

GENETIC DIVERSITY OF FELIDS' CYTOCHROME B[†]

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Abstract

The mitochondrion cytochrome b gene has been widely used for genetic diversity work such as species identification, taxonomic and phylogenetic studies. Cytochrome b gene of three felid families; Asian golden cat (*Catopuma temminckii*), flat-headed cat (*Prionailurus planiceps*), and fishing cat (*Prionailurus viverrinus*) have been cloned, sequenced, translated and compared with the cytochrome b gene and protein of *Felis catus* (domestic cat) accession number U20753. From 1,140 bp of the cytochrome b gene, 232 positions showed variation among these sequences. However, only 30 of 379 amino acids full-length cytochrome b peptide varied. Phylogenetic analysis indicated that *C. temminckii* is more closely related to *F. catus* than the other two *Prionailurus*. The differences in the cytochrome b gene will later be used for probe design for mitochondrial DNA identification in interspecies cloning research.

Keywords: Genetic diversity, Cytochrome b, Felid species

Introduction

The oocyte is valuable. This is an unmistakable fact underscored by the scarcity of oocytes available from species where they could be most useful, i.e., human, for biomedical applications, and endangered or extinct species, for their conservation and rescue. One of the possibilities presented by somatic cell nuclear transfer (SCNT) is called interspecies cloning, where the recipient ooplast and donor nucleus are derived from different species.

Interspecies somatic cell nuclear transfer (iSCNT) or interspecies cloning involves transferring cell nuclei of one species into enucleated

oocytes of another species. In cloned animals where somatic cell nuclei and oocytes are from the same or closely related species, the mitochondrial DNA (mtDNA) of the oocyte is dominantly inherited. However, in interspecies cloned embryos where nuclear donor and oocyte are from two distantly related species, the distribution of the mtDNA species is not known. The fate of foreign mtDNA following nuclear transfer (NT) is still controversial. Mitochondrial DNAs are essential for cellular function including regulation of energy biosynthesis. At present, mtDNA has been a key determinant in deciding

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the developmental fate of cloned embryos to term and the survival of subsequent offspring. The mtDNAs of cloned embryos should be derived mainly from recipient cytoplasm with a limited contribution/transmission from the donor cell. In the cloned embryos to term, the resultant animals exhibit one of the three patterns of mtDNA transmission 1) homoplasmy derived from the recipient oocytes, 2) homoplasmy derived exclusively from the donor somatic cells, or 3) heteroplasmy from the fusion partners (St John *et al.*, 2004). Varying changes in distribution, proliferation and activity during early and subsequent stages of development have been observed (Katayama *et al.*, 2006). The genotypes and quantities of mtDNA in cloned embryos and animals may illustrate mosaicism depending on the species, embryonic stage, donor cells used, organs in which it is detected and individual-to-individual variations (Chen *et al.*, 2002; Takeda *et al.*, 2003; Hua *et al.*, 2008).

The successful of offspring productions with iSCNT have been shown in gaur (Lanza *et al.*, 2000), mouflon (Loi *et al.*, 2001), zebu (Meirelles *et al.*, 2001), African wild cat (Gomez *et al.*, 2004), and yak (Li *et al.*, 2006). In felids species, cloned domestic cat embryos have been successfully produced as a model for wild felids (Shin *et al.*, 2002; Skrzyszowska *et al.*, 2002; Gomez *et al.*, 2003, 2004; Kitiyanant *et al.*, 2003; Wen *et al.* 2003; Yin *et al.*, 2005). Leopard cat (Lorthongpanich *et al.*, 2004) and African wild cat (Gomez *et al.*, 2004) embryos have also been produced from enucleated domestic cat oocytes. The birth of cloned domestic cats (Shin *et al.*, 2002; Yin *et al.*, 2005) and cloned iSCNT African wild cats (Gomez *et al.*, 2004) exhibits the effectiveness of interspecies cloning.

In some previous reports, the mtDNA of SCNT animals were exclusively derived from the recipient oocytes (Takeda *et al.*, 1999, Meirelles *et al.*, 2001). Conversely, it has been reported that in panda-rabbit iSCNT embryos, mtDNA from both panda fibroblasts and rabbit oocytes coexisted in early blastocysts. However, the mtDNA from the recipient rabbit oocytes was eliminated after implantation (Chen *et al.*, 2002). In cloned calves, some calves and fetuses have

been reported to show proliferation of mtDNA from donor cells during embryogenesis (Takeda *et al.*, 2003). These differential mtDNA outcomes provide an opportunity to investigate and define the involvement of mitochondrial persistence and inheritance in relation to development and survival frequencies in interspecies cloned embryo production. In this study, cytochrome b genes of three felid families: Asian golden cat (*Catopuma temminckii*), flat-headed cat (*Prionailurus planiceps*), and fishing cat (*Prionailurus viverrinus*), have been cloned, sequenced, translated and compared with the cytochrome b gene and protein of *Felis catus* (domestic cat) accession number U20753. This will be used for the study of mtDNA nuclear transfer in interspecies cat cloning.

Materials and Methods

Mitochondrial DNA Extraction

Mitochondrial DNA of three felid families: Asian golden cat (*C. temminckii*), flat-headed cat (*P. planiceps*) and fishing cat (*P. viverrinus*) were extracted from fibroblast using the method described by Sambrook and Russell (2001).

Polymerase Chain Reaction (PCR) and PCR Product Purification

To amplify the complete sequence (1,140 bp) of the cytochrome b gene, primers CytoB15008/CytoB16260 (5'-CCATCGTTGTA TTTCAACTATAAGA AC-3' and 5'-GCTTCT TCCTTGAAGTCTTAGGG-3') were used for the amplification of the target region. We designed this primer set based on the domestic cat mtDNA sequence (accession number U20753). The amplification of the cytochrome b region was performed in a final volume of 50 µl containing 0.5µg DNA extract, 1x Green GoTaq® Flexi buffer (Promega), 200 mM each dNTP (Promega), 1.25 U of GoTaq DNA polymerase (Promega), and 0.5 µM of each primer. The amplification was carried out in a DNA Thermal cycler (GeneAmp® PCR System 2400, PERKIN ELMER) for an initial denaturation at 95°C for 2 min followed by 45 cycles of 95°C for 30 sec, 50°C for 30 sec and

72°C for 2 min followed by final extension at 72°C for 5 min. One microliter of PCR product was electrophoresed on 1% agarose gel and stained with ethidium bromide, and the DNA band in the gel was then visualized under an ultraviolet illuminator. The remaining PCR products (49µl) were purified by the QIAquick PCR Purification kit (Qiagen, Servicios Hospitalarios, Barcelona, Spain).

Sequencing of the PCR Product

DNA fragments were ligated into pGEM-T Easy Vector (Promega). Ligation mixes were transformed into competent *Escherichia coli* DH5 α using electroporation method. Transformants were selected by using ampicillin and the presence of the pGEM-T Easy containing insert was determined by screening for blue/white colonies using X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) and IPTG (isopropyl- β -D-thiogalactopyranoside). The presence of insert was confirmed by restriction analysis using *Eco*RI and sent to Macrogen, Korea for DNA sequence analysis using T7 promoter and SP6 primers. At least 3 clones of each sample were sequenced. The cytochrome b sequences were compared with those in the database at GenBank by using blast program (NCBI) and were used to

determine genetic diversity.

Construction of Phylogenetic Tree

For the phylogenetic and molecular evolutionary analyses, the alignment was performed by using the CLUSTALW and Maximum Parsimony algorithms of *MEGA* version 4 (Tamura *et al.*, 2007). The following sequences (with GenBank accession numbers in parentheses) were analyzed: *C. temminckii* (FJ594957), *P. planiceps* (FJ594958), *P. viverrinus* (FJ594959), *F. catus* (U20753), *L. pardinus* (EF689048), *L. canadensis* (AY928671), and *P. bengalensis* (AB210238).

Results and Discussion

The felid cytochrome b specific primer set (CytoB15008/CytoB16260) used in this experiment was designed based on the domestic cat mtDNA sequence (accession number U20753) reported from Gene Bank. The PCR products of 1,252 bp were detected in all felid species after ethidium bromide staining (Figure 1). The identities of these bands were further confirmed by sequencing (data not shown) by Macrogen, Korea.

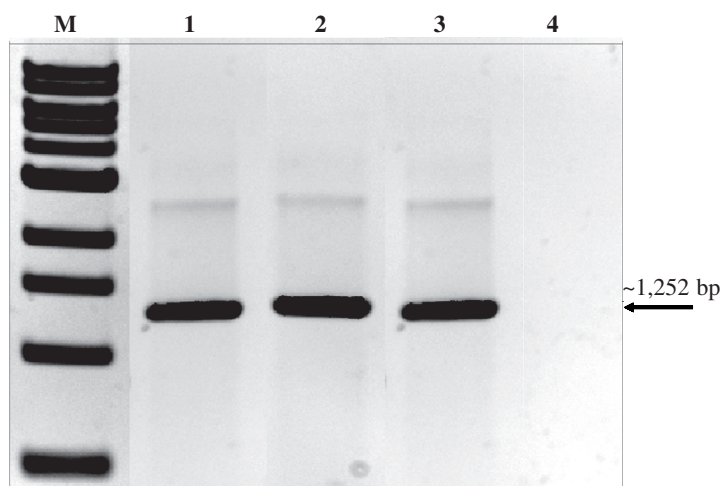


Figure 1. Ethidium-bromide-stained 1% agarose gel displaying amplification cytochrome b gene of three felid families, ~1,252 bp. Lane: M; 1Kb marker, 2; Asian golden cat (*C. temminckii*), 2; flat-headed cat (*P. planiceps*), 3; fishing cat (*P. viverrinus*) and lane 4; negative control, without genomic DNA in PCR

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Felis_catus      ATGACCAACATTCGAAAAATCACACCCCTTACCAAAATTAATTAACACTC
Catopuma_temminckii ATGACCAACATTCGAAAAATCACACCCCTTATTTAAATTAATTAACACTC
Prionailurus_viverrinus ATGACCAACATTCGAAAAATCACACCCCTTATCAAAATCATCAACCACTC
Prionailurus_planiceps ATGACCAACATTCGAAAAATCACACCCCTTATTTAAATCATCAACCACTC
*****
Felis_catus      ATTTCATCGACCTACCTGCCCATCTAACATCTCAGCATGATGAAACTTCG
Catopuma_temminckii ACTTCATTGATCTACCTGCCCATCTCAACATCTCAGCGTATGAAACTTTG
Prionailurus_viverrinus ATTTCATGATCTACCCACCCATCTAATATCTCAGCATGATGAAACTTCG
Prionailurus_planiceps ATTTCATCGATCTGCCTGCCCATCTCAACATCTCAGCATGGTGGAACTTCG
*****
Felis_catus      GCTCCCTTCTAGGAGTCTGCCAATCTTACAAATCTCACCGGCCTCTTT
Catopuma_temminckii GATCCCTGCTAGGAGTCTGCCAATCTTACAAATCTTCACTGGCCTTTTC
Prionailurus_viverrinus GCTCCCTGCTAGGAGTATGCCAATCTTACAAATCTTACCGGCCTTTTC
Prionailurus_planiceps GCTCCCTGCTAGGAGTATGCCAATCTTACAAATCTTACCGGCCTCTTC
*****
Felis_catus      TTGGCCATACACTACACATCAGACACAACAACCGCCTTTTCATCAGTTAC
Catopuma_temminckii CTAGCCATACACTACACATCGGACACAATAACCGCCTTCTCATCAGTCAC
Prionailurus_viverrinus CTAGCCATACACTACACATCAGATACAACACCGCCTTCTCATCAGTCAC
Prionailurus_planiceps TTAGCCATACACTACACACAGATACAACAACCGCCTTCTCATCAGTCAC
*****
Felis_catus      CCACATCTGTGCGGACGTAAATTTATGGCTGAATCATCCGATATTTACACG
Catopuma_temminckii CCATATCTGCGCGGACGTCAACTACGGCTGAATTTATTCGATACATACATG
Prionailurus_viverrinus TCACATCTGTGCGGACGTAAACTACGGCTGAATCATCCGGTACATACACG
Prionailurus_planiceps CCACATCTGTGCGGACGTCAACTACGGCTGAATTTATCCGATACATGACAG
*****
Felis_catus      CCAACGGAGCTTCTATATCTTTATCTGCCGTACATACATGTAGGACGG
Catopuma_temminckii CCAACGGAGCTTCCATATCTTTATCTGCCGTATACATGTAGGACGA
Prionailurus_viverrinus CCAACGGAGCTTCCATATCTTTATCTGTCTATACATGCAGTGGGCCGA
Prionailurus_planiceps CCAACGGAGCTTCCATATTTTATCTGCCGTATACATGCAGTAGGCCGA
*****
Felis_catus      GGAATATACTACGGCTCCTACACCTTCTCAGAGACATGAAACATTGGAAT
Catopuma_temminckii GGAATGTACTACGGCTCCTACACCTTCTCAGAAACATGAAATATCGGAAT
Prionailurus_viverrinus GGAATATATTTATGGTTCTTACACCTTCTCAGAAACATGAAACATTGGAAT
Prionailurus_planiceps GGAATATATTTACGGCTCCTATACCTTCTCTGAAACATGAAACATTGGAAT
*****
Felis_catus      CATACTATTTATTTACAGTCATAGCCACAGCTTTTATGGGATACGTCCTAC
Catopuma_temminckii TCTACTACTATTTACAGTTATAGCCACAGCTTTTATAGGATACGTCCTTAC
Prionailurus_viverrinus CATACTGCTATTCGAGCCATAGCCACAGCTTTTCATAGGTACGTACTAC
Prionailurus_planiceps CGTACTACTATTCGAGTCATAGCTACAGCCTTTCATGGGCTACGTACTAC
*****
Felis_catus      CATGAGGCCAAATGTCTTCTGAGGAGCAACCGTAATCACTAACCTCCTG
Catopuma_temminckii CATGAGGCCAAATATCTTCTGAGGAGCAACCGTAATTACCAACCTCCTA
Prionailurus_viverrinus CATGAGGCCAAATATCTTCTGAGGAGCAACCGTAATCACTAACCTCCTA
Prionailurus_planiceps CATGAGGCCAAATATCTTCTGAGGAGCAACCGTAATTACCAACCTCCTA
*****
Felis_catus      TCAGCAATTCATACATCGGACTGAAGTGTAGTAATGAATCTGAGGGGG
Catopuma_temminckii TCAGCAATCCCATACATCGGAAGTAACTTAGTAGAATGAATCTGAGGGGG
Prionailurus_viverrinus TCAGCAATCCCATACATTCGAGCCAACTTAGTAGAATGGGTCTGAGGGGG
Prionailurus_planiceps TCAGCAATCCCATATATTTGAACCGACCTAGTAGAATGAATCTGAGGGGG
*****
Felis_catus      CTTCTCAGTAGACAAAGCCACCCTAACACGATTCTTTGCTTTCCACTTCA
Catopuma_temminckii CTTTTCAGTAGATAAAGCCACCCTAACACGATTCTTTGCTTTCCACTTFA
Prionailurus_viverrinus TTTCTCAGTAGACAAAGCCACCCTAACACGATTCTTCGCTTTCCACTTTCA
Prionailurus_planiceps CTTTTCAGTAGACAAAGCCACCCTAACACGATTCTTCGCTTTCCACTTTFA
*****
Felis_catus      TTCTTCCATTTCATTATCTCAGCCTTAGCAGCAGTACACCTCTTATTCCTT
Catopuma_temminckii TCCTCCCATTTCAATTATCTCAGCCTTAGCAGCAGTACACCTCTTATTCCTT
Prionailurus_viverrinus TTCTCCCATTCATTATTTTCAGCCTTAGCAGCAGTACACCTCTTATTCCTC
Prionailurus_planiceps TCCTTCCGTTTCATCTCTCAGCCTTAGCAGCAGTACACCTCTTATTTCTC
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Figure 2. The full nucleotide sequences of cytochrome b gene. Sequences were aligned using blast program from NCBI database. The star symbol indicates the same nucleotide sequence

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Felis_catus
Catopuma_temminckii
Prionailurus_viverrinus
Prionailurus_planiceps
CATGAAACAGGATCTAACAAACCCCTCAGGAATTACATCCGATTCAGACAA
CACGAAACAGGATCTAACAAACCCCTCAGGAATTACATCCGATTCAGACAA
CATGAAACAGGATCCAAATAACCCCTCAGGAGTCATATCCGACTCGGACAA
CATGAAACAGGATCCAAACAAACCCCTCAGGAATTATATCCGACTCAGATAA
** ***** ** ***** ** ***** ** ***** ** *****
AATCCCATTCACCCCATACTATACAATCAAGACATCCTAGGTCTCTCTAG
AATCCCATTCACCCGTACTACACAATCAAGACATCCTAGGTCTCTCTAG
AATCCCATTCACCCCATATTATACAATCAAGACATCCTAGGCTCTCTAG
AATCCCATTCACCCCATACTACACAATCAAGGATGTCTAGGCTCTCTAA
***** ** ***** ** ***** ** ***** ** *****
TACTAGTTTAAACACTCATACTACTCGTCTATTTTTACCAGACCTGCTA
TATTAAATCTTACACTTATATGCTCGTCTATTTTACACCAGACCTGCTA
TACTAATCTTAAACACTTATACTACTTGTCTATTCTCACCAGACCTGCTA
TACTAATCTTAACTACTACTACTCGTCTATTCTCACCAGACCTACTA
***** ** ***** ** ***** ** ***** ** *****
GGAGACCCAGACAATACATCCCGCAACCCCTTTAAATACCCCTCCCCA
GGAGACCCAGACAATACATTCCTGCCAACCCCTCTAAATACCCCTCCCCA
GGAGACCCAGATAACTACACTCCCGCTAATCTTTTAAACACCCCTCCCCA
GGAGACCCAGACAATACATCCCGCTAATCTCTGAAACACTCTCTCCCCA
***** ** ***** ** ***** ** ***** ** *****
TATTAAACCTGAATGATACTTCTTATTCGCATACGCAATTCTCCGATCCA
TATTAAACCTGAGTGGTACTTCTTATTTGCGTACGCAATCTCTCCGATCCA
TATCAAGCCCGAATGATACTTCTTATTCGCATACGCAATTCTCTCCGATCTA
TATTAAACCCGAATGATACTTCTTATTCGCATACGCAATTCTCTCCGATCCA
***** ** ***** ** ***** ** ***** ** *****
TCCCAAACAACCTAGGGGAGTCTTAGCCCTAGTACTCTCCATCTCTAGTA
TCCCAAACAACCTAGGAGGAGTCTTAGCCCTAGTACTCTCCATCTCTAGTC
TCCCTAATAAACTGGGAGGGTCTTAGCCCTTGGTACTCTCCATCTCTAATC
TCCCTAACAATTTGGGAGGGTCTTAGCCCTAGTACTCTCCATCTCTAATC
***** ** ***** ** ***** ** ***** ** *****
CTAGCAATCATTCCAATCTCCACACCTCCAACAACGAGGAATAATGTT
CTAGCAATCATCCCAATCTCCACACCTCCAACAACGAGGAATAACATTT
CTAGCAATTATTCCAGTCTCCACACCTCCAACAACGAGGAATAATATT
CTGGCAATTATTCCCAATCTCCACACCTCCAACAACGAGGAATAATATT
** ***** ** ***** ** ***** ** ***** ** *****
TCGACCACTAAGCCAATGTCTATTCTGACTCTAGTAGCGGATCTCTCTAA
TCGACCACTAAGCCAGTGTCTATTCTGATTTCTAGTAGCAGACCTCTCTGA
CCGACCTCTAAGCCAATGCTTATTCTGACTCTAGTAGCAGACCTCTCTAA
CCGACCTTTAAGCCAATGCTTATTCTGACTCTAGTAGCGGACCTCTCTAA
***** ** ***** ** ***** ** ***** ** *****
CCCTAACATGAATCGGTGGCCAACTGTAGAATATCCATTCATCACCATC
CCTTAACATGAATTTGGTGGCCAAACCGTAGAATATCCCTTTATCACCATC
CCCTAACATGAATTTGGCGGCCAAACCGTAGAATATCCCTTTATCACCATC
TCCTAACATGAATTTGGCGGTCAACCGTAGAGTACCCCTTCATCACCATC
* ***** ** ***** ** ***** ** ***** ** *****
GGCCAACCTAGCCTCCATCTTATTTCTCAACCTCTCTAATCTAATACC
GGCCAACCTAGCCTCCATCTTATCTTCTCAATTTCTACTGGTCTAATACC
GGCCAGCTAGCCTCCATCTTATCTTTCTCAACCTCTCTAGTCTAATACC
GGCCAACCTAGCTTCATCTCTTATCTTCTCAACCTCTCTAGTCTAATACC
***** ** ***** ** ***** ** ***** ** *****
CATCTCAGGCATTATTGAAAACCGCCTACTCAAATGAAGA
CATTTCAGGCATTATCGAAAACCGCCTCTCTAAATGAAGA
CATTTCAGGCATTATTGAAAACCGCCTCTCTCAAATGAAGA
CATCTCAGGCATTATTGAAAACCGCCTCTCAAATGAAGA
*** ***** ** ***** ** *****

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Figure 2. (Continued)

From the three felid families: Asian golden cat (*C. temminckii*), flat-headed cat (*P. planiceps*), and fishing cat (*P. viverrinus*), the complete (1,140 bp) cytochrome b and protein sequences were determined (Figures 2 and 3). From 1,140 bp of the cytochrome b gene, 232 positions showed variation among these sequences. However, only 30 of 379 amino acids full-length cytochrome b peptide varied. The three felid species cytochrome b sequences were sent to Genbank. The Accession numbers of Asian golden cat (*C. temminckii*), flat-headed cat (*P. planiceps*), and fishing cat (*P. viverrinus*) were obtained as FJ594957, FJ594958 and FJ594959, respectively. From our mtDNA based phyloge-

netic analysis, it is apparent that the mtDNA sequences identified in this report form two distinct clusters belonging to group I that includes *F. catus*, *C. temminckii*, *L. canadensis* and *L. pardinus* and are distant from *P. planiceps* and *P. viverrinus*, a member of group II (Figure 4). This result indicated that flat-headed cat (*P. planiceps*) and fishing cat (*P. viverrinus*) are more closely related to lynx than the Asian golden cat (*C. temminckii*). And the Asian golden cat is more closely related to domestic cat (*F. catus*).

From this experiment, the designed felid cytochrome b specific primer set was able to amplify cytochrome b gene from felid and even

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Felis_catus      MTNIRKSHPLTKIINHSDIDLPAAPSNI SAZZNFGSLLGVCILILQILTGLF
Prionailurus_planiceps MTNIRKSHPLIKIINHSDIDLPAAPSNI SAWWNFGSLLGVCILILQILTGLF
Prionailurus_viverrinus MTNIRKSHPLIKIINHSDIDLPAAPSNI SAZZNFGSLLGVCILILQILTGLF
Catopuma_temminckii  MTNIRKSHPLIKIINHSLIDLPAAPSNI SAZZNFGSLLGVCILILQILTGLF
*****
Felis_catus      LAIHYSDTTTFSSVTHICRDVNYGZII RYLHANGASIFFICLYIHVGR
Prionailurus_planiceps LAIHYPDTTTFSSVTHICRDVNYGZII RYMHANGASIFFICLYIHVGR
Prionailurus_viverrinus LAIHYSDTTTFSSVTHICRDVNYGZII RYIHANGASIFFICLYMHVGR
Catopuma_temminckii  LAIHYSDTTTFSSVTHICRDVNYGZII RYIHANGASIFFICLYMHVGR
*****
Felis_catus      GIYGSYTFSETZNIGIILLFTVIATAFMGYVLPZQMSFZGATVITNLL
Prionailurus_planiceps GIYGSYTFSETZNIGVLLFAVIATAFMGYVLPZQGISFZGATVITNLL
Prionailurus_viverrinus GIYGSYTFSETZNIGIILLFAAIATAF IGYVLPWQGISFZGATVITNLL
Catopuma_temminckii  GMYGSYTFSENMKYRNSTTIYSYSHSFYRIRLTPRPNILLRSNRNYQPP
*
Felis_catus      SAIPYIGTELVEZIZGGFSVDKATLTRFFAFHFILPFIISALAAVHLLFL
Prionailurus_planiceps SAIPYIGTDLVEZIZGGFSVDKATLTRFFAFHFILPFIISALAAVHLLFL
Prionailurus_viverrinus SAIPYIGANLVEWVZGGFSVDKATLTRFFAFHFILPFIISALAAVHLLFL
Catopuma_temminckii  ISNPIHRNZLSRMNLRGLFSRZSHPNTILCLPLYPPIHYLSLSSSTPPIP
*
Felis_catus      HETGSNNPSGITSDSDKIPFHPYTTIKDILGLLVLLTILLLVLFSPDLL
Prionailurus_planiceps HETGSNNPSGIIISDSKIPFHPYTTIKDVLGLLILILLLVLFSPDLL
Prionailurus_viverrinus HETGSNNPSGVIISDSKIPFHPYTTIKDILGLLVLLTILLLVLFSPDLL
Catopuma_temminckii  SRNRIZQPLRNYIRLRQNPFPVLHNQRHPSPSINSYTYIARSITYTRPA
..
Felis_catus      GDPDNYIPANPLNTPPHIKPEZYFLFAYAILRSIPNKLGGVLAIVLSILV
Prionailurus_planiceps GDPDNYIPANPLNTPPHIKPEZYFLFAYAILRSIPNKLGGVLAIVLSILI
Prionailurus_viverrinus GDPDNYTPANPLNTPPHIKPEZYFLFAYAILRSIPNKLGGVLAIVLSILI
Catopuma_temminckii  RRPRQLHSCQPSKYPSYZTZ—VVLICVRNPPHHPKQTRRSPSLST
*
Felis_catus      LAIIPILHTSKQRGIMFRPLSQCLFZLLVADLLTLTIZGGQPVEHPFITI
Prionailurus_planiceps LAIIPILHTSKQRGIIIFRPLSQCLFZLLVADLLTLTIZGGQPVEYFPFITI
Prionailurus_viverrinus LAIIPVLHTSKQRGIIIFRPLSQCLFZLLVADLLTLTIZGGQPVEYFPFITI
Catopuma_temminckii  LHSSPSNHPNPPHLQITRNISTTK—PVLILLSSSRPDLNMNMWPT
*
Felis_catus      QQLASILYFSTLLILIPISGIIENRLLKZR-----
Prionailurus_planiceps QQLASILYFSTLLVLIPISGIIENRLLKZR-----
Prionailurus_viverrinus QQLASILYFSTLLVLIPISGIIENRLLKZR-----
Catopuma_temminckii  RRTSLYHHRPTGLHLILLNSTGPNTHFRHYRKPPPMZMK
:

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Figure 3. The full amino acid sequences of cytochrome b gene. Sequences were aligned using blast program from NCBI database. The star symbol indicates the same nucleotide sequence

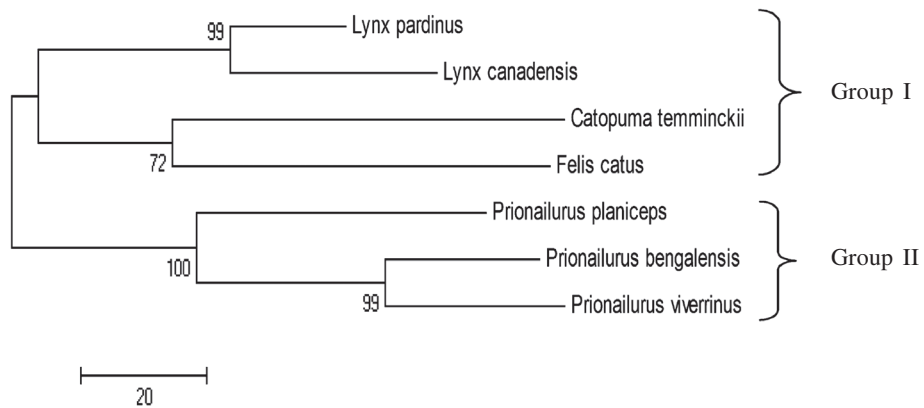


Figure 4. Phylogenetic tree of some of the members of the cat family. Tree was generated based on a CLUSTALW alignment and the using Maximum parsimony method (MEGA). Numbers above the branches represent the percentages of 1,000 bootstrap replications

more distantly related family. Later on, the differences in the cytochrome b gene of each species will be used in the content on mtDNA in iSCNT.

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