibrinogen intron 7 and ND4 trees using *Paguma larvata* (masked palm civet) because no sequences of these two genes could be obtained from spotted hyena. In MP analysis, a branch-and-bound search strategy was employed with all characters treated as equal weights and gaps as missing data. The best-fitting models of sequences evolution for ML and Bayesian analyses 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. Bayesian analyses started with randomly generated trees and four Markov chains under default heating values were run for 2×10^6 generations, with sampling at intervals of 100 generations. To ensure that the analyses were not trapped on local optima, the dataset was run three times independently. We determined the burn-in period by checking for likelihood stationarity. To test for nodal reliabilities, heuristic bootstrap analyses (Felsenstein, 1985; 1000 replicates for MP and 100 for ML, respectively) and posterior probabilities were applied, with groups appearing in 50% or more of the trees in bootstrap analysis and sampled with 80% or more frequency in Bayesian analysis retained.

2.3. Combination with other available datasets

Available sequences data from four other mt genes 12SrRNA (352–354 bp), cytochrome *b* (cytb; 289 bp), 16SrRNA (372–375 bp), and ND5 (318 bp) (Janczewski et al., 1995; Johnson and O'Brien, 1997; Masuda et al., 1996) for the 15 taxa in the ND2 and ND4 datasets, as well as those from two other nuclear genes IRBP exon (1274 bp) and TTR intron (784–839 bp) (Yu et al., 2004b) for the same 12 taxa as those of present β-fibrinogen intron 7 dataset, were also obtained from the GenBank database and combined in a variety of ways for the analyses (9 in total).

Phylogenetic reconstruction for six mt sequences (i.e., 12SrRNA + cytb + 16SrRNA + ND5 + ND2 + ND4), three nuclear sequences (i.e., β-fibrinogen + IRBP + TTR), and combined sequences data were performed following the aforementioned methods (MP, ML, and Bayesian algorithms). Outgroup sequences for *Crocuta crocuta* taxa were retrieved from GenBank in each data analysis, excepting those of β-fibrinogen and ND4 data as explained above. Partitioned Bremer support analysis

(PBS; Bremer, 1988, 1994), as implemented by TreeRot.v2 (Sorenson, 1999), was used to measure the respective contribution of each gene to the total nodal Bremer support.

Three of 14 ingroup taxa (*P. onca*, *Puma concolor*, and *Acinonyx jubatus*) in mtDNA dataset were not represented by nuclear data, and as a result, their nuclear characters in the combined analyses using all evidence were coded as missing data. We also tested for incongruent phylogenetic signal between nuclear and mt genes using the partition homogeneity test (Farris et al., 1994, 1995) in PAUP*4.0b8 (Swofford, 2001).

3. Results

3.1. Novel findings of SINE element in β-fibrinogen intronic segment

As mentioned above (see Section 2), two 239 bp large insertions (Gol-ins and Pal-ins) have occurred uniquely in *Profelis temminckii* (positions 96–334) and *Otocolobus manul* taxa (positions 587–825) β-fibrinogen intron 7, respectively. A BLAST search from GenBank database revealed that 225 bp of Gol-ins and Pal-ins exhibited the highest similarities to carnivoran-specific short interspersed element, i.e., CAN SINE family. The results from the RepeatMasker program (A.F.A. Smit and P. Green, unpublished data) confirmed that they belong to the subfamily SINEC_Fc of CAN SINE. These two SINE-like insertions contained RNA polymerase III promoter near the 5′ end, a AT-rich region at the 3′ end and perfect direct terminal repeats, characteristics of tRNA-derived SINEs families (Coltman and Wright, 1994; Okada, 1991a,b). Here, we unexpectedly find another illustration of species-specific SINE family members located in intronic regions and it appeared that the Gol-ins and Pal-ins were derived from two independent insertion events. Evolutionary mechanisms underlying in these two repeated elements are unclear but definitely merit further investigation.

3.2. Base composition and substitution patterns

Base composition was AT-rich biased in β-fibrinogen (60.8%), ND2 (61.8%), and ND4 (60.1%) genes. No evidence of base frequencies heterogeneity across taxa was observed for any genes. Nuclear β-fibrinogen gene showed the lowest transition/transversion ratio (ti/tv = 1.94), contrasted with obvious tendency of transitions in the mtDNA genes (5.25 in ND2 and 8.41 in ND4). TN93 distances within the ingroup taxa ranged from 0 to 4.6% for β-fibrinogen (2.5% on average), from 1.4 to 18.4% for ND2 (12.9% on average), and from 0.6 to 19.3% for ND4 (15.3% on average). Comparisons of sequence divergences revealed faster rates of evolution in the ND2 and ND4 than in the β-fibrinogen gene at about 5.16 and 6.12 times, respectively. The results from DAMBE program (Xia, 2000) confirmed a lack of multiple substitutions in each gene and codon position (plot not shown). Therefore, all substitutions in β-fibrinogen and those of the codon positions in ND2 and ND4 genes were used in the analyses.

Sequence characteristics of individual genes are summarized in Table 3. The six previously published datasets were included for comparison. The nine DNA sequences including four protein-coding (ND2, ND4, ND5, and cytb) and two rRNA genes (12S and 16S) from mt genome, as well as two introns (β-fibrinogen and TTR) and one exon (IRBP) from nuclear genome, range in size from 289 (cytb) to 1368 (ND4), with ND5 showing the

Table 3
Summary statistics for nuclear and mitochondrial gene segments used in this study

	Nuclear datasets			Mitochondrial datasets					
	β-Fibrinogen intron	IRBP exon	TTR intron	ND2	ND4	ND5	cytb	12SrRNA	16SrRNA
Aligned sites (%)	657	1274	841	1038	1368	318	289	354	380
A%	28.4	18.2	28.6	36.7	32.3	33.5	27.8	36.5	33.6
C%	20.2	31.8	21.9	28.9	28.1	27.4	28.8	22.3	22.4
G%	19	32	19.5	9.3	11.8	7.8	15.5	17	20.1
T%	32.4	18	30	25.1	27.8	31.2	27.9	24.2	23.8
Variable sites	133 (20.24%)	102 (8.01%)	98 (11.65%)	418 (40.27%)	536 (39.18%)	136 (42.77%)	103 (35.64%)	63 (17.8%)	84 (22.11%)
Parsimony-informative sits	28 (4.26%)	21 (1.65%)	13 (1.55%)	251 (24.18%)	385 (28.14%)	80 (25.16%)	66 (22.84%)	26 (7.34%)	37 (9.74%)
ti:tv ratio	1.94	3.93	1.74	5.25	8.41	5.48	7.09	3.28	3.98
Mean TN distance (%) within ingroup	2.5 (0–4.6%)	1.1 (0.2–2.3)	1.1 (0–1.9)	12.9 (1.4–18.4)	15.3 (0.6–19.3)	14.8 (0–23.2)	12.3 (1.8–17.5)	3.9 (0.6–6.1)	5.6 (1.4–7.9)
Proportion of invariable sites (I)	0	0.8081	0.5457	0.4931	0.5612	0	0	0	0
Gamma-shape parameter (α)	0.401	∞	∞	1.419	1.8889	0.3913	0.1808	0.1615	0.1752

highest percentage of variable sites (42.77%) and IRBP the lowest (8.01%). ND4 had the most parsimony-informative sites (28.14%) while TTR had the least (1.55%). Pairwise divergences comparisons revealed that among nuclear genes the substitution rate of β-fibrinogen was about two times higher than those of both IRBP and TTR. Among mt genes, ND4 showed the fastest rate of substitution, followed by ND5, ND2, cytb, 16S, and 12S. As expected, estimates of ti/tv ratio were higher for mt than nuclear DNA genes. In addition, mt sequences were generally much more divergent than nuclear sequences (8.2 times in substitution rate on average), and they also showed greater rate heterogeneity among sites (lower estimates of α) (Table 3). Among the nine examined genes, the relative rate estimation of cytb over β-fibrinogen gene has often been measured in previous avian studies. For the feline species examined in this study the value was 4.92, compared with 2.79 and 3.7 in various woodpeckers (Prychitko and Moore, 1997, 2000) and 5.6 among pigeons and doves (Johnson and Clayton, 2000).

3.3. Phylogenetic analyses based on nuclear characters

ML bootstrap trees based on analyses of individual β-fibrinogen dataset and combined nuclear dataset (β-fibrinogen + IRBP + TTR; 2772 bp) are shown in Figs. 1A and B, respectively. The topologies of two gene trees are identical in all respects except that the weakly supported sister-group relationship between *Otocolobus manul* and *Lynx lynx* (58%) inferred in the β-fibrinogen tree was not supported in the combined nuclear tree. All other nodes, however, received stronger supports from the combined data. The 11 cat species were separated into two large clades: one containing all representatives of *Panthera* and its closest sister species *Neofelis nebulosa* while the other contained the remaining pantherine cats and the two species of domestic cat lineage. MP bootstrap and Bayesian analyses recovered similar, but less-resolved trees compared to the ML analysis (β-fibrinogen MP tree length = 157, CI = 0.879, and RC = 0.626; combined MP tree length = 380, CI = 0.903, and RC = 0.650) and notably differed in placing *P. temminckii* as the earliest diverging cat species in both MP trees (52–53%). *O. manul* and *L. lynx* were paired together in all analyses of β-fibrinogen dataset (MP, 61%; ML, 58%; Bayesian, 97%). Combined nuclear data analyses consistently identified closest affinity between *P. uncia* and *P. pardus* (MP, 93%; ML, 81%; Bayesian, 99%) within *Panthera*, with *P. leo* placed as the sister taxon to them (MP, 96%; ML, 83%; Bayesian, 100%). The genus *Panthera* and *N. nebulosa* formed a monophyletic group in all nuclear analyses.

3.4. Phylogenetic analyses based on mitochondrial characters

ML bootstrap trees derived from analyses of individual ND2 and ND4 datasets are shown in Figs. 2A and B, respectively. The topologies of the two gene trees contrasted in several aspects; however, in no case was a node with a strong bootstrap value for one gene contradicted by a well-supported node for the other gene. The ND4

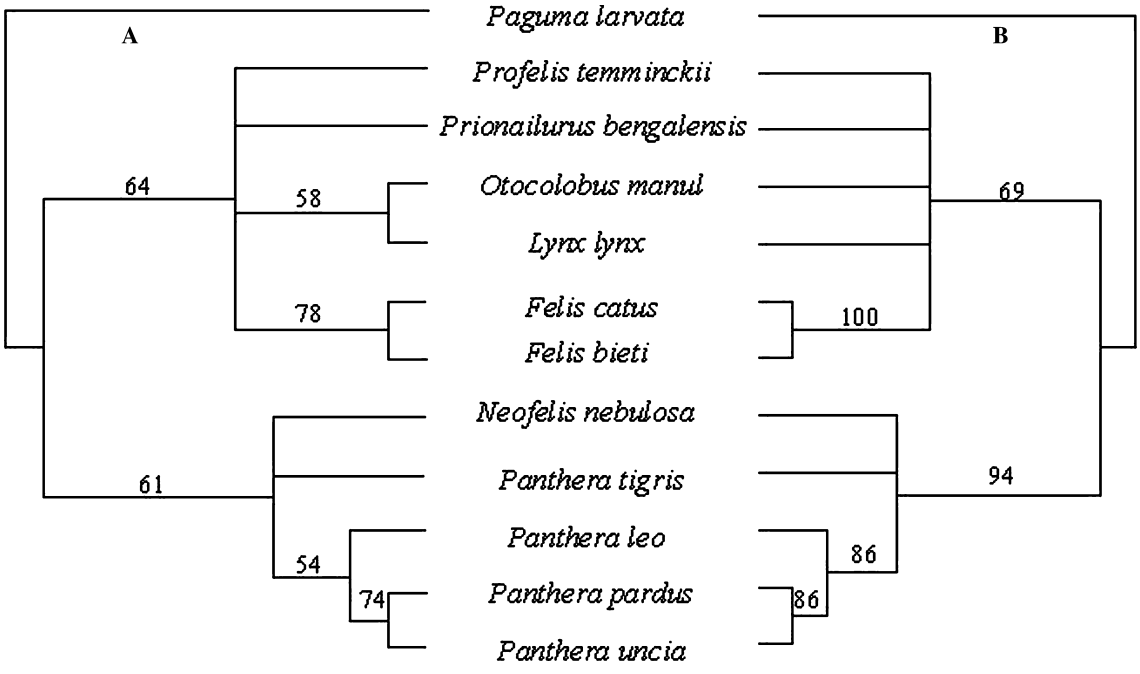

Fig. 1. Bootstrap maximum likelihood (ML) trees for β-fibrinogen intron gene (A; $-\ln l = 1679.5955$) and combined β-fibrinogen, IRBP and TTR genes (B; $-\ln l = 5857.64$ 61) from 11 species of felids, using *Paguma larvata* as outgroup. The numbers above branches indicate bootstrap values, of which only those greater than 50% are shown. The maximum parsimony (MP) and Bayesian analyses obtained similar tree topologies.

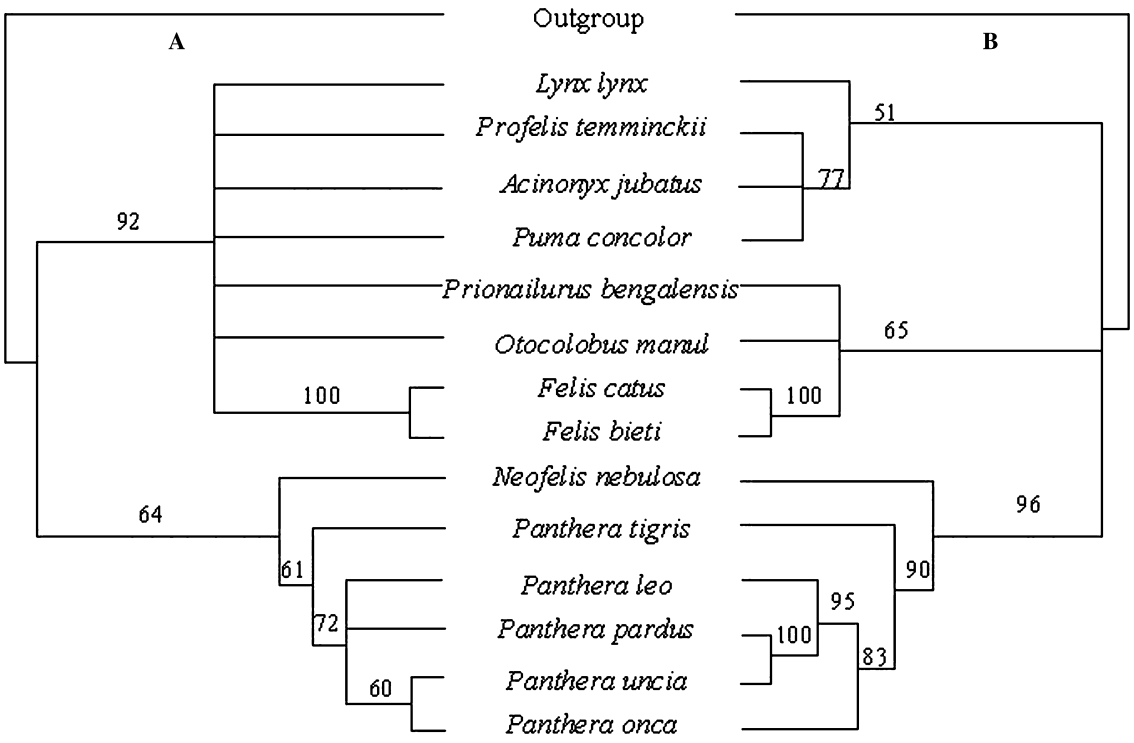

Fig. 2. Bootstrap maximum likelihood (ML) trees for mt ND2 (A; $-\ln l = 5032.1025$) and ND4 genes (B; $-\ln l = 6799.0806$) for 14 species of felids, using *Crocuta crocuta* and *Paguma larvata* as outgroups, respectively. The numbers above branches indicate bootstrap values, of which only those greater than 50% are shown. The maximum parsimony (MP) and Bayesian analyses obtained similar tree topologies.

tree tended to have overall greater resolution and higher nodal supports but lower RC indices. In ND4 analyses, complete resolution among five *Panthera* members and their close relationship with *N. nebulosa* were supported. *P. uncia* paired with *P. pardus* (100%) rather than with *P. onca* (60%) as identified in ND2 data. The precise placements of the other cats were ambiguous in the two gene trees as demonstrated by the presence of polytomies and low bootstrap values. MP (tree length = 882, CI = 0.569, and RC = 0.251) and Bayesian trees from ND2 data analyses provided a distinct picture from Fig. 2A, with *N. nebulosa* either being the most basal taxon of all the felids in MP tree (80%) or an uncertain taxon in a polytomy at the base of Bayesian tree. In addition, *O. manul* was weakly placed as the sister taxon to the two species of domestic cat lineage in MP tree (52%). *P. temminckii*, *P. concolor*, and *A. jubatus* were grouped together in the Bayesian tree although with low posterior probability (82%). In the ND4 gene analyses, MP and Bayesian trees were largely congruent to Fig. 2B (MP tree length = 1305, CI = 0.490, and RC = 0.235). Internal relationships within *Panthera* and its sister-group of *N. nebulosa* remained well-supported while other cat species either constituted a monophyletic clade under the Bayesian method (91%), as was also seen in Fig. 2B, or collapsed into a large polytomy under MP method.

Four mt genes (12SrRNA, cytb, 16SrRNA, and ND5) for the same set of cat species were combined with mt sequences from this study and analyzed simultaneously (≈3750 bp). The topologies obtained by various analytic methods were identical (MP tree length = 2808, CI = 0.531, and RC = 0.228) and exhibited much improved resolution and nodal support than either mtDNA gene region alone. ML analysis of the combined mt data is presented in Fig. 3A. This multiple gene tree is completely resolved and strongly supported for all nodes except for the position of *P. temminckii* (53%). Similar support values for this node were also obtained by MP (50%) and Bayesian (75%) analyses. The 14 cat species split into two large groups in all analyses, one containing the monophyletic *Panthera* genus and *N. nebulosa* (MP, 56%; ML, 81%; Bayesian, 84 %) and the other containing the remaining pantherine cats and two domestic cat lineage species (MP, 94%; ML, 98%; Bayesian, 100%). In the former group, *N. nebulosa* occupied the most basal position followed by *P. tigris*, *P. onca*, *P. leo*, and last two most recently diverged sister species *P. pardus* and *P. uncia*. In the latter, all felids were further subdivided into two clades: one contained *P. temminckii*, *P. concolor*, *A. jubatus* plus *L. lynx* (MP, 66%; ML, 93%; Bayesian, 99%) and the other contained *O. manul*, *P. bengalensis* plus the two domestic cat species (MP, 50%; ML, 79%; Bayesian, 100%). *P. concolor* and *A. jubatus* were strongly paired together, with *P. temminckii* and *L. lynx* successively joining the first subclade. In the other subclade *O. manul* consistently

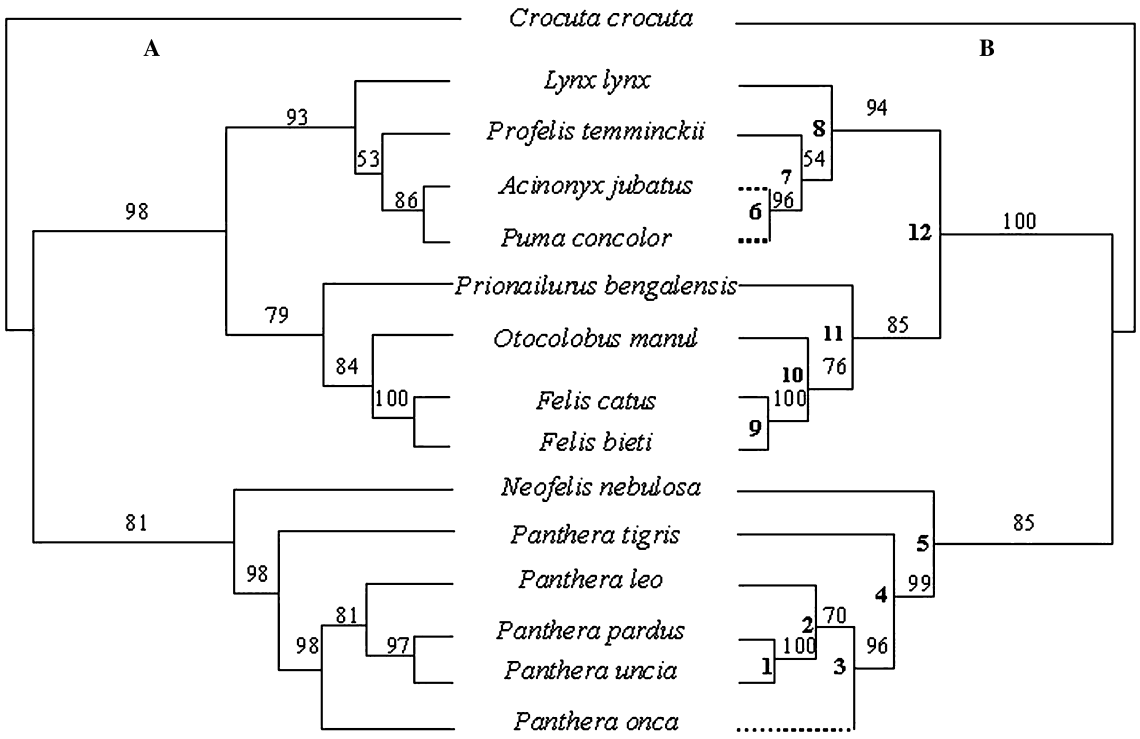

Fig. 3. Bootstrap maximum likelihood (ML) trees for combined mt (A; $-\ln l = 16744.44364$) and combined mt and nuclear genes (B; $-\ln l = 22187.8346$) from 14 species of felids, using *Crocuta crocuta* as outgroup. The numbers above branches indicate bootstrap values, of which only those greater than 50% are shown. The maximum parsimony (MP) and Bayesian analyses obtained nearly identical tree topologies.

showed a robust association with two domestic cat representatives and *P. bengalensis* was identified as the sister taxon to them.

3.5. Phylogenetic analyses based on entire characters

The partition homogeneity test (Farris et al., 1994, 1995) indicated the homogeneity of phylogenetic signal between our nuclear and mt datasets ($p = 0.98$) allowing a total of about 6500 bp characters to be combined for tree reconstruction. All analyses resulted in essentially identical topologies as those based on combined mt data alone with similar or slightly improved support for most nodes and higher RC index (0.243 vs. 0.228). The ML bootstrap tree from this dataset is presented in Fig. 3B. Broken lines in the tree denote the branches connecting three taxa *P. concolor*, *A. jubatus*, and *P. onca*, from whom nuclear sequences were missing.

Partitioned Bremer values (Table 4) reveals that the majority of phylogenetic information from the combined gene-based topologies was contributed by the mt characters, with ND4 gene holding the highest percentage (43.83%), followed by 16SrRNA (16.05%), ND2 and cytb equally (11.11%), 12SrRNA (8.64%), and ND5 (8.02%), in sharp contrast to the nuclear characters (1.23% in total). The relatively weak influence of nuclear partition upon analyses of complete set of genes was unsurprising, given their extremely low degree of genetic divergence. The majority of variations and informative sites in the total dataset are from mt regions, especially protein-coding genes. Nuclear genes only account for 20% of the variable and 7% of the informative characters, though they exhibit lower levels of homoplasy. It thus seemed that mt genes are superior to nuclear genes for tracing phylogenetic relationships among closely related felines. In our view, the hypotheses based on combined mt or full datasets (Fig. 3) represent the best current estimate of feline phylogeny in the present phylogenetic reconstruction because all nodes are resolved and highly supported.

4. Discussion

4.1. Monophyly and interspecific relationships of panthera genus

Traditionally, *Panthera* is composed of five recognized extant species. However, interspecific relationships within the genus have been equivocal probably due to extremely recent speciation (1–2 MYA; Kurten and Anderson, 1980; Wayne et al., 1989) resulting in short internodes that have not been resolved because of insufficient sequence data used in previous studies (see Fig. 4). In this paper, although individual genes examined failed to provide a well-supported phylogeny, the

Table 4
Results of partitioned Bremer support (PBS) analyses for each node on the total evidence MP tree (combined nuclear and mt dataset)

Nodes[a]	Mitochondrial datasets							Nuclear datasets[b]			Combined nuclear	Combined nuclear and mt gene trees (Fig. 4B)
	cytb	12SrRNA	16SrRNA	ND5	ND2	ND4	Combined mt	β-Fibrinogen	IRBP	TTR		
1	0	−3	0	−4	−4	39	28	0	1	0	1	29
2	−2	1	0	1	−2	12	10	0	0	0	0	10
3	5	1	4	−4	−6	9	9	0	0	0	0	9
4	−2	2	5	6	7	5	23	0	0	0	0	23
5	1	2	2	2	−9	2	0	0	0	0	0	0
6	0	3	0	0	−1	1	3	0	0	0	0	3
7	0	1	2	−1	−6	5	1	0	0	0	0	1
8	0	2	1	0	0	0	3	0	1	1	2	5
9	13	2	9	1	30	0	55	0	0	0	0	55
10	1	3	0	13	1	−4	14	−1	0	0	−1	12
11	1	1	0	1	0	−1	2	0	0	0	0	2
12	1	−1	3	−2	8	3	12	0	0	0	0	12
Total	18 (11.11%)	14 (8.64%)	26 (16.05%)	13 (8.02)	18 (11.11%)	71 (43.83%)	160 (98.77%)	−1	2 (1.23%)	1 (0.62%)	2 (1.23%)	162

[a] Nodes are numbered as in Fig. 3B.
[b] Nuclear sequence data of three samples (*P. onca*, *Puma concolor*, and *Acinonyx jubatus*) were unavailable, the PBS scores for the nuclear datasets would be underestimated in some aspects.

analyses of full data and combined mt data (Fig. 3), consistently produced a robust, well-resolved tree. Our phylogenies strongly uphold the monophyly of *Panthera*. The internal affinities within the genus are shown to be novel, and our results differ from all previous hypotheses (Fig. 4). Though one of the species, *P. onca*, was not included in present nuclear datasets, the branching orders of the other four (Fig. 1) were mostly congruent between mt, nuclear and combined analyses, the exception being in the poorly supported result obtained from the ND2 gene (Fig. 2A). In conclusion, our analyses clearly indicate that *P. tigris* is the sister taxon to the other members of *Panthera*, within the clade containing the other members of the genus, *P. unica* and *P. pardus* are sister species, next joined by *P. leo* and finally by *P. onca.*

The most interesting and novel finding of this study is the strong sister-group pairing of *P. uncia* and *P. pardus.* Morphological studies and some previous molecular work favored placing *P. uncia* as the most basal taxon in the genus (Figs. 4B–D). A recent analysis of chemical signal, however, suggested it is the closest relative of *P. tigris* (Fig. 4E). *P. pardus* has also been alternatively hypotheized as the sister taxon of *P. leo* (Figs. 4A, C, E, and F) or of *P. onca* from protein electrophoresis data (O'Brien et al., 1987), or based on combined morphology, karyology and molecular sequences as a diverging taxon after *P. uncia* (Fig. 4D). Our analyses also question fossil evidence that suggests a close relationship of *P. leo* with *P. tigris* or *P. onca* (Hemmer, 1971; Neff, 1982).

Taking all evidence together, we can find that no two analyses have come to a completely identical result regarding affinities among these five species, with conclusions varying depending on the analytic methods and character type used. However, given the remarkable congruence between genes of a distinct linkage group and the large size of our dataset, as well as the robust support of many nodes, we consider our gene tree the preferred interpretation of *Panthera* species relationships. Both Wilcoxon signed-ranks (Templeton, 1983) and Shimodaira–Hasegawa tests (as implemented in PAUP*) suggest that the combined mt or full data topology we recovered (Fig. 3) were significantly the best estimate for present dataset. None of six prior hypotheses (Fig. 4) was supported ($p < 0.001$, data not shown).

4.2. Phylogenetic resolutions of non-panthera species in pantherine lineage

As with *Panthera*, dispute also exists over the taxonomy and phylogeny of the non-*Panthera* pantherines, most recently reviewed by Mattern and McLennan (2000) and Bininda-Emonds (2001). Seven species, each representing one genus of non-*panthera* pantherines (Ewer, 1973; Nowak, 1999), were sampled

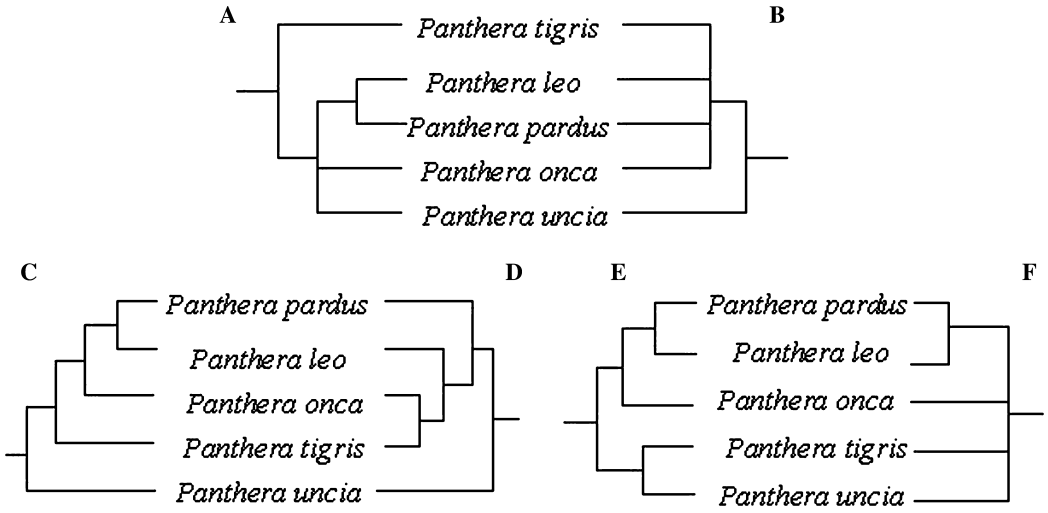

Fig. 4. Competing hypotheses of phylogenetic relationships within *Panthera* deduced from (A) partial 12SrRNA and cytb genes (Janczewski et al., 1995), (B) partial 16SrRNA and ND5 genes (Johnson and O'Brien, 1997), (C) supertree construction of data sources spanning 25 years of systematic research (Bininda-Emonds et al., 1999), (D) partial 12SrRNA, 16SrRNA, ND5, and cytb genes with morphological and karyological characters (Mattern and McLennan, 2000), (E) chemical signals (Bininda-Emonds, 2001), and (F) complete mtDNA control region (Jae-Heup et al., 2001).

in this study. Although precise relationships among them were not well resolved in separate gene analyses, the combined mt and full data, however, provided insightful understanding of these pantherines' evolution.

4.2.1. Close association of Neofelis nebulosa to Panthera

The clustering of *N. nebulosa* with *Panthera* was well corroborated by the present data. It also supported the increasing evidence that *N. nebulosa* was included as the most basal member within *Panthera* genus (Bininda-Emonds et al., 1999; Johnson and O'Brien, 1997; Mattern and McLennan, 2000; Yu et al., 2004b). The possible sister species status of *N. nebulosa* and *P. tigris*, as proposed by Janczewski et al. (1995) from mt 12SrRNA and cytb, is not supported by our results. Such a grouping requires an extra 40 steps and significantly worse ($p < 0.0001$) for the combined mt and full datasets. MP analyses of the ND2 gene alone, however, revealed that *N. nebulosa* was less closely linked with *Panthera* and occupied a basal position in the tree (bootstrap value = 82%). This ideosyncratic pattern of relationship can be explained by the "long branch attraction" effect between *N. nebulosa* and the outgroup taxon. PHYLTEST (Kumar, 1996) results have observed a significantly accelerated rate of evolution in *N. nebulosa* relative to the other felids ($p < 0.05$; data not shown). It has been shown that among tree-building methods, ML is superior to MP in producing reliable trees effectively in cases of long-branch attraction, and it is likely to be so in this case. A reversion of *N. nebulosa* to the traditional placement in the ND2 MP tree only requires seven additional steps and no statistically significant difference between these alternative topologies was found ($p = 0.1083$).

4.2.2. Phylogenetic position of Otocolobus manul

Within non-*Panthera* pantherines, one cat species that long has been considered difficult to place in phylogenetic studies is *Otocolobus manul* (Pallas's cat). Studies of morphology (Wayne et al., 1989), albumin immunological distance (AID; Collier and O'Brien, 1985), karyology (Wurster-Hill and Centerwall, 1982), mt cytb (Masuda et al., 1996), and ND5 genes (Johnson and O'Brien, 1997) identified the lineage resulting in *O. manul* as an early divergence among the domestic cat lineages. However, sequences from mt 12S and 16SrRNA genes (Johnson and O'Brien, 1997; Masuda et al., 1996), together with nuclear characters (Pecon Slattery and O'Brien, 1998; Yu et al., 2004b), provided novel evidence for the inclusion of it in the pantherine lineage.

Our individual solutions were equivocal on this issue, in which the position of *O. manul* was poorly resolved and showed no particular affinities to any taxon except for those in β-fibrinogen analyses and one of ND2 analysis. All β-fibrinogen trees diagnosed the association of *O. manul* with *L. lynx* with low bootstrap values (61% in MP; 58% in ML) but high posterior probability (97%), indicative of probable inclusion of it within the pantherine lineage. Of note, Pecon Slattery and O'Brien (1998) obtained the same relationship from introns of two Y-linked genes. A recent analysis by us, also based on nuclear genes (i.e., IRBP and TTR), failed to recover the linkage between *O. manul* and *L. lynx*; however, we argued for a closer relationship of it with the pantherine cats (Yu et al., 2004b). The combination of three nuclear genes (i.e., β-fibrinogen, IRBP, and TTR), unexpectedly showed no support of any view in this respect (Fig. 2B). In contrast, *O. manul* was supported as the closest sister taxon to two species of domestic cats lineage in ND2 MP (52%) and both combined mt and full datasets analyses

with strong bootstrap supports (96 and 92% in MP; 84 and 76% in ML) and high posterior probabilities (99 and 90%). Our analyses support placing the elusive *O. manul* inside the domestic cat lineage, although this was not supported by nuclear data.

4.2.3. Relatedness of Acinonyx jubatus and Puma concolor

The result of sister grouping between two distinctive monotypic genera, *A. jubatus* and *P. concolor* in our combined mt dataset was another important point in obtained phylogenies, although sequences from both taxa were missing in the nuclear data. The exact placements of these two species have been problematic. Bininda-Emonds (2001), for example, has recommended particular attention of them in future felid systematics. Several previous studies of DNA sequences (Johnson and O'Brien, 1997; Mattern and McLennan, 2000; Pecon Slattery and O'Brien, 1998) defined a monophyletic *Puma* group within the cat family composed of *A. jubatus*, *P. concolor*, and *H. yagouaroundi*, the former two of which were sampled here.

Our result confirmed a closer grouping of *Puma* and *Acionyx* to each other than either was to the other felids, a relationship also has been suggested by mt cytb and 12SrRNA gene (Janczewski et al., 1995) and by fossil evidence (Adams, 1979). Conflicting views which place *A. jubatus* as either the sister taxon to the *Panthera* group (Salles, 1992), or as the earliest genus to diverge within pantherine lineage (Collier and O'Brien, 1985) were not upheld by any of our analyses, nor was the hypothesis supporting *P. concolor* as either a close relative to the *Panthera* group (Salles, 1992) or as an divergent lineage within modern felids (Bininda-Emonds, 2001).

4.3. Utility of different mt and nuclear genes in phylogeny of cat family

Our results demonstrate that individual genes, including the six previously published gene data (tree not shown), and the combined nuclear dataset, fail to recover a satisfying phylogeny. This lack of resolution is largely due to insufficient phylogenetic information in individual loci. The concatenation of all mt genes, comprising four protein-coding and two rRNA genes, however, provides decisive resolution of the relationships among the taxa examined, as does the dataset of combined mtDNA and three nuclear genes. It is apparent that having more sites results in better resolution, and strong historical signals appear when multiple genes with distinct substitution dynamics are combined, as a consequence of their differential resolving powers at multiple taxonomic levels.

Newly established ND4 and ND2 datasets in present study proved especially informative in phylogenetic reconstruction of these recently evolved pantherine cats. Both genes evolved at a rapid rate and no sign of significant saturation were observed. Separate analyses of the ND2 and ND4 genes produced congruent tree topologies to those obtained from the combined mt and full data. The ND4 gene was found to contribute the most phylogenetic signal while ND2, equivalent to cytb, only lesser than ND4 and 16SrRNA to the final tree (Fig. 4; see Table 4). ND4 gene alone appeared enough to resolve *Panthera* relationships (including *N. nebulosa*) in terms of complete resolution and strong nodal supports (Fig. 2B), whereas it did not serve well in the rest of the pantherines. The other five mt genes included in this study (ND2, ND5, cytb, 12S, and 16SrRNA) were more helpful in the recovery of relatively basal divergences and affinities among genera, thus making the combination of all mt genes ideal for providing persuasive evidence of the resolution to all parts of the trees. Among the six mt genes examined, ND4 was shown the most appropriate while ND5 the least for the tree construction of closely related species. ND2, cytb, and the two rRNA genes were best able to resolve nodes in the middle range of divergence.

Nuclear genes have been shown to have a lower substitution rate and are less subject homoplasy than the mt genes. Our study also provides valuable information on the utility of three nuclear genes, β-fibrinogen, IRBP, and TTR, in the phylogenetic resolution of these pantherine cats. IRBP exon and TTR intron have been widely used for resolving relationships at a variety of taxonomic levels, particularly in carnivoran species (Flynn and Nedbal, 1998; Flynn et al., 2000; Sato et al., 2003, 2004; Yoder et al., 2003; Yu et al., 2004a,b). The β-fibrinogen intron gene was newly explored in the present study to reconstruct phylogeny of felids, encouraged by the fact that this region has been shown to contain valuable phylogenetic signals in various groups of avian taxa that diverged as anciently as 90 MYA as recently as 2–5 MYA (Prychitko and Moore, 1997, 2003). Although a basal split dividing the sampled taxa into two large clusters and some affinities within the clusters were revealed by ML analysis of the β-fibrinogen intron gene region (Fig. 1A), the β-fibrinogen region displayed low sequence divergence resulting in extremely short internodes, and consequently had less resolving power in recovering relatively recent branching patterns in pantherines. It is expected that this novel nuclear marker would be better suited for resolving supergeneric (e.g., interfamilial) relationships among carnivores and other mammals.

5. Supplementary materials

The sequences reported in this paper have been deposited in the GenBank database. Accession Nos. AY634369–AY634385 and AY634387–AY634405.

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References

Adams, D.B., 1979. The cheetah: native American. Science 205, 1155–1158.

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.

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.

Bininda-Emonds, O.R.P., 2001. The utility of chemical signals as phylogenetic characters: an example from the felidae. Biol. J. Linnean Soc. 72, 1–15.

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.

Chung, D.W., Que, B.G., Rixon, M.W.A., Mace Jr., M., Davie, E.W., 1983. Characterization of complementary deoxyribonucleic acid and genomic deoxyribonucleic acid for the β chain of human fibrinogen. Biochemistry 22, 3244–3250.

Collier, G.E., O'Brien, S.J., 1985. A molecular phylogeny of the Felidae: immunological distance. Evolution 39, 437–487.

Coltman, D.W., Wright, J.M., 1994. Can SINEs: a family of tRNA-derived retroposons specific to the superfamily Canoidea. Nucleic Acids Res. 22, 2726–2730.

Creer, S., Malhotra, A., Thorpe, R.S., 2003. Assessing the phylogenetic utility of four mitochondrial genes and a nuclear intron in the Asian pit viper genus, *Trimeresurus*: separate, simultaneous, and conditional data combination analyses. Mol. Biol. Evol. 20, 1240–1251.

Eastman, E.M., Gilula, N.B., 1989. Cloning and characterization of a cDNA for the β chain of rat fibrinogen: evolutionary conservation of translated and 3′-untranslated sequences. Gene 79, 151–158.

Ewer, R.F., 1973. The Carnivores. Cornell Univ. Press, Ithaca, NY.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1994. Testing significance of congruence. Cladistics 10, 315–320.

Farris, J.S., Kallersjo, M., Kluge, A.G., Bult, C., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

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.

Giannasi, N., Malhotra, A., Thorpe, R.S., 2001. Nuclear and mtDNA phylogenies of the *Trimeresurus* complex: implications for the gene versus species tree debate. Mol. Phylogenet. Evol. 19, 57–66.

Hemmer, H., 1971. In: Zur Dharakerisierung und stratigraphischen beduting von *Panthera gombaszoegensis* (Kretzoi, 1938). Neues Jahrbuch fur Geologie und palaontologie, Monatchefte, pp. 701–711.

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

Jae-Heup, K., Eizirik, E., O'Brien, S.J., Johnson, W.E., 2001. Structure and patterns of sequence variation in the mitochondrial DNA control region of the great cats. Mitochondrion 1, 279–292.

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, S98–S116.

Johnson, K.P., Clayton, D.H., 2000. Nuclear and mitochondrial genes contain similar phylogenetic signal for pigeons and doves (Aves: Columbiformes). Mol. Phylogenet. Evol. 14, 141–151.

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, Arizona, USA.

Kurten, B., Anderson, E., 1980. Pleistocene Mammals of North America. Columbia University Press, NY.

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

Leyhausen, P., 1979. Cat Behavior. Garland Press, NY.

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

Martin, L.D., 1980. Functional morphology and the evolution of cats. Trans. Neb. Acad. Sci. 13, 141–154.

Mattern, M.Y., McLennan, D.A., 2000. Phylogeny and speciation of Felids. Cladistics 16, 232–253.

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.

Moyle, R.G., 2004. Phylogenetics of barbets (Aves: Piciformes) based on nuclear and mitochondrial DNA sequence data. Mol. Phylogenet. Evol. 30, 187–200.

Neff, N.A., 1982. The Big Cats: The Paintings of Guy Coheleach. Abrams Press, NY.

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

O'Brien, S.J., Collier, G.E., Benveniste, R.E., Nash, W.G., Newman, A.K., Simonson, J.M., Eichelberger, M.A., Seal, U.S., Janssen, D., Bush, M., Wildt, D.E., 1987. Setting the molecular clock in Felidae: the great cats, Panthera. In: Tilson, R.L., Seal, U.S. (Eds.), Tigers of the World. Noyes, NJ, pp. 10–27.

Okada, N., 1991a. SINEs: short interspersed repeated elements of the eukaryotic genome. Trends Ecol. Evol. 6, 358–361.

Okada, N., 1991b. SINEs. Curr. Opin. Genet. Dev. 1, 498–504.

Pecon Slattery, J., Johnson, W.E., Goldman, D., O'Brien, S.J., 1994. Phylogenetic reconstruction of South American felids defined by protein electrophoresis. J. Mol. Evol. 39, 296–305.

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.

Prychitko, T.M., Moore, W.S., 1997. The utility of DNA sequences of an intron from the β-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). Mol. Phylogenet. Evol. 8, 193–204.

Prychitko, T.M., Moore, W.S., 2000. Comparative evolution of the mitochondrial cytochrome b gene and nuclear β-fibrinogen intron 7 in woodpeckers. Mol. Biol. Evol. 17, 1101–1111.

Prychitko, T.M., Moore, W.S., 2003. Alignment and phylogenetic analysis of β-fibrinogen intron 7 sequences among avian orders reveal conserved regions within the intron. Mol. Biol. Evol. 20, 762–771.

Reeves, R.H., O'Brien, S.J., 1984. Molecular genetic characterization of the RH-114 gene family of endogenous feline retroviral sequences of the domestic cat. J. Virol. 52, 164–171.

Rpnquist, F.R., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1752–1754.

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

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

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 Univ., Boston, MA.

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.

Templeton, A.R., 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37, 221–244.

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.

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 Univ. Press, Ithaca, NY, pp. 465–495.

Weibel, A.C., Moore, W.S., 2002. A test of a mitochondrial gene-based phylogeny of woodpeckers (Genus Picoides) using an independent nuclear gene. Mol. Phylogenet. Evol. 22, 247–257.

Werdelin, L., 1985. Small Pleistocene felines of North America. J. Vert. Paleo. 5, 194–210.

Wurster-Hill, D.H., Centerwall, W.R., 1982. The interrelationships of chromosome banding patterns in Procyonids, Viverrids and Felids. Cytogenet. Cell Genet. 34, 178–192.

Xia, X., 2000. DAMBE: Data Analysis in Molecular Biology and Evolution. Kluwer Academic, Boston.

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., 2004a. Phylogeny of the bears (Ursidae) based on nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 32, 480–494.

Yu, L., Li, Q.W., Ryder, O.A., Zhang, Y.P., 2004b. Phylogenetic relationships within mammalian order Carnivora indicated by sequences of two nuclear DNA genes. Mol. Phylogenet. Evol. 33, 694–705.