http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (16): 2639-2645, 2007
ISSN 1028-8880

Genetic Relationships among Wild Felidae in Thailand Using AFLP Markers

[1]Nattapong Srisamoot, [2]Arunrat Chaveerach, [1]Suporn Nuchadomrong, [1]Nison Sattayasai,
[3]Prapansak Chaveerach, [2]Alongkoad Tanomtong and [4]Krit Pinthong
[1]Department of Biochemistry, [2]Department of Biology, Faculty of Science,
Khon Kaen University, Khon Kaen, 40002, Thailand
[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine,
Khon Kaen University, Khon Kaen, 40002, Thailand
[4]Biology Program, Faculty of Science and Technology,
Surin Rajabhat University, Surin, 32000, Thailand

Abstract: The cytogenetics of eight Felidae species in Thailand were investigated by the colchicines-hypotonic fixation-air drying technique followed by a conventional technique. All species studied have an identical number of 38 diploid chromosomes, indicating a close genetic relationship among species. At a deep study level, the genetic relationships of eight Felidae species were accessed by the AFLP method. Blood samples were collected from sources locating in their original regions for DNA extraction. With ten successful primer combinations, a total of 4208 scorable bands were generated. Of these bands, 18.91% are polymorphic. Percentages of Polymorphic Bands (PPB) for each primer combination range from 15.00 to 23.59%. The generating bands were used for dendrogram construction. The average genetic similarity values among all Felidae species are 68.20% (between *Panthera tigris* and *Neofelis nebulosa*) to 85.53% (between *Prionailurus bengalensis* and *Prionailurus viverrinus*). The dendrogram shows that the eight Felidae species were clustered together and the subfamily Pantherinae and Felinae with *Neofelis nebulosa* are distinguished. The Felinae, *Prionailurus bengalensis*, *Prionailurus viverrinus*, *Catopuma temminckii*, *Felis chaus*, *Pardofelis marmorata* and *Neofelis nebulosa* were clustered together with 91% bootstrap support and the Pantherinae, *Panthera pardus* is clustered with *Panthera tigris* with 92% bootstrap support. In summary, the ten successful primer combinations can be used to determine genetic differences among eight Thailand Felidae species.

Key words: AFLP, genetic relationships, subfamily Felinae, subfamily Pantherinae, wild Felidae

INTRODUCTION

All members of Felidae were considered from major population in Appendix I and II of CITES and on the IUCN Red list of threatened, endangered and extinct species (Baillie *et al*., 2004). Thailand has many charismatic felid species in the world, including tiger (*Panthera tigris*), leopard (*Panthera pardus*), clouded leopard (*Neofelis nebulosa*), asian leopard cat (*Prionailurus bengalensis*), fishing cat (*Prionailurus viverrinus*), flat-headed cat (*Prionailurus planiceps*), asian golden cat (*Catopuma temminckii*), jungle cat (*Felis chaus*) and marbled cat (*Pardofelis marmorata*). Nine species of Felidae in Thailand can be divided into two subfamilies, Felinae and Pantherinae, based on morphological classification (Wilson and Reeder, 2006). Habitat loss has occurred violently throughout Southeast Asia over the past 20 years and these felid species are vulnerable to population pressures and habitat fragmentation. Forest destruction has negatively affected on wild animals such as Felidae. It reduces habitat for wild animals and causes population fragmentation due to the loss of genetic heterogeneity and thus they become vulnerable to environmental change and risk extinction.

Molecular genetic data for Felidae in Thailand, such as genetic relationships, is directly needed for conservation. The phylogenetic relationships among the Felidae have been addressed by several studies that employed both morphological and molecular techniques. Early efforts included comparative karyology (Modi and O'Brien, 1988; Wurster-Hill and Centerwall, 1982), cross-species chromosome painting (Tian *et al*., 2004), the genomic occurrence of two felid endogenous retroviruses (Benveniste and Todaro, 1974;

Corresponding Author: Arunrat Chaveerach, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand Tel: 66 4334-2908 Fax: 66 4336-4169

Benveniste *et al*., 1975; Reeves and O'Brien, 1984), albumin immunological distance (Collier and O'Brien, 1985), comparative morphology (Salles, 1992), allozyme electrophoresis (O'Brien *et al*., 1987; Pecon-Slattery *et al*., 1994) and two-dimensional protein electrophoresis (Pecon-Slattery *et al*., 1994). More recently, efforts to resolve phylogenetic relationships have focused on the nuclear DNA sequence (Flynn *et al*., 2005; Sato *et al*., 2006; Zhang *et al*., 2006) and mitochondrial genome (Janczewski *et al*., 1995; Johnson *et al*., 1996; Lopez *et al*., 1994; Uphyrkina *et al*., 2002; Johnson *et al*., 2004; Flynn *et al*., 2005; Koepfli *et al*., 2006). However, few analyses have included information from Amplified Fragment-Length Polymorphism (AFLP).

AFLP has become a popular marker technique in genetic relationships because it combines restriction digestion and amplification of DNA fragments, with no prior knowledge about the target genome required (Vos *et al*., 1995). Abundant polymorphism and reproducibility have been proved to be advantages of the AFLP technique (Pejic *et al*., 1998; Powell *et al*., 1996; Russell *et al*., 1997). The high frequency of identifiable polymorphic AFLP markers, coupled with their reproducibility, make this technique an attractive tool for detecting polymorphism and determining genetic relationships (Gupta *et al*., 1999). Moreover, the genetic relationships within Felidae in Thailand have never been addressed using AFLP analysis.

In this study we investigated the cytogenetics data and AFLP was applied to estimate genetic relationships of eight Felidae species in Thailand, without *Prionailurus planiceps* because of lack of sample.

MATERIALS AND METHODS

Sample collection: Blood samples of *Prionailurus bengalensis*, *Prionailurus viverrinus*, *Catopuma temminckii*, *Felis chaus*, *Pardofelis marmorata*, *Neofelis nebulosa*, *Panthera tigris* and *Panthera pardus* were taken from sources locating in their original regions for DNA extraction as shown in Table 1 in 2003-2004. AFLP fingerprints were done at Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand in 2004-2006. As both morphological and molecular evidence place the family Viverridae as a successive sister-group to the Felidae (Mattern and McLennan, 2000), so the Viverridae, *Paguma larvata* (Masked-palm civet) was chosen as an outgroup in our analysis.

Cytogenetic analysis: The cultured cells were examined by the colchicines-hypotonic fixation-air drying technique followed by a conventional technique (Tanomtong *et al*., 2005). Chromosomal checks were performed with 20 cells of each individual by light microscopy.

DNA extraction: Genomic DNA was isolated from blood samples using protinase K digestion and treatment with phenol/chloroform (Sambrook *et al*., 1989). The quality and quantity of extracted DNA was checked by 0.8% agarose gel electrophoresis and spectrophotometry.

AFLP procedure: The procedure of the AFLP method (Vos *et al*., 1995) was performed according to the protocol of the kit (AFLP^R Analysis System I, Invitrogen, USA).

Table 1: Lists of individual number of each species collected from different original regions and sources

Species	Individual	Original regions (Thailand)	Sources
Prionailurus bengalensis	1	Northeastern	Nakhon Ratchasrima Zoo
	2	Northeastern	Nakhon Ratchasrima Zoo
	3	Southern	Song Khla Zoo
Prionailurus viverrinus	1	Northeastern	Nakhon Ratchasrima Zoo
Catopuma temminckii	1	Southern	Song Khla Zoo
	2	Northern	Chiangmai Zoo
	3	Northern	Chiangmai Zoo
Felis chaus	1	Northeastern	Nakhon Ratchasrima Zoo
	2	Northeastern	Nakhon Ratchasrima Zoo
Pardofelis marmorata	1	Southern	Song Khla Zoo
Neofelis nebulosa	1	Northeastern	Nakhon Ratchasrima Zoo
Panthera tigris	1	Northern	Chiangmai Zoo
	2	Northeastern	Nakhon Ratchasrima Zoo
	3	Northeastern	Nakhon Ratchasrima Zoo
Panthera pardus	1	Northeastern	Nakhon Ratchasrima Zoo
	2	Northeastern	Nakhon Ratchasrima Zoo
	3	Southern	Song Khla Zoo
	4	Southern	Song Khla Zoo
Paguma larvata	1	Northeastern	Nakhon Ratchasrima Zoo

After adaptor ligation and preselective amplification, selective amplification was conducted with 24 primer combinations and 10 primer combinations: *Eco*RI-AAC/*Mse*I-CAA, *Eco*RI-AAC/*Mse*I-CTG, *Eco*RI-AAC/*Mse*I-CTT, *Eco*RI-AAG/*Mse*I-CAA, *Eco*RI-ACA/ *Mse*I-CAG, *Eco*RI-ACA/ *Mse*I-CAT, *Eco*RI-ACA/ *Mse*I-CTA, *Eco*RI-ACA/ *Mse*I-CTC, *Eco*RI-ACA/ *Mse*I-CTG and *Eco*RI-ACA/ *Mse*I-CTT were successful. The PCR products amplified with different primer combinations were loaded onto 6.0% denaturing polyacrylamide gels and electrophoresed for 3 h and detected by Silver Quest™ Silver Staining Kit (Invitrogen, USA).

Data analysis: The AFLP bands were visually scored as either present (1) or absent (0) for each accession and each primer combination without band intensity consideration. With the 0/1 data, a pair-wise genetic distance value was generated using Pearson correlation coefficients and these values were then converted to a genetic distance matrix. Based on the genetic distance matrix, cluster analyses were performed and the corresponding dendrogram was constructed for Felidae using arithmetic means analysis (UPGMA). Unbiased measures of genetic similarity index (S) were also calculated between each pair of the species. All these analyses were done by the Fingerprinting™ II Software (BIO-RAD, USA).

RESULTS

Results from lymphocyte culture of whole blood and conventional staining of the eight Felidae species, namely *Prionailurus bengalensis*, *Prionailurus viverrinus*, *Catopuma temminckii*, *Felis chaus*, *Pardofelis marmorata*, *Neofelis nebulosa*, *Panthera tigris* and *Panthera pardus* indicate that all species have an identical number of 38 diploid chromosomes (2n) consisting of 36 autosomes and 2 sex chromosomes. A representative chromosome of the eight Felidae species from a male of *Prionailurus bengalensis* is shown in Fig. 1.

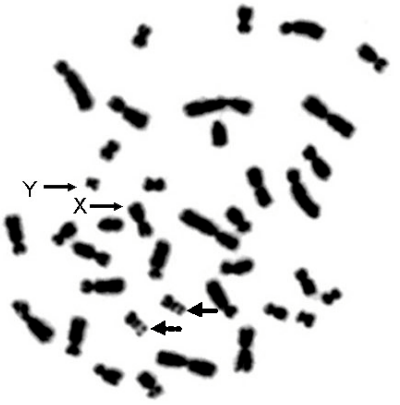

Fig. 1: Diploid chromosome number of male *Prinailurus bengalensis*, 2n = 38 consisting of 36 autosomes and 2 sex chromosomes. The satellite chromosomes are indicated bv dash arrows

A total of 4208 scorable bands, with sizes ranging from approximately 100-1500 base pairs (bp), were generated using ten *Eco*RI/*Mse*I AFLP primer combinations with the entire collection of 18 Felidae individuals (Table 2). The number of scorable bands for each primer combination ranged from 320 to 518 (mean 420.8). Of these bands, 18.91% are polymorphic. Percentages of Polymorphic bands for each primer combination range from 15.00 to 23.59%. The most informative primer combination is the *Eco*RI-ACA/*Mse*I-CTC pair, which produced the highest number of bands (518), while primer *Eco*RI-AAC/*Mse*I-CTG combinations produced a minimum number of bands (320). Representative AFLP profiles of 18 Felidae individuals generated by the *Eco*RI-ACA/*Mse*I-CTG primer combination are shown in Fig. 2.

The dendrogram constructed by the UPGMA method demonstrate that the 8 Felidae species are clustered together and the subfamilies Pantherinae and Felinae can be separated into two groups, whereas the outgroup *Paguma larvata* is segregated from the Felidae with a high bootstrap value (Fig. 3). In subfamily Felinae, *Prionailurus bengalensis*, *Prionailurus viverrinus*,

Table 2: A summary of AFLP primers, number of bands scored, number of polymorphic bands and percentages of polymorphisms for amplification profiles of eight felidae species in Thailand

AFLP primers	No. of bands scored	No. of polymorphism bands	Percentage of polymorphism
*Eco*RI-AAC/*Mse*I-CAA	375	58	15.47
*Eco*RI-AAC/*Mse*I-CTG	320	48	15.00
*Eco*RI-AAC/*Mse*I-CTT	438	76	17.35
*Eco*RI-AAG/*Mse*I-CAA	427	81	18.97
*Eco*RI-ACA/*Mse*I-CAG	411	75	18.25
*Eco*RI-ACA/*Mse*I-CAT	348	54	15.52
*Eco*RI-ACA/*Mse*I-CTA	407	96	23.59
*Eco*RI-ACA/*Mse*I-CTC	518	94	18.15
*Eco*RI-ACA/*Mse*I-CTG	481	101	21.00
*Eco*RI-ACA/*Mse*I-CTT	483	113	23.40
Total	4208	796	18.91

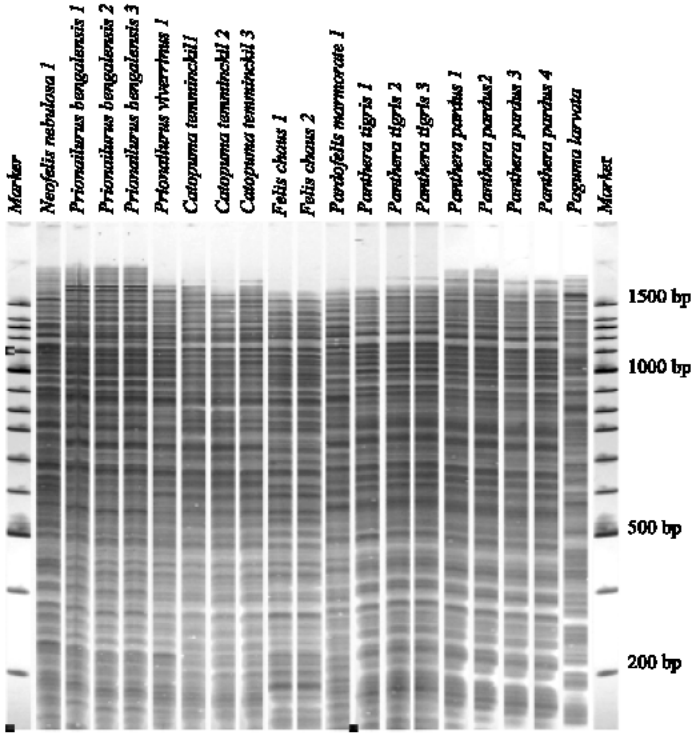

Fig. 2: Banding patterns of AFLP analysis among 18 Felidae accessions generated by the *Eco*RI-ACA/*Mse*I-CTG primer combination

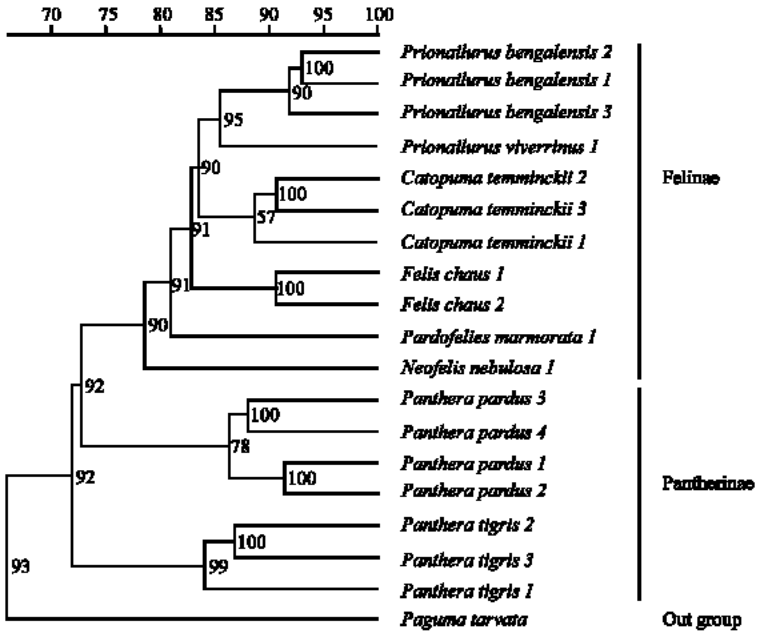

Fig. 3: Dendrogram of eight Felidae species constructed based on UPGMA clustering

Catopuma temminckii, *Felis chaus* and *Pardofelis marmorata* are clustered together with 91% bootstrap support attaching with *Neofelis nebulosa* of Pantherinae. In subfamily Pantherinae, *Panthera pardus* is clustered with *Panthera tigris* without *Neofelis nebulosa* with 92% bootstrap support. Moreover, *Neofelis nebulosa*

Table 3: The average similarity index values based on the AFLP analysis of the 8 felidae species and Masked-palm civet

Species	*Prionailurus bengalensis*	*Prionailurus viverrinus*	*Catopuma temminckii*	*Felis chaus*	*Pardofelis marmorata*	*Neofelis nebulosa*	*Panthera pardus*	*Panthera tigris*	*Paguma larvata*
Prionailurus bengalensis	92.13								
Prionailurus viverrinus	85.53	100							
Catopuma temminckii	82.79	85.43	89.27						
Felis chaus	81.58	83.05	83.42	90.60					
Pardofelis marmorata	83.30	80.30	80.43	79.75	100				
Neofelis nebulosa	76.97	78.80	78.53	82.50	74.10	100			
Panthera pardus	73.11	72.43	72.23	74.05	70.70	71.23	87.38		
Panthera tigris	71.78	74.27	74.20	72.10	68.73	68.20	71.30	84.93	
Paguma larvata	68.33	65.30	63.87	62.75	61.90	59.80	71.76	63.27	100

produces a sister species to the Felinae group with high bootstrap value (90%). The average genetic similarity index (S) of 8 species ranged from 68.20% between *Panthera tigris* and *Neofelis nebulosa* to 85.53% between *Prionailurus bengalensis* and *Prionailurus viverrinus* (Table 3).

DISCUSSION

In the present study, the cytogenetic data demonstrate that eight Felidae species have an identical diploid chromosome number (2n = 38), agreeing with Hsu and Rearden (1965), Wurster-Hill and Benirschke (1968), Wurster-Hill (1969), Wurster-Hill and Gray (1973) and Pathak and Wurster-Hill (1977) and reflecting the close genetic relationships among species of Felidae. However, the chromosome number does not provide deep information of determination genetic relationships among Felidae, so the AFLP molecular marker was used to elucidate. AFLP markers were successfully used to survey genetic variation and relationships among eight species of Felidae in Thailand. The ability to determine genetic variation among species at molecular level is directly related to the number of polymorphisms detected and their reproducibility. From resulting bands from ten primer combinations, a low level of polymorphism was found among Felidae species agreeing with the identical chromosome number. *Prionailurus viverrinus* is very closely related to *Prionailurus bengalensis* based on the highest S value, 85.53%, while the lowest S value is between *Panthera tigris* and *Neofelis nebulosa*. The S values between individuals in a species were generally high (>80%) (data not shown).

The dendrogram shows that 8 species of Felidae are separated into two groups, subfamilies Pantherinae and Felinae which includes *Neofelis nebulosa* possessing a relatively high S value to Felinae species than to other Pantherinae, which is contrary to morphological classification, but agree with the shape and size according to Felinae. The separation of Felidae into two groups of big cats (Pantherinae) and small cats (Felinae) is generally supported by most studies. A clear size dichotomy does seem to exist in Felidae (Bininda-Emonds *et al*., 2001). *Neofelis nebulosa* which is the smallest of the big cats with a body size of 16-23 kg (Mattern and McLennan, 2000) and the shape of a small cat has teeth and skull structure which is clearly that of a Pantherinae (Postanowicz, 2006). Support for monophyletic groups composed of Pantherinae species is abundant from morphological characters (Neff, 1982; Peters and Hast, 1994; Salles, 1992).

Based on present study, we found a close relationship between *Pardofelis marmorata* and *Neofelis nebulosa* with high S value according to the phylogenetic tree recently constructed by Bininda-Emonds *et al.* (1999) and Ortolani (1999). The dendrogram shows that *Catopuma temminckii* has a stronger genetic relationship to *Prionailurus bengalensis* and *Prionailurus viverrinus* than *Felis chaus*, while Bininda-Emonds *et al.* (1999), Mattern and McLennan (2000) and Pecon-Slattery *et al.* (2004) reported that *Felis chaus* is more closely related to *Prionailurus bengalensis* and *Prionailurus viverrinus* than *Catopuma temminckii*. Present results agreeing with the phylogenetic relationships of Felidae based on analyses of combination 16S rRNA with NADH-5 mtDNA gene segments by Johnson and O'Brien (1997) and the phylogeny of body and tail color pattern, tail tip markings, eye contour and eye patches constructed by Ortolani (1999) shows that *Catopuma temminckii* comprises a sister species to *Prionailurus bengalensis* and *Prionailurus viverrinus*, while *Felis chaus* forms a sister species to these groups. However, *Prionailurus bengalensis* and *Prionailurus viverrinus* are classified to the Asian leopard cat group whereas *Catopuma temminckii*, only one species in Thailand, is classified to the Bay cat group (Johnson and O'Brien, 1997). In order to resolve the genetic relationships between the Asian leopard cat group and *Catopuma temminckii* properly, it is necessary to study a larger sample. However, large sample studies of wildlife are most difficult, causing the small sample number studied in our research, so that the method for producing reproducible bands and abundant polymorphisms like AFLP has been used. Thus, these results suggest that genetic analysis based on the AFLP

technique has a good capacity for the study of genetic relationships, especially in Felidae. As AFLP analysis does not require prior genetic information for the taxa, this technique should be of value in genetic analysis of wild Felidae. Moreover, the ten successful primer combinations can be used to determine genetic differences among the eight Felidae species in Thailand.

ACKNOWLEDGMENTS

This study was supported by research fund from Thailand Zoological Park Organization under the Royal Patronage of His Majesty the King.

REFERENCES

Baillie, J.E.M., C. Hilton-Taylor and S.N. Stuart, 2004. 2004 IUCN Red List of Threatened Species. A Global Species Assessment. IUCN, Gland, Switzerland and Cambridge, UK. XXIV +, pp: 191.

Benveniste, R.E. and G.J. Todaro, 1974. Homology between type-C viruses of various species as determined by molecular hybridization. Proc. Nat. Acad. Sci. USA., 70: 3316-3320.

Benveniste, R.E., C.J. Sherr and G.J. Todaro, 1975. Evolution of type C viral genes: Origin of feline leukemia virus. Science, 190: 886-888.

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

Bininda-Emonds, O.R.P., D.M. Decker-Flum and J.L. Gittleman, 2001. The utility of chemical signals as phylogenetic characters: An example from the felidae. Biol. J. Linn. Soc., 72: 1-15.

Collier, G.E. and S.J. O'Brien, 1985. A molecular phylogeny of the felidae: Immunological distance. Evolution, 39: 437-487.

Flynn, J.J., J.A. Finarelli, S. Zehr, J. Hsu and M.A. Nedbal, 2005. Molecular phylogeny of the carnivora (Mammalia): Assessing the impact of increased sampling on resolving enigmatic relationships. Syst. Biol., 54: 317-337.

Gupta, P.K., R.K. Varshney, P.C. Sharma and B. Ramesh, 1999. Molecular markers and their applications in wheat breeding. Plant Breed., 118: 369-390.

Hsu, T.C. and H.H. Rearden, 1965. Futher karyological studies on felidae. Chromosoma, 16: 365-371.

Janczewski, D.N., W.S. Modi, J.C. Stephens and S.J. O'Brien, 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., P.A. Dratch, J.S. Martenson and S.J. O'Brien, 1996. Resolution of recent radiations within three evolutionary lineages of felidae using mitochondrial restriction fragment length polymorphism Variation. J. Mammal. Evol., 3: 97-120.

Johnson, W.E. and S.J. O'Brien, 1997. Phylogenetic reconstruction of the felidae using 16S rRNA and Nadh-5 mitochondrial genes. J. Mol. Evol., 44: S98-S116.

Johnson, W.E., J.A. Godoy, F. Palomares, M. Delibes, M. Fernandes, E. Revilla and S.J. O'Brien, 2004. Phylogenetic and phylogeographic analysis of iberian lynx populations. J. Hered., 95: 19-28.

Koepfli, K., S.M. Jenks, E. Eizirik, T. Zahirpour, B.V. Valkenburgh and R.K. Wayne, 2006. Molecular systematics of the hyaenidae: Relationships of a relictual lineage resolved by a molecular supermatrix. Mol. Phylogenetic. Evol., 38: 603-620.

Lopez, J.V., N. Yuhki, R. Masuda, W. Modi and S.J. O'Brien, 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.

Mattern, Y.M. and A.D. McLennan, 2000. Phylogeny and speciation of felids. Cladistics, 16: 232-253.

Modi, W.S. and S.J. O'Brien, 1988. Quantitative Cladistic Analysis of Chromosomal Banding Among Species in Three Orders of Mammals: Hominoid Primates, Felids and Arvicolid Rodents. In: Chromosome Structure and Function. Gustafson, J.P. and R. Appels (Eds.), Needleman SB, Plenum Press, New York.

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

O'Brien, S.J., G.E. Collier, R.E. Benveniste, W.G. Nash, A.K. Newman, J.M. Simonson, M.A. Eichelberger, U.S. Seal, D. Janssen, M. Bush and D.E. Wildt, 1987. Setting The Molecular Clock in Felidae: The Great Cats, Panthera. In: Tigers of the World. Tilson, R.L. and U.S. Seal (Eds.), Noyes, NJ, pp: 10-27.

Ortolani, A., 1999. Spots, stripes, tail tips and dark eyes: Predicting the function of carnivore colour patterns using the comparative method. Biol. J. Linn. Soc., 67: 433-476.

Pathak, S. and D.H. Wurster-Hill, 1977. The distribution of constitutive heterochropmatin in carnivores. Cytogenet. Cell Genet., 18: 245-254.

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

Pecon-Slattery, J., A.J. Pearks Wilkerson, W.J. Murphy and S.J. O'Brien, 2004. Phylogenetic assessment of itrons and SINEs within the Y chromosome using the cat family Felidae as a species tree. Mol. Biol. Evol., 21: 2299-2309.

Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplick, P. Castiglion, G. Taramino and M. Motto, 1998. Comparative analysis of genetic similarity among maize Inbred lines detected by RFLPs, SSRs and AFLPs. Theor. Applied Genet., 97: 1248-1255.

Peters, G. and M.H. Hast, 1994. Hyoid Structure, Laryngeal Anatomy and Vocalization in Fields (Mammalia: Carnivora: Felidae). Z. Saugetierkunde, 59: 87-104.

Postanowicz, R., 2006. Online. Lioncrusher's domain-clouded leopard. Retrieved on 24/09/06 from http://www.lioncrusher.com/animal.asp?animal=46.

Powell, W., M. Morgate, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski, 1996. The comparison of RFLP, RAPD, AFLP and SSR markers for germplasm analysis. Mol. Breed., 2: 225-238.

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

Russell, J.R., J.D. Fuller, M. Macaulay, B.J. Hatz, A. Jahoor, W. Powell and R. Waugh, 1997. Direct comparison of level of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Applied Genet., 95: 714-722.

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

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor, NY: Cold spring harbor laboratory.

Sato, J.J., M. Wolsan, H. Suzuki, T. Hosoda, Y. Yamaguchi, K. Hiyama, M. Kobayashi and S. Minami, 2006. Evidence from nuclear DNA sequences sheds light on the phylogenetic relationships of pinnipedia: Single origin with affinity to musteloidea. Zool. Sci., 23: 125-146.

Tanomtong, A., S. Khunsook, W. Kaensa and R. Banjongrat, 2005. Cytogenetics of pig-tailed macaque (*Macaca nemestrina*) by conventional staining. Naresuan University J., 13: 1-11.

Tian, Y., W. Nie, J. Wang, M.A. Ferguson-Smith and F. Yang, 2004. Chromosome evolution in bears: Reconstructing phylogenetic relationships by cross-species chromosome painting. Chromosome Res., 12: 55-63.

Uphyrkina, O., D. Miquelle, H. Quigley, C. Driscoll and S.J. O'Brien, 2002. Conservation genetics of the far eastern leopard (*Panthera pardus orientalis*). J. Hered., 93: 303-311.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP AFLP: A new technique for DNA fingerprinting. Nucl. Ac. Res., 23: 4407-4414.

Wilson, D.E. and D.M. Reeder, 2006. Mammal Species of the World, 3rd Edn. Johns Hopkins University Press.

Wurster-Hill, D.H. and K. Benirschke, 1968. Comparative cytogenetic studies of the order carnivora. Chromosoma, 24: 336-382.

Wurster-Hill, D.H., 1969. Cytogenetic and Phylogenetic Studies in Carnivora. In: Comparative Mammalian Cytogenetics., Benirschke, K. (Ed.), New-York.

Wurster-Hill, D.H. and C.W. Gray, 1973. Giemsa banding patterns in the chromosomes of the twelve species of cats (Felidae). Cytogenet. Cell Genet., 12: 377-379.

Wurster-Hill, D.H. and W.R. Centerwall, 1982. The interrelationships of chromosome banding patterns in procyonids, viverrids and felids. Cytogenet. Cell. Genet., 34: 178-192.

Zhang, W., Z. Zhang, F. Shen, R. Hou, X. Lv and B. Yue, 2006. Highly conserved D-loop-like nuclear mitichondrial sequences (Numts) in tiger (*Panthera tigris*). J. Genet., 85: 107-116.