Biological Forum – An International Journal **14(1): 861-869(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Use of Taxidermy Samples to Generate Forensically Informative Nucleotide Sequences of Globally threatened Species of Small cats including the Species from India by using 12SrRNA and Cytochrome b gene

Archana Bahuguna[1*] and Priyanka Negi[2]

[1]High Altitude Regional Center, Zoological Survey of India, Saproon, Solan, (Himachal Pradesh), India.
[2]Department of Zoology, Eternal University, Baru Sahib, Sirmaur, (Himachal Pradesh), India.

(Corresponding author: Archana Bahuguna)*
(Received 04 November 2021, Accepted 05 January, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: **Although the research on carnivore ecology, including molecular studies are commendable but the molecular study on small cats remain very limited in India as well as in other Asian countries for providing scientific proof for Wildlife forensic and for molecular ecology. As it is difficult to get the samples of small cats for molecular study because of small population size and rare status thus the present study using the taxidermy samples highlight the importance of museum specimens as DNA source material for threatened taxa. Most of the research on cats being skewed more towards big cats and small cats are almost ignored. Small cats are known to be widely spread across globe and they are more specialized than big cats in terms of resource selection. Major challenges to felid populations are habitat loss, the use of felids for the pet, poaching for wildlife parts and products, disappearance of natural prey and cat human conflicts leading to decrease in population of cat species and making them vulnerable to extinction. The present study indicates the use of taxidermy samples for providing molecular reference material for forensic purpose. In the present study genetic data was created by using DNAsp, MEGA X, Mesquite, Mr Bayes for delimiting the species on the basis of two genetic markers *i.e*. 12SrRNA (25 partial gene sequences of 15 species) and Cytochrome b genes (53 partial gene sequences of 17 species). Thus total 78 gene sequences of two markers were analyzed belonging to 21 species of small cats. Out of 78, 19 gene sequences (belonging to 5 species) were generated from taxidermy samples. Phylogenetic study with *Canis lupus* gene sequence as an out group delimited the species by using both the genes and also generated 17 haplotypes for 12SrRNA gene for 15 species (25 gene sequences) and 36 haplotypes (53 gene sequences) for 19 species of small cats thus will be helpful to know the origin of seized material.**

Keywords: 12SrRNA, Cytochrome b, small cats, wildlife forensic, taxidermy samples.

INTRODUCTION

There are three subfamilies of Felidae the Pantherinae, the Felinae and the Acinonychinae, differing from each other by the ossification of the hyoid apparatus and by the cutaneous sheaths which protect their claws (Pocock 1917) . However with the development of molecular biology the living species of Felidae are divided in two subfamilies: the Pantherinae and Felinae, with the Acinonychinae incorporated into Felinae. Pantherinae *i.e*. lions and tigers includes five *Panthera* and two *Neofelis* species, while Felinae (small cats, Pumas and Cheetah) includes the other 34 species into ten genera, with eight lineages with broad geographic occurrence in terrestrial habitats except treeless tundra and polar ice caps (Buckley-Beason *et al.* 2006; Feldhamer, *et al.*, 1999; Johnson *et al.*, 2006; Li *et al*. 2016; Clutton-Brock and Wilson, 2001; Denis, 1964; Grzimek, 2003, Kelsey-Wood, 1989; O'Brian, 2001; Vaughan, *et al*., 2000). But few species of small cats have limitation of habitat e.g. habitat of sand cats, *Felis margarita* of sandy and stony deserts, domestic and feral cats, *F. catus* are ubiquitous globally and are especially prevalent in urban and suburban areas (Grzimek, 2003; Nowell and Jackson, 1996). Felids are apex predators and control the prey base. They are considered as a keystone species in their native habitats. They primarily prey on rodents, hares, and rabbits, thus controlling pest populations throughout their range of distribution (Grzimek, 2003). Threat to felid populations are habitat loss, their use as pet, in fashion trades, disappearance of natural prey and cat- human conflicts. They are noted to be excellent

model to study intricate color pattern and variations in mammals as studied by Kaelin *et al.* (2021). Study by Bahuguna (2018) on *Prionailurus* revealed that cytochrome b and 12SrRNA are effective genetic markers in providing the species molecular characterization.

However it is also noticed that the population of most of the cat species are decreasing thus making them vulnerable to extinction due to natural disasters, epidemics, poaching and inbreeding depression. Populations of 29 out of 36 recognized species of felids are in declining trend as reported by IUCN (Wozencraft, 2005) and 5 of the remaining species are data deficient. The present study describes the characteristic species specific genetic data of 21 species by using 12SrRNA and cytochrome b partial gene sequences of Felidae based on MEGA X, DNA sp, Network ver.10 and also species delimitation by using Mr Bayes 3.2.7. Tracer v1.6 was used for ESS vales *i.e* effective sample size and Fig tree was used to view tree. This study is useful for molecular characterization of the small cat species for wildlife forensic as well as for molecular ecology studies.

MATERIALS AND METHODS

Sample collection. Taxidermy samples (Table 1, n=9) of 5 species of small cats were collected from Mammal Section of Zoological Survey of India, Kolkatta and the samples were washed with sterile Milli-Q water and ethanol 70% (v/v) respectively. The taxidermy samples thus collected treated with MilliQ water and hydrated before digestion by incubating the samples for 24 hrs. in 1ml TE solution (Tris 10mM, EDTA 1mM at pH 7.6) as given by Moraes-Barros and Morgnate (2007). After this treatment DNA was extracted by using HiPur A^{TM} Forensic Sample Genomic DNA Purification Kit (HIMEDIA) following manufacturer protocol.

Amplification of genes 12SrRNA gene sequences were amplified using a set of primer pair, L1091 and H1478 (Kocher *et al.,* 1989). Polymerase Chain Reaction (PCR) was performed with reaction mixture of 25µl (10X PCR-with $MgCl_2$, 2.5µl; 10mM dNTP's, 2.5µl; 5 pmol primer, 0.45µl each; 15ng of DNA template; 1.5U Taq enzyme). Each cycle of PCR consisted of initial denaturation of 94^{o}C for 4 minutes and then each cycle of denaturation for 1 min at 94^{o}C, hybridization for 1 min at 55^{o}C and extension for 1 min at 72^{o}C followed by final elongation for 10 min at 72^{o}C. The cycle (35 times) was done in Eppendorf mastercycler X50. The PCR products were sequenced using ABI's AmpliTaq FS dye terminator cycle sequencing chemistry on an automated ABI 3100 Genetic Analyser. Negative controls were used in all DNA extraction and PCR amplification to control potential contamination.

Table 1: Details of taxidermy samples, collected from Mammal section, Zoolgical Survey of India used as DNA source material for 19 gene sequences generated in the present study.

Sr. No.	Species	Registration number	Sex	Locality	Date of collection
1.	*Caracal caracal* (Schreber)	7140	Male	Donated by Zoological Garden, Kolkatta	1967
2.	*Caracal caracal* (Vigors & Horsfield)	3120	Male	Donated by Zoological Garden, Kolkatta	1965
3.	*Pardofelis marmorata* (Martin)	3982	Male	Zoological garden, Kolkatta	1886
4.	*Pardofelis marmorata* (Martin)	4216	Male	Zoological garden, Kolkatta	1886
5.	*Pardofelis marmorata* (Martin)	7339	Female	Zoological garden, Kolkatta	1983
6.	*Otocolobus manul nigripectus* (Pallas)	8057	Male	Zoological garden, Kolkatta	1985
7.	*Catopuma temminkii* (Vigors&Horsfield)	3769	Male	Tippera hills (Bengal), Zoological Garden, Kolkatta	1878
8.	*Catopum temminkii* (Vigors & Horsfield)	8566	-	Sikkim	1877
9.	*Felis silvestris* Schneider	8013	-	Baena, east of Mahangarh, Rajasthan	1950

Cytochrome b sequences were amplified by using a set of primer pair of L14841 and H15149 (Kocher *et al.*, 1989). The PCR reaction was performed in Eppendorf mastercycler X50, with volume of 25 µl reaction mixture consisting10 mM PCR buffer-with $MgCl_2$, 2.5 ml; 10 mM dNTP's, 2.5 ml; 5 pmol primer, 0.45 ml each; 15 ng of DNA template; and 1.5 U Taq enzyme.

For cytochrome b, PCR cycle comprised of denaturation at 94°C for 4 min and further denaturation for 1 min at 94°C, hybridization for 1 min at 55°C (50°C for cytochrome b), and extension for 1 min at 72°C, followed by final elongation for 10 min at 72°C. The cycle was repeated for 35 times.

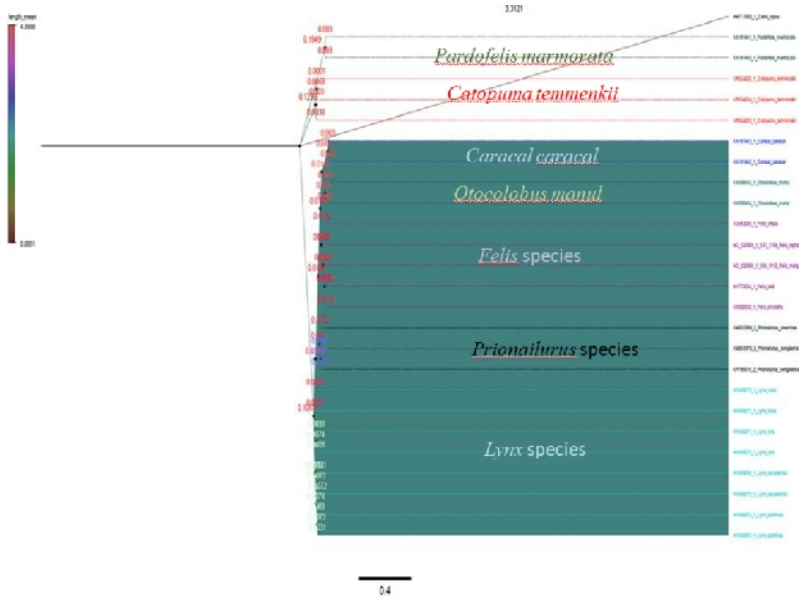

Fig. 1. Phylogenetic tree with branch values of 12StRNA partial genes sequences (n= 25) of species of small cats by using Mr Bayes 3.2.7.

All PCR products thus obtained were sequenced by using ABI's AmpliTaq FS dye terminator cycle sequencing chemistry on an automated ABI 3100 Genetic Analyzer. All experiments were done in a PCR workstation (Bangalore GeNeiTM). Negative controls were used to check potential contamination. Gene sequences of 12SrRNA gene and cytochrome b gene thus obtained were used for conducting sequence alignment by Bioedit for submission to NCBI.

Data analysis. Chromas 1.6 (Technelysium Pty Ltd., South Brisbane Australia) was used for editing the sequences. The quarry sequences were compared using GenBank BLAST (http://www.ncbi.nlm.nih.gov/BLAST). CLUSTAL W was used to compare DNA sequence data implemented in BioEdit v 7.0.9.0 software (Hall 1999) with out-group *Canis lupus*. All sequences were proof read and analyzed by using MEGA - X (Kumar *et al.*, 2018) and were aligned by using Clustval W (Thompson *et al.* 2003). MEGA- X and DNA sp were used for information on the haplotypes, haplotype diversity, nucleotide diversity, conservation sites, variable sites, parsimony informative sites. Mesquite 2.74 (Maddison and Maddison 2010) was used for getting the nexus file of aligned sequences and for getting the delimitation of the gene sequences through phylogentic tree by using Baeysian inference analysis of Mr Bayes 3.2.7. Tree was visualized by Fig tree and saved. Tracer was also used for getting the ESS value. Bayesian analyses were executed using a random starting tree and program's default distribution for model parameter. The analyses were done twice and each analysis comprised of 3 million generation. The results were sampled every 1000th generation. Convergences were assessed by calculating the effective sample sizes (ESS) using Tracer v1.6. The first 25% of the sampled trees were discarded as 'burn in' and the remaining 75% of the sampled trees were used to calculate the Bayesian posterior probabilities (BPP). The cytochrome b gene sequence of *Canis lupus* was used as an out group for rooting the trees. NETWORK ver 10 was used to generate Haplotype network and genetic data was obtained by using MEGA X and DNAsp.

RESULTS AND DISCUSSION

A. Genetic Data for 12SrRNA

Genetic data for 12SrRNA partial gene sequences analyzed were 25 of 15 species of small cats with outgroup *Canis lupus* gene sequence (accession nos. of NCBI, given in Table 2), indicated 18 haplotypes (Table 2, Fig. 2) with haplotype diversity, Hd: 0.9767 with outgroup and 17 haplotypes with Haplotype diversity, Hd 0.9733 without out group. Out of 25 gene sequences 12 sequences generated by using taxidermy samples and rest 13 were uploaded from NCBI. Number of conservation sites were 253, number of variable sites noted to be 102, number of singletons 3 and number of parsimony informative sites were 99. Tajima's D thus calculated was 0.02140 with statistical significance: Not significant, $P > 0.10$. Total number of mutations, Eta was 119 and total number of singleton mutations, Eta(s) noted to be 208. Theta (per site) from Eta 0.09493, Theta (per sequence) from Eta 0.094933. Average number of pairwise nucleotide differences was k: 26.1133 with nucleotide diversity, Pi: 0.07865. Fu and Li's D test statistic was reported to be 1.57630 with statistical significance $P < 0.05$, Fu and Li's F test statistic: 1.00635, Statistical significance $P < 0.05$. The average nucleotide frequencies are: T(U) =23.9% , C= 22.9 % , A =35.7 % and G =17.5.

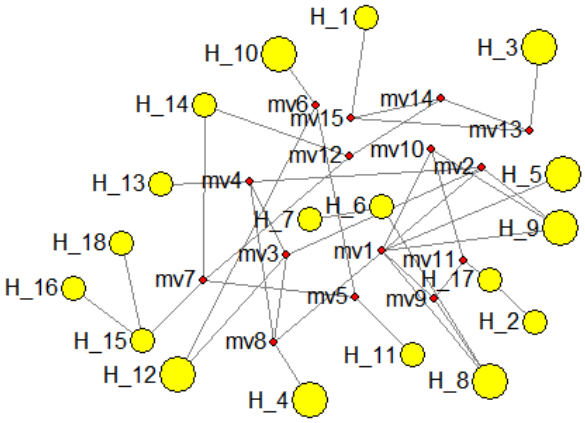

Fig. 2. Haplotype Median network of partial 12SrRNA gene sequences of species of Small cats by using NETWORK ver 10 with outgroup.

Table 2: Haplotype numbers (as given in Figs. 2, 4) with accession numbers of NCBI and the corresponding species.

Haplotype nos.	Haplotypes with accession nos of the gene sequences (Cytochrome b)	Species	Haplotypes with accession nos. of gene sequences (12SrRNA)	Species
1	KX181470.1* KX181469.1* KX181471.1*	*Pardofelis marmorata*	KR024225* KR024224.1 *	*Catopuma temmenkii*
2	KX181465.1*	*Otocolobus manul*	KX181460.1* KX181461.1*	*Pardofelis marmorata*
3	KF990330.1	*Otocolobus manul*	[KX181463.1* KX181462.1*	*Caracal caracal*
4	AY170102.1 KJ193344.1	*Felis silvestris*	AY499271.1 AY499270.1	*Lynx lynx*
5	KF754937.1 KF754936.1	*Pardofelis marmorata*	[AY499280.1	*Lynx candenensis*
6	KF754935.1	*Pardofelis marmorata*	AY499279.1	*Lynx candenensis*
7	KF754934.1	*Pardofelis marmorata*	AY499269.1 AY499268.1	*Lynx pardinus*
8	KF754933.1	*Pardofelis marmorata*	AY499278.1 AY499277.1	*Lynx rufus*
9	KF754950.1 KF754947.1 KF754946.1	*Catopu-ma temminckii*	KX098455.1* KX098454.1*	*Otocolobus manul*
10	KF754949.1	*Catopuma temmenkii*	KM093869.2*	*Prionailurus viverrinus*
11	KF754948.1	*Catopuma temmenkii*	KM093870.2 * KP789016.2	*Prionailurus bengalensis*
12	KF754945.1	*Catopuma temmenkii*	KU963205.1	Felis chaus
13	KJ193345.1	*Leptailurus serval*	NC_028309.1	*Felis nigrpes*
14	KX181466.1* KX181467.1* KX181468.1*	*Caracal caracal*	NC_028308.1	*Felis margarit*
15	MK487798.1 MK487797.1 MK487796.1	*Caracal caracal*	AY773084.1	*Felis beiti*
16	MK487795.1	*Caracal caracal*	KR024223.1*	*Catopuma temmenkii*
17	KJ193341.1	*Caracal caracal*	KX002032.1*	*Felis silvestris*
18	KU253484.1	*Leopardus pardus*	AY499278.1	*Lynx lynx*
19	KT626622.1	*Leopardus pardus*	AY4999277.1	*Lynx lynx*

20	NC028321.1	*Leopardus guigna*		
21	NC028318.1	*Leopardus wiedii*		
22	NC028317.1	*Leopardus tigrinus*		
23	AY499334.1, AY499333.1	*Lynx Canadensis*		
24	AY319512.1	*Lynx Canadensis*		
25	MK040982.1	*Lynx lynx*		
26	MK040981.1,MK040980.1 MK040979.1	*Lynx lynx*		
27	EF689048.1, EF689047.1, KF561246.1	*Lynx pardinus*		
28	NC_028304.1	*Prionailurus rubiginosus*		
29	KX866325.1	*Prionailurus bengalensis*		
30	KX866324.1, KX866323.1 KX866322.1	*Priionailurus bengalensis*		
31	JN811057.1	*Prionailurus viverrinus*		
32	JN811056.1	*Prionailurus viverrinus*		
33	JN811055.1	*Prionailurus viverrinus*		
34	JN811054.1	*Prionailurus viverrinus*		
35	JN811053.1	*Prionailurus viverrinus*		
36	FJ594958.1	*Prionailurus planiceps*		

*Nineteen partial gene sequences from India, source taxidermy samples.

Genetic data for Cytochrome b Genetic data for all 53 partial gene sequences (selected region 1-350) of cytochrome b examined belonging to 19 species, indicated 36 Haplotype (Table 2, Fig. 4) with Haplotype diversity of Hd: 0.9826. Fu and Li's D test statistic: 0.63550 Statistical significance: Not significant, P > 0.10, Fu and Li's F test statistic: 0.62282, Statistical significance: Not significant, P > 0.10, Number of polymorphic (segregating) sites, S: 105, Total number of mutations, Eta: 132, Total number of singleton mutations, Eta(s): 21, Nucleotide diversity, Pi: 0.09089, Theta (per site) from Eta: 0.08311. Average nucleotide difference 31.80987, Theta (per sequence) from Eta: 29.08742, Theta (per site) from Eta: 0.08311, Tajima's D: 0.33280 Statistical significance: Not significant, P > 0.10, Fu and Li's D test statistic: 0.63550, Statistical significance: Not significant, P > 0.10, Fu and Li's F test statistic: 0.62282

Statistical significance: Not significant, P > 0.10.

Conservation threshold, CT: 0.75. There is no overlapping of haplotypes among the species. The nucleotide frequencies are A = 29.3%, T/U = 26.6%, C = 30.4%, and G = 13.7%.

Phylogenetic analysis for delimitation of the species and geographic signature through Haplotype networking: Wildlife trade is recognized as an impediment to conservation. CITES listed 23 species or subspecies under Appendix I, with all remaining species placed under Appendix II as the trade would derive many species to extinction. The North American Endangered Species Act lists 8 species or subspecies of North American felids as threatened or endangered (Grzimek, 2003; Nowell and Jackson, 1996). The threats generally involve human activity, persecution as dangers to human life, killing for trade, for their parts for medicinal value etc., capture for food and decline of prey base and usually through over-hunting. The family Felidae is well represented in India, with 15 species residing here, making it the richest in cats worldwide. But all of them are under threat mainly due to poaching and habitat fragmentation. In the present study 53 gene sequences (17 species) were examined for Cytochrome b gene and 25 gene sequences (15 species) examined for 12srRNA genes. Thus total 78 partial gene sequences of both markers were used and out of 78, 19 partial gene sequences were generated by using taxidermy samples as DNA reference source. Seven clades were formed for the species examined by using Cytochrome b genes and 12S rRNA genes (Figs. 1, 3) and it was noted that both markers are useful for delimiting the species but the trees are non congruent and the number of clades formed were same in both trees (Figs. 1, 3). Cytochrome b gene analysis showed ESS value 1618, LnL mean -2554.0231 with SD of mean 0.315 but 12SrRNA had ESS value 5, LnL mean -2272.4658 with SD of mean 3958 (Fig. 5). All species of *Lynx* formed a separate clade. *Catopuma temmenkii* also formed a separate clade. *Felis silvestris* also grouped in separate clade. However *Leptailurus serval* formed a clade with *Pardofelis aurata* as depicted in phylogeny of cytochrome b (Fig. 3), African golden cat, *Caracal aurata*, endemic to the rainforests of West and Central Africa, earlier known as *Pardofelis aurata* is threatened due to deforestation and bush meat hunting and listed as Vulnerable on the IUCN Red List (Bahaa-el-din *et al*. 2015). It is a close relative of both the caracal and

the serval (Johnson *et al.* 2006). Previously, it was placed in the genus *Pardofelis* (Wozencraft 2005). Phylogenetic studies showed that the African golden cat is closely related with the caracal (*Caracal caracal*) (Johnson and O'Brien 1997, Johnson *et al.* 2006). These two species, together with the serval (*Leptailurus serval*), form the Caracal lineage, one of the eight lineages of Felidae. This lineage evolved nearly 8.5 million years ago (Johnson and O'Brien 1997, Johnson *et al.* 2006). Because of this close relationship, the African golden cat has been placed into the genus *Caracal* (Bahaa-el-din *et al.* 2015). *Leopardus* species and *Otocolobus manul* also formed a separate clade. *Prionailurus* species also nested in a separate clade. This indicates that 12SrRNA and Cytochrome b genes can be used for delimiting the species of small cats.

Old World lineage of small cats belongs to the genus *Felis* , including the wildcats, sand cat, jungle cat, and the domestic cat. Li *et al.* (2016) studies on molecular data reported that the Felinae and Pantherinae diverged about 11.5 million years ago and that the eight felid lineages diverged sequentially from 4.23 to 10.67 million years ago. *Felis* diverged from the Leopard Cat lineage about 7.25 million years ago. The most basal lineage of the Felinae is the Caracal lineage, followed by the Ocelot lineage, the Lynx and Bay Cat lineages, the Puma lineage, and finally the Leopard Cat and Domestic Cat lineages. Li *et al.* 2016 suggested that some genera and species have been difficult to be placed in the felid phylogeny. As mentioned by them *Otocolobus* has been associated as a basal member of the Domestic Cat lineage, but in the latest phylogeny it is basal in the Leopard Cat lineage. In the present study too, the species was noted to be nested differently in the phylogeny of two markers (Figs. 1, 3). The African and Asiatic golden cats were formerly considered to be close, but they occupy different lineages, *i.e*. the Asiatic golden cat and the bay cat form a distinct genus. It was noted that African golden cat is closest to the caracal (both in the genus *Caracal*), which has no close relationship to the lynxes. The marbled cat (*Pardofelis*) is also found in the Bay Cat lineage, but is more distantly related to *Catopuma* (Kitchener *et al.*, 2017). In addition to major threats to cats the reduced population sizes and increase vulnerability to extinction due to natural disasters, epidemics, and inbreeding depression are also major threats to the species of small cats.

The use of genetic analysis to identify the species poached and from traded products are the most common application in wildlife DNA forensics. As reported by Ogdem *et al.* (2009), for enforcement of Wildlife Protection laws and to enforce CITES regulations there is need to find out geographic signature of a sample, in addition to identify the species. Within some species, populations may be so much isolated from one another that there is effectively no exchange of genetic material between them. Genetic differences will gradually accumulate over evolutionary time to a point where members of an isolated region share the same types of genetic marker (alleles) within their population, but exhibit different alleles to that of any other population. Markers that exhibit such discrete variation are very useful for identifying populations and therefore for assigning an individual to a geographic region with a high degree of confidence (Ogdem *et al.* 2009). For geographically distinct populations, mitochondrial DNA (mtDNA) can be used to identify origin of seized material, similar to species identification. The hypervariable mtDNA control region, sequence types *i.e*. 'haplotypes' correspond to specific populations of the species. Thus NETWORK 10 was used for getting the haplotypes of the species. Wu *et al.* (2005) applied this technique to the Chinese sika deer *Cervus nippon* that is classified into 4 subspecies. Two sub-species of Chinese sika deer were extirpated from the wild due to hunting and now exist only in captivity. The remaining two subspecies that exist in the wild are very seriously threatened and heavily protected by Chinese law. In order to enforce this conservation legislation, a method to discriminate one of the wild subspecies from one of the domesticated subspecies was developed, which was based on mtDNA control region haplotype variation. Thus emphasized the importance of the technique to use haplotype to know the origin of the seized material. The present study also generated 17 haplotypes for 12SrRNA gene for 15 species and 36 haplotypes for 19 species of small cats thus will be helpful to know the origin of seized material.

Table 3: Genetic data of 12SrRNA for species of small cats examined by using partial gene sequence of 358 bp.

No. of Species examined	N.	C &CT	V	S	H	Hd	Π	D	K	InDel p	Pi
15 species	25 (without outgroup)	253	102	3	17	0.777	0.12410	-2.10983	9.587	0	99

N Number of sequences, C Conserved sites, V Number polymorphic sites, S number of Singleton sites, H total number of haplotypes, h haplotype diversity, π nucleotide diversity, D Tajima's d and average number of nucleotide difference (k), Insertion deletion polymorphism InDel p, Pi Parsi mony informative sites

Table 4: Genetic data of Cytochrome b for species of small cats examined by using partial gene sequence 350 bp.

No. of Species examined	N.	C &CT	V	S	H	Hd	Π	D	K	InDel p	Pi
17 species	53 (without outgroup)	230 & CT: 0.75	105	15	36	0.983	0.09089	0.33280	31.80987	0	105

N Number of sequences, C Conserved sites, V Number polymorphic sites, S number of Singleton sites, H total number of haplotypes, h haplotype diversity, π nucleotide diversity, D Tajima's d and average number of nucleotide difference (k), Insertion deletion polymorphism InDel p, Pi Parsi mony informative sites.

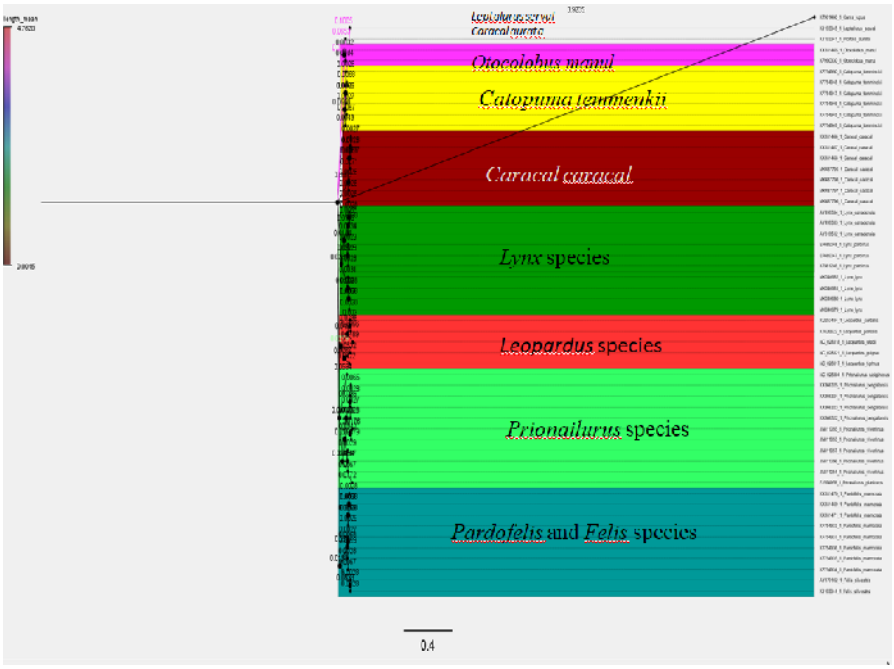

Fig. 3. Phylogenetic delimitation with branch length values of cytochrome b partial gene sequences (n=53) of small cats by using Mr Bayes 3.2.7.

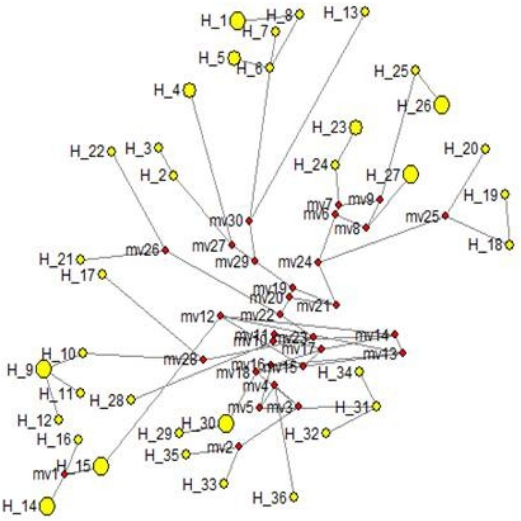

Fig. 4. Haplotype Median network for 53 cytochrome b gene sequences examined of 17 species of small cats by using NETWORK 10.

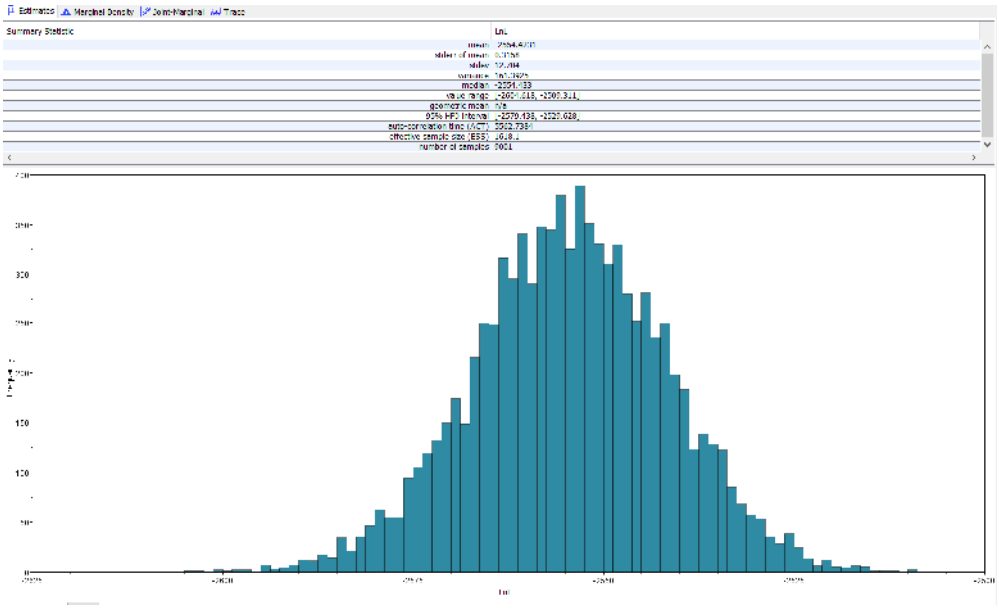

Fig. 5. Marginal density with ESS value of the Cytochrome b genes analysed by using Tracer v1.6.

CONCLUSION

As the decline of small cats is occurring worldwide due to habitat fragmentation, and poaching, the present study is useful to provide the strong scientific proofs for the court and also useful for molecular ecology. The study inferred that taxidermy samples could be used effectively for generating molecular reference data for threatened taxa. Geographical signatures developed by haplotype networking could help in tracking the origin of seized materials and for implementation of CITES and other Wildlife protection laws and regulation.

Funding: Regular official funding by Zoological Survey of India, Kolkatta, India (Govt. of India, Ministry of Environment, Forest and Climate Change) for research work.

Data availability: Partial gene sequences from taxidermy samples developed by the corresponding author are available at NCBI.

Conflict of Interest. None.

REFERENCES

Bahaa-el-din, L., Mills, D., Hunter, L. and Henschel, P. (2015). "Caracal aurata". IUCN Red List of Threatened Species.

Bahuguna, A. (2018). Forensically informative nucleotide sequencing (FINS) for species and subspecies of genus *Prionailurus* (Mammalia: Carnivora: Felidae) through mitochondrial genes (12SrRNA and Cytochrome b) by using old taxidermy samples. *Mitochondria Part B*, *3*(2): 615-619.

Buckley-Beason, V. A., Johnson, W. E., Nash, W. G., Stanyon, R., Menninger, J. C., Driscoll, C. A, Howard, J. G., Bush, M., Page, J. E., Roelke, M. E., Stone, G., Martelli, P. P., Wen, C., Ling, L., Duraisingam, R. K., Lam, P. V. and O'Brien, S. J. (2006). Molecular evidence for species-level distinctions in clouded leopards. *Current Biology*, *16*: 2371-2376.

Clutton-Brock, J. and Wilson, D. (2001). Cats. Pp. 208-215 (Eds Burnie, D., Wilson, D.) Smithsonian Institution: Animals. New York, NY: DK Publishing.

Denis, A. (1964). Cats of the World. Boston: The Riverside Press Cambridge.

Feldhamer, G., Drickamer, L., Vessey, S. and Merritt, J. (1999). *Mammalogy: Adaptation, Diversity, and Ecology*. Second Edition. Boston, MA: McGraw Hill.

Grzimek, B. (2003). Cats (Felidae). Pp. 369-392 (Eds H Michael, K Devra, G Valerius, M Melissa) Family: Felidae. *14*(2) Edition. Farmington Hills, Michigan: The Gale Group.

Hall, T. A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*: 95-98. IUCN 2020. The IUCN Red List of Threatened Species. Version 2020-2. <www.iucnredlist.org>.

Johnson, W. E. and O'Brien, S. J. (1997). "Phylogenetic reconstruction of the Felidae using 16S rRNA and NADH-5 mitochondrial genes". *Journal of Molecular Evolution*, *44* (Supplement 1): S98–S116. Bibcode: 1997JMolE..44S.98J.

Johnson, W. E., Eizirik, E., Pecon-Slattery, J., Murphy, W. J., Antunes, A., Teeling, E. O'Brien, S. J. (2006). "The Late Miocene Radiation of Modern Felidae: A Genetic Assessment". *Science*, *311*(5757): 73–77.

Kaelin, C. B., McGowan, K. A. and Barsh, G. S. (2021). Developmental genetics of color pattern establishment in cats. *Nature communications*, *12*(5124): 1-12.

Kelsey-Wood, D. (1989). *The Atlas of Cats of the World: Domesticated and Wild*. Neptune City, NJ: T.F.H. Publications.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., and Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, *86*(16): 6196-6200.

Kumar, S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*: 1547-1549.

Kitchener, A. C., Breitenmoser-Würsten, C., and Eizirik, E. (2017). "A revised taxonomy of the Felidae: The final report of the Cat Classification Task Force of the IUCN Cat Specialist Group". *Cat News* (Special Issue 11): 60−61.

Li G., Davis B. W., Eizirik E. and Murphy W. J. (2016). Phylogenomic evidence for ancient in the genomes of living cats (Felidae). *Genome Research*, *26*: 1-11.

Maddison, W. P. and Maddison. D. R. (2010). Mesquite: a modular system for evolutionary analysis. Version 3.61 http://www.mesquiteproject.org.

Moraes-Barros, N.de and Morgant, J. S. (2007). A simple protocol for the extraction and sequence analysis of DNA from study skin museum collections. *Genet. Mol. Biol*., *30* (4):1181-1185.

Nowell, K. and Jackson, P. (1996). Wild Cats: Status Survey and Conservation Action Plan. IUCN/SSC Cat Specialist Group, Gland, Switzerland.

O'Brian, S. (2001). Cat Family. Pp. 8-39 The Encyclopedia of Mammals. (Ed. D Macdonald). Oxford, UK: Andromeda Oxford Limited.

Ogdem, R., Dawnay, N. and McEwing, R. (2009). Wildlife DNA forensics—bridging the gap

between conservation genetics and law enforcement, *Endang Species Res*: 1-17.

Pocock, R. I. (1917). "The classification of the existing Felidae". *Annals and Magazine of Natural History*. Series *8*. XX(119): 329–350.

Thompson, J. D., Gibson, T. J. and Higgins, D. G. (2003). Multiple sequence alignment using Clustal W and ClustalX. *Current protocols in bioinformatics*, (1), 2-3.

Vaughan, T., Ryan, J. and Czaplewski, N. (2000). Mammalogy. Fourth Edition. Philadelphia, PA: Brooks/Cole-Thomson Learning.

Wozencraft, W. C. (2005). Mammal Species of the World: A Taxonomic and Geographic Reference(3rd ed.).P 544 (Eds Wilson, D.E.; Reeder, D.M.)). "Genus *Profelis*". Johns Hopkins University Press.

Wu, H., Wan, Q.H., Fang, S.G. and Zhang, S.Y. (2005). Application of mitochondrial DNA sequence analysis in the forensic identification of Chinese sika deer subspecies. *Forensic Sci. Int.*, *148*: 101–105.

How to cite this article: Archana Bahuguna and Priyanka Negi (2022). Use of Taxidermy Samples to Generate Forensically Informative Nucleotide Sequences of Globally threatened Species of Small cats including the Species from India by using 12SrRNA and Cytochrome b gene. *Biological Forum – An International Journal*, *14*(1): 861-869.