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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

Li Yu [a,b,c], Ya-ping Zhang [a,b,*]

[a] *Laboratory of Cellular and Molecular Evolution, and Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China*
[b] *Laboratory for Conservation and Utilization of Bio-Resource, Yunnan University, Kunming 650091, China*
[c] *Graduate School, Chinese Academy of Sciences, Beijing, China*

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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.

* Corresponding author. Fax: +86 871 5195430.
E-mail address: zhangyp1@263.net.cn (Y.-p. Zhang).

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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.